Clinical Neurophysiology 130 (2019) 1900–1907

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Can theta burst stimulation safely influence auditory hearing thresholds in healthy young adults?

Nuno Pinto [a,b,*,1], Iris Oliveira [a,1], Joana Ferreira [a,1], Jorge Gama [a,c,2], Maria Vaz Pato [a,d,1]

[a] CICS-Health Sciences Research Centre, University of Beira Interior, Covilhã 6200-506, Portugal
[b] Dr. Lopes Dias School of Health – Polytechnic Institute of Castelo Branco, 6000-767, Portugal
[c] University of Beira Interior – Department of Mathematics, Covilhã 6200-506, Portugal
[d] Sousa Martins Hospital, Guarda Local Health Unit, Guarda 6300-858, Portugal

ARTICLE INFO

Article history:
Accepted 13 July 2019
Available online 29 July 2019

Keywords:
Transcranial magnetic stimulation
Theta burst stimulation
Auditory threshold
Audiometry
Safety
Auditory cortex

HIGHLIGHTS

- Theta-burst stimulation (TBS) of auditory cortex was not associated with hearing impairment of other side effects.
- Intermittent TBS (iTBS) of auditory cortex resulted in lowering of hearing threshold.
- This effect occurred most prominently at 500 Hz and 4000 Hz.

ABSTRACT

Objective: This TBS sham-controlled study aimed to evaluate the effects of intermittent TBS (iTBS) and continuous TBS (cTBS) upon ipsilateral hearing thresholds after stimulation on the left auditory cortex.
Methods: Sixty healthy adults, aged between 19 and 32 years (median of 23 years), were randomly distributed into three groups and underwent iTBS, cTBS or sham stimulation. Each double-blind experimental session comprised two pure tone audiometric evaluations per subject, before and after stimulation. To assess volunteer safety, a follow-up of at least 48 hours was implemented.
Results: The iTBS group mean thresholds displayed a tendency to decrease after stimulation, predominantly in the 500 Hz–6000 Hz interval and group comparisons revealed significant differences between the iTBS and sham groups for 500 Hz ($p = 0.041$) and between the iTBS and cTBS groups for 4000 Hz ($p = 0.038$). Neither relevant side effects nor any significant hearing threshold impairment after active or sham stimulation were found.
Conclusions: A single stimulation session led to an effective neuromodulation of the auditory cortex, reflected in lower thresholds when using iTBS.
Significance: These encouraging results with this safe noninvasive tool suggest that iTBS may have the potential to positively influence hearing thresholds.

1. Introduction

Repetitive transcranial magnetic stimulation (rTMS) is a neuromodulation tool, capable of influencing neural networks through the application of repetitive and patterned stimuli (Wassermann and Zimmermann, 2012; Lefaucheur et al., 2014). It can be used in several clinical applications, and is a promising technique for the treatment of auditory related disorders such as tinnitus, auditory hallucinations, and hearing loss (Rossi et al., 2009; Mennemeier et al., 2013; Schraven et al., 2013). However, noise levels achieved with the coils at higher intensities have the theoretical ability to impair hearing if long stimulation procedures

* Corresponding author at: University of Beira Interior – Faculty of Health Sciences, Avenida Infante D. Henrique, 6200-506 Covilhã, Portugal.
E-mail addresses: nfcpinto@gmail.com (N. Pinto), a30345@fcsaude.ubi.pt (I. Oliveira), a21912@fcsaude.ubi.pt (J. Ferreira), jgama@ubi.pt (J. Gama), mariavazpato@gmail.com (M. Vaz Pato).
[1] University of Beira Interior – Faculty of Health Sciences, Avenida Infante D. Henrique, 6200-506 Covilhã, Portugal.
[2] University of Beira Interior – Department of Mathematics, Rua Marquês D'Ávila e Bolama, 6201-001 Covilhã, Portugal.

https://doi.org/10.1016/j.clinph.2019.07.019
1388-2457/

are used (Rossi et al., 2009; Andoh and Zatorre, 2011; Schraven et al., 2013). Accordingly, exposure to excessive noise during stimulation, with sound levels that can exceed the 120 dB barrier, poses a health risk concerning possible sensorineural hearing loss, underlining the importance of using hearing protection (Schraven et al., 2013). So far, studies following safety guidelines suggest that rTMS is relatively safe and well-tolerated (Rossi et al., 2009). Even in short duration sessions discomfort, minor hearing losses and hypersensitivity to noise have been described, but rapidly disappear in most cases (Rossi et al., 2009; Schönfeldt-Lecuona et al., 2012; Schraven et al., 2013; Lefaucheur et al., 2014; Zhang and Ma, 2015).

Theta burst stimulation (TBS) is an optimized rTMS paradigm, using significantly shorter duration sessions and lower stimulation intensities (Huang et al., 2005; Cárdenas-Morales et al., 2010). TBS paradigms may be capable of inducing more pronounced and enduring effects in cortical excitatory and inhibitory phenomena when compared with rTMS (Cárdenas-Morales et al., 2010; Clavagnier et al., 2013). TBS benefits from shorter duration protocols (typically 40–190 seconds for TBS vs around 30 minutes for rTMS), achieving similar therapeutic efficacy (namely in depression) (Blumberger et al., 2018), allowing better time management in laboratory application (Huang et al., 2007, 2009; Bakker et al., 2015). These effects are attributed to changes in synaptic strength associated with long-term potentiation and long-term depression phenomena induced by a single TBS session (Cacace et al., 2018; Tse et al., 2018). However, the exact neural mechanism that underlies the auditory cortical modulation and the possible degree of cortical reorganisation remains unknown (Jäncke et al., 2002; Zhang and Ma, 2015).

Hearing related disorders have been studied with rTMS/TBS and treatment protocols have been developed, especially in tinnitus, both in human and animal studies (Lefaucheur et al., 2014; Zhang and Ma, 2015; Mulders et al., 2016). Interventions are based on the premise that primary and secondary auditory cortices can be modulated and that the stimulation has the ability to promote cortical plasticity (Lefaucheur et al., 2014; Zhang and Ma, 2015; Mulders et al., 2016). Auditory cortical stimulation must comply with the anatomical specificities of this area. Human ear is able to discern a spectrum of frequencies between 20 Hz and 20000 Hz and these are spread according to a tonotopic distribution which, in the primary auditory cortex (PAC), occurs identically in both hemispheres in Heschl's gyrus, with bilateral ear representation. Thus a unilateral intervention may modulate this frequency range, with results being dependent on correct PAC targeting (Pérez-González and Malmierca, 2014; Gardumi et al., 2017; Yuan et al., 2018). Although promising, scientific evidence in this area requires a greater number of studies in rTMS and especially in TBS in order to ensure patient hearing safety, particularly relevant in the ear closer to the coil, and to identify the most effective protocols to effectively intervene.

With this TBS sham-controlled study in a group of healthy young adults, we aimed to assess ipsilateral hearing safety after TBS exposure over the left PAC and also to evaluate the effects of both iTBS and cTBS over the ipsilateral hearing thresholds.

2. Methods

2.1. Subjects and study design

Sixty healthy adults agreed to participate in this prospective double-blind sham-controlled study, recruited among students enrolled at the Faculty of Health Sciences, University of Beira Interior (FHS-UBI), Covilhã, Portugal. After answering a confidential screening questionnaire, students were included in the study if they met the following inclusion criteria: age between 18 and 35 years with no hearing complaints. Exclusion criteria were as follows: altered initial pure tone audiometry, previous ear diseases, tinnitus or other hearing related complains, brain injury or suspected diagnosis of organic brain damage, previous severe head trauma, epilepsy or convulsions, presence of major medical illness (including neuropsychiatric diseases), recent intake of any drugs or medication, pregnancy, implanted devices or foreign metal articles in the head or chest areas, sleep deprivation, alcoholism and history of drug abuse (Rossi et al., 2009). Participants were instructed to rest as usual, avoid being exposed to excessive noise and avoid taking alcoholic beverages or other toxic/stimulant substances 24 hours prior to the application of the technique.

Volunteers were randomly allocated to three equally sized separate groups according to stimulation type: intermittent TBS group (iTBS group), continuous TBS group (cTBS group), and sham group (placebo stimulation), with 20 volunteers per group. A sealed envelope randomisation protocol was used.

After being fully informed about all procedures, subjects signed a written informed consent and anonymity was ensured. Study protocols were approved by the Faculty of Health Sciences-UBI Ethics Committee (CE-FCS-2011-001), in conformity with the Declaration of Helsinki.

2.2. Theta burst stimulation (TBS)

TMS protocols were performed in accordance with safety and ethics recommendations of the 2009 guidelines (Rossi et al., 2009), in the FHS-UBI TMS laboratory, using a MagVenture MagPro1G3 X100 5.0.1, with a Dantec™ Keypoint.net v.2.03 for motor threshold determination. All procedures were carried out under medical supervision. Using a butterfly coil MCF-B70, stimuli were applied according to classic TBS protocols, using biphasic pulses, with a total of 600 pulses sent in 3 pulse bursts, repeated at 5 Hz, either in iTBS or cTBS. In continuous mode, bursts occurred without interruption for 40 seconds, and in the intermittent mode bursts were delivered only for 2 seconds (sets of 10 bursts), repeated every 10 seconds for a total of 190 seconds (Huang et al., 2005). Coil handle was positioned parallel to the midline (Lefaucheur et al., 2012).

Stimulation target site was the left primary auditory cortex and it was found for each individual: in order to set the stimulation coil over the left PAC, a procedure based on the 10/20 international system for electrode placement was used to find that specific site. Starting from the T3 position, we measured 2.5 cm towards Cz (following the coronal plane) and then measured 1.5 cm posteriorly, perpendicular to the plane T3-Cz (Langguth et al., 2006; Lorenz et al., 2010; Minami et al., 2011; Schecklmann et al., 2011). The primary motor cortex (PMC) was used as a marker for stimulus intensity and was identified by the single pulse vs visible thumb-twitch relation. Active motor threshold (AMT) was defined as the lowest stimulation intensity over the left PMC capable of inducing a consistent contralateral *abductor pollicis brevis* (APB) motor evoked potential (150–200 μV), while maintaining minimal voluntary contraction, on more than half of the pulses applied (Huang et al., 2005; Di Lazzaro et al., 2008; Sandrini et al., 2011). For real stimulation over the left PAC, intensity was defined as 80% AMT. For sham stimulation, the same coil was used, maintaining scalp contact, using a 90-degree tilted position (magnetic field pointing downwards), also emitting sound of randomly cTBS or iTBS, simulating actual stimulation, even though this technique is not capable of effective neural activation (Di Lazzaro et al., 2008; Rossi et al., 2009; Sandrini et al., 2011). Subjects were instructed to use disposable earplugs (Ohropax® Germany - noise reduction rating of 22–27 dB; 125–8000 Hz) during active or sham stimulation. All volunteers were relaxed, seating in a comfortable reclining armchair

during active or sham stimulation and stimuli application was always performed by the same technician.

2.3. Threshold audiometry

Audiological measurements were implemented in a noise isolated room. Standard pure tone audiometry was performed using a calibrated clinical screening audiometer – MAICO Audiometer GmbH®, ST 20 model (steps of 10 dB) – evaluating the following frequencies: 250 Hz, 500 Hz, 1000 Hz, 2000 Hz, 3000 Hz, 4000 Hz, 6000 Hz and 8000 Hz. The audiometry protocol followed the Guidelines for Manual Pure-Tone Threshold Audiometry from the American Speech-Hearing-Language Association (ASHA) (Campbell et al., 2005).

2.4. Experimental design

Preceding every volunteer participation, procedure explanation was presented and informed consent was obtained, followed by audiological evaluations and TMS stimulation. Therefore, each experimental session comprised two audiometric evaluations of the left ear per subject (stimulation ipsilateral ear), before and after real or sham stimulation: a pre-TBS audiometry and a post-TBS audiometry. TBS or sham protocols were applied immediately after the first audiometry, following the protocol mentioned earlier (AMT determination and PAC stimulation). In order to standardise procedures and minimise changes in immediate TBS sound impact, the second audiometry always occurred 5 minutes after the end of the TBS/Sham stimulation. All experimental sessions took place at the same time of the day and each volunteer was subjected to only one session of real or sham TBS over the left PAC, according to his/her previous randomised group allocation. Both volunteer and team member that performed the audiometry were blind to the stimulation type used (cTBS, iTBS or Sham). Audiometries and stimulation sessions were always performed in two completely separate rooms, and only the team member in charge of performing the TBS/Sham session was aware of the actual stimulation type (sham, iTBS or cTBS) applied to each volunteer. The researcher responsible for the audiometry had no information about what type of stimulation was performed. None of the volunteers had been previously submitted to rTMS/TBS and was not aware of the stimulation type performed, thus contributing to the blinding method success.

In order to assess volunteer safety and control eventual side effects, a follow-up of at least 48 hours was implemented (focusing on self-reported unwanted effects).

2.5. Statistical analysis

Statistical analysis was performed with IBM® SPSS Statistics® 25.0, using a mixed-design repeated measures ANOVA. ANOVA assumptions were verified using the Shapiro-Wilk normality test and the Levene test, allowing the latter to evaluate homogeneity variance. Due to sample size and the fact that the normality assumption was not validated, analysis was also performed with a non-parametric version of repeated measures ANOVA (Nonparametric Longitudinal Data in Factorial Experiments, with the "nparLD" package, version 2.1, for the statistical program R). However, since results obtained with both analyses were similar and compatible, we opted to present only the results obtained by the parametric version, which can be more easily interpreted. Only one repeated measures ANOVA with a single repetition (post) and one factor (group) was used. There is no random effect other than that of the volunteers. For comparisons between the intensity average pairs of the iTBS, cTBS and Sham groups or for the pre and post TBS, the Sidak test (Student's t-test for independent samples or paired – Least Significant Difference-Sidak's correction) was used for each group. Hypotheses tests were considered significant when test value (p-value, p) did not exceed the significance level of 5% ($p < 0.05$).

3. Results

Among the 60 volunteers (median age of 23 years) who agreed to participate in this study, 44 (73.3%) were females aged 19–28 years and males between 21 and 32 years. Sex and age distributions showed no significant differences (Mann-Whitney U, $p = 0.773$), with a median age of 23 years in both sexes and similar averages of 23.10 years (SD = 1.96 years) and 23.87 years (SD = 2.85 years) for the female and male sex, respectively.

The iTBS group consisted of 14 females and 6 males; in the other two groups, distribution consisted of 15 females and 5 males. Age distribution of the three groups was not significantly different (Kruskal-Wallis, $p = 0.273$), the age medians in the three groups were 23 years and averages were approximately 23.30 years (SD = 0.73 years), 23.85 years (SD = 3.03 years) and 22.65 years (SD = 2.23 years) in the iTBS, cTBS and Sham groups, respectively.

Due to the reduced number of male volunteers in each group, it was decided not to consider the influence of sex on the variables related to the audiometry.

During the stimulation procedure, no incidents occurred and in the 48 h hour follow-up, only two volunteers reported mild focal discomfort related to the cTBS stimulation site and one volunteer submitted to iTBS mentioned a mild headache. No other major adverse events were reported and none of the volunteers dropped out.

Pre-TBS audiometry and post-TBS audiometry mean threshold intensities per stimulation group are shown in Fig. 1.

Regardless of the group, mean auditory thresholds of all the volunteers evaluated for the range of frequencies tested showed that the highest threshold was found at 250 Hz (17.33 ± 7.78 dB) and the best (lowest) thresholds were found between 2000 Hz (9.17 ± 9.49 dB) and 6000 Hz (3.50 ± 6.84 dB), as previously described in other studies (Johnson, 2012). Fig. 1 also seems to show that group behaviour was not similar, with the iTBS group results displaying a trend towards a threshold decrease after stimulation, mainly for the 500 Hz–6000 Hz interval (mean difference between −2 and −4dB), except for 8000 Hz, in which there was a slight increase (+0.5 dB). The cTBS group showed mixed results, with slight threshold increases in 2000 Hz and 8000 Hz (mean difference between +0.5 and +2 dB), slight decreases after stimulation in the 250 Hz, 500 Hz, 1000 Hz and 3000 Hz (mean difference between −0.5 and −2.5 dB), and unaltered thresholds in the 4000 Hz and 6000 Hz. We can also observe that the sham group did not show a clear trend, with variations between 0.5 and 1.5 dB, except for the 8000 Hz, in which there was a decrease (−4.5 dB). Moreover, it should be noted that when an increased threshold occurred after active TBS or sham, these variations were of small degree.

Table 1 presents the stimulation effect and interaction regarding group type (repeated measures ANOVA) and Table 2 shows the pre-TBS audiometry and post-TBS audiometry mean difference for each group (iTBS, cTBS, and sham).

Pre-post iTBS group threshold mean difference showed statistically significant differences in frequencies between 500 Hz and 4000 Hz (500 Hz $p < 0.001$; 1000 Hz $p = 0.026$; 2000 Hz $p = 0.005$; 3000 Hz $p = 0.004$; 4000 Hz $p = 0.004$), with lower thresholds after stimulation. No significant differences were found in cTBS group threshold mean differences. Sham group results showed no statistically significant differences between 250 Hz and 6000 Hz. How-

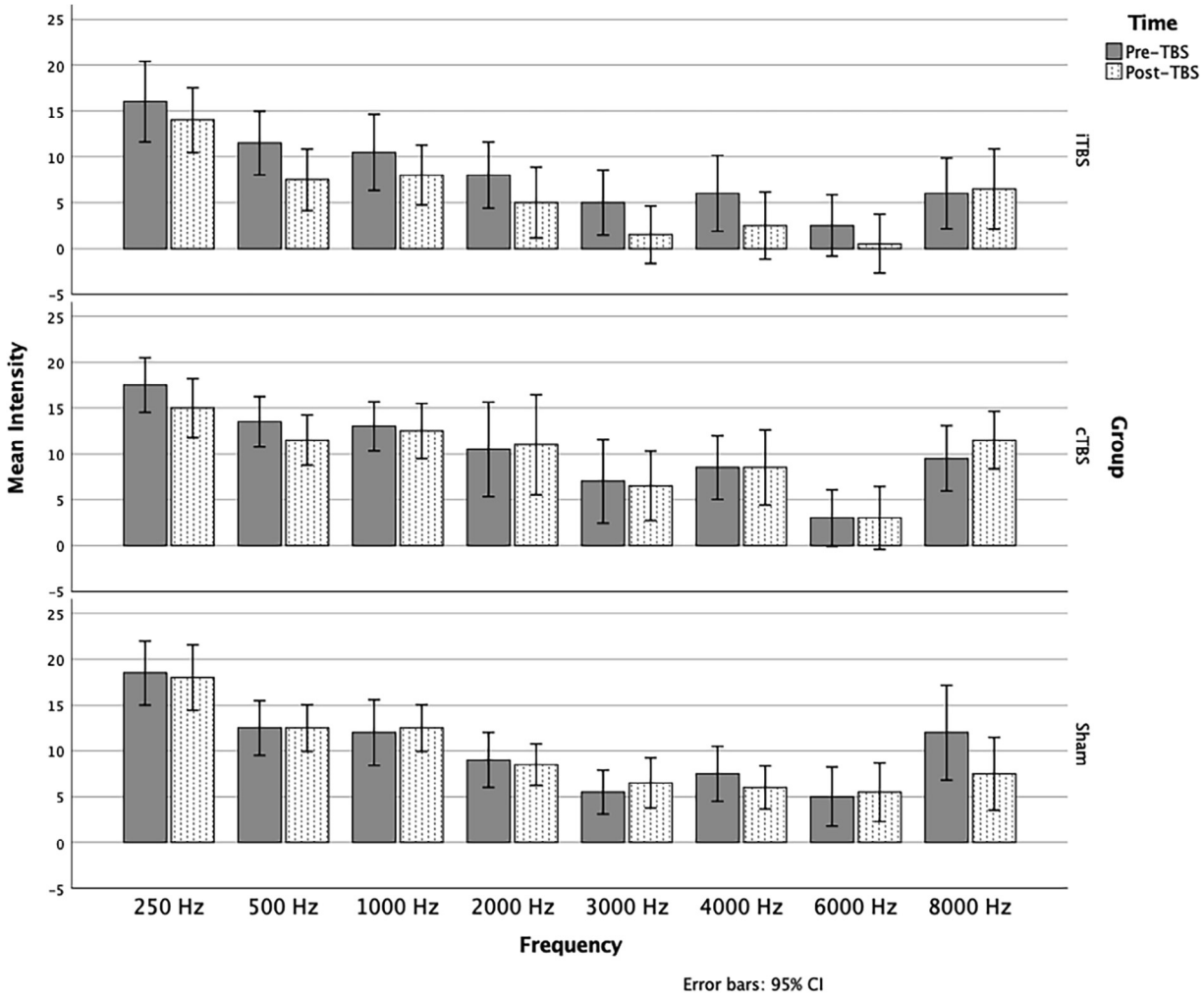

Fig. 1. Audiometry mean threshold intensities (dB HL) per frequency (Hz) and stimulation group.

Table 1
Stimulation effect and interaction versus group type – univariate repeated measures ANOVA.

Frequency (Hz)	Pre vs post TBS *p-value*	Between groups effect *p-value*	Interaction stimulation-group *p-value*
250	**0,025**	0.349	0,506
500	**0,001**	0.197	**0,028**
1000	0,192	0.193	0,152
2000	0,098	0.280	0,055
3000	0,140	0.248	**0,026**
4000	**0,015**	0.169	0,107
6000	0,470	0.187	0,298
8000	0,489	0.197	**0,019**

$p < 0.05$.

ever, results in the 8000 Hz of the sham group revealed a significant difference (p = 0.009).

Group comparisons between the pre-TBS audiometry and post-TBS audiometry mean differences are shown in Table 3.

Baseline audiometry records showed no statistically significant differences between the iTBS, cTBS and sham groups, at any of the evaluated frequencies, thereby revealing no inconsistencies between groups at baseline.

On the other hand, post-stimulation results showed statistically significant mean differences between the iTBS and sham groups for 500 Hz (p = 0.041) and also between the cTBS and iTBS groups for 4000 Hz (p = 0.038).

As can be seen in Table 3, and also in Fig. 1, none of the stimulated groups had a significant worsening of the mean threshold, after active or sham stimulation, supporting that the technique is safe to use, as long as you use adequate protection.

4. Discussion

Our study, using a sham-controlled protocol, revealed neither relevant side effects nor any significant hearing threshold impairment of the ipsilateral ear after iTBS, cTBS or sham stimulation over the PAC, thereby contributing to better understanding the possible safety limitations in these protocols. Further analysis showed that iTBS seems to have a greater capacity to influence hearing thresholds when compared with cTBS and sham stimulation, resulting in lower thresholds after stimulation between 500 Hz and 4000 Hz. Direct group comparison showed significantly lower thresholds at 500 Hz after iTBS compared to Sham and at 4000 Hz also after iTBS compared to cTBS stimulation. Our data suggest that this specific TBS method can be a safe approach to influence hearing sensitivity through non-invasive neurostimulation.

One of our main objectives was to assess hearing safety of the ipsilateral ear after exposure to one session of TBS over the left primary auditory cortex. Even though rTMS and TBS stimulation may involve some health side effects, they are considered safe tech-

Table 2
Audiometry results: pre-TBS vs post-TBS mean difference for each group.

	iTBS group		cTBS group		Sham group	
Frequency (Hz)	Intensity: mean diff. pre-post (dB HL)	p-value[1]	Intensity: mean diff. pre-post (dB HL)	p-value[1]	Intensity: mean diff. pre-post (dB HL)	p-value[1]
250	2.0	0.116	2.5	0.051	0.5	0.691
500	4.0	**<0.001**	2.0	0.056	0.0	1.000
1000	2.5	**0.026**	0.5	0.649	−0.5	0.649
2000	3.0	**0.005**	−0.5	0.629	0.5	0.629
3000	3.5	**0.004**	0.5	0.668	−1.0	0.392
4000	3.5	**0.004**	0.0	1.000	1.5	0.198
6000	2.0	0.098	0.0	1.000	−0.5	0.676
8000	−0.5	0.763	−2.0	0.231	4.5	**0.009**

$p < 0.05$.
[1] LSD test with Sidak's correction.

Table 3
Group comparisons: mean differences between the pre-TBS audiometry and post-TBS audiometries.

Frequency (Hz)	Intensity: mean diff. cTBS-iTBS	p-value[1]	Intensity: mean diff. cTBS-Sham	p-value[1]	Intensity: mean diff. iTBS-Sham	p-value[1]
250[i]	1.5	0.908	−1.0	0.970	−2.5	0.683
250[f]	1.0	0.964	−3.0	0.495	−4.0	0.251
500[i]	2.0	0.715	1.0	0.951	−1.0	0.951
500[f]	4.0	0.134	−1.0	0.942	−5.0	**0.041**
1000[i]	2.5	0.655	1.0	0.966	−1.5	0.897
1000[f]	4.5	0.082	0.0	1.000	−4.5	0.082
2000[i]	2.5	0.739	1.5	0.927	−1.0	0.977
2000[f]	6.0	0.098	2.5	0.748	−3.5	0.506
3000[i]	2.0	0.802	1.5	0.904	−0.5	0.996
3000[f]	5.0	0.079	0.0	1.000	−5.0	0.079
4000[i]	2.5	0.663	1.0	0.967	−1.5	0.900
4000[f]	6.0	**0.038**	2.5	0.641	−3.5	0.363
6000[i]	0.5	0.994	−2.0	0.740	−2.5	0.587
6000[f]	2.5	0.603	−2.5	0.603	−5.0	0.082
8000[i]	3.5	0.539	−2.5	0.770	−6.0	0.118
8000[f]	5.0	0.171	4.0	0.344	−1.0	0.974

$p < 0.05$.
[i] Pre-stimulation.
[f] Post-stimulation.
[1] LSD test with Sidak's correction.

niques, and major risks when applying these techniques following accepted safety protocols are negligible, both in children and adults (Rossi et al., 2009; Oberman et al., 2011; Kukke et al., 2017). Special attention should be taken when undergoing PAC stimulation because secondary effects can occur both from neural stimulation and from noise related to rTMS at higher intensities (Rossi et al., 2009). Although higher stimulation can achieve the 120 dB SPL threshold, thereby risking exposure to excessive noise and possible sensorineural hearing loss, sound levels usually do not go above 60–70 dB SPL (Schraven et al., 2013). While some studies found hearing impairment related to cochlear effects (Tringali et al., 2012), some tinnitus patients reported worsening of hyperacusis after rTMS (Rossi et al., 2009), or headaches, tinnitus worsening and increased sensitivity to noise after TBS (Plewnia et al., 2012), other studies did not report any hearing decline or significant complaint after 20 sessions of TBS stimulation (Schraven et al., 2013). The temporal cortex is not a frequent location where to apply TBS, but mixed results concerning secondary effects after few stimulation sessions in that location can be found. Poreiz et al., used a 3 session TBS (iTBS + cTBS + imTBS) protocol in tinnitus patients and reported complaints of discomfort, headaches and three patients suffered a worsening in tinnitus-related complaints (Poreisz et al., 2009). On the other hand, De Ridder et al., applied one session of modified cTBS in 46 tinnitus patients and reported no significant side effects (De Ridder et al., 2007). Our results showed neither significant global threshold increase after either iTBS, cTBS or sham stimulation at all tested frequencies, nor other relevant side effects (such as tinnitus or perceived hearing loss). These results are particularly relevant since our objective was to test the ipsilateral ear immediately after stimulation, in order to evaluate the ear closest to the stimulation coil and more likely to reveal any changes linked to excessive noise from the coil. The fact that there was no threshold worsening, none of the volunteers mentioned any hearing related complaints and none of the volunteers dropped out, suggests that TBS stimulation over PAC can be a safe procedure if safety guidelines are followed. Furthermore, we observed an encouraging trend towards threshold reduction in some frequencies with iTBS. In terms of side effects, these can be considered negligible (Rossi et al., 2009), because our volunteers described only two cases of mild focal discomfort and one case of mild headache related to active stimulation. Our data clearly support the scarce information to date that TBS can be a safe technique when applied to PAC, suggesting good auditory tolerance.

The other main objective focused upon the study of the auditory effects of both iTBS and cTBS in ipsilateral hearing thresholds using a placebo-controlled protocol, after stimulating the left PAC. Regarding auditory information processing, evaluated by positron emission tomography or functional magnetic resonance imaging, it is important to mention the apparent existence of a left hemisphere dominance, either at rest (Geven et al., 2014) or after auditory stimulation of only one (pure-tone or speech) (Millen et al., 1995) or both ears (Bernal et al., 2004). Thus, we opted to primarily

stimulate the left cortex because it seemed to be the hemisphere in which we would probably have the higher chance of influencing hearing capabilities, either in terms of improving the hearing thresholds, or in terms of inducing a negative change related to the stimulation procedure. TMS modulatory capacity has been tested and proven in auditory related research. Inhibitory properties using the temporal or temporoparietal cortices were found in studies using schizophrenic patients with auditory hallucinations (reduction) and especially studying patients with tinnitus stimulating the hypermetabolic areas (PAC stimulation for tinnitus reduction) (Langguth et al., 2006). TBS experiments, though scant and mostly using cTBS protocols, have been used to treat or improve tinnitus symptoms, namely hearing thresholds, but results have been controversial. Positive results using one stimulation session over the auditory cortex (De Ridder et al., 2007) and 20 sessions over the auditory temporoparietal cortex (Soekadar et al., 2009) showed improved tinnitus symptoms. In contrast, Poreisz et al., and Plewnia et al., showed no significant positive results when stimulating the temporoparietal cortex, but concluded that the protocols used were safe (Poreisz et al., 2009; Plewnia et al., 2012). Disparities between stimulation protocols and study designs can explain these results as some studies did not use placebo/sham-controlled designs and used diverse protocols – different session numbers, different stimulation locations and slight differences in used intensities (De Ridder et al., 2007; Poreisz et al., 2009; Soekadar et al., 2009; Plewnia et al., 2012). Evaluation of our results revealed a statistically significant reduction in mean hearing thresholds between the 500 Hz and 4000 Hz interval for the iTBS group after stimulation. However, for the 250 Hz, 6000 Hz and 8000 Hz frequencies, there was no significant change in the thresholds. Since we are working with groups with a relatively small number of volunteers per group and our audiometer only operates in 10 dB steps, these results should be evaluated in direct comparison with the cTBS and Sham groups. Group comparison using Sidak test showed significantly lower thresholds only at 500 Hz ($p = 0.041$) comparing iTBS vs Sham groups (mean threshold in the Sham group remained stable and the iTBS threshold significantly decreased) and at 4000 Hz ($p = 0.038$) when comparing iTBS vs cTBS groups (mean threshold in the cTBS group remained unaltered and iTBS threshold significantly diminished). In addition to highlighting the safety of the technique, these results suggest that iTBS may influence auditory capability, by specifically decreasing auditory thresholds at some frequencies. Cortical modulation processes and respective synaptic hearing circuits are still to be clarified in their totality; however, our results can be understood on the basis of probable neuron modulation in distinct zones of the tonotopic map of the primary auditory cortex. Since the observed significant changes are located at different frequencies but mostly between 500 Hz and 6000 Hz, they are also supported by the hypothesis of stimulated neurons integrating different sound frequencies among themselves. In the PAC, tonotopic organisation manifests itself equally in both hemispheres, in the form of two gradients in Heschl's gyrus, which make up a pattern of high, low and high frequencies again (Gardumi et al., 2017; Yuan et al., 2018). Even if the stimulation coil has a slight target area deviation, it is believed that this can be within an acceptable margin of error because it is known that figure-8 coils can produce a magnetic field directly over an area extending around 3 cm of length and 2 cm of width (Langguth et al., 2006). Thus, we can consider that even if there are some deviations, stimulation will still be performed in PAC although probably more focused on low to mid frequency areas. This would account for more effective results seen with some frequencies but not with all. Perhaps results would be more homogeneous if a neuronavigation system was be used to identify the target zone, thus promoting a more accurate stimulation of the PAC. Another limitation when analysing these results is that it is still unclear what the underlying mechanisms mediating potential iTBS benefits are, since they can be explained by more than one hypothesis. A possible hypothesis for the results is that of the cortical plasticity aptitude: functional magnetic resonance imaging (fMRI) studies have demonstrated that the auditory cortex has the capacity to reorganise and change its expression of excitatory and inhibitory neurotransmitters (Zhang and Ma, 2015). Neurotransmitter modulation by transcranial magnetic stimulation is a known outcome, namely in the upregulation (increased levels) of the excitatory neurotransmitter glutamate related to excitatory stimulation (Yang et al., 2014; Croarkin et al., 2016; Dlabac-de Lange et al., 2017), or in the down-regulation in glutamate in the left hemisphere, directly linked to a reduced loudness level of tinnitus awareness, found by Cacace et al., These authors used a 5 day inhibitory protocol over the left auditory cortex (Cacace et al., 2018). Even though we cannot confirm these theories, we believe they may explain some of our data. Our findings also support some previous studies reporting that excitatory stimulation can influence hearing, namely the work by Andoh et al., in which 10 Hz rTMS applied to the Heschl's gyrus originated improved auditory performance, but only in females (Andoh and Zatorre, 2011).

Results for the cTBS group showed that, for all frequencies evaluated, no significant statistical change ($p > 0.05$) was found in hearing thresholds after stimulation. This lack of significant results is still observed when we compare the mean differences of the cTBS group with the iTBS and Sham groups, highlighting the frequent variations in the thresholds, either decreasing or increasing. These results suggest that a single session of the cTBS protocol used in this investigation does not significantly modulate neuronal auditory activity. This indicates that effects and possible efficacy of iTBS and cTBS techniques may be distinct, at least when applied over the auditory cortex. The result for 8000 Hz obtained in the group undergoing sham stimulation, failed to reach statistical significance when compared with the results of the excitatory and inhibitory groups - thus, its significance may be negligible. The reasons that led to a threshold decrease at 8000 Hz in the sham group may only be speculated, but a placebo effect is a possibility, mainly because it is a very high frequency in the auditory spectrum, which is therefore more difficult to assess. Several studies (Chan, 2014; Požgain et al., 2014; Morral et al., 2017) support the multifactorial nature inherent to the mechanisms underlying the placebo effect; however, in this specific case, it is possible that the main mechanism may be related to an intrinsic expectation that the volunteers had that TBS could improve their hearing capabilities.

Future work should focus upon the study not only of the ipsilateral thresholds but also of the contralateral ones. Ipsilateral vs contralateral dominance and which side can be the most effective for auditory cortex stimulation can be a controversial issue because there are several contradictory studies. For instance, some studies in tinnitus gave stimulation primacy to the left cortex independently of the complaints being lateralized to the right side, to the left side or bilateral. In contrast, other studies reported better results when stimulating the contralateral cortex related to the existing complaints (Khedr et al., 2010; Lefaucheur et al., 2012; Zhang and Ma, 2015). Another limitation to our rationale is that we do not know how long iTBS effects last. It would still be interesting to study whether two daily sessions, separated by at least 15 minutes (Tse et al., 2018), could have an enhanced or more prolonged effect. It should also be mentioned that although some studies use increasing stimulus intensity, our objective was to comply with standard safety guidelines, even more so because stimulation outside the primary motor area may yield some inaccuracies related to distance-adjusted intensities (Stokes et al., 2005).

As for study design, despite all participants officially declaring that they did not take any drugs, we were not able to include in

our protocols a screening test to evaluate their presence, thus limiting our control over this experiment. Even though our design was simple and all our subjects were completely naïve regarding stimulus characteristics and effects, we should also have included a blinding assessment in order to increase result reliability.

In this area, very few studies used TBS, and most studies used the technique mainly in patients, neglecting its effects in normal healthy volunteers. No study focused on the use of both iTBS and cTBS, comparing the results with sham stimulation. Our study protocol is therefore unique and to the authors' knowledge this is the first approach to a healthy volunteer placebo-controlled research using both cTBS and iTBS over the left PAC, showing diverse effects between these two stimulation modalities, thus contributing to a better understanding of this type of noninvasive neurostimulation over the auditory cortex. Our results using only a single session point to an effective neuromodulation of the PAC, reflected in lower thresholds when using iTBS. It can be assumed that several sessions could be more effective, as most of the protocols that formed the basis of various previous studies applied several sessions (between 3 to 20 sessions) (Loo et al., 2001; Khedr et al., 2008, 2010; Plewnia et al., 2012; Barwood et al., 2013; Schraven et al., 2013; Zhang and Ma, 2015; Cacace et al., 2018), as is currently used in depression therapy. It is also noteworthy that threshold improvement occurred manly around the human speech/voice frequency range (500–2000 Hz) (Williams et al., 2005; Anjos et al., 2014). This possible hearing improvement in the low to mid frequency range can be particularly important if similar stimulation protocols can be used in sensorineural hearing loss, which is often attributed to hereditary factors and congenital conditions (Shah et al., 2005), specifically if trying to enhance patient speech perception. These interventions may aim to improve life quality in patients; however, this possible use for TBS should be approached carefully, after replication of this method in further studies with a larger number of healthy subjects and, finally, after patient investigation.

5. Conclusions

iTBS, a safe, non-invasive neuromodulation tool has the potential to positively influence hearing thresholds in healthy young adults. The same iTBS protocols amy be reproduced in older adults with minor sensorineural hearing loss, presbycusis or other hearing loss cases. This would allow improvement of patients' hearing capacity by modulating PAC to become more sensitive to the auditory stimuli, thereby helping patients to improve their auditory assessment of the world and increasing patients' quality of life.

Author contributionsss

N.P. and M.V.P conceived, supervised all work and wrote the main manuscript text. N.P., I.O., J.F., conducted the experiment(s). Statistical analysis conducted by J.G. All authors analysed the results and reviewed the manuscript.

Data availability

Data sets analysed during the current study are available on request.

Declaration of Competing Interest

There are no conflicts of interest and the authors have not received any specific grant.

Acknowledgements

The authors thank Prof. Luís Taborda Barata for the support and writing assistance. We also thank all volunteers for participating in our study.

References

Andoh J, Zatorre RJ. Interhemispheric connectivity influences the degree of modulation of TMS-induced effects during auditory processing. Front Psychol 2011;2:1–13.
Anjos WT, Labanca L, de Resende LM, Costa-Guarisco LP. Correlation between the hearing loss classifications and speech recognition. Cefac 2014;16:1109–16.
Bakker N, Shahab S, Giacobbe P, Blumberger DM, Daskalakis ZJ, Kennedy SH, et al. rTMS of the dorsomedial prefrontal cortex for major depression: safety, tolerability, effectiveness, and outcome predictors for 10 Hz versus intermittent theta-burst stimulation. Brain Stimul 2015;8:208–15.
Barwood CHS, Wilson WJ, Malicka AN, McPherson B, Lloyd D, Munt K, et al. The effect of rTMS on auditory processing in adults with chronic, bilateral tinnitus: a placebo-controlled pilot study. Brain Stimul 2013;6:752–9.
Bernal B, Altman NR, Medina LS. Dissecting nonverbal auditory cortex asymmetry: an fMRI study. Int J Neurosci 2004;114:661–80.
Blumberger DM, Vila-Rodriguez F, Thorpe KE, Feffer K, Noda Y, Giacobbe P, et al. Effectiveness of theta burst versus high-frequency repetitive transcranial magnetic stimulation in patients with depression (THREE-D): a randomised non-inferiority trial. Lancet 2018;391:1683–92.
Cacace AT, Hu J, Romero S, Xuan Y, Burkard RF, Tyler RS. Glutamate is down-regulated and tinnitus loudness-levels decreased following r TMS over auditory cortex of the left hemisphere: A prospective randomized single-blinded sham-controlled cross-over study. Hear Res 2018;358:59–73.
Campbell J, Graley J, Meinke D, Vaughan L, Aungst RMT. Guidelines for manual pure-tone threshold audiometry Available from. ASHA 2005. http://www.asha.org/policy/GL2005-00014/.
Cárdenas-Morales L, Nowak Da, Kammer T, Wolf RC, Schönfeldt-Lecuona C. Mechanisms and applications of theta-burst rTMS on the human motor cortex. Brain Topogr 2010;22:294–306.
Chan TE. Regulating the placebo effect in clinical practice. Med Law Rev 2014;23:1–26.
Clavagnier S, Thompson B, Hess RF. Long lasting effects of daily theta burst rTMS sessions in the human amblyopic cortex. Brain Stimul 2013;6:860–7.
Croarkin PE, Nakonezny PA, Wall CA, Murphy LL, Sampson SM, Frye MA, et al. Transcranial magnetic stimulation potentiates glutamatergic neurotransmission in depressed adolescents. Psychiatry Res Neuroimaging 2016;247:25–33.
De Ridder D, van der Loo E, Van der Kelen K, Menovsky T, van de Heyning P, Moller A. Theta, alpha and beta burst transcranial magnetic stimulation: brain modulation in tinnitus. Int J Med Sci 2007;4:237–41.
Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P, et al. The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. J Physiol 2008;586:3871–9.
Dlabac-de Lange JJ, Lieburg EJ, Bais L, van de Poel-Mustafayeva AT, de Lange-de Klerk ESM, Knegtering H, et al. Effect of bilateral prefrontal rTMS on left prefrontal NAA and Glx levels in schizophrenia patients with predominant negative symptoms: an exploratory study. Brain Stimul 2017;10:59–64.
Gardumi A, Ivanov D, Havlicek M, Formisano E, Uludağ K. Tonotopic maps in human auditory cortex using arterial spin labeling. Hum Brain Mapp 2017;38:1140–54.
Geven LI, de Kleine E, Willemsen ATM, van Dijk P. Asymmetry in primary auditory cortex activity in tinnitus patients and controls. Neuroscience 2014;256:117–25.
Huang Y-Z, Chen R-S, Rothwell JC, Wen H-Y. The after-effect of human theta burst stimulation is NMDA receptor dependent. Clin Neurophysiol 2007;118:1028–32.
Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron 2005;45:201–6.
Huang Y-Z, Rothwell JC, Lu C-S, Wang J, Weng Y-H, Lai S-C, et al. The effect of continuous theta burst stimulation over premotor cortex on circuits in primary motor cortex and spinal cord. Clin Neurophysiol 2009;120:796–801.
Jäncke L, Wuestenberg T, Schulze K, Heinze HJ. Asymmetric hemodynamics responses of the human auditory cortex to monaural and binaural stimulation. Hear Res 2002;170:166–78.
Johnson K. Acoustic and auditory phonetics. Third. Chichester, West Sussex: Wiley-Blackwell; 2012.
Khedr EM, Rothwell JC, Ahmed MA, El-Atar A. Effect of daily repetitive transcranial magnetic stimulation for treatment of tinnitus: comparison of different stimulus frequencies. J Neurol Neurosurg Psychiatry 2008;79:212–5.
Khedr EM, Abo-Elfetoh N, Rothwell JC, El-Atar A, Sayed E, Khalifa H. Contralateral versus ipsilateral rTMS of temporoparietal cortex for the treatment of chronic unilateral tinnitus: comparative study. Eur J Neurol 2010;17:976–83.
Kukke SN, Brewer CC, Zalewski C, King KA, Damiano D, Alter KE, et al. Hearing safety from single- and double-pulse transcranial magnetic stimulation in children and young adults. J Clin Neurophysiol 2017;34:340–7.
Langguth B, Zowe M, Landgrebe M, Sand P, Kleinjung T, Binder H, et al. Transcranial magnetic stimulation for the treatment of tinnitus: a new coil positioning method and first results. Brain Topogr 2006;18:241–7.
Lefaucheur J-P, Brugières P, Guimont F, Iglesias S, Franco-Rodrigues A, Liégeois-Chauvel C, et al. Navigated rTMS for the treatment of tinnitus: A pilot study

with assessment by fMRI and AEPs. Neurophysiol Clin Neurophysiol 2012;42:95–109.
Lefaucheur J-P, André-Obadia N, Antal A, Ayache SS, Baeken C, Benninger DH, et al. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). Clin Neurophysiol 2014;125:2150–206.
Loo C, Sachdev P, Elsayed H, McDarmont B, Mitchell P, Wilkinson M, et al. Effects of a 2- to 4-week course of repetitive transcranial magnetic stimulation (rTMS) on neuropsychologic functioning, electroencephalogram, and auditory threshold in depressed patients. Biol Psychiatry 2001;49:615–23.
Lorenz I, Müller N, Schlee W, Langguth B, Weisz N. Short-term effects of single repetitive TMS sessions on auditory evoked activity in patients with chronic tinnitus. J Neurophysiol 2010;104:1497–505.
Mennemeier M, Munn T, Allensworth M, Lenow JK, Brown G, Allen S, et al. Laterality, frequency and replication of rTMS treatment for chronic tinnitus: pilot studies and a review of maintenance treatment. Hear Res 2013;295:30–7.
Millen SJ, Haughton VM, Yetkin Z. Functional magnetic resonance imaging of the central auditory pathway following speech and pure-tone stimuli. Laryngoscope 1995;105:1305–10.
Minami SB, Shinden S, Okamoto Y, Watada Y, Watabe T, Oishi N, et al. Repetitive transcranial magnetic stimulation (rTMS) for treatment of chronic tinnitus. Auris Nasus Larynx 2011;38:301–6.
Morral A, Urrutia G, Bonfill X. Efecto placebo y contexto terapéutico: un reto en investigación clínica. Med Clin (Barc) 2017;149:26–31.
Mulders WHAM, Vooys V, Makowiecki K, Tang AD, Rodger J. The effects of repetitive transcranial magnetic stimulation in an animal model of tinnitus. Sci Rep 2016;6:38234.
Oberman L, Edwards D, Eldaief M, Pascual-Leone A. Safety of theta burst transcranial magnetic stimulation: a systematic review of the literature. J Clin Neurophysiol 2011;28:67–74.
Pérez-González D, Malmierca MS. Adaptation in the auditory system: an overview. Front Integr Neurosci 2014;8:1–10.
Plewnia C, Vonthein R, Wasserka B, Arfeller C, Naumann A, Schraven SP, et al. Treatment of chronic tinnitus with theta burst stimulation: A randomized controlled trial. Neurology 2012;78:1628–34.
Poreisz C, Paulus W, Moser T, Lang N. Does a single session of theta-burst transcranial magnetic stimulation of inferior temporal cortex affect tinnitus perception? BMC Neurosci 2009;10:54.
Pozgain I, Pozgain Z, Degmecic D. Placebo and nocebo effect: a mini-review. Psychiatr Danub 2014;26:100–7.
Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 2009;120:2008–39.
Sandrini M, Umiltà C, Rusconi E. The use of transcranial magnetic stimulation in cognitive neuroscience: A new synthesis of methodological issues. Neurosci Biobehav Rev 2011;35:516–36.
Schecklmann M, Volberg G, Frank G, Hadersdorfer J, Steffens T, Weisz N, et al. Paired associative stimulation of the auditory system: a proof-of-principle study. Malmierca MS, editor. PLoS ONE 2011;6. e27088.
Schönfeldt-Lecuona C, Cárdenas-Morales L, Moreno-Aguirre A, Dorn K, Langguth B, Brühl AB, et al. Effect of 1 Hz repetitive transcranial magnetic stimulation over the auditory cortex on audiometry and otoacustic emissions. Brain Topogr 2012;25:241–7.
Schraven SP, Plontke SK, Rahne T, Wasserka B, Plewnia C. Hearing safety of long-term treatment with theta burst stimulation. Brain Stimul 2013;6:563–8.
Shah RK, Blevins NH, Karmody CS. Mid-frequency sensorineural hearing loss: aetiology and prognosis. J Laryngol Otol 2005;119:529–33.
Soekadar SR, Arfeller C, Rilk A, Plontke SK, Plewnia C. Theta burst stimulation in the treatment of incapacitating tinnitus accompanied by severe depression. CNS Spectr 2009;14:208–11.
Stokes MG, Chambers CD, Gould IC, Henderson TR, Janko NE, Allen NB, et al. Simple metric for scaling motor threshold based on scalp-cortex distance: application to studies using transcranial magnetic stimulation. J Neurophysiol 2005;94:4520–7.
Tringali S, Perrot X, Collet L, Moulin A. Repetitive transcranial magnetic stimulation (rTMS) noise: A relevance for tinnitus treatment? Brain Stimul 2012;5:655–6.
Tse NY, Goldsworthy MR, Ridding MC, Coxon JP, Fitzgerald PB, Fornito A, et al. The effect of stimulation interval on plasticity following repeated blocks of intermittent theta burst stimulation. Sci Rep 2018;8:8526.
Wassermann EM, Zimmermann T. Transcranial magnetic brain stimulation: Therapeutic promises and scientific gaps. Pharmacol Ther 2012;133:98–107.
Williams GB, Kun LE, Gould HJ, Thompson JW, Stocks RMS. Hearing loss as a late complication of radiotherapy in children with brain tumors. Ann Otol Rhinol Laryngol 2005;114:328–31.
Yang X-R, Kirton A, Wilkes TC, Pradhan S, Liu I, Jaworska N, et al. Glutamate alterations associated with transcranial magnetic stimulation in youth depression. J ECT 2014;30:242–7.
Yuan G, Liu G, Wei D, Wang G, Li Q, Qi M, et al. Functional connectivity corresponding to the tonotopic differentiation of the human auditory cortex. Hum Brain Mapp 2018;39:2224–34.
Zhang D, Ma Y. Repetitive transcranial magnetic stimulation improves both hearing function and tinnitus perception in sudden sensorineural hearing loss patients. Sci Rep 2015;5:1–10.