Short-term Effects of Single rTMS Sessions on Auditory Evoked Activity in Chronic Tinnitus Patients.

Running title: Short-Term Effects of rTMS on Auditory Evoked Activity

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Subjective tinnitus is the perception of a sound without any external source. Repetitive transcranial magnetic stimulation has been examined as a treatment tool for chronic tinnitus for several years trying to target hyperactivity / abnormal synchronization within the auditory cortex putatively underlying the auditory phantom percept. However, its exact impact on auditory cortical activity remains largely unknown. This study’s objective was to systematically examine changes in auditory responses (N1, auditory steady state response) measured by means of magnetoencephalography after single sessions of stimulation with different transcranial magnetic stimulation paradigms. Subjects with chronic tinnitus (n=10) underwent five sessions of repetitive transcranial magnetic stimulation in which they received one of five different stimulation protocols (1Hz, individual alpha frequency, continuous theta burst stimulation, intermittent theta burst stimulation, and sham) in randomized order using a single-blind study design. Cortical steady state responses to 40 Hz amplitude-modulated tones were measured before and after each magnetic stimulation protocol. The results demonstrate a reduction of the cortical response to the auditory steady state stimulus after magnetic stimulation, whereas the N1 response was slightly enhanced or remained unchanged. Furthermore, the reduction of the auditory steady state response was driven by effects of intermittent theta burst stimulation, continuous theta burst stimulation, and 1Hz stimulation. Correspondingly, behavioral measures demonstrated the greatest reduction of tinnitus loudness after the respective rTMS protocols. The current study offers an interesting insight into the effects of repetitive transcranial magnetic stimulation on auditory cortical activity. The results of the study are discussed in context of the current limitations of transcranial magnetic stimulation for the treatment of chronic tinnitus.
Keywords: Tinnitus, Transcranial Magnetic Stimulation, Auditory Steady State Response, N1, Magnetoencephalography

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Introduction

Tinnitus, the subjective perception of a sound in the absence of any physical sound source, is characterized by simple acoustical features, for instance, a pure tone or a narrow-band noise. Up to 15% of the general population experiences such a phantom sound (Eggermont and Roberts, 2004). In most cases tinnitus is associated with hearing loss induced by noise exposure or the aging process as has been demonstrated in animal models of hearing loss (Rajan and Irvine, 1998, Salvi et al., 2000). It is widely assumed that deprivation of afferent input caused by hearing damage leads to reduced inhibition in central auditory structures, which results in hyperexcitability of circumscribed regions of the central auditory system. This is reflected in an increase in the spontaneous firing rate (SFR) in cortical and subcortical auditory structures (Eggermont and Roberts, 2004; Kaltenbach, 2006). Furthermore, rapid increases in synchronized firing in the deprived frequency regions after noise trauma (Norena and Eggermont, 2003), as well as enhanced burst-firing in inferior colliculus, primary, and secondary auditory cortex after salicylate or quinine injection (substances known to trigger tinnitus) have been demonstrated in animal studies (e.g., Chen and Jastreboff, 1995; Kenmochi and Eggermont, 1997; Norena and Eggermont, 2003). This aberrant neuronal activity within the auditory pathways may have sufficient postsynaptic impact to be interpreted as a sound at higher auditory processing stages.

The direct measurement of neuronal spiking in humans is only possible using invasive methods. For the assessment of altered neuronal activity in the human central auditory system evoked potentials have been examined, which are elicited by a large population of synchronously active neurons. An interesting technique in this context is the so-called auditory steady state response (aSSR), an evoked oscillatory response driven by the modulation frequency of a given stimulus (Regan, 1982). In tinnitus an increase of the aSSR amplitude has been reported (Diesch et al., 2004; Wienbruch et al., 2006), as well as a
flattened tonotopic gradient as compared to normal hearing controls (Wienbruch et al., 2006).

Since the aSSR has its main generators in the primary auditory cortex (A1) (Bidet-Caulet et al., 2007; Galambos et al., 1981; Liegeois-Chauvel et al., 1994), the results imply an enhanced excitability of neuronal cell assemblies in primary auditory areas of tinnitus sufferers, which may stem from reduced inhibition leading to an increased ongoing synchronization. In a recent treatment study Okamoto and colleagues demonstrated a reduction of the perceived tinnitus loudness after individualized auditory stimulation accompanied by reduced aSSR amplitudes (Okamoto et al., 2010). Transient auditory evoked potentials, the most dominant one being the N1, originating mainly from secondary auditory (A2) and association cortices (Liegeois-Chauvel et al., 1994), have also been investigated previously in tinnitus subjects. However, results from these studies have been inconsistent.

An early study found a reduction in ERP amplitudes (N1, P2, and P3) in the tinnitus group compared to hearing loss and age-matched controls (Attias et al., 1993). More recent studies have reported increased N1 either for sounds with frequencies at the audiometric edge (Dietrich et al., 2001) or at lower (non deprived) frequencies (Weisz et al., 2005). One single study using magnetic source imaging reported abnormal tonotopic organization in tinnitus patients with a linear correlation between the deviation of the N1 generator of the tinnitus frequency and the subjective tinnitus strength (Muhlnickel et al., 1998).

Thus, there is an increasing amount of evidence from animal as well as human studies that tinnitus is related to enhanced excitability of auditory cortical regions (Sun et al., 2009, Dong et al., 2010) - putatively leading to spurious spontaneous synchronization (Weisz et al., 2007a). Despite the current knowledge about its pathophysiological mechanisms, tinnitus treatment is still elusive. Transcranial magnetic stimulation (TMS) - a minimal invasive method for depolarizing cortical neurons based on the principle of electromagnetic induction (Barker et al., 1985) - has recently gained popularity as a research tool as well as a possible treatment for chronic tinnitus. The rhythmic application of series of single stimuli is referred
to as repetitive TMS (rTMS), a method that has been demonstrated to induce long-term potentiation (LTP) or depression (LTD)-like changes of cortical excitability, which outlast the stimulation period (Siebner and Rothwell, 2003). Based on its ability to focally modulate cortical excitability rTMS has been investigated as a therapeutic tool in disorders characterized by functionally altered cortico-subcortical networks, such as depression, schizophrenia, stroke or tinnitus (Ridding and Rothwell, 2007). After-effects of rTMS depend on a complex interplay of various factors, including stimulation frequency, number of pulses, and stimulation intensity, but also the history of synaptic activity of the stimulated brain region (Ridding and Rothwell, 2007).

Based on the finding that 1 Hz rTMS in general reduces cortical excitability (Chen et al., 1997), 1 Hz rTMS over the temporo- or temporoparietal cortex has been studied extensively as a treatment tool for chronic tinnitus during recent years. Even if it has overall demonstrated statistically significant reductions of tinnitus, the effect sizes are only moderate (~20% symptom reduction) and interindividual variability is high (for an overview see (Kleinjung et al., 2007a; Londero et al., 2006). Thus, despite being conceptually an ideal tool for tackling tinnitus, the clinical impact of the currently used stimulation protocols is limited. Recently, theta burst stimulation (TBS) has been introduced as a new stimulation paradigm. Single sessions of TBS, consisting of bursts of three pulses of TMS at 50 Hz repeated at theta frequency (5 Hz), have been demonstrated to induce more pronounced and longer lasting effects on motor cortex excitability as compared to tonic stimulation with rTMS (Huang and Rothwell, 2004). However, the effects of various rTMS protocols on cortical excitatory and inhibitory networks have been investigated mainly in the motor system where a direct behavioral impact can be recorded by means of EMG. It is uncertain whether this knowledge can be directly transferred to other cortical areas such as the auditory cortex (Sparing et al., 2005; Franca et al., 2006; Speer et al., 2003).
Surprisingly, the influence of rTMS on auditory responses in tinnitus patients has not yet been investigated although an enhancement of the aSSR has been demonstrated in chronic tinnitus patients compared to controls. Our current knowledge of the influence of rTMS on auditory cortex activity can only be indirectly inferred from rTMS studies in non-auditory modalities, particularly the motor system. Learning more about the impact of rTMS on auditory cortical activity is necessary in understanding how this technique may alleviate tinnitus symptoms, how to further improve its efficacy, and about current limitations of this method. Thus, the primary goal of the present study is an advancement of our understanding of the short-term influence of different rTMS protocols on the hyperactivity within the auditory cortex in tinnitus patients and thus on short-term changes of tinnitus loudness. Furthermore, the frequency selectivity of the rTMS effects will be investigated. However, we do not aim at replicating clinical improvements related to rTMS, which would require larger sample sizes.

Based on the current notion relating tinnitus to hyperexcitability in auditory cortical regions we hypothesize that enhanced auditory cortical activity (reflected in the aSSR and the N1) is reduced after rTMS.

METHODS

Subjects

Ten patients with chronic tinnitus participated in the study (seven males, three females). The mean age was 49.8 years (21-70 years); the mean tinnitus duration was 1.8 years (0.5-3 years). Five patients reported unilateral tinnitus (4 left sided tinnitus, 1 right sided tinnitus), 5 patients experienced bilateral tinnitus. Mean tinnitus severity according to the German version of the Tinnitus Questionnaire (Goebel and Hiller, 1998) was relative low (mean: 29.9, range:
Patients were recruited via advertisements in the local newspaper and flyers at the University of Konstanz. None of them had any experience with rTMS before. All patients have been investigated thoroughly regarding a previous personal or family history of epileptic seizures. Patients with relevant neurological or psychiatric co-morbidity (assessed using the Mini International Neuropsychiatric Interview (Sheehan et al., 1998)), those with contraindications for TMS (e.g., epilepsy, cardiac pacemaker, pregnancy, neurodegenerative diseases), as well as patients taking anticonvulsant or tranquilizer medication were excluded from the study. Since rTMS in chronic tinnitus has been demonstrated to be more promising with short tinnitus duration (De Ridder et al., 2005; Kleinjung et al., 2007b) we only included patients with maximum tinnitus duration of 4 years. All participants were informed about the content of the study prior to participation and signed a written informed consent. The study conformed to the Declaration of Helsinki and was approved by the Ethics Committee of the University of Konstanz.

- Insert Table 1 about here -

**Measurement of tinnitus loudness**

Before the first and after the second magnetoencephalography (MEG) measurement (see figure 1) patients had to rate their tinnitus on a visual analogue scale (VAS) assessing the current loudness on a scale ranging from 0 (minimal) to 10 (maximal) (*How loud is your tinnitus?*).

**MEG procedure and data acquisition**
During the MEG measurement, the participants were stimulated with three 40 Hz amplitude-modulated (AM) tones (250 Hz, 1000 Hz, 4000 Hz) appearing in randomized order and presented monoaurally to the ear affected by the tinnitus (right-sided in the case of bilateral tinnitus). In the following the AM tones will be referred to as ‘low frequency tone’ (250 Hz), ‘middle frequency tone’ (1000 Hz), and ‘high frequency tone’ (4000 Hz). The auditory stimulation procedure consisted of 210 stimuli (i.e., 70 stimuli per frequency), each stimulus lasting 800 ms (75 ms rise / fall time). The inter-stimulus interval varied randomly from 2800 to 3100 ms. Data were recorded with a 148-channel whole-head magnetometer system (MAGNES 2500 WH, 4D Neuroimaging, San Diego, USA), installed in a magnetically shielded room (Vakuumschmelze Hanau, Germany). The head position within the MEG helmet had to be assessed, thus positions of five index points and individual head shapes were sampled using a digitizer. Participants lay in a supine position and were requested to keep their eyes open during the measurement and to focus on a fixed point on the ceiling. To ensure that all stimuli were perceived at the same subjective loudness level, each stimulus was individually matched to the loudness of a reference tone (1 kHz, 50 dB SL) for each patient. The auditory stimuli were conducted to the patient’s ear via a flexible tubing sound delivery system. The whole procedure, including sending markers to the data acquisition system, was implemented in Psyscope X B53 for Mac OS X (http://psy.ck.sissa.it).

MEG measurements were conducted before and after rTMS; the interval between the end of stimulation and the start of the second MEG measurement did not exceed 3 minutes. Since we measured resting state brain activity for 5 minutes first (reported in a companion paper), the measurement of evoked data was started about 8 minutes after the end of rTMS.

rTMS stimulation
Five rTMS sessions were applied with a minimum interval of one week between sessions using a randomized, single-blind, sham-controlled design. Biphasic magnetic pulses were administered with a figure-of-eight coil (coil winding diameter 2 x 75mm; Magnetic Coil Transducer C-B60, Medtronic) connected to a MagPro X 100 TMS stimulator (Medtronic A/S, Skovlunde, Denmark). Patients were seated in a comfortable chair. The coil was placed over left Heschl’s Gyrus by moving 2.5 cm upwards from T3 on the line between T3 and Cz and then 1.5 cm in posterior direction perpendicularly to the line T3-Cz (in case of right-ear or bilateral tinnitus), analogously over right Heschl’s Gyrus (in case of left-ear tinnitus). This procedure has been proven to position the TMS coil reliably over the auditory cortex (Langguth et al., 2006) The handle of the coil was pointing upwards. During stimulation the coil was held by a mechanical arm.

The following stimulation protocols were applied in randomized order: 1 Hz rTMS (1 train with 1000 pulses), individual alpha frequency rTMS (IAF, 20 trains with 50 pulses and 25 seconds inter-train-interval, frequency ranging between 8 and 12 Hz), intermittent theta burst stimulation (iTBS, 10 trains of 10 bursts at a frequency of 5 Hz with an 8 second inter-train-interval interval and bursts consisting of three pulses at 50 Hz), continuous theta burst stimulation (cTBS, bursts at a frequency of 5 Hz with bursts consisting of three pulses at 50 Hz), sham stimulation (45° coil angulation (one wing), applying the IAF protocol). The patients were blind to the TMS condition.

The intensity of the stimulation was expressed as a percentage of the maximum output of the stimulator (0-100%) and was adjusted according to the resting motor threshold (RMT) – a common procedure in rTMS studies (Pridmore et al., 1998). The RMT was measured by delivering single pulses at the optimal place over the motor cortex to produce visible hand muscle contractions. Resting motor threshold was defined as the lowest stimulation intensity for producing a visible hand muscle contraction in at least five out of ten trials. For 1Hz, IAF, and sham stimulation an intensity of 110% RMT was applied, whereas an intensity of 80%
RMT was employed for iTBS and cTBS (according to Huang et al., 2005). Earplugs were provided to the patients to prevent hearing damage due to the loud clicking sound of the TMS. For an overview of the study outline please see figure 1.

Data analysis

Continuous data were epoched (-2000 to 2000 ms relative to sound onset) and downsampled to 300 Hz. Epochs containing artefacts such as eye-blinks were excluded via visual inspection. Since the experimental procedure required the participant to leave the MEG (within one experimental session, as well as between sessions), all comparisons were performed in source space using the 'lcmv' beam former (Van Veen et al., 1997). A multisphere model was fitted to the headshape collected in the first measurement yielding a grid of dipoles with a 10 mm resolution. This assured that the same grid was used across all measurements for a single subject. The lead field for each grid point was calculated for each measurement separately, however. Time windows representing the early transient response (oversimplified called the N1-period here due to the dominance of the N1m; 0-300 ms) and the steady state field (SSF) (400-700 ms) were defined by inspection of the grand average of the sensor-level activity across all participants. Furthermore, we determined a pre-stimulus baseline period of equal length (-300-0 ms). The general source analysis strategy for one measurement was to first calculate spatial filters for each grid point via the lcmv-beamformer, by using a common time-period that encompassed baseline and activation periods. In order to optimize spatial filters for the relevant activity, epochs were low-pass filtered (20 Hz) for the N1 and band-pass filtered (30-50 Hz) for the SSF prior to calculation of the sensor-level covariance. After deriving the common spatial filter, dipole moments were estimated for the
aforementioned activation and baseline periods and relative changes of brain activity were calculated: (activation-baseline) / baseline. These measures of evoked brain activity were then interpolated onto individually collected MRIs and subsequently spatially normalized to the Montreal Neurological Institute (MNI) brain using SPM2 (www.fil.ion.ucl.ac.uk/spm/software/spm2).

We defined two regions of interest (ROI) for data analysis – namely, the auditory cortices ipsi- and contralateral to the TMS stimulation side (figure 2). Therefore, source activity (as described above) was averaged across all pre MEG measurements for each subject. Subsequently, source activations were averaged across all participants of each stimulation side separately. The whole procedure was applied for the N1 and the aSSR, respectively. A cluster of voxels with high activation was defined in each hemisphere by applying a threshold (85% of the maximum). The masks derived from the N1 and the aSSR were combined for both hemispheres resulting in two auditory regions of interest. Thereafter, the activity from the respective ROIs was extracted and averaged across all voxels of interest, leading to one single value each ipsi- and contralateral to TMS stimulation side for each patient. This procedure was applied for each stimulation parameter, for each of the three AM tones, for each measurement time and for N1 and aSSR, respectively, resulting in 60 values per patient, hence 600 values overall.

All aspects of analysis of the MEG data were performed using the Fieldtrip toolbox (http://fieldtrip.fcdonders.nl) in Matlab 7.6.0 (The MathWorks, Natick, MA).

- Insert Figure 2 about here -

Statistical analyses
Statistical analyses were performed using R version 2.6.0 for Mac OS X (www.r-project.org). Normalized power was computed by means of a (post-pre)/pre (relative to rTMS intervention) ratio to minimize variance resulting from strong interindividual variability. Bilateral auditory evoked responses were not clearly identifiable in all subjects. This may be attributed to the use of monaural rather than binaural auditory stimulation. Datasets without a clear N1 or aSSR were treated as outliers and removed from the data. Outliers were defined according to the boxplot criterion as a data point falling more than 1.5 times the interquartile range above the third quartile or below the first quartile (Hoaglin, 1986). With the remaining values a linear mixed effect models statistic (LME) was computed, which is an appropriate method for representing data from repeated measures on the same statistical units and is furthermore particularly suitable for analyses with missing values due to removal of outliers (Pinheiro, 2000). The following variables were entered as fixed effects: Stimulation protocol, auditory response, tone frequency and region of interest. Subjects were defined as random effects. LME analysis was performed using the nlme-library of R (Pinheiro, 2000). If significant results were detected in the ‘omnibus’ lme statistic, post-hoc planned contrasts (paired t-tests) were computed. Since auditory cortical activity underlies natural changes we compared auditory activity after active rTMS protocols to auditory activity at baseline, but also to auditory activity after sham stimulation (as a control variable). The significance level was set to 0.05 in all analyses.

Results

None of the patients reported relevant side-effects of rTMS, apart from transient mild discomfort due to cutaneous sensations and muscle contractions. One patient reported periods of complete absence of tinnitus lasting for several minutes after 1Hz stimulation. Three patients reported a very loud tinnitus after IAF stimulation lasting for several hours up to a
few days. None of the other stimulation parameters were associated with spontaneously reported increase of tinnitus loudness.

Methods

Effects of rTMS on auditory cortical activity

The results of an ‘omnibus’ lme statistic demonstrate a significant main effect for the factor auditory response (N1 or aSSR) (mean N1 = 0.077, mean aSSR = -0.256, F = 16.159, p = 0.0001). Since the N1 and the aSSR display an opposite reaction pattern after rTMS we divided the data according to the auditory response for further analyses.

A significant stimulation X ROI interaction for the auditory steady state response (F = 3.310, p = 0.011) was found. Post hoc analyses for each stimulation parameter ipsi- and contralateral were performed. Intermittent theta burst stimulation led to a significant reduction of the aSSR compared to baseline (t = -3.813, p = 0.0005) as well as compared to sham (t = -3.525, p = 0.0005) in the stimulated auditory cortex (ipsilateral to rTMS). Furthermore, 1Hz rTMS resulted in a significant reduction of the aSSR ipsilateral to rTMS compared to sham (t = -1.687, p = 0.049) and a trend was revealed for the aSSR after cTBS ipsilateral to rTMS compared to sham (t = -1.533, p = 0.064) (figure 3). The aSSR contralateral to the stimulation side was reduced significantly compared to baseline after sham stimulation (t = -2.997, p = 0.003) (figure 3).

Furthermore, a trend was revealed for a stimulation X frequency interaction regarding the N1 (F = 1.972, p = 0.051) (figure 4). Post hoc analyses revealed a significant enhancement of the N1 for the low frequency tone after IAF stimulation (t = 2.402, p = 0.014) as well as after
sham (t = 2.12, p = 0.024) compared to baseline. Compared to sham as a control variable we found a significantly reduced N1 after iTBS (t = -1.696 p = 0.049) as well as after cTBS (t = -1.82, p = 0.038). Regarding the middle frequency tone a trend was revealed for a reduction of the N1 after sham (t = -1.444, df = 17, p = 0.083) compared to baseline. For the high frequency tone a significant reduction of the N1 was found after sham (t = -2.564, p = 0.010), as well as a significant enhancement of the N1 after iTBS (t = 1.872, p = 0.041) compared to baseline. Compared to sham we found a significant greater N1 after iTBS (t = 3.136, p = 0.002) as well as after cTBS (t = 2.062, p = 0.024). A trend was revealed for a greater N1 after 1Hz stimulation (t = 1.508, p = 0.070) compared to sham.

No significant interaction effects were revealed for the aSSR regarding the different tone frequencies.

Effects of rTMS on tinnitus loudness

Regarding the behavioral data (measurement of tinnitus loudness (VAS)), an lme model revealed a significant effect for the factor stimulation (F = 3.665, p = 0.013). Post hoc tests demonstrated a significant reduction of tinnitus loudness after cTBS (t = -3.312, p = 0.005) compared to baseline (compared to sham: t = -1.496, p = 0.080) as well as after 1Hz stimulation (t = -2.008, p = 0.037) compared to baseline (compared to sham: t = -1.750, p = 0.048). Trends were revealed for a reduction of tinnitus loudness after iTBS (t = -1.583, p = 0.073) compared to baseline (compared to sham: t = -1.325, p = 0.100) and for an
enhancement of tinnitus loudness after IAF stimulation (t = 1.609, p = 0.071) compared to baseline (compared to sham: t = 1.301, p = 0.108).

Besides, linear relationships between the behavioral data and the aSSR were computed. We detected a positive correlation between the aSSR in the stimulated auditory cortex (ipsilateral to rTMS) and tinnitus loudness after rTMS (r = 0.475, p = 0.0006).

**Discussion**

The current study examined the short-term influence of different rTMS stimulation protocols on auditory cortical activity (aSSR, N1) in chronic tinnitus patients measured by means of MEG. In general, we found a significant reduction of the aSSR after rTMS. The results demonstrate that significant changes after active stimulation occur mainly in the directly stimulated auditory cortex ipsilateral to the coil placement. Intermittent theta burst stimulation and 1Hz stimulation had the greatest effects on the aSSR compared to sham stimulation, however, for cTBS a trend was revealed as well. The effects of rTMS on the N1 regarding the different tone frequencies demonstrated quite a complex pattern: Sham stimulation enhanced the N1 for the low frequency tone and concurrently reduced the N1 for the middle as well as for the high frequency tone. This pattern greatly differed from the active stimulation protocols in which only IAF stimulation enhanced the N1 for the low frequency tone and iTBS enhanced the N1 for the high frequency tone.
Regarding the behavioral results 1Hz stimulation and cTBS resulted in a significant reduction of tinnitus loudness; a trend was revealed for iTBS. This corresponds nicely to the aSSR data, exhibiting a reduction of the aSSR precisely after these stimulation protocols. Thus, our data are in line with a recent treatment study demonstrating a reduction of the perceived tinnitus loudness after individualized auditory stimulation accompanied by reduced aSSR amplitudes (Okamoto et al., 2010).

Stronger effect of rTMS on the auditory steady state response

Our results demonstrate a greater influence of the active stimulation protocols on the aSSR compared to the N1. This phenomenon suggests that rTMS effects occur preferentially in A1, which is supposed to play an important role for the generation of the aSSR (Bidet-Caulet et al., 2007; Galambos et al., 1981; Liegeois-Chauvel et al., 1994) and only to a lesser extent in A2. The coil was localized directly above the ear according to the international 10/20 system – a position that was validated before by means of neuronavigation to mainly target the A1 (Langguth et al., 2006). Hence, our results indirectly confirm this coil positioning method. For the first time neurophysiological data are provided for the debate about the exact cortical region in which rTMS exerts clinical effects in tinnitus patients (Langguth et al., 2010). It has been argued that A1 is difficult to reach by TMS, since it is located far from the brain surface in the sylvian fissure in lateromedial direction. Furthermore, patients with low frequency tinnitus (cortical activation is expected to be more superficially located) did not respond better to rTMS than those with high frequency tinnitus (Frank et al., 2010). Our results also did not demonstrate any frequency specific effects regarding the aSSR. Thus, even if our data suggest that the rTMS effects occur predominantly in A1, this may not be a direct stimulation effect but rather transsynaptically mediated via more
superficial cortical areas, either by cortico-cortical connections or by cortico-thalamo-cortical transmission.

Intermittent theta burst stimulation turned out to be the parameter leading to the greatest reduction of the aSSR, although iTBS has been demonstrated to induce excitatory effects on the motor cortex (Huang et al., 2005). Thus, our results underscore that specific rTMS protocols may have different effects on various cortical areas (Poreisz et al., 2008; Speer et al., 2001). Furthermore, it has to be considered that rTMS effects were investigated in tinnitus patients who differ from healthy controls presumably by increased auditory cortex activity. Since the excitability state of the stimulated area has been repeatedly shown to have important impact on rTMS effects (Lang et al., 2004; Siebner and Rothwell, 2003; Siebner et al., 2004; Potter-Nerger et al., 2009) the reduction of aSSR after iTBS may reflect iTBS-induced homeostatic plasticity. The homeostatic plasticity rule predicts that the greater the ongoing activity, the less effective are processes leading to LTP, while processes leading to LTD are enhanced (Bienenstock et al., 1982). Our results of strongly reduced auditory excitability after iTBS are also in line with recent animal data demonstrating a significant increase of the inhibitory transmitter γ-aminobutyric acid (GABA) in the rat cortex acutely (within 30-45 minutes) after stimulation with iTBS (Trippe et al., 2009). However, due to the pioneering aspect of our work these assumptions are mere speculative and would have to be validated by means of examining a healthy control group.

Regarding the N1 we found different results. In general there is a slight enhancement of the N1 after rTMS. After both sham and IAF rTMS the N1 for the low frequency tone was significantly enhanced, whereas the N1 for the middle and high frequency tone was reduced after sham. In contrast, after the burst protocols (cTBS, iTBS) a converse pattern was observed. At this stage it is unclear why such a distinct frequency specific pattern was observed both for sham as well as for IAF stimulation. Since for sham and IAF rTMS the same stimulation protocol was applied, both produced acoustic artifacts (due to the clicking
sound of the TMS machine) in the individual alpha frequency. Furthermore, the effects on the N1 were not laterality specific, thus, it is tempting to speculate that the observed changes of the N1 after these protocols are a mere consequence of the rhythmic sound stimulation at the individual alpha frequency. Massive sound stimulation at the individual alpha frequency may be capable to induce alpha entrainment and as a consequence inhibitory effects (Mathewson et al., 2009; Sauseng et al., 2009). This explanation is further supported by the fact that the N1 is assumed to be generated in secondary auditory areas (Liegeois-Chauvel et al., 1994), which have not been directly magnetically stimulated in the current study, but which were activated by the TMS-related sound stimulation. If sham and IAF stimulation have a specific frequency effect on the N1 due to acoustic artifacts in the alpha frequency, it remains unclear whether the effects of the burst protocols on the N1 are also a mere consequence of the acoustic stimulation or whether they result from the magnetic effects on corticocortical connections to A2. Disregarding the exact mechanisms at this point the sham findings for the N1 raise awareness that neurophysiological effects may not only be induced directly by magnetic stimulation, which necessitates further research.

Spatial Resolution of rTMS regarding different tone frequencies

The influence of rTMS on auditory cortical activity regarding the three different tone frequencies (i.e., the fine tuning within the auditory cortex) clearly demonstrates diverse results. As mentioned above, the converse pattern is strongest pronounced for the N1 after sham stimulation suggesting primarily that the specific sound stimulation of the TMS at the alpha frequency is responsible for the frequency-specific effects on the N1. In contrast, the lme model did not reveal any significant interaction effects regarding the aSSR for the different tone frequencies. Hence, our results demonstrate an impact of rTMS on auditory cortex excitability, however this is rather non-frequency-specific. Assuming that in tinnitus
patients not the entire auditory cortex exhibits hyperactivity / -synchrony but circumscribed parts affected by deprivation (Weisz et al., 2007a), calls for more precise targeting of neuronal assemblies along the tonotopic map. The pivotal question is if an improvement of the spatial resolution of TMS regarding the auditory cortex would lead to greater treatment effects (e.g., Silvanto and Pascual-Leone, 2008; Silvanto et al., 2007).

Limitations

The sample size of the current study was small because of the time-consuming measurements (2.5 hours per session, 5 sessions per person). Nevertheless, due to the numerous MEG measurements (100 recordings) providing the data, the statistical power should be sufficient and the data reliable. Anyway, our study should be regarded as a precursor in this research field and thus conclusions from the current results for the general tinnitus population are limited and require further research with greater sample sizes and non-tinnitus controls (regarding protocols that exhibited the greatest neurophysiological effects, for instance, iTBS).

An important aspect regarding transcranial magnetic stimulation of the auditory cortex is the concomitant massive (non-intentional) sound stimulation of auditory cortical areas due to the loud clicking sound of the TMS machine when the pulse is generated. By tilting the coil for sham stimulation the magnetic field produced by the TMS is greatly reduced, thus a depolarization of neurons is impossible. However, auditory stimulation by the clicking sound of the TMS still exists. The IAF protocol was applied for sham stimulation, which turned out to be the loudest stimulation paradigm (about 93 dB subtracting 30 dB attenuation by means of earplugs). During active rTMS depolarization caused by the magnetic pulse and depolarization related to the processing of the clicking sound may interact and contribute to
synaptic plasticity due to associative learning mechanisms (Stefan et al., 2000). This learning process is missing in the case of sham stimulation since there is no induced current. Yet, at least for the visual modality it has been demonstrated that by entrainment of the visual cortex via 10 Hz stimulus presentation, functional inhibition can be observed on a short-term basis (Sauseng et al., 2009). It is unknown whether a prolonged (several minutes) stimulation of, for instance, visual or auditory stimuli would lead to longer-lasting changes in respective sensory brain regions. If this was the case, however, then this massive sound stimulation at the IAF may have resulted in considerable inhibitory effects. It is important to emphasize that in both cases (associative learning, IAF-entrainment) sham cannot be considered as a completely "inactive" control condition.

Another problem we had to face, especially concerning the data analysis, was the application of TMS (and accompanying the application of the sound stimulation) on different sides, since we decided to stimulate contralateral to the tinnitus side. Our approach to divide the activity according to regions of interest ipsi- and contralateral to rTMS stimulation side functioned as a good compromise for the current study. Yet, statements for subgroups of tinnitus (e.g., laterality, gender) cannot be drawn. Furthermore, it still remains a matter of debate to what extent tinnitus related changes are predominantly located in the auditory cortex contralateral to the perceived tinnitus (Weisz et al., 2007b) or whether alterations of neural activity in the central auditory system are independent from the perceived tinnitus laterality (Arnold et al., 1996; Langguth et al., 2006).

Moreover, it has not been possible to measure auditory cortical changes directly after rTMS since there was a delay of about 8 minutes until the measurement started again. The loudness rating on the VAS has been conducted after the second MEG measurement, thus loudness has been gathered only 20 minutes after rTMS, which clearly limits conclusions on the prompt effects of rTMS on tinnitus. However, studies regarding rTMS effects on the
human motor cortex (Chen et al., 1997; Huang et al., 2005) demonstrate that even effects of single rTMS sessions are lasting for up to 15-60 minutes. Accordingly, we were still able to demonstrate changes regarding the aSSR as well as the tinnitus loudness after active rTMS protocols compared to sham stimulation, which further argues for extended effects even of single rTMS sessions. Nevertheless, differences between burst and tonic stimulations have to be interpreted with care, since burst stimulation was demonstrated to have longer lasting effects on the human motor cortex (Huang et al., 2005).

**Conclusions**

The results of the current study demonstrate stronger effects of rTMS on the aSSR compared to the N1, which possibly argues for a greater influence on A1 using a coil placement approach that was supposed to target the A1 (Langguth et al., 2006).

The results of the present study are promising: Auditory cortex excitability (reflected in the aSSR) is reduced after iTBS, cTBS and 1Hz and these particular protocols were followed by a reduction of tinnitus, thus confirming our assumptions outlined in the introduction. Yet, the spatial resolution of rTMS regarding the frequency specificity of the auditory cortex is low, which is reflected in unspecific results regarding the stimulation with three different tone frequencies.

Since the current study does not claim to be a therapy study regarding a treatment of tinnitus further research concerning longer rTMS periods distributed across several days is required.

Besides the above-mentioned limitations the current study is the first to demonstrate rTMS effects on evoked auditory cortical activity. A greater number of corresponding studies will improve insights into possible mechanisms of different TMS protocols on auditory
activity potentially leading to advances in applying this technique as a treatment of chronic


tinnitus.
References


Ridding MC, Rothwell JC. Therapeutic use of rTMS. Nat Rev Neurosci. 2007


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Speer AM, Willis MW, Herscovitch P, Daube-Witherspoon M, Shelton JR, Benson BE, Post RM, Wassermann EM. Intensity-dependent regional cerebral blood flow during 1-Hz repetitive transcranial magnetic stimulation (rTMS) in healthy volunteers studied with


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<th>SUBJECT</th>
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<th>GENDER</th>
<th>TINNITUS SIDE</th>
<th>TINNITUS DURATION IN YEARS</th>
<th>TINNITUS FREQUENCY</th>
<th>TINNITUS SEVERITY</th>
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EXPERIMENTAL DESIGN

Measure of tinnitus loudness (VAS)

MEG: auditory stimulation
(250 Hz, 1000 Hz, 4000 Hz in randomized order)

TMS

1 Hz
IAF
iTBS
Sham

MEG: auditory stimulation
(250 Hz, 1000 Hz, 4000 Hz in randomized order)

Measure of tinnitus loudness (VAS)
Influence of different rTMS protocols on the auditory steady state response

- Sham
- 1Hz
- cTBS
- iTBS
- IAF

Contra: dark bars
Ipsi: light bars

*** p < 0.001
** p < 0.01
* p < 0.05
+ p < 0.1
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<th>iTBS</th>
<th>cTBS</th>
<th>sham</th>
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<td><em>aSSR contralateral to rTMS</em></td>
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<td>-0.40</td>
<td>-0.08</td>
<td>-0.29</td>
<td>-0.54**</td>
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Influence of different rTMS protocols on the N1 regarding different tone frequencies

- **low freq**
- **middle freq**
- **high freq**

- **+ p < 0.1**
- *** p < 0.05**
- **** p < 0.01
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<th>iTBS</th>
<th>cTBS</th>
<th>sham</th>
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<td>0.37*</td>
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<td>-0.50**</td>
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Change of tinnitus loudness (VAS) after different stimulation protocols

- sham
- 1Hz
- cTBS
- iTBS
- IAF

** p < 0.01
* p < 0.05
+ p < 0.1
Figure 1. Outline of experimental sessions: Tinnitus loudness was assessed followed by an MEG measurement of auditory cortical activity while patients were stimulated with three different AM tones (70 stimuli in each condition) each lasting 800 ms. Subsequently, one of five rTMS protocols (including a sham stimulation) were applied in a randomized order. After TMS the MEG procedure and the tinnitus loudness measurement were repeated.

Figure 2. Regions of interest of right and left auditory cortices, respectively, computed by averaging auditory cortical activity across all PRE measurements. A threshold with 85% of the maximum was defined and values below this threshold were set to 0, whereas values above the threshold were set to 1.

Figure 3. Comparison between the effects of different stimulation protocols on the aSSR regarding different stimulation sides (ipsi- vs. contralateral) (shaded bars represent sham control). Intermittent theta burst stimulation is the only active stimulation parameter significantly reducing the aSSR in the stimulated auditory cortex (ipsilateral to rTMS) compared to baseline as well as compared to sham. However, compared to sham 1Hz also significantly reduced the aSSR. For cTBS a trend was revealed. Stars demonstrate significance (** p ≤ 0.001, * p ≤ 0.01, + p ≤ 0.1), bars represent standard errors.

Figure 4. Comparison between the effects of different stimulation protocols on the N1 regarding different tone frequencies (250 Hz, 1000 Hz, 4000 Hz) (shaded bars represent sham control). Stars demonstrate significance (** p ≤ 0.01, * p ≤ 0.05, + p ≤ 0.10), bars represent standard errors.

Figure 5. Comparison between tinnitus loudness (VAS) measured after different active rTMS protocols compared to baseline and to sham. Tinnitus loudness was significantly reduced after 1Hz stimulation and cTBS compared to baseline, a trend was revealed for the reduction of tinnitus loudness after iTBS. Compared to sham tinnitus loudness was reduced significantly after 1Hz stimulation, trends were revealed for cTBS and iTBS. Stars demonstrate significance (** p ≤ 0.01, * p ≤ 0.05, + p ≤ 0.1), bars represent standard errors.

Figure 6. Positive correlation between the aSSR ipsilateral and tinnitus loudness after rTMS (r = 0.475, p = 0.0006). Colored points represent the different stimulation protocols (red = cTBS, blue = iTBS, green = 1Hz, yellow = lAF, black = sham), dashed colored lines demonstrate the respective individual correlations for cTBS (red), iTBS (blue), and 1 Hz stimulation (green).