Repetitive transcranial magnetic stimulation for the treatment of chronic subjective tinnitus:

Optimization of treatment effects

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Preface

Enjoy the silence – unless you suffer from tinnitus. Our everyday lives are characterized by a constant exposure to sensory (over-) stimulation. Moments of quietness have become increasingly important for many of us in order to compensate for this overload, to calm down, to just let our senses get some rest. It is therefore not surprising that – if patients suffering from tinnitus are asked what their biggest problem about the phantom sound is – the answer often is that they miss the sound of silence and have problems to relax. Up to now, there is no cure for tinnitus and many patients have to try to live with the constant noise within their ears or head. From a scientific point of view, the way back to silence is still supposed to be a long one. The current thesis adds knowledge to tinnitus research in order to move one step closer towards a cure by investigating repetitive transcranial magnetic stimulation (rTMS) as a treatment option for chronic subjective tinnitus.

After some background information about tinnitus pathophysiology and the method of rTMS is given, three studies are presented which have the common aim to optimize rTMS treatment effects in tinnitus sufferers. In a concluding discussion, the results are put in a wider scientific context. As all three studies have already been published in peer-reviewed journals within the last four years, every study is presented as a stand-alone manuscript. The manuscripts were adapted so that the thesis as a whole meets the formatting requirements as described in the 6th Edition of the Publication Manual of the American Psychological Association. All data are given as mean ± standard deviation unless otherwise specified. Decimal numbers were rounded to two decimal places with the exception of *p*-values smaller than .01 where three decimal places are reported. Tables and Figures were renumbered and placed after the section in which they were first mentioned (e.g. all tables mentioned in the methods of study 1 were placed after this section). Furthermore, the three separate reference sections were merged into one reference list at the end of the thesis.

Danksagung

An dem Gelingen meiner Dissertation waren einige Menschen beteiligt, die mich in dieser herausfordernden Phase meines Lebens begleitet und unterstützt haben und ohne deren Zutun ich vermutlich niemals an dem Punkt angelangt wäre, an dem ich heute stehe. Diesen Menschen möchte ich an dieser Stelle von ganzem Herzen Danke sagen. Leider kann ich nicht jeden beim Namen nennen und hoffe, dass die Gemeinten wissen, dass dies auch an sie gerichtet ist.

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Contents

Abstract

Subjective tinnitus is a highly prevalent and for many patients very debilitating condition for which there is still no cure. As tinnitus has been shown to be associated with changes of neural activity in different areas of the cortex, repetitive transcranial magnetic stimulation (rTMS) has been used as a treatment tool in order to interfere with these changes. Up to now, treatment success is limited and strategies to enhance treatment effects are clearly needed. The three studies of this cumulative dissertation address the question how rTMS treatment of patients suffering from chronic subjective tinnitus can be optimized. Study 1 tested whether treatment response was associated with grey matter (GM) changes and whether pre-treatment GM volume might be a potential predictor for treatment success. Although transient GM changes in the insulae and the bilateral inferior frontal cortex were observed, these changes were not correlated to treatment outcome. It was shown, however, that GM volume in the frontal cortex and the lingual gyrus might be possible predictors for treatment response. While traditionally, rTMS targeted the auditory cortex of tinnitus patients, study 2 and study 3 examined a new protocol which stimulated three sites successively in order to better interfere with cortical networks involved in tinnitus pathophysiology: the left dorsolateral prefrontal cortex and the left and right temporoparietal cortices. Study 2 was a pilot study which tested the new protocol in a one-arm open label study and compared the results with a historical control group of patients receiving traditional single-site stimulation. The results suggested that the triple-site protocol might show better long-term effects. As a consequence, the new protocol was explored in more detail in study 3 in order to replicate the result in a randomized controlled parallel group trial. In this study, the superiority of the multisite protocol was only seen on a descriptive but not on a statistical significant level. In a concluding discussion, the methods used in this work and future approaches for the enhancement of rTMS treatment effects are discussed.

Zusammenfassung

Subjektiver Tinnitus ist ein weit verbreitetes und für viele Betroffene sehr beeinträchtigendes Symptom, für das bis dato keine Heilungsmethode existiert. Da gezeigt wurde, dass Tinnitus mit Veränderungen der neuronalen Aktivität in verschiedenen kortikalen Arealen einhergeht, wird seit einiger Zeit die repetitive transkranielle Magnetstimulation (rTMS) als Behandlungsmöglichkeit erforscht, die an genau diesen neuronalen Veränderungen anzusetzen versucht. Der Erfolg dieser Behandlungsmethode ist bislang begrenzt. Deshalb beschäftigen sich die drei Studien der vorliegenden kumulativen Dissertation mit der Frage, wie die rTMS Behandlung bei chronischem Tinnitus optimiert werden kann. In Studie 1 wurde untersucht, ob der Behandlungserfolg mit Veränderungen in der grauen Substanz einhergeht und ob das Volumen der grauen Substanz vor Behandlungsbeginn möglicherweise ein potenzieller Prädiktor für den Behandlungserfolg darstellt. Auch wenn vorübergehende Veränderungen der grauen Substanz bilateral in der Insula und dem inferioren frontalen Kortex gefunden wurden, so korrelierten diese Veränderungen jedoch nicht mit dem Behandlungsergebnis. Es zeigte sich aber, dass das Volumen der grauen Substanz im frontalen Kortex und dem Gyrus lingualis potenzielle Prädiktoren für den Behandlungserfolg darstellen könnten.

Während ursprünglich der auditorische Kortex von Tinnituspatienten als Zielort für die rTMS diente, wurde in Studie 2 und Studie 3 ein neuartiges Stimulationsprotokoll untersucht. Dieses Protokoll sieht eine sukzessive Stimulation von drei kortikalen Arealen vor und verfolgt dabei das Ziel, die an der Pathophysiologie des Tinnitus beteiligten neuronalen Netzwerke besser beeinflussen zu können. Stimuliert wurden der linke dorsolaterale präfrontale Kortex sowie der linke und rechte temporoparietale Kortex. Studie 2 war eine Pilotstudie in welcher dieses neue Protokoll in einem einarmigen, nicht-verblindeten Studiendesign untersucht wurde und dann mit einer Kontrollgruppe aus einer früheren Studie

verglichen wurden. Die Patienten der Kontrollgruppe hatten die traditionelle linkstemporale Stimulation erhalten. Die Ergebnisse legten nahe, dass das neue Stimulationsprotokoll bessere Langzeiteffekte erzielt als das traditionelle Protokoll. Studie 3 wurde schließlich konzipiert, um dieses vielversprechende Ergebnis in einer randomisierten, kontrollierten Studie mit Parallelgruppen zu replizieren. In dieser Studie war das neue Protokoll zwar auf deskriptiver Ebene überlegen, dieser Unterschied war jedoch nicht statistisch signifikant.
In einer zusammenfassenden Diskussion werden die Methoden, die in dieser Arbeit Verwendung fanden sowie zukünftige Möglichkeiten zur Verbesserung der rTMS-Behandlung diskutiert.

Contributions

Study 1	**Structural brain changes following left temporal low-frequency rTMS in patients with subjective tinnitus**
Study idea	Landgrebe, Langguth, Lehner
Study design	Landgrebe, Langguth
Data acquisition	Landgrebe, Langguth, Hajak, Poeppl
Statistical analysis	Lehner
Manuscript writing	Lehner
Manuscript revision	all authors
Study supervision	Langguth, Schecklmann
Study 2	**Multisite rTMS for the treatment of chronic tinnitus: stimulation of the cortical tinnitus network – a pilot study**
Study idea	Langguth, Schecklmann
Study design	Langguth, Lehner, Schecklmann
Data acquisition	Lehner, Kreuzer, Poeppl, Vielsmeier
Statistical analysis	Lehner
Manuscript writing	Lehner
Manuscript revision	all authors
Study supervision	Langguth
Study 3	**Triple-site rTMS for the treatment of chronic tinnitus: a randomized controlled trial**
Study idea	Langguth, Schecklmann
Study design	Langguth, Lehner, Schecklmann
Data acquisition	Lehner
Statistical analysis	Lehner
Manuscript writing	Lehner
Manuscript revision	all authors
Study supervision	Greenlee, Langguth, Rupprecht, Schecklmann

Abbreviations

ANCOVA	analysis of covariance
ANOVA	analysis of variance
BDI	Beck's Depression Inventory
dB HL	decibel hearing level
DLPFC	dorsolateral prefrontal cortex
EEG	electroencephalography
FWE	family wise error
fMRI	functional magnetic resonance imaging
GM	grey matter
GÜF	Geräuschüberempfindlichkeitsfragebogen
Hz	hertz
IFG	inferior frontal gyrus
kHz	kilohertz
LOCF	last observation carried forward
MDI	Major Depression Inventory
MEG	magnetoencephalography
MNI	Montreal Neurological Institute

MP-RAGE	magnetization prepared rapid acquisition gradient echo
MRI	magnetic resonance imaging
PET	positron emission tomography
RMT	resting motor threshold
ROI	region of interest
rTMS	repetitive transcranial magnetic stimulation
SAP	statistical analysis plan
SPM	statistical parametric mapping
THI	Tinnitus Handicap Inventory
TQ	Tinnitus Questionnaire
VAT	ventral attention network
VBM	voxel based morphometry
WHO-QoL	World Health Organization Quality of Life

Introduction

Tinnitus

Definition.

Tinnitus is the perception of a sound or noise in the absence of an external acoustic stimulus. If there is a sound source within the body e.g. altered blood flow or muscle movement (Langguth, Kreuzer, Kleinjung, & De Ridder, 2013), the sound can also be heard by the examiner and is therefore called objective. In the much more prevalent subjective tinnitus, the sound is only heard by the patient and no inner-body source for this percept can be identified. In many cases, tinnitus is experienced acutely for only a few minutes or hours and the person recovers spontaneously. The phantom sound is considered chronic if it is perceived for at least three to six months (Hall et al., 2011; Landgrebe et al., 2008). Acute tinnitus is a very common symptom which about 25% of the adults in the US have experienced at least once (Shargorodsky, Curhan, & Farwell, 2010). About 10-15% of the population experience tinnitus in its chronic form (Axelsson & Ringdahl, 1989; Hoffman, H. J. & Reed, 2004). In the current thesis, only patients with chronic subjective tinnitus were treated. Therefore, the term *tinnitus* always means *chronic subjective tinnitus* unless otherwise specified.

Pathophysiology I: from peripheral damage to auditory cortex.

While traditionally, tinnitus was thought to be a pure otological symptom, the current state of research clearly suggests that tinnitus has many possible causes involving both peripheral and central sensory pathways and that in many patients there is not a single trigger but rather multiple influences which may eventually result in a phantom sound (Langguth et al., 2013). Still, tinnitus is supposed to be usually initiated by peripheral mechanisms such as cochlear damage. This assumption is backed by etiological studies which clearly indicate that

hearing loss is a dominant risk factor for tinnitus (Axelsson & Ringdahl, 1989; Hoffman, H. J. & Reed, 2004). Furthermore, even in patients with normal audiograms, some form of auditory deafferentation might exist (Schaette & McAlpine, 2011; Weisz, Hartmann, Dohrmann, Schlee, & Norena, 2006). This peripheral damage causes altered auditory input to the central auditory pathway where plastic changes are supposed to occur in order to compensate for this altered input (Eggermont & Roberts, 2004; Roberts, L. E. et al., 2010). The exact mechanisms by which the auditory pathway might (mal-)adapt to hearing loss and thus create a phantom sound are not yet completely understood. There are different models which try to explain this process such as the thalamocortical-dysrhythmia model which suggests that deafferentation due to hearing loss leads to a reduced lateral inhibition and therefore to increased firing rates in surrounding areas of the dorsal and ventral cochlear nucleus and the inferior colliculus. This is supposed to cause a reorganization of the tonotopic maps and increased neural synchrony in those auditory structures. As a consequence a phantom sound occurs (De Ridder, Vanneste, Langguth, & Llinas, 2015; Roberts, L. E. et al., 2010).

The influence of deafferentation is not limited to peripheral or subcortical structures but also affects the auditory pathway at level of the auditory cortex. Studies using different methods to measure central neural activity in tinnitus patients report some sort of hyperactivity in the temporal cortex. For example, studies employing electroencephalography (EEG) or magnetoencephalography (MEG) have shown an increase of gamma activity in the auditory cortex (Weisz, Dohrmann, & Elbert, 2007) which is correlated with tinnitus loudness and which parallels findings of gamma band activity in physiological processing of auditory signals (van der Loo et al., 2009; Weisz et al., 2007). This tinnitus-related increased gamma band activity might be the consequence of reduced inhibitory alpha band activity which has also been observed over temporal areas of tinnitus patients (Schlee et al., 2014;

Weisz, Moratti, Meinzer, Dohrmann, & Elbert, 2005). Also studies using neuroimaging methods clearly indicate that the activity in the auditory cortex of tinnitus patients is altered. For example, enhanced activity of the left auditory cortex has been shown (Arnold, Bartenstein, Oestreicher, Romer, & Schwaiger, 1996) as well as elevated sound evoked activity in the primary auditory cortex of tinnitus patients (Gu, Halpin, Nam, Levine, & Melcher, 2010). All in all, the involvement of the auditory cortex in the generation of tinnitus seems now to be unambiguous. However, activity in the auditory cortex alone may well be insufficient for the generation of a conscious auditory percept.

Excursus: conscious auditory perception in healthy humans.

Studies in patients suffering from unresponsive wakefulness syndrome i.e. patients who awaken from coma but remain unresponsive (Laureys et al., 2010) have provided important information about healthy sensory processing in general and auditory processing in particular. It has been shown that if a an auditory stimulus was presented to those patients in comparison to controls, the auditory cortices were activated in both groups whereas patients showed a lack of activation of the temporoparietal junction and also a lack of functional connectivity between the auditory cortex and higher-order areas like parts of the parietal and cingulate cortex (Laureys et al., 2000). This indicates that besides the activation of the sensory pathway itself, activity of a fronto-parieto-cingular network is additionally required for a conscious auditory percept. This network is assumed to modulate the activity in sensory cortices via top-down amplification or inhibition (Dehaene & Changeux, 2004).

Pathophysiology II: from auditory cortex to a conscious, distressing percept.

Although there is no objective sound source for tinnitus, it still is a conscious auditory percept. It can therefore be assumed that – just as for any other auditory percept – the

involvement of higher-order areas should play a role in tinnitus as well. Indeed, neuroimaging studies suggest that alterations of activity in the central nervous system of tinnitus patients exceed the auditory cortex and are also present in distant, non-auditory cortical regions such as frontal and parietal areas (Adjamian, Sereda, & Hall, 2009; Lanting, de Kleine, & van Dijk, 2009). There are some preliminary results which back the hypothesis that the simultaneous activity of the auditory cortex and a fronto-parieto-cingular network are crucial for a conscious tinnitus percept to appear (De Ridder, Elgoyhen, Romo, & Langguth, 2011). For instance, Schlee, Hartmann, Langguth, and Weisz (2009) observed an increased long-range gamma coupling between temporal, frontal, parietal and cingulate cortices in resting state MEG of tinnitus patients as compared to healthy controls. Furthermore, it was found that "the more the activity in the temporal cortices was driven by other brain regions the stronger the subjective distress" (Schlee, Mueller, et al., 2009, p.6) stressing the top-down influence of non-auditory brain regions. Besides this fronto-parieto-cingular network, another subnetwork is supposed to play a role in tinnitus: an unspecific "distress network" which is thought to represent the affective component of the phantom sound and which is most likely composed of the anterior cingulate cortex, the insula, amygdala and parahippocampus (van der Loo, Congedo, Vanneste, Van De Heyning, & De Ridder, 2011; Vanneste et al., 2010). This concept of different neural subnetworks, which are considered to represent separable characteristics of the tinnitus percept (De Ridder et al., 2014), are precious input for researchers trying to find new treatment options and are therefore of particular importance for the current work.

Treatment of tinnitus.

To date, there is no cure for chronic subjective tinnitus and often there is also no clear indication for a specific, causally oriented treatment option. Possible treatment approaches

include psychological interventions such as counselling or cognitive behavioural therapy (CBT). There is evidence that CBT is able to improve tinnitus patients' quality of life but is not able to reduce tinnitus intensity (Cima et al., 2012; Martinez-Devesa, Perera, Theodoulou, & Waddell, 2010). In addition, different pharmacological treatments and auditory stimulation techniques are being examined like hearing aids, cochlea implants or sound therapy (for an overview, see Fernandez, Shin, Scherer, & Murdin, 2015; Langguth et al., 2013). Finally, brain stimulation techniques such as transcranial direct current stimulation (Song, Vanneste, Van de Heyning, & De Ridder, 2012), transcranial random noise stimulation (Vanneste, Fregni, & De Ridder, 2013) or repetitive transcranial magnetic stimulation (rTMS) have also been introduced as possible treatment options (Langguth, de Ridder, et al., 2008).

Repetitive transcranial magnetic stimulation

Explanation of the method.

Transcranial magnetic stimulation is a non-invasive brain stimulation technique, which uses electromagnetic induction to induce electric currents in the brain. To this end, a coil of wire is placed above the scalp while a current pulse is produced within the coil resulting in a magnetic field. This magnetic field induces an electrical field able to depolarize axons, which lie perpendicular to the induced current (Hallett, 2000; Siebner, Hartwigsen, Kassuba, & Rothwell, 2009). The effects of rTMS are state-dependent which means that TMS effects differ according to the initial activation state of the stimulated neurons (Dayan, Censor, Buch, Sandrini, & Cohen, 2013). Probably, neurons are most prone to becoming depolarized by a TMS pulse if their membrane potential is just below the threshold (Siebner et al., 2009).

The stimulation depth of TMS is dependent on the type of coil and reaches one to six centimetres. The strength of the induced electrical field decreases with increasing stimulation

depth. Therefore, TMS is only able to depolarize superficial cortical neurons (Siebner & Ziemann, 2007). However, due to axonal projections and network connections within the brain, TMS also exerts indirect influence on activity in cortical areas distant from the stimulation site. Again, these effects are state-dependent with TMS exerting stronger impact on cortico-cortical connections which are in an activated state (Siebner et al., 2009).

TMS and the human brain: 30 years in a nutshell.

In 1985, Anthony Barker was the first to use TMS in order to stimulate the motor cortex of humans (Barker, Jalinous, & Freeston, 1985). He found that if the coil is placed over the motor cortex, single TMS pulses can induce neural activation in the underlying cortical area resulting in movements of the respective contralateral limb. Thereafter, TMS was established as a diagnostic tool to measure motor evoked potentials (Kobayashi & Pascual-Leone, 2003; Rossini et al., 1994) and other parameters of motor cortex excitability (Siebner & Ziemann, 2007). Since its introduction, TMS has been used to investigate causal links between brain structures and inter-regional functional interactions (Dayan et al., 2013).

After it had been shown that the repeated application of TMS pulses (rTMS) resulted in changes of the cortical excitability of the motor cortex, which outlasted the stimulation period (Fitzgerald, Fountain, & Daskalakis, 2006; Rossini et al., 2015), it became possible to use rTMS as a therapeutic tool which allowed the investigator to actively modulate cortical excitability. The precise direction of the rTMS effect is thought to depend on the frequency of the applied TMS pulses. While low-frequency (1 Hz) rTMS was shown to inhibit the cortical excitability of the motor cortex, high-frequency rTMS increased it (Dayan et al., 2013). The specific biophysical mechanisms underlying this modulating effect are not completely understood but it is supposed that rTMS initiates long-term potentiation or long-term depression-like effects (Dayan et al., 2013).

rTMS for the treatment of chronic tinnitus.

As a painless, safe technique to manipulate cortical activity with only little adverse effects (Rossi, Hallett, Rossini, & Pascual-Leone, 2009), rTMS has been explored as a treatment option for neurological and psychiatric disorders associated with changes of cortical excitability (Lefaucheur et al., 2014). After it had been shown that low-frequency rTMS of the left temporal cortex could reduce auditory hallucinations in patients suffering from schizophrenia (Hoffman, R. E. et al., 2000) it was hypothesized that this might also be useful in the treatment of other phantom percepts such as subjective tinnitus. The hyperactivity observed in the auditory cortex of tinnitus patients posed a promising target for the modulatory effects of low-frequency rTMS. Indeed, some studies reported a transient reduction of tinnitus intensity after the auditory cortex of tinnitus patients was targeted with about 200 pulses of 1 Hz TMS (Folmer, Carroll, Rahim, Shi, & Hal Martin, 2006; Lefaucheur et al., 2014) and this effect was shown to be dose-dependent with more TMS pulses resulting in longer lasting effects (Plewnia et al., 2007). Since 2003 (Langguth et al., 2003) many studies have investigated the effect of repeated rTMS sessions for the treatment of tinnitus. Usually, five to ten daily rTMS sessions have been used with each session consisting of about 1000 pulses of low-frequency rTMS of the left temporal or left temporoparietal cortex. The results are mixed with some sham-controlled studies reporting beneficial effects while others do not. Three recently published reviews differ in their conclusions drawn: While Meng et al. (2011) state that there is only limited support for the use of low-frequency rTMS for the treatment of tinnitus, Soleimani et al. (2015) report medium to large effect sizes especially at follow-up assessments and Lefaucheur et al. (2014) conclude that there is a possible therapeutic efficacy. In any case, treatment effects are burdened by high inter-individual variability, the reported effects are transient and complete disappearance of the phantom

sound is rare (Lefaucheur et al., 2014). Therefore, efforts to enhance treatment success are clearly indicated.

To this end, different strategies have been proposed and tried such as the combination of rTMS with pharmacological interventions (Kleinjung et al., 2009, 2011), the investigation of different firing modes such as theta burst stimulation (Plewnia et al., 2012; Schecklmann et al., 2016) or the identification of clinical and demographical parameters to identify potential treatment responders (Frank, G. et al., 2010; Lehner et al., 2012). All those efforts have not yet resulted in significant improvements of rTMS treatment. Another approach to optimize treatment effects is to modify the cortical area which is stimulated. While rTMS has traditionally been applied to the left auditory cortex, the new insights in tinnitus pathophysiology indicate that stimulation of the auditory cortex alone might not be sufficient to achieve a long lasting improvement of tinnitus severity. If several separable networks including both auditory and non-auditory brain structures are involved in the generation of chronic tinnitus (De Ridder et al., 2011), targeting also non-auditory cortical areas could be a promising approach to optimize treatment effects.

Scope of the present thesis

This cumulative dissertation is composed of three studies all of which have a common aim: They intend to investigate how the effects of rTMS treatment of tinnitus can be enhanced. In all three studies, patients suffering from chronic subjective tinnitus were treated with ten daily sessions of rTMS.

Study 1 focused on the neural mechanisms by which the well-investigated standard stimulation protocol of low-frequency rTMS of the auditory cortex exerts its effects. If we understand why some patients benefit from rTMS treatment while others do not, this might enable us to better tailor the treatment protocols to the individual patient's needs. Therefore, study 1 analyzed whether structural changes in the cortical grey matter (GM) of tinnitus patients' brains might be able to explain the high inter-individual variability of treatment effects. More precisely, the study intended to answer the questions whether (a) there are any GM changes detectable after low-frequency rTMS of the auditory cortex, (b) these changes are associated with treatment success, and (c) the GM volume before the first treatment session might be useful as a predictor for treatment outcome. Accordingly, high-resolution images of the patients' brains were acquired before and after ten daily rTMS treatment sessions. Those images were analyzed by means of voxel based morphometry (VBM) and correlated with clinical measures of treatment outcome.

In **Study 2 and 3** an optimization of treatment protocol was developed which takes the already existing knowledge about tinnitus pathophysiology into account. Assuming that both auditory and non-auditory cortical networks are involved in tinnitus generation and chronification, the protocol targets three central hubs of these networks. **Study 2** is a pilot study in which this triple-site protocol was examined for the very first time in a one-arm open label study in order to find out whether triple-site stimuluation was safe and applicable in daily clinical routine and whether it had potential to augment treatment effects. To this end,

the protocol was compared to a historical control group which had been treated with standard low-frequency rTMS of the left temporal cortex. In order to replicate the results of this pilot study, **study 3** was conducted in which the triple-site protocol was compared to the standard stimulation protocol in a randomized controlled, parallel-group clinical trial.

Study 1: Structural brain changes following left temporal low-frequency rTMS in patients with subjective tinnitus

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Abstract

Repetitive transcranial magnetic stimulation (rTMS) of the temporal cortex has been used to treat patients with subjective tinnitus. While rTMS is known to induce morphological changes in healthy subjects, no study has investigated yet whether rTMS treatment induces GM changes in tinnitus patients as well, whether these changes are correlated with treatment success and whether GM at baseline is a useful predictor for treatment outcome. Therefore, we examined magnetic resonance images of 77 tinnitus patients who were treated with rTMS of the left temporal cortex (10 days, 2000 stimuli/day, 1 Hz). At baseline and after the last treatment session high-resolution structural images of the brain were acquired and tinnitus severity was assessed. For a subgroup of 41 patients, additional brain scans were done after a follow-up period of 90 days. GM changes were analysed by means of VBM. Transient GM decreases were detectable in several brain regions, especially in the insula and the inferior frontal cortex. These changes were not related to treatment outcome though. Baseline images correlated with change in tinnitus severity in the frontal cortex and the lingual gyrus, suggesting that GM at baseline might hold potential as a possible predictor for treatment outcome.

Introduction

Subjective tinnitus is the phantom perception of a sound in the absence of a corresponding objective sound source. With about 25% of adults in the US having experienced a ringing in the ears at least once (Shargorodsky et al., 2010), transient tinnitus is a common phenomenon. About 10-15% of the world population experience tinnitus in its chronic form (Axelsson & Ringdahl, 1989). While the majority of those 10-15% gets used to their tinnitus and is able to lead a normal life, in 1-3% of the general population tinnitus is experienced as extremely bothersome and debilitating. It can severely affect patients' everyday lives and is often accompanied by psychiatric comorbidities such as depressive syndromes or sleep disturbances (Axelsson & Ringdahl, 1989; Langguth, 2011). In order to improve existing treatment options and also to generate new treatment strategies for subjective tinnitus, it is mandatory to broaden knowledge on the neural mechanisms underlying the tinnitus percept.

More than 15 years ago it has been suggested (Jastreboff, 1990; Moller, 1997) and demonstrated (Arnold et al., 1996) that tinnitus is related to alterations in the central nervous system. Furthermore, recent functional neuroimaging studies suggest (Adjamian et al., 2009; Lanting et al., 2009; Song, De Ridder, Van de Heyning, & Vanneste, 2012; Vanneste & De Ridder, 2012) that, apart from the auditory cortex, widespread neural networks involving many different brain areas seem to be involved in the generation and maintenance of the phantom sounds as well as in the distress accompanied by the tinnitus percept (De Ridder et al., 2011; Langguth et al., 2013). In addition to functional alterations within the brain, tinnitus has also been shown to be related to structural brain changes (Schecklmann et al., 2013). Studies using high-resolution magnetic resonance imaging (MRI) to compare the GM volume and cortical thickness of tinnitus patients with healthy control subjects have revealed alterations in the auditory cortex (Aldhafeeri, Mackenzie, Kay, Alghamdi, & Sluming, 2012;

Boyen, Langers, de Kleine, & van Dijk, 2013; Schneider et al., 2009) and in subcortical parts of the central auditory pathway like the thalamus (Muhlau et al., 2006) and the right inferior colliculus (Landgrebe et al., 2009). Furthermore, alterations in GM volume and cortical thickness were also found in non-auditory brain locations (Aldhafeeri et al., 2012; Diesch, Schummer, Kramer, & Rupp, 2012; Landgrebe et al., 2009; Leaver et al., 2011, 2012; Muhlau et al., 2006).

The knowledge that subjective tinnitus is associated with neural alterations suggests the therapeutic use of brain stimulation techniques such as rTMS. The early finding that the auditory cortex is overly active in tinnitus patients (Arnold et al., 1996) led to the idea to use low-frequency rTMS to modify the cortical hyperactivity in patients with phantom sounds (Eichhammer, Langguth, Marienhagen, Kleinjung, & Hajak, 2003). Ever since then low-frequency rTMS has been investigated in an increasing number of studies (for a review, see Langguth & De Ridder, 2013) showing that rTMS is effective with high inter-individual variability. However, it is still difficult to identify predictors for treatment success (Lehner et al., 2012). The idea to use and improve rTMS as a treatment for tinnitus is further pursued though. To gain deeper insight into the mechanisms of rTMS treatment – and consequently to facilitate improvement of the therapeutic approach -- the complementary use of both longitudinal neuroimaging and clinical assessment to measure rTMS effects in tinnitus patients is an important next step in tinnitus research (Langguth et al., 2012). The number of studies addressing this issue is limited so far. Some studies investigated the effect of low-frequency rTMS treatment on auditory evoked potentials and auditory steady state responses using EEG and MEG (Lefaucheur et al., 2012; Lorenz, Muller, Schlee, Langguth, & Weisz, 2010; Yang et al., 2013). Two studies using single-photon emission computed tomography and functional magnetic resonance imaging (fMRI) found changes of neural activity in the temporal lobe, the right cingulate gyrus and the uncus (Lefaucheur et al., 2012; Marcondes et

al., 2010). While those studies have provided first insight in the functional alterations that are associated with low-frequency rTMS of the auditory cortex, there is no study which adds knowledge about structural alterations induced by rTMS treatment in tinnitus patients. Until now, only one study examined the effect of low-frequency rTMS over the left auditory cortex in healthy subjects using VBM (May et al., 2007). The results suggest that five days of rTMS treatment lead to GM changes in the auditory cortex and the thalamus.

Based on all those results the current study was conducted with the following three research questions in mind: (1) Is there a change in GM detectable in tinnitus patients after 10 sessions of rTMS treatment and after a follow-up period of 90 days? (2) Is there a relationship between the clinical outcome and the GM changes? (3) Can structural imaging be used as a predictor for outcome? To answer these questions we evaluated MRI scans of patients suffering from subjective tinnitus which were done routinely before and after low-frequency rTMS of the temporal cortex.

Materials and Methods

Subjects.

Data from 77 patients (59 male, 18 female) with chronic tinnitus were included in the analyses. Patients with cardiac pacemakers, history of seizures or any severe somatic, neurologic or psychiatric disorder were excluded. The decision whether a patient was suffering from any severe somatic, neurologic or psychiatric disorder was made by the physician, who decided about study inclusion based on the global clinical impression. One criterion for a severe somatic, neurologic or psychiatric disorder was the need for an immediate therapeutic action for the treatment of this disorder. Another criterion was current hospitalisation because of such disorder.

All patients were treated with rTMS and underwent MRI scanning before (baseline) and after (day 12) 10 sessions of rTMS treatment. In a subgroup of 41 patients an additional measurement was done after a follow-up period of three months (day 90). The total sample of 77 patients was therefore divided into two independent subgroups of one sample with two scans (n = 36) and one sample with three scans (n = 41). Demographical and clinical characteristics for both subgroups are shown in Table 1. Audiological data and a measure of hyperacusis were not available for all patients and could therefore not be included in the further analyses. Standardized pure tone audiometry data was available for 57 patients and revealed a mean hearing loss of 20.38 ± 12.14 decibel hearing level (dB HL, average of all thresholds measured bilaterally ranging from 125 Hz to 8 kHz). As a screening measure of hyperacusis patients were asked whether "sounds cause pain or physical discomfort" (Schecklmann, Landgrebe, & Langguth, 2014). Of the 61 patients who answered this question 35 said *yes* and are therefore supposed to suffer from hyperacusis. Independent samples *t*-tests and Chi^2-tests revealed no significant difference between the two independent subgroups concerning all variables reported in Table 1.

Repetitive transcranial magnetic stimulation.

rTMS treatment consisted of 10 treatment sessions on 10 consecutive working days. Patients were either treated in the context of several clinical trials (Kleinjung et al., 2008, 2009; Langguth et al., 2014) or rTMS was done as compassionate use treatment between 2006 and 2009. Patients were stimulated over the left temporal cortex (1Hz, 2000 stimuli/day, 110% resting motor threshold, RMT) which was either localized by using a standard procedure targeting the primary auditory cortex based on the 10-20-system (Jasper, 1958; Langguth et al., 2006) or by using neuronavigation based on individual MRI or PET (positron emission tomography) images. In the latter cases, the area of increased activation within the

primary auditory cortex was used as target area. Even if these two methods may have resulted in slightly different targets, the spatial difference is smaller than the spatial accuracy of rTMS treatment with the used figure-of-eight coil. For rTMS treatment, a Medtronic system with a figure-of-eight coil was used (90 mm outer diameter; Medtronic, Minneapolis, MN). The coil was held with a mechanical arm and placed over the left temporal cortex with the handle of the coil pointing upwards. During treatment, the patients were seated in a comfortable treatment chair. The resting motor threshold (RMT) was measured once before the first treatment session and was defined as the minimal intensity at which at least four out of eight magnetically evoked potentials were ≥ 50 μV in amplitude in the right abductor digiti minimi muscle (Rossini et al., 1994). All patients were treated at the Tinnitus Centre at the University of Regensburg, Germany, and provided written informed consent. The treatment protocol has been approved by the local ethics committee.

Clinical assessment.

For the assessment of demographical and clinical characteristics, the Tinnitus Sample Case History Questionnaire was used (Langguth et al., 2007). Tinnitus severity was assessed using the German version of the Tinnitus Questionnaire (TQ; Goebel & Hiller, 1994; Hallam, Jakes, & Hinchcliffe, 1988) and a numeric rating scale, which measured how loud the tinnitus was perceived ("How strong or loud is your tinnitus at present?"). This scale was rated from 0 (not loud at all) to 10 (extremely strong or loud). These measures were assessed before the first treatment session (baseline), after the last treatment session (day 12) and – for the subgroup of 41 patients with three images -- after the follow-up period of three months (day 90).

MRI.

A Siemens Sonata 1.5-Tesla whole body scanner (Siemens AG, Erlangen) with a standard 8-channel birdcage head coil was used to collect the anatomical images. For each subject and each time point, a high-resolution T1-weighted image was acquired using a magnetization-prepared-rapid-acquisition-gradient-echo (MP-RAGE) sequence (repetition time 1880 ms; echo time 3.42 ms; flip angle 15°; matrix size 256x256; number of slices 176; voxel size 1x1x1mm^3).

Data processing and statistical analysis.

For statistical analyses of the clinical data, PASW statistics 18 (SPSS Inc., Chicago, IL) was used. To test for changes in tinnitus severity an analysis of variance (ANOVA) with the within-subjects factor time (baseline, day 12, day 90) was calculated for both the TQ and the loudness scale. In case of significant results, post-hoc paired *t*-tests were done. For the group of 36 patients with only two assessments, paired *t*-tests were used to compare the TQ and loudness on baseline and day 12. All statistical tests were two-tailed. The level of significance was set at .05.

Processing and statistical analysis of the anatomical data were performed with the SPM8 software package (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm). All anatomical images were visually examined for the presence of morphological abnormalities or artefacts. Preprocessing of the anatomical data was done using the standard procedure of the VBM toolbox (VBM8 Version 435, Structural Brain Mapping Group, http://dbm.neuro.uni-jena.de/vbm) for longitudinal data and involved intra-subject realignment, bias correction, segmentation and normalization to the Montreal Neurological Institute (MNI) space. The default options of the standard procedure were not changed. As modulation is not necessary for longitudinal data,

unmodulated images were used. Afterwards, a quality check was done using VBM8 before smoothing data with a Gaussian kernel of 8mm full-width at half maximum. Only GM images were used for further analyses. For the statistical analyses all voxels with a GM value below 0.1 were excluded to avoid edge effects around the border between grey and white matter. All analyses were done for the overall group of 77 patients (baseline and day 12 scans) as this group provided the highest statistical power. Additionally, all analyses were also done for the independent subgroups with two (n = 36) and three (n = 41) MRI scans. The following whole-brain analyses were performed:

(1) GM images acquired at every time point were compared by estimating a flexible factorial model in SPM8 with the factors subject and time (baseline, day 12, day 90).

(2) To test for correlations between the GM changes over time and changes in the clinical outcome parameters, difference images were calculated using the image calculator implemented in SPM8 (day 12 – baseline, day 90 – baseline) and correlated with the corresponding difference in the TQ and loudness scores.

(3) To find out whether GM images might be useful as a predictor for clinical outcome, baseline images were correlated with the difference in the TQ score (day 12 – baseline). Please see Table 2 for an overview of all analyses done.

For all analyses, the significance threshold was set to $p < .001$ (uncorrected) at voxel level and $p < .05$ (family-wise error, FWE, corrected) at cluster level. Due to the non-isotropic smoothness of VBM data, correction for non-stationarity was applied. Anatomical Automatic Labeling (Tzourio-Mazoyer et al., 2002) and the SPM Anatomy Toolbox (Eickhoff et al., 2005) were used for anatomic labelling of significant clusters.

Table 1

Demographical data and clinical characteristics for both independent subgroups

	VBM data at baseline, day 12 and day 90 (n = 41)	VBM data at baseline and day 12 (n = 36)	group comparison	*p-value*
gender	32 m (78 %); 9 f (22 %)	27 m (75 %); 9 f (25 %)	$\chi^2(1,77) = 0.10$	.75
age (years)	50.72 ± 13.37	50.79 ± 13.28	$t(75) = -0.02$	.98
tinnitus laterality	10 % right, 15 % left 75 % bilateral	14 % right, 14 % left 72 % bilateral	$\chi^2(2,77) = 0.32$	.85
tinnitus duration (years)	8.97 ± 8.36	7.57 ± 6.74	$t(75) = 0.80$	.43
TQ (baseline)	36.61 ± 17.78	39.56 ± 18.21	$t(75) = -0.72$	.48
loudness (baseline)	6.32 ± 2.04	6.00 ± 2.11	$t(75) = 0.67$	.44
mean hearing threshold [dB HL]	21.67 ± 11.49 (n = 29)	19.06 ± 12.85 (n = 28)	$t(55) = 0.81$	.42
hyperacusis	51% (n = 39)	68% (n = 22)	$\chi^2(2,61) = 2.31$	.32

Note. TQ: Tinnitus Questionnaire; loudness: How STRONG or LOUD is tinnitus at present (0 Not at all, 10 Extremely strong or loud); mean hearing threshold: average of all thresholds measured bilaterally ranging from 125 Hz to 8 kHz).

Table 2

Overview over all VBM analyses

	Statistics		
Research question	*n* = 41 (3 scans)	*n* = 36 (2 scans)	*N* = 77 (2 scans)
GM changes after rTMS?	*Flexible factorial models with factors subject + time*		
	time points: baseline, day 12, day 90	time points: baseline, day 12	time points: baseline, day 12
Correlation between GM changes and clinical outcome parameters?	*Correlation of difference in the TQ and loudness rating with difference images*		
	time difference: day 12 – baseline day 90 – baseline	time difference: day 12 - baseline	time difference: day 12 - baseline
GM as predictor for treatment response?	*Correlation of difference in the TQ with baseline images*		

Results

Clinical outcome.

The paired *t*-tests comparing the TQ and the loudness differences between baseline and day 12 in the overall group of 77 patients revealed a significant decrease in the TQ score ($t(76) = 2.47$, $p = .016$) and a marginally significant decrease in the loudness rating ($t(76) = 1.75$, $p = .085$). The paired *t*-tests comparing the TQ and the loudness differences between baseline and day 12 in the subgroup with only two scans ($n = 36$) revealed a significant decrease in the TQ score ($t(35) = 2.29$, $p = .028$) and no significant change in the loudness rating ($t(35) = -0.10$, $p = .92$). The ANOVA comparing the TQ scores of the subgroup with three scans ($n = 41$) revealed no significant effect of time ($F(1.70, 67.82) = 1.74$, $p = .19$). The ANOVA comparing the loudness scores of all three time points suggested a significant difference between at least two time points ($F(2, 80) = 3.52$, $p = .034$). Post-hoc paired t-tests revealed a significant decrease from baseline to day 12 ($t(40) = 2.53$, $p = .015$) and a marginally significant decrease from baseline to day 90 ($t(40) = 2.01$, $p = .052$). There was no significant change from day 12 to day 90 ($t(40) = -0.37$, $p = .71$). See Figure 1 for a line chart showing the development of the TQ and loudness scores over time.

VBM.

(1) The flexible factorial models revealed significant GM concentration decreases from baseline to day 12 in the left and right insula as well as in the left and right inferior frontal gyrus (please see Figure 2 and Table 3 for MNI coordinates and statistical details). These GM changes were visible in both the $n = 41$ and the overall patient sample with 77 patients. It was not detected in the $n = 36$ sample though. If data of this group was analysed with a more relaxed statistical threshold ($p < .05$ (uncorrected) at voxel level and $p < .05$ FWE corrected at cluster level), GM decreases were found in the right inferior frontal gyrus

(x = 40, y = 39, z = 19, *Z* = 3.07, *p* =.059). Please see Figure 3 for the mean GM concentration of the relevant clusters for all groups and all time points.

In addition, GM decreases were found in the left temporal pole and the left ventromedial prefrontal cortex. These GM changes were only visible in the *n* = 41 sample though. The contrast between baseline and day 12 in the overall patient sample (*N* = 77) additionally revealed decreased GM in the left inferior/medial temporal gyrus (Table 3). This was also visible in the *n* = 41 group (x = -62, y = -36, z = -20, *Z* = 4.08, *p* =.016) if analysed with a more relaxed statistical threshold ($p < .001$ (uncorrected) at voxel level and uncorrected at cluster level). In the *n* = 36 group, no significant GM decreases were visible. Overall, no GM increases from baseline to day 12 were visible in neither group. Neither GM increases nor decreases were found from baseline to day 90.

(2) The correlation analyses between the difference images and the difference in the TQ/ loudness ratings revealed no significant results.

(3) The correlation analyses between the TQ difference and the baseline images revealed a positive correlation of the TQ with GM concentration in the left medial temporal pole and the right posterior cingulate cortex in the *n* = 36 group (Table 3). The correlations in the *n* = 41 group did not reach statistical significance. Furthermore, in the overall patient group, a positive correlation between the TQ difference and the baseline images was found in the left and right lingual gyrus. Additionally, a marginally significant positive correlation was detected in the right inferior/middle frontal gyrus. Using a more relaxed statistical threshold ($p < .05$ (uncorrected) at voxel level and $p < .05$ FWE corrected at cluster level), a marginally positive correlation in the lingual gyrus (x = -4, y = -91, z = 13, *Z* = 3.78, *p* =.064) and in the inferior/middle frontal gyrus (x = 40, y = 44, z = 21, *Z* = 3.34, *p* =.093) was also found in the *n* = 41 group.

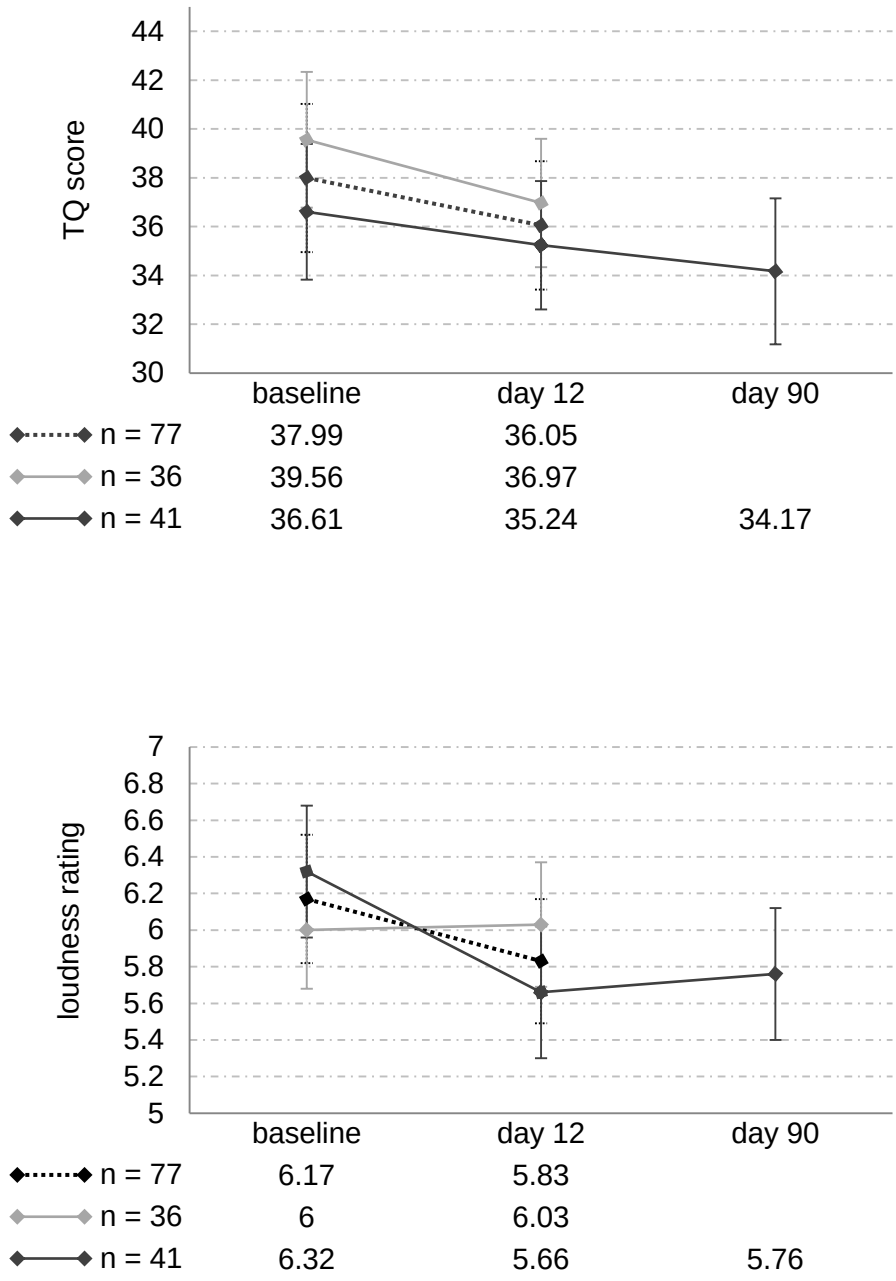

Figure 1. Line charts showing the time course of the TQ scores and loudness ratings for both independent subgroups and the overall group. Error bars represent the standard errors.

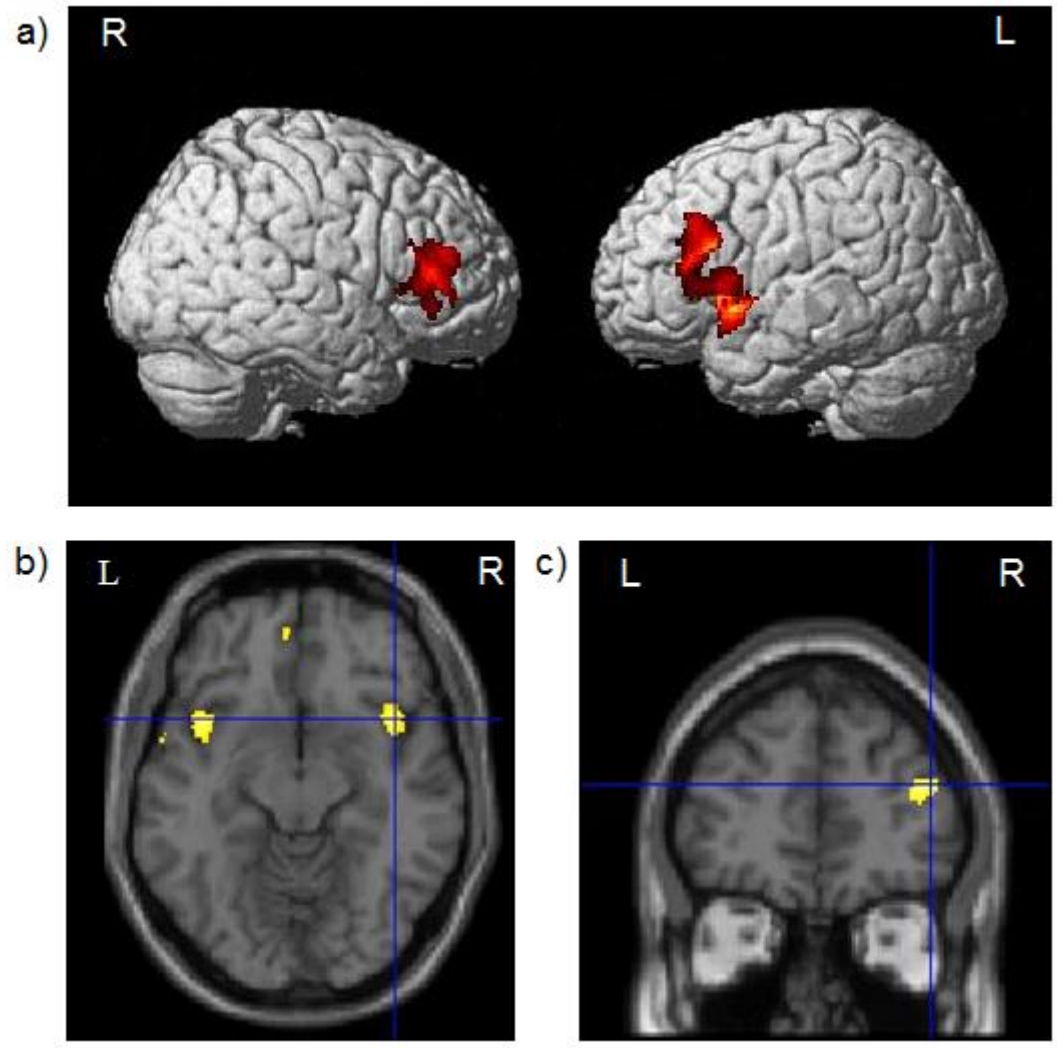

Figure 2. GM decreases from baseline to day 12 in (a) the right and left inferior frontal gyrus and (b) the insula bilaterally. (c) Positive correlation of the TQ difference with the GM concentration at baseline in the right frontal gyrus. R: right, L: left.

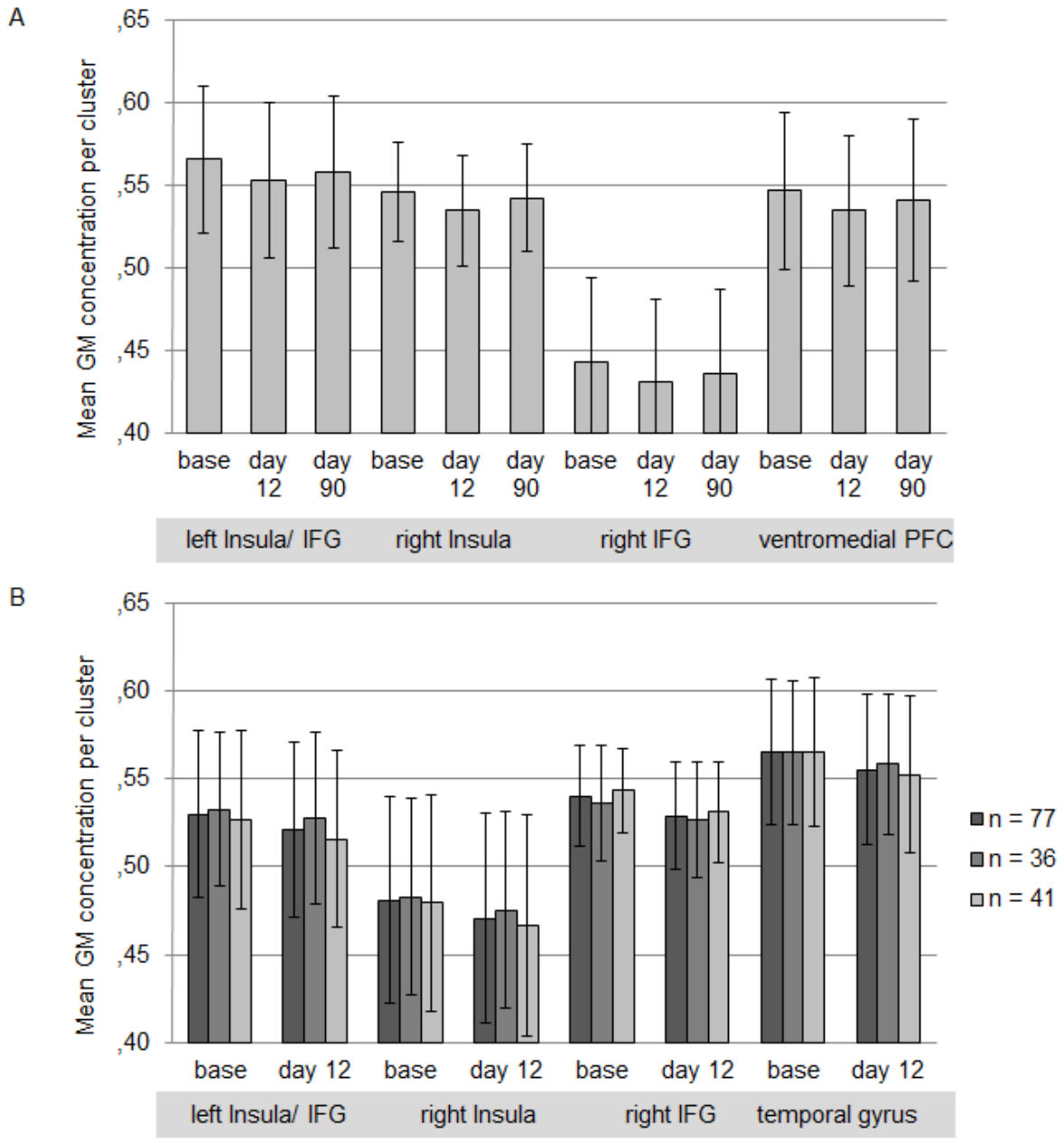

Figure 3. Mean GM concentration for each time point for the clusters with significant GM changes in (A) the subgroup of 41 patients, (B) the total group of 77 patients. For the clusters of (B) the mean GM concentration is also shown for the two independent subgroups. Error bars represent the standard deviations.

Table 3

Results of all VBM analyses

Laterality	Anatomical region	Cluster size in voxels	MNI coordinates x	y	z	Peak voxel *Z*-Score	Cluster level *p*
GM decrease from baseline to day 12 (n = 41)							
L	Temporal pole, Insula, Inferior frontal gyrus	1121	-56	8	-18	4.93	< .001
R	Insula (extending into temporal pole)	565	33	10	-18	4.79	.001
R	Inferior frontal gyrus	475	51	33	12	4.49	.009
L	Ventromedial prefrontal cortex	355	-4	52	-8	3.72	.026
GM decrease from baseline to day 12 (n = 77)							
L	Inferior frontal gyrus, Insula	1439	-46	12	-5	4.41	< .001
R	Insula (extending into temporal pole)	684	42	16	-11	4.44	.001
R	Inferior frontal gyrus	616	51	34	12	4.74	.001
L	Inferior/medial temporal gyrus	558	-57	-42	-17	4.21	.045
Positive correlation of TQ difference with baseline images (n = 36)							
L	Medial temporal pole	460	-32	6	-33	4.67	.014
R	Posterior cingulate cortex	430	6	-45	31	4.19	.036
Positive correlation of TQ difference with baseline images (N = 77)							
R+L	Lingual gyrus	534	4	-72	0	4.49	.037
R	Inferior/ middle frontal gyrus	413	52	30	19	3.86	.089

Note. FWE-corrected at cluster level $p < 0.05$; L, left; R, right; MNI, Montreal Neurological Institute.

Discussion

In order to improve rTMS treatment for patients suffering from subjective tinnitus, it is of particular importance to understand the neural alterations rTMS induces in tinnitus patients' brains in general – and in treatment responders' brains in particular. The current study aimed at investigating the structural brain changes after rTMS treatment and the connection between these changes and clinical outcome. We examined GM alterations after 10 sessions of low-frequency rTMS of the left temporal cortex. Besides the result that tinnitus severity and loudness were significantly reduced after rTMS treatment, the main findings of the present study were the following: 1) Transient GM decreases from baseline to day 12 were observed in several cortical areas. Neither GM increases nor GM changes from baseline to day 90 were detectable. 2) There was no correlation between GM changes and clinical outcome. 3) GM images at baseline correlated with treatment outcome suggesting that GM at baseline might be related to treatment response.

GM changes from baseline to day 12.

Bilateral GM decreases from baseline to day 12 were detectable in the insula and the inferior frontal gyrus (IFG). Those results were identical in the $n = 41$ group and the overall patient sample. On a more relaxed statistical threshold, the GM decreases in the right inferior frontal cortex were also visible in the $n = 36$ group. As can be seen in Figure 3, this group also shows the tendency for GM decreases in both the right and left insula and frontal cortex. However, the difference is too small to reach statistical significance. Together with the anterior parts of the insula, the IFG is supposed to be a part of the ventral attention network (VAT) – a mostly right-lateralized network responsible for a stimulus-driven "bottom-up" reorientation of attention to salient stimuli (Corbetta, Patel, & Shulman, 2008). An altered connectivity between the VAT and the auditory and visual cortices in patients with

bothersome tinnitus has recently been shown (Burton et al., 2012). Furthermore, the insula has been reported to be part of a salience network (Seeley et al., 2007) and both the IFG and the anterior insula are supposed to be involved in conflict-processing (Roberts, K. L. & Hall, 2008). If tinnitus is perceived as a permanent salient stimulus it continuously attracts attention and conflicts with other salient stimuli. It is therefore not surprising that as part of the VAT, alterations in the structure (Aldhafeeri et al., 2012) and function (Song, De Ridder, et al., 2012) of the IFG have been repeatedly reported in tinnitus research. While the insula is also a part of the VAT, it additionally plays an important role as part of a non-specific distress network (De Ridder et al., 2011). A relation between the insula and tinnitus distress has been consistently found in EEG-studies (van der Loo et al., 2011; Vanneste et al., 2010) and in studies examining structural brain alterations: Decreased GM volume in the insula was reported in highly distressed patients (Schecklmann et al., 2013) as well as a positive correlation between tinnitus distress and the cortical thickness in the anterior insula (Leaver et al., 2012).

Notably, the GM decreases in the IFG and the insula seen in the current study were observed for the whole group independently of treatment outcome, indicating that these changes are rather related to the intervention than to its clinical effect. The same is true for the remaining GM decreases observed. While GM alterations in the left temporal pole and the ventromedial prefrontal cortex were only visible in the small sample and are therefore not further discussed, the GM decrease in the inferior and middle temporal gyrus was only seen in the overall sample and – on a more relaxed statistical threshold – in the $n = 41$ sample. Again, the $n = 36$ sample showed the same tendency (see Figure 3) but not in a significant degree. Similar to the IFG and the insula, the medial temporal cortex has been previously reported to show functional alterations in tinnitus patients (Song, De Ridder, et al., 2012; Vanneste, van de Heyning, & De Ridder, 2011). However, GM changes in the medial

temporal cortex might be rather linked to hearing loss than to tinnitus (Boyen et al., 2013) and the same might be true for the inferior temporal cortex. Again, the morphological changes observed in the current study are not correlated with changes in the TQ or loudness scores. These results clearly suggest that rTMS leads to GM changes indeed, but that these changes are an expression of 'treatment' rather than an expression of 'treatment outcome'. All in all, those results are to be seen as preliminary and replications are clearly needed as the GM decreases were only statistically significant in the overall sample and one subsample but not in the second, smaller subgroup of 36 patients.

Besides the GM decreases reported above, no GM increases were found from baseline to day 12 – a finding which is not in line with the results of May et al. (2007) who found GM increases in the left superior temporal area after 5 days of rTMS stimulation of the temporal cortex. The absence of such a GM increase in the current study is presumably not a problem of too little statistical power as it was neither found in the subsamples nor in the larger sample with 77 patients. One of the main differences between the current study and the study of May et al. is that the latter applied rTMS to healthy subjects while we used rTMS as a treatment for patients with subjective tinnitus. Maybe, tinnitus brains react differently to low-frequency magnetic stimulation in comparison to control subjects. Knowing that there are both structural and functional alterations in the tinnitus brain in comparison to healthy controls (Adjamian et al., 2009; Lanting et al., 2009) and knowing that the effect of 1 Hz rTMS is state-dependent (Lefaucheur et al., 2012; Weisz, Steidle, & Lorenz, 2012) the different study outcomes might be reconcilable.

GM changes from baseline to day 90.

Interestingly enough, no GM decreases (nor increases) were seen from baseline to day 90 which suggests that the decreases seen on day 12 are temporary in nature. This

observation is in line with the results of May et al. (2007) who also found that the changes induced by rTMS are transient. It remains to be seen at which point in time the regression of the GM changes happens exactly. Whether the observed transient nature of the rTMS effect on GM may also reflect a transiency of clinical effects of rTMS treatment should be explored in further studies. Notably, previous long-term follow-up investigations in tinnitus patients have suggested long-lasting effects over periods up to four years in the majority of rTMS responders (Burger et al., 2011; Khedr, Rothwell, & El-Atar, 2009).

GM changes and clinical outcome.

Obviously, rTMS treatment of the temporal cortex leads to alterations in cortical regions known to be important for subjective tinnitus. These alterations do not seem to directly cause change in tinnitus distress though. As we investigated 77 patients, the lacking correlations do probably not arise from too little statistical power. Rather, it has to be considered that VBM might not be a method sensitive enough to capture neural changes that are related to the slight change of tinnitus distress or loudness which can be obtained using rTMS. This might be different for TMS treatment protocols with larger treatment effects and this might also be different for neuroimaging methods more sensitive to function rather than structure – such as fMRI or EEG. The only study investigating functional changes induced by rTMS using fMRI measurements could in fact not detect a relationship between changes in brain activity and clinical outcome (Lefaucheur et al., 2012). However, with only six patients the study might have lacked the required power to detect such an effect.

Taken together, the key message is that rTMS treatment of tinnitus patients affects brain structures different to the stimulation site which points to the importance of interconnections between distant cortical areas. It is well-known that TMS effects are not limited to the stimulated area and that functional changes can also be seen in remote cortical

brain areas (Bestmann & Feredoes, 2013; Siebner & Rothwell, 2003). What is true for functional changes might also be right for structural changes. While May et al. (2007) found GM increases in the stimulated area, they also reported the trend of GM increases in the temporal cortex contralateral to the stimulation site as well as in the thalamus bilaterally. Together with the results of the current study this emphasizes the importance of having in mind that magnetic stimulation of one cortical hotspot results in functional and presumably also structural alterations in a whole network of interconnected areas.

In summary, the bilateral alterations in the IFG and insulae after rTMS – although not seen on a significant level in the $n = 36$ group subgroup – further support the notion of functional connectivity between the left temporal cortex and the VAT in tinnitus patients. Whereas rTMS induces transient alterations in these areas and also in the inferior and medial temporal cortex, these changes do not determine the clinical effects.

Baseline GM images as predictor for treatment outcome.

Concerning the question whether GM images can serve as predictors for treatment response, the current results suggest that there are some cortical areas in which patients who will benefit from rTMS treatment have less GM at baseline than patients who will not benefit. In the right IFG and the lingual gyrus bilaterally, a positive correlation between GM at baseline and the TQ change was detected which means that an improvement in the TQ (implicated by negative values) is related to less GM at baseline. These results were seen in the overall patient group and in tendency also in the $n = 41$ group. Though a positive correlation was also found in the left medial temporal pole and the right posterior cingulate cortex, these results were only visible in the $n = 36$ sample and are therefore not further discussed. As mentioned above, the right IFG is part of the VAT and important for attention shifts to salient stimuli. The question arises however, what "reduced GM volume in the right

IFG" actually means in terms of the function of the VAT. One could speculate that the VAT had been less sensitive to salient stimuli (e.g. the tinnitus) prior to rTMS treatment. As a consequence, a reduction of tinnitus severity might have been easier to accomplish in those patients. This is speculation though and – after replication – a challenging question for future research. The lingual gyrus has never been reported to play an important role for subjective tinnitus. However, functional and structural alterations in nearby occipital regions have been observed in tinnitus patients (Boyen et al., 2013; Maudoux et al., 2012), even if one of those studies suggests that GM decreases in occipital regions might be rather due to hearing loss than due to tinnitus (Boyen et al., 2013). Overall, these findings have to be considered as preliminary as the mentioned correlations reached statistical significance only in the overall patient group but not in the two independent subsamples. Therefore, replications are needed to confirm those results. Furthermore, there is some evidence that patients who benefitted from treatment once also benefit from a second treatment phase (Langguth, Landgrebe, Hajak, & Kleinjung, 2008; Mennemeier et al., 2008, 2013). For that reason, future studies should also try to shed light on the question whether there are characteristics in the brain which predispose an individual to benefit from rTMS treatment in general while others do not.

Limitations.

The current study has a number of limitations which should be considered in future studies. First, as just mentioned, hearing level was not available for all patients and could therefore not be integrated in the analyses. Although hearing loss is not supposed to be a predictor for response to rTMS treatment (Lehner et al., 2012), previous studies have shown that hearing loss is an important confounder concerning GM changes in tinnitus patients (Boyen et al., 2013; Husain et al., 2011; Melcher, Knudson, & Levine, 2013). To be able to

thoroughly interpret research results, future work should try to include pure tone audiogram including high-frequency audiogram (Boyen et al., 2013; Husain et al., 2011; Melcher et al., 2013) for all patients. Second, the lacking correlation between treatment outcome and GM changes might have been due to the small treatment effects. As is already known from previous studies, the effect of rTMS treatment is small. Therefore, an even higher number of patients might have been necessary to ensure sufficient power for all analyses. The third and main limitation of the current study is the lack of a placebo condition. Without a patient group treated with sham stimulation we cannot definitely determine whether the observed GM changes were specific to rTMS treatment or unspecific effects. In the study of May et al. (2007), healthy control subjects showed no GM changes after sham rTMS as opposed to subjects treated with active rTMS. This finding has not been replicated for tinnitus patients yet.

Conclusions

To the best of our knowledge this is the first study to combine clinical assessment and longitudinal structural MRI scans to measure rTMS effects in tinnitus patients. The major result of the study is that ten days of low-frequency rTMS treatment of the temporal cortex lead to transient GM decreases in cortical regions different from the stimulated area. This highlights the importance of considering that the brain is organised in networks and that this organisation highly influences the outcome of an intervention. Transient GM decreases were seen bilaterally in the insula, the IFG and the left inferior/middle temporal gyrus, indicating functional connectivity between the stimulation site in the left temporal cortex and the VAT in tinnitus patients. Although these cortical areas are known to be important in the generation and maintenance of tinnitus, the GM decreases were independent from treatment success. Thus, they were rather related to the TMS intervention per se, and not to its clinical effect.

However, treatment outcome correlated with GM at baseline indicating reduced GM in the right IFG and the lingual gyrus in patients benefiting from treatment. Thus, baseline GM images might hold potential to be further investigated as predictor for rTMS response in the future.

Study 2: Multisite rTMS for the Treatment of chronic tinnitus: Stimulation of the cortical tinnitus network – a pilot study

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Abstract

Low-frequency rTMS of the auditory cortex has been shown to significantly reduce tinnitus severity in some patients. There is growing evidence that a neural network of both auditory and non-auditory cortical areas is involved in the pathophysiology of chronic subjective tinnitus. Targeting several core regions of this network by rTMS might constitute a promising strategy to enhance treatment effects. This study intends to test the effects of a multisite rTMS protocol on tinnitus severity. Forty-five patients with chronic tinnitus were treated with multisite stimulation (left dorsolateral prefrontal, 2000 stimuli, 20 Hz; left temporoparietal, 1000 stimuli, 1 Hz; right temporoparietal, 1000 stimuli, 1 Hz). Results were compared with a historical control group consisting of 29 patients who received left temporal stimulation (2000 stimuli, 1 Hz). Both groups were treated on ten consecutive working days. Tinnitus severity was assessed at three time points: at baseline, after the last treatment session (day 12) and after a follow-up period of 90 days. A change of tinnitus severity over time was tested using repeated measures ANOVA with the between-subjects factor treatment group. Both groups improved similarly from baseline to day 12. However, there was a difference on day 90: The multisite stimulation group showed an overall improvement whereas patients receiving temporal stimulation returned to their baseline level of tinnitus severity. These pilot data suggest that multisite rTMS is superior to temporal rTMS and represents a promising strategy for enhancing treatment effects of rTMS in tinnitus. Future studies should explore this new protocol with respect to clinical and neurobiological effects in more detail.

Introduction

Subjective tinnitus is the perception of sound or noise without presence of an internal or external sound source. Affecting about 10–15 % of adults in general population samples (Hoffman, H. J. & Reed, 2004), tinnitus represents a highly prevalent condition which is often accompanied by psychological distress ranging up to comorbid depressive disorders, anxiety and sleeping problems (Langguth, 2011). As the neurobiological mechanisms of these phantom sounds are not yet completely understood, treatment of chronic subjective tinnitus proves to be difficult. However, within the last few years, new insights concerning neural alterations in chronic subjective tinnitus have been gained, which can now be used to develop innovative treatment approaches. Both animal studies (Roberts, L. E. et al., 2010) and neuroimaging studies in humans (Adjamian et al., 2009; Lanting et al., 2009) have shown tinnitus-related hyperactivity in the auditory cortex. Repetitive transcranial magnetic stimulation (rTMS) is able to influence cortical excitability and has successfully been used for the treatment of other hyperexcitability symptoms such as auditory hallucinations (Hoffman, R. E. & Cavus, 2002). It has therefore been introduced as a new treatment option for chronic subjective tinnitus several years ago (Eichhammer et al., 2003). In the meantime many clinical studies have examined the effects of five to 10 sessions of low-frequency rTMS on tinnitus severity. The majority of these studies demonstrated significant reductions of tinnitus severity after unilateral stimulation of the temporal or temporoparietal cortex in some patients (Plewnia, 2011). Although these findings are considered promising, the results are burdened by only small to moderate effect sizes and large inter-individual variability. Thus optimization strategies are to be found to enhance the efficacy of this innovative treatment approach.

Recent results from imaging studies indicate that in tinnitus patients alterations of neural activity are not restricted to the auditory cortex but have also been observed in non-

auditory cortical areas. For instance, the reduction of tinnitus loudness after administration of lidocaine results in reduced regional cerebral blood flow in temporal as well as frontal, parietal and limbic areas (for a review see Lanting et al., 2009). Furthermore, magnetoencephalographic measurements have shown that in tinnitus patients spontaneous neural activity in both temporal and frontal cortices is characterized by a decrease in alpha power and an enhancement in delta and gamma power (Weisz et al., 2005, 2007). These activity changes in auditory and non-auditory structures seem to be related to each other as recent studies suggest that also the functional connection between those areas plays a crucial role in subjective tinnitus. Schlee, Hartmann et al. (2009) applied resting-state MEG to analyze the connectivity between temporal, parietal, frontal and cingulate cortices in tinnitus patients and healthy controls. In tinnitus patients a decreased connectivity between those areas in the alpha band as well as increased inter-areal coupling in the gamma frequency range was found. Furthermore, the gamma network turned out to be more widespread in patients who had been experiencing their tinnitus for more than four years. Those results indicate that a network consisting of functionally connected auditory and non-auditory structures is involved in the development and the chronic manifestation of subjective tinnitus.

These data do not only provide an explanation for the limited effects of past studies examining single-site rTMS for the treatment of chronic tinnitus but also offer a starting point for strategies to enhance treatment effects. If a network of several cortical areas forms the neural basis for chronic tinnitus, stimulation of the auditory cortex might not be sufficient to achieve a long lasting improvement of tinnitus severity. An extension of rTMS stimulation to non-auditory areas might therefore represent a good strategy to optimize treatment effects. There are promising results from a pilot study which compared standard low-frequency left temporal stimulation to a combined stimulation protocol (high-frequency left dorsolateral prefrontal plus low-frequency left temporal stimulation, Kleinjung et al., 2008). Immediately

after ten days of treatment no difference in the efficacy of both stimulation protocols was observed. After a follow-up period of three months, however, patients in the 'combined stimulation' group showed a more pronounced overall symptom improvement than patients who had been treated with left temporal stimulation. The superior long term effects of combined stimulation were confirmed by a recent retrospective study with larger sample sizes (Lehner et al., 2012). Based on these studies and the knowledge about altered functional connectivity between auditory and non-auditory brain regions, we hypothesized that treatment effects can be enhanced if the whole network (Schlee, Hartmann, et al., 2009) is targeted by rTMS. Here we aimed to test a new stimulation protocol which covers a majority of the central hubs of increased tinnitus related connectivity. Since this network comprises temporal, parietal, frontal and cingulate brain regions all of which can be stimulated with a varying number of stimuli and frequencies, there are many possibilities of how an optimal stimulation protocol could look like. We chose a multisite protocol, which combined high-frequency stimulation of the left dorsolateral prefrontal cortex (DLPFC) and bilateral low-frequency stimulation of the temporoparietal cortices for several reasons. High-frequency stimulation over the DLPFC has been shown to mediate activity changes in the anterior cingulate cortex via functional connections between the DLPFC and the anterior cingulate cortex (Speer et al., 2000). Furthermore, combined stimulation of the left DLFPC and left temporal cortex has already been successfully applied to tinnitus patients with combined stimulation resulting in more pronounced treatment effects than temporal stimulation alone (Kleinjung et al., 2008). The decision to treat temporoparietal cortices over both hemispheres was based on two ideas. (a) Four core regions of the tinnitus network (bilateral temporal and parietal) could be reached by stimulating only two areas (bilateral temporoparietal, Tracy et al., 2010). (b) Furthermore, results from past studies about low-frequency rTMS over the temporoparietal cortex are ambiguous concerning the question of which hemisphere to treat.

While some studies suggest that stimulation over temporoparietal cortex contralateral to the tinnitus percept is superior to ipsilateral stimulation (Khedr et al., 2010), other studies indicate that stimulation of the left temporoparietal cortex is effective irrespective of tinnitus laterality (Khedr, Rothwell, Ahmed, & El-Atar, 2008; Rossi et al., 2007). These conflicting results bear an uncertainty of targeting the appropriate hemisphere – a problem which could be avoided by bilateral stimulation of both temporoparietal cortices. The objective of the current pilot study was to obtain a first estimate of this new protocol's effectiveness in order to find out if the idea of network stimulation should be explored in more detail in the future.

Materials and Methods

Subjects and rTMS treatment.

Data from 74 patients were analysed. All patients had suffered from disturbing tinnitus for at least three months. Forty-five patients (31 men, 14 women, mean age 53.54 ± 12.82 years) (all data in the text and tables are given as mean ± standard deviation) were treated with the multisite stimulation protocol which consisted of high-frequency stimulation of the left DLFPC (20 Hz, 2000 stimuli/day) followed by left and right temporoparietal stimulation (1 Hz, 1000 stimuli/day over each hemisphere), resulting in 4000 stimuli/day and 40.000 stimuli in total. The sequence of stimulation was the same for all patients (DLPFC first, then left and then right temporoparietal cortex). As the treatment protocol was examined for the very first time, we intended to gather pilot data and test the safety and feasibility of the new protocol. Therefore, data were collected in a one-arm open label study from February to November 2011.

For a preliminary comparison with left temporal rTMS we used a historical control group consisting of 29 patients (23 men, 6 women, mean age 46.48 ± 15.09) who had been treated with 1 Hz stimulation over the left temporal cortex (2000 stimuli/day, 20.000 stimuli

in total) in the course of a former study between August 2009 and April 2011 (Kreuzer et al., 2011).

All participants received 10 sessions of rTMS on ten consecutive working days at the Tinnitus Center at the University of Regensburg, Germany. Both groups met identical inclusion and exclusion criteria (see Table 4). Demographical and clinical data of both groups were comparable except for age and baseline scores for THI (Tinnitus Handicap Inventory) and Beck's Depression Inventory (BDI) with the multisite group being older and scoring higher on the THI and BDI (see Table 5). Additionally, the multisite group tended to score higher in two rating scales (strong/ loud and ignoring). Many patients were treated with centrally acting drugs (for detailed information see Table 6). The medication was kept constant in all cases during the stimulation period and in almost all patients throughout the follow-up period. All patients provided written informed consent after comprehensive explanation of the procedures.

For both treatment protocols, a Medtronic system with a figure-of-eight coil (Cool B-65 Butterfly, Medtronic, Minneapolis, MN, USA) was used. Stimuli were applied with an intensity of 110% RMT but never higher than 60% of maximal stimulator output. RMT was defined as the minimal intensity at which at least five of ten motor evoked potentials were 50 µV in amplitude in the right M. abductor digiti minimi. Temporal and temporoparietal cortices were localized by using the 10-20 localization system (Hoffman, R. E. et al., 2000; Jasper, 1958; Langguth et al., 2006). The DLPFC was localized by centring the coil 6 cm anterior from the part of the motor cortex that had already been targeted for defining the RMT (Frank, E. et al., 2011). Both treatment protocols had been approved by the local ethics committee.

Clinical assessment.

For the assessment of demographical and clinical characteristics patients completed the Tinnitus Sample Case History Questionnaire (Langguth et al., 2007) when they visited the Tinnitus Center for the first time during tinnitus consultation hours. Hearing level [dB HL] is reported as an average of all thresholds measured bilaterally in pure-tone audiogram ranging from 125 Hz to 8 kHz. Tinnitus severity was assessed by the German versions of the TQ (Goebel & Hiller, 1994), the THI (Kleinjung, Fischer, et al., 2007; Newman, Jacobson, & Spitzer, 1996) and five additional items which measured (a) how loud, (b) uncomfortable, (c) annoying or (d) unpleasant tinnitus was perceived and e) how easily it could be ignored. Those items were rated on a numeric scale ranging from 0 to 10 with higher values indicating a more severe tinnitus. For assessment of depressive symptoms patients completed the BDI (Beck & Steer, 1984). These psychometric measures were assessed at three time points during the study: at baseline (immediately before treatment beginning), at the end of treatment (day 12) and after a follow-up period of three months (day 90).

Statistical analysis.

For statistical analyses PASW statistics 18 (SPSS Inc., Chicago, IL) was used. All data were entered into the database of the Tinnitus Research Initiative (Landgrebe et al., 2010). Data management was conducted according to the Data Handling Plan (TRI-DHP V07, 09.05.2011). Data analysis was conducted according to the Standard Operating Procedure (TRI-SA V01, 09.05.2011) thereby following a study-specific Statistical Analysis Plan (SAP) that was written according to the SAP template (TRI-SAP 007, 09.02.2012). All documents can be accessed under (http://database.tinnitusresearch.org/). Missing values were replaced by using a last observation carried forward (LOCF, or if this was not possible backward) procedure. Two baseline-scores for the TQ and one for the BDI and the rating

scale strong/ loud were missing. For day 12, two scores were missing for the THI and one for all other variables measured. For day 90, ten scores had to be replaced for the TQ, nine for the THI and eight for all other variables (drop-outs on day 90).

To test for changes in tinnitus severity from baseline to day 12 and day 90, an ANOVA with within-subjects factor time (baseline, day 12, day 90) and between-subjects factor group (left temporal vs. multisite stimulation) was calculated for all eight variables (TQ, THI, BDI, five rating scales). In order to find out if the group differences in age and some of the baseline values of tinnitus severity (see Table 5) exerted an influence on the results, two additional analyses were conducted: First, an analysis of covariance (ANCOVA) was done with age, the baseline scores for the THI, BDI and the two rating scales "uncomfortable" and "ignoring" being entered as covariates. Second, we created matched groups by selecting 25 patients of each group which were matched for all variables measured (all group comparisons had a *p*-value of $\geq .11$) and which were then compared using an ANOVA.

All further analyses were then based on the ANOVA which was done for all 74 patients. If no significant interaction effect resulted, no further analysis was carried out for the respective variable. In case of significant interaction effects, different post-hoc tests were calculated. For the within-group comparisons of tinnitus severity, groups were analysed separately by using paired t-tests (baseline vs. day 12, baseline vs. day 90, day 12 vs. day 90). For the between-group comparisons, difference values were calculated for each pair of time points (e.g., TQ on day 12 minus TQ at baseline) with negative values indicating an improvement in time. Those difference values of both groups were then used for the between-group comparisons with independent sample t-tests. Finally, responder rates were calculated with responders defined as patients having improved by five points or more in the TQ score

(Adamchic et al., 2012; Frank, G. et al., 2010; Kleinjung, Steffens, et al., 2007). Responder rates were then compared between groups by using a Chi^2 test.

Former studies suggest that adding more stimulation sites results in better long-term effects of rTMS treatment (Kleinjung et al., 2008; Lehner et al., 2012). A better long-term effect is therefore expected for the multisite protocol as well. However, as the treatment groups in the current study differed both in the number of stimuli and in the number of stimulation sites, the question arises which of the two parameters is responsible if better long-term effects are observed. To provide a first answer to this question, a third group of patients (N = 193) was added to the analysis who received the same number of stimuli as the multisite group (4000 stimuli/day) but over two stimulation sites instead of three (110% motor threshold, 2000 stimuli at 20 Hz over left dorsolateral prefrontal cortex plus 2000 stimuli at 1 Hz over temporal cortex). Details on this group have been reported elsewhere (Lehner et al., 2012). An ANOVA with within-subjects factor time (baseline, day 90) and between-subjects factor group (left temporal vs. multisite stimulation vs. temporal plus frontal stimulation) was calculated for the TQ which was the only outcome parameter available for the third group. Independent sample t-tests were used as post-hoc tests with the TQ changes (score on day 90 minus score on baseline) as dependent variable. All statistical tests were two-tailed and unadjusted for multiple comparisons following a pilot study approach. The level of significance was set at .05.

Table 4

Inclusion and exclusion criteria for both treatment groups

Inclusion criteria	Exclusion criteria
• Age older than 18 years • Subjective chronic tinnitus • Duration of tinnitus more than 3 months	• Contraindication for rTMS (epilepsy, cardiac pacemaker, head injury, evidence of significant brain malformation or neoplasm, cerebral vascular events, neurodegenerative disorder affecting the brain or prior brain surgery, metal objects in and around the body that cannot be removed, pregnancy) • Objective tinnitus • Treatable cause of the tinnitus • Involvement in other treatments for tinnitus at the same time • Clinically relevant psychiatric comorbidity • Clinically relevant unstable internal or neurological comorbidity • Alcohol or drug abuse • Prior treatment with rTMS

Table 5

Demographical data and clinical characteristics for both treatment groups

	left temporal rTMS (*N* = 29)	multisite rTMS (*N*= 45)	group comparison	*p*
Gender	M (79 %) F (21 %)	M (69 %) F (31 %)	$\chi^2(1, 74) = 0.97$	.324
Age (years)	46.48 ± 15.09	53.54 ± 12.82	$t(72) = -2.15$	.035*
Hearing threshold [dB HL]	19.58 ± 13.69	21.16 ± 12.55	$t(72) = -0.51$	.610
tinnitus laterality	right (14 %) left (10 %) both ears (76 %)	right (7 %) left (11 %) both ears (82 %)	$\chi^2(2, 74) = 1.05$	.593
Tinnitus duration (years)	7.11 ± 6.41	8.34 ± 7.34	$t(72) = -0.74$	.464
Tinnitus severity (baseline):				
TQ	39.03 ± 18.03	44.98 ± 16.09	$t(72) = -1.48$	.14
THI	40.55 ± 20.15	51.47 ± 22.33	$t(72) = -2.13$	.037*
BDI	8.55 ± 7.47	12.27 ± 6.61	$t(72) = -2.24$	.028*
Strong/ loud	6.48 ± 2.17	6.76 ± 1.77	$t(51.33) = -0.57$	.57
Uncomfortable	6.76 ± 2.20	7.64 ± 1.71	$t(49.27) = -1.84$	.072#
Annoying	6.48 ± 2.36	6.87 ± 1.90	$t(72) = -0.77$	.44
Unpleasant	6.48 ± 2.15	6.62 ± 2.08	$t(72) = -0.28$	.78
Ignoring	6.28 ± 2.51	7.24 ± 2.06	$t(72) = -1.81$	.074#

Note. *$\alpha < .05$; #$\alpha < .10$.

Table 6

Centrally acting medication for both treatment groups (number of patients treated with each drug are specified in brackets)

	Left temporal rTMS (*N* = 29)	Multisite rTMS (*N* = 45)
Antidepressants	Opipramol (1)	Opipramol (2)
	Agomelatine (1)	Agomelatine (8)
	Mirtazapin (2)	Mirtazapin (6)
	Duloxetin (1)	Duloxetin (1)
	Venlafaxin (1)	Citalopram (3)
		Escitalopram (1)
		Trimipramin (1)
		Bupropion (1)
		Amitriptylin (3)
		Doxepin (2)
		Lithium (1)
Anticonvulsants	Pregabalin (2)	Pregabalin (2)
	Carbamazepin (1)	
Neuroleptica	Quetiapin (1)	Quetiapin (2)
		Risperidon (1)
Others	Gingko (1)	Gingko (2)
	Zopiclon (1)	Sifrol (2)
	Metoprolol (1)	Metoprolol (3)
	Baclofen (1)	Bisoprolol (4)

Results

Adverse events.

Both the left temporal and the multisite stimulation protocol were well tolerated by the patients. No serious adverse effects were observed. Five patients in the multisite group complained about transient headache as a side effect. One patient reported an elevated loudness of his tinnitus. All side effects recovered within the observation period and were comparable to those seen in the group treated with left temporal rTMS (eight patients reported headache, three reported elevated loudness of the tinnitus). No patient dropped out of the study during the treatment phase, but a total of eight patients did not show up for the final visit on day 90, five of them being in the multisite group (drop-outs on day 90).

Statistical analysis.

ANOVAs revealed a significant interaction effect between time and group for six of the eight variables analysed (see Table 7). No significant interactions were found for THI and BDI. Those variables were therefore not included in the following analyses. However, on a descriptive level those variables showed a comparable pattern to the variables with significant effects.

For left temporal rTMS within-group comparisons with post-hoc paired t-tests revealed significant to marginally significant changes from baseline to day 90 for the rating scales “strong/ loud”, “ignoring” and “unpleasant” (see Table 8). The difference from day 12 to day 90 was significant to marginally significant for all rating scales, which were entered into the post-hoc tests. As can be seen in Figure 4, both the changes from baseline to day 90 and day 12 to day 90 were due to a worsening of the tinnitus. For multisite rTMS, the change from baseline to day 12 was significant for the TQ and the rating scale “uncomfortable”. The changes from baseline to day 90 were significant to marginally significant for the TQ and

three of the rating scales (see Table 8). The change from day 12 to day 90 was only significant for the rating scale "unpleasant". For the multisite rTMS group all significant changes were due to an improvement in tinnitus severity (see Figure 4).

Between-group comparisons revealed that the change in tinnitus severity from baseline to day 12 was comparable between both treatment groups as no comparison reached statistical significance (see Table 9). However, changes from baseline to day 90 and from day 12 to day 90 were significantly different between groups for all variables entered into the between-group comparisons. To control for the group differences an ANCOVA and an ANOVA for matched groups were calculated. In comparison to the ANOVA including all 74 patients, the results reveal some differences between the tests (see Table 7) but do not suggest systematic changes caused by age or tinnitus severity. With respect to the responder rates, there was no difference between groups on day 12 (31% in the left temporal stimulation group, 49 % in the multisite stimulation group, $\chi 2(1, N = 74) = 2.31$, $p = .13$). On day 90, the responder rates differed however (20% in the temporal stimulation group, 49% in the multisite stimulation group, $\chi 2(1, N = 74) = 5.96$, $p = .015$). The ANOVA comparing the TQ changes on day 90 between multisite stimulation, left temporal stimulation and left temporal plus left frontal stimulation revealed an marginally significant interaction effect between time and treatment ($F(2, 226) = 2.99$, $p = .052$). Post-hoc t-tests showed a significant difference between multisite and left temporal stimulation ($t(72) = -2.23$, $p = .029$), a marginally significant difference between left temporal and temporal plus frontal stimulation ($t(182) = -1.95$, $p = .053$) and no significant difference between multisite and temporal plus frontal stimulation ($t(198) = -1.20$, $p = .23$). Figure 5 shows the TQ scores for all three time points and all three groups.

Table 7

Results from two-way repeated measures analyses of variance: interaction effects (time x group)

	ANOVA		ANCOVA[+]		ANOVA matched groups	
	F (*df*)	*p*	*F* (*df*)	*p*	*F* (*df*)	*p*
TQ	4.05 (1.47, 105.65)	.032*	2.32 (1.44, 96.52)	.12	2.73 (1.51, 72.29)	.086[#]
THI	3.10 (1.47, 105.74)	.065[#]	1.56 (1.48, 98.96)	.22	3.58 (1.58, 75.96)	.043*
BDI	1.07 (1.78, 128.01)	.34	0.19 (1.80, 120.69)	.80	3.16 (2, 96)	.047*
Ratings:						
strong/ loud	4.28 (1.79, 129.07)	.019*	3.04 (1.82, 122.18)	.056[#]	2.46 (2, 96)	.091[#]
uncomfortable	4.24 (1.76, 126.93)	.020*	4.45 (1.84, 123.38)	.016*	5.97 (1.76, 84.55)	.005*
annoying	3.97 (1.71, 123.35)	.027*	2.50 (1.73, 115.67)	.095[#]	1.56 (2, 96)	.22
ignoring	5.35 (2, 144)	.006*	3.44 (2, 134)	.035*	4.60 (2, 96)	.012*
unpleasant	4.12 (1.82, 130.67)	.022*	3.16 (1,82, 122.12)	.050*	3.32 (2, 96)	.040*

Note. *$\alpha \leq .05$; [#] $\alpha < .10$; [+]Covariates: age and baseline scores for THI, BDI, uncomfortable and ignoring.

Table 8

Results from post-hoc tests: paired t-test for within-group comparisons

	left temporal rTMS (*df* = 28)						multisite rTMS (*df* = 44)					
	12 - baseline		90 - baseline		90 - 12		12 - baseline		90 - baseline		90 - 12	
	t	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*
TQ	0.98	.34	-0.98	.34	-1.62	.12	2.29	.027*	2.32	.025*	1.25	.22
strong/ loud	0.92	.38	-1.85	.074#	-2.34	.027*	0.77	.45	1443	.16	1.27	.21
uncomfortable	0.92	.37	-0.75	.46	-1.75	.092#	2.69	.010*	2.88	.006*	1.22	.23
annoying	0.22	.83	-1.60	.12	-1.98	.057#	1.03	.31	1.82	.076#	1.66	.10
ignoring	0.61	.55	-2.37	.025*	-2.74	.011*	1.46	.15	2.01	.050*	0.93	.36
unpleasant	0.68	.50	-1.71	.096#	-2.19	.037*	-0.63	.55	1.06	.30	2.53	.015*

Note. *$\alpha < .05$; #$\alpha < .10$.

Table 9

Results from post-hoc tests: independent samples t-test for between group comparisons (df = 72)

	12 - baseline		90 - baseline		90 - 12	
	t	*p*	*t*	*p*	*t*	*p*
TQ	0.87	.39	2.23	.029*	2.04	.045*
strong/ loud	-0.09	.93	2.31	.024*	2.75	.007*
uncomfortable	1.10	.28	2.64	.010*	2.13	.037*
annoying	0.51	.61	2.42	.018*	2.61	.011*
ignoring	0.51	.61	2.87	.005*	2.76	.007*
unpleasant	-0.88	.38	1.70	.093$^{\#}$	3.33	.001*

Note. $^{*}\alpha < .05$; $^{\#}\alpha < .10$.

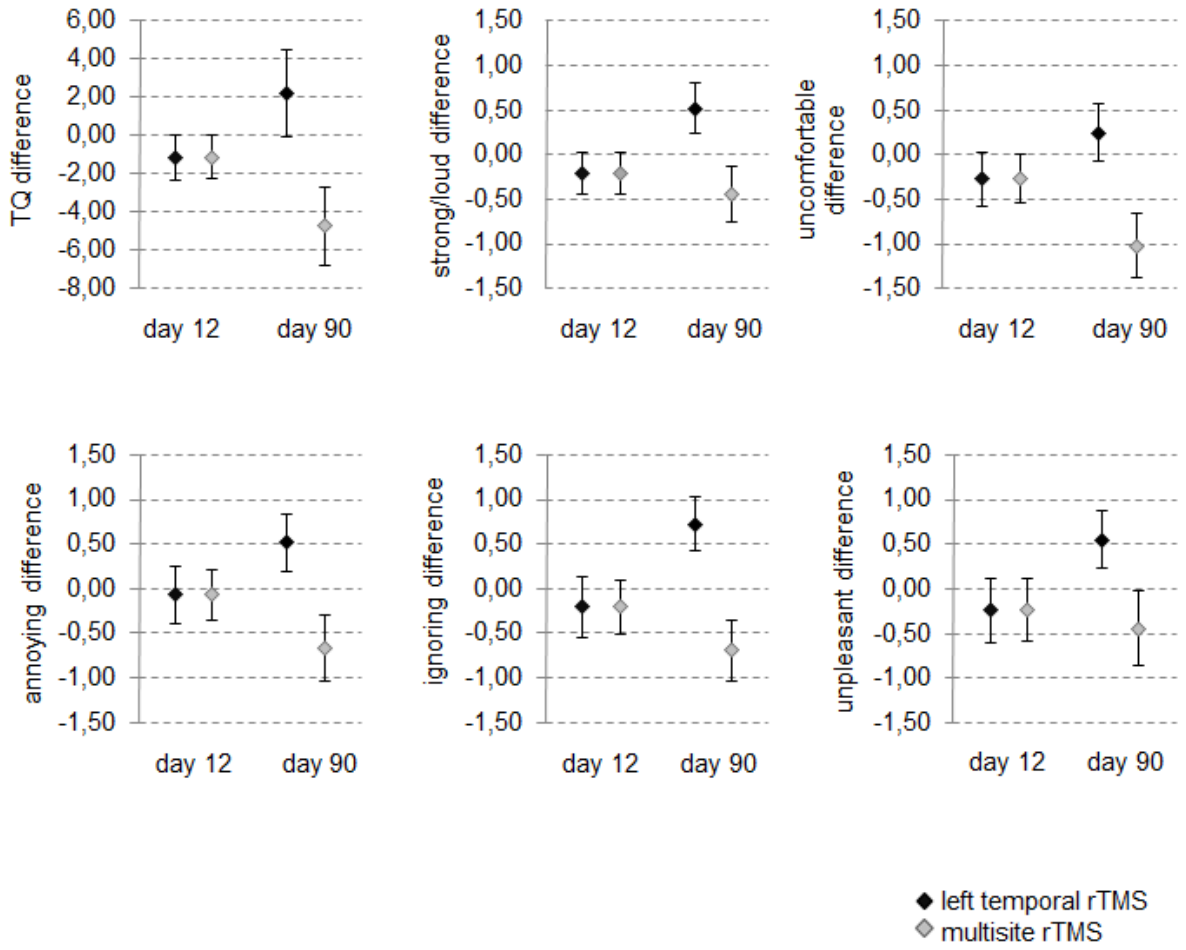
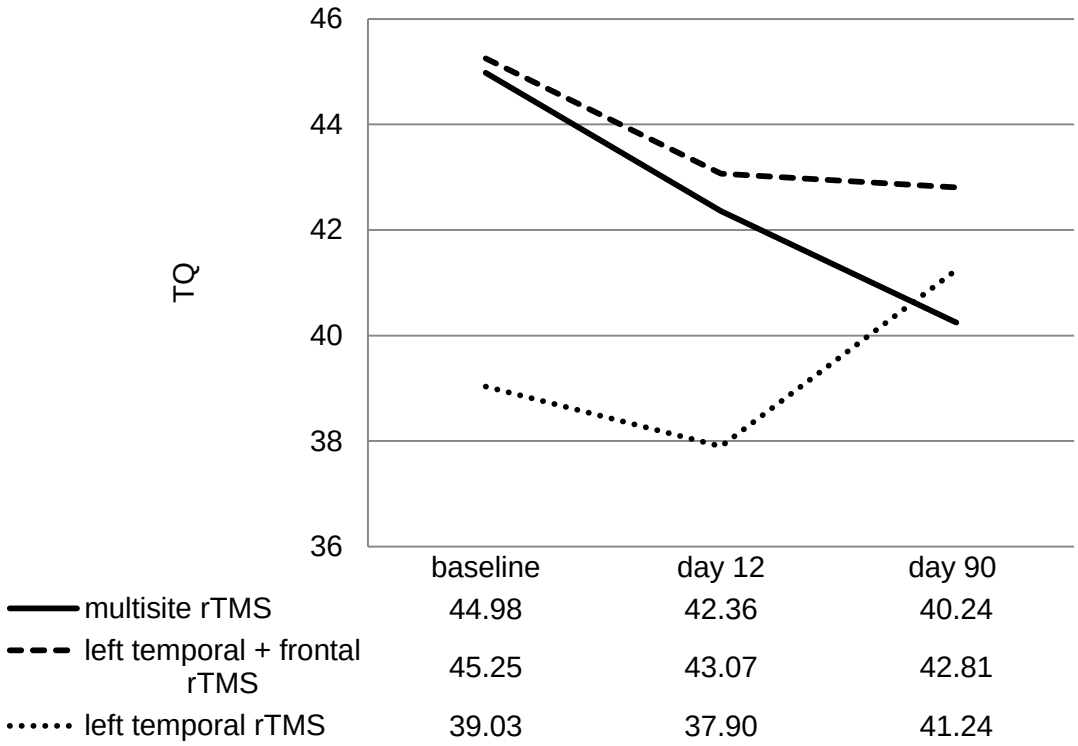


Figure 4. Point diagrams showing the relative changes of TQ scores and rating scales from baseline to day 12 / day 90 for both groups separately (*M* ± *SE*, negative values describe an improvement of tinnitus severity).



	baseline	day 12	day 90
multisite rTMS	44.98	42.36	40.24
left temporal + frontal rTMS	45.25	43.07	42.81
left temporal rTMS	39.03	37.90	41.24

Figure 5. Line chart showing the time course of the TQ for both treatment groups in comparison to a group receiving left temporal plus left frontal rTMS.

Discussion

The current study aimed to make a rough estimate of the safety, feasibility, tolerability and effectiveness of a new stimulation protocol which was designed on the basis of the tinnitus network as described by Schlee, Hartmann et al. (2009). The main finding of this study is that ten days of rTMS treatment with this new multisite stimulation protocol resulted in a reduction of tinnitus severity that outlasted the follow-up period of 90 days. This long-term improvement appears especially prominent if compared to the control group, which was treated over the left temporal cortex only. In the short run (day 12), both protocols caused a similar improvement of tinnitus severity. In the control group, this improvement was rather short-lasting however, with tinnitus severity returning to its baseline level on day 90. Therefore, no improvement was detectable from baseline to day 90 in this group. In contrast, in the multisite stimulation group tinnitus severity remained largely stable from day 12 to day 90 with even some further (but mostly non-significant) improvement, resulting in an overall significant improvement of tinnitus severity from baseline to day 90. Responder rates on day 12 and day 90 underpin these results with both groups having similar responder rates on day 12 but significantly higher responder rates in the multisite stimulation group on day 90. These results are in line with the results of Kleinjung et al. (2008) who compared left temporal stimulation to a combined frontal plus left temporal stimulation. Similar to the current results, both protocols were similarly effective on day 12 whereas on day 90, combined stimulation was slightly superior to left temporal stimulation. This superiority did not reach statistical significance though, whereas the multisite protocol of the current study turned out to be significantly superior. However, in the current study the patients receiving left temporal stimulation were treated with 2000 stimuli/day whereas patients receiving multisite stimulation were treated with 4000 stimuli/day. Therefore the question arises whether the superior long-term effect of the multisite protocol is due to the higher number of

stimuli or due to the fact that more stimulation sites were treated. There is evidence that the effect of rTMS might be dose-dependent with more stimuli resulting in more tinnitus reduction (Plewnia et al., 2007). However, this has only been shown in single sessions of rTMS so far and currently no study has examined if the long-term outcome of rTMS treatment in tinnitus patients is dose-dependent as well. Nevertheless, it cannot be ruled out that multisite stimulation was superior to left temporal stimulation only because of the higher number of stimuli. For this reason, another historical control group was added to the analysis. Those patients were treated with 4000 stimuli/day over two stimulation sites. The comparison of the long-term effects for all three groups suggests that both the higher number of stimuli and the higher number of stimulation sites add to the better long-term effects of the multisite protocol (see Figure 5). Although TQ changes on day 90 do not differ significantly between multisite and temporal plus frontal stimulation, the difference between multisite and left temporal stimulation is significant indeed while the difference between left temporal plus frontal and left temporal stimulation is only marginally significant. Therefore, a large portion of the superiority of the multisite over the left temporal protocol might be due to a larger amount of stimuli but it also seems to matter, where these stimuli are applied (see Figure 5). It has to be considered however that this is an exploratory study and that this result should be interpreted with caution. Future studies applying a dose-matched control protocol with randomized group allocation and using multiple outcome parameters should try to replicate the results.

We are well aware about the limitations of the present study. Due to the exploratory nature of this pilot study, our findings have to be considered preliminary and need confirmation by randomized controlled trials. Because of the exploratory purpose of this pilot study, the various dependent variables were analysed separately without adjusting for multiple comparisons. Moreover results were compared with a historical control group. Even

if all patients of both the multisite and the left temporal group met identical inclusion and exclusion criteria (Table 4) and even if the treatment was performed in both groups at the same institution and by the same team a group-specific selection bias cannot be excluded. Groups differed in their concomitant medication though. However, in all patients medication was kept constant during the treatment period and in most of the patients also during the follow-up period. Therefore, no systematic variation of drugs took place. Although we cannot exclude the possibility that some drugs might have had an additional effect on treatment outcome or might have interacted with rTMS treatment, neither our data nor former studies (Kleinjung et al., 2009, 2011) do indicate such effects.

Also, there were group differences regarding age and some of the baseline measures of tinnitus severity which might have affected treatment outcome. We tried to control for those differences by including the variables concerned as covariates and by creating matched groups. The results suggest that the effect of multisite stimulation being superior to left-temporal rTMS is rather stable and largely independent of those group differences. Nevertheless, replication of the results using a study design with randomized group allocation is necessary to confirm the results of the current study.

Despite these limitations the assumption that multisite stimulation is able to disrupt altered activity within the tinnitus network and thereby leads to an improvement of tinnitus severity constitutes a plausible explanation for its better long-term effects. The auditory and the non-auditory areas are supposed to assume different roles concerning the tinnitus percept (De Ridder et al., 2011). Therefore, different outcomes would be expected depending on if the auditory cortex is stimulated exclusively or if non-auditory areas are stimulated in addition. As activity in the auditory cortex is correlated with tinnitus loudness (van der Loo et al., 2009) it can be speculated that the loudness of the sound might be coded in auditory structures whereas the involvement of non-auditory brain areas is considered essential for

conscious perception of this sound. According to Dehaene and Changeux (2004), a conscious auditory percept requires activity of a "global workspace" in form of a fronto-parieto-cingular network which is assumed to modulate the activity in sensory cortices via top-down amplification or inhibition (Dehaene & Changeux, 2004). Consequently, left temporal stimulation might be able to influence activity within the auditory cortex and therefore result in a temporary improvement of tinnitus severity. But as the altered top-down influence of the global workspace may still be present, the activity within the auditory cortex might be elevated again (Schlee et al., 2011), causing a rebound of tinnitus severity to its baseline level. In contrast, multisite stimulation targets both the auditory cortex and the global workspace on several central nodes, possibly resulting in a disruption of the whole network and consequently in a long-term improvement of tinnitus severity.

Results from the study of Schlee, Mueller et al. (2009) suggest that this top-down modulation might in fact play a role in chronic tinnitus. They found that in tinnitus patients the prefrontal cortex serves as an important cortical hub, which exerts strong influence on other cortical regions whereas the temporal cortex is a core region as well but is strongly influenced by other brain regions. Moreover, this external modulation of the temporal cortex was positively correlated with tinnitus distress: The more the temporal cortex was driven by other areas, the more distress was reported by the patients.

After all, this explanation of a successfully disrupted tinnitus network as a reason for more pronounced long-term effects of multisite stimulation remains speculative, of course. In order to clarify this issue future research should combine rTMS treatment with functional neuroimaging methods and compare functional alterations that appear after left temporal vs. after multisite stimulation. If multisite stimulation interferes with the tinnitus network whereas left temporal stimulation does not, different functional changes should be detectable.

Instead of altered network activity being the reason for the observed results of this pilot study there is also the possibility of single stimulation sites being responsible for the better long-term effect of multisite stimulation. For instance, high-frequency stimulation of the left DLPFC is well known to exert antidepressant effects (Gross, Nakamura, Pascual-Leone, & Fregni, 2007). As tinnitus patients do often suffer from comorbid depression (Langguth, 2011) it is possible that patients felt less bothered by their tinnitus or could better cope with it due to an improvement of their depressive symptoms. This explanatory approach is considered unlikely though as no significant interaction effect between time and group concerning the BDI was observed. This indicates that depressive symptoms did not change differently after treatment with multisite or with left temporal stimulation, respectively. This would have been expected though if depressive symptoms were responsible for the better long-term effects of multisite stimulation. Therefore, the superiority of the applied multisite stimulation paradigm cannot be ascribed to a sole reduction of depressive symptoms. Bilateral stimulation over temporoparietal cortices alone is also highly unlikely to be responsible for the observed effect, since two recent studies (Hoekstra et al., 2012; Plewnia et al., 2012) did not find tinnitus reduction after bilateral stimulation of the temporal or temporoparietal cortices.

In this study, a stimulation protocol was designed which was assumed to have great potential to represent a clinically effective treatment for tinnitus patients. With the clinical effectiveness being our primary goal the most promising treatment protocol was used right away without testing different protocols consisting of only two stimulation sites beforehand. Thus, no definite answer can be given regarding the question which of those possible mechanisms is finally responsible for the longer lasting effects of multisite stimulation compared to left temporal stimulation.

All in all, this study was conducted to obtain an estimate of the effectiveness, feasibility and safety of the new protocol in order to find out if the idea of network stimulation should be further explored in future studies. The multisite protocol turned out to be feasible in clinical routine and was well tolerated by all patients. As the protocol caused only slight side effects (like transient headache) which are already known from low-frequency temporal stimulation, our data suggest that the multisite protocol is as safe as the well-known temporal stimulation protocol. Additionally, the pilot data show promising long-term effects of the multisite stimulation indeed. Subsequent studies are now needed to analyse both clinical and neurobiological effects of this new stimulation paradigm in more detail. Those studies should take the limitations of the current trial into account by using randomized group allocation, by matching the number of stimuli in both treatment groups and by including measures of neurobiological changes to allow for an analysis of the neural changes different treatment protocols are inducing in tinnitus brains.

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Study 3: Triple-site rTMS for the treatment of chronic tinnitus: a randomized controlled trial

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Abstract

Recent research indicates that tinnitus is related to alterations of neural networks including temporal, parietal, and prefrontal brain regions. The current study examines a rTMS protocol which targets three central nodes of these networks in a two-arm randomized parallel group trial. Overall, 49 patients with chronic tinnitus were randomized to receive either triple-site stimulation (left DLPFC stimulation, 1000 pulses, 20 Hz plus left and right temporoparietal stimulation, 1000 pulses each, 1 Hz) or single-site stimulation (left temporoparietal stimulation, 3000 pulses, 1 Hz). Both groups were treated in 10 sessions. Tinnitus severity as measured by the TQ was assessed before rTMS (day1), after rTMS (day12) and at two follow-up visits (day 90 and day 180). The triple-site protocol was well tolerated. There was a significant reduction in tinnitus severity for both treatment groups. The triple-site group tended to show a more pronounced treatment effect at day 90. However, the measurement time point x group interaction effect was not significant. The current results confirm former studies that indicated a significant reduction of tinnitus severity after rTMS treatment. No significant superiority of the multisite protocol was observed. Future approaches for the enhancement of treatment effects are discussed.

Introduction

Chronic subjective tinnitus is defined as the perception of sound or noise without presence of a corresponding internal or external sound source. It is a highly prevalent (Hoffman, H. J. & Reed, 2004) and for many patients very stressful condition which impairs their everyday lives and mental well-being (Langguth, 2011). There is no cure for tinnitus yet and the development of effective causally oriented treatment options is highly dependent on a more detailed understanding of tinnitus pathophysiology. Traditionally, tinnitus research focused on the peripheral and central auditory system (Eggermont & Roberts, 2004) but in the past years, it has shifted to a more global perspective also considering non-auditory cortical areas (Adjamian et al., 2009; De Ridder et al., 2011; Lanting et al., 2009). It has been shown that tinnitus is accompanied by alterations of functional connectivity within and between several neural networks including temporal, parietal and frontal cortices (Maudoux et al., 2012; Schlee, Hartmann, et al., 2009; Schmidt, Akrofi, Carpenter-Thompson, & Husain, 2013). It is supposed that the tinnitus reaches awareness only if there is a co-activation between the auditory cortex and a "perception network" including parietal and frontal cortices (De Ridder et al., 2011). Correlations of neural processes with clinical tinnitus data suggest that different aspects of the tinnitus percept are encoded by separable networks indeed. For instance, tinnitus loudness was shown to correlate with gamma activity in the auditory cortex (van der Loo et al., 2009) while tinnitus distress has been linked to a general distress network including the anterior cingulate cortex, the DLFPC, the insula and posterior cingulate cortex (Vanneste, Congedo, & De Ridder, 2014; Vanneste et al., 2010). It has also been shown that those networks seem to be functionally interconnected in highly distressed patients (Vanneste et al., 2014). The current study seizes the idea of this network perspective with the aim of improving rTMS treatment for chronic subjective tinnitus.

Having the auditory pathway in mind, many clinical studies have examined the effects of unilateral low-frequency rTMS of the auditory cortex as a treatment for chronic tinnitus (for a review, see Lefaucheur et al., 2014). The results of those studies are mixed and the effect size is small emphasizing the need for more effective treatment protocols. One possibility to enhance treatment effects is to increase the number of stimulated areas. In the last years, this optimization strategy has also been increasingly investigated for other neurological or psychiatric indications for rTMS treatment like major depression or Parkinson's disease. Whereas this approach revealed mixed results in depression treatment (Chen et al., 2014) the bilateral stimulation of the primary motor cortex has been shown to be superior to unilateral stimulation for the treatment of Parkinson's disease (Lefaucheur et al., 2014). The targeted extension of the stimulated areas might therefore represent a promising approach for future rTMS research and might also be useful for the treatment of chronic tinnitus.

The combined stimulation of left temporal and left frontal cortices has already been tested in tinnitus patients. Indeed, patients receiving the combined stimulation protocol showed better long-term symptom improvement than patients who had been treated with single-site temporal stimulation (Kleinjung et al., 2008). Another study indicated that the combined protocol appeared in trend to be superior, but the difference was not statistically significant (Langguth et al., 2014). Based on these results and on the knowledge about the altered functional connectivity between different networks in the tinnitus brain, a new, triple-site protocol was recently tested in a pilot, single-arm study (Lehner, Schecklmann, Poeppl, et al., 2013). This protocol added another target to the combined protocol of Kleinjung et al. (2008) resulting in three stimulation sites: bilateral low-frequency rTMS of the temporoparietal cortex plus high-frequency rTMS of the DLPFC. The triple-protocol targets the most important hubs of the tinnitus network as defined by Schlee, Hartmann et al. (2009).

In the pilot study, this protocol showed better long-term effects than a historical control group which was treated with unilateral temporal stimulation and – on a descriptive level – also better long-term effects than a historical control group treated with combined left temporal plus left DLPFC stimulation (Lehner, Schecklmann, Poeppl, et al., 2013). The current study intends to replicate the results of the pilot study in a randomized controlled trial. We determined whether the stimulation of multiple hubs of the neural networks involved in tinnitus is superior to the standard single-site stimulation protocol.

Materials and Methods

Design.

The presented data come from a two-arm randomized, double-blind parallel-group trial whose design and methods were published in detail in Lehner, Schecklmann, Kreuzer et al. (2013, 2014). The study was registered at Clinical Trials on July 23, 2012 (NCT01663324) and has been approved by the ethics committee of the University of Regensburg (10-101-0169). The study was done in accordance with the approved guidelines. All data were collected at the Department of Psychiatry and Psychotherapy, University of Regensburg between July 2012 and January 2015 (last follow-up visit).

Subjects.

The study was designed to find an interaction effect between group (single-site vs. triple-site) and time (day1, day 12). Based on our pilot data (Lehner, Schecklmann, Poeppl, et al., 2013) a small effect size of $f = 0.1$ for this interaction effect was assumed. Although small, such an effect is still an important step in tinnitus management. If the study sample size is determined to provide sufficient power (0.8) for detection of such an effect in a repeated measures ANOVA (with $\alpha = 0.05$), a total of 42 tinnitus patients have to be

examined. Due to the complex and time-consuming study design, a higher patient drop-out rate than usual was assumed. A total of 50 patients (25 per group) aged between 18 and 70 years were therefore enrolled in the study (see Table 10). One patient dropped out of the single-site stimulation group after two rTMS sessions due to an increase in tinnitus loudness. Due to this drop-out, data of 49 patients (35 male, 14 female, age 47.11 ± 12.13 years) are reported. All patients suffered from chronic subjective tinnitus with at least moderate handicap as measured with the THI (score ≥ 38, Newman et al., 1996). Tinnitus was present in all patients for at least six months. Study exclusion criteria were prior treatment with rTMS, clinical relevant unstable psychiatric, somatic or neurologic comorbidity and all standard exclusion criteria for rTMS treatment. Patients were recruited during routine clinical tinnitus consultations and via announcements in print-media and on the homepage of the tinnitus clinic at the Regensburg University. All patients gave written informed consent.

Questionnaires and outcome measures.

For the assessment of demographical and clinical characteristics patients completed the Tinnitus Sample Case History Questionnaire (Langguth et al., 2007). All questionnaires listed below were administered on the first treatment day ("day 1"), last treatment day ("day 12") and during two follow-up visits ("day 90" and "day 180"). Tinnitus severity was assessed using the THI, the TQ (Goebel & Hiller, 1994) and numeric rating scales for tinnitus loudness and annoyance (ranging from 0 = not at all loud/annoying to 10 = extremely loud/annoying). Furthermore, quality of life was measured using the WHO-QoL (World Health Organization Quality of Life) assessment. Depressive symptoms and hyperacusis were assessed using the Major Depression Inventory (MDI) and a German hyperacusis questionnaire (Geräuschüberempfindlichkeitsfragebogen, "GÜF"; Nelting & Finlayson, 2004). On day 1 and day 12, the hearing level [dB HL] was measured using pure-tone

audiometry. It is reported as an average of all thresholds measured bilaterally ranging from 125 Hz to 8 kHz. The comparison between pre and post treatment hearing level served as safety parameter. The primary outcome parameters were defined as (a) the change of tinnitus severity from day 1 to day 12 as measured by the TQ score and (b) as the number of treatment responders (as defined by a reduction of at least five points in the TQ score). The change in the remaining questionnaires over the four measurement time points (THI, MDI, GÜF, WHO-QoL), the rating scales and the treatment responders on day 90 and day 180 served as secondary outcome parameters.

rTMS treatment.

On the first treatment day, patients were randomized by random group allocation (http://www.random.org) to receive either single site or triple-site rTMS treatment. All patients received ten treatment sessions on ten consecutive working days. Non-blinded study staff assigned patients to the interventions and applied treatment. These persons were not involved in patient management, assessment or data analysis. The triple-site rTMS protocol consisted of high-frequency stimulation of the left DLPFC (20 Hz, 20 trains, 25s inter-train interval, 1000 pulses/day) followed by left temporoparietal and right temporoparietal stimulation (1 Hz, 1000 pulses/day each). The three sites were stimulated successively and always in the same order: DLPFC first, then left temporoparietal cortex and right temporoparietal cortex at the end. The single-site group was treated with 3000 pulses/day of the left temporoparietal cortex. Low-frequency rTMS of the left temporoparietal cortex has been the standard approach for rTMS tinnitus treatment during the past years(Lefaucheur et al., 2014). Both treatment groups received 3000 pulses per session at an intensity of 110% of the RMT, but – for safety reasons – never higher than 60% of the maximal stimulator output. The RMT was measured before the first treatment sessions and was defined as the minimal

intensity at which at least five of ten motor evoked potentials were 50µV in amplitude in the right abductor digiti minimi. Treatment was performed with a Medtronic MagPro Option stimulator (Medtronic, Minneapolis, MN, USA) and a 70mm figure-of-eight coil. The temporoparietal cortices were targeted using the 10-20 system by placing the coil between the temporal (T3/T4) and the parietal (P3/P4) electrode sites (Hoffman, R. E. et al., 1999; Jasper, 1958). For targeting the DLPFC, the coil was centered 6 cm anterior from the site over the motor cortex that had been used for defining the RMT.

Placebo control group.

As the goal of the study was to test superiority of the triple-site stimulation over the standard approach (temporoparietal stimulation), an active stimulation protocol was chosen as control protocol instead of a placebo stimulation, as proposed by recent reviews (Duecker & Sack, 2015; Lefaucheur et al., 2014). In order to additionally offer a descriptive comparison to placebo stimulation, data of a placebo control group from a previous rTMS study (Langguth et al., 2014) is presented. Those patients were treated with a sham-coil system (90mm outer diameter, coil MC-B70, Medtronic, Minneapolis, MN) on ten consecutive working days. The coil was localized at the auditory cortex by using a PET-guided neuronavigational system. From the 44 available placebo-datasets (Langguth et al., 2014), 25 were chosen in order to create a group which matched the triple- and single-site groups with respect to the baseline TQ score, age, gender, tinnitus laterality and tinnitus duration (see Table 10). With respect to outcome measures, only the TQ at day 1, day 12 and day 90 was available. A follow-up period of 180 days is not common in previous published trials and is thus unique for this study. As data of this group were collected earlier by different study staff and under different circumstances, they will not be submitted to

statistical analyses. They are meant to provide a qualitative reference point for the possible effects of sham stimulation.

Statistical analysis.

For statistical analyses IBM SPSS Statistics for Windows (Version 22.0, Armonk, NY: IBM Corp.) was applied. Missing values were replaced by using a LOCF procedure, if at least one measurement after rTMS was available. Patients without post rTMS measurements were not included in the analysis (drop-outs). Concerning the missing values, data of four patients had to be replaced using LOCF on day 90 and data of two patients had to be replaced on day 180. As some of the questionnaires were not filled in correctly, there were some additional missing values for specific questionnaires. Data for two patients were missing on day 12 for the MDI and the GÜF questionnaires and data of one additional patient was missing on day 90 for the THI. On day 180, data of two additional patients were missing for the TQ and data for one patient were missing for the rating scales (loudness and annoyance) and for the MDI. In order to test whether the LOCF procedure had an effect on our results, all statistical tests were done twice: for the whole dataset with LOCF and for the smaller subset of data without LOCF. All statistical tests yielded the same results when conducted without LOCF replacement of missing values.

The change of the TQ score from day 1 to day 12 (primary outcome) was tested using an ANOVA with the within-subjects factor measurement time point (day 1, day12) and the between-subjects factor group. To test for changes in tinnitus severity over all four measurement time points an ANOVA with the within-subjects factor measurement time point (day 1, day 12, day 90, day 180) and between-subjects factor group (single-site vs. triple-site stimulation) was calculated for all questionnaires. The prerequisites for use of ANOVAs were checked for all dependent variables: The homogeneity of variances was tested with Levene's

Test. The result was non-significant for all variables except for the MDI on day 12. The F_{max}-Test revealed, that an adaptation of the level of significance was not necessary (F_{max} = 2.04). The sphericity of data was checked with Mauchley Tests. In case of significant Mauchley-Tests, Greenhouse-Geisser corrections were applied.

Number of treatment responders on day 12 (primary outcome), day 90 and day 180 were compared using Chi² tests. Treatment responders were defined as patients with a reduction in the TQ score of at least five points (Adamchic et al., 2012). For safety reasons, we compared the hearing level of all patients from pre to post treatment using a paired t-test with the within-subjects factor time (day 1, day 12).

Table 10

Demographical data and clinical characteristics for both treatment groups and the placebo control group

	single-site rTMS (N = 24)	triple-site rTMS (N = 25)	placebo (N = 25)	group comparisons
age (years)	48.89 ± 10.05	45.39 ± 13.83	52.80 ±13.32	$F(2,71) = 2.18$; $p = .12$
gender	17 m, 7 f	18 m, 7 f	18 m, 7 f	$\chi^2(2,74) = .01$; $p > .99$
mean hearing threshold [dB HL]	33.79 ± 13.48	27.71 ± 10.46		$t(47) = 1.77$; $p = .083$
tinnitus laterality (r/l/l>r/r>l/both/inside head)	2/5/4/4/8/1	5/6/3/5/5/1	3/6/4/3/7/2	$p = .98$ (Fisher's Exact Test)
duration (months)	120.14 ± 118.02	103.93 ± 118.78	95.64 ± 85.46	$F(2,71) = 0.32$; $p = .73$
Questionnaire scores on day 1				
TQ	44.42 ± 16.66	45.56 ± 13.75	45.24 ± 15.90	$F(2,71) = 0.04$; $p = .97$
THI	50.17 ± 22.26	47.36 ± 17.94		$t(47) = 0.49$; $p = .63$
MDI	6.25 ± 3.97	7.68 ± 5.60		$t(47) = -1.03$; $p = .31$
GÜF (n = 47)	15.70 ± 8.40	16.54 ± 9.34		$t(45) = -0.33$; $p = .75$
WHO-QoL Domain 1	16.23 ± 2.50	15.31 ± 2.38		$t(47) = 1.32$; $p = .19$
WHO-QoL Domain 2	15.29 ± 2.19	14.13 ± 2.56		$t(47) = 1.70$; $p = .096$
WHO-QoL Domain 3	16.21 ± 2.41	15.15 ± 2.95		$t(47) = 1.38$; $p = .18$
WHO-QoL Domain 4	17.08 ± 1.54	16.45 ± 2.09		$t(47) = 1.18$; $p = .24$

Note. Mean hearing threshold (in dB HL): average of all thresholds measured bilaterally ranging from 125 Hz to 8 kHz; Tinnitus laterality is defined in categories: r: right-sided, l: left-sided, l>r: both sides but louder on the left side; r>l: both sides but louder on the right side; both: both sides; inside head: Tinnitus is perceived in the middle of/ inside the head; TQ: Tinnitus Questionnaire; THI: Tinnitus Handicap Inventory; MDI: Major Depression Inventory; GÜF: Geräuschüberempfindlichkeitsfragebogen (German Hyperacusis Questionnaire); WHO-QoL: World Health Organization-Quality of Life.

Results

Adverse events.

Both the left temporoparietal and the triple-site stimulation protocol were well tolerated by the patients. No serious adverse effects were observed. There was no significant change of the hearing level from pre to post rTMS treatment ($t(48) = -1.38$, $p = .17$). The adverse events for both treatment groups are listed in Table 11.

Statistical analysis.

Concerning the primary outcome (change in the TQ score from day 1 to day 12), the effect of measurement time point was significant ($F(1,47) = 23.97$, $p < .001$) with the TQ score decreasing from 45.00 (± 15.10) to 40.41 (± 15.61). The effect of group was not significant ($F(1, 47) = 0.06$, $p = .80$) and there was no significant interaction effect between measurement time point and group for the change in the TQ score from day 1 to day 12 ($F(1, 47) = 0.003$, $p = .96$). Furthermore, there was no significant difference between groups in the responder rates on day 12 (10 responders in each group, $\chi 2(1, N = 49) = 0.01$, $p = .91$). Concerning the secondary outcome measures (ANOVAs comparing all four measurement time points for all questionnaires) significant effects of measurement time point were observed for the TQ (see Figure 6), the THI and the rating scale "annoyance" (see Table 12). The measurement time point effect for the rating scale "loudness" was marginally significant. For the TQ, post-hoc t-tests revealed significant differences from day 1 to day 12 ($t(48) = 4.94$, $p < .001$), from day 1 to day 90 ($t(48) = 2.26$; $p = .029$) and to day 180 ($t(48) = 2.67$, $p = .010$). The same differences were significant for the THI (day 1 to day 12: $t(48) = 3.13$, $p = .003$; day 1 to day 90: $t(48) = 3.00$, $p = .004$; day 1 to day 180: $t(48) = 2.89$, $p = .006$) and the rating scale "annoyance" (day 1 to day 12: $t(48) = 2.11$, $p = .040$; to day 90 $t(48) = 2.40$, $p = .20$; to day 180: $t(48) = 2.31$, $p = .025$). No significant effects of group were

observed (see Table 12). The interaction effects measurement time point x group were not significant either. There was no significant difference between groups in the responder rates on day 90 (9 responders in the single-site group, 13 responders in the triple-site group, $\chi 2(1, N = 49) = 1.04, p = .31$) or on day 180 (10 responders in the single-site group, 14 responders in the triple-site group, $\chi 2(1, N = 49) = 1.01, p = .32$).

Descriptive comparison with the placebo control group.

In Figure 6, the change of the TQ score from day 1 to all subsequent measurement time points is shown for all three groups separately with negative values indicating a reduction of tinnitus severity. For the placebo group, only data for day 12 and day 90 were available. On a descriptive level, both study groups show more reduction of the TQ score than the placebo group on day 12. On day 90, the triple-site group shows the most pronounced reduction of the TQ, followed by the single-site group. Please note that the group x measurement time point interaction effect was not significant for the two treatment groups. For the placebo group nearly no change of the TQ score was visible on day 90.

Table 11

Adverse events for both treatment groups

	single-site rTMS	triple-site rTMS
transient adverse events	-	-
muscular tension	1	-
headache	6	3
blurred vision	1	-
increase in tinnitus loudness	3	-
mood swings	1	-
dizziness	-	1
feeling of heaviness in the legs	-	1
ongoing adverse events	-	-
increase in tinnitus loudness	3*	-
broadening of the frequency range of the tinnitus	-	1

Note. * One of those three patients dropped out after two days of treatment.

Table 12

Results from repeated measures analyses of variance

	main effect: measurement time point			main effect: group			interaction effect: measurement time point x group		
	F (*df*)	*p*	Eta^2	*F* (*df*)	*p*	Eta^2	*F* (*df*)	*p*	Eta^2
TQ	*F*(2.23, 104.96) = 4.94	.007*	0.094	*F*(1, 47) = 0.003	.95	>0.001	*F*(2.23, 104.96) = 0.66	.54	0.013
THI	*F*(2.48, 116.33) =5.02	.005*	0.095	*F*(1, 47) = 0.55	.46	0.012	*F*(2.48, 116.33) =1.09	.35	0.021
MDI	*F*(2.49, 116.83) = 0.92	.43	0.019	*F*(1, 47) = 0.46	.50	0.10	*F*(2.49, 116.83) = 1.14	.33	0.023
GÜF	*F*(2.34, 105.26) = 1.99	.13	0.041	*F*(1, 45) = 0.004	.95	> 0.001	*F*(2.34, 105.26) = 1.33	.27	0.027
loudness	*F*(2.20, 103.58) = 2.38	.092#	0.048	*F*(1, 47) = 0.02	.89	>.001	*F*(2.20, 103.58) = 0.39	.70	0.008
annoyance	*F*(2.50, 117.28) = 3.17	.035*	0.063	*F*(1, 47) = 0.52	.48	0.011	*F*(2.50, 117.28) = 0.40	.72	0.008
WHO-QoL									
domain 1	*F*(3, 141) = 0.78	.51	0.016	*F*(1, 47) = 0.94	.34	0.020	*F*(3, 141) = 0.70	.56	0.014
domain 2	*F*(2.48, 116.66) = 0.16	.89	0.003	*F*(1, 47) = 1.15	.29	0.024	*F*(2.48, 116.66) = 1.78	.17	0.036
domain 3	*F*(2.28, 107.21) = 0.63	.60	0.013	*F*(1, 47) = 2.33	.13	0.047	*F*(2.28, 107.21) = 0.14	.89	0.003
domain 4	*F*(2.58, 121.37) = 0.65	.58	0.013	*F*(1, 47) = 1.24	.27	0.026	*F*(2.58, 121.37) = 0.40	.59	0.012

Note. *$\alpha \leq .05$; # $\alpha < .10$.

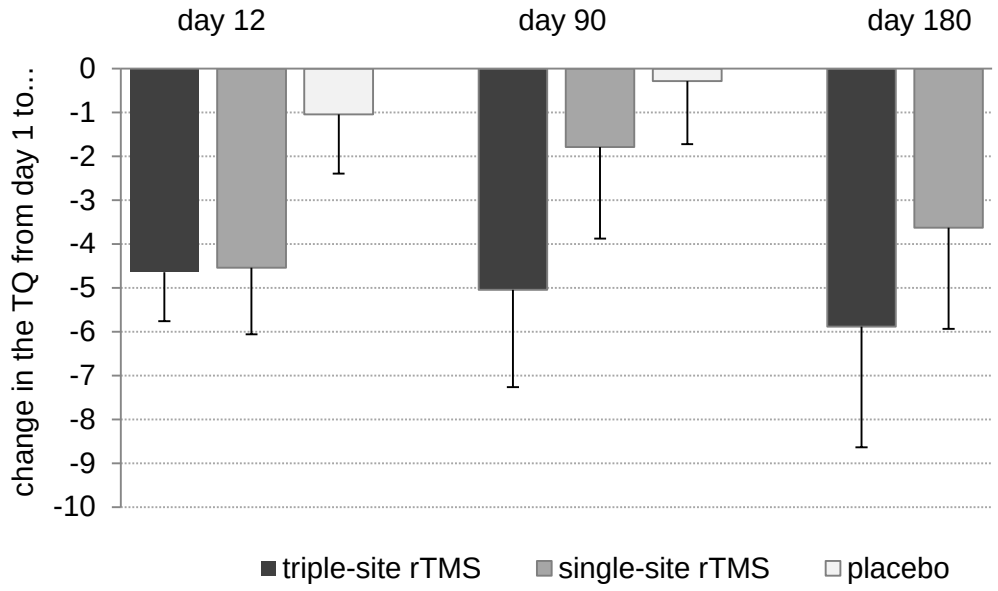

Figure 6. Reduction in the TQ sum score from day 1 to all subsequent measurement time points (for the placebo group only data for day 12 and day 90 were available). Error bars represent standard errors.

Discussion

Recent studies have suggested that alterations of the connectivity between and within widespread neural networks including frontal, parietal and temporal areas are associated with chronic tinnitus (De Ridder et al., 2011, 2014; Schlee, Hartmann, et al., 2009; Vanneste et al., 2014). The current study aimed to use this knowledge about tinnitus pathophysiology for a new treatment option by stimulating three central hubs of these neural networks involved in tinnitus. Results indicate that both the single-site and the triple-site protocols led to a significant reduction of tinnitus severity which emphasizes the potential of rTMS for the treatment of tinnitus. However, the superiority of the triple-site protocol was modest at best (Figure 6) and the effect sizes were small (see Table 12). At first glance these results do not agree with an earlier study from our group (Lehner, Schecklmann, Poeppl, et al., 2013). On a descriptive level however, the present results resemble those of the pilot study and a superiority of the triple-site stimulation can be observed 90 days (see Figure 6) and in trend 180 days after rTMS. The single-site group reported a reduction in tinnitus severity on day 90. This matches exactly what was observed in the pilot study. One possible reason for the lack of statistical significance of the current results in comparison to the pilot data might be that data of the pilot study were not matched with respect to the number of applied pulses. In the pilot study the triple-site group received 4000 pulses per session, the single-site group received only 2000 rTMS pulses per session. There is evidence that treatment with more pulses results in a more pronounced effect both for the treatment of depression (Gershon, Dannon, & Grunhaus, 2003) and the treatment of tinnitus (Plewnia et al., 2007). Therefore, the higher dose of the triple-site stimulation might have contributed to its superiority in the pilot study. As the number of pulses was kept constant in the current study design, this lacking dose-effect might be one reason for the unexpected non-significant outcome. This makes clear that future studies investigating multisite stimulation should take the number of

pulses into account. If multi-site stimulation involves a higher number of pulses, a possible superiority of multi-site stimulation could be simply the consequence of a higher dose.

Moreover the relative small sample sizes of our study for detecting a differential effect of two active protocols has to be considered in the interpretation of data. The observed effect size of $Eta^2 = 0.013$ for the interaction effect between measurement time point and group concerning the TQ is small but might still be in a range of clinical relevance. Although tiny, this effect suggests that there might be some advantage of multisite protocols to evoke a more sustained reduction of tinnitus severity.

The tendency towards a better, albeit modest, long-term effect of the triple-site protocol, which was observed in the current study, is in line with other studies that administered combined treatment protocols (Kleinjung et al., 2008; Kreuzer et al., 2011) and indicates the potential of the concept to stimulate multiple sites of a pathologically altered brain network. The idea of stimulating several hubs of the neural networks involved in tinnitus can and should encourage new concepts of multisite-treatment protocols. There are diverse variables which can be varied in future protocols: How many areas should be stimulated in which frequency and in which order? We chose to stimulate all patients in the triple-site group with the same stimulation sequence (first DLPFC, then left and right temporoparietal cortex) in order to stick to the protocol of the pilot study (Lehner, Schecklmann, Poeppl, et al., 2013) and in order to be able to use a sample size small enough to enable us to also include EEG and fMRI measurements (Lehner, Schecklmann, Kreuzer, et al., 2013). Future studies could randomize the order of the stimulated sites in order do find out which sequence of stimulated areas might be most effective. Moreover, it might be more effective if stimulation sites were not treated successively but simultaneously or with a particular timing between the magnetic pulses over different stimulation sites. More knowledge about tinnitus pathophysiology is needed to define treatment protocols which are

able to effectively interfere with the tinnitus-specific alterations. Recent studies already provide important information for potential future treatment protocols by presenting increasingly refined knowledge about the neural networks involved in the tinnitus percept. While the current study was motivated by the finding of frequency-specific changes of functional connectivity between temporal, parietal, frontal and cingulate cortices in tinnitus patients (Schlee, Hartmann, et al., 2009), more recent studies define separate distress and loudness networks with e.g. increased electroencephalographic alpha activity in prefrontal areas and increased beta activity in the dorsal anterior cingulate cortex (Vanneste et al., 2014). However, the results of such studies are still mixed with respect to the network hubs considered to be important and the frequencies with which alterations of connectivity can be perceived. Combining treatment studies with brain imaging can help to specify in more detail which changes of functional connectivity are correlated with treatment response and should therefore be targeted (Langguth et al., 2012). Another promising approach to improve (multi-site) rTMS treatment is customizing brain stimulation to each patient. As tinnitus is a heterogeneous condition the information which neural networks are altered in the tinnitus brain in general may be less relevant than the alterations which are present in the individual tinnitus patient at the very moment we intend to apply rTMS treatment. It is well-known that the effect of rTMS is dependent on the status of the brain at the time the stimulus is applied (Siebner et al., 2009). It might be therefore a promising task for future studies to identify the optimal treatment protocol for each patient and eventually also for each treatment session separately. A further approach to improve rTMS treatment might be related to increases of the dosage of rTMS. This can be done either by increasing the applied pulses per day or the number of treated days. Here, we stimulated with 3000 pulses per day showing remarkable changes in tinnitus distress which were higher in comparison to a recent meta-analysis (Soleimani et al., 2015) and a retrospective analysis of over 500 patients (Lehner et al., 2012),

where a lower number of pulses per day was used (Soleimani et al., 2015). A higher number of treatment sessions is common in the rTMS treatment of patients suffering from major depression. In these patients, rTMS treatment for four to eight weeks (George & Post, 2011; George, Taylor, & Short, 2013) has been approved by the Food and Drug Administration in the United States and may also represent a promising approach in improving treatment effects in patients with tinnitus.

Conclusions

We report a tendency towards a modest, sustained long-term effect of the triple-site stimulation protocol in comparison to the single-site protocol. This descriptive advantage shows that innovative treatment protocols carry potential for a more effective treatment of subjective tinnitus. Future work could aspire to apply novel protocols based on emerging knowledge about tinnitus pathophysiology and, above all, about the individual tinnitus brain.

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Contributions

BL and MS conceived the idea of the study. BL, MS and AL contributed to designing of the trial. AL collected and analysed the data. BL, MS, MWG and RR supervised the study. AL drafted the manuscript. All authors contributed to the interpretation of the results. All authors approved the final version.

Additional Information

The authors declare no competing financial interests with respect to the study.

Concluding Discussion

Methodical considerations

Study design.

Study 1 was not an experimental study but a pooled, retrospective analysis of longitudinal imaging data of tinnitus patients who underwent MRI before and after the treatment phase of 10 sessions of rTMS. Up to now, this is the only study that examined the effect of rTMS treatment with respect to GM changes in tinnitus patients. Due to its longitudinal character, every patient acted as his own control which is strongly recommended for studies measuring brain volume (Steen, Hamer, & Lieberman, 2007). The main limitation of the study design used is the lack of a placebo-control group. It has already been addressed in the discussion of study 1 that this limitation considerably restricts the interpretation of the results as it cannot be excluded that the perceived GM changes were due to unspecific effects independent of rTMS treatment. Furthermore, the correlation of TQ difference with the baseline GM volume in the lingual gyrus and parts of the frontal gyrus might also be independent of rTMS effects. Maybe, GM volume in these structures is not a predictor for rTMS outcome but a predictor for an improvement of tinnitus severity independent of any intervention. A special characteristic of study 1, which should also be mentioned, is that data coming from different clinical trials were pooled. The main reason for using pooled data was, that the effects of low-frequency rTMS are known to be rather small (Meng et al., 2011) and hence the GM changes were also expected to be small. Therefore, a large sample size was considered to be of particular importance for the current study. Being able to present data of a large sample of 77 patients some of which were scanned three times was only possible by aggregating data of different trials. Although this kind of analysis does not meet the high standard of a randomized controlled trial, the pooling of imaging data is considered an important next step in neuroimaging research (Poline et al., 2012). Large neuroimaging

databases have been formed within the last years e.g. for schizophrenia research (Wang et al., 2016) or for Alzheimer disease (Neu, Crawford, & Toga, 2016). Indeed, an international workgroup of researchers has been formed (COST TINNET Workgroup 3; http://tinnet.tinnitusresearch.net/index.php/2015-10-29-10-22-16/wg-3-neuroimaging) that is trying to establish an international database for imaging data of tinnitus patients as well. Therefore, pooled analyses can be considered an acceptable and important way to collect imaging data now and in the future. Moreover, there is longitudinal imaging data waiting to be analysed which was collected in the course of study 3. These data were collected in the course of a randomized controlled trial and is eligible to replicate the results of the pooled analysis of study 1.

Study 2 was a one-arm open-label study in which the triple-site treatment protocol was explored for the very first time. The study focused on the question whether the new protocol was safe, feasible in the clinical routine and tolerable. This pilot study was done in order to find out if the protocol should be investigated in a more elaborate controlled trial or if the concept of triple-site stimulation should be rejected. As Landgrebe et al. stated "only 'promising' interventions justify the performance of a RCT [randomized controlled trial]. The choice of a promising intervention is difficult and can be based on clinical pilot data (…)" (Landgrebe et al., 2012, p. 5). In order to provide a rough estimate of the new stimulation protocol's effectiveness, the pilot data were compared to data of a control group from a previous trial which was stimulated with the standard low-frequency stimulation protocol. This approach entailed some confounding factors which have already been addressed in the discussion of study 2 but should again be mentioned for the sake of completeness: First, the number of TMS pulses was not kept constant in this pilot study. While the control group was treated with 2000 pulses per session, the triple-site group received 4000 pulses per session. As the effect of rTMS is known to be dose-dependent (Plewnia et al., 2007), it is important to

apply the identical number of pulses in cases where two treatment protocols are compared. Furthermore, many patients in study 2 were medicated with centrally acting drugs which – even if held constant during the rTMS treatment phase – might have influenced the rTMS treatment effect. The conclusions drawn from study 2 should therefore be considered preliminary as systematic biases cannot be excluded.

As study 3 was done in order to replicate the results of study 2, particular attention was paid to those limitations. Study 3 was designed as two-arm parallel-group trial with randomized group allocation. Both treatment groups received the same number of pulses per session. Furthermore, patients could only be included if they were not taking centrally acting drugs. Still, this study does not meet the gold standard of a randomized, placebo-controlled trial as no placebo control group was chosen. As has already been addressed in the discussion of study 3, this was done because the intention of the trial was to find out whether the triple-site protocol was superior to the standard protocol used in tinnitus treatment (Lehner, Schecklmann, Kreuzer, et al., 2013, 2014). It is indisputable, of course, that placebo-controlled trials are clearly needed to control for non-specific effects such as patient expectation or spontaneous improvement (Landgrebe et al., 2012). However, a placebo condition complicates patient recruitment, especially if rTMS is provided as outpatient treatment. This is the reason why placebo-controlled trials often require large and expensive multi-center studies in order to be able to obtain sufficiently large patient samples (e.g. Landgrebe et al., 2008). Considering that there are countless possibilities of how rTMS treatment effects might be enhanced (see discussion below) and that many possible protocols wait to be tested, it becomes clear that small, less costly trials are necessary in order to find out which treatment protocols are promising enough to initiate an expensive placebo-controlled trial.

Another disadvantage of an actively controlled trial is that it is more difficult to find a significant difference to a treatment group than to a placebo group. However, if the goal of a study is to improve rTMS treatment, the new treatment protocol should be superior to the already existing standard stimulation protocol and therefore this standard protocol should be chosen as control condition. Although conservative, this is what was done in study 3. A final shortcoming of the control group is that the triple- and single-site protocols differed in more than one parameter. While the number of pulses was kept constant, the number of stimulation targets and the frequency of stimulation were (partly) different. Therefore, all those parameters could have contributed to the tendency of a better long-term effect of the triple-site stimulation protocol. In order to disentangle those effects a step-wise approach would be necessary in which only one parameter is changed at a time.

Study samples.

As tinnitus is a very heterogeneous condition with respect to its causes, clinical characteristics (Langguth, Kleinjung, & Landgrebe, 2011) and comorbid symptoms such as depression, anxiety (Langguth, Landgrebe, Kleinjung, Strutz, & Hajak, 2010), sleep disturbances (Schecklmann et al., 2015) or hyperacusis (Schecklmann et al., 2014), a thorough clinical diagnosis of the individual patient is necessary to ensure optimal care. It has to be determined whether the patient's tinnitus is objective or subjective, whether it has a pulsatile character and whether there are causes which might benefit from special treatment options like hearing aids, cochlear implants or microvascular decompression (Langguth et al., 2013). All patients treated in the course of this thesis underwent an in-depth diagnostic procedure including audiological measurements, examination by an otorhinolaryngologist, psychiatrist and other specialists if necessary. Only if tinnitus was subjective and if no clear

indication for a specialized treatment was present, patients were possible candidates for treatment with rTMS.

Recruitment and rTMS treatment of all patients was done in a multidisciplinary tinnitus centre offering outpatient care. Usually, patients have to wait several months for their first appointment in the clinic and in many cases those patients have already undergone many different treatment attempts before consulting the tinnitus clinic. Therefore, patient selection is clearly biased towards patients who have had their tinnitus for a very long time, who feel highly distressed and who have shown to be treatment-resistant to many other treatment attempts. Therefore, the current results may not generalize to other patient populations.

Outcome measurement.

In order to be able to define treatment outcomes in clinical trials, valid metric variables are needed which are sensitive to change. The term "subjective tinnitus" implies that there is no objective measurement of the phantom sound. Every assessment is dependent on the patient's ability to verbally describe his or her tinnitus and to perceive whether it has changed during a therapeutic intervention. Therefore, there is an ongoing debate about optimal outcome measures for clinical trials in tinnitus. There are several characteristics which can be measured (e.g. tinnitus distress, loudness, minimal masking level) and also different possibilities of how these characteristics can be measured (e.g. using different questionnaires). Recently, an international initiative was founded in order to define an international standard of outcome measurement (Hall et al., 2015). For the current thesis, two outcome measures were chosen which were used in each of the three studies reported and therefore providing comparability between the presented results: tinnitus distress and tinnitus loudness. Tinnitus distress was quantified using the German version of the TQ (Goebel & Hiller, 1994; Hallam et al., 1988). As the internal consistency of the TQ is very high (r = 94)

and the questionnaire is supposed to be sensitive to change, it was chosen as the primary outcome measure in all three studies. The TQ comprises 52 items. The sum score ranges from zero to 84 and can be used to assess tinnitus severity with higher scores indicating more pronounced tinnitus severity. Changes of at least five points in the TQ are thought to be of clinical relevance (Adamchic et al., 2012). Therefore, patients were defined as treatment responders in all three presented studies if they had improved at least five points in the TQ. The subjective loudness of the tinnitus was also measured in each of the studies using a rating scale ("How strong or loud is tinnitus at present?") ranging from 0 ("not at all") to 10 ("extremely strong or loud").

Depending on the aim of the three studies, some additional outcome parameters were used. As study 2 was a pilot study, four additional rating scales were measured in order to cover extra dimensions of tinnitus which might reflect treatment response to the innovative treatment protocol. All of those rating scales seemed to measure the same trend in study 2 (please see Figure 4). Therefore, only tinnitus loudness was reported in study 3.

In addition to the TQ, the German version of the THI was additionally used to measure tinnitus severity in both study 2 and 3 (Kleinjung, Fischer, et al., 2007; Newman et al., 1996). The THI is an internationally and widely used questionnaire for tinnitus distress and thus enables the results to be put in context of worldwide clinical trials. This was considered to be of particular importance for a newly developed treatment protocol like the triple-site protocol. The THI is comprised of 25 items. The sum score ranges from zero to 100, again with higher scores indicating more tinnitus distress.

Finally, a quality of life assessment was added as outcome measure to study 3 as we hypothesized that an improvement of tinnitus should be reflected in patients' daily lives. As a reliable, valid and open-access questionnaire, the WHO-QoL was considered to be suitable for quantifying this supposed change in quality of life (O'Carroll, Smith, Couston, Cossar, &

Hayes, 2000; Skevington, Lotfy, & O'Connell, 2004). The WHO-QoL measures four domains of quality of life: physical health, psychological health, social relationships and environment. As no changes in any of those domains were seen in study 3, it can be supposed that treatment effects appear to have been too weak to induce measurable quality of life changes.

Hearing loss as confounding factor.

With hearing loss being probably the most important risk factor for tinnitus (Axelsson & Ringdahl, 1989) it is a very important confounding factor in tinnitus research. Hearing loss itself is supposed to lead to e.g. plastic changes and hyperactivity in the central auditory pathway (Salvi, Wang, & Ding, 2000; Zhao, Song, Li, & Li, 2016). It is therefore a challenge to disentangle hearing loss- and tinnitus-related effects when trying to understand tinnitus pathophysiology. Consequently, one important limitation of study 1 is that the mean hearing threshold could not be included in the GM analyses as it was not available for all patients.

Differential GM changes due to hearing loss and tinnitus have indeed been described (Husain et al., 2011). As study 1 was a longitudinal study in which every patient acted as his own control, it can be supposed that the hearing threshold was most probably stable for the period of 10 days (from before to after rTMS treatment). Therefore, hearing loss should have had no influence on GM changes. Furthermore, hearing loss does not seem to be correlated with treatment response (Lehner et al., 2012). Nonetheless, it cannot be excluded that hearing loss may represent a confounding factor for GM changes due to rTMS. In the course of study 3, all patients underwent both pure tone audiometry and magnetic resonance imaging (Lehner, Schecklmann, Kreuzer, et al., 2013). The imaging data have not been analysed yet but will be helpful in the process of disentangling rTMS induced GM changes in patients with and without hearing loss.

rTMS as a treatment tool for chronic subjective tinnitus

Summary: contribution of the current thesis to tinnitus research.

rTMS treatment protocols consist of many parameters all of which can be changed in order to enhance treatment effects: coil localization, frequency, stimulation intensity, number of pulses, number of treatment sessions and a countless amount of possible combinations of multiple stimulation targets with variable stimulation order. In the current thesis, two different approaches have been used to optimize rTMS treatment effects in tinnitus patients.

The goal of study 1 (Lehner, Langguth, et al., 2014) was to gain more knowledge concerning structural brain changes underlying rTMS treatment effects and to identify potential predictors for treatment outcome. With respect to the first question, no correlations between GM changes and changes in tinnitus severity were observed. With respect to the second question it was shown, however, that GM volume might be useful as a predictor for treatment outcome. This result is of particular interest as before study 1, we conducted another analysis in which we tried vainly to identify demographical or clinical characteristics which were able to predict treatment outcome (Lehner et al., 2012). Therefore, neural predictors such as GM volume are clearly needed. In study 2 (Lehner, Schecklmann, Poeppl, et al., 2013) and study 3 (Lehner, Schecklmann, Greenlee, Rupprecht, & Langguth, 2016), an innovative triple-site stimulation protocol was examined for the very first time in order to find out whether this protocol was safe and feasible and whether it was superior to the standard single-site stimulation protocol. While a superiority of the new protocol was indicated in the pilot study (study 2), this result could not be replicated on a statistically significant level in a randomized controlled trial (study 3). However, on a descriptive level, a better long-term effect of the triple-site protocol was observed. These results indicate that a higher number of stimulation targets alone may not to be sufficient to significantly disrupt the altered neural networks involved in tinnitus and to enhance long-term treatment effects. In the

following paragraphs, these results are discussed in a broader scientific context and implications for future research will be made.

Study 1: VBM to measure and predict rTMS treatment effects.

The results of study 1 indicate that changes of GM volume do not correlate with changes in tinnitus severity induced by rTMS treatment. Therefore, it was concluded that VBM might not be sensitive enough to detect neural mechanisms which are related to treatment response (Lehner, Langguth, et al., 2014). A region of interest (ROI) approach instead of whole brain analyses might have yielded more promising results. A study by Furtado et al. (2013) investigated the relation of brain volume and rTMS treatment effects in patients suffering from major depression using a ROI approach. This study reported an association between the antidepressant response and volume changes of amygdala and hippocampus. However, the antidepressant effects in that study were larger than the effects on tinnitus severity observed in study 1 and the correlation with amygdala volume was reported to be only near significant (Furtado et al., 2013). Most likely, larger treatment effects are necessary in order to be represented by GM volume changes which are large enough to be measurable using VBM. Therefore, the rTMS effects on tinnitus severity might still be too small to induce detectable GM changes.

When it comes to the question whether pre-treatment GM volume may be a predictor for treatment response, the results of study 1 are more promising with GM volume in the frontal cortex and the lingual gyrus being correlated with treatment outcome. Although this is the only study addressing this question in tinnitus patients so far, very recent studies in post-stroke patients and patients suffering from auditory hallucinations have also reported relations between brain volume at baseline and treatment outcome. It was shown, for example, that white matter volume in the cortical region just below the TMS coil predicted

the response to a motor learning task in post-stroke patients (Brodie, Borich, & Boyd, 2014) and that in responders to rTMS treatment, there was better pre-treatment volume preservation of parts of the internal capsule (Carey et al., 2014). Nathou et al. (2015) found that GM density and scalp-to-cortex distance in the area below the TMS coil and also in the primary hand motor cortex were predictors for treatment effects in patients suffering from auditory hallucinations. Consequently, it might be promising for future studies to investigate GM volume in general and GM volume of the region below the TMS-coil in particular as potential predictor for treatment outcome using ROI analyses. The longitudinal imaging data which was collected in the course of study 3 (Lehner, Schecklmann, Kreuzer, et al., 2013) is waiting to be analysed. In these data, different TMS targets were used. ROI analyses of the baseline GM volumes in the target areas will be able to add knowledge concerning this issue.

Study 2 and 3: reasons for high inter-individual variability.

The high inter-individual variability of rTMS effects which have been observed in tinnitus patients (Lefaucheur et al., 2014) have also been reported in other contexts (Dayan et al., 2013) and are most likely due to the complex mechanisms in the brain that underlie rTMS treatment effects. As has already been mentioned in the introduction, TMS is state-dependent: A magnetic pulse is assumed to have a differential effect on the cortex depending on its activational state (Dayan et al., 2013; Siebner et al., 2009). Although high-frequency rTMS has been reported to exert excitatory effects and low-frequency rTMS to induce inhibitory effects (Dayan et al., 2013), it is debatable, whether this classification does accurately reflect reality and whether these effects which were observed stimulating the motor cortex can be generalized to other cortical areas. As far as the temporal cortex is concerned, there is evidence that a more complex interaction between the activational state of the stimulated area and the frequency of TMS pulses has to be considered to explain rTMS effects (Weisz et al.,

2012). Furthermore, morphological differences such as the scalp-to-cortex distance have been shown to affect the resultant stimulation intensity that reaches the cortex (Stokes et al., 2005). Hence, even in healthy humans, there are numerous variables which have an impact on a TMS pulse's effect on the brain and which contribute to the variable outcome which the apparently same rTMS protocol produces in different individuals.

In contrast to the healthy brain, there are even more variables which may alter the effect of TMS pulses in tinnitus patients. Tinnitus is a heterogeneous symptom with multiple possible causes and diverse clinical characteristics (Langguth et al., 2013) all of which might have a differential effect on brain structure and function. This heterogeneity of tinnitus patients may be also reflected by the heterogeneous treatment outcome. Patients with a specific type of tinnitus might respond to rTMS in general or to triple-site rTMS in particular while others do not. To date, there are no clinical, demographical (Lehner et al., 2012) or even neural predictors for rTMS outcome. Thus, no tinnitus-specific inclusion or exclusion criteria were available for the studies presented in this thesis. Tinnitus patients with diverse tinnitus characteristics were included. It is therefore not surprising that the triple-site protocol suffers from the same high inter-individual variability of treatment effects which has already been observed in former studies. The clinical relevant change of five points in the TQ sum score (Adamchic et al., 2012) was only reached in 50% (study 2) and 56% (study 3) of patients if measured after the follow-up period of 90 days. Albeit innovative, the triple network-stimulation might only be effective for certain tinnitus patients whereas others might have benefited from slightly different stimulation parameters.

Future directions to optimize rTMS treatment.

Besides finding predictors for treatment outcome or examining innovative treatment protocols, there are some additional possible parameters in rTMS treatment which can be

varied in order to improve treatment effects – some of which might be very promising and are therefore worth mentioning. One parameter which has rarely been investigated is the number of treatment courses. Usually, rTMS treatment consists of five to ten treatment sessions (Lefaucheur et al., 2014) and then stops. In parallel to study 3, we analysed data of patients who underwent at least two rTMS treatment courses in order to find out whether a repetition of rTMS treatment might help to maintain or even enhance treatment effects (Lehner, et al., 2015). The results revealed that both the first and the second treatment course resulted in a significant improvement of tinnitus severity especially in patients who worsened in between the two treatment courses. Therefore, the repetition of rTMS might be a good tool to maintain treatment effects.

Furthermore, a higher number of sessions per treatment course might also be promising to enhance treatment effects. In patients suffering from major depression, 15 to 20 sessions per rTMS treatment are common practice and quite effective as "the efficacy of HF [high-frequency] rTMS of the left DLPFC in depression is definite" (Lefaucheur et al., 2014, p. 2178). It would therefore be a reasoned next step to schedule more than 10 sessions in the treatment of tinnitus patients as well – especially because of the known dose-dependency of rTMS in tinnitus patients (Plewnia et al., 2007). In order to investigate whether more treatment sessions lead to better treatment success, we are currently collecting data for a study which compares the effects of 10 versus 20 treatment sessions.

While in past studies, it was mostly the impact of parameters such as frequency or stimulation target which was investigated (e.g. Khedr et al., 2008, 2010), a different type of coil might also help to optimize treatment effects. For all studies of the current thesis, the standard figure-of-eight coil was used. Recently, a new coil, the "double cone coil" was invented which is said to have an expanded stimulation depth and is therefore able to stimulate deeper cortical areas (Hayward et al., 2007). As deeper brain structures such as the

anterior cingulate cortex have been shown to be involved in tinnitus distress (Vanneste et al., 2010), this new coil might turn out to be a precious tool in tinnitus treatment. To date, studies using this coil for the treatment of tinnitus are rare and results are mixed. Just as for the figure-of-eight coil, the effects of the double-cone-coil on either the parietal (Vanneste, van der Loo, Plazier, & De Ridder, 2012) or the frontal cortex (Vanneste, Plazier, Van de Heyning, & De Ridder, 2011) seem to be dependent of stimulation frequency and number of sessions (Vanneste & De Ridder, 2013). In parallel to study 3, we collected data to compare the effect of two combined treatment protocols: left temporoparietal stimulation plus either left DLPFC stimulation with the standard figure-of-eight coil or medial frontal stimulation with the new double-cone-coil (Kreuzer et al., 2015). In this study, no superiority of stimulation with the new coil was observed. It will be a challenge for future research to find the optimal stimulation target and frequency for the double-cone-coil in order to benefit from its ability to reach deeper brain structures.

Of course, an optimization of stimulation protocols does still hold much potential to enhance treatment effects. To this end, more refined knowledge about the pathophysiology of tinnitus will be mandatory. The design of the triple-site treatment protocol was based on the concept of a tinnitus network as proposed by Schlee, Hartmann et al. (2009). Although the idea that tinnitus is the result of activity in, and connectivity between, multiple cortical networks might still hold true, the different networks might not be of equal importance in different tinnitus patients. For future "network stimulation protocols" it will be of relevance to find out, whether other network hubs than those in the triple-site protocol should be targeted to interrupt the altered (network) activity. For instance, besides the already described subnetworks which may contribute to tinnitus generation and maintenance (De Ridder et al., 2011), recent findings concerning the chronification of pain might provide an important hint for future treatment protocols. It has been shown that an initially greater functional

connectivity between the prefrontal cortex and the nucleus accumbens predicts whether pain persisted or recovered (Baliki et al., 2012). As tinnitus and chronic pain are known to be similar (De Ridder et al., 2011; Moller, 1997), those mechanisms might also play a role in tinnitus. If this is the case, treatment protocols targeting the limbic system could help to prevent the chronification of the phantom sound. The observed tendency towards a better long-term effect of the triple-site protocol could also be explained by this limbic involvement. High-frequency stimulation of the DLPFC has been shown to induce activity changes in the basal ganglia (Speer et al., 2000) which might have led to longer lasting treatment effects if the circuitry involved in the chronification of tinnitus was manipulated. This is mere speculation though and further studies are needed to find out whether a more directed stimulation of the basal ganglia (e.g. by using the double-cone-coil) leads to better treatment effects and whether the corticostriatal connectivity plays a role in the chronification of tinnitus at all.

Another approach to optimize simulation protocols might be the simultaneous rather than the successive stimulation of different network hubs. Recently, a multi-channel stimulator was presented which is able to stimulate different targets with different treatment protocols simultaneously (Roth, Levkovitz, Pell, Ankry, & Zangen, 2014). Using such a stimulator, a simultaneous triple-site stimulation would be possible where the exact timing of pulses between e.g. the temporoparietal cortex and the DLPFC would be an interesting parameter to investigate.

Finally, an important optimization approach will be the identification of predictors for treatment response. The results of study 1 indicate that there might be certain characteristics in the brain which define whether a patient will benefit from rTMS treatment. Furthermore, the above mentioned study investigating repeated rTMS courses found that patients who responded to the first treatment course did not necessarily respond to the second treatment

course and the same was true for nonresponders (Lehner et al., 2015). Therefore, there do not seem to be hard categories such as "rTMS responders" per se but this preparedness to respond to rTMS might change over time – an assumption which is in line with the known state-dependent effect of rTMS (Dayan et al., 2013). This also explains why it is difficult to find clinical or demographical variables which predict treatment response: Probably, it is not those variables which are important but rather the effect they have on brain activity and this effect might be different in every tinnitus patient and every point in time. Methods which are able to measure the "state" a tinnitus brain is in – such as fMRI or EEG – might help to identify state-dependent predictors for rTMS response even better than e.g. VBM analyses. In the course of study 3, longitudinal EEG and fMRI data were collected. Maybe, these data will be able to provide some knowledge about which activity patterns predict a response to single- or triple-site rTMS treatment. All in all, a more individualized treatment approach which adapts rTMS treatment to an individual's patient needs is a promising albeit distant goal for future research. This approach will need to take tinnitus-specific and also tinnitus-unspecific morphological and activational brain differences into account. Tailoring rTMS protocols to the state of an individual's brain at a specific point in time with having a specific "target state" in mind would therefore be the ultimate goal for rTMS treatment – both in tinnitus patients and in other psychiatric and neurological symptoms. A very important step towards this goal is to find out, which patients benefit from which protocol and why.

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