Electric Field Navigated Transcranial Magnetic Stimulation for Chronic Tinnitus: A Randomized, Placebo-Controlled Study

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Abbreviations:

BDI	Beck's Depression Inventory	rTMS	Repetitive transcranial magnetic stimulation
dB	Decibel	STG	Superior temporal gyrus
GIC	Global Impression of Change	THI	Tinnitus Handicap Inventory
E-field	Electric field	TMS	Transcranial magnetic stimulation
Hz	Hertz	VAS	Visual Analog Scale
JSEQ	Jenkins Sleep Evaluation Questionnaire	V/m	Volts per meter (E-field strength)
MRI	Magnetic resonance image		
NRS	Numeric Rating Scale		
PTA	Pure tone average		
RCT	Randomized controlled trial		
RMT	Resting motor threshold		

ABSTRACT

Objective: Repetitive transcranial magnetic stimulation (rTMS) may alleviate tinnitus. We evaluated effects of electric field (E-field) navigated rTMS targeted according to tinnitus pitch. No controlled studies have investigated anatomically accurate E-field-rTMS for tinnitus.

Design: Effects of E-field-rTMS were evaluated in a prospective randomized placebo-controlled 6-month follow-up study on parallel groups. Patients received 10 sessions of 1-Hz rTMS or placebo targeted to the left auditory cortex corresponding to tonotopic representation of tinnitus pitch. Effects were evaluated immediately after treatment and at 1, 3 and 6 months. Primary outcome measures were visual analog scores (VAS 0-100) for tinnitus intensity, annoyance and distress, and the Tinnitus Handicap Inventory (THI).

Study sample: Thirty-nine patients (mean age 50.3 years).

Results: The mean tinnitus intensity (F_3=15.7, p<0.0001), annoyance (F_3=8.8, p=0.0002), distress (F_3=9.1, p=0.0002) and THI scores (F_4=13.8, p<0.0001) decreased in both groups over time with non-significant differences between the groups. After active rTMS, 42% and 37 % of the patients showed excellent response at 1 and 3 months against 15 % and 10 % in the placebo group (p=0.082 and p=0.065).

Conclusions: Despite the significant effects of rTMS on tinnitus, differences between active and placebo groups remained non-significant, due to large placebo-effect and wide inter-individual variation.

Clinical trial reference: ClinicalTrials.gov (ID NCT 01929837)

Introduction

In tinnitus, a disturbing sound is perceived in the absence of external auditory stimuli. It concerns approximately 10 - 15 % of the population. Many people habituate to the phantom sound, but 1 – 2 % of all people suffer from chronic disabling tinnitus causing anxiety, concentration or sleep difficulties, and distress in daily living (Langguth, et al. 2013). Severe tinnitus in depressed patients can even lead to suicide (Dobie. 2003). Tinnitus, like pain, is entirely a subjective experience only measurable by self-evaluation (Henry, et al. 2005).

Recent research suggests that tinnitus results from maladaptive plasticity in the central auditory network associated with hearing deficit. Abnormal hyperactivity is generated within the auditory cortex and auditory brainstem nuclei because of cortical deafferentation and functional reorganization following injury to the cochlea or auditory nerve (Henry, et al. 2014). Neuroimaging studies have further demonstrated increased activity also in non-auditory areas like the frontal lobes and the cerebellum (Leaver, et al. 2011). Thus, tinnitus is currently considered as a complex disorder involving multiple brain networks while its exact pathophysiology remains unclear.

The treatment of chronic tinnitus is challenging as there is no curative therapy or effective pharmacological treatment. Psychological therapies and masking devices or hearing aids can reduce tinnitus distress and improve quality of life. Anti-depressive medications may diminish tinnitus by modulating mood and anxiety in depressed patients (Tunkel, et al. 2014).

In transcranial magnetic stimulation (TMS) magnetic pulses are given with a coil on the scalp to induce electric currents in the brain, enabling non-invasive, painless stimulation of the underlying cortex. Repetitive TMS (rTMS) can either increase or decrease cortical activity by causing long-

term potentiation or long-term depression-like effects in synaptic transmission (Hoogendam, et al. 2010). Low frequency (≤ 1 Hz) rTMS is known to reduce neural activity in the brain regions stimulated, and has thus been used to treat the hyperactive auditory cortex in tinnitus patients (Plewnia, et al. 2007). In addition to these focal alterations, rTMS induces widespread functional changes in brain networks via long-range neural connections (Hoogendam, et al. 2010, Moisset, et al. 2016).

rTMS has been shown to be effective and safe in the treatment of neuropathic pain and depression (Cruccu, et al. 2016, Lefaucheur, et al. 2014). Several studies on rTMS for tinnitus demonstrate moderate benefit (Anders, et al. 2010, Eichhammer, et al. 2003, Folmer, et al. 2015, Khedr, et al. 2008, Kleinjung, et al. 2005, Marcondes, et al. 2010, Mennemeier, et al. 2011, Plewnia, et al. 2007, Rossi, et al. 2007). Some recent controlled studies have not shown significant differences between active and placebo treatments (Hoekstra, et al. 2013, Langguth, et al. 2014, Piccirillo, et al. 2013). However, a recent systematic review and meta-analysis observed moderate efficacy of low-frequency rTMS for tinnitus; the odds ratio of therapeutic success, assessed with Tinnitus Handicap Inventory (THI) (Newman, et al. 1996), was 15 times greater in the active group (Soleimani, et al. 2016). As there are still many open questions, the recent guidelines do not recommend rTMS for routine treatment of tinnitus patients (Tunkel, et al. 2014). Further, tinnitus decrease after rTMS is usually incomplete and transitory with large inter-individual variations (Burger, et al. 2011, Lefaucheur, et al. 2014), and there is very scarce data on the long-term efficacy of rTMS.

Electric field (E-field) navigated rTMS visualizes in real time the strength and the direction of the induced E-field on the brain 3D-MRI, and the cortical target with an anatomical accuracy of a few millimetres (Picht, et al. 2011). We hypothesize that targeting the treatment anatomically accurately with E-field navigation should give better results than non-navigated rTMS, as has been shown for

the treatment of depression and pain (Fitzgerald, et al. 2009, Ayache, et al. 2016). While most rTMS studies on tinnitus have been done without neuronavigation, there appears to be one open study utilizing E-field navigated rTMS for tinnitus (Sahlsten, et al. 2015) showing significant treatment effect. We also presume that targeting rTMS treatment to the representation area roughly corresponding to the perceived tinnitus pitch would more specifically inhibit the cortical hyper-excitability. An additional goal of this study was to evaluate the long-term effects of E-field navigated rTMS on chronic tinnitus.

Methods

This prospective randomized, single-blind placebo-controlled study on parallel groups was approved by the Ethical Committee for the Hospital District of South-Western Finland (73/1800/2013). Patients gave their written informed consent. The study took place in the Departments of Ear, Nose and Throat Diseases (ENT) and Clinical Neurophysiology at Turku University Hospital (TUCH) during 2013 – 2015. The trial was registered on ClinicalTrials.gov (ID NCT 01929837).

Patients

All tinnitus patients born between 1948 and 1995 treated in the department of ENT in TUCH between January 2009 and March 2013 were reviewed using patient archives. Inclusion criteria were chronic (6 months-10 years), uni- or bilateral tinnitus in the age group of 18-65 years. Figure 1 shows the flow chart. The chosen 42 patients underwent complete audiological and otological investigations and a 3D-MRI to rule out possible treatable causes for their tinnitus and provide anatomical guidance for navigated rTMS. There were no tumours in the MRIs, but four patients had

mild leucoaraiosis, six had mild atrophy and three had small benign cyst(s) in the brain. Patients were randomized using random permuted block design for either active or placebo stimulation.

Thirty-nine patients (27 males and 12 females) ages 23-65 years (mean age 50.3 years, SD 11.8) with mean tinnitus duration of 5.1 (SD 2.5) years completed the study and were included in the data analysis. All patients were right-handed, except for one in the placebo group. Table 1 lists the characteristics of the 19 patients in the active group and 20 patients in the placebo group. Comparing baseline characteristics of both groups, their only statistically significant difference was a mean tinnitus loudness match in the left ear ($p=0.03$).

Evaluation scales

An audiogram (both air and bone thresholds) was measured and a pure tone average (PTA) of 500-4000 Hz calculated for both ears at the baseline and after the serial treatment. Patients verbally described the pitch of tinnitus in each rTMS session. In addition, the loudness (dB) and the pitch (Hz) of tinnitus were psycho-acoustically measured with a clinical audiometer at the baseline, after the treatment, and 1 and 3 months after the rTMS. At the same time points, the patients evaluated their symptoms with the Tinnitus Handicap Inventory (THI); scoring between 0 (slight tinnitus) and 100 (catastrophic tinnitus) (Newman, et al. 1996) and Visual Analog Scale (VAS, between 0 (no tinnitus) and 100 (the worst tinnitus the patient could imagine)) for tinnitus intensity, annoyance, and distress in everyday life. Additionally, the patients rated their Global Impression of Change (GIC) on a scale between -3 (very much worse) and +3 (very much improved), 0 meaning no change. Beck's Depression Inventory (BDI) (Steer, et al. 1999) and Jenkins Sleep Evaluation Questionnaire (JSEQ) (Jenkins, et al. 1988) were used to follow up mood and sleep. The final control was a telephone interview at 6 months including NRS (0-10) concerning tinnitus intensity,

THI and GIC. Structured psychiatric interviews (SCID Axis I and II) were performed at the baseline.

rTMS

The TMS equipment used was NBS System 4.0 (Nexstim Ltd, Helsinki, Finland). It visualizes the induced E-field superimposed on the patient's brain 3D-MRI in real time during stimulation. It also computes an estimate of the strength of the E-field (in V/m) at the cortical stimulation target. The user can easily control the cortical target and the direction of the electric current vector during stimulation (Figure 2). This "hot spot" of stimulation has been shown to have an anatomical accuracy of a few millimetres (Picht, et al. 2011). A medical doctor or a trained technician monitored the stability of the coil throughout the treatment session.

During the first session, the resting motor threshold (RMT) was determined at the right M1 cortex representing the left hand thenar muscles using a figure-of-eight coil giving biphasic magnetic pulses (Valmunen, et al. 2009). The RMT was defined as the lowest intensity (% of maximum device output) capable of eliciting > 50 μV compound muscle action potential in 50 % of the trials. Details of the RMT determination are given elsewhere (Sahlsten, et al. 2015, Valmunen, et al. 2009).

Patients received 10 treatment sessions over 2 weeks (5 daily weekday sessions). Each session consisted of 4000 pulses at a continuous 1 Hz rate given to the left superior temporal gyrus (STG) at 100 % of the RMT. In the active group, all patients received 10 full sessions, except for one patient in whom one session was only 2800 pulses (due to her late arrival). In the placebo group, 17 patients received 10 full sessions of rTMS, 2 patients received 8, and 1 patient received only 6 full sessions (due to patient-related issues and technical problems in the NBS system). In the active

group, the intensity was lowered from 100 % to 95-80 % for 5 patients because of annoying facial contractions. The left side was chosen based on the previous literature, which mostly indicates that stimulation of the left auditory area is efficient, irrespective of tinnitus location (Burger, et al. 2011, Lehner, et al. 2012) , although contradictory evidence exists as well (Kim, et al. 2014). For placebo stimulation, a 15-cm plastic block was attached to the coil without the patient seeing it. The added distance effectively lowered the E-field to the cortex to negligible amounts of 1-4 V/m. Patients used ear plugs during the treatment.

Figure 2 shows the stimulation-induced E-field and current vector at the "hot spot". In the active group, the induced E-field on the cortex varied between 48-143 V/m between patients and sessions. Since different pitches are tonotopically represented within the auditory cortex: high frequencies are represented on the posterior and lower frequencies on the anterior areas (Moerel, et al. 2014), we hypothesized that targeting rTMS treatment to the representation area roughly corresponding to the perceived tinnitus pitch would more specifically inhibit the cortical hyper-excitability. We targeted the more posterior regions of the auditory cortex when tinnitus was high pitched (the most posterior point in Figure 2); if the pitch lowered during treatment, we gradually moved forward by 0,5-1 cm steps to the more anterior target points. In the active group, the treatment was started in the posterior part of the auditory cortex for 12/19 patients and moved forward for 8 patients, as the serial treatment proceeded. For lower pitch tinnitus, the treatment was started at the most anterior point or the middle in 7/19 patients and moved into more posterior locations in 4 patients.

Statistical analysis

Sample size calculations were based on our pilot study where the mean change in tinnitus intensity was -2.6 (SD=1.4) measured on the NRS scale of 0-10 (Sahlsten, et al. 2015). Placebo response was estimated to be half of the mean change seen in the pilot study, approximately -1.3. Selecting

statistical power at 80 % and significance level at 0.05 (two-tailed) led to a sample size of 19 per group.

All the data are presented as mean with standard deviation (SD) when describing the raw data or estimated mean with standard error (SE) when describing the hierarchical linear mixed model analysis results. Possible baseline difference was tested using two-sample t-tests or a Wilcoxon rank sum test. Responders to rTMS were patients who showed at least 30 % reduction in tinnitus intensity, annoyance or distress in the VAS scores at any assessment point, as it is considered clinically meaningful alleviation in recent RCTs on pain (Dworkin, et al. 2008). The reduction of 6 or more THI scores was also considered clinically significant change (Zeman, et al. 2011). An excellent responder was defined as having reductions in all three VAS scores and the THI scores at any assessment point. Comparison of the number of responders between the treatment groups was performed with Fisher's exact test separately at each time point.

Normal distribution of variables was evaluated from studentized residuals visually and tested using the Shapiro-Wilk test. Logarithmic transformation was performed to THI and JSEQ to fulfill the normality assumption. To study whether the mean change of VAS scores (including NRS intensity), THI and JSEQ occurred over the study period and whether the mean changes were different between the groups, a hierarchical linear mixed model (HLMM) was used, including time as within-factor, group as between-factor (group) and their interaction. In addition, model included relevant clinical background factors (sex, hearing deficit, use of medicine for central nervous system, location and duration of tinnitus, age group, smoking, depression, tinnitus handicap inventory grading). From the final analysis model, all non-significant factors ($p>0.10$) were removed. However, the same final model for all VAS scores was executed and randomized treatment group was kept in the model even though it was not significant. Time factor was handled as categorical to

be able to estimate all possible shapes of mean changes over time. Compound symmetry covariance structure was used for time. Adjusted mean (SAS least square means) values with 95 % CI are shown from the final model as well as degrees of freedom together with F values (F_{df}) for main results from the final model. Additionally, as we wanted to evaluate whether baseline value of VAS scores associated to the treatment effect, we built up another model where baseline was handled as a covariate (instead of one time point) with the same background factors as in the model explained above.

To evaluate effect size in our study, Cohen's *d* (with 95% confidence interval), was calculated separately for the mean change in tinnitus intensity (at all post-treatment control times compared to the baseline) in both groups. All tests were two-sided with a significance level set at 0.05. The analyses were performed using SAS System, Version 9.3 for Windows (SAS Institute Inc., Cary, NC, U.S.).

Results

Primary Outcome Measures

Overall, significant decrease over 3 months was observed in mean intensity (HLMM,: F_3=15.7, p<0.0001), annoyance (F_3=8.8, p=0.0002) and distress (F_3=9.1, p=0.0002) VAS scores for tinnitus in the entire study population. However, there were no significant differences between the active and placebo group over time (F_3=0.8, p=0.50 for intensity, F_3=0.3, p=0.82 for annoyance, F_3=0.9, p=0.46 for distress, Figure 3, Supplement Table 1). Because of this, post hoc paired comparisons could not be performed although tinnitus intensity was lower in the active group immediately after the treatment and at the 1-month control (Figure 3 a). The VAS scores (intensity, annoyance and distress) improved immediately after the treatment and, at the 1- and 3-month controls in both

groups. The mean tinnitus intensity in NRS units decreased to 5.8 (SE 0.4) at 6 months compared to the baseline (the first telephone interview) 6.8 (SE 0.3) (F_1=14.2, p=0.0006), but the difference between the groups remained non-significant (F_1=4.0, p=0.053).

The effect size in Cohen's *d* for intensity calculated between the baseline and post-treatment time points ranged from 0.92 (95% CI 0.35-1.48) immediately after active rTMS to 0.82 (CI 0.32-1.33) at 3 months and was also high in the placebo group, ranging from 0.69 (CI 0.18-1.19) immediately after rTMS to 0.78 (CI 0.32-1.24) at 3 months. The baseline VAS scores (p=0.86 for intensity and distress, p=0.33 for annoyance) were not associated with rTMS treatment efficacy. Patient sex, hearing deficit, use of medication for central nervous system (depression medication excluded), and location or duration of tinnitus were not associated with treatment results (all p-values > 0.10 and therefore these factors were excluded from the final model). The duration of tinnitus was associated with tinnitus annoyance and distress; the longer the duration of tinnitus, the higher the VAS scores (p=0.011 for annoyance and p=0.011 for distress). Considering tinnitus intensity, in both the active and placebo groups, older patients (age group >60 years) benefitted more from the treatment than did younger patients (p=0.0013).

There was a significant reduction (F_4=13.8, p<0.0001) in the THI scores in the total group over time, but no significant difference between the two treatment groups over time (F_4=1.3, p=0.28) (Figure 4, Supplement Table 2). The reduction in the median THI scores was maintained for up to 6 months, when they were still lower than at the baseline (HLMM: comparison between the baseline and 6 months p<0.0001). The change in the THI scores was associated with depression in both groups; depressed patients experienced less reduction (F_4=4.1, p=0.0035).

The rate of excellent responders (clinically meaningful reductions both in all VAS and THI scores) did not differ significantly between the active and placebo treatment groups, although there were

more excellent responders in the active group at the 1- (42 % vs. 15 %, Fisher's exact test p=0.082) and 3-month (37 % vs. 10 %, p=0.065) controls (Supplement Figure 1). Considering only a 30 % reduction in tinnitus intensity after rTMS as a response, there were 53 % responders in the active and 30 % in the placebo group (p=0.20) whereas using the 6-point reduction in the THI scores resulted in 58 % responders in the active and 65 % in the placebo group after the rTMS (p=0.75) (Supplement Table 3).

Based on the GIC scale, 5/19 (26 %) patients in the active group and only 1 (5 %) patient in the placebo group felt they had benefitted clinically significantly (GIC $\geq$ +1) from the treatment after the serial rTMS (Supplement Figure 2).

Secondary Outcome Measures and Other Findings

There were no rTMS treatment induced changes in hearing in the whole group or between the groups in either ear (Supplement Table 4 a).

There were no changes in psycho-acoustically measured loudness or pitch of tinnitus in the whole group or between the groups during the follow-up (Supplement Table 4 b and 4 c).

There was a minor improvement in the BDI and JSEQ scores after treatment in the whole group and in both treatment groups (HLMM: time effect BDI: F_2=16.8, p<0.0001, JSEQ: F_2=5.5, p=0.0062), but there were no interaction effects between the groups over time (Supplement Table 5).

After the follow-up, the patients were asked about their opinion about the treatment they had received; in the active group, 9/19 (47%) patients guessed correctly that they had received active rTMS, and in the placebo group, 6/20 (30%) thought having received active rTMS.

There were no major side effects, but some patients reported local irritation due to muscle twitching at the stimulation side and mild temporary side-effects, like headaches.

Discussion

This randomized controlled trial (RCT) showed improvement for the VAS scores (intensity, annoyance, distress) and THI scores both in the active rTMS group and the placebo group. The therapeutic efficacy did not differ between the active and placebo treatment groups, though. The THI scores and NRS intensity were still lower compared to the baseline in both groups at the 6-month control. E-field navigated rTMS proved to be safe, well-tolerated, and easy to use.

The absence of significant differences between the treatment groups may partly be due to wide inter-individual variation in efficacy and a large placebo-effect. There was a high effect size of the placebo rTMS, showing Cohen's *d* values up to 0.78. As patient counseling and psychological therapies have a positive effect on tinnitus patients (Tunkel, et al. 2014), it may well be that some of the high placebo effect is due to the nature of the study; the prompt clinical examination, MRIs and frequent visits to the clinic with caring personnel. In an anti-depressant medication trial, up to 40 % of the tinnitus patients benefitted from placebo (Dobie, et al. 1993). In addition, placebo effects are associated with the release of neurotransmitters, such as endogenous opioids and dopamine; also, rTMS exerts its effects at least partly by boosting endogenous dopamine-opioid axis (Lefaucheur, et al. 2014). These previous findings may partly explain similar therapeutic effects in both groups of the present study.

In line with our study, other RCTs also conclude that active rTMS is no more effective than placebo stimulation for tinnitus (Piccirillo, et al. 2013, Hoekstra, et al. 2013, Plewnia, et al. 2012, Langguth, et al. 2014). All these studies used some navigation method (although not E-field navigation) and low frequency stimulation with 100-110 % of RMT (except for a continuous theta burst stimulation with 80 % of RMT in (Plewnia, et al. 2012)). However, the treatment protocols differed from the present study involving bilateral stimulation (Hoekstra, et al. 2013, Plewnia, et al. 2012), or combined stimulation at the temporo-parietal or frontal cortex (Langguth, et al. 2014, Plewnia, et al. 2012). The number of pulses per session was lower (900-1500 pulses) in two studies (Piccirillo, et al. 2013, Plewnia, et al. 2012) and the same as here in the other two (Hoekstra, et al. 2013, Langguth, et al. 2014). The number of the treatment sessions varied from 5 to 20 across the studies. The median improvement in tinnitus questionnaires after active rTMS treatment ranged from -2 to -10, as in the current study. There was a slight or significant improvement in tinnitus in all these studies, but the overall effect of active rTMS did not prove superior to the placebo.

Contrary to the studies above, several RCTs show significant improvement of tinnitus favoring rTMS over placebo treatment (Anders, et al. 2010, Khedr, et al. 2008, Marcondes, et al. 2010, Folmer, et al. 2015, Mennemeier, et al. 2011). The mean reduction of the THI scores in the active rTMS group has been somewhat larger than in our present results (Khedr, et al. 2008, Marcondes, et al. 2010). In one study (Anders, et al. 2010), THI and Tinnitus Questionnaire scores decreased in the active group, but no significant changes in the VAS scores occurred in either group. In our study, there was a reduction in all scores (both THI and VAS), but it was not restricted to the active group. In our study 10/19 (53 %) patients in the active group were responders (≥30 % reduction) based on the VAS intensity score. This is somewhat better than in one previous trial showing positive efficacy based on the 43 % responder rate (at least a 33 % drop in tinnitus loudness) and a significant VAS decrease only after active, not placebo treatment (Mennemeier, et al. 2011). In

another study (Folmer, et al. 2015), 56 % of the patients in the active rTMS group were responders according to the Tinnitus Functional Index after the serial rTMS. This result is in line with the present responder rate 58 %, when based only on the reduction of the THI scores.

Different explanations are possible for the opposing outcomes of these parallel RCTs. The rTMS system, navigation method, treatment targets, and especially the stimulation protocols differed between the studies. Tinnitus severity has been suggested to be a positive predictor of rTMS outcome (Lehner, et al. 2012) and may have affected the present results in patients with rather mild symptomatology. It is also suggested that longer duration of tinnitus (De Ridder, et al. 2005) and hearing loss (Kleinjung, et al. 2007) may reduce the efficacy of rTMS treatment. In this study, the duration of tinnitus or hearing deficit was not associated with the treatment results. Results differ greatly across the studies, and we can agree with the statement that there are no good demographic or clinical predictors for treatment outcome (Lehner, et al. 2012).

Rather small study groups may also explain these contradictory results. Results of the present study differed from our open methodological pilot study (Sahlsten, et al. 2015). The responder rate (≥ 30 % reduction in NRS) in the pilot study was 62 % for tinnitus intensity and 69 % for annoyance. Open design, small group, and clearly more severe tinnitus with baseline mean intensity for NRS scores 7.1/10 and annoyance 7.0/10, compared to mean VAS scores 59/100 and 52/100 in the present study. The power calculations of the present study were based on the results of this pilot study (Sahlsten, et al. 2015), which may have led to type 2 error i.e., too small groups to show a significant difference between the active and placebo group. This is in line with the recent meta-analysis on 720 patients concluding moderate efficacy of low-frequency rTMS for tinnitus (Soleimani, et al. 2016).

In addition to tinnitus severity, the amount of pulses differed between our two studies; in the pilot study, there were 1800-3000 pulses/session, compared to 4000 pulses in the present study. In a large meta-analysis, depression improved significantly better in rTMS studies with fewer stimuli per session (Kedzior, et al. 2014). Stimulating the brain with too many stimuli at one time might lead to neural network saturation and consequently, to a reduction in the therapeutic effect of rTMS (Kedzior, et al. 2014). Furthermore, for rTMS in depression, results may become better after a longer serial treatment (> 2 weeks), so our two-week protocol may have been too short to induce long-lasting effects.

There was a minor improvement in the BDI points after the treatment of the total group, but no difference between the groups. Most patients were not depressed in either group, so the treatment effects on single patients cannot be explained by a positive impact on clinical depression.

There were no changes in the measured loudness or pitch of tinnitus in either group during follow-up. The results of psycho-acoustic testing of tinnitus perception have been shown to have little if any correlation with the degree of tinnitus impact (Henry, et al. 2005). Thus, there is need for multimodal assessment of rTMS efficacy as different outcome measures elucidate distinct aspects of potential treatment effects.

The rTMS treatment results are characterized by high inter-individual variability (Lehner, et al. 2012). In line, in our study, there were excellent responders in the active group, with 3 patients experiencing very little tinnitus or even total silence after the treatment series, whereas 3 other patients did not receive any benefit from the treatment. Different individual features, including genetics, may have an impact on the efficacy of the treatment (Hoogendam, et al. 2010, Jääskeläinen, et al. 2014).

The most efficient protocol, location, and side for rTMS stimulation in tinnitus remain uncertain (Langguth, et al. 2010). Individual fMRI or PET imaging of the most hyperactive region of the cortex could be useful when choosing the optimal target (Plewnia, et al. 2007).The role of stimulating multiple cortical targets (Lehner, et al. 2013) and the optimal protocol for rTMS maintenance therapy also need more research.

In conclusion, despite clear decrease in tinnitus scores during the study period, active E-field navigated rTMS to the left auditory cortex was not shown to be more effective than the placebo in this study. A large placebo effect and rather small study group in combination with wide inter-individual variation in the efficacy may explain the present results, as well as the previous contradictory findings in therapeutic rTMS for tinnitus. In the future, RCTs with proper patient selection and characterization, significantly larger patient groups, and standardized treatment protocols are necessary to determine the true value of neuronavigated rTMS for tinnitus.

Conflicts of Interest

Dr Sahlsten has received travel grant from Nexstim for an international congress on therapeutic use of rTMS. Dr Joutsa has received a lecturer honorarium from Boehringer-Ingelheim, travel grants from Abbvie and research grants from Lundbeck and the Orion Research Foundation. Dr Taiminen has received a lecturer honorarium from Astra-Zeneca, Bayer, Bristol-Myers Squibb, Efeko, Eisai, GlaxoSmithKline, Lilly, Lundbeck, Nexstim, Orion Pharma, Pfizer, Schering-Plough and UCB. PhD Holm has received a travel grant from ResMed. Prof Jääskeläinen has received a lecturer honorarium from Nexstim, Orion Pharma, Pfizer and Ratiopharm. Others involved in the study declared no conflicts of interest.

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Figure legends

Figure 1. Patient recruitment process. The number of the patients at each step are shown. Exclusion criteria were magnetically active, metallic intra-corporeal appliances (e.g. cochlear implants and cardiac pace makers), epilepsy or increased risk of seizure (e.g. brain tumour, stroke, alcohol abuse), active bipolar disorder, severe heart disease, migraine, and pregnancy. Pulsatile tinnitus and objective tinnitus were excluded. Patients were contacted by phone to ask whether they still had refractory tinnitus with an average intensity at least 4/10 on the Numeric Rating Scale (NRS) from 0 (no tinnitus) to 10 (the worst tinnitus the patient could imagine). None of the patients had been treated with TMS.

Figure 2. The red arrow in the figure shows the induced electric field vector on the cortical target site at the left STG. The exact stimulation spot ("hot spot") is at the joining of the red and the blue arrows. The brightness of the arrow reflects the optimal tangential position of the coil. The calculated electric field on the cortex is also shown numerically as V/m on the screen (not shown in the figure).

Several stimulation targets were used on the STG: the most anterior was situated close to the posterior end of the sulcus centralis (for lower pitch tinnitus) and the most posterior one was situated approximately 2 cm more posteriorly (for high pitch tinnitus). The stimulation spot was

moved forward or backward on the STG during consecutive sessions according to the achieved results (diminution of intensity and changes in the pitch of tinnitus).

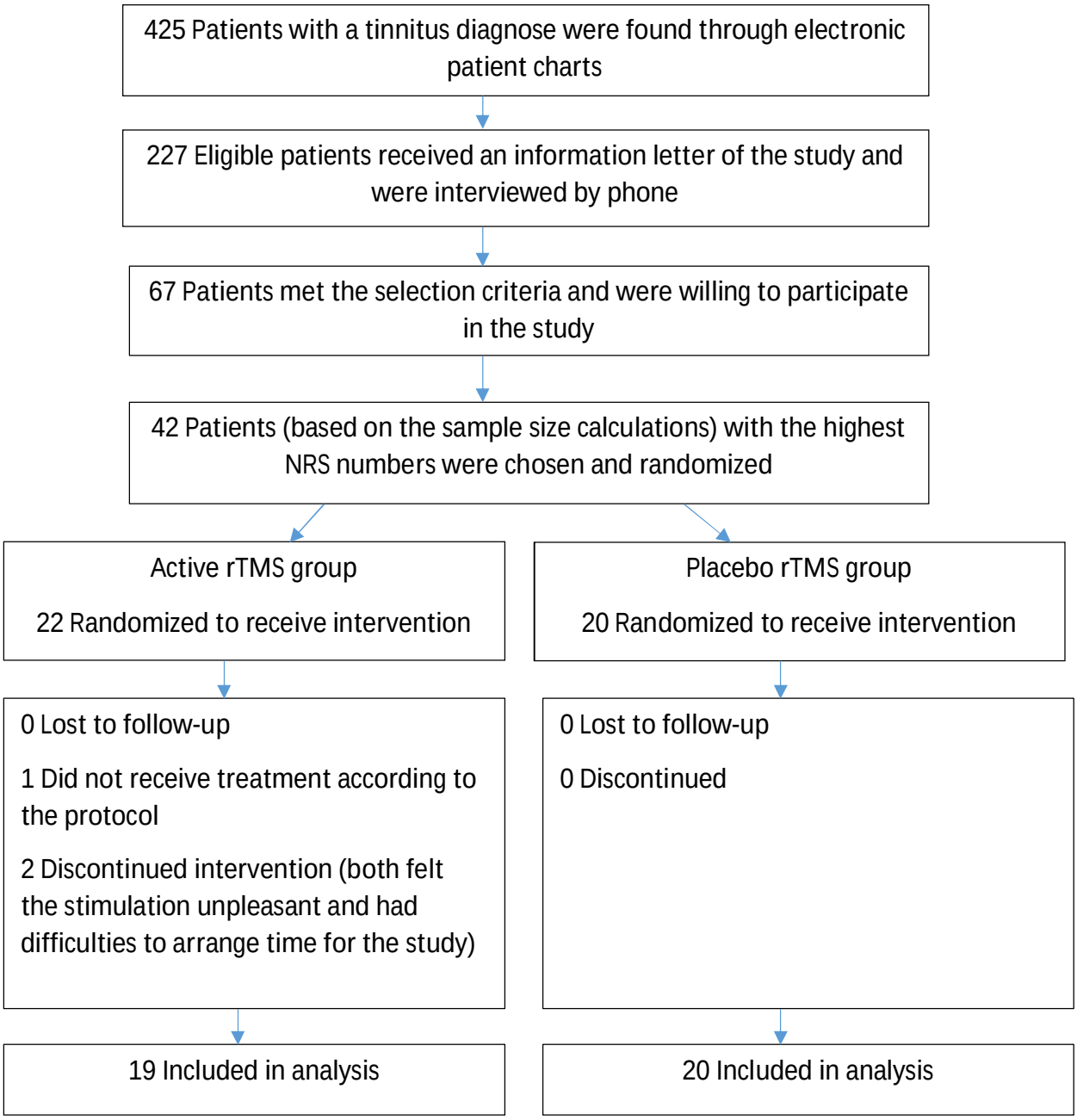
Figure 3. The effect of the serial rTMS on patients's self-rated tinnitus intensity (a), annoyance (b) and distress (c) in the active rTMS and placebo group in VAS (0-100), in time from the beginning of the treatment, the adjusted means (+-SE). There was no significant difference between the mean changes over time between the treatment groups in any VAS score (F_3=0.8, p=0.50 for intensity, F_3=0.3, p=0.82 for annoyance, F_3=0.9, p=0.46 for distress). See Supplement Table 1 for details.

Figure 4. The effect of the serial rTMS on the median THI scores (+- quartiles) in the active rTMS and placebo group in time from the beginning of the treatment. There was a significant reduction (F_4=13.8, p<0.0001) in the THI scores in the whole group over time. The median points reduced from 30 (quartiles 14 – 44) to 18 (quartiles 6 - 34) after the serial treatment, but there was no significant difference between the two treatment groups over time (F_4=1.3, p=0.28). See Supplement Table 2 for details.

Supplement Figure 1. The percentage of the excellent responders in the active rTMS and placebo group are shown in time from the beginning of the treatment. The excellent responder was defined to have at least 30 % reduction in all VAS (intensity, annoyance, distress) scores and the reduction of 6 or more in THI scores. There was no significant difference between the groups, but there was a trend toward more responders in the active group at the 1 month (p=0.082) and 3 months (p=0.065) assessment points. See Supplement Table 3 for details of the responder rates.

Supplement Figure 2. The number of the patients who felt they had benefitted clinically significantly from the treatment, based on the GIC scale (+1 - +3) in time from the beginning of the rTMS treatment.

Figure 1.


425 Patients with a tinnitus diagnose were found through electronic patient charts
227 Eligible patients received an information letter of the study and were interviewed by phone
67 Patients met the selection criteria and were willing to participate in the study
42 Patients (based on the sample size calculations) with the highest NRS numbers were chosen and randomized
Active rTMS group
22 Randomized to receive intervention
Placebo rTMS group
20 Randomized to receive intervention
0 Lost to follow-up
1 Did not receive treatment according to the protocol
2 Discontinued intervention (both felt the stimulation unpleasant and had difficulties to arrange time for the study)
0 Lost to follow-up
0 Discontinued
19 Included in analysis
20 Included in analysis

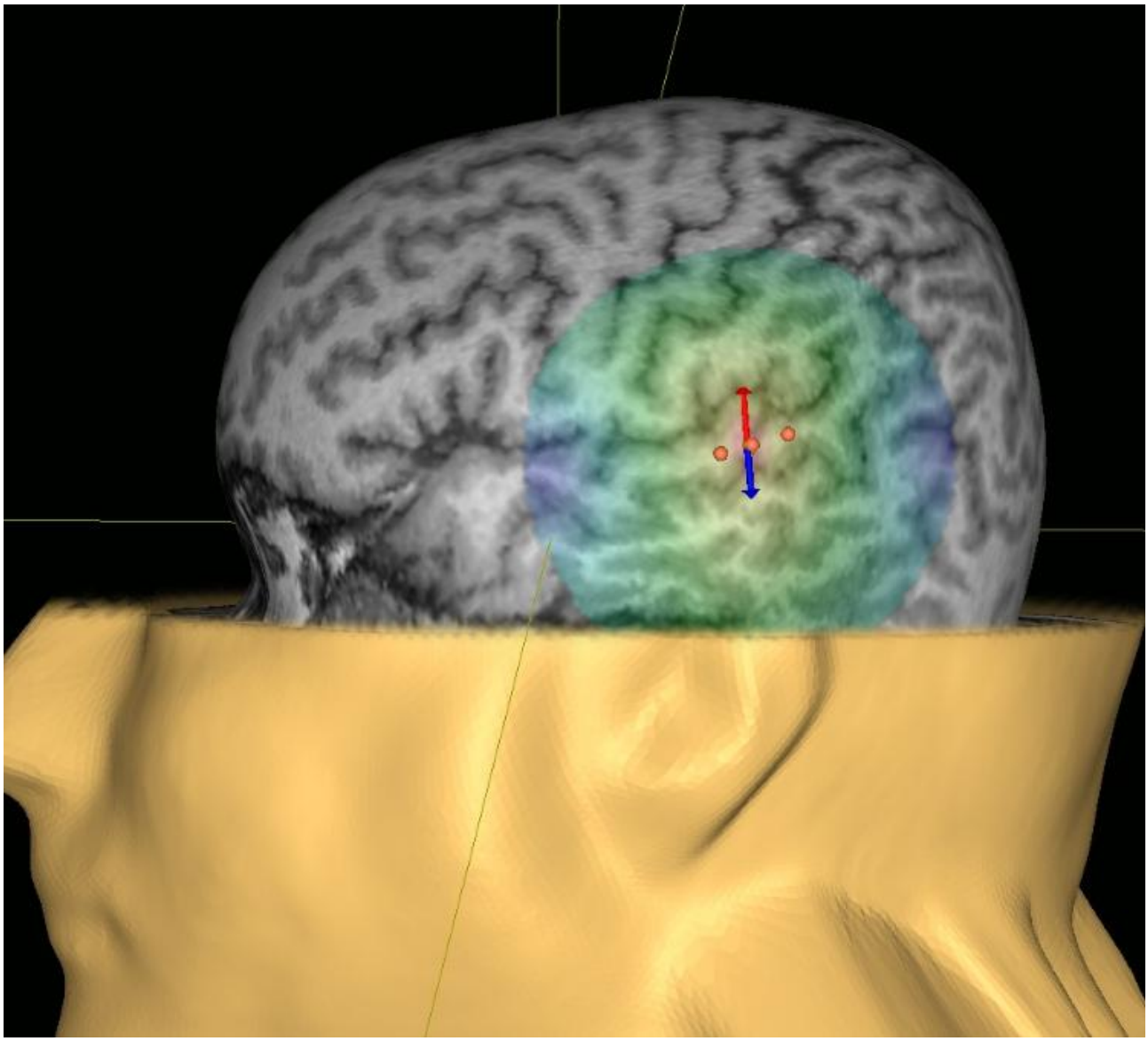


Figure 3 a.

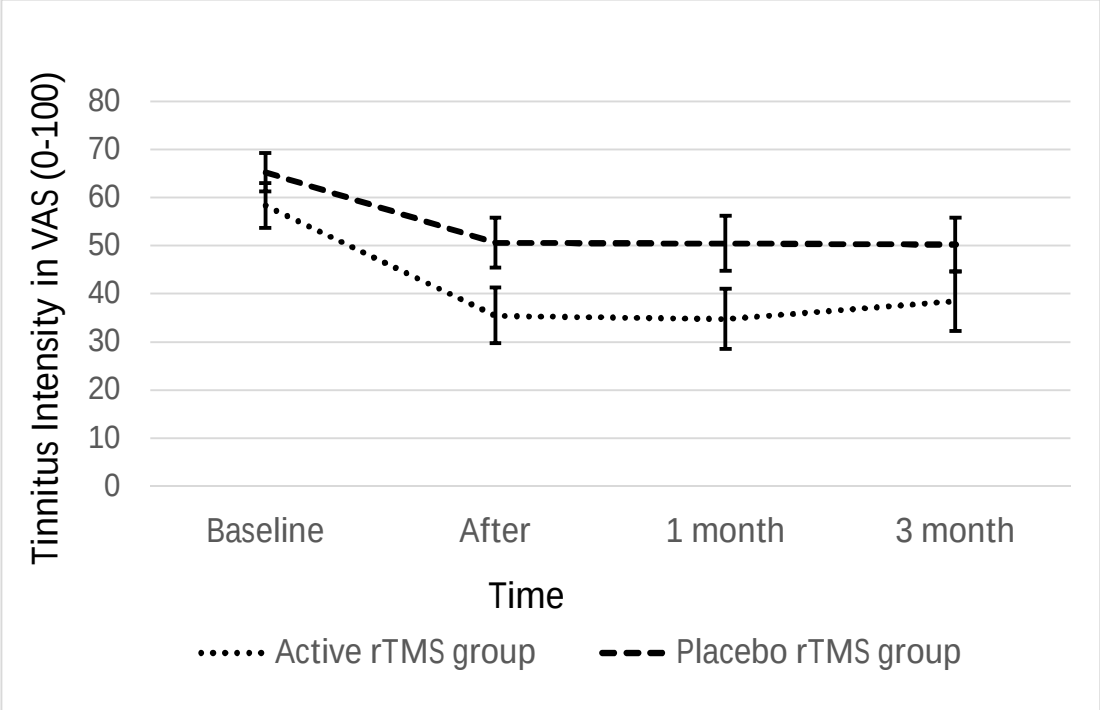


Figure 3 b.

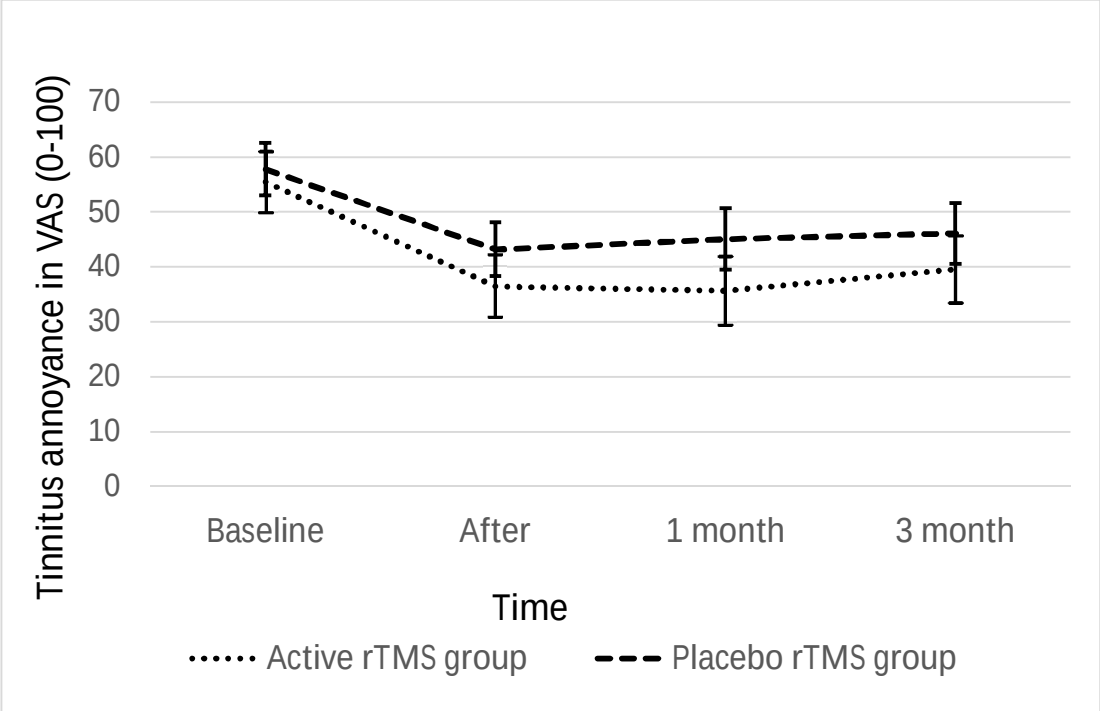


Figure 3 c.

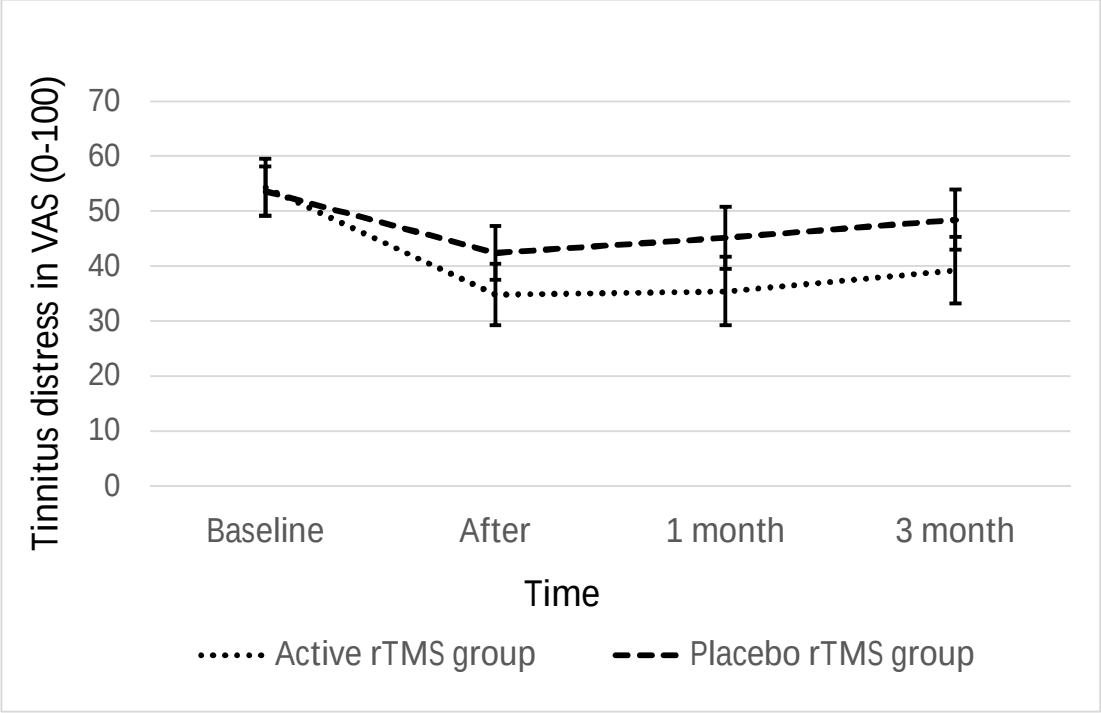

Tinnitus distress in VAS (0-100)
70
60
50
40
30
20
10
0
Baseline
After
1 month
3 month
Time
Active rTMS group
Placebo rTMS group

Figure 4.

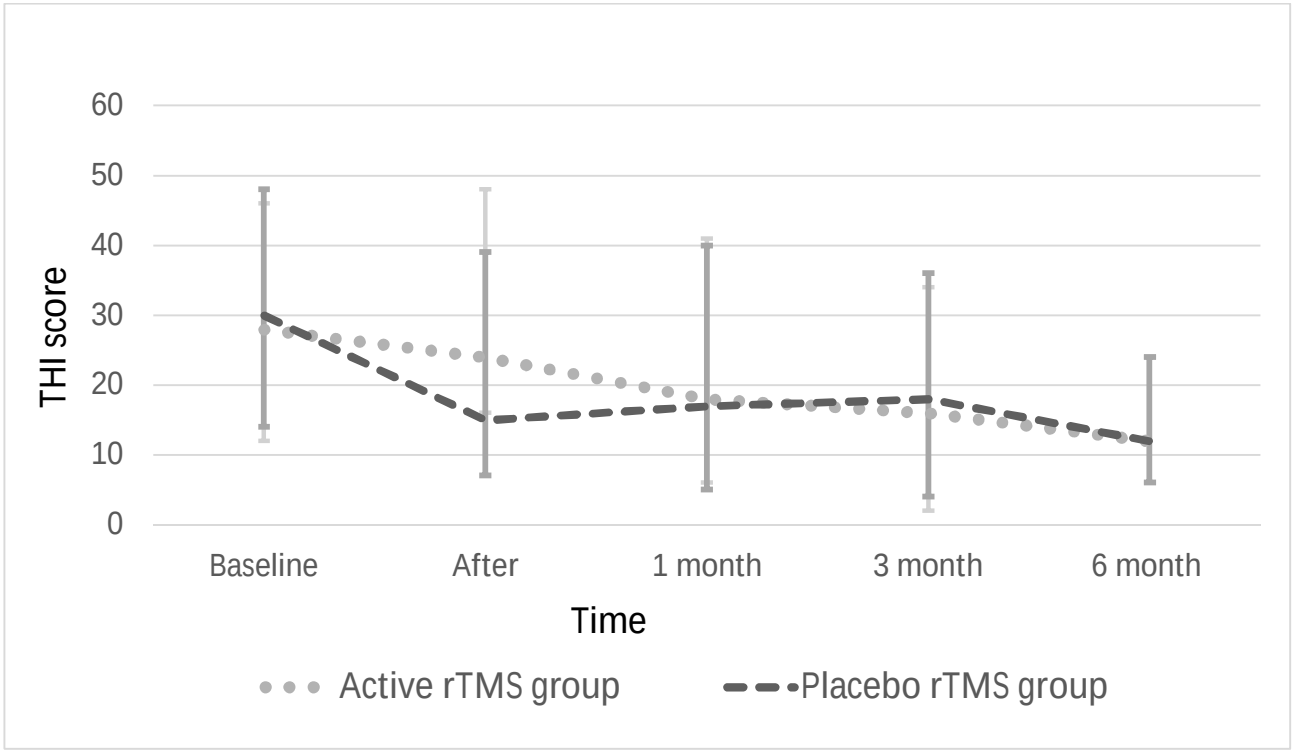

60
50
40
30
20
10
0
THI score
Baseline
After
1 month
3 month
6 month
Time
Active rTMS group
Placebo rTMS group

Table 1. The Characteristics of the patients

Characteristics		Active rTMS group (n=19)	Placebo rTMS group (n=20)	P value for between-group comparison
Men, No.		13	14	0.92
Age, mean (SD),years		48.9 (13.1)	51.5 (10.7)	0.72
Age group, No. of patients	< 50 years	7	9	0.72
	50-60 years	7	5	
	> 60 years	5	6	
No. of smoking patients		2	2	1.00
Duration of tinnitus, mean (SD), years		5.4 (2.5)	4.9 (2.7)	0.57
Tinnitus location, No. of patients	Right ear	6	1	0.06
	Left ear	2	6	
	Both ears	11	13	
Baseline (first phone contact) NRS tinnitus intensity, mean (SD)		6.5 (1.1)	6.5 (1.0)	0.94
Baseline VAS tinnitus intensity, mean (SD)		56.3 (14.5)	61.9 (14.1)	0.23
Baseline VAS tinnitus annoyance, mean (SD)		52.1 (22.6)	51.0 (20.4)	0.87
Baseline VAS tinnitus distress, mean (SD)		52.1 (20.4)	47.4 (19.9)	0.47
Baseline Tinnitus Handicap Inventory score, median (quartiles)		28.0 (16.0-40.0)	30.0 (14.0-48.0)	0.68
Baseline Beck Depression Inventory score, median (quartiles)		5.0 (2.0-9.0)	4.0 (0-10.5)	0.69
SCID (psychiatric interview), No. of depressed patients		8	6	0.43
Baseline Jenkins Sleep Evaluation score, median (quartiles)		8.0 (4.0-10.0)	4.5 (2.0-10.0)	0.80
Baseline Hearing Right Ear, PTA (500-4000 Hz), mean (SD)		14.0 (11.5)	16.0 (12.6)	0.46
Baseline Hearing Left Ear, PTA (500-4000 Hz), mean (SD)		18.5 (19.8)	18.5 (12.7)	0.98
Baseline Tinnitus loudness match Right ear, mean (SD), dB sensation level		34.2 (21.9)	26.1 (23.0)	0.27
Baseline Tinnitus loudness match Left ear, mean (SD), dB sensation level		24.5 (26.1)	43.9 (26.9)	0.03
Baseline Tinnitus pitch match Right ear, median (min-max), kHz sensation level		6.0 (3-8)	4.0 (0.5-8)	0.098
Baseline Tinnitus pitch match Left ear, median (min-max), kHz sensation level		6.0 (1-8)	6.0 (0.5-8)	0.50
Baseline Tinnitus pitch, No. Of patients	Low pitch	1	2	1.00
	High pitch	18	17	
	Medium pitch	0	1	
Resting motor threshold, mean (SD), %		38.3 (11.1)	34.4 (12.3)	0.31