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ORIGINAL RESEARCH

Brain responses evoked by high-frequency repetitive transcranial magnetic stimulation: An event-related potential study

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Background
Many recent studies have used repetitive transcranial magnetic stimulation (rTMS) to study brain-behavior relationships. However, the pulse-to-pulse neural effects of rapid delivery of multiple TMS pulses are unknown largely because of TMS-evoked electrical artifacts limiting recording of brain activity.

Objective
In this study, TMS-related artifacts were removed with independent component analysis (ICA), which allowed for the investigation of the neurophysiologic effects of rTMS with simultaneous electroencephalographic (EEG) recordings.

Methods
Repetitive TMS trains of 10 Hz, 3 seconds (110% of motor threshold) were delivered to the postcentral gyrus and superior parietal lobule in 16 young adults. Simultaneous EEG recordings were made with a TMS-compatible system. The stereotypical pattern of TMS-related electrical artifacts was identified by ICA.

Results
Removal of artifacts allowed for identification of a series of five evoked brain potentials occurring within 100 milliseconds of each TMS pulse. With the exception of the first potential, for both areas

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targeted, there was a quadratic relationship between potential peak amplitude and pulse number within the TMS train. This was characterized by a decrease, followed by a rise in amplitude.

Conclusions

ICA is an effective method for removal of TMS-evoked electrical artifacts in EEG data. With the use of this procedure we found that the physiologic responses to TMS pulses delivered in a high-frequency train of pulses are not independent. The sensitivity of the magnitude of these responses to recent stimulation history suggests a complex recruitment of multiple neuronal events with different temporal dynamics.

Keywords transcranial magnetic stimulation; rTMS; electroencephalography; event-related potential; independent component analysis

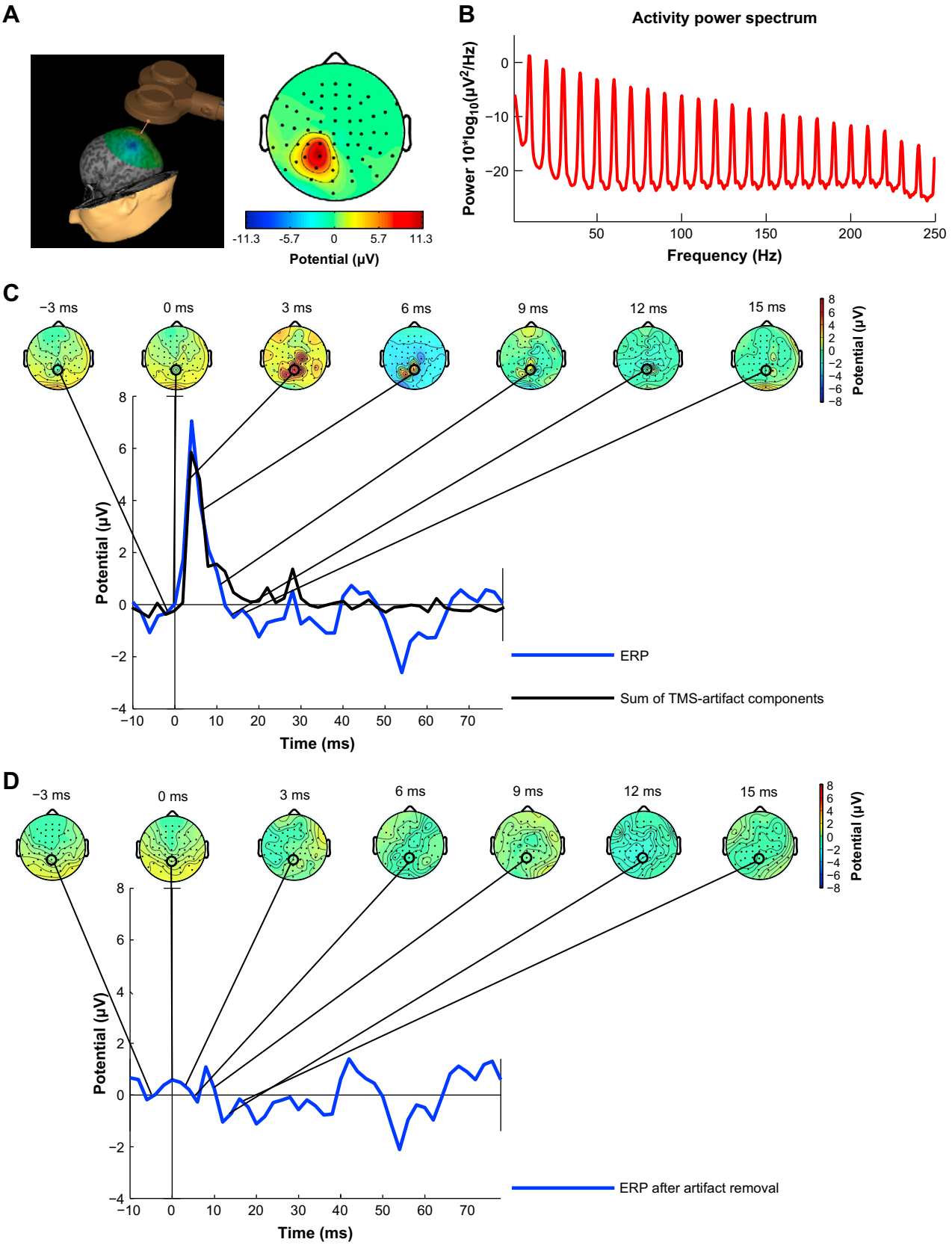
The ability to noninvasively alter neuronal processing with transcranial magnetic stimulation (TMS) has provided an important tool with which to study mind-brain relationships.[1,2] Repetitive TMS (rTMS) has generally been thought to produce a "virtual lesion," thereby impairing task performance.[3,4] As such, rTMS provides a powerful method to examine whether a given brain area plays a causal role in task performance.[5,6] However, some studies have produced seemingly paradoxical rTMS-related improvements in performance, with, for example, rTMS to the parietal cortex producing improved performance on tests of mental rotation[7] and working memory.[8,9] These findings indicate that rTMS may have more complex effects on brain function than initially thought.[3,4,10]

Indeed, recent neuroimaging and electrophysiologic studies have shown that the physiologic effects of TMS are neither short-lived nor limited to the brain area that is targeted.[11-14] For example, previous electroencephalogram (EEG) studies have shown that delivery of a single pulse of TMS elicits a sequence of event-related potential (ERP) components.[15,16] To understand how rTMS modulates brain activity, and hence task performance, it is important to combine it with neuroimaging methods such as EEG.[17-20] The combination of rTMS and EEG is particularly promising given the high temporal resolution, as illustrated by recent combined rTMS-EEG studies that have revealed changes in neuronal oscillations poststimulation.[19,20] However, cortical activity changes *during* rTMS stimulation have not been previously investigated. This is likely related to the fact that each TMS pulse produces artifacts in the recorded data arising from the large current produced by the magnetic discharge of each pulse. Although recent TMS-compatible EEG systems have minimized such artifacts and allowed for analysis of neural activity to within 10 milliseconds of the TMS pulse,[21] at higher intensities of stimulation the artifact is more prominent.[22]

From in vitro and in vivo studies of microstimulation of neurons, it is known that multiple stimuli delivered close in time can lead either to a potentiated (e.g., paired-pulse facilitation and synaptic augmentation[23,24]) or depressed (e.g., paired-pulse depression,[24,25] synaptic depression[26]) response, depending on the cell type and details of the stimulation protocol. Several recent studies suggest that rTMS may enhance cortical excitability. For example, studies of TMS of the motor cortex have shown evidence of potentiation of the motor-evoked potential (MEP) (paired-pulse TMS,[27] 5 Hz rTMS),[28] and of the TMS-evoked ERP, following a 5-Hz rTMS train.[29] Other studies, however, using rTMS-evoked MEPs[30,31] and long-interval paired-pulse stimulation,[32,33] have described evidence for TMS-induced cortical inhibition. Some of this variability across studies may be due to individual differences in baseline cortical activity,[34] as well as to complex, cumulative effects of rTMS that may depend on the number of pulses delivered.[20] Thus, rTMS effects are likely to include both decreases and increases in cortical excitability.

The current study used simultaneous rTMS and EEG to examine whether rTMS-evoked brain responses change over the course of a train of pulses during a cognitive task. To this end, rTMS was applied at 10 Hz to the superior parietal lobule (SPL) and postcentral gyrus (PCG) during the delay period of a visual working memory task, while EEG was recorded.[9] Several studies have used similar rTMS parameters to investigate brain-behavior relations, and we have used this precise stimulation protocol to produce both impairment[35] and improvement[9] of behavioral performance. Furthermore, the two target areas were chosen because our previous studies have shown that SPL and PCG rTMS produce differential behavioral effects. The relationship between the effects of rTMS and task performance will be presented elsewhere. Here, we use these data to examine the possibility of pulse-to-pulse interactions or cumulative effects of multiple successive pulses with rTMS[20] by measuring the strength of TMS-induced brain responses.

Recently, to be able to study the neurophysiologic effects of rTMS, there have been several attempts to remove rTMS-related EEG artifacts post hoc. Some groups have avoided the artifact by limiting their analysis to the late effects of rTMS.[36] Another group sought to remove the artifact by subtracting the rTMS response in the EEG during a rest condition from that measured during task performance.[37] However, both these methods preclude the study of the immediate effects of TMS on brain activity. An alternative class of approaches uses offline digital signal processing to remove


A
-11.3
-5.7
0
5.7
11.3
Potential (μV)
B
Activity power spectrum
Power 10*log10(μV²/Hz)
Frequency (Hz)
C
−3 ms
0 ms
3 ms
6 ms
9 ms
12 ms
15 ms
Potential (μV)
Time (ms)
ERP
Sum of TMS-artifact components
D
ERP after artifact removal

TMS-related artifacts from the EEG signal without affecting underlying neural activity.[38,39] In the current study, we used independent component analysis (ICA) as a method of removing the TMS-related artifact. Because the electrical artifact is temporally and spatially predictable, ICA provides a relatively simple method with which to remove it without affecting the underlying neurophysiologic activity.

Methods

Subjects

Sixteen young adults (12 male, mean age = 22.5 years [SD = 3.8]) participated in the study. Subjects reported no neurologic, psychiatric, or other excluding medical conditions as determined by a psychiatrist or clinical psychologist who administered a structured psychiatric diagnostic interview (MINI[40]) and mood assessment (HAM-D[41]). The study was approved by the local ethics committee. All subjects provided informed consent and were compensated monetarily.

Behavioral task

Subjects performed a delayed-recognition working memory task. Half the trials, randomly distributed, required memory for spatial locations, whereas the other half of trials required memory for shapes. In half the trials, randomly distributed and orthogonal to the factor of trial type, a 3-second, 10-Hz rTMS train was applied to the target brain area time-locked to the onset of the 3-second delay period. Because the behavioral context is being treated as incidental to the effects of rTMS, the data presented here are collapsed across tasks.

For safety reasons, the number of TMS pulses delivered to each subject must be limited.[42] To obtain data from an adequate number of trials of each condition, the experiment therefore consisted of two sessions that took place on separate days. In each session, a different brain area was targeted. The order of the brain area stimulated was counterbalanced across subjects. In each session, subjects performed 192 memory trials (96 per trial type), divided into eight task blocks. During half of these trials (n = 96), randomly distributed, rTMS was applied.

rTMS

rTMS was applied to two different brain areas: the SPL and the PCG. The SPL was chosen based on a previous finding from our group that rTMS of SPL leads to an improvement in task performance.[9] The area representing the leg in the somatosensory cortex of the PCG served as a control region for the behavioral study.[9,35] Both areas were identified based on individual anatomy from whole-brain anatomical magnetic resonance images (MRIs) that were obtained for each subject before the study (GE Signa VH/I, 256 sagittal slices, 0.5 mm × 0.5 mm × 0.8 mm). An infrared-based frameless stereotaxy system was used to accurately target each brain area with the TMS coil (eXimia NBS, Nexstim, Helsinki, Finland). For all subjects, rTMS was applied to the left hemisphere.

In $rTMS_{present}$ trials, a 10-Hz rTMS train was delivered during the entire 3-second delay period (30 pulses). rTMS was applied at 110% of resting motor threshold (as measured with an electromyogram at the first dorsal interosseus muscle). Minor adjustments to the stimulation intensity were made to correct for scalp-to-cortex distance differences between each target brain area and the motor cortex.[43] For each brain area targeted (and, hence, for each session), a total of 2880 pulses were delivered. TMS was delivered with a Magstim Standard Rapid magnetic stimulator fit with a 70-mm figure-of-eight stimulating coil (Magstim, Whitland, United Kingdom). Because $rTMS_{present}$ and $rTMS_{absent}$ trials were randomly intermixed, the intertrain interval varied. A minimum of 17.1 seconds separated each train. White noise was played in the background during all trials.

EEG recordings

EEG was recorded with a 60-channel carbon cap and TMS-compatible amplifier (Nexstim, Helsinki, Finland). This amplifier is designed to avoid saturation by the TMS pulse by using a sample-and-hold circuit that keeps the output of the amplifier constant from 100 microseconds prestimulus to 2 milliseconds poststimulus.[21] To reduce residual TMS-related artifacts, the impedance at each electrode was kept below 3 kΩ. The right mastoid was used as the reference and eye movements were recorded using two additional electrodes. Data were acquired at 1450 Hz sampling rate with 16-bit resolution.

Preanalysis

Data were processed offline using the EEGLAB toolbox[44] running in a MATLAB environment (Mathworks, Natick,

Figure 1 Representative transcranial magnetic stimulation (TMS)-artifact component identified by independent component analysis (ICA). (**A**) This panel illustrates that a typical artifact was limited to the electrodes immediately below or next to the location of stimulation. In this example, the superior parietal lobule (SPL) was targeted (near electrode P1). (**B**) Power spectrum of the artifact reveals a strong, sharp peak at 10 Hz and corresponding harmonics. **C** and **D** illustrate the effectiveness of ICA in removing TMS-related electroencephalographic artifacts for a representative subject. Shown are scalp topographies time-locked to the onset of the TMS pulse before (**C**) and after (**D**) removal of seven ICA components representing the TMS-related artifact. Also shown is the summed time course of activity of these seven artifact components (black line, **C**), as well as the TMS-induced evoked responses after artifact removal (blue line, **D**). As can be seen in this figure, the artifact was most prominent in the first 10 milliseconds after the TMS pulse.

Massachusetts). The data were first down-sampled to 500 Hz and then bandpass filtered between 0.1 and 500 Hz. After this, the data were cleaned of large movement-related artifacts, and channels with excessive noise were reinterpolated by using spherical spline interpolation.[45] Before analysis, the data were rereferenced to an average of all 60 channels.

Removing TMS artifacts with ICA

Because of smearing of the electrical signals by the skull, physiologic EEG signals, even if from a very localized source, are detected by many electrodes on the scalp. TMS-related electrical artifacts, on the other hand, originate from outside the skull and, with TMS-compatible EEG systems, localize to only the few electrodes surrounding the TMS coil.[16] TMS-related artifacts are also temporally predictable. They reliably occur with delivery of each pulse and typically last a few milliseconds.[21,46] Thus, they are readily distinguishable from physiologic signals.

ICA, a method that separates statistically independent sources from a mixed signal, is ideally suited to separate the electrical artifacts from physiologic data in the EEG recordings. It is a technique that has already been successfully applied to removal of other non-neurophysiologic EEG artifacts.[47,48] In this study, the residual TMS artifacts, as well as other stereotypical artifacts, such as eye blinks and some muscle contractions, were identified and removed by using ICA as implemented in EEGLAB.[48] Two rounds of ICA were performed on the data. This was to ensure that less prominent TMS artifacts would also be detected. The first ICA was performed on continuous EEG data, and the second ICA was performed on data from only the epoch during which rTMS was applied. TMS artifact components were identified by using three characteristics. First, as described previously, the artifact should be relatively spatially localized (Figure 1A). Second, the power spectrum of an ICA artifact component should have a strong peak at 10 Hz (accompanied by peaks at every harmonic of 10 Hz) because rTMS was delivered at 10 Hz. Although physiologic EEG signals often also have a peak at around 10 Hz (the α-band peak), this peak typically covers a wider frequency range, does not have strong harmonics, and has a general 1/frequency pattern in the power spectrum. In contrast, the TMS artifact typically consists of a sharp peak at the frequency of stimulation (and corresponding harmonics), which is superimposed on a flat power spectrum (Figure 1B). A third criterion concerned the component activity. With the TMS-compatible EEG system, the artifact, if present, is limited to the first 10-15 milliseconds after the pulse.[21,46] Because the timing of the TMS train was known, a component reflecting the ICA artifact therefore had to peak within a few milliseconds of the start of each TMS pulse in the train (Figure 1C and D).

For several subjects, ICA also produced one or more components that seemed to contain elements of both neurophysiologic data and TMS artifact. If such a component was identified after the first ICA, it was kept for further analysis, with the idea that the second ICA may separate the two. If such components were still present after the second ICA step, they were then discarded. Although removal of physiologically relevant data with removal of ICA components is concerning, it should be noted that relatively few components that included both TMS artifact and physiologic activity were removed. Of further importance, these components typically explained relatively little variance in the data (see the Results section).

ERP analysis

To examine whether TMS-evoked brain responses changed as a function of pulse position in the train, we used the global field power (GFP).[49] GFP indexes the overall strength of the electric field of evoked potentials and is computed as the square root of the sum of squares of the average-referenced activity over all channels.

As a first analysis, we collapsed the data across time by averaging the GFP across the 100-millisecond time window after each pulse (GFP_{mean}). This allowed us to assess changes in the amplitude of rTMS-evoked ERPs without making any assumptions about their temporal or topographic distribution. GFP_{mean} values were calculated both for the ICA artifact-corrected data and for data for which ICA components (including those representing TMS artifacts) were not removed. In this way, we could examine whether the ICA procedure removed physiologically important information that may have affected our results. Subsequent analyses looked at the temporal pattern of the GFP after each pulse and more specifically determined the timing of rTMS-related changes in brain activity. GFP_{mean} and GFP values were submitted to separate repeated-measures analyses of variance (ANOVAs) with pulse number as a within-subject variable. Whenever a significant main effect of pulse number was found, polynomial tests were run to assess whether evoked potential amplitudes changed as a function of pulse number. In addition, to determine whether there are differences in the scalp distribution of the responses to SPL versus PCG rTMS, we performed a 2-way (electrode × rTMS target) ANOVA on vector normalized measured potentials.[50]

Results

TMS artifact removal

Before ICA artifact removal, of the 192 trials collected per brain area, an average (± SD) of 12.3% ± 8.0% was removed because of large movement-related artifacts. Overall, of a mean of 55.0 ± 4.0 components produced by ICA, an average of 7.4 ± 3.4 TMS artifact-related components were identified and removed after the first

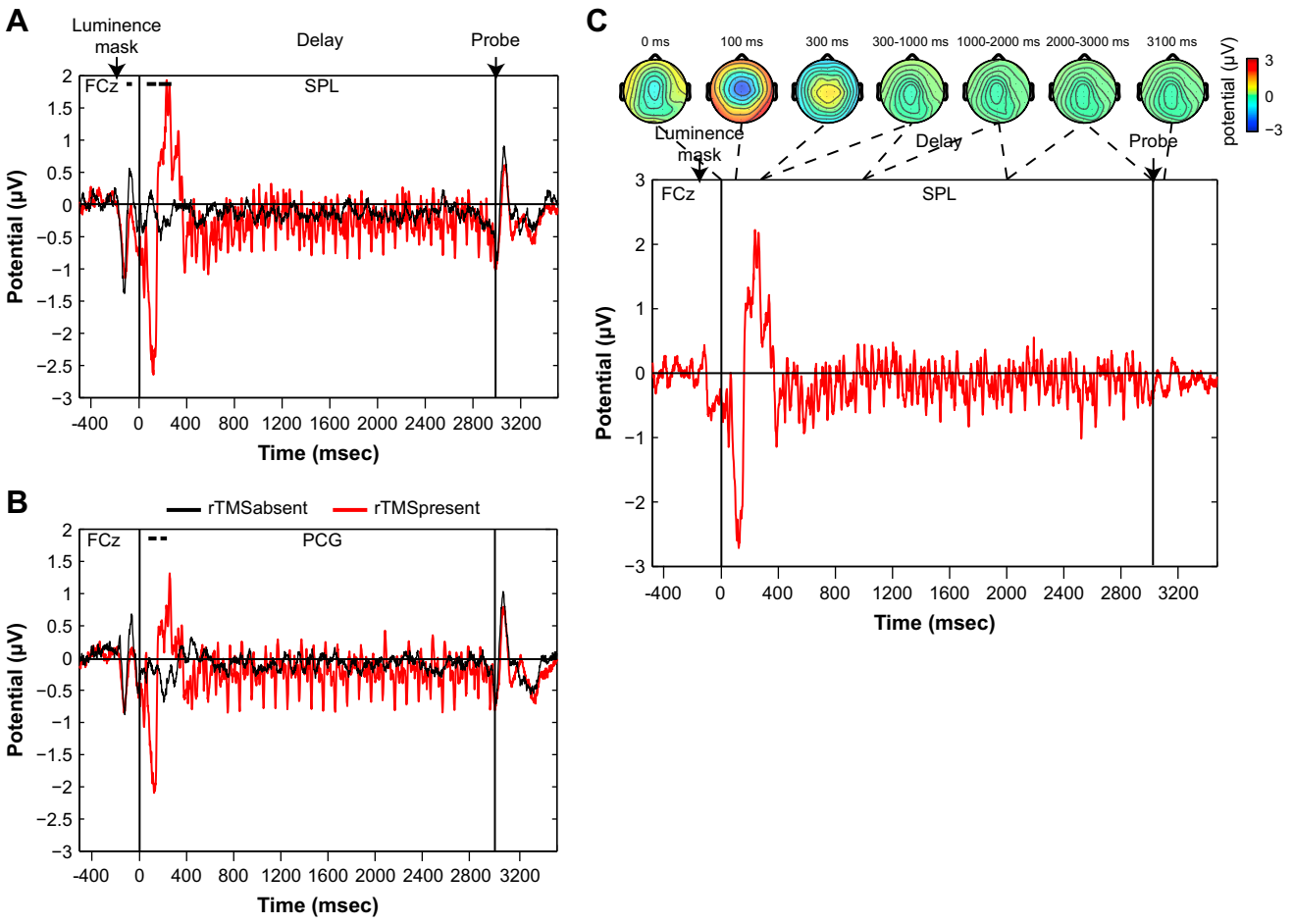


Figure 2 Delay-period neuronal activity, averaged across all subjects, at electrode FCz in the absence (black line) and presence (red line) of 10 Hz repetitive transcranial magnetic stimulation (rTMS), shown separately for superior parietal lobule (SPL) (**A**) and postcentral gyrus (PCG) (**B**) stimulation. As can be seen, the onset of the rTMS train was associated with a strong negativity around 100 milliseconds, followed by a positivity between 200 and 400 milliseconds. Lines above the graph indicate period for which the $rTMS_{present} - rTMS_{absent}$ contrast revealed a significant difference in neuronal activity (paired t test, $P < .05$) for at least 20 consecutive samples (40 milliseconds). (**C**) The difference in delay-period activity in $rTMS_{present}$ versus $rTMS_{absent}$ trials with scalp topographies (shown for SPL rTMS).

round of ICA. The second round of ICA led to the removal of an additional 5.8 ± 2.2 TMS artifact components. An average of 1.6 ± 1.4 components that contained both physiologic data and TMS artifact were removed. These components explained 13.9% ± 16.7% of the variance of the epoched dataset.

Response during rTMS

Figure 2 shows the effects of rTMS on brain activity during the delay period of the task. As can be seen in this figure, both when SPL and PCG were targeted, the onset of the rTMS train was associated with an early negativity around 100 milliseconds, followed by a broader positivity around 200-400 milliseconds that was not present in $rTMS_{absent}$ trials. These responses were strongest over midline frontal scalp regions (Figure 2C). The fact that this initial volley of responses was maximal over sites that were not directly under the TMS coil and did not vary as a function of stimulation site indicates that it may reflect processes related to a general orienting response to the onset of the rTMS train. After this initial sequence of responses, the delivery of each pulse in the rTMS train was associated with a specific pattern of evoked potentials, which depended on the site of stimulation (described in detail later in the text). We chose to only focus on brain responses elicited after the fourth pulse, to avoid contamination of our analysis by the initial orienting response. In addition, the 30th pulse was excluded from our analyses, because the brain response to this last pulse overlapped with the brain response elicited by the onset of the probe stimulus that was presented immediately after the delay period ended.

ERP as a function of pulse number

Collapsed across time

We first examined changes in the amplitude of rTMS-evoked ERPs without making any assumptions about the temporal or topographic distribution of the response using GFP_{mean}. When rTMS was applied to the SPL, the GFP_{mean} elicited

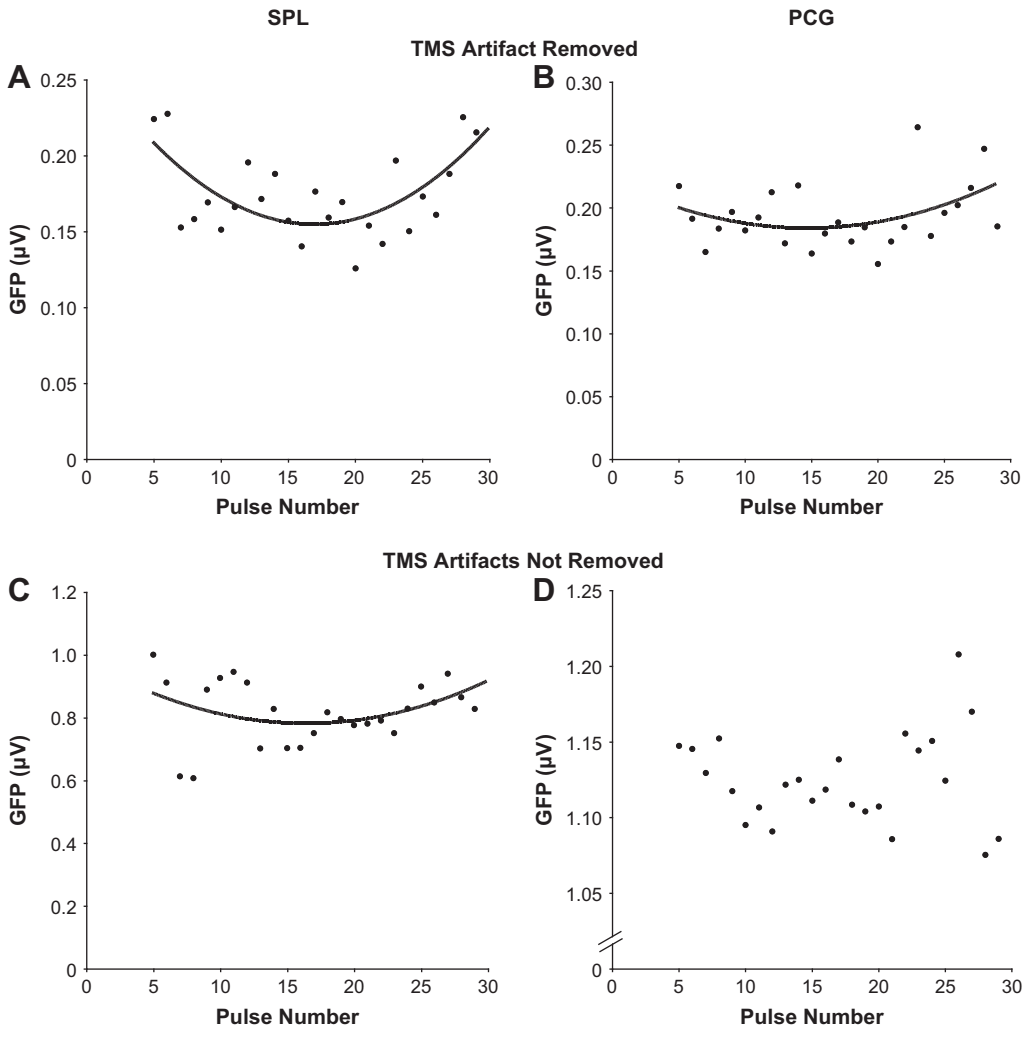


Figure 3 Mean changes in the amplitude of transcranial magnetic stimulation (TMS) pulse-evoked brain responses across the repetitive TMS (rTMS) train with and without removal of independent component analysis (ICA) components. Shown are global field power $(GFP)_{mean}$ values averaged across the 100-millisecond time window after each pulse for pulses 5-29. **A** and **B** show GFP_{mean} values after removal of ICA components representing TMS-related artifacts for superior parietal lobule (SPL) and postcentral gyrus (PCG) rTMS, respectively. **C** and **D** represent the same data without the removal of these components. Significant quadratic relationships between pulse number and GFP_{mean} amplitude are displayed.

by each pulse showed a significant effect of pulse number [$F(24) = 4.09$; $P < .0001$]. This was driven by a significant quadratic trend [$F(1,15) = 20.11$; $P < .0005$], reflecting a dip in amplitude halfway through the train (Figure 3A). When the PCG was targeted, a significant effect of pulse number on GFP_{mean} was observed [$F(24) = 6.28$; $P < .0001$]. Here, too, the data showed a dip in response amplitude halfway through the rTMS train as indexed by a significant quadratic trend [$F(1,15) = 7.75$; $P < .05$; Figure 3B].

It is possible that the removal of TMS-artifacts by using ICA may have affected our measurement of the rTMS-evoked physiologic responses. Therefore, we repeated the previously discussed analysis on data for which ICA components were *not* removed. Because the amplitude of the TMS-related artifact should be identical for each pulse, any changes in GFP_{mean} as a function of pulse number should be due to differences in physiologic response. The effects of rTMS on GFP_{mean} values calculated for the uncorrected data, although more noisy, were very similar to those found after artifact correction. When the SPL was targeted, the uncorrected data also showed a significant effect of pulse number [$F(24) = 3.72$; $P < .0001$], driven by a quadratic relationship between pulse number and GFP_{mean} [$F(1,15) = 18.46$; $P < .001$; Figure 3C]. With PCG rTMS, we again found a significant main effect of pulse number [$F(24) = 2.16$; $P < .005$]. This was driven by a marginally significant tertiary relationship between GFP_{mean} and pulse number [$F(1,15) = 3.67$; $P = .07$; Figure 3D].

In addition to these relatively fast effects of rTMS on evoked brain activity, which were measured within the 3-second delay period, it was also possible that rTMS had more slowly developing physiologic effects. To test for this possibility, we looked at changes in the GFP_{mean} across the eight task blocks into which the experimental session was

divided. Specifically, GFP_{mean} values were averaged across the 360 pulses present in each task block. These values were entered as dependent variables in repeated-measures ANOVAs that assessed effects of task block on rTMS-evoked brain activity, separately for SPL and PCG rTMS. For both SPL and PCG rTMS, we found no significant effect of task block (both Fs < 1.34). We repeated this analysis with the same data, but without removal of ICA components and once again found no significant effect of task block on GFP_{mean} amplitude for either SPL or PCG (both Fs < 1.12). These findings are not indicative of more slowly developing effects of rTMS on neural function.

Temporal pattern of the response

As described previously, we found that the GFP_{mean} changed quadratically with pulse number. To more precisely determine the timing of these rTMS-related effects, we next looked at the temporal pattern of the TMS-evoked response after each pulse. As can be seen in Figure 4, both SPL and PCG rTMS elicited a sequence of brain responses as reflected by the different peaks in the GFP function. These peaks are indicative of high variance between channels and reflect a maximum of the total underlying brain activity that contributes to the surface potential field.[49] For both areas targeted, five prominent GFP peaks (peaks I-V) were identified. The five peaks occurred at approximately 4, 26, 42, 60, and 84 milliseconds postpulse. The scalp topographies of the ERPs corresponding to each peak are also displayed in Figure 4. With the SPL targeted, peak I was maximal at approximately the site of stimulation (near electrode Pz). With PCG targeted, peak I was maximal at electrode C6, contralateral to the site of stimulation. The scalp topographies corresponding to each subsequent peak appeared similar for SPL and PCG rTMS, with maximal activity over central scalp regions. However, analyses directly comparing the scalp topographies of the different potential peaks between SPL and PCG rTMS revealed significant differences in evoked responses. Specifically, the spatial distribution of the first and fourth potential evoked by SPL and PCG rTMS were significantly different [peak I: $F(59,885) = 2.11$; $P < .0001$; peak IV: $F(59,885) = 1.65$; $P < .005$].

To determine whether there is a relationship between pulse number and TMS-evoked brain responses, for each subject and peak separately, we first calculated mean GFP using a three-sample (6-millisecond) window around the peak. These values were entered as dependent variables into a repeated-measures ANOVA with pulse number as a within-subject variable, separately for each GFP peak and target site. Figure 5 plots the mean amplitude of each GFP peak for each pulse, peak and target site separately. As can be seen from this figure, except for peak I, GFP amplitudes generally changed across the pulse train in a quadratic manner, similar to GFP_{mean} amplitudes. These rTMS effects are described in more detail in Table 1.

Discussion

The current study examined the neurophysiologic effects of high-frequency rTMS using simultaneous EEG recordings. Our data show that ICA can provide an effective method for removal of TMS-related artifact from EEG data with minimal effect on the measurement of neuronal responses. We found that TMS-evoked brain responses were affected by high-frequency repetitive stimulation, with most evoked responses decreasing in amplitude across the first few pulses, then increasing for the remaining pulses of the rTMS train. This novel finding suggests that rTMS evokes a series of neuronal events that evolves as a function of recent stimulation history.

TMS artifact removal

The current data illustrate the usefulness of ICA in removing TMS-related artifacts from EEG data. ICA successfully identified TMS artifacts, which generally occurred directly below the coil, and within 10-15 milliseconds of pulse delivery. After artifact removal we observed the stereotypical sequence of brain potentials after each TMS pulse in the train that has been described in previous, single-pulse TMS studies.[14,15,22] Nevertheless, it is possible that our method for artifact removal also removed some brain activity. ICA is a statistical algorithm that is useful for removing artifacts that are linearly and independently mixed with signals of interest. Thus, because the TMS artifact and the immediate brain response induced by TMS are highly temporally correlated, ICA may not be able to separate the two sources. Although we did identify and remove components that seemed to contain both artifact and neuronal activity, relatively few such components were identified, and these components explained little variance in the data. Furthermore, analysis of the data without artifact removal revealed, although less clearly, the same general pattern of responses as when the artifact was removed with ICA. We thus believe that ICA can remove TMS-related artifacts with only minimal effects on the measurement of neuronal responses. It is important to note, however, that this method is not likely to remove TMS-related neuronal activity that is less well defined in terms of timing and scalp topography and that, in some contexts, may also be considered artifactual (such as the brain response associated with TMS-related auditory stimuli). Inclusion of a control, such as a controlled auditory stimulation condition during $rTMS_{absent}$ trials, may be useful in this respect. Nonetheless, the ICA method allows for detection of subtle effects of rTMS on evoked EEG response, as well as performance of more complex analyses (i.e., spectral analysis) without contamination by the artifact. It should be noted that it may be necessary to run ICA twice, to ensure that ICA will also identify relatively weak TMS artifacts.

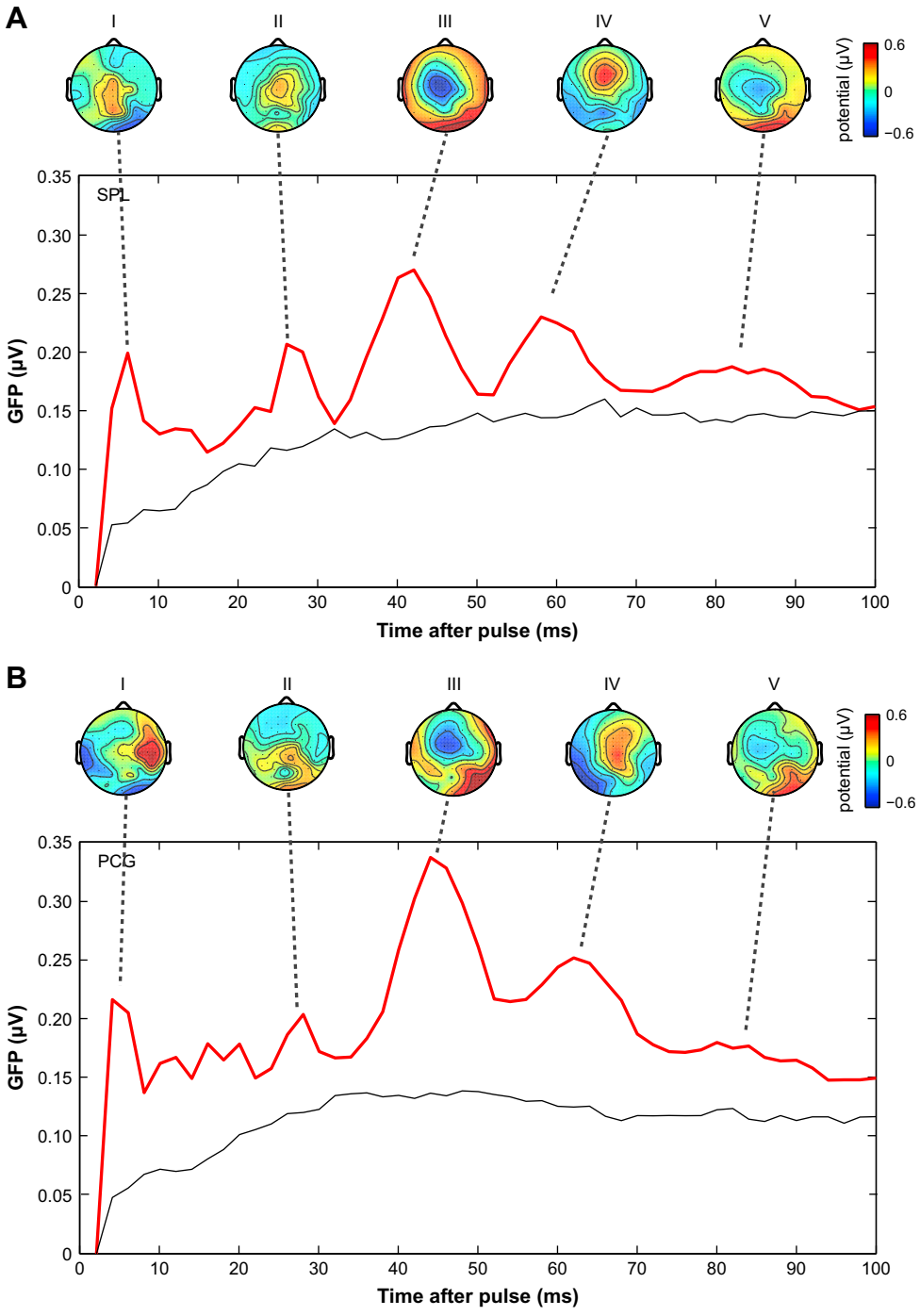


Figure 4 Mean spatiotemporal pattern of brain potentials evoked by repetitive transcranial magnetic stimulation (rTMS). Global field power (GFP) waveforms computed more than 100 milliseconds, averaged across subjects, are shown separately for rTMS to the superior parietal lobule (SPL) (**A**) and postcentral gyrus (PCG) (**B**), averaged across pulses 5-29. For each GFP peak, the corresponding scalp topography is plotted.

Effect of repetitive pulses on TMS-evoked ERPs

After an initial orienting response, each pulse in the rTMS train elicited a sequence of ERPs that were very similar both in time course and scalp topography across the rTMS train. However, for both brain areas targeted (SPL and PCG), we observed a quadratic relationship between the amplitude of evoked response and pulse number, characterized by a dip in the response halfway through the rTMS train. Except for the initial peak after the TMS pulse, this pattern was observed for each peak of the TMS-related ERP. These data extend findings of several previous studies that have reported enhanced MEPs immediately after the delivery of a sequence of pulses,[28,51] by showing that rTMS

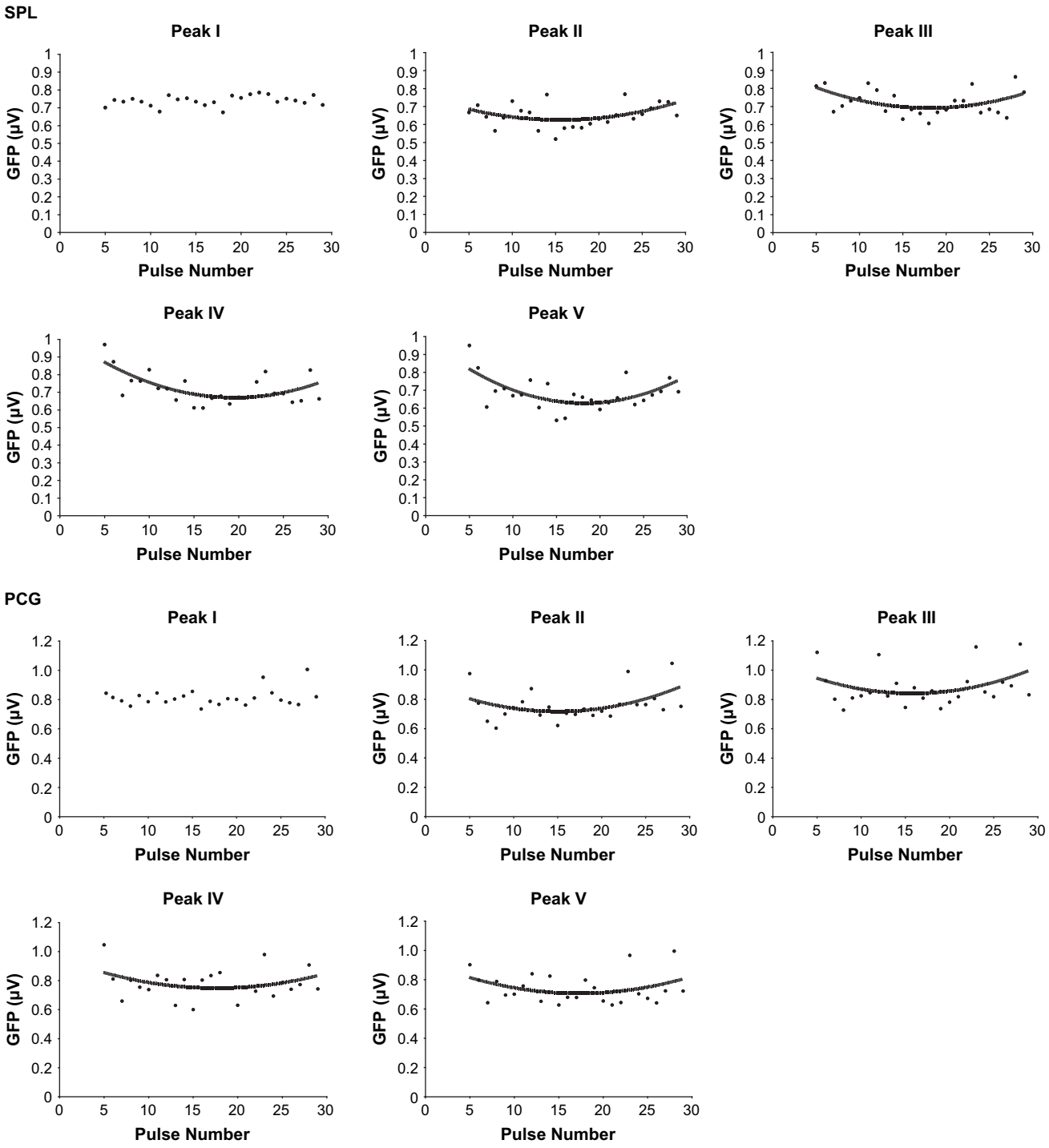


Figure 5 Effects of pulse number in the repetitive transcranial magnetic stimulation (rTMS) train on TMS-evoked brain response amplitudes. Shown are global field power (GFP) peak amplitudes for pulses 5-29 in the rTMS train, separately for superior parietal lobule (SPL) (top panel) and postcentral gyrus (PCG) (bottom panel) rTMS. With the exception of peak I, for both SPL and PCG rTMS a quadratic relationship between pulse number and peak amplitude is evident for all peaks.

may modulate pulse-to-pulse neural excitability during the stimulation train. Studies with paired-pulse delivery of TMS have shown that the physiologic response to a pulse is affected by the delivery of a prior pulse, even with an interpulse interval of several hundred milliseconds.[33,52] The pattern of results observed in the current study corroborates these findings and shows that the physiologic response to a pulse delivered in a high-frequency train of pulses also depends on the number of pulses that have previously been delivered. Together, these data suggest that the physiologic effects of rTMS are more complex than simple linear summation of the neural response. Furthermore,

Table 1 Relationship between GFP amplitude and pulse number reported separately for each peak in the TMS-evoked response

Brain area targeted	Peak no.	Effect of pulse number $F(24)$	Polynomial relationship between peak amplitude and pulse number
SPL	I	0.66; ns	None
	II	1.56; $P < .05$	Quadratic; $F(1,15) = 10.93$; $P < .005$
	III	1.52; $P = .06$	Quadratic; $F(1,15) = 6.26$; $P < .05$
	IV	2.56; $P < .0001$	Linear; $F(1,15) = 4.71$; $P = .05$ Quadratic; $F(1,15) = 12.50$; $P < .005$
	V	2.37; $P < .0005$	Quadratic; $F(1,15) = 8.97$; $P < 0.01$
PCG	I	2.11; $P < .005$	None
	II	3.31; $P < .0001$	Quadratic; $F(1,15) = 7.90$; $P < .05$
	III	3.65; $P < .0001$	Quadratic; $F(1,15) = 4.21$; $P = .06$
	IV	2.91; $P < .0001$	Quadratic; $F(1,15) = 3.00$; $P = .10$
	V	3.15; $P < .0001$	Quadratic; $F(1,15) = 3.46$; $P = .08$

GFP = global field power; TMS = transcranial magnetic stimulation; SPL = superior parietal lobule; ns = not significant; PCG = postcentral gyrus.

they provide additional support for the notion that rTMS has more complex effects than production of a "virtual lesion" with induction of neural noise.[10]

The current findings also demonstrate that, as with single-pulse TMS,[15,16] the effects of rTMS are not limited to the period immediately after the pulse or localized to a circumscribed brain area. Thus, rTMS-related improvements in behavior[7-9] may be better explained by electrophysiologic changes involving multiple brain areas, rather than simple inhibition or potentiation of localized neural activity.

The neurophysiologic processes underlying TMS-evoked brain responses are not well understood,[53] although there is some evidence that these responses reflect the immediate change in current induced by the magnetic pulse.[16] One can therefore only speculate about the cellular basis of the observed quadratic pattern in the TMS-evoked response across our train of 30 pulses. There are at least two possible explanations for the observed effects. One is that rTMS leads to both depression and potentiation. This is conceivable given prior work with repeated stimulation showing dynamic patterns of synaptic response caused by an interaction between synaptic depression and potentiation.[54] Notably, Mongillo et al.[55] described a role for residual calcium levels in transient synaptic potentiation. Their model proposes an interaction between the decrease of available resources with each spike (leading to depression of response) and an increase in synaptic efficacy associated with an increase in residual synaptic calcium levels with each spike. Thus, the pattern of the amplitude of the synaptic response with each spike is determined by the time constants relating to these two opposing effects. There has been at least one experimental demonstration that the time constant for the depressing effect is lower than that of the potentiating effect (which was found to be around 1 second[54]), a pattern that, at least qualitatively, fits the timing of our findings of initial depression, followed by subsequent enhancement of the TMS-evoked response.

A second explanation for the pattern of findings described in this study is that because TMS affects a large population of neurons, it is possible that the initial decrease and subsequent increase in response amplitudes reflect stimulation of two different groups of cell types. Specifically, it is possible that the initial depression of ERP amplitudes was due to a depression of response in excitatory synapses, whereas the subsequent enhancement of ERP amplitudes was due to a delayed depression of the response in inhibitory synapses (or similarly an initial potentiation of inhibition followed by later potentiation of excitation). In line with this possibility, Quartarone et al.[56] suggest that rTMS may affect specific subpopulations of neurons differently, an effect that cannot be distinguished by EEG because of its relatively low spatial resolution.

A question that remains for future studies is whether the pulse-to-pulse changes in rTMS response depend on the frequency of stimulation. Is there something specific about the timing of 10-Hz rTMS that leads to the observed changes in amplitude with subsequent TMS pulses? On the basis of previous paired-pulse TMS studies showing that neuronal excitability is highly dependent on the inter-pulse interval, one may predict that the timing between pulses (and hence frequency of rTMS) will affect cortical excitability from one pulse to the next. Future studies are necessary to examine the effects of stimulation frequency and other parameters, such as intensity or number of stimuli delivered, on brain activity, and may allow for a further characterization of the effects of rTMS on brain function.

Conclusions

We found that TMS-evoked brain responses were affected by repetitive stimulation, with an initial depression of the TMS ERP, followed by a late potentiation. This finding suggests that 10-Hz rTMS may evoke multiple cellular

mechanisms. Furthermore, this study showed that ICA provides a relatively simple and effective method for TMS-related artifact removal from EEG data.

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