

COGNITIVE NEUROSCIENCE

The effect of task-irrelevant visual backgrounds on human transcranial magnetic stimulation-evoked electroencephalography responses and cortical alpha activity

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Abstract

Brain responses evoked by transcranial magnetic stimulation (TMS) in task-free experimental contexts are known to depend on psychophysiological states such as sleep, vegetative state and caffeine-induced arousal. Much less is known about how TMS-evoked responses depend on task-irrelevant steady perceptual input. Here, we examined ongoing alpha activity and the mean amplitude of EEG potentials in response to occipitally applied TMS as a function of task-irrelevant visual backgrounds. Responses to TMS were robustly modulated by photographs of natural scenes and man-made environments. These effects began as early as during the N100 and continued for several hundred milliseconds after the stimulation. There was also a more general effect of background along with other stimuli, such as blank backgrounds, sinusoidal gratings and moving dot-patterns. This effect was observable from ongoing alpha activity as well. Based on these results we conclude that different types of steady perceptual input modulate visual cortex reactivity and/or connectivity and it is possible to measure these modulations by combining TMS with electroencephalography.

Introduction

Transcranial magnetic stimulation (TMS) in combination with electroencephalography (EEG) has been used repeatedly to study state-dependent reactivity and connectivity of the human brain. TMS-evoked responses during wakefulness are characterized by complex patterns of recurrent activity lasting for several hundred milliseconds and involving a distributed network of cortical areas (Massimini *et al.*, 2005; Garcia *et al.*, 2011). In striking contrast to this are the locally confined, strong but short-lived responses to TMS during quiet sleep (Massimini *et al.*, 2005) or vegetative state (Rosanova *et al.*, 2012). Negativity of the slow TMS-evoked potential increases with caffeine-induced change in alertness (Murd *et al.*, 2010) and decreases in quiet sleep (Stamm *et al.*, 2011).

Transcranial magnetic stimulation can also be deployed to probe reactivity/connectivity during more transient states such as variable rhythmical baseline activity. Posterior alpha oscillations have long been associated with visual receptiveness. This view is strengthened by results showing that phosphenes are more likely to be perceived if TMS is applied at a time when alpha power is low (Romei *et al.*, 2008) or when its phase is optimal (Dugué *et al.*, 2011). It is impor-

tant to note that the same results are also obtained with generic sensory stimuli (van Dijk *et al.*, 2008; Mathewson *et al.*, 2009). This indicates that TMS taps into primal processes of perception and should thus interact with those processes in meaningful ways.

The exact mechanism by which TMS exerts its influence on neural processes is still a matter of considerable debate, but whether by direct cortico-cortical connections (Massimini *et al.*, 2005) or by cortico-subcortico-cortical interactions (Logothetis *et al.*, 2010), TMS is capable of influencing other regions besides the site of stimulation. With this in mind, we wanted to take the state-dependency concept one step further. Whereas responses to TMS should first and foremost depend on the level of processing going on in the target region, the current state of other associated areas might also have a bearing on the outcome. We thus asked whether the responses to TMS could be modulated by the information currently processed in the visual cortex.

In our experiment subjects performed a simple visual detection task while the task-irrelevant background image was varied. We selected several classes of background images on the basis of expected differences in engaging visual cortical areas (e.g. sine-wave gratings or photographs). Furthermore, each background class was represented by two dissimilar subcategories (e.g. high- and low-frequency gratings). As different background images might modulate the state of alertness in the visual system and thus influence receptiveness to TMS (van Dijk *et al.*, 2008; Romei *et al.*,

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2008; Mathewson *et al.*, 2009; Dugué *et al.*, 2011) we first analysed differences in ongoing alpha activity as a function of visual backgrounds. We then ascertained whether the TMS-evoked response could differentiate between our experimental conditions as well. We put forward the hypothesis that TMS-evoked responses will differ between classes of task-irrelevant visual backgrounds.

Materials and methods

Subjects

Ten subjects [five male, aged 19–32 years (mean 25.2, SD = 3.9)] participated in the TMS-EEG experiments. Two subjects were left-handed. All had normal or corrected to normal vision. Subjects gave written informed consent prior to participation. The study was approved by the ethics committee of the University of Tartu and the experiments were undertaken in compliance with national legislation and the Declaration of Helsinki.

Stimuli

We selected several classes of background images on the basis of expected differences in engaging visual cortical areas. Because V1 is involved in brightness and spatial-frequency processing (Rossi & Paradiso, 1999; Nauhaus *et al.*, 2012) we used black and white backgrounds and high- vs. low-spatial-frequency sine wave gratings. We also used backgrounds involving rightward and leftward motion (V2 and V5 are known to be involved in motion discrimination – Sack *et al.*, 2006; Silvanto & Muggleton, 2008; Lu *et al.*, 2010) and photographs of natural scenes vs. scenes depicting man-made environments. The processing of these photographic backgrounds should be more elaborate in comparison with the other backgrounds because of, for example distributed object processing in occipital and temporal cortices (Ishai *et al.*, 2000) including areas V4/V8 and the lateral occipital complex (Lerner *et al.*, 2001). Thus, the processing of photographic background should incorporate the most extensive network of brain areas.

Visual background stimuli were divided into four general classes, each with two subcategories: blank backgrounds (black or white), gratings (high or low frequency), photographs (of natural scenes or man-made environments) and moving dot-patterns (to the left or to the right). Figure 1 depicts examples of each of the different background types. Backgrounds covered the whole monitor ($18 \times 25^\circ$ of visual angle). Space-average luminance of the individual backgrounds was the following: 0.8 cd/m² for black, 73.9 cd/m² for

white, 21.8 cd/m² for high-frequency gratings, 24.6 cd/m² for low-frequency gratings, 11.8 cd/m² for photographs of natural scenes, 11.7 cd/m² for photographs of man-made environments and 1.2 cd/m² for moving dot-patterns. The spatial frequency of high- and low-frequency sine-wave gratings was 4 and 0.4 cycles per degree of visual angle, respectively, with a Michelson contrast of 0.3. The orientation of the gratings varied randomly. The moving dot patterns consisted of a black background with randomly placed white dots (3×3 pixels each). The velocity of the pattern was 1.3° of visual angle per second. The backgrounds depicting natural scenes or man-made environments comprised four photographs, two for each type. Furthermore, each of the four photographs was presented with high as well as with low contrast. The root mean square (RMS) contrast for high-contrast images was 0.22 cd/m² and that for low-contrast images was 0.14 cd/m².

A fixation cross was placed in the middle of the screen (0.4°). At random times the fixation cross was replaced by a square-Landolt stimulus (0.5°) for 500 ms. The fixation cross and Landolt were either black or white, depending on the background, to ensure their visibility.

Task and design

Subjects were seated in a dimly lit room, 80 cm from the screen. Stimuli were presented on a SUN CM751U monitor (1024×768 pixels) at 100 Hz refresh rate. The experiment comprised 48 trials in total. There were 32 trials for white, black, high-frequency and low-frequency backgrounds (eight trials for each background) and 16 trials for rightward movement, leftward movement, natural scenes and man-made environments (four trials for each background). On each trial one of the different backgrounds was presented for 30 s. The presentation order of the backgrounds was fully randomized for each subject. The subjects were instructed to fixate on a cross in the middle of the screen and to pay attention to randomly occurring Landolt squares. Their task was to report via a button press whether they detected a Landolt on a given trial and, if so, on which side the gap of the last Landolt (in case there were several) was. On six of the trials three Landolts occurred. Two Landolts occurred on 18 trials and one Landolt occurred also on 18 trials. On six trials no Landolts occurred at all. The order of these trials was fully randomized and the occurrence of Landolts was evenly distributed over time. This was accomplished by dividing each trial into three 10 s long segments and allowing for only one Landolt to occur at a random time within each segment, but the likelihood of occurrence was still equal for all three segments. The task was designed to be quite easy and, indeed, subjects gave a correct answer on most of the trials. Errors were made for 2.7% of the trials on average (ranging from 0 to 8%).

TMS stimulation

The target area for magnetic stimulation was determined on individual anatomical images prior to the experiment. Target location in V1 was placed over the calcarine fissure by visual inspection. Precise stimulation of the target area was controlled with a navigated brain stimulation system (Nexstim Ltd, Helsinki, Finland).

A TMS-system (Nexstim Ltd) with figure-of-eight coil was used for stimulation. Single pulses with duration < 1 ms were applied. The intensity was 30% of the maximum output of the apparatus (maximal output 0.7 T in cortex). The relatively low pulse intensity guaranteed that subjects did not experience phosphenes. TMS was delivered with an inter-stimulus interval varying haphazardly between 2.5 and 3.5 s.

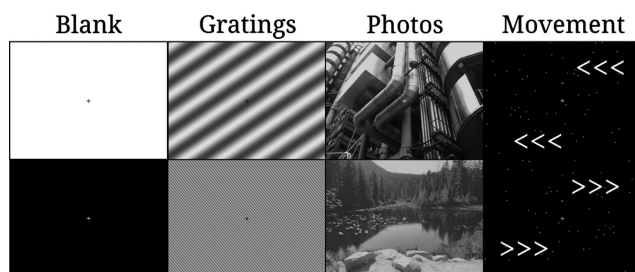


FIG. 1. Different backgrounds used in the experiment. The orientation of the gratings was random. All photographs were presented with both high and low contrast. The upper photograph is an example of man-made environments with high contrast. The lower photograph depicts a natural scene with low contrast. Randomly placed white dots moved either leftward or rightward ('<<<' indicates direction of movement). A fixation cross was present throughout the trials.

EEG recordings

We used a Nexstim eXimia EEG-system with 60 carbon electrodes cap (Nexstim Ltd). The following 11 electrodes were prepared for recording: F7, F8, Fcz, T7, Cz, T8, TP9, CP5, CP6, TP10, P1 and P2 of the extended 10–20 system. No occipital electrodes were prepared for recording because of the expected movement/pressure artifacts from the coil. The impedance at all electrodes was kept below 15 k Ω . The EEG signals were referenced to an additional reference electrode placed on the forehead and sampled at 1450-Hz sampling rate. All signals were amplified with a gain of 2000; the bandwidth of the signal was ca. 0.1–350 Hz. As our system only allows one pair of eye-electrodes the vertical electrooculogram was recorded in addition to the EEG.

EEG preprocessing

EEG data were analysed with FieldTrip (<http://fieldtrip.fcdonders.nl>; version 01-10-2012). For the analysis of TMS-evoked potentials (TEPs), data were epoched around TMS-stimulation (–200 to +1000 ms). Epochs were baseline corrected with a 200-ms time period before TMS-stimulation onset and linear trends were removed from the data. For the frequency analysis of pre- and post-stimulus activity, 1-s epochs prior to TMS-stimulation (–1000 ms to 0 ms) and 1-s epochs after TMS-stimulation (+100 ms to +1100 ms) were investigated. Again, linear trends were removed from the data. All epochs were inspected manually for artefacts. Epochs containing eye movements and other artefacts were removed from the data. To exclude any effects the main detection task may have had on TMS-evoked responses all epochs where a Landolt stimulus occurred were also removed from further analysis.

Although our EEG system is TMS-compatible and the pulse-induced artefact is greatly reduced by the hardware it is evident that remnants of the artefact are still present in the data. Filtering over these artefacts can distort the TEPs and lead to erroneous results. For this reason we chose to conduct all EEG analyses on raw unfiltered data. For nicer plots, however, data were additionally processed in the following way. First, the TMS-induced artefacts were removed by replacing data from TMS onset until 70 ms after stimulation with zero values. Second, data were filtered with a 10-Hz low-pass zero phase shift Butterworth filter. After that, condition-specific averages were created in the conventional way.

EEG analysis

Electrodes were clustered into four groups: a parietal group (electrodes P1 and P2), a frontal group (electrodes F7, F8 and Fcz) and two temporal groups (left electrodes T7, TP9 and CP5; right electrodes T8, TP10 and CP6). The data from single electrodes within each group were pooled together for the analyses.

The experimental conditions were (1) high luminance uniformly coloured achromatic background, (2) low luminance uniformly coloured achromatic background, (3) background filled with a high-spatial-frequency sine wave grating, (4) background filled with a low-spatial-frequency sine wave grating, (5) dark background covered by random luminous dots moving left, (6) dark background covered by random luminous dots moving right, (7) backgrounds depicting natural scenes including plants and animals, (8) backgrounds depicting man-made environments including furniture, electronic equipment or industrial plant milieu.

On average, the following number of epochs were available after artefact rejection: for white backgrounds: mean = 56.5, SD = 12.7;

for black backgrounds: mean = 58.4, SD = 12.2; for high-frequency backgrounds: mean = 60, SD = 11.1; for low-frequency backgrounds: mean = 51.9, SD = 11.2; for dot-patterns moving to the left: mean = 28.5, SD = 4.9; for dot-patterns moving to the right: mean = 26.8, SD = 5.1; for photographs depicting natural scenes: mean = 28.1, SD = 9.8; for photographs depicting man-made environments: mean = 29.2, SD = 7.2. Very similar quantities were available for the frequency analyses of pre- and post-stimulus activity.

To assess differences for specific frequencies across electrode groups and experimental conditions, frequency representations of 1-s epochs prior to TMS and 1-s long epochs shortly after TMS were obtained with single Hanning tapers for frequencies 4–40 Hz. After the frequency transformation, epochs were averaged according to the experimental conditions and electrodes were grouped together. For the analysis of alpha activity, peak alpha was determined individually for each subject on their grand averages over all trials (mean = 10.8, SD = 1.03). For an exploratory analysis of a wider frequency spectrum frequencies from 4 to 40 Hz (in steps of 2 Hz) were tested.

Mean amplitude was used for analysis of TEP components. First, component peaks were identified from a grand average over all conditions and all subjects (Fig. 2 shows their latencies and peak amplitudes) and, second, the length of the period for mean amplitude was determined for each peak by visual inspection. The following criteria were applied: for N1, 108 ± 30 ms; for P2a, 182 ± 40 ms; for P2b, 275 ± 40 ms; for late negativity, 400–600 ms mean amplitude.

We chose to analyse mean amplitude of TEP components (and neither peak amplitude nor peak latency) because this measure is not biased by unequal signal-to-noise ratios across conditions (Luck, 2005). Therefore, it is not necessary to equalize the number of epochs for all conditions. On the contrary, it is even advisable to include all available epochs because this can only enhance statistical power without increasing the risk for type II errors (Luck, 2005).

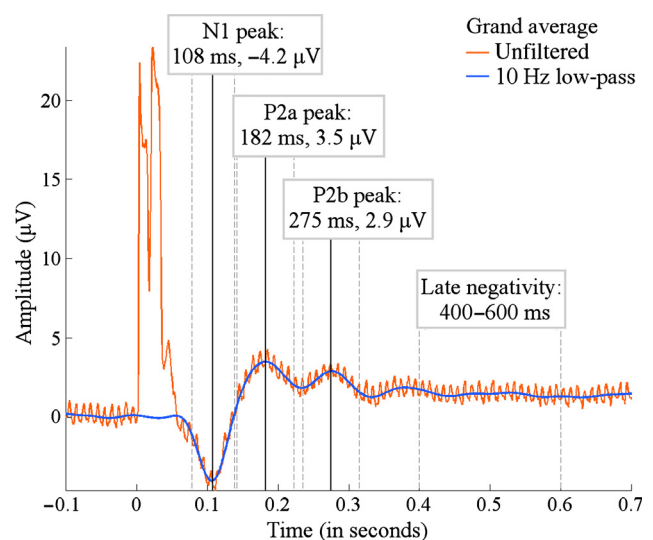


FIG. 2. The grand average TEP over all conditions and all subjects. Both unfiltered data and its filtered version (10 Hz low-pass) are shown. Before filtering, TMS-induced artefacts were removed by replacing data from TMS onset until 70 ms after stimulation with zeros. Solid black lines mark the component peaks. Peak latencies and amplitudes are described in the connected boxes. Dashed lines mark the areas for mean amplitude per component. [Color figure version available online.]

Although mean amplitude is not biased by an unequal number of epochs between the conditions, we still repeated all the TEP analyses with an equal number of epochs to demonstrate the validity of our results. For each subject the condition with the minimum number of epochs was determined. An equal number of epochs were randomly selected also from all other conditions and the analyses were performed as before.

If not indicated otherwise, all *post-hoc* contrasts were corrected with the Holm–Bonferroni method. This correction method is always more powerful than the classical Bonferroni procedure and valid for arbitrary assumptions, although it still remains quite conservative (Wright, 1992).

Results

Frequency analysis of pre- and post-TMS activity

Do TMS-evoked responses depend on the nature of information the visual cortex currently processes? Before we can answer this question we must first determine if there are any differences in the ongoing brain activity originating from varying sensory input alone. After all, it is possible that differences in EEG responses are mainly attributable to differences in sensory input and TMS does not actually contribute any additional information to these effects. Specifically, it has been demonstrated in several contexts that pre-stimulus alpha activity reflects the state of alertness in the visual system and can modulate subsequent processing of the external stimuli (van

Dijk *et al.*, 2008; Romei *et al.*, 2008; Mathewson *et al.*, 2009; Dugué *et al.*, 2011). As alertness could also be different between the conditions in our study, we asked whether the power of ongoing alpha activity could differentiate between our experimental conditions.

We analysed individual peak alpha-power of 1-s periods before TMS and 1-s periods after TMS. A four-way repeated-measures analysis of variance (ANOVA) with epoch (before or after TMS), electrode group, background class and subcategory as factors revealed a main effect of electrode group ($F_{3,27} = 18.8$, $P = 8.7\text{e-}07$) and a main effect of background class ($F_{3,27} = 7.2$, $P = 0.001$), but there was also a significant interaction between electrode group and background class ($F_{9,81} = 5.6$, $P = 5.4\text{e-}06$). The main effect of subcategory was not significant ($F_{1,9} < 1$). Neither were significant any of the other interactions (max $F_{3,27} = 2.4$, $P = 0.1$). Importantly, there were no reliable differences between pre- and post-TMS epochs, indicating that alpha activity was modulated by the different visual stimuli but not by TMS. To find out which electrode groups bore the effect of background class, three-way repeated-measures ANOVAs with epoch (before or after TMS), background class and subcategory as factors were carried out for each electrode group separately. The results are shown in Table 1 and Fig. 3 illustrates the differences.

As can be seen from Table 1, *post-hoc* analyses for all electrode groups showed reliable differences between background classes. There were no reliable differences between subcategories and the interactions were also not significant (see Table S1 for a comprehensive collection of uncorrected *post-hoc* contrasts). There was a marginally significant effect of epoch for frontal and left temporal

TABLE 1. Experimental effects on alpha power, separately for each electrode group

	Frontal	Left temporal	Right temporal	Parietal
Epoch	$F_{1,9} = 8$, $P = 0.08$	$F_{1,9} = 7.5$, $P = 0.08$	$F_{1,9} < 1$	$F_{1,9} < 1$
Background class	$F_{3,27} = 3.2$, $P = 0.04$	$F_{3,27} = 5.2$, $P = 0.01$	$F_{3,27} = 6.4$, $P = 0.007$	$F_{3,27} = 7.9$, $P = 0.007$
Subcategory	$F_{1,9} < 1$	$F_{1,9} < 1$	$F_{1,9} < 1$	$F_{1,9} < 1$
Interactions	Max. $F_{3,27} = 1.4$, $P = 0.53$	Max. $F_{1,9} = 2.8$, $P = 0.52$	Max. $F_{3,27} = 2$, $P = 0.52$	Max. $F_{3,27} = 1.3$, $P = 0.53$

Each column contains the ANOVA results for one of the electrode groups. Rows are for the main effects of epoch, background class and subcategory. Because there were no significant interactions only the interaction with the highest F -value is shown. Cells containing significant effects are in bold. P -values are corrected for multiple comparisons.

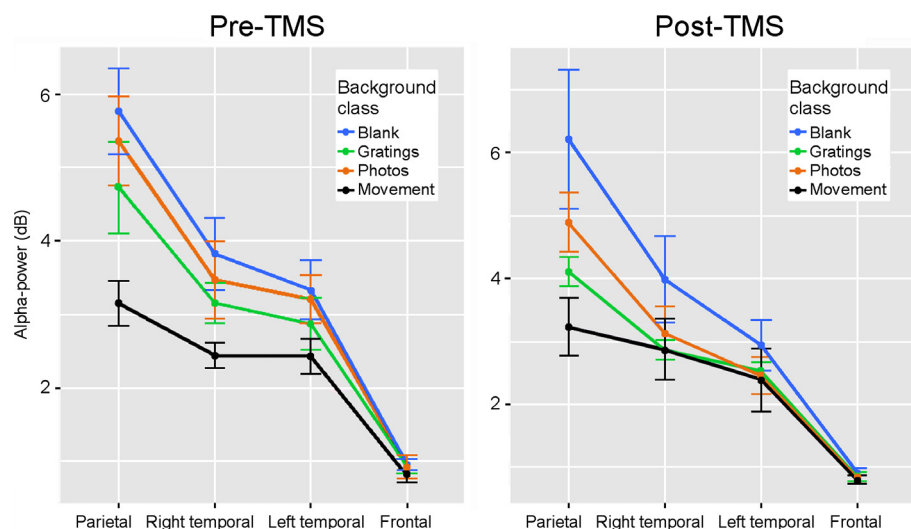


FIG. 3. Mean alpha power (\pm SEM) per electrode group and background class, separately for the pre-TMS baseline epoch (left) and post-TMS epoch (right). Peak alpha power was determined individually for all subjects. [Color figure version available online.]

electrodes. For these electrode groups alpha power tended to be higher during the epoch prior to TMS ($m_{\text{pre}} = 0.91$ vs. $m_{\text{post}} = 0.85$ for frontal electrodes and $m_{\text{pre}} = 2.96$ vs. $m_{\text{post}} = 2.59$ for left temporal electrodes). To find out which pairs of background classes are reliably different from each other, alpha power was pooled over epochs and *post-hoc* contrasts were tested for each pair of background classes. Due to the significant interaction between electrode groups and background classes we performed the *post-hoc* contrasts separately for each electrode group as well as for averages over all electrode groups. The results are shown in Table 2.

Differences in alpha power were most reliable between blank backgrounds and moving dot-patterns. This contrast was significant for the parietal and the right temporal electrode group, but marginally significant for the frontal and the left temporal electrode group. Differences between blank backgrounds and gratings were reliable only for the parietal electrode group. Pooling over electrode groups also revealed a marginally significant difference between photographs and moving dot-patterns.

In addition to the analysis of alpha activity, we also conducted an explorative investigation of frequencies between 4 and 40 Hz (in steps of 2 Hz) with all electrode groups. Four-way repeated-measures ANOVAs with epoch, electrode group, background class and subcategory as factors were conducted for each of the 19 frequencies and the *P*-values were corrected for multiple comparisons with the Holm–Bonferroni method. Differences between electrode groups were significant for frequencies 4–14 Hz (max $F_{3,27} = 24.6$, $P = 1.3\text{e-}06$). For these frequencies power was always strongest on parietal electrodes and weakest on frontal electrodes. Differences between background classes were significant for 14 Hz ($F_{3,27} = 8.6$, $P = 0.007$). The interaction between electrode groups and background classes was significant for 10 Hz ($F_{9,81} = 4.3$, $P = 0.002$). The difference between subcategories was marginally significant for 16 Hz ($F_{1,9} = 15.1$, $P = 0.07$). 16 Hz power tended to be lower for black backgrounds, low-frequency gratings, photographs of natural scenes and leftward moving dot-patterns ($m = 0.88$) compared with the complementary set of subcategories ($m = 0.95$). The interaction between epochs and electrode groups was significant for 18 Hz ($F_{3,27} = 6.8$, $P = 0.028$). 18 Hz power was lower on frontal electrodes after TMS ($m_{\text{pre}} = 0.76$ vs. $m_{\text{post}} = 0.7$) but higher on other electrode groups ($m_{\text{pre}} = 1.28$ vs. $m_{\text{post}} = 1.3$). Detailed results from this explorative analysis of the baseline activity can be found in Table S2.

Analysis of TMS-evoked responses

Now that we have established the baseline differences between conditions we turn to the central question of the study at hand. Do TMS-evoked responses differ depending on the background image the subject currently perceives? Because we found differences in ongoing alpha activity we have good reason to expect some differ-

ences in TMS-evoked responses as well. Thus, the critical question is rather if the analysis of TMS-evoked responses is able to enhance these differences or bring out differences which are not already evident from alpha activity. Although previous research suggests that early visual event-related components might be generated by a phase reset of ongoing alpha oscillations (Hanslmayr *et al.*, 2007), several authors have argued that phase resetting probably constitutes only one of the mechanisms underlying event-related potential generation (see Sauseng *et al.*, 2007 for a critical review). Thus, TMS-evoked responses might carry important additional information.

Our hypothesis was that general classes of backgrounds have an effect on the TMS-evoked responses. To test this hypothesis, four general classes of backgrounds and their subcategories were contrasted over the TEP components and electrode groups (Fig. 4A shows the corresponding time courses of TEPs; Table S3 shows the time courses separately for each electrode group and Table S4 shows the time courses over a 2-s period centred on TMS). A four-way repeated-measures ANOVA with backgrounds, subcategories, TEP components and electrode groups as factors yielded an expected main effect of TEP component ($F_{3,27} = 22.2$, $P = 1.9\text{e-}07$), a main effect of electrode group ($F_{3,27} = 9$, $P = 0.0003$) and an interaction between TEP component and electrode group ($F_{9,81} = 15.6$, $P = 1.9\text{e-}14$). The main effect of background class was marginally significant ($F_{3,27} = 2.9$, $P = 0.055$). The main effect for subcategory was also marginally significant ($F_{1,9} = 4.4$, $P = 0.066$). There was, however, a significant interaction between background class and subcategory ($F_{3,27} = 3.6$, $P = 0.027$).

Thus, as with the analysis of alpha activity there is again an effect of background class and for at least one class there are also differences between subcategories. To determine if the effect of background class is based on any specific differences, *post-hoc* contrasts were tested (Table 3). The pairwise comparisons of background classes did not confirm significant differences for any of the compared pairs. For the current sample differences in mean amplitude were largest between blank backgrounds and moving dot-patterns for all TEP components. Note that this fits with the findings from alpha activity where the difference between blank and moving backgrounds was significant. However, contrary to the differences in alpha activity, which were most reliable on parietal electrodes, the effect of background class on TEPs seems to be present on all electrode groups as we did not find any interaction between electrode groups and background classes.

Post-hoc comparisons between subcategories within background classes revealed a pronounced difference between TMS-evoked responses to natural photographs compared with man-made photographs. Mean TEP for photographs depicting natural scenes was more negative for all TEP components. All other comparisons between subcategories within background classes were far from significant (Fig. 4B–E shows the pair-wise time courses of TEPs; Table S4 shows the results separately for each electrode group).

TABLE 2. Alpha power contrasts of background classes, separately for each electrode group

	Blank vs. gratings	Blank vs. photos	Blank vs. movement	Gratings vs. photos	Gratings vs. movement	Photos vs. movement
Frontal	$T_9 = 1.8$, $P = 0.49$	$T_9 = 1.7$, $P = 0.49$	$T_9 = \mathbf{3.2}$, $P = \mathbf{0.07}$	$T_9 = 0.4$, $P = 0.72$	$T_9 = 1.6$, $P = 0.49$	$T_9 = 1.4$, $P = 0.49$
Left temporal	$T_9 = 2.4$, $P = 0.16$	$T_9 = 2.8$, $P = 0.11$	$T_9 = \mathbf{3.2}$, $P = \mathbf{0.06}$	$T_9 = -0.7$, $P = 0.51$	$T_9 = 1.3$, $P = 0.44$	$T_9 = 2.4$, $P = 0.16$
Right temporal	$T_9 = \mathbf{3.1}$, $P = \mathbf{0.07}$	$T_9 = 1.9$, $P = 0.26$	$T_9 = \mathbf{3.4}$, $P = \mathbf{0.05}$	$T_9 = -1.2$, $P = 0.52$	$T_9 = 1.2$, $P = 0.52$	$T_9 = 2.7$, $P = 0.1$
Parietal	$T_9 = \mathbf{3.4}$, $P = \mathbf{0.04}$	$T_9 = 1.7$, $P = 0.39$	$T_9 = \mathbf{3.6}$, $P = \mathbf{0.03}$	$T_9 = -1.2$, $P = 0.39$	$T_9 = 1.6$, $P = 0.39$	$T_9 = 2.7$, $P = 0.1$
Pooled over all electrode groups	$T_9 = \mathbf{3.7}$, $P = \mathbf{0.03}$	$T_9 = 2.1$, $P = 0.19$	$T_9 = \mathbf{3.7}$, $P = \mathbf{0.03}$	$T_9 = -1.1$, $P = 0.33$	$T_9 = 1.5$, $P = 0.33$	$T_9 = \mathbf{2.8}$, $P = \mathbf{0.08}$

Each column contains the results of one paired *t*-test between a pair of background classes. Rows are for different electrode groups. Cells containing significant effects and trends are in bold. *P*-values are corrected for multiple comparisons.

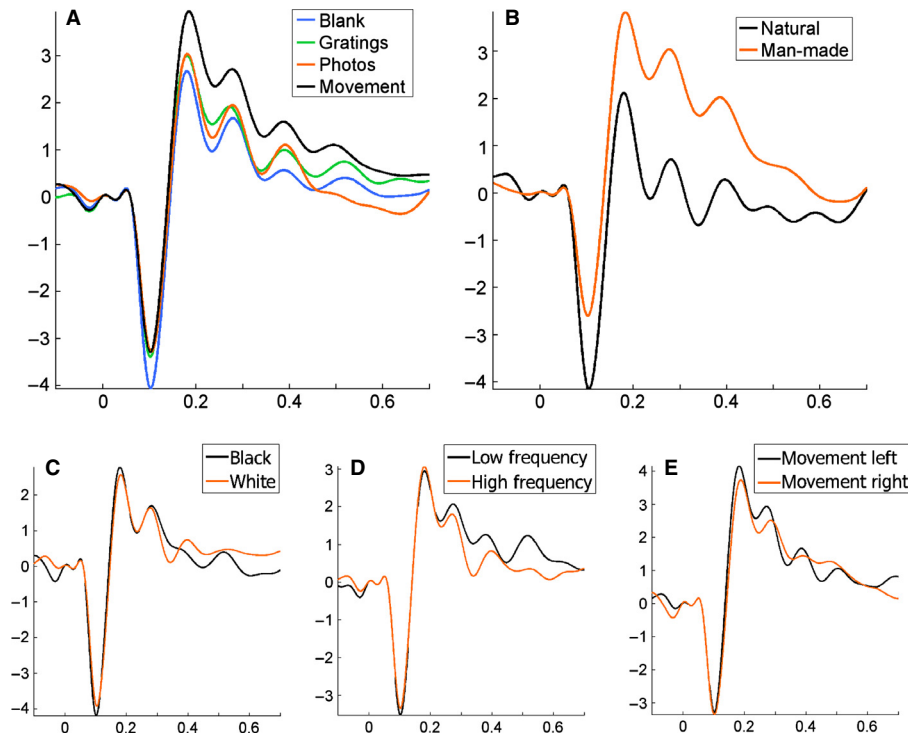


FIG. 4. EEG responses to TMS recorded while subjects were seeing different types of task-irrelevant visual backgrounds. Potentials are pooled together over frontal, parietal and temporal electrodes. (A) Four main classes of backgrounds were used in this experiment: blank backgrounds (black and white), gratings (high- and low-frequency sine-waves), photographs (of natural scenes and man-made environments) and moving dot-patterns (to the left or to the right). (B–E) TMS-evoked responses divided by the two subcategories belonging to each of the four main background classes.

TABLE 3. Pairwise comparisons of TEPs by background classes and their subcategories

	Black/white	High frequency/low frequency	Natural/man-made	Movement left/movement right
Black/white	$T_9 = 0.1, P = 1$	$T_9 = -1.4, P = 0.62$	$T_9 = -1.5, P = 0.62$	$T_9 = -2.4, P = 0.24$
High frequency/low frequency		$T_9 = -0.2, P = 1$	$T_9 = 0.3, P = 0.77$	$T_9 = -1.8, P = 0.53$
Natural/man-made			$T_9 = -4, P = \mathbf{0.012}$	$T_9 = -1.5, P = 0.62$
Movement left/movement right				$T_9 = 0.3, P = 1$

Contrasts on the main diagonal are between subcategories within one background class. Other contrasts are between different background classes. *P*-values are corrected for multiple comparisons. Cells containing significant effects are in bold.

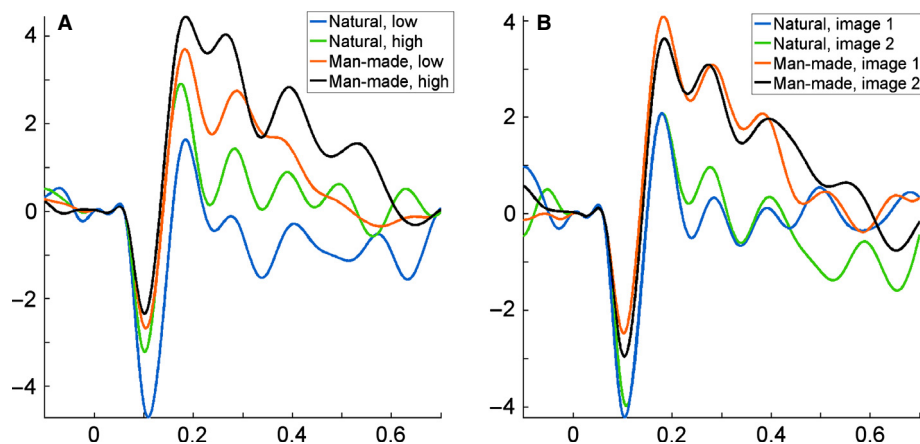


FIG. 5. TMS-evoked EEG responses recorded when photographs of natural scenes and man-made environments were seen, pooled together over frontal, parietal and temporal electrodes. (A) Both categories of photographs were shown with high as well as with low contrast. There was a substantial effect of image category, but contrast did not produce reliable differences. (B) Both image categories were represented by two individual photographs. The difference between natural scenes and man-made environments was evident for both exemplars of the respective categories.

TABLE 4. Pairwise comparisons of TEPs by background classes and their subcategories after accounting for the covariance with alpha power

	Black/white	High frequency/low frequency	Natural/man-made	Movement left/movement right
Black/white	$T_9 = 0.3, P = 1$	$T_9 = -0.6, P = 1$	$T_9 = -0.5, P = 1$	$T_9 = -1.8, P = 0.57$
High frequency/low frequency		$T_9 = -0.4, P = 1$	$T_9 = 0.02, P = 1$	$T_9 = -1.5, P = 0.74$
Natural/man-made			$T_9 = -3.5, P = \mathbf{0.026}$	$T_9 = -1, P = 1$
Movement left/movement right				$T_9 = 0.2, P = 1$

Contrasts on the main diagonal are between subcategories within one background class. Other contrasts are between different background classes. *P*-values are corrected for multiple comparisons. Cells containing significant effects are bold.

Mean amplitude of TEPs is not biased by different signal-to-noise ratios, i.e. by an unequal number of epochs between conditions (Luck, 2005). As long as variance is comparable between the conditions (which is true for our data; see Table S5) all available epochs should be included in the TEP calculation because it increases statistical power. Nonetheless, we repeated the above analyses with equalized signal-to-noise ratios (for details see Methods) and found very similar results for our experimental factors (see Table S6 for results).

To investigate further the dissociation between natural scenes and man-made environments, two additional *post-hoc* analyses were carried out. First, the backgrounds within both categories were divided into high- and low-contrast images (see Fig. 5A). A four-way repeated-measures ANOVA with backgrounds (natural scenes vs. man-made environments), luminance contrasts (high vs. low), TEP components and electrode groups as factors revealed a significant main effect of TEP component ($F_{3,27} = 20.9, P = 6.5\text{e-}07$) and electrode group ($F_{3,27} = 5.2, P = 0.009$). The main effect for background was also significant ($F_{1,9} = 17.1, P = 0.0032$), but the main effect for contrast was not significant ($F_{1,9} = 3.6, P = 0.18$) nor was the interaction between background and contrast ($F_{1,9} < 1$). Thus, contrast does not explain the difference in EEG responses to TMS for natural scenes and for man-made environments.

Second, because both categories were represented by only two different photographs we also checked if the difference between natural scenes and man-made environments was present for all four photographs (see Fig. 5B). A four-way repeated-measures ANOVA with backgrounds (natural scenes vs. man-made environments), individual photographs (exemplar 1 vs. exemplar 2), TEP components and electrode groups as factors again revealed a significant main effect of TEP component ($F_{3,27} = 20.7, P = 6.5\text{e-}07$), electrode group ($F_{3,27} = 5.5, P = 0.009$) and background ($F_{1,9} = 19.8, P = 0.0032$). There was no main effect of individual photographs ($F_{1,9} < 1$) and no interaction between background categories and individual photographs ($F_{1,9} < 1$), although there was a tendency to an interaction between electrode groups, backgrounds and individual photographs ($F_{3,27} = 3.3, P = 0.073$). However, further inspection revealed that this trend originated solely from the different ordering of individual photographs of background categories for different electrode groups, and not from changes in the main effects (see Table S7 for separate ANOVAs for each sensor group). These analyses were, again, repeated with equalized signal-to-noise ratios, but the main results did not change (see Tables S8 and S9 for results). Thus, these results demonstrate conclusively that the difference between natural scenes and man-made environments was not a mere side-effect of some aberrant images.

Relationship between ongoing alpha activity and TMS-evoked responses

Our previous results indicate that different classes of backgrounds modulate the power of ongoing alpha activity as well as responses

to TMS. But is there a relationship between these two measures? If we assume that responses to TMS are modulated by the current state of the brain then it is to be expected that differences in ongoing alpha activity are also reflected in the TMS-evoked responses. We therefore tested if alpha activity can account for the observed differences in TMS-evoked responses.

We performed a two-way repeated-measures analysis of covariance with background class and subcategory as factors and alpha power as a covariate. Data were pooled over epochs (before and after TMS), TEP components and electrode groups to simplify the analysis. After accounting for the effect of alpha power there was still a significant interaction between background class and subcategory ($F_{3,26} = 3.4, P = 0.034$). *Post-hoc* comparisons with adjusted values confirmed that this interaction was based on the significant difference between TMS-evoked responses to photographs of natural scenes compared with photographs depicting man-made environments (Table 4). The main effect of subcategory was marginally significant ($F_{1,8} = 4.3, P = 0.073$). Contrary to the previous results, the main effect of background class was no longer marginally significant ($F_{3,26} = 71.8, P = 0.17$). Thus, it seems that alpha activity does indeed covary with TMS-evoked responses to different background classes, but it cannot explain the difference in TMS-evoked responses to photographs of natural scenes and scenes depicting man-made environments.

Discussion

We set out with the hypothesis that different types of background classes modulate responses to TMS. Our results confirmed the hypothesis. There was an effect of background class on TMS-evoked brain responses. Although the effect of background image was already evident from ongoing alpha activity – and may thus be originating solely from differences in sensory input – it is nonetheless noteworthy that these differences are also reflected in the TMS-evoked EEG responses. In addition to the expected effect of background class, we also found a pronounced difference in TMS-evoked responses for different types of photographs. Stimulation during natural scenes led to an initially stronger negative-going TEP around 100 ms and stayed relatively more negative over the course of several hundred milliseconds after the pulse. The response during scenes depicting man-made environments was overall more positive. Importantly, this difference between natural scenes and man-made environment was not evident in the ongoing oscillatory activity.

Thus, the main message of the present investigation is twofold. First, EEG responses to TMS can depend on the informational content processed by the visual cortex and associated areas. Second, and surprisingly, differences in TMS-evoked responses are clearest between photographs of natural scenes and man-made environments. If no background image effects would have been found, it would be easy to deny the possibility of probing differences in visual cortex reactivity/connectivity with TMS – either due to the robustness of

the TMS-evoked perturbations compared with the intricate patterns of naturally occurring neural processes or because of the principal non-specificity of the TMS-evoked activity. However, because the effect of task-irrelevant visual backgrounds was found for different categories of photographs we must conclude that artificially produced, task-independent neural activity can be influenced by the type of modal information processing going on in the brain.

The TMS-evoked activation observed in this study is comparable with the results of other studies using occipital TMS. For example, visual cortex responses to TMS were analysed by Casali *et al.* (2010). The spatial distribution and time course of the responses revealed a reproducible profile of reactivity and connectivity, characterized by a selective propagation of TMS-evoked activation from occipital to frontal areas that reached a maximum at 70–100 ms. Similar values were found for responses to occipital TMS when quiet sleep and caffeine-induced effects were studied (Murd *et al.*, 2010; Stamm *et al.*, 2011). The N100 latency found in the present work conforms to this time window. Furthermore, our results show that the amplitude of this early peak is modulated by the different types of backgrounds used in our experiment.

In addition to the pronounced effect of different categories of photographs we also observed an effect of general background classes, but several factors make it difficult to draw any definite conclusions from this result. First, *post-hoc* contrasts did not reveal significant differences between any specific pair of background classes. This by itself should not trouble us, because we did not have any expectancy about specific differences between background classes in the first place. Our hypothesis was a general one stating that there is an overall effect of background class. Second, we must consider that an analysis of ongoing alpha activity revealed very similar results concerning the four background classes and the effect of background class vanished when alpha-power was added as a covariate in an analysis of covariance. It may thus be that responses to TMS, in particular, reflect stimulus-driven differences in the ongoing alpha-oscillations.

Nonetheless, a few critical remarks about the above-mentioned explanation must be made. First, the interval between TMS pulses used in the current study was very short (2.5–3.5 s; otherwise an interval of 5 s is recommended) and it is thus possible that there were cumulative effects of TMS over the course of the experiment. Consequently we must ask whether our assessment of the ongoing activity could have been distorted by these cumulative effects. Perhaps our experimental conditions allowed for different amounts of accumulation or adaption of the TMS effect. This would explain why there were differences between experimental conditions already in the pre-TMS baseline activity. However, it would not explain why differences between categories of photographs were only evident in the TEPs.

The baseline differences between background classes were specific to alpha frequencies. Alpha activity is most often associated with the state of alertness in the visual system (van Dijk *et al.*, 2008; Romei *et al.*, 2008; Mathewson *et al.*, 2009; Dugué *et al.*, 2011). Therefore, the effects of background classes found in this study may stem from the capacity of different visual information to increase or decrease the general alertness of the brain. Moving dot-patterns, for example, were associated with the lowest alpha activity in the baseline and might have thus increased alertness. Note, however, that background differences in TEPs were also evident during the late slow potential negativization. In previous studies (e.g. Murd *et al.*, 2010; Stamm *et al.*, 2011) negativization was associated with higher alertness and consciousness. In the current study moving dot-patterns were associated with the most positive

responses which would indicate a decrease in alertness. Evidently, this is in conflict with the conclusion drawn from the data on ongoing alpha activity. Another difference between these two sets of results is their topographical distribution. Differences in ongoing alpha activity were most reliable on parietal electrodes, but differences in TEPs were comparable on all of the analysed electrode groups. One possible way to explain these contradictions is to assume a confounding effect of alertness: there is indeed an effect of background class on baseline activity (unrelated to TMS), but this effect is different from the effect of background classes on the TMS-evoked responses. Further research must address this possibility.

Regardless of the somewhat confusing circumstances concerning our four general background classes the effect of natural scenes vs. man-made environments demonstrates clearly that responses to TMS can depend on the informational content currently processed by the visual cortex and associated areas. There are three possible reasons why informational content represented by the visual cortex could influence responses to TMS. First, it could modulate the reactivity of the stimulated target area. It is well known that feedback information to V1 plays an important role in perceptual processes (Pascual-Leone & Walsh, 2001; Silvanto *et al.*, 2005). It is possible that feedback to V1 differs depending on the informational content of the photographs and this could alter the reactivity of V1. Note, however, that although our TMS was aimed at V1, other areas, in particular V2, might have been stimulated as well (Thielscher *et al.*, 2010). Therefore, we should not limit our discussion only to V1 *a priori*. Second, there may be differences in effective connectivity between the processing of different categories of photographs. Thus, the effect of TMS could manifest itself in other regions than the stimulated target area because of differences in signal propagation. Third, the differences in TMS-evoked responses may not be related to the target region or any of the connected visual areas at all. Given our current experimental design it is not possible to distinguish between these alternative possibilities. As we did not include a control stimulation site we do not know if the results from the TEP analyses are specific to V1 or not. Further research must definitely amend this shortcoming.

On a related subject, it remains an open question which particular aspects of the photographs were responsible for the difference in TMS-evoked responses. Because we did not find any effects related to brightness, spatial frequency or movement direction it might seem that the reason why natural scenes lead to relatively more brain-potential negativization has to do with some higher-order conceptual processes. This might be quite reasonable considering that EEG potentials are inherently sensitive to category-related differences (Kiefer, 2001; Rossion *et al.*, 2003). On the other hand, the effect of natural scenes vs. man-made environments could also stem from differences in pre-categorical image-statistical characteristics for these two types of environments.

Recently, there has been a growing interest in the particular properties of real-world stimuli. It has long been known that the structure of our natural environment is characterized by certain statistical regularities (see Simoncelli & Olshausen, 2001; for an overview). Therefore, it seems highly plausible that our brain has adapted to this statistical structure of the world to optimize perceptual inference (Kersten *et al.*, 2004). Some compelling evidence in support of this notion has indeed been found. During maturation, spontaneous neural activity in V1 becomes increasingly more similar to the activity evoked by real movies but not by drifting sinusoidal gratings (Berkles *et al.*, 2011). Scholte *et al.* (2009) reported that Weibull statistics of real-world images can explain up to 71% of the early

variability in the EEG responses to these images. In light of such results, it is legitimate to argue that realistic images comprise ideal stimuli for the visual system and thus it is not surprising if they evoke richer and more differentiated responses compared with artificial stimuli. This line of argument matches well with our current results. We found the most pronounced effect between different categories of realistic images. Nonetheless, to ascertain whether this effect is mainly related to visual processing of the background image or whether they are non-specific to the actual physical parameters of the image, further studies are needed.

Last but not least, we have to consider that very little is known about the dynamics of the visual processes during prolonged exposure to a stimulus. Most studies have used brief presentation times to study the event-related responses to stimuli. We do not know how well the implications from those results apply to our current study. For example, after an initial stage of intense processing of a new image the visual system may settle into a sustained mode which is quite different from the initial one. Attentional processes in the form of graded saliency maps may become more important than the processing of low-level image statistics (e.g. Bogler *et al.*, 2011). If it is so, different categories might lead to distinctly different saliency representations and thereby also to different responses to TMS. As there is a vast prevalence of feedback information from higher category-selective areas to lower sensory areas similar to the active black-board concept (Bullier, 2001) it may well be that this feedback determines the differential reactivity of the visual cortex for different types of input. Regardless, the present work demonstrates that TMS can in principle be used to study such intricate modulations of cortical reactivity.

Supporting Information

Additional supporting information can be found in the online version of this article:

Tables S1. All alpha-power contrasts.

Table S2. Explorative frequency-analysis of baseline activity.

Table S3. TEPs for background classes and electrode groups.

Table S4. TEPs for background classes and electrode groups plus an extra long pre-stimulus epoch.

Table S5. TEPs for subcategories and electrode groups.

Table S6. Tests of homogeneity of variance.

Table S7. Main analysis with equalized signal-to-noise ratios.

Table S8. Effect of individual photographs.

Table S9. *Post-hoc* analyses for photographs with equalized signal-to-noise ratios.

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Abbreviations

ANOVA, analysis of variance; EEG, electroencephalography; TEP, TMS-evoked potential; TMS, transcranial magnetic stimulation.

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