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Effects of Repetitive Transcranial Magnetic Stimulation on Spike Pattern and Topography in Patients with Focal Epilepsy

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Abstract Repetitive Transcranial Magnetic Stimulation (rTMS) is a non-invasive method for brain stimulation. Group-studies applying rTMS in epilepsy patients aiming to decrease epileptic spike- or seizure-frequency have led to inconsistent results. Here we studied whether therapeutic trains of rTMS have detectable effects on individual spike pattern and/or frequency in patients suffering from focal epilepsy. Five patients with focal epilepsy underwent one session of rTMS online with EEG using a 6 Hz prime/1 Hz rTMS protocol (real and sham). The EEG was recorded continuously throughout the stimulation, and the epileptic spikes recorded immediately before (baseline) and after stimulation (sham and real) were subjected to further analysis. Number of spikes, spike-strength and spike-topography were examined. In two of the five patients, real TMS led to significant changes when compared to baseline and sham (decrease in spike-count in one patient, change in topography of the after-discharge in the other patient). Spike-count and topography remained unchanged the remaining patients. Overall, our results do not indicate a consistent effect of rTMS stimulation on interictal spike discharges, but speak in favor of a rather weak and individually variable immediate effect of rTMS on focal epileptic activity. The individuation of most effective stimulation patterns will be decisive for the future role of rTMS in epilepsies and needs to be determined in larger studies.

Keywords Repetitive TMS · Epilepsy · Spike Pattern

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Introduction

Repetitive Transcranial Magnetic Stimulation (rTMS) is a non-invasive and generally well-tolerated method for brain stimulation, based on principles of electromagnetic induction. Small intracranial electrical currents are generated by a strong fluctuating extracranial magnetic field (Barker et al. 1985; Kobayashi and Pascual-Leone 2003; Bae et al. 2007). In contrast to single- and paired-pulse TMS, rTMS can induce changes of cortical excitability and neuronal activity outlasting the duration of the stimulation itself (Hallett 2000; Silvanto and Pascual-Leone 2008) Stimulation with frequencies of 1 Hz and lower decreases cortical excitability (Chen et al. 1997). This effect of rTMS has been applied with therapeutic attempt in several pathologies such as depression, pain, tinnitus and stroke, as well as in epilepsy patients to inhibit epileptic activity (Theodore 2003).

The pathophysiology of focal epilepsy is thought to be based on cortical imbalance between excitatory and inhibitory mechanisms within a certain brain area (Valentin

et al. 2008). Paroxysmal synchronous depolarization within such areas of imbalance can be recorded with EEG or MEG on the scalp surface as interictal epileptic spikes (ES) and, in case of failure of surrounding inhibitory mechanisms, can eventually result in epileptic seizures. Studies using low frequency rTMS (0.3–1 Hz) in epilepsy patients initially reported promising results with beneficial effects on seizure frequency and/or number of ES after stimulation (Tergau et al. 1999; Theodore et al. 2002). However, subsequent trials on low frequency rTMS in epilepsy patients were discordant regarding clinical effectiveness. On the one hand, several case reports and open label studies have reported beneficial, some even long lasting, reduction of seizures and/or ES (Daniele et al. 2003; Fregni et al. 2005a; Santiago-Rodriguez et al. 2008) or complete arrest of seizure signs in a patient with epilepsia partialis continua (Misawa et al. 2005). Others, on the other hand, failed to demonstrate significant effects on seizure rates (Kinoshita et al. 2005).

Discordant results have also been obtained in sham controlled studies. A significant reduction of ES and long lasting (>2 months) significant seizure reduction was found in 21 patients with cortical malformations after 5 days of 1 Hz rTMS in the real rTMS group only by Fregni et al. (2006b). In contrast, Theodore et al. (2002) used 1 Hz rTMS and found a non-significant reduction of seizures after real stimulation in their 24 patients. Tergau et al. (2003) studied 17 patients and did not find significant differences after 1 Hz-stimulation, but a significant decrease of seizure rates using 0.3 Hz. However, the latter effect was observed during the stimulation period only, and only when compared to baseline but not to placebo (Tergau et al. 2003). Cantello et al. (2007) included 43 patients and used 0.3 Hz-stimulation on five consecutive days showing non-significant reduction of seizures and ES in the active stimulation group.

The available data fail to hold up to the first promising results as to the use of rTMS in epilepsy treatment. A beneficial influence of low frequency rTMS on epileptic activity seems to be present at least in some subgroups of patients, but it remains unclear what accounts for the success in some and not in other patients.

The majority of the abovementioned studies evaluated the effect of rTMS on the number of spikes and seizures only. The present study intended to go one step further by addressing the question whether therapeutic trains of rTMS have detectable effects on the spatio-temporal pattern of epileptic discharges. Analysis was thereby performed on the individual level (single-subject analysis) in order to explore effect variability. To capture potentially discrete effects on spikes, we not only looked at whether a single session of rTMS leads to immediate changes in the number of ES, but also in their electric field strength, their topography and their dominant map sequence (i.e. the propagation pattern). The rationale for exploring effects of inhibitory rTMS on these ES-parameters is based on the assumption that rTMS can achieve reorganization of cortical excitability, with immediate inhibitory effects outlasting the stimulation (Maeda et al. 2000; Silvanto and Pascual-Leone 2008). In addition, the inhibitory effects of low frequency rTMS have been attributed to the trans-synaptic activation of GABAergic inhibitory interneurons to the recurrent inhibition of the targeted cortical neurons through axonal collaterals (Pascual-Leone et al. 1994). As GABAergic interneurons are contributing to the hyperpolarization (the so-called after-discharge) subsequent to the rapid synchronized discharges forming the spike peak, we hypothesized that a focal inhibitory stimulation would lead to demonstrable changes of the spike pattern and topography.

Materials and Methods

Patients

Five patients participated in this study (2 female, 3 male; mean age of 26.2 years, range: 18–35.6 years). All patients except for patient #2 had symptomatic focal epilepsy and had been referred to the presurgical evaluation center of the Neurology Department of the University Hospital of Geneva. The patients suffered from therapy-refractory focal epilepsy and were candidates for the work-up procedure to determine the possibility of surgical treatment. The patient's characteristics such as age at onset of the epilepsy and at evaluation, aetiology of the epilepsy, seizure frequency, EEG focus and stimulation parameters are listed in Table 1.

All patients were informed in detail about the purpose and the proceedings of the study. All participants had given their written informed consent prior to participation. The local ethics committee approved the study. The entry criterion were frequent and unifocal ES in the awake EEG, no change of antiepileptic medication in the 8 weeks prior to the stimulation and age over 18 years.

rTMS Protocol

We used a protocol that has been shown to enhance the inhibitory effects of 1 Hz stimulation in healthy participants (Iyer et al. 2003) and involves a priming sequence of 6 Hz TMS trains applied at 90% motor threshold immediately preceding the 10 min of 1 Hz stimulation with 110% motor threshold. This enhancement of inhibitory effects is likely due to homeostatic meta-plasticity. Hoemostatic meta plasticity, in this case, describes the enhancement of the inhibitory effect of 1 Hz rTMS, if

Table 1 Patient characteristics and stimulation parameters

Patient no.	Sex	Age	Age of onset (years)	Aetiology of epilepsy	Seizure frequency	Focus	N of elect	Protocol	Stimulation intensity (6 Hz/ 1 Hz)
1	F	22.8	0	Neonatal meningoencephalitis	1-2/week	P3	64	(1) 6 Hz/1 Hz sham (2) 6 Hz/1 Hz real	56/70
2	M	22.9	15	Idiopathic	5-10/year	F7-FT 7	64	(1) 6 Hz/1 Hz sham (2) 6 Hz/1 Hz real	50/62
3	M	35.6	15	Head trauma	10/month	FCz	31	(1) 6 Hz/1 Hz sham (2) 6 Hz/1 Hz real	54/70
4	F	18.0	8	Hippocampus sclerosis	4-6/week	T7- TP9	64	(1) 6 Hz/1 Hz real (2) 6 Hz/1 Hz sham	53/64
5	M	31.6	12	Congenital brainmalformation	∼1-3/ day	P4	31	(1) 1 Hz sham (2) 1 Hz real (3) 6HZ/1 Hz real	55/62 55/62

Note that all patients received a 6 Hz-primed/1 Hz stimulation protocol given as real or sham-stimulation except for patient #5. He participated in an1 Hz (sham and real) session and received the 6 Hz-primed/1 Hz stimulation protocol on a different day in real only. The choice of protocol is described in more detail in the method section. The stimulation intensities are given in % of stimulator output and were adapted to the patient's individual motor threshold (90%/110%)

F female, *m* male, *P* parietal, *O* occipital, *F* frontal, *C* central, *T* temporal, *R* right, *L* left)

preceded by a transient increase of excitability, for example via anodal transcranial direct current stimulation (Siebner et al. 2004) or due to pharmacologically different blood levels of valproate (Fregni et al. 2006a). The 6 Hz primes were applied for 10 min, intermittently in 20 trains of 30 pulses, with 25 s breaks between each train. The 1 Hz stimulation was applied continuously over 10 min immediately after the 6 Hz priming. This resulted in a total of 1200 pulses applied within 20 min. Motor threshold was assessed for the dominant hand after mounting of the EEG, so that the distance between head and coil introduced by the electrodes was taken into consideration for the motor threshold estimation.

We used a Magstim Rapid Transcranial Magnetic Stimulator (Magstim Co., Whitland Wales, UK) with a figure of eight coil of 7 cm diameter for each circular loop (maximum field strength: 2.2 T). The spot targeted with rTMS was the area underneath the electrode recording the largest negative amplitude of ES. The EEG focus was identified in the clinical EEG recordings beforehand and verified in the recordings of the stimulation session offline. Table 1 contains the electrodes with focus identification for each patient. In case of a phase inversion of the epileptic activity between two electrodes, the location between these two positions was targeted (patients #2 and 4). For SHAM stimulation the coil was held perpendicularly to the patients head over the same spot. The patients were informed that two different ways of application of the magnetic stimulation would be tested without giving any further information on effectiveness of the one or the other.

Patients #1, 2, 3 and 4 received two blocks of 6 Hz/1 Hz rTMS (REAL and SHAM), with a 30 min break in between. In patients #1, 2 and 3 we applied rTMS first in SHAM, then in the REAL condition. In patient #4, we inverted this order, i.e. REAL rTMS was applied before SHAM. Patient #5 underwent two sessions of rTMS stimulation on different days. In addition to the 6 Hz/1 Hz rTMS protocol, this patient received 1 Hz rTMS session at 100% MT for 10 min (i.e. 600 pulses), to test whether 1 Hz stimulation without the priming sequence of 6 Hz leads to a different effect than the 6 Hz/1 Hz stimulation protocol. The 1 Hz stimulation session preceded the 6 Hz/1 Hz session by one week, and in the latter session, SHAM stimulation could not be performed due to time limitations.

EEG Recording

The patients were seated in a comfortable chair. A TMS compatible EEG system was mounted. Patients #3 and 5 were recorded with a 31-channel system with conductive plastic-body electrodes coated with a thin layer of silver epoxy (Ives EEG solutions, Inc. Burlington, Ontario, Canada) at 200 Hz sampling rate. The other three patients were recorded with a 64-channel system at 250 Hz sampling rate (BrainAMP MR; Brain Products GmbH, Gliching, Germany). Impedances were kept below 10 kΩ in all cases. Data were recorded against a vertex reference, and re-referenced to the average reference for analysis. The electrodes remained on the patient's head throughout the whole EEG recording session, which lasted about 2 h

starting from 30 min before the first to 30 min after the last rTMS stimulation (EEG recorded continuously).

Offline Analysis of EEG Data

The 30 min EEG before and after each rTMS application were analyzed. The EEG blocks will be referred to as *BL* (Baseline), *REAL* (after REAL rTMS) and *SHAM* (after SHAM rTMS).

Each patient's EEG was analyzed by the same M.D./ clinical neurophysiologist experienced in reading clinical EEG (V.B.) Prior to this analysis, a different co-author (L.S.) cut the EEG blocks into 10 min sections and renamed them without disclosing which condition the section belonged to. As analyses were performed on the data before or after TMS, no EEG section contained TMS artifacts that could have provided clues as to which protocol was used (SHAM, REAL TMS). This way the analyzer was kept blind to patient and TMS condition per each section analyzed.

To distinguish potential early from late effects of the stimulation on spike number and or configuration, we split the EEG of each condition in two halves. The resulting conditions are labeled $BL\text{-}T_1$, $BL\text{-}T_2$, $SHAM\text{-}T_1$, $SHAM\text{-}T_2$, $REAL\text{-}T_1$ and $REAL\text{-}T_2$. It is conceivable that a given effect on spike patterns might be short lasting, appearing immediately or within a certain delay. Splitting the recorded ES in two halves should avoid a weakening effect when averaging all ES of one condition.

Number of Epileptic Spikes

All epileptic spikes (ES) occurring in the EEG blocks were marked at the maximal negative amplitude. The ES were marked first for pure spike count, disregarding any artifact contamination from movements or eye blinks. In a second step, artifact-free ES were selected and exported for subsequent spike pattern analysis. The total occurrence of ES is quantified as the number of ES per 1 min in Fig. 1. We used a 3×2 factorial ANOVA to test for changes in spike numbers per patient, using condition (BL, SHAM, REAL) and block time (T_1 and T_2) as factors.

Comparison of Spike Patterns

All artifact-free spikes were exported as single epochs of 100 ms before and 300 ms after the spike's negative maximum. Before exporting, the data were band-pass filtered from 0.3 to 70 Hz, including a notch filter at 50 Hz. All spike pattern analyses were performed comparing two pairs of conditions ($BL\text{-}T_1$ vs. $SHAM\text{-}T_1$, $BL\text{-}T_1$ vs. $REAL\text{-}T_1$, $SHAM\text{-}T_1$ vs. $REAL\text{-}T_1$; $BL\text{-}T_2$ vs. $SHAM\text{-}T_2$, $BL\text{-}T_2$ vs. $REAL\text{-}T_2$, $SHAM\text{-}T_2$ vs. $REAL\text{-}T_2$).

To test for differences in electric field strength over time, we randomly selected for each patient and condition 40 single spike epochs. This was done to adjust to the patient/condition with the smallest number of artifact-free epochs. These data were subjected to an unpaired *t*-test on the single epochs' global field power (GFP) performed time-point by time-point along the spike potential (Lehmann and Skrandies 1980; Murray et al. 2008) per each of the six comparisons (three comparisons on data of the first (T1) and three on the second half of the recordings (T2)).

To probe for differences regarding the spatial distribution of the electrical field measured on the scalp surface (which is independent and orthogonal to the above GFP analysis), we performed a topographical ANOVA (T-ANOVA), as previously used in event-related potential analyses (Pourtois et al. 2005; De Santis et al. 2007). It measures the topographical dissimilarity of a recorded potential time-point per time-point and gives an index of configuration differences between two electric fields at a given moment. It is based on a non-parametric randomization test comparing the global map dissimilarity between two conditions. The global map dissimilarity corresponds to the GFP of the difference of the normalized maps and is thus a measure of topographic difference independent of field strength (Lehmann and Skrandies 1980; Koenig 2009). The randomization test includes all single spikes of each condition [for full methodological details and formula see (Murray et al. 2008)]. The same EEG epochs as for the GFP analysis were entered for this T-ANOVA. For both, the *t*-test on GFP and the T-ANOVA, effects with *P*-values lower than 0.05 were considered significant only if lasting 20 ms or longer (e.g. Guthrie and Buchwald 1991).

Additionally, we characterized the temporal dynamics of each patients' average spike potential of each condition by performing a spatio-temporal segmentation analysis to identify the most dominant map topographies that contribute to the average potential. Specifically, we applied a modified hierarchical cluster analysis as implemented in Cartool (Topographic Atomize & Agglomerate Hierarchical Clustering (T-AAHC)). Cluster analyses have proven to be a powerful tool for identifying differences in dominant maps between conditions and have been used for characterization of seizure and spike propagation (Lantz et al. 2001; Lantz et al. 2003) and in the analysis of event-related potentials (for reviews see, Michel et al. 2001, 2009). The method identifies periods of topographic stability within and between different conditions and defines the sequence of map topographies optimally summarizing the average data (Michel et al. 2004; Murray et al. 2008). In the current study this method was used to reduce the data to the most dominant maps and to test for possible differences in the presence and sequence of these maps between the conditions (Lantz et al. 2003). The numbers of clusters was set to
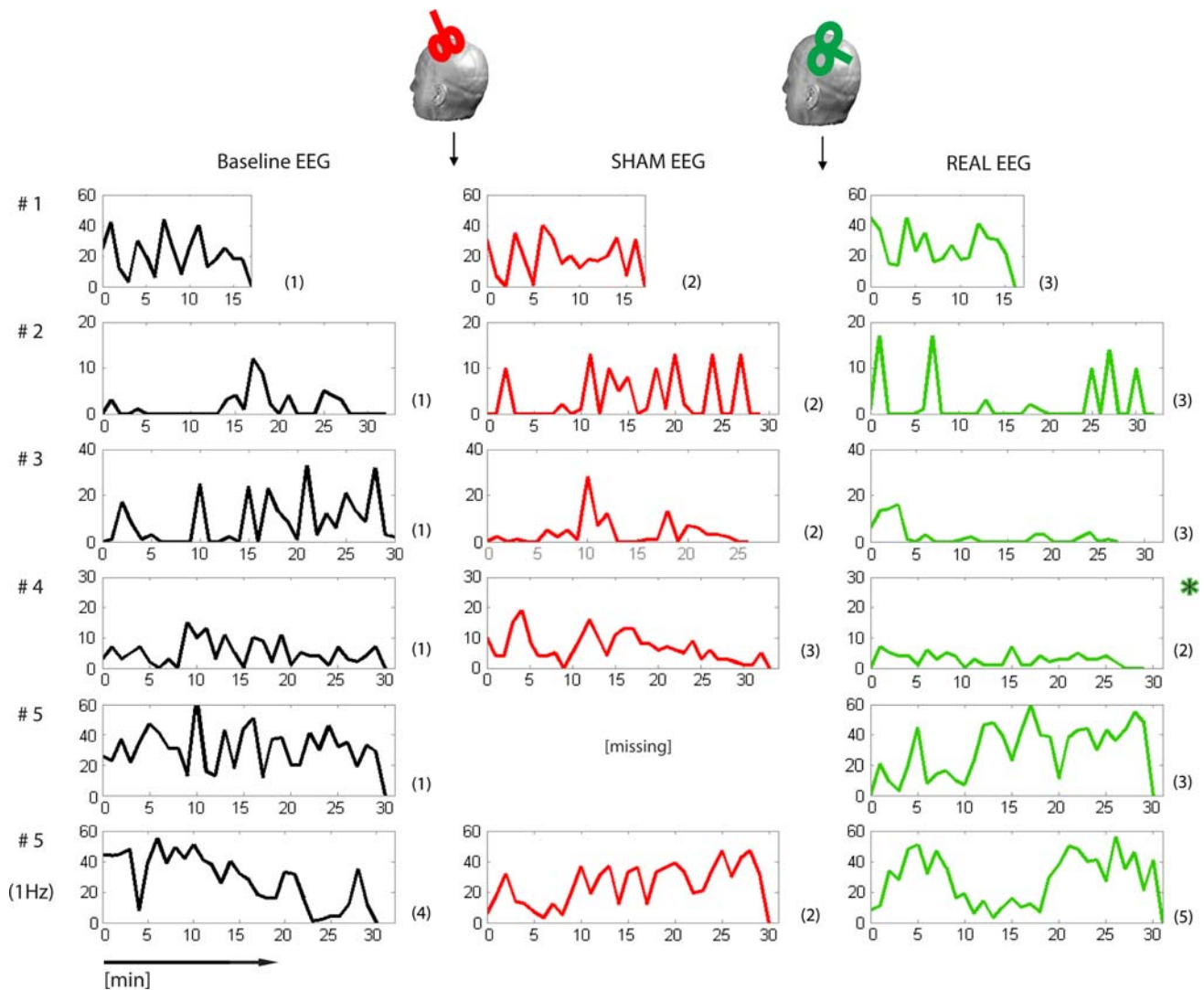
Fig. 1 Evolution of spike count over block time. Individual spike rate per minute (y-axis) over time of the recording (y-axis) which was 30 min (except in patient 1, who discontinued the last recording session following real TMS after 17 min). TMS was applied between the shown blocks of EEG spike count as indicated by the two head models. Note that for the sake of consistent display, we show the SHAM block before REAL, although block order was counterbalanced (see text or number on lower right corners for block order). Patient #5 had a total of 5 blocks of EEG due to the additional 1 Hz rTMS session performed at another day (no sham in 6 Hz/1 Hz). The green asterisk indicates the only patient (#4) who showed a significant effect (decrease) in number of spikes after real rTMS

a maximum of 10, the minimum duration of one cluster to 20 ms. The average spike of BL, SHAM, and REAL per patient, grouped for T1 and T2, were segmented. A cross-validation criterion was used to determine the optimal number of maps (Pascual-Marqui et al. 1995).

Results

The stimulation procedure was well tolerated by all patients. Because patient #1 wished to discontinue the last post-TMS EEG session after 17 min of recording (due to being too hungry to continue, but not reporting any discomfort when explicitly asked) and because patient #2 did not show sufficiently enough ES to allow grouping into sub-blocks, the splitting of the EEG blocks into the first and second 15 min after TMS-administration (T$_1$ and T$_2$) was only possible for patients #3–5.

Number of Epileptic Spikes (ES)

The evolution of ES over time is shown for each patient in Fig. 1 as plots of ES per minute over time for each condition. Baseline is shown in black, SHAM in red and REAL in green. The figure illustrates the strong variability in spike rate over time in all patients.

For the 6 Hz/1 Hz-protocol, statistical comparisons between the three treatment conditions (BSL, SHAM, REAL) over time of recordings (0–15 min vs. 15–30 min) for patients #3–5 (no factor time of recording for patients #1–2) revealed a significant effect of REAL TMS on the number of ES only in patient #4 (main effect of treatment, $F(2,84) = 9.84$, $P < 0.001$) with a significant reduction of ES after REAL rTMS as compared to SHAM ($F(1,56) = 21.4$, $P < 0.001$) and as compared to BSL ($F(1,56) = 6.37, P = 0.015$). This was independent of time, i.e. present for both the 0–15 min and 15–30 min recording periods following TMS (no interaction treatment x time, $F < 1, P = 0.77$). For all other patients, no significant effect on spike rate specific to REAL TMS could be identified.

Comparing the different protocols in patient #5 did not reveal any main effects of protocol (6 Hz/1 Hz vs. 1 Hz; $F < 1$, n.s.) or treatment (BSL vs. REAL, $F < 1$, n.s.) nor an interaction between these factors ($F < 1$, n.s.) or between these factors and time ($F(1,112) = 1.8$, n.s.). Thus, there was no evidence that either protocol was effective in changing spike rate in this patient.

Comparison of Spike Patterns

Figures 2, 3, 4, 5, 6, 7 show the results of the detailed spike pattern analysis (one figure per patient) and are structured in the following way:

The top row shows the average spike potential of each condition in butterfly display (all EEG traces superposed) with the corresponding GFP per condition below. The data are aligned to the spike's negative maximum (0 ms) and are shown for an epoch spanning from 100 pre-spike maximum to 300 ms post-spike maximum. As indicated by the vertical grey line, this time line also applies to the statistical analyses and segmentation output shown below.

The statistical results are displayed in the two black panels, depicting P-values lower than 0.1 for (i) the unpaired t-test on global field power and (ii) the T-ANOVA for comparison of topography. If significant differences were identified, we considered them specific to real TMS only if they appear concomitantly (same time periods) in the comparisons SHAM vs. REAL and BL vs. REAL. Differences

Fig. 2 Results of spike pattern analysis for patient #1. *Top*: average spike potential of each condition in a butterfly plot with the GFP curve below (−100 pre- to +300 ms post-spike maximum; *black*: Baseline (BL), *red*: SHAM stimulation, *green*: REAL stimulation). *Middle*: Results of the statistical analysis: (1) unpaired *t*-test on global field power, (2) T-ANOVA for comparison of topography. *P*-values lower than 0.1 are shown. The horizontal red line indicates the level of significance at $P = 0.05$ (*red*: BL vs. SHAM, *blue*: BL vs. REAL, *yellow*: SHAM vs. REAL). *Bottom*: Results of the topographic cluster analysis defining segments with different map topographies. Each condition's GFP (BL, SHAM and REAL) is shown with the sequentially identified segments, each segment coded in a different color. The template maps are displayed below, framed in the color of the corresponding segment (negative amplitudes in *blue*, positive in *red*, maps seen from top, nose up, left ear left). More details are given in the result section
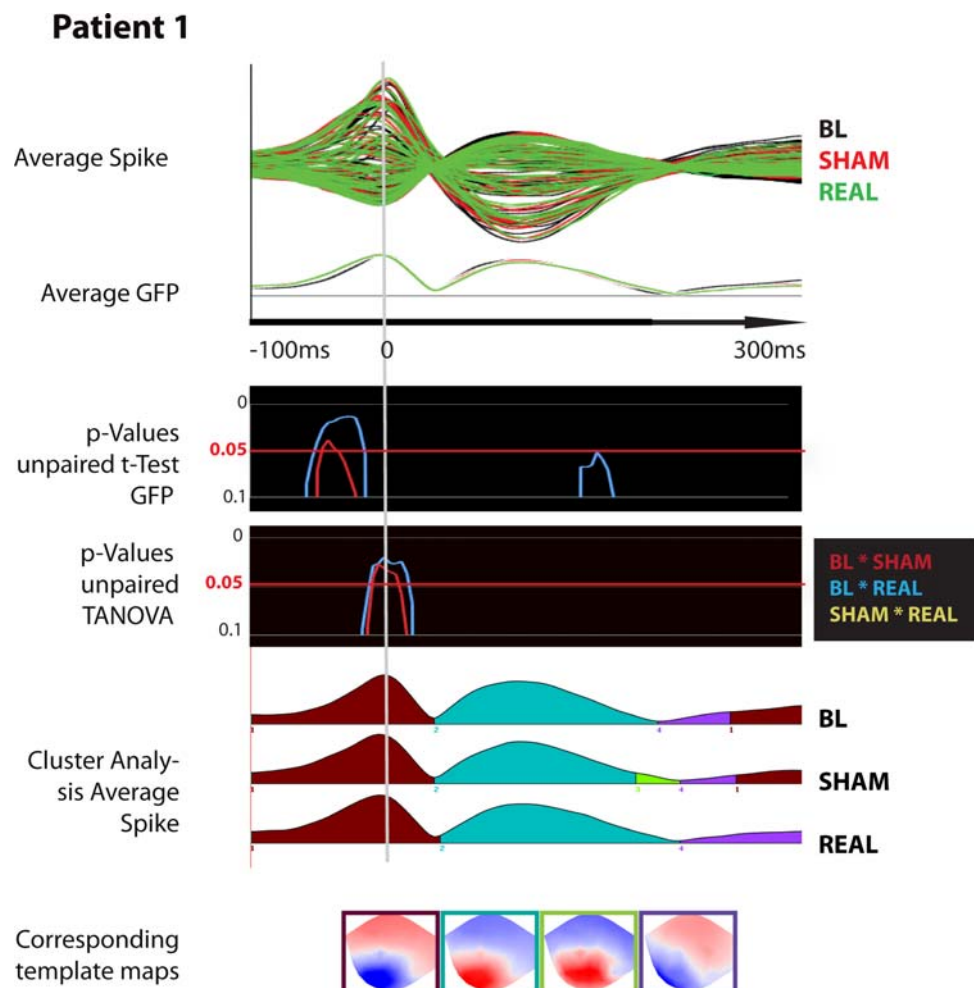
Fig. 3 Results of spike pattern analysis for patient #2. For details of the figure see legend of Fig. 2
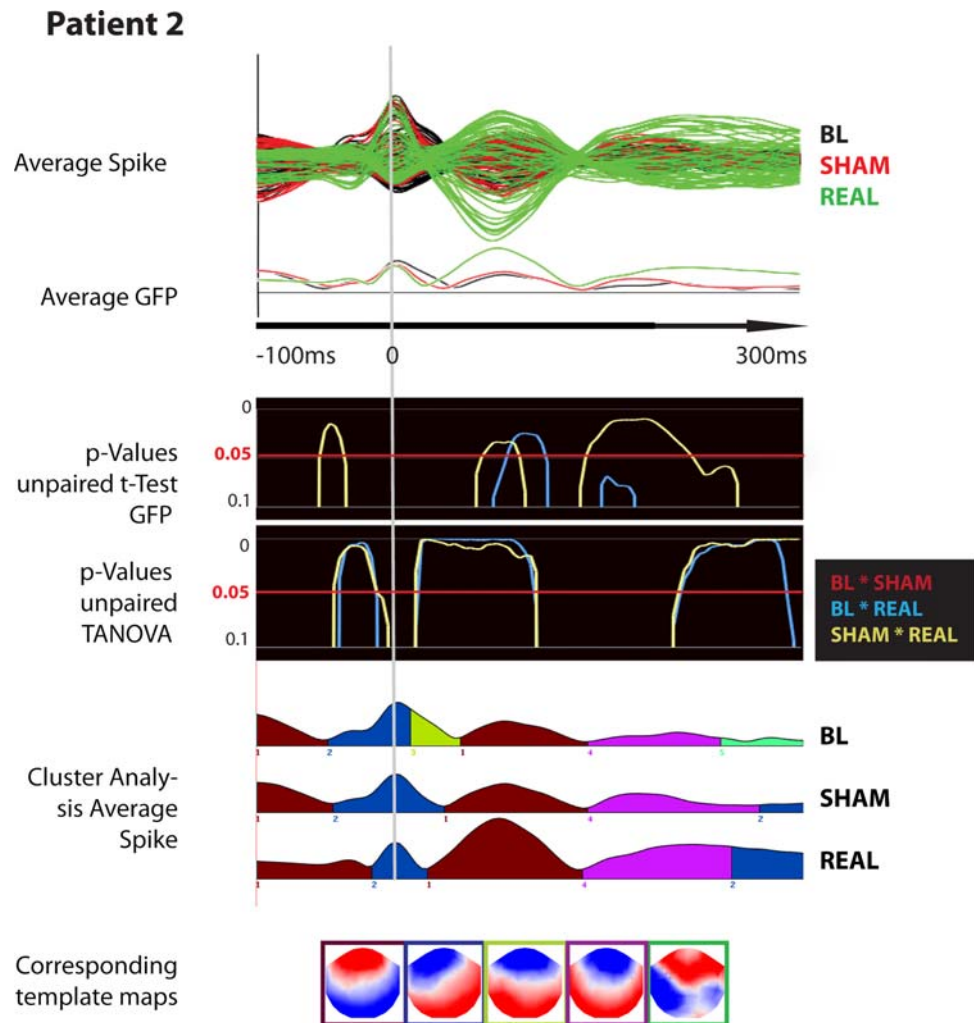
are considered unspecific, however, if in the same time window SHAM also differs from BL.

The result of the segmentation analysis (T-AAHC) is illustrated below. Each condition's GFP (BL, SHAM and REAL) is shown with the sequentially identified segments, each segment coded by a different color. The segments correspond to template maps best defining the given time period. The template maps are displayed below, framed in the color of the corresponding segment and listed in the order of their appearance.

For patients #1 and 2 all spikes of one condition are summarized (see Methods for details). For the other patients, the results are split according to first and second half of the recordings (T_1 and T_2).

Average Spike Potential and Global Field Power

Except for patient #5, all patients showed typical epileptic spike and wave complexes with a rapid peak followed by a slower after-discharge potential. Patient #5 showed slow

sharp waves with one single GFP peak only. On visual inspection, most of the average spike traces appeared relatively unchanged over conditions in the same patient, with one exception: Patient #2 showed a clear increase in the amplitude of the spike's after-discharge in REAL as well as a clear increase in amplitude at the end of the epoch (140–220 ms) in the same condition (see Fig. 3, upper row, Average GFP).

The statistical comparisons of GFP across conditions revealed several intervals of significant differences in most patients (see Figs. 2, 3, 4, 5, 6, 7, P-values of unpaired t-tests on GFP). However, only few differences qualified as being specific to real TMS. In patient #2, the differences that are visible in the averaged spike traces are also statistically reliable. In a time window during the after-discharge (64–92 ms), an increase in amplitude in both REAL vs. SHAM and REAL vs. BL was observed (Fig. 3). Likewise, patient #4 showed differences specific to real TMS in the late after-discharge (94–142 ms and 222–270 ms) in T_1 but with reduced amplitude in REAL as
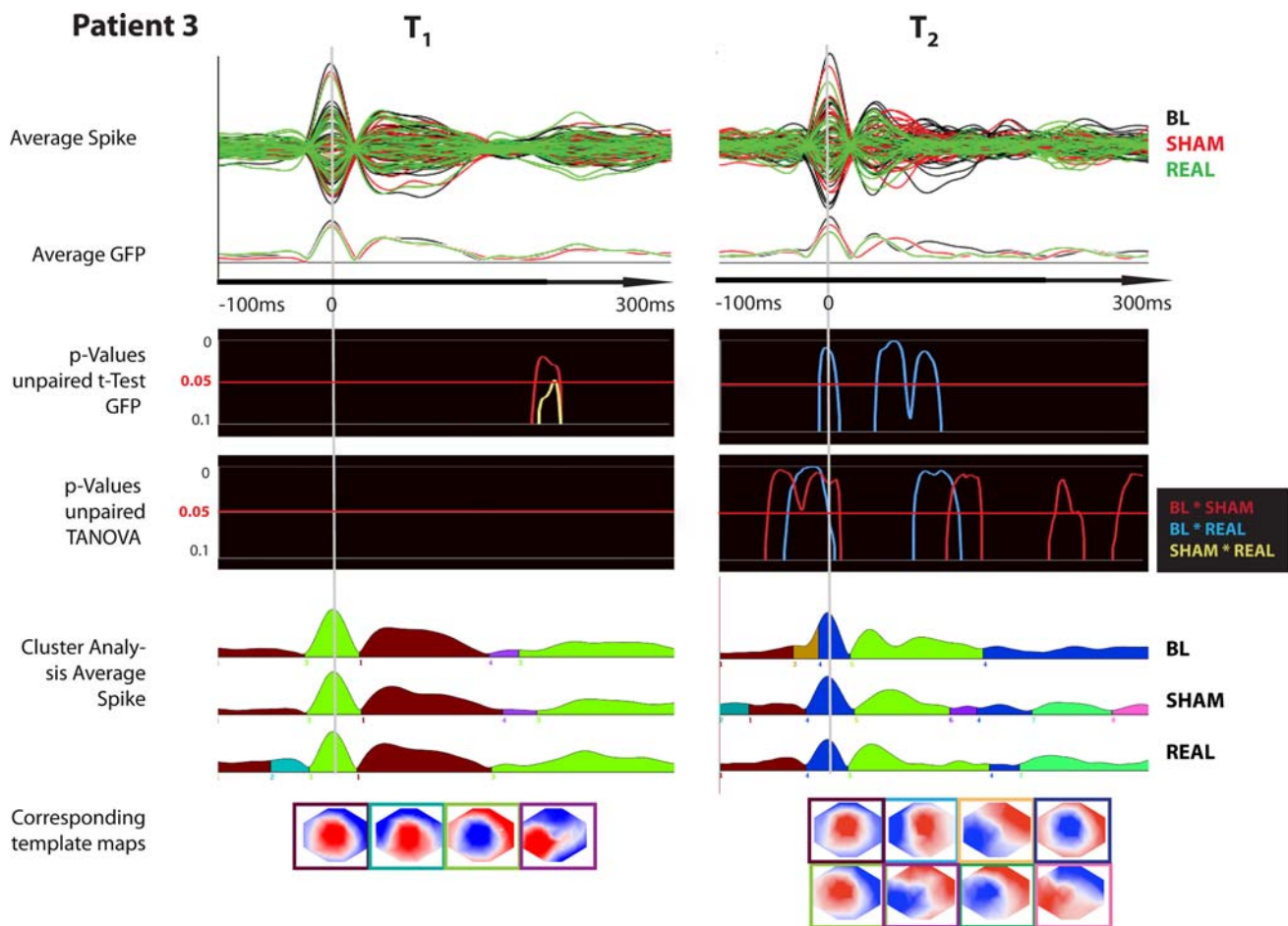
Fig. 4 Results of spike pattern analysis for patient #3. For details of the figure see legend of Fig. 2

compared SHAM and BL (Fig. 5). In T_2, patient #4 showed one short time period in the post-peak phase (170–194 ms), but only in the SHAM vs. REAL comparison, which is therefore considered unspecific (Fig. 5).

No other patient showed effects on GFP that could be classified as specific to real rTMS. In patient #1, all observed differences were unspecific, as none appeared in SHAM vs. REAL (Fig. 2). In patient #3, all differences were short (T_1) and/or did not occur for SHAM vs. REAL ($T_{1 \text{ and }} T_2$) (Fig. 4). In patient #5, again no difference was observed for SHAM vs. REAL ($T_{1 \text{ and }} T_2$) (Figs. 6, 7). Although the 6 Hz/1 Hz stimulation lacks the SHAM condition, the increase in REAL vs. BL in this protocol in T2 (Fig. 7) cannot be interpreted as specific, as similar long-lasting effects were also observed in this patient for REAL vs. BL in the 1 Hz-protocol in the absence of concomitant differences between REAL vs. SHAM (Fig. 6, T_1).

Segmentation Results and Topographical Differences

The topographies were relatively similar over the three conditions in most patients, as suggested by the

segmentation results and confirmed by the T-ANOVA. Only in patient #2 was there a difference in topography that was specific to real TMS. This patient showed a reduction in duration of the spike-segment during REAL as compared to both BL and SHAM (see segmentation result; early blue segment/map 2, Fig. 3) (with one additional segment in the BL condition (bright green frame) that is not identified in SHAM or REAL). The topographic differences around the spike-peak were substantiated by the T-ANOVA showing significant differences for the comparisons of REAL vs. BL and REAL vs. SHAM for both the rising (−44 to −22 ms) and falling phase (16–104 ms) of the spike. The latter period of significance furthermore extended into the period of the after-discharge (Fig. 3). Similarly, significant differences between REAL vs. BL and REAL vs. SHAM were observed at the end of the spike period (208–300 ms). Here, the segmentation shows one additional map in BL and a different duration of the last map in SHAM, explaining the topographic difference (Fig. 3).

No other patient showed effects on topography that could be classified as specific to real rTMS. Patient #1
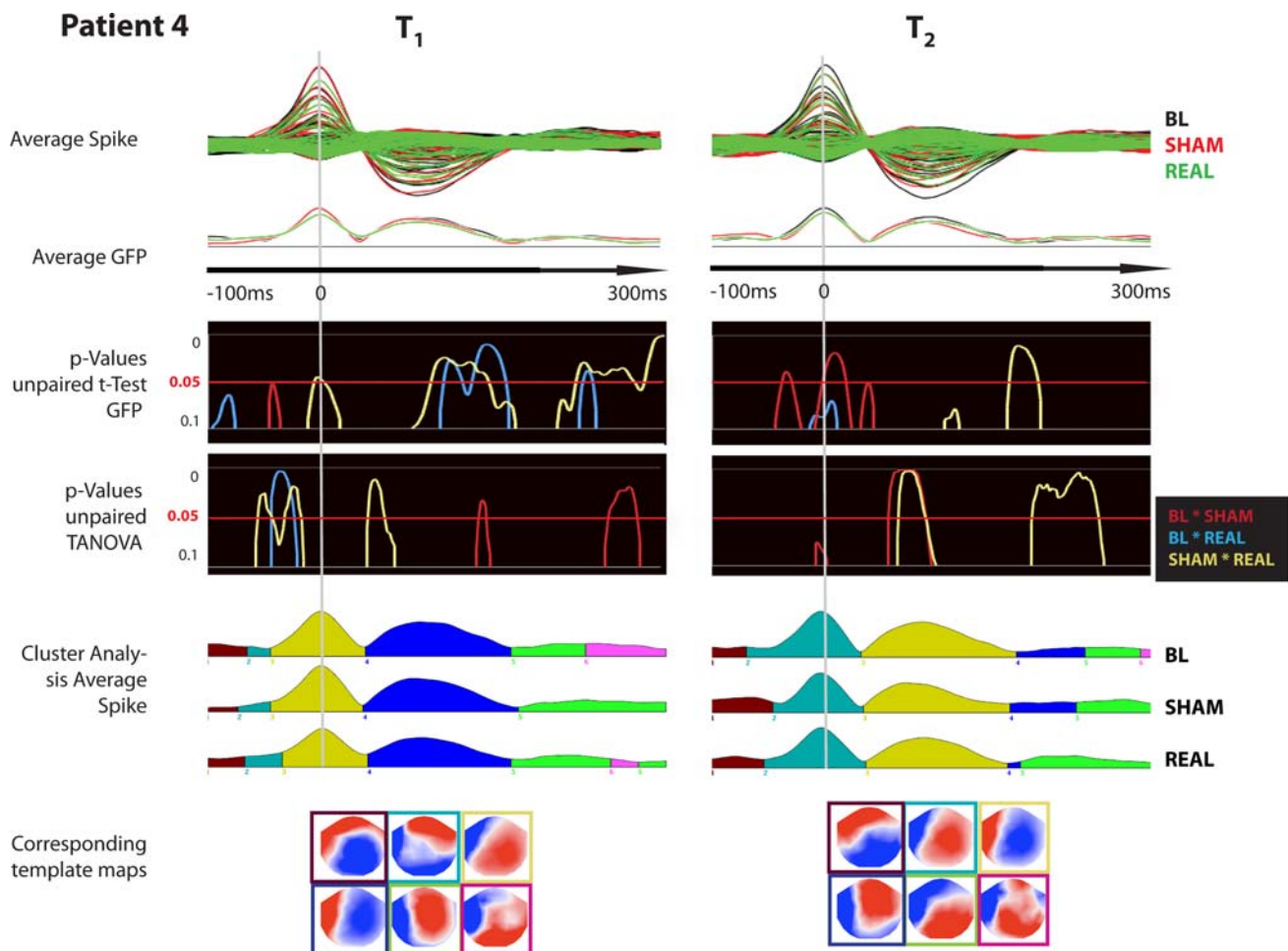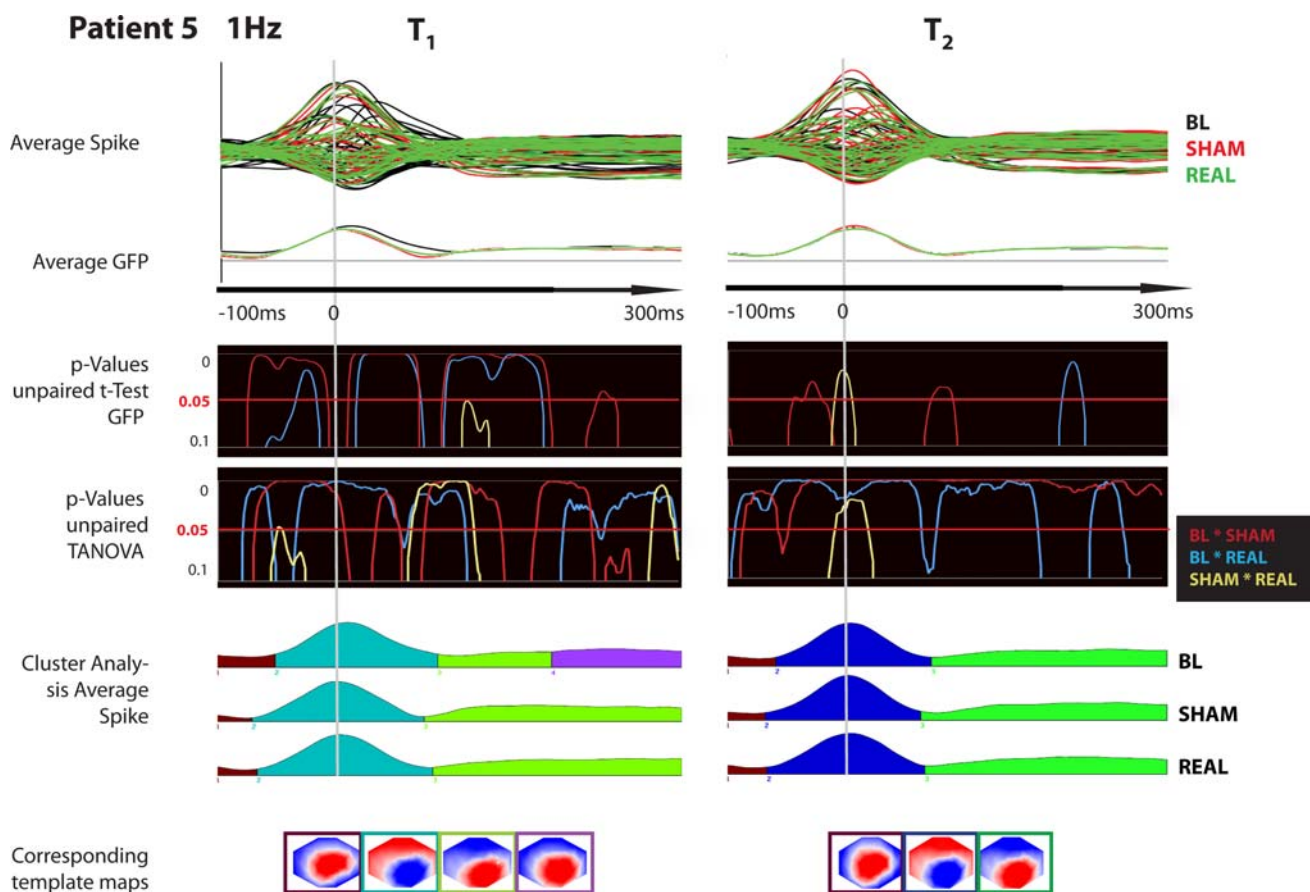
Fig. 5 Results of spike pattern analysis for patient #4. For details of the figure see legend of Fig. 2

showed nearly identical segmentation results in all conditions except for some additional segments towards the end of the epoch, which however were not significant in the T-ANOVA (Fig. 2). Patient #3 showed one segment appearing in REAL only (light blue segment/map 2 in T_1 before spike-onset, Fig. 4) but which again failed to reach significance in the T-ANOVA. Further segments/differences observed in T_2 were also unspecific to real rTMS. Patient #4 showed nearly identical segmentation results in all conditions, with no differences specific to REAL. The segmentation in patient #5 showed the same segments in all conditions in the 6/1 Hz and in the 1 Hz stimulation (with the exception of one map observed at the end of the epoch in BL only, T_1, Fig. 6). The T-ANOVAs did not identify any time periods of specific differences, although the 6 Hz/1 Hz stimulation was lacking the SHAM condition. Together with the segmentation results and the highly similar maps identified over conditions and even protocols, the identifiable differences between BL and REAL in 6 Hz/1 Hz stimulation in this patient are interpreted as unspecific changes as well.

Discussion

We analyzed immediate effects of rTMS on spike-number and spike-patterns using protocols that have previously been shown to be inhibitory in nature (6 Hz primed 1 Hz, 1 Hz). We performed an extensive analysis with which even subtle changes in spike-patterns should be detectable. As described in the Introduction, our hypothesis was that the focal inhibitory stimulation would lead to demonstrable changes in the patients' cortical signs of focal over-excitability, especially in the phase of the after-discharge of the epileptic spikes.

Table 2 summarizes the results of spike count, GFP, topographical analysis and segmentation for all the patients. Highlighted are those results that are indicative of differences specific to REAL stimulation (i.e. those appearing concomitantly in both SHAM vs. REAL and BL vs. REAL contrasts without changes in BL vs. SHAM). Our principal finding is that none of the patients showed differences on all measures, and no measure was consistently affected across all patients. Real rTMS decreased the
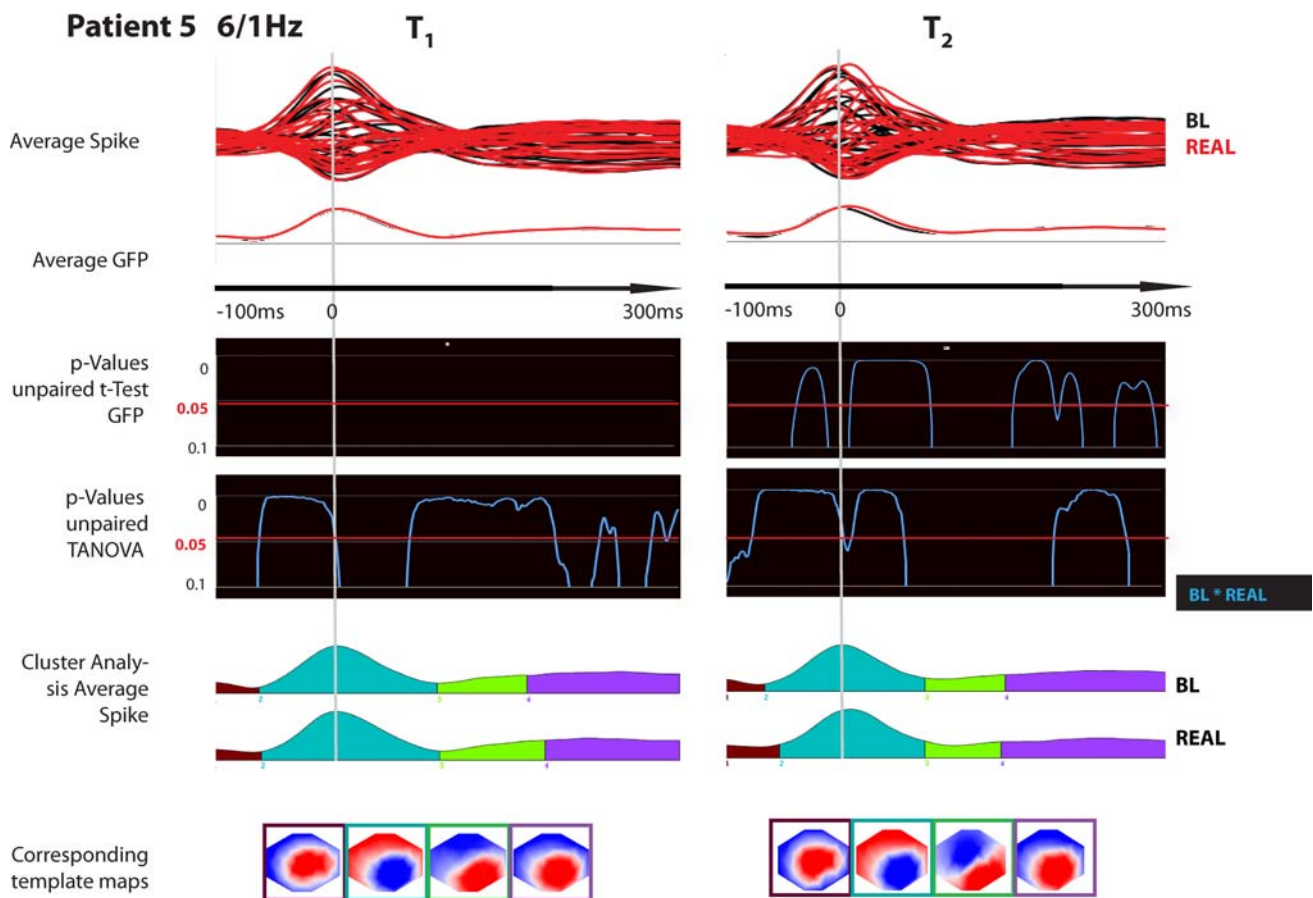
Fig. 6 Results of spike pattern analysis for patient #5 with the 1 Hz stimulation only. For details of the figure see legend of Fig. 2

number of spikes in one patient (#4), but left it unchanged in the other 4 patients (Fig. 1). Specific effects of real TMS on spike-patterns were found in two patients (#2 and #4). One patient showed reduced ES-amplitude (#4) in the late after-discharge phase, in line with his reduced ES-count and thus beneficial rTMS effects. The other patient (#2), in contrast, showed enhanced amplitude of after-discharges but unchanged ES-count, with the amplitude-increase in REAL being rather the opposite of what one would have expected from an inhibitory stimulation. Furthermore, these few identified differences found on GFP and topography were rather weak (Figs. 2, 3, 4, 5, 6, 7). Given the numerous reports of clinical efficacy of low frequency rTMS in epilepsy patients, these are surprising results.

To our knowledge, our study is the first to evaluate immediate effects of rTMS on the configuration of interictal epileptic activity. A direct impact of rTMS on EEG signals has been previously demonstrated in healthy subjects. Many studies have shown rTMS-induced changes in the amplitude or topography of somatosensory or visual event-related potentials (Enomoto et al. 2001; Bohotin et al. 2002; Fumal et al. 2003; Schutter and van Honk 2003; Thut et al. 2003). Others have looked at ipsilateral and

contralateral TMS-evoked potentials (TEPs) in EEG in response to single TMS pulses over sensorimotor (Komssi et al. 2002) or prefrontal cortex (Kahkonen et al. 2005; Esser et al. 2006) as a method to estimate cortical excitability in non-motor regions.

Studies on the immediate effects of rTMS on spike pattern in epileptic patients are rare. A case with absence epilepsy and typical spike-wave-discharges was recently reported in whom rTMS was applied only during frequent ES. This protocol led to a reduced duration of the spike-wave discharges, but they did not outlast the rTMS stimulation itself (Conte et al. 2007). The lack of methodologically comparable studies combined with the variability in TMS-outcome makes the interpretation of our results challenging. Several hypotheses for the lack of consistent immediate effects of rTMS on ES are conceivable, including: (1) rTMS induced changes are too subtle to be detected by our methods or are present only after several stimulation sessions on consecutive days; (2) The chosen rTMS protocol did not lead to the expected inhibitory effects; or (3) The impact of TMS is too dependent on individual susceptibility, so no major group effect or consistent effect across several patients emerges.

Fig. 7 Results of spike pattern analysis for patient #5 with the 6 Hz priming followed by 1 Hz stimulation. For details of the figure see legend of Fig. 2

Table 2 Summary of effects of REAL rTMS on epileptic spike number (ES count), field strength (GFP), map topography and dominant map configuration (segmentation)

Patient No.	ES Count	GFP	Topo-graphy	Segmen-tation	Comment
1	x	x	x	x	
2	x	+	+	x	
3	x	x	x	x	
4	−	−	x	x	
5 (6/1 Hz)	x	x	x	x	no SHAM
5 (1 Hz)	x	x	x	x	

A minus (−) indicates inhibitory, a plus (+) facilitative effects specific to real rTMS and an X (x) the absence of any effect. The only patient with a reduction of epileptic spike (ES) count is patient #4. In him, ES-amplitude (GFP) was also reduced but no spike pattern effects were detectable. Patient 2 showed facilitative effects with an enhancement in ES-amplitude (GFP), accompanied by topographic changes. Patient #5 had two TMS stimulation sessions; the 6 Hz/1 Hz protocol was applied in real only

Regarding the possibility that effects are too subtle or require multiple sessions, it is worth noting that despite the observation of some clinical effects after single stimulation sessions (Fregni et al. 2005b; Misawa et al. 2005), most studies examining clinical effects of rTMS in epilepsy patients have been performed with stimulation on several consecutive days (Tergau et al. 1999; Menkes and Gruenthal 2000; Theodore et al. 2002; Daniele et al. 2003; Kinoshita et al. 2005; Fregni et al. 2006b; Joo et al. 2007; Santiago-Rodriguez et al. 2008). These findings support the assumption that the beneficial rTMS-effects on seizure- and/or ES-count in epilepsy patients required more than one stimulation session. To account at least for a small short-lasting effect in the range of minutes due to our single-session protocol, we split the spike pattern analysis of each EEG block in two halves (T_1 and T_2, patients #3–5). This approach failed also to reveal any differential early versus late effects.

As for the effectiveness of our protocol in eliciting an inhibitory effect, we would note that adding a priming sequence to increase the cortical excitability is based on concordant literature of homeostatic plasticity in healthy participants (Iyer et al. 2003; Lang et al. 2004; Siebner et al. 2004; Fregni et al. 2006a). The lack of consistent results in our patient group could be due to the fact that the

6 Hz priming might be less effective in focal epilepsy patients than in healthy participants; the former already having an elevated level of excitability within the epileptic focus. Further increasing the excitability with the 6 Hz priming might have set the "starting point" for the subsequent 1 Hz stimulation too high to lead to a detectable inhibitory effect. To test this possibility, we explored the effect of pure 1 Hz rTMS in an additional stimulation session in patient #5. This, however, did not lead to a decrease in ES-count or to a change in ES-pattern, as compared to the 6 Hz primed 1 Hz-stimulation in the same patient. To determine if priming leads to more or less effects of the following stimulation rTMS, more subjects and/or different combinations of priming/stimulation frequencies will need to be examined.

Inter-individual variability is an important and consistent variable in medicine, and concerns any treatment, including established drug treatment. The antiepileptic effect of rTMS might be variable over individuals, as also suggested by the many contradictory rTMS-trial outcomes; with some placebo controlled studies in larger numbers of patients failing to prove superiority of REAL vs. SHAM stimulation protocols for both, number of spikes, and number of seizures (Theodore et al. 2002; Tergau et al. 2003; Cantello et al. 2007) and others providing evidence for beneficial effects (Fregni et al. 2005b; Fregni et al. 2006b; Santiago-Rodriguez et al. 2008). Apart from personal/genetic factors, other aspects of the focus itself may come into play.

One possibility is the impact of whether the epilepsy is unifocal or multifocal. Reports of beneficial effects of rTMS rather favor unifocal epilepsy patients to be the most susceptible to inhibitory effects (Fregni et al. 2006b). However, only one of our patients with unifocal epilepsy (#4) experienced a decrease in number of spikes, indicating that this variable alone may sufficiently predict the response to rTMS.

Another possible contributing factor is the depth of the focus. Theodore et al. have shown that an effect on spike-count can be obtained more reliably in patients with neocortical than in patients with deep foci (Theodore et al. 2002). This is well explained by the fact that the magnetic field is reaching only a depth of about 2–4 cm within the brain tissue (Wagner et al. 2008). However, patient #4 was the only patient with a rather deep epileptic focus (mesial temporal due to hippocampus sclerosis), but the only one who positively responded to inhibitory rTMS. The most likely explanation for this beneficial effect is that we have stimulated a cortical propagation node through which rTMS reached the deep epileptic focus.

In summary, the results all together do not indicate a consistent detectable influence of rTMS stimulation on the spike pattern in the EEG-recordings 30 min after stimulation. Our results speak in favor of a rather weak immediate effect of rTMS on focal epileptic activity that depends on the individual susceptibility of the patient. The identification of most effective stimulation patterns in each patient will therefore likely be decisive for the future therapeutic role of rTMS in epilepsies and possibly also other disorders. Theta burst stimulation protocols have recently proven to lead to more profound and longer lasting effects in healthy subject than conventional TMS (Huang et al. 2005; Nyffeler et al. 2008; Silvanto and Pascual-Leone 2008). These protocols might be more promising for epileptic patients, which however, remains to be evaluated.

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