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Effects of single-pulse transcranial magnetic stimulation (TMS) on functional brain activity: a combined event-related TMS and evoked potential study

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Abstract

Objective: To further evaluate the potential of slew-rate limiting amplifiers to record electrophysiological signals in spite of concurrent transcranial magnetic stimulation (TMS), and to explore the effects of single-pulse TMS on electroencephalographic (EEG) correlates of functional brain activity.

Methods: Visual-evoked potentials (VEPs) to checkerboards were recorded in 7 right-handed subjects, while single-pulse TMS was applied to the occipital pole either at visual stimulus onset, during the build-up or at the expected peak of the early VEP component P1 (VIS&TMS). Timing of TMS was individually adjusted based on each subject's VEP-latency. A condition of TMS without concurrent visual stimulation (TMS_{alone}) served for subtraction purposes (VIS&TMS minus TMS_{alone}) to partial out TMS-related contaminations of the EEG signal.

Results: When TMS was applied at visual stimulus onset, VEPs (as calculated by subtraction) perfectly matched control VEPs to visual stimulation alone. TMS at around P1, in contrast, modified the targeted (P1) and the subsequent VEP component (N1), independently of whether TMS was given at build-up or peak.

Conclusions: The retrieval of regular VEPs with concomitant TMS at visual stimulus onset suggests that the employed EEG system and subtraction procedure are suited for combined EEG-TMS studies. The VEP changes following TMS at around P1 provide direct clues on the temporal dynamics of TMS pulse effects on functional activity in the human brain. Our data suggest effects of relatively long duration (~100 ms) when TMS is applied while functional neuronal activity evolves.

Keywords: Electroencephalography; Visual-evoked potential; Transcranial magnetic stimulation; EEG-guided TMS; Single-pulse; occipital cortex

1. Introduction

Transcranial magnetic stimulation (TMS) can be used to transiently induce electric activity in relatively circumscribed brain regions and to depolarize large numbers of neurons (Barker et al., 1985, 1987). By this means, 'noise' can be induced in the cerebral network, which interferes with performance in various sensory, motor and cognitive tasks, provided proper timing and location of TMS pulse delivery (Pascual-Leone et al., 1999, 2000; Walsh and Cowey, 2000). For instance, a single magnetic pulse can impair visual perception if applied to the occipital pole at a certain delay from visual stimulus onset. Initially reported by Amassian et al. (1989), the effect has been corroborated in several subsequent studies showing impaired visual perception of small letters (e.g. Amassian et al., 1998; Beckers and Homberg, 1991; Corthout et al., 1999a,b) and objects (e.g. Miller et al., 1996; Kammer and Nusseck, 1998) as well as induction of scotomas in large-field, patterned stimuli (Kamitani and Shimojo, 1999). These studies suggest a period between roughly 60 and 140 ms after visual stimulus onset as critical for the induction of the most effective visual suppression, although other, earlier periods of suppression have been reported (e.g. Corthout et al., 1999a,b). The suppressive effect of occipital TMS on

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visual stimulus detection has been attributed to interference with visual activity in striate (V1) or peri-striate (V2/V3) cortex (e.g. Amassian et al., 1989; Corthout et al., 1999a; Kamitani and Shimojo, 1999). As has been pointed out by Paulus et al. (1999), the critical period for visual suppression matches the latency of early, visual-evoked potentials (VEPs) derived from electroencephalography (EEG). VEPs reveal two early peaks at around 75 and 100 ms post-stimulus (C1, P1), whose generators have been estimated to be located in V1 and/or V2/V3 in recent functional magnetic resonance imaging (fMRI)-EEG studies (Bonmassar et al., 2001; Di Russo et al., 2002). It is therefore tempting to infer that the effects of single-pulse TMS on visual perception must be related to neurophysiological changes that should be demonstrable by studying TMS effects on functional occipital activity, namely VEPs. No such study has however been published so far.

To date, there is a growing number of studies describing the behavioral consequences of a single TMS pulse when applied in the course of task execution for chronometry and mapping of brain functions (Pascual-Leone et al., 1999, 2000; Théoret and Pascual-Leone, 2003). However, relatively little is known about the consequences of a TMS pulse on the associated (task-related) neuronal activity, in particular when applied during its evolution. The duration of these effects can provide additional clues on the temporal resolution of single-pulse TMS, important for the study of the chronometry of information processing.

The aim of the present study was to investigate TMS pulse effects on VEPs following pulse delivery to the occipital pole at the time of specific VEP components. For this purpose, we adapted an EEG system originally tailored to the magnetic environment within the MRI scanner (Ives et al., 1993) for combined EEG-TMS studies. A slew-rate limiting amplifier design was used to avoid the main artifact problem (Epstein, 1995), which consists of magnetically elicited voltage peaks causing standard EEG systems to saturate for several hundreds of milliseconds. To test for TMS-compatibility of the EEG device, we delivered TMS pulses at visual stimulus onset and compared the corresponding VEPs with standard VEPs to visual stimulation alone (without concurrent TMS). As the magnetic pulses lie well outside the critical period for functional interference (visual suppression), any deviation from the standard VEPs would be likely to be due to TMS-related contaminations of the EEG-signal (e.g. artifacts) rather than due to interference with the VEP generation, hence indicating poor performance of the EEG device. To explore the temporal dynamics of TMS effects on functional visual activity, occipital TMS pulses were delivered within the critical period for visual suppression. In order to increase the probability of interference with visual activity, we applied TMS either at the build-up times or peak times of the early VEP component P1. The time points of TMS pulse delivery were individually adjusted relative to each subject's VEP response (EEG-guided timing of TMS).

2. Methods

2.1. Subjects

Seven right-handed healthy subjects (1 woman, 6 men) with an average age of 32 years (range: 29–36 years) participated in this study. All had normal or corrected to normal vision and no history of neurological or psychiatric disorders. Written informed consent was obtained from all subjects prior to participation in the study that had been approved by the Institutional Review Board of the Beth Israel Deaconess Medical Center.

2.2. Experimental design

The design and the rationale for analysis are illustrated in Fig. 1. Before the actual experiment, subjects were presented with visual stimuli alone (flashed checkerboards, ISI = 2 s, $n = 150$, Pre-Trials). The corresponding VEPs were computed immediately thereafter allowing for individual timing of TMS pulse delivery relative to each subject's VEP components. For the actual experiment, all possible conditions were presented in one experimental block, during which EEG was continuously recorded. The conditions were characterized by 3 trial types (A–C). Their order of presentation was randomized and the inter-trial interval was 2 s. Trial type A consisted of visual stimulation alone (VIS_{alone}, $n = 100$). In trials of type B, visual stimulation was associated with delivery of a TMS pulse (VIS&TMS). The TMS pulse was applied at 3 different time points, i.e. either (1) at visual stimulus onset ($n = 100$), (2) at the beginning of the build-up phase of P1 ($n = 100$), or (3) at the expected peak of P1 ($n = 100$). Mean delay from visual stimulus onset was 100 ms (SE: ±5.4 ms) for TMS during build-up and 118 ms (SE: ±4.5 ms) for TMS at peak. Trials of type C consisted of single TMS pulses alone (TMS_{alone}, $n = 100$). In all trials with TMS, magnetic stimulation was applied to the left occipital pole (see Section 2.5).

2.3. Rationale for the experimental design

While trials of type A provided control VEPs, trials of type B were expected to evoke VEPs that may have been modified by TMS interference with the generators of these potentials. In addition, trials of type B, as well as those of type C, were expected to be associated with TMS-related, neuronal responses that represent possible sources of VEP contaminations (see Fig. 1, lower part). These responses consist of magnetically induced activity in the cortex underlying the coil and spreading to anatomically connected areas (Ilmoniemi et al., 1997; Kähkönen et al., 2001; Paus et al., 2001; Komssi et al., 2002), auditory evoked activity related to the coil click (Nikouline et al., 1999; Tiitinen et al., 1999), and tactile evoked activity related to the coil tap on the scalp (Nikouline et al., 1999; Paus et al., 2001; Komssi

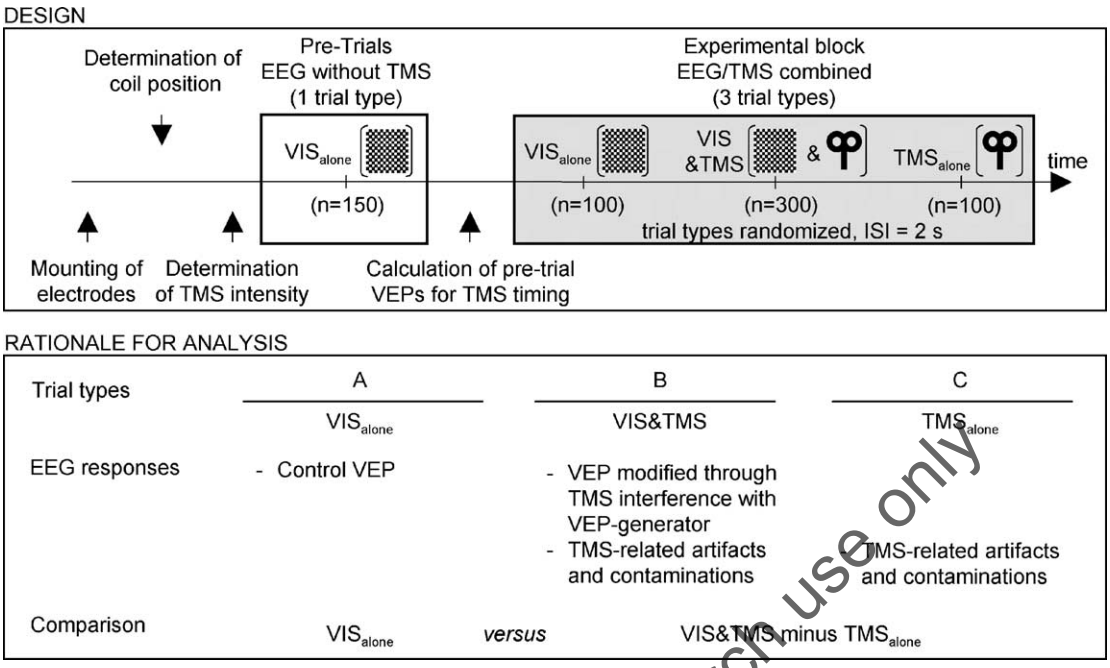

Fig. 1. Summary of experimental design, rationale for subtraction procedure and comparisons. Following mounting of the electrodes and determination of coil position and TMS intensity, subjects were presented with visual stimuli (checkerboards) in 150 trials while EEG was recorded (Pre-Trials). The pre-trials were used to assess each subject's VEP-latencies, which served to individually guide TMS pulse delivery in time. In the actual experiment (Experimental block), EEG responses to visual stimuli were recorded either following visual stimulation alone (VIS_{alone}) or following visual stimulation combined with the delivery of a TMS pulse (VIS&TMS). For subtraction purposes, we also delivered TMS pulses without concurrent visual stimulation (TMS_{alone}). The order of these trials was randomized within the experimental block. Through subtraction of evoked potentials (VIS&TMS minus TMS_{alone}), we attempted to partial out magnetically elicited artifacts and TMS-induced contaminations of the EEG signal, i.e. TMS effects unrelated to interference with the VEP generators. The critical test consisted of comparisons between the control VEPs to visual stimulation alone (conventionally calculated) and the VEPs associated with TMS (calculated by subtraction).

et al., 2002). Further, possible sources of contaminations are the mechanical forces induced on the electrodes under the stimulation coil (Virtanen et al., 1999). We expected that these responses to TMS would be partialed out by subtraction of C from B (VIS&TMS minus TMS_{alone}), i.e. that the residual signal would correspond to VEPs modified through TMS interference with their generators. To explore the single TMS pulse effects, we thus compared EEG responses to control trials A with those to trials (B minus C) (i.e. VIS_{alone} versus (VIS&TMS minus TMS_{alone})).

2.4. Visual stimulation and task

The visual stimulus was a black&white checkerboard ($11.5 \times 11.5°$ of total visual angle; $0.76 \times 0.76°$ for each check), flashed for 80 ms below a central, black fixation cross ($0.5 \times 0.5°$). The fixation cross and the upper end of the checkerboard were separated by 1.25°. The visual stimulus was comparable in size and presentation time to the large-field, patterned gratings used in a previous TMS study on visual suppression (Kamitani and Shimojo, 1999). Stimuli were presented on a white computer screen in a dimly lit room. The cross stayed on the screen during the entire experimental block. Subjects were asked to fixate the central cross while viewing the stimuli and to avoid eye movements including eye blinks and saccades. Visual stimuli were presented on a 16-inch Apple monitor (Apple Computers, Cuppertino, CA) driven by a Power Mac computer (model: 9600/200, Apple Computers), running PsyScope (Cohen et al., 1993).

2.5. Transcranial magnetic stimulation

TMS pulses were applied to the left occipital pole. A Magstim Super Rapid Transcranial Magnetic Stimulator (Magstim Company, Dyfed, UK) and a 70 mm figure-of-8 coil were used (2.2 T maximum field strength). Intensity of TMS was set to 120% of individual phosphene threshold at the stimulation site. This equaled 78% (SE: ±3%) of stimulator maximum output. Phosphene threshold was defined as the minimal intensity that was capable of evoking phosphenes in at least 3 of 6 consecutive trials in the blindfolded subjects. With the eyes open, no subject reported perceiving phosphenes at these stimulation intensities.

The site of stimulation was determined using a functional coil positioning procedure (alignment of TMS-induced phosphenes with visual stimulus position). It has been shown that phosphenes overlap in space with scotomas (perceptual suppression) and can thus serve as a guide to maximize TMS effects on visual functions (Kammer, 1999). Magnetic stimulation can evoke phosphenes when applied

over area V1 (e.g. Meyer et al., 1991; Sparing et al., 2002) but also over areas V2/V3 (Kammer et al., 2001) and V5/MT (Cowey and Walsh, 2000; Pascual-Leone and Walsh, 2001). We targeted a left occipital spot as close as possible to the midline, from where single TMS pulses evoked phosphenes that were positioned within the contralateral, right visual field and overlapped with the checkerboard position. The TMS sites (coil center) were located 1.3 ± 0.48 cm (mean ± SE) to the left and 3.6 ± 0.35 cm above the Inion. With respect to the international 10-10 EEG coordinate system, the coil center was placed −4.2 ± 1.5% to the left and 10.3 ± 1.1% above the Inion (0/0%), i.e. between O1 (−10/10%) and Oz (0/10%). In 4 of the 7 subjects, the position of the coil center was determined relative to their brain off-line to the experiment using individual MR images and a frameless stereotaxic system (Brainsight, Rogue Research, Montreal, Canada). The data indicate that the coil was located over areas of the left occipital pole caudal to V5/MT (see Fig. 2 for an exemplar subject). Because we targeted a site where phosphenes were restricted to the contralateral visual field, TMS most likely affected primarily V2/V3. In fact, it has recently been demonstrated by coregistration of TMS coil positions and retinotopic maps delineated from functional magnetic resonance imaging that TMS over V2/V3 generates unilateral phosphenes, while TMS over V1 induces phosphenes in both visual fields (Kammer et al., 2001).

For functional coil positioning, subjects fixated a small light spot (visual angle: 0.05°) presented in darkness against the black monitor. The light spot marked the position of the fixation cross, presented during visual stimulation. The coil position was adjusted until subjects reported seeing TMS-induced phosphenes that were located to the lower right of the light spot and overlapped the black monitor (the checkerboard almost covered the full screen). Total light-deprivation was held below 10 min for each subject, as prolonged light-deprivation (~45 min) may cause changes in visual cortex excitability (Boroojerdi et al., 2000).

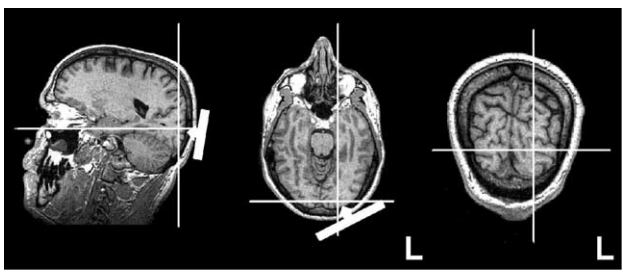

Fig. 2. TMS site of one exemplar subject relative to his MR image. The cross-hairs highlight the cortical point located radially inward from the center of the coil as extrapolated using a frameless stereotaxic system (see coil outline on sagittal and transverse slices). Scalp coil positions have been determined functionally and in each subject separately, i.e. TMS was applied to an occipital spot close to midline where magnetic stimulation evoked contralateral phosphenes overlapping with the position of the visual checkerboard.

The coil was oriented with the handle pointing upward inducing currents in the rostro-caudal direction.

2.6. EEG recordings and hardware layout

EEG was sampled continuously at 200 Hz from 45 scalp electrodes with a unit based on a 128-channel data acquisition system developed for invasive EEG recordings (Ives et al., 1991). Forty-eight of the 128 channels were activated, which allowed for recording of an additional 3 bipolar leads, two for monitoring of horizontal and vertical eye movements (EOG leads) and one for marking time points of visual and magnetic stimulation (external marker channel). The EEG electrodes were homogeneously distributed on the scalp according to the international 10–10 electrode system (Fig. 3). We used conductive plastic-body electrodes, which were internally coated with a thin layer of silver epoxy and have previously been shown to be compatible with rapid rate TMS (Ives et al., 1998). The electrode design prevents overheating of the EEG electrodes, which could lead to scalp burning (Pascual-Leone et al., 1990; Roth et al., 1992). Both EEG and EOG signals were recorded using a bipolar montage, because pre-tests suggested that the magnetically elicited artifacts were smaller when recorded from a bipolar than a referential setting. Electrodes were wired into 6 8-channel preamplifier modules with interconnections such that EEG signals could be recalculated off-line against a common reference (i.e. converted from bipolar to referential mode). The amplifiers are designed not to saturate due to rapid and strong changes in magnetic fields and have previously been used for simultaneous EEG-fMRI recordings (Ives et al., 1993). Their slew rate is curtailed to permit frequencies to be recorded only up to 70 Hz (as opposed to just using low-pass filters). This is accomplished by adjusting the gain such that the gain-bandwidth product is restricted to about 70 Hz.

A Power Macintosh computer, running PsyScope (see Section 2.4), controlled timing of both visual and magnetic stimulation. The computer fed the markers for visual stimulus onset directly into the EEG marker channel, while markers for TMS onset were fed first in a synchronization box (connected to the EEG system) before being forwarded from the box to the TMS device and the EEG marker channel. The box allowed the TMS pulse to be gated by the clock of the EEG data acquisition system. As a result, TMS pulses were perfectly synchronized to the sampling rate of the EEG system, which in pre-tests proved to be essential for subtraction in order to retrieve contamination-free visual-evoked potentials (at least for the present system with a relatively low sampling rate).

2.7. EEG averaging

Sweeps free of eye-movements and eye–blinks were averaged to individual evoked potentials (EP). The sweeps of checkerboard conditions (VIS_{alone}, VIS&TMS) were

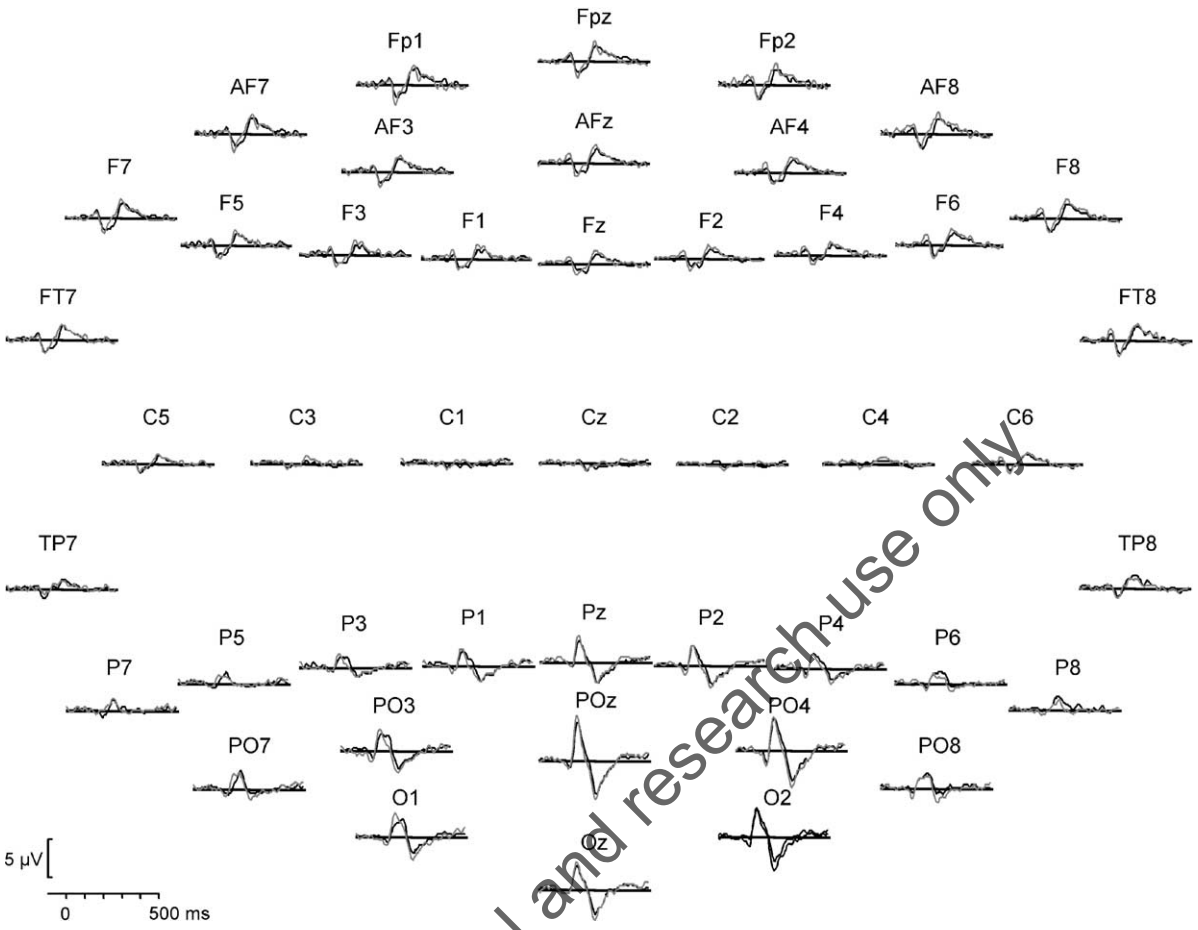

Fig. 3. VEPs to checkerboards after single-pulse TMS at checkerboard-onset (calculated by subtraction, gray line) superimposed on control VEPs to the same checkerboards (no TMS pulse and no subtraction applied, black line). Note the close correspondence between the two traces suggesting that the system and subtraction procedure is able to retrieve visual-evoked potentials that are devoid of TMS-related artifacts and contaminations despite concomitant TMS pulse delivery. Grand-mean data is shown. Negative is down.

aligned to visual stimulus onset (−100 ms pre- to 500 ms post-stimulus), while the TMS-sweeps without concurrent visual stimulation (TMS_{alone}) were brought to match these sweeps in length (600 ms) and time-frame of TMS pulse onset for subtraction purposes. For each individual EP of the VIS&TMS conditions (TMS at stimulus onset, P1 build-up, or P1 peak), we calculated a corresponding individual EP to TMS_{alone}. These EPs were subtracted, after baseline correction to the initial 100 ms (pre-stimulus period in VIS_{alone} and VIS&TMS conditions) and recalculation against the average of the 45 scalp electrodes. The resulting potentials often contained a residual artifact at TMS onset extending over one or maximal two time frames (5–10 ms), which was removed from the EEG traces by interpolation between the preceding and following time frame.

2.8. Analysis of EP map topography

The data were subjected to temporal segmentation and spatial map fitting procedures in order to explore the evolution of EP map topographies over time and across conditions (for recent reviews see Michel et al., 1999, 2001; for recent applications see Morand et al., 2000; Ducommun et al., 2002; Schnider et al., 2002). Changes in map topographies over time or across conditions denote changes in underlying source configurations (i.e. in the spatial distribution of the active neuronal populations).

2.8.1. Temporal segmentation

We used a *k*-means spatial cluster-analysis to search for the EP map topographies and the number of EP maps best explaining the whole data set (Pascual-Marqui et al., 1995). The procedure is based on the observation that EP map topographies typically remain stable for a certain time period (e.g. Lehmann, 1987). This allows the reduction (clustering) of all map topographies of the EP time series (total n maps = n time frames × n conditions) to a relatively small number of maps adequately explaining the whole data set. The optimal number of clusters/maps explaining the whole data set (segmentation maps) is defined using a cross-validation criterion. Temporal segmentation was applied to grand-mean data.

2.8.2. EP map fitting and statistical analysis

Segmentation maps were fitted to individual EPs in order to assess variability within and across subjects for statistical testing. We applied a fitting procedure that is based on spatial correlation coefficients (Brandeis et al., 1992) and provides information on the proportion of global variance that is explained by a given segmentation map (global explained variance, GEV). To characterize the evolution of EP map topographies over time, we subdivided the EP map series in time windows of interest and compared for each window GEV across segmentation maps using analysis of variance (ANOVA; repeated measure, within-subject factor: *(segmentation-)* Maps) and post hoc paired *t* tests. TMS effects were probed by comparing control VEPs to visual stimulation alone versus VEPs to combined visual and magnetic stimulation. We searched for segmentation maps that differentially explain these two VEP responses (for each of the 3 VIS&TMS conditions separately), again using ANOVAs (repeated measure) on GEV. ANOVA factors (all within-subject) consisted of *(time-)* Windows, TMS (VIS vs. VIS&TMS) and *(segmentation-)* Maps.

2.9. Waveform analysis

Area-under-curve (AUC), peak amplitude and peak latency were determined within the latency range of each VEP component (latency ranges were identical with the time windows of interest for map analysis, see Sections 2.8 and 3) and were compared across study conditions using ANOVA with TMS (VIS vs. VIS&TMS), Side (left vs. right electrodes) and Electrodes (PO7/PO8 vs. PO3/PO4 vs. O1/O2) as within-subject factors.

3. Results

Fig. 3 illustrates the grand-mean VEP to checkerboards after delivery of a TMS pulse at checkerboard-onset (as calculated by the subtraction procedure, see materials and methods) and the grand-mean VEP to the same checkerboards when no TMS pulse (and no subtraction) is applied. Note the close correspondence between the two traces over all electrodes. The example illustrates that the EEG system is able to record electrophysiological signals immediately after TMS pulse delivery, i.e. that the amplifiers do not saturate due to the magnetic pulse. It also suggests that the subtraction procedure is suited to separate contamination caused by the TMS pulse (induced activity in the magnetically stimulated cortex, auditory and tactile evoked activity, residual artifacts) from EEG responses to visual stimulation (VEPs).

For further validation, we subjected the entire data set of grand-mean VEPs to analysis of map topography, illustrated in Fig. 4. The data set consists of 3 conditions with different timing of TMS pulse delivery (1, TMS at visual stimulus onset; 2, TMS during build-up of P1; 3, TMS at peak of P1) and their corresponding control conditions where no TMS pulse, i.e. only visual stimulation was applied. The checkerboard evoked 3 components (see Fig. 4A, upper part) that correspond to classical VEP components C1, P1 and N1 (e.g. Di Russo et al., 2002), occurred 60–95 ms (window w2), 95–180 ms (window w3) and 180–310 ms post-stimulus (window w4), respectively, and were topographically characterized by occipital negativity (C1, N1) or occipital positivity (P1) (Fig. 4A, lower part). Cluster analysis of the entire VEP map series (temporal segmentation) revealed that the total data set can be described by 7 EP maps (segmentation maps: M1–M7, Fig. 4B, upper part), explaining 90.98% of the global variance. Fitting of these 7 maps to individual VEPs in time windows w1-w4 revealed that the components C1, P1 and N1 can be characterized by 3 of those 7 maps (M5–M7). As shown in Fig. 4B (lower part) and confirmed by ANOVA, M5 explains significantly more variance than any other map in w2(C1) and in w4(N1) (main effect Maps: both $F(6,36) > 18.5$, $P < 0.0001$; post hoc *t* tests: all $t > 3.2$, $P < 0.01$ for M5). M6 and M7 dominate in w3(P1) (Maps: $F(6,36) = 5.8$, $P = 0.002$; all $t > 2.17$, $P < 0.07$ for M6 and M7).

Analysis of TMS-effects revealed no significant deviation from the control VEP topography in any of the 4 windows for TMS at visual stimulus onset (Fig. 4B, upper panel; 4 × 2 × 7 ANOVA: interaction Windows × TMS × Maps not significant, no significant interaction TMS × Maps in any window). In contrast, TMS at P1 (Fig. 4B, middle and lower panels) significantly modified VEP topography in w3 and w4 independently of whether the pulse was given at build-up or peak times (Windows × TMS × Maps: both $F(18,108) > 1.72$, $P < 0.047$; TMS × Maps significant in w3 and w4: all $F(6,36) > 3.63$, $P < 0.0064$). As compared to the control VEP, TMS at P1 led to a significant increase in proportion of M6 topography in w3(P1) (all $F(1,6) > 4.13$, $P < 0.08$) to the detriment of M7 (all $F(1,6) > 7.93$, $P < 0.031$). This corresponds to a signal decrease over the targeted region (as M6 shows less left occipital positivity than M7, see Fig. 4B). In w4(N1), M5 was less frequently found following TMS at P1 than in the control condition (all $F(1,8) > 9.1$, $P < 0.02$).

TMS at P1 also led to topographically specific changes with respect to waveform, while TMS at visual stimulus onset had no effect. Area-under-curve (AUC) of both the targeted (P1) and the subsequent component (N1) were differentially affected over left and right occipital electrodes by TMS at P1, independently of whether TMS was applied at P1 build-up or peak (interaction TMS × Side: all $F(1,6) > 8.3$, $P < 0.03$). As compared to the control VEP, AUC of P1 was reduced over left (both $F(1,6) > 6.8$, $P < 0.04$) but not right occipital electrodes (both $F < 1$, not significant). Topographically specific changes for N1 consisted in an increase of AUC over left (both $F > 6.7$, $P < 0.04$) but not right occipital contacts (both $F < 1$, not significant). P1 amplitude, on the other hand, was not affected by TMS at P1, while N1-amplitude

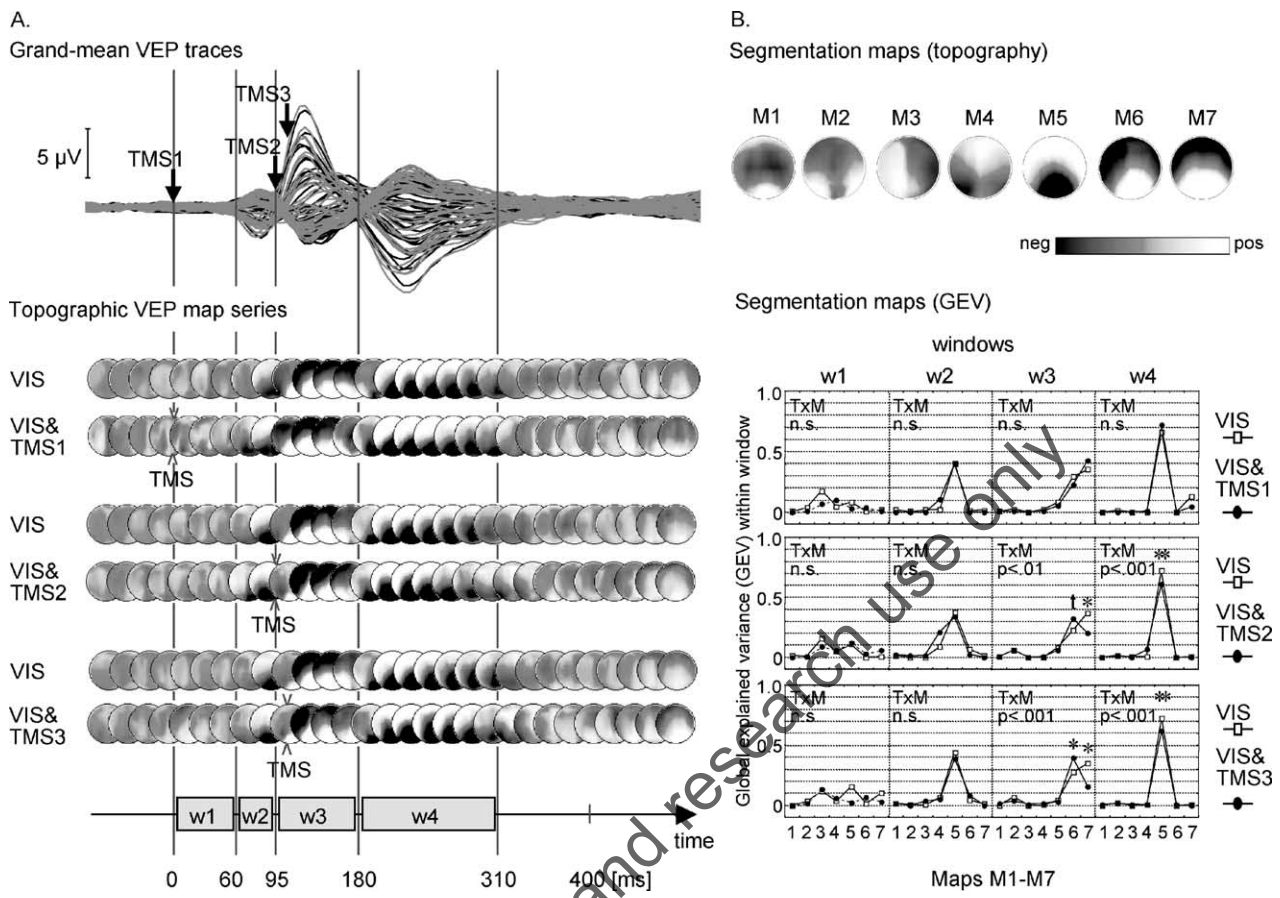

Fig. 4. (A) Grand-mean VEPs over time and conditions. Upper part: Traces (all 45 electrodes superimposed) of all averaged TMS- (in gray, subtraction data) and all averaged control-conditions (in black). The arrows indicate time points of magnetic stimulation (TMS). TMS pulses were delivered either at visual stimulus onset (TMS1), during build-up of the P1-component (TMS2) or at its peak (TMS3). Negative is down. Lower part: Time series of VEP map topographies in the 3 TMS-conditions (VIS&TMS1, VIS&TMS2, VIS&TMS3, subtraction data) and their corresponding control conditions (VIS = VIS_{alone}). Maps are shown in 20 ms steps. White/black indicates positive/negative potentials. Note that the EEG response to the checkerboards is characterized by the 3 classical VEP components C1, P1 and N1 occurring in time windows w2, w3 and w4 with distinct map topographies. (B) Map topographies optimally explaining the full data set (segmentation maps: M1–M7) as well as their representation (global explained variance) over time windows and conditions as revealed by spatio-temporal map clustering/fitting. Note that TMS at visual stimulus onset did not induce any significant VEP change in comparison to control VEP topography (upper panel: VIS vs. VIS&TMS1). Significant deviations from control VEP topography were observed when the magnetic pulse was delivered at around P1 (middle and lower panels). Note that TMS affected map topography of the targeted component (P1) in w3 but also of the subsequent component (N1) in w4. The modifications in map topography are interpreted to reflect interference with the cortical generators of the visual-evoked potential.

showed differential effects over left and right electrodes (TMS × Side: both $F(1,6) > 5.8$, $P < 0.05$; amplitude increase due to TMS: left > right electrodes). There was no other consistent effect of TMS on any other component or measure, including latency of VEP components.

4. Discussion

Our main findings are twofold. First, the data demonstrate that the employed EEG system and analysis procedure permit the retrieval of VEPs that are free of TMS-related artifacts and contaminations despite concomitant delivery of a TMS pulse at high intensity (approximately 80% of maximum stimulator output). Second, following a magnetic pulse over the occipital pole targeting VEP component P1 (EEG-guided TMS), we observed topographically specific VEP changes that lasted for up to 100 ms after the TMS pulse delivery. This is taken as an indication that TMS interfered with the neural sources underlying the visual evoked potential. The findings suggest that the effects of a sub-millisecond TMS pulse (pulse width of 220 μs) can resonate for up to 100 ms within targeted brain regions of the visual system.

In contrast to our study, most previous combined EEG-TMS studies applied TMS at rest while EEG was used to study the spreading of TMS-induced cortical activity for tracing of cortico-cortical connectivity (Ilmoniemi et al., 1997; Kähkönen et al., 2001; Komssi et al., 2002) or to investigate the temporal dynamics of the initial cortical response (Paus et al., 2001). Similar to our study, Schürmann et al. (2001) applied TMS pulses during the evolution of evoked potentials, i.e. to the functionally activated brain. However, these authors studied

somatosensory-evoked potentials (SEPs) following TMS over the somatosensory cortex and focused exclusively on short-latency SEPs (P25). All these studies used sample-and-hold circuits to avoid the main TMS artifact problem (Virtanen et al., 1999). We approached the principal artifact problem through a slew-rate limiting amplifier design (Epstein, 1995) and adopted a subtraction procedure (similar to Tiitinen et al., 1999) to partial out TMS-related contaminations that may confound the VEPs. Our results show that normal VEP-traces are retrieved through subtraction for TMS at visual stimulus onset (Fig. 3). These VEPs associated with simultaneous visual and magnetic stimulation were not significantly different from control VEPs to visual stimulation alone. That is, the VEPs calculated by subtraction appeared to be free of magnetically induced artifacts that are expected to occur immediately after TMS pulse delivery (Virtanen et al., 1999; Paus et al., 2001) as well as free of contaminations due to TMS-induced cortical activity which peaks predominantly at latencies < 50 ms and not later than 100 ms from the TMS pulse delivery (Ilmoniemi et al., 1997; Kähkönen et al., 2001; Paus et al., 2001; Komssi et al., 2002). Also, auditory contaminations can be ruled out given the absence of differences at latencies of 100–200 ms at which the loud coil click, propagated through air and bone, induces auditory potentials (Nikouline et al., 1999).

Accordingly, the VEP changes after TMS at P1-latencies (on average 100 ms and 118 ms post-stimulus for TMS at build-up and peak) are interpreted to reflect interference with functional visual activity, potentially reflecting an electrophysiological correlate of altered visual processing. This would correspond to previous behavioral findings showing that a single TMS pulse at 60–140 ms following visual stimulus onset affects visual perception, while TMS at earlier time-points is ineffective (e.g. Amassian et al., 1998; Kammer and Nusseck, 1998; Kamitani and Shimojo, 1999). Attenuation of functional neuronal activity by a single TMS pulse could result on the cellular level from TMS-induced inhibitory postsynaptic potentials, which are likely to predominate over TMS-elicited excitatory potentials in analogy to the cat motor cortex (Amassian et al., 1998). Alternatively, TMS-induced cellular activity could represent 'physiological noise' that interferes with functional signals independently of its quality (inhibitory or excitatory) (Kammer and Nusseck, 1998).

Our finding that a single pulse not only affected the targeted VEP component (P1) but also the subsequent component (N1) suggests that the effect of a sub-millisecond pulse resonates for at least 100 ms within the visual system. This is in accordance with a recent EEG-TMS study reporting neuronal responses occurring for up to 100 ms following single-pulse TMS over the resting brain (Paus et al., 2001). Our finding also agrees with previous studies probing electromyographic (EMG) responses to TMS by applying single- or paired-pulse protocols over motor sites. Sustained EMG-activity due to voluntary hand muscle contraction, for instance, can be suppressed for up to 300 ms by a single TMS pulse applied over contralateral hand motor cortex (Inghilleri et al., 1993; Hallett, 1995). Paired-pulse studies have shown that the motor evoked potentials (MEP) elicited in a relaxed muscle by contralateral M1 stimulation can be modulated in amplitude through the delivery of a preceding (conditioning) pulse to the same or a functionally connected site for inter-pulse-intervals of up to 200 ms (e.g. Valls-Sole et al., 1992; Sanger et al., 2001). Within the visual system, the conditioning effect of an initial pulse on a test pulse also appears to last for at least 100 ms (Amassian et al., 1993, 1998). As shown by Amassian et al. (1998), a single, occipital pulse applied at 50 ms after visual stimulus onset can depress visual perception, if followed by a second pulse given at 150 ms latency, although each pulse on its own has no perceptual outcome (lies outside the critical time window for visual extinction).

The TMS effects of long duration observed in the present study might have been brought about by a relatively long-lasting, TMS-induced disruption of the neurophysiological mechanisms that govern functional cortical activation ($\sim$100 ms), although the TMS pulse itself is of sub-millisecond duration. Of interest in this regard is our finding that a TMS pulse had differential effects when applied at visual stimulus onset than when applied at the P1 latency, as TMS at visual stimulus onset failed to affect the early VEP components peaking as early as 75 ms post-stimulus. This would be consistent with the possibility that the impact of a TMS pulse varies as a function of the state of the brain, which differs in many aspects between the time point of visual stimulation and the early VEP component P1 (e.g. in level and type of neuronal activity). Our data would imply that TMS over the functionally activated occipital cortex at P1 is more effective than occipital TMS at visual stimulus onset, at least in terms of the duration of its effect. Similar state-dependent effects have been demonstrated for both TMS over motor and visual sites. Their neurophysiological basis are however unknown. EMG responses induced by TMS over motor cortex are enhanced during motor execution and motor imagery as compared to baseline conditions (e.g. Kasai et al., 1997; Hashimoto and Rothwell, 1999; Fadiga et al., 1999). Similarly, phosphenes are more easily induced by occipital TMS under visual mental imagery as compared to rest (Sparing et al., 2002). Computer simulations also suggest enhanced sensitivity of neocortical neurons to TMS when background activity is increased (Kamitani et al., 2001). Alternatively, the long-lasting effects on VEPs may result from transient effects on the P1-generator(s) rather than from long-lasting interference with the neurophysiological bases of functional brain activation per se. If P1 signals early visual input/visual information processing which primes later visual processes, e.g. those reflected in N1 (serial information processing), the effects on P1 could spread to N1, even in case of transient interference with P1.

In summary, we conclude that slew-rate limiting amplifiers allow the recovery of EEG correlates of functional brain activity (e.g. VEPs) in spite of concomitant TMS pulse delivery, if a subtraction procedure is used to partial out TMS-induced artifacts and contaminations. Using this technique, we found that a single TMS pulse at the occipital pole has relatively long-lasting effects on electrophysiological correlates of visual functions. Investigating the effect of a single TMS pulse on functional brain activity can provide more detailed information on mechanisms of functional suppression and TMS pulse action than previously possible, in particular when TMS is applied while functional neuronal activity evolves. It remains to be determined under what conditions the alteration of functional neuronal activity translates into perceptual effects, as the neurophysiological changes in the magnetically stimulated cortex do not necessarily mirror perceptual consequences.

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