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Steady-state auditory evoked fields reflect long-term effects of repetitive transcranial magnetic stimulation in tinnitus

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HIGHLIGHTS

- Long-term effects of rTMS on steady-state auditory evoked fields (SSAEFs) in tinnitus are unknown.
- We report that SSAEFs remained decreased for one month after rTMS.
- SSAEFs may serve as a biomarker for long-term effects of rTMS on tinnitus.

ABSTRACT

Objectives: Evidence of plastic changes in tinnitus has been demonstrated in functional brain imaging. Although repetitive transcranial magnetic stimulation (rTMS) has been shown to decrease steady-state auditory evoked fields (SSAEFs) in tinnitus, the long-term consequence remained unknown. In addition, association between plastic changes as reflected by hemispheric asymmetry and tinnitus handicap inventory (THI) before and after rTMS have not been addressed.
Methods: Twelve tinnitus patients received rTMS and 12 received sham stimulation. Another 12 healthy participants served as the normal hearing controls. Patients responded to the THI before the 1st session and at one month after the final session of rTMS/sham stimulation. Changes in brain activity were assessed by measuring SSAEFs.
Results: SSAEFs remained decreased one month after rTMS compared to before treatment, along with a significant reduction in THI score. There was no significant effect between the index of hemispheric asymmetry and THI score.
Conclusions: The current study objectively demonstrated the long-term effects of rTMS on tinnitus using SSAEFs. A longitudinal study to develop an index using SSAEFs to assess the subjective severity of tinnitus is warranted.
Significance: This study suggests the possible use of SSAEFs to assess the long-term effects of rTMS on tinnitus.

1. Introduction

Tinnitus, a symptom of unknown pathogenesis, causes various degrees of distress and prognoses. It affects about 10–15% of adults, and has a profound impact on the quality of life in 1–3% of the general population (Heller, 2003; Vio and Holme, 2005; Tunkel et al., 2014). Treatment includes a variety of modalities and/or medications, however the outcomes tend to be unsatisfactory. The pathophysiology possibly involves cochlear damage followed by functional reorganization of the auditory cortices, which in turn may lead to impaired optimization between inhibition and excitation of central auditory processing (Lockwood

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et al., 2002; Osipova et al., 2006; Leaver et al., 2016). However, the precise localization of tinnitus lesion(s) and the modulation of the auditory pathway after insults have yet to be elucidated.

Our previous studies suggested that any lesion along the neural axis may induce subsequent changes in neuroplasticity at the point above (Li et al., 2003, 2006, 2012, 2013), which can then initiate a cascade of functional reorganization. While the cochlea has generally been considered to be the most probable site of lesions causing tinnitus, it is possible that neural deficit(s) may also be noted at a higher level of the auditory system. Plastic changes within diverse areas of the central auditory pathway have been reported with various techniques of functional brain imaging in patients with tinnitus (Lockwood et al., 1998; Muhlnickel et al., 1998; Smits et al., 2007). We previously verified that steady-state auditory evoked fields (SSAEFs) are increased in patients with tinnitus (Wang et al., 2015). The fields have also been reported to be positively correlated with the grade of disturbance for tinnitus (Diesch et al., 2004, 2010). The few reports characterizing auditory brain activation may connote a central signature which may reflect previous insults and/or disease activity of tinnitus.

Low-frequency repetitive transcranial magnetic stimulation (rTMS), a non-invasive tool used to modulate neuronal activity in stimulated and/or adjacent areas of the brain (Chen et al., 1997, Siebner et al., 2003, May et al., 2007), can relieve the symptoms of tinnitus to some extent in terms of decreasing questionnaire scores for chronic tinnitus (Theodoroff and Folmer, 2013). It is thus reasonable to infer that significant differences with respect to plastic changes may be found in relevant regions of the brain for patients responsive to rTMS. However, few studies have investigated the functional organization of the central auditory pathway in patients with tinnitus following rTMS (Lorenz et al., 2010; Marcondes et al., 2010; Muller et al., 2013; Yang et al., 2013; Schecklmann et al., 2014). Although an instant (i.e. within minutes) effect of rTMS on decreasing SSAEFs in patients with tinnitus has been shown (Lorenz et al., 2010), the long-term consequences are unknown. While our previous researches showed that functional reorganization as represented by laterality indices of auditory evoked fields could predict the treatment outcomes of idiopathic sudden sensorineural hearing loss (ISSNHL) (Li et al., 2012, 2013), the association between plastic changes and inventory scales of tinnitus before as well as after rTMS has never been investigated.

Since the positive effects of rTMS could still be found months or even years after therapy in terms of reduction in questionnaire scores (Kleinjung et al., 2005, Theodoroff and Folmer, 2013), we thus hypothesized that rTMS should have a persisting effect on brain activity in tinnitus. By using magnetoencephalography (MEG), changes in brain activity were assessed by measuring SSAEFs in patients with tinnitus before and 1 month after rTMS/sham treatment. To verify the possible association between brain activity and subjective senses in the patients, the degree of distress was evaluated using the Mandarin edition of the Tinnitus Handicap Inventory (THI) (Newman et al., 1996; McCombe et al., 2001). The aim of this study was to explore the long-term effect of rTMS on auditory evoked fields in the brains of patients with tinnitus.

2. Methods

2.1. Subjects (Table 1)

The baseline characteristics and confounders of subjects are the same in either group. Twelve right-handed adult patients with chronic left (n = 2), right (n = 5), or bilateral (n = 5) tinnitus (7 males; 40–73 years of age, mean = 57 years) were treated with rTMS, and 12 right-handed adult patients with chronic left (n = 3), right (n = 5), or bilateral (n = 4) tinnitus (7 males; 40–65 years of age, mean = 54 years) served as sham controls. To represent a true sample of the larger group of tinnitus patients as a whole, the inclusion criterion was intractable tinnitus lasting for more than 6 months combined with various degrees of hearing impairment after an insult (and treatment) of ISSNHL, without history of long-term noise exposure, major co-morbidities, or medications with side effects leading to tinnitus. The patients were randomized into two groups matched in terms of age, gender, and severity of tinnitus (moderate to catastrophic grading of THI scoring). They were blinded to the type of treatment they received. THI scoring was evaluated by the same experienced audiologist in our department. Twelve right-handed healthy volunteers with normal hearing (6 males; 30–64 years of age, mean = 48 years) served as controls. The controls had normal pure tone audiometry results (≦25 dB HL for all frequencies). None of the subjects had implanted pacemakers, neurological deficits, or a history of trauma. The patients were evaluated by Mini-International Neuropsychiatric Interview questionnaire to exclude psychiatric disorders on recruitment, and completed the THI before the first session and 1 month after the final session of rTMS/sham treatment. Written informed consent was obtained from each participant, and the study protocol was approved by the Institutional Ethics and Research Committee of Cheng Hsin General Hospital and Taipei Veterans General Hospital, with the concomitant approval of the Ministry of Health and Welfare of Taiwan.

2.2. rTMS/sham procedure (Rossini et al., 2015)

A trained psychiatrist (CTL) performed rTMS using a Magstim Super Rapid Magnetic Stimulator (Magstim Company, Ltd., Wales, United Kingdom), with four booster modules equipped with a 70-mm air-cooled figure of eight-shaped coil. For the rTMS group, the coil was held by a handle lateroposteriorly to the head and oriented sagittally to the surface of the scalp (Li et al., 2016). The ear would not be covered by a wing of the coil according to such a coil orientation (Theodoroff and Folmer, 2013). For the sham group, the coil was oriented at 90° to the skull with the small side of the butterfly coil. The patients sat upright in a comfortable chair with their eyes open, and foam earplugs were used during rTMS/sham treatment to diminish the noise from the discharging coil. The stimulation site on the left primary auditory cortex, which is the same area for the measurement of SSAEFs, was identified using a navigation system (Brainlab™, Feldkirchen, Germany). Although this study includes patients with right, left, and bilateral tinnitus, the left primary auditory cortex was selected for stimulation because many previous studies succeeded in treatment of tinnitus by rTMS to stimulate this area regardless of the location of the perceived tinnitus (Rossi et al., 2007, Khedr et al., 2008, Anders et al., 2010, Marcondes et al., 2010). The importance of coil orientation has been shown to be always related to the orientation of the target cortex by one previous research (Raffin et al., 2015). To increase the accuracy and consistency of coil placement to the left primary auditory cortex, we used brain-navigation computer software and an infrared system (Brainsight, Rogue Research, Inc., Montreal, QC) to accurately guide the figure of eight-shaped coil to target the left primary auditory cortex, which was defined as the Brodmann area (BA) 41 on each patient's brain MRI (Li et al., 2010). The primary auditory cortex was targeted according to frameless stereotaxy based on patients' magnetic resonance imaging (MRI) data. First, we identify Heschl's gyrus. We then target at the inner half one of the Heschl's gyrus. Since the pulse of TMS activates a cylindrical volume (around 1 cm of radius), instead of a single point, this method should be able to target the primary auditory cortex. During the initial treatment visit, the patients of both rTMS and sham group had their motor threshold (MT) determined at rest in the contralateral (i.e. right) *abductor pollicis brevis* (APB) muscle as described previously (Pascual-Leone et al., 1992, Li et al., 2010, 2016). The patients received about 30 min of rTMS/sham treatment each weekday for five sessions during a 1-week period. They were given 1 Hz in each session at 110% of MT. In other words, 1800 pulses per session and a total of 9000 pulses were delivered throughout the five sessions of treatment.

2.3. MEG paradigm

MEG measurements were performed in a magnetically shielded room using a whole-head 306-channel neuromagnetometer (Vectorview™ 4-D Neuroimaging, Helsinki, Finland). The measurements were taken before the first session and 1 month after the final session of rTMS/sham treatment. Measurements were performed in the normal hearing controls once during the study period. The subjects sat upright with their eyes open during the measurements. Auditory stimuli [1000 Hz, amplitude-modulation (AM) frequency 37 Hz, modulation depth 100%, 180 s duration, and loudness matched individually to 50 dB SL at the exit end of the plastic tube] were delivered binaurally via molded earpieces using an analog-to-digital conversion card NI USB-6259 (National Instruments, Austin, Texas, USA) controlled by LabView™ (National Instruments, Austin, Texas, USA). Triggers were given each second and reserved for the subsequent processing of the signals. Trials with electro-oculographic amplitudes exceeding 150 μV were rejected. MEG signals were sampled at 400 Hz and band-pass filtered at 0.03 to 100 Hz. Two sessions (separated by 2 min of rest) were averaged before further analysis. An equivalent current dipole (ECD) model consisting of bilateral sources was used to explain the MEG signals (Hari and Makela, 1988). First, an initial guess of an independent source was made in both hemispheres, respectively. Each ECD was applied to a subset of 40–60 sensors around the maximum peak in one hemisphere with a goodness-of-fit (g) larger than 90% for acceptance. Since the accuracy of dipole localization depends on the signal-to-noise ratio (Jacobson, 1994), we included a sensor only when the peak amplitude of the signal was stronger than two standard deviations above the baseline. After the ECD with the highest g value was identified, all channels were taken into account for further analysis so that it best explained the recorded magnetic field globally (Hari and Makela, 1988). T1-weighted magnetic resonance imaging (MRI) of the subjects' brains were acquired using a 3.0 T Bruker MedSpec S300 system (Bruker, Kalsrube, Germany) for MEG-MRI co-registration. No obvious abnormalities (e.g., vascular lesions, tumor growth, etc.) were found in any of the brain MRI scans.

2.4. Data analysis

Each epoch was analyzed using complementary ensemble empirical mode decomposition according to our previous study (Wang et al., 2015). The time window for each epoch was 1 s, separated by the triggers given on recording. A band-pass filter was set at 0.03 to 100 Hz. Since two sessions (each 180 s duration) were averaged before further analysis, totally 360 epochs would be used for the average and data normalization. To avoid possible bias, the analyzers were blinded to the connection between the patients and the type of treatment they received. The inter-hemispheric differences in peak dipole strength of the SSAEFs observed in the left and right hemispheres of all of the participants, and those in stimulated (i.e. left) and non-stimulated (i.e. right) hemispheres before and after rTMS/sham treatment in the patients with tinnitus, were evaluated using the Wilcoxon signed-rank test. Among-group differences (controls, rTMS group, and sham group) in the strength of the SSAEFs between hemispheres (right versus right, and left versus left) were evaluated using the Kruskal-Wallis test. A laterality index (ratio of SSAEF strength over the right hemisphere to that over the left hemisphere) was used to assess the degree of hemispheric asymmetry in the patients with tinnitus. Since this is a sham-controlled study design, a 2-way ANOVA with time (before and after) and group (rTMS and sham) factors was done to show whether there were significant differences between rTMS and sham groups before going on for analysis in individual group. The prognostic relevance of the hemispheric asymmetry as expressed in the relationship between the laterality index and THI score was evaluated using Spearman's rank correlation. Statistical significance was set as a p value less than 0.05.

3. Results

All of the patients completed the rTMS/sham treatment, and no side effects were reported.

3.1. Differences in THI score and strength of SSAEFs before and 1 month after rTMS/sham treatment in the patients with tinnitus (Table 1; Fig. 1)

The 2-way ANOVA showed that there were significant differences on THI for the interaction between time (before and after) and group (rTMS and sham) factors ($F = 5.089$, $p = 0.029$)

3.2. The patients with tinnitus receiving rTMS treatment

The THI score was significantly lower 1 month after rTMS treatment ($m = 51$, $sd = 18.9$) than before rTMS treatment ($m = 80$, $sd = 12.9$, $p = 0.002$). The SSAEFs were significantly weaker over the stimulated (i.e. left) hemispheres 1 month after rTMS treatment ($m = 11.3$ nAm) than before rTMS treatment ($m = 17.1$ nAm, $p = 0.002$). Over the non-stimulated (i.e. right) hemispheres, the SSAEFs were also significantly weaker one month after rTMS treatment ($m = 15.1$ nAm) than before rTMS treatment ($m = 22.5$ nAm, $p = 0.002$).

3.3. The patients with tinnitus receiving sham treatment

There were no significant differences between THI score after sham treatment ($m = 64$, $sd = 16$) and before sham treatment ($m = 72$, $sd = 15.8$, $p = 0.091$), between SSAEFs over stimulated (i.e. left) hemispheres after sham treatment ($m = 15.7$ nAm) and before sham treatment ($m = 15.8$ nAm, $p = 0.875$), and between SSAEFs over non-stimulated (i.e. right) hemispheres after sham treatment ($m = 22.9$ nAm) and before sham treatment ($m = 24.5$ nAm, $p = 0.092$).

3.4. Correlation between laterality index and THI (Fig. 2)

There was no correlation between laterality index and THI in the patients with tinnitus. However, the laterality index before rTMS was positively correlated with THI score before rTMS with a borderline effect ($r = 0.559$, $p = 0.059$).

3.5. Among-group differences (controls, rTMS group, and sham group) in the strength of SSAEFs between hemispheres (right versus right, and left versus left; Table 1 and Fig. 3)

Over the left hemisphere, there was no significant difference in SSAEFs among the controls ($m = 12.7$ nAm) and patients with tinnitus before rTMS/sham treatment ($m = 17.1$ nAm for the rTMS group, $m = 15.8$ nAm for the sham group, $p = 0.192$). Over the right hemisphere, however, there was a significant difference in SSAEFs among the controls ($m = 17.2$ nAm) and patients with tinnitus before rTMS/sham treatment (m = 22.5 nAm for the rTMS group, m = 24.5 nAm for the sham group, p = 0.022). Post hoc analysis showed a significant difference between the controls and patients with tinnitus before rTMS (p = 0.033), and also between the controls and patients with tinnitus before sham treatment (p = 0.008), i.e. the SSAEFs in the patients with tinnitus before both rTMS and sham treatment were significantly stronger than those in the controls.

There was no significant difference in SSAEFs among the controls (m = 12.7 nAm) and patients with tinnitus after rTMS/sham treatment (m = 11.3 nAm for the rTMS group, m = 15.7 nAm for the sham group, p = 0.062) over the left hemispheres. However, over the right hemispheres there was a significant difference in SSAEFs among the controls (m = 17.2 nAm) and patients with tinnitus after rTMS/sham treatment (m = 15.1 nAm for the rTMS group, m = 22.9 nAm for the sham group, p = 0.004). Post hoc analysis showed that there was a significant difference between the controls and patients with tinnitus after sham treatment (p = 0.006), and also between the patients with tinnitus after rTMS and those after sham treatment (p = 0.004), i.e. the SSAEFs in the patients with tinnitus after sham treatment were significantly stronger than those in both the controls and patients with tinnitus after rTMS.

3.6. Within-group differences (controls and patients with tinnitus) in the strength of SSAEFs between hemispheres (right versus left; Table 1)

In the controls, the SSAEFs were stronger over the right hemispheres (m = 17.2 nAm) than the left hemispheres (m = 12.7 nAm, p = 0.003). No matter before or after rTMS treatment, the SSAEFs in the patients with tinnitus were stronger over the right hemispheres (before: m = 22.5 nAm; after: m = 15.1 nAm) than those over the left hemispheres (before: m = 17.1 nAm, p = 0.002; after: m = 11.3 nAm, p = 0.002). No matter before or after sham treatment, the SSAEFs in the patients with tinnitus were stronger over the right hemispheres (before: m = 24.5 nAm; after: m = 22.9 nAm) than those over the left hemispheres (before: m = 15.8 nAm, p = 0.002; after: m = 15.7 nAm, with a borderline effect p = 0.051).

4. Discussion

4.1. Decreased SSAEFs in patients with tinnitus 1 month after rTMS

A major and novel finding in this study is the decreased SSAEFs in the patients with tinnitus 1 month after rTMS (Table 1 and Fig. 1). When measured 4 weeks later, the dipole moment strength of the SSAEFs was significantly reduced after 5 days of rTMS treatment compared with that before the treatment. This phenomenon was not found in the sham group, and the SSAEFs for the patients with tinnitus after sham treatment were significantly stronger than those for both the controls and patients with tinnitus after rTMS (Fig. 3). To the best of our knowledge, this is the first study to report a rTMS effect persisting for as long as 1 month in terms of decreased SSAEFs for patients with tinnitus.

SSAEFs in patients with tinnitus have been reported to be enhanced compared to those in controls in both hemisphere regardless of tinnitus laterality (Diesch et al., 2004; Wienbruch et al., 2006; Diesch et al., 2010; Wang et al., 2015), which might be due to the evidence of increased cortical or brainstem/thalamic neural synchrony found both in animal models (Roberts et al., 2010) and human beings (Weiss et al., 1995). This is consistent with the findings of the current study, in that the SSAEFs of our patients with tinnitus before both rTMS and sham treatment were significantly stronger than those in the controls (Table 1 and Fig. 3). One possible explanation may be a loss of optimal interaction for effective information processing (Reale and Kettner, 1986; Reale et al., 1987), since an increase in auditory evoked responses associated with tinnitus has been postulated to be associated with both inhibition down-regulation and excitation up-regulation in the cortices and subcortical regions of the auditory pathway (Gerken, 1996; Mossop et al., 2000; Salvi et al., 2000; Syka, 2002, Eggermont and Roberts, 2004; Parra and Pearlmutter, 2007). Reason(s) for the increased fields in the patients with tinnitus could thus be ascribed at least in part to the synchronous entrainment by the AM envelope of the external stimuli for more neurons in the auditory cortex (Wienbruch et al., 2006). In addition, activity emerging inside the brain may add to the interactions, as steady-state auditory evoked responses (SSAERs) have been suggested to mirror not only external signals (i.e. stimuli spectra) but also internally generated ones, among them tinnitus-associated events in the cortical regions (Diesch et al., 2010). Even input from non-auditory structures such as emotion-related activities of the limbic system (Lockwood et al., 1998) could influence the SSAEFs and contribute to the hyper-excitation.

The mechanisms underlying the enduring effect in reducing SSAEFs by rTMS in the patients with tinnitus of our study remained elusive. Low-frequency rTMS (e.g. 1 Hz used in our study) is known to lower the excitability in locally-stimulated and functionally-linked regions of the brain (Hoffman and Cavus, 2002). Since there is functional connectivity between hemispheres as evidenced by activity including SSAEFs (Schlee et al., 2008, Minami et al., 2015), it is not surprised that rTMS treatment was done over one side and SSAEFs were changed in both hemispheres. The remote effect found in this study can also be noted in a recent research. The cortical region mostly affected by transcranial direct current stimulation (tDCS) over bilateral hemispheres in terms of the inhibition 40 Hz SSAEFs was located far away from the stimulation site and covered a large area of the right temporal cortex (Pellegrino et al., 2019). Although it is commonly seen that the synergetic areas in different hemisphere have reciprocal changes, the functional connectivity between the right and left auditory cortex became significantly stronger after electrical stimulation (Minami et al., 2015) probably in a synchronous way (Roberts et al., 2010; Weiss et al., 1995) instead of a reciprocal way for tinnitus.

The decrease in excitability caused by rTMS has been reported to result from long-term depression (LTD) of synaptic transmissions, which is similar to that induced by electrical stimulation (Hoffman and Cavus, 2002). By using MEG, immediate changes (within minutes) in cortical activity with specific respect to steady-state responses following rTMS have been shown in patients with tinnitus (Lorenz et al., 2010). Although the duration for the effect of LTD produced by electrical stimulation has been reported to last for several days to ten weeks in animals as evidenced by activity recorded in the brain (Weiss et al., 1995; Heynen et al., 1996; Adamec, 1999; Manahan-Vaughan et al., 1999; Froc et al., 2000), there is currently no evidence of a persistent effect for rTMS regarding evoked signals observed in modalities of functional brain imaging in humans. The current study provides an objective clue in terms of SSAEFs for the long-term effect of rTMS on tinnitus as suggested in previous researches, in which an improvement lasting up to 6 months has been noted with respect to questionnaire scores (Kleinjung et al., 2005; Kim et al., 2014).

4.2. Prognostic relevance of hemispheric asymmetry to tinnitus severity

In this study, there was no correlation between hemispheric asymmetry and severity of tinnitus. Although a borderline effect between the initial laterality index on binaural stimulation and ini-

Table 1
Amplitude of SSAEFs dipole moment for controls and patients with tinnitus before as well as 1 month after rTMS/sham treatment.

Hemisphere	Control					Patient (rTMS)							Patient (sham)						
	Left Q (nAm)	PTA	Gender	Age	Right Q (nAm)	Left Q (nAm)	PTA	Gender	Age	Tnnitus Laterality	THI	Right Q (nAm)	Left Q (nAm)	PTA	Gender	Age	Tnnitus Laterality	THI	Right Q (nAm)
						pre-rTMS							*pre-sham*						
1	19.2	20	F	30	22.6	10.8	55	M	40	R	86	17.1	14.4	45	M	61	B	82	26.8
2	15.7	15	M	39	21.9	14.0	50	M	42	B	80	26.1	27.8	55	F	63	R	80	32.0
3	8.6	25	F	58	12.6	24.8	30	F	53	R	66	28.8	20.6	40	M	53	R	70	29.6
4	11.7	10	M	47	18.7	19.7	35	M	61	R	78	23.1	10.7	55	M	49	B	60	18.4
5	15.1	20	M	49	17.6	34.7	55	F	48	R	84	42.7	18.5	50	F	58	L	88	25.2
6	9.2	20	F	47	15.5	23.5	40	F	73	L	58	25.7	13.4	45	F	43	R	50	20.8
7	11.9	15	M	50	12.6	13.2	45	F	61	B	74	18.7	22.4	30	M	53	R	96	22.6
8	8.2	25	F	52	19.1	13.3	30	M	60	B	100	19.3	13.4	35	M	51	B	62	15.0
9	6.2	25	F	51	14.7	16.8	55	M	57	B	92	18.9	9.2	50	M	55	L	86	25.5
10	12.6	25	M	48	14.6	8.7	45	M	69	R	64	9.8	14.7	55	M	65	R	82	16.2
11	20.9	20	M	45	20.2	13.8	40	F	63	B	76	19.2	12.8	45	F	52	B	54	43.6
12	13.1	20	F	64	16.3	12.4	50	M	63	L	96	20.1	11.2	35	F	40	L	52	18.4
m	12.7	20		48	17.2	17.1	44		57		80	22.5	15.8	45		54		72	24.5
SD	4.4	4.8		8.5	3.4	7.4	9.3		10.1		12.9	8.1	5.5	8.5		7.5		15.8	8.0
p1(pre-post)						0.002	0.157				0.002	0.002	0.875	0.317				0.091	0.092
p2(among group)													0.192						0.022
p3(right-side dominance)					0.003							0.002							0.002
						post-rTMS							*post-sham*						
1						8.8	55				58	9.6	14.6	45				70	24.1
2						12.2	50				50	24.7	30.2	55				52	31.8
3						22.3	35				36	26.8	18.9	40				58	27.7
4						7.9	35				38	10.2	10.8	55				68	17.5
5						10.5	55				56	12.7	17.8	50				60	24.9
6						7.5	40				36	11.6	13.8	50				62	21.0
7						11.1	45				36	17.1	20.9	35				98	20.2
8						12.1	30				98	14.9	12.8	35				58	16.8
9						15.8	55				70	16.9	10.0	50				73	20.3
10						7.6	45				30	8.9	15.9	50				80	28.1
11						10.7	45				50	17.7	11.3	50				60	26.0
12						9.1	50				54	10.6	11.3	35				32	15.9
m						11.3	45				51	15.1	15.7	46				64	22.9
SD						4.2	8.5				18.9	5.8	5.7	7.6				16.0	5.0
p2(among group)													0.062						0.004
p3(right-side dominance)												0.002							0.051

Threshold for statistical significance using Wilcoxon signed rank test and Kruskal-Wallis test was set at P < 0.05. Left, left hemisphere; Right, right hemisphere; Q (nAm), dipole moment strength of SSAEFs; PTA, threshold for pure tone audiometry at 1000 Hz (dB HL); Age, y/o; m, mean; sd, standard deviation; laterality, tinnitus referral; THI, score for Tinnitus Handicap Inventory; P_1, significance of difference for SSAEFs, THI, and PTA between pre- and post-rTMS/sham treatment; P_2, significance of difference for SSAEFs among control, rTMS group, and sham group; P_3, significance of difference between responses of left hemispheres vs. those of right hemispheres.

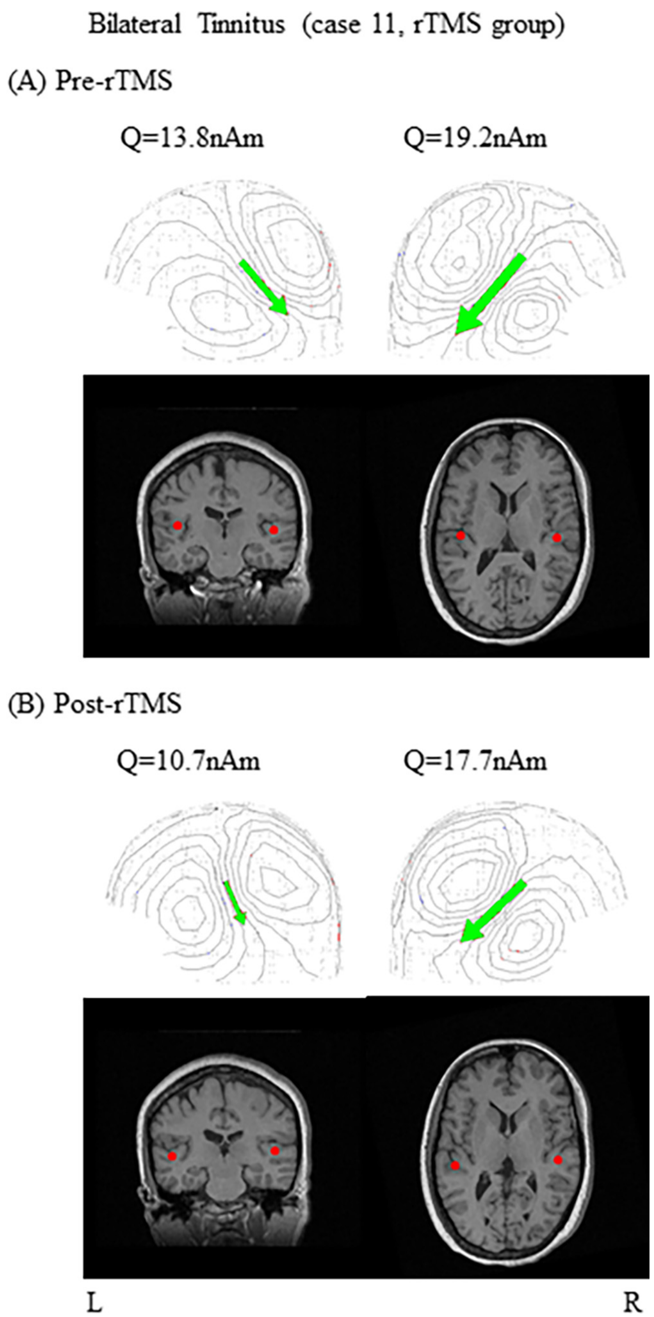

Fig. 1. Dynamics of neuromagnetic responses to binaural stimulation pre- and post- rTMS in patients with tinnitus. Patient 11 (female, bilateral tinnitus) was studied before rTMS and 1 month after rTMS. (A) SSAEFs of pre-rTMS examinations. The patient demonstrated a pattern of right-side dominance for SSAEFs in the initial examination. (B) SSAEFs of post-rTMS examinations. In the final examination, the patient again demonstrated a pattern of right-side dominance for SSAEFs, as paralleled by a reduced ECD 1 month after rTMS. Dipole sources (red dots) were localized at the auditory cortices of bilateral temporal lobes in her MRI scans. MRI views are displayed according to neurological convention, i.e., the subject's right hemisphere is on the right side of the images.

tial THI in the patients with tinnitus of the rTMS group was noted, no conclusion can be proposed on the basis of the negative results (Fig. 2). Our previous researches revealed for the first time that hemispheric asymmetry as represented by the laterality index of auditory evoked fields could predict the treatment outcomes of ISSNHL (Li et al., 2012, 2013). Possibly due to the small sample size of this study and/or unknown factors interfering with treatment effect of rTMS, there was no evidence of an association between

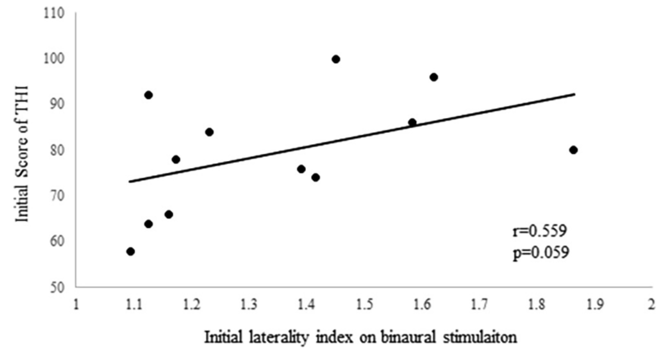

Fig. 2. The relationship between laterality index and THI score. When the laterality index was correlated to THI, no significant correlation was revealed, including a borderline effect between the initial laterality index on binaural stimulation and initial THI in the patients with tinnitus of the rTMS group ($r = 0.559$, $p = 0.059$). Since the sample size is small, no conclusion can be proposed on the basis of the negative results. r, correlation coefficient; statistical significance was thresholded at $p < 0.05$.

pre-rTMS laterality index and THI score 1 month after rTMS, although a correlation between SSAEFs and disturbance of tinnitus reflected by visual analogue scale scores was reported immediately after rTMS in one previous study (Lorenz et al., 2010). While the exact cause of the link between neuromagnetic patterns and tinnitus perception remains to be elucidated, it may be associated with tinnitus-related plastic changes in auditory cortices (Diesch et al., 2004). The degree of reorganization with specific respect to tonotopic deviations has been reported to be associated with the self-stated intrusiveness of tinnitus (Muhlnickel et al., 1998). An increase of the SSAEFs in patients with tinnitus has also been reported to reflect at least partly the severity of tinnitus as mirrored by questionnaire scores (Diesch et al., 2004, 2010). Those previous studies suggest the possibility on the development of an objective index based on SSAEFs for the subjective severity of tinnitus.

4.3. Right-side dominance for SSAEFs in the patients with tinnitus and controls

In this study, the SSAEFs were always stronger over the right hemispheres in the patients with tinnitus (no matter before or after rTMS/sham treatment) and controls (Table 1). This finding of right-side dominance for SSAEFs in both normal hearing controls and patients with tinnitus is consistent with one of our previous studies (Wang et al., 2015) and several other studies (Ross et al., 2005; Osipova et al., 2006). The mechanisms underlying this right-side dominance of SSAEFs have been well proposed. It was hypothesized that temporal changes in acoustic stimuli were managed predominantly over the auditory cortex of the left hemisphere, while spectral information of sound was processed mainly over the right hemisphere (Zatorre et al., 2002). Since SSAEFs have been associated with overlaid responses in the brain evoked by auditory stimuli containing different frequencies (i.e. spectrums) (Osipova et al., 2006), it is reasonable to conjecture that cortical activity be deviated to the right hemisphere.

4.4. Limitations of the study

Although the primary goal of this study was to explore the long-term effect of rTMS on evoked responses of the auditory cortex in neuromagnetic domains rather than its therapeutic effect on tinnitus, there are some limitations. Due to the budget limit, the sample size was small and the follow-up was not long enough and can only

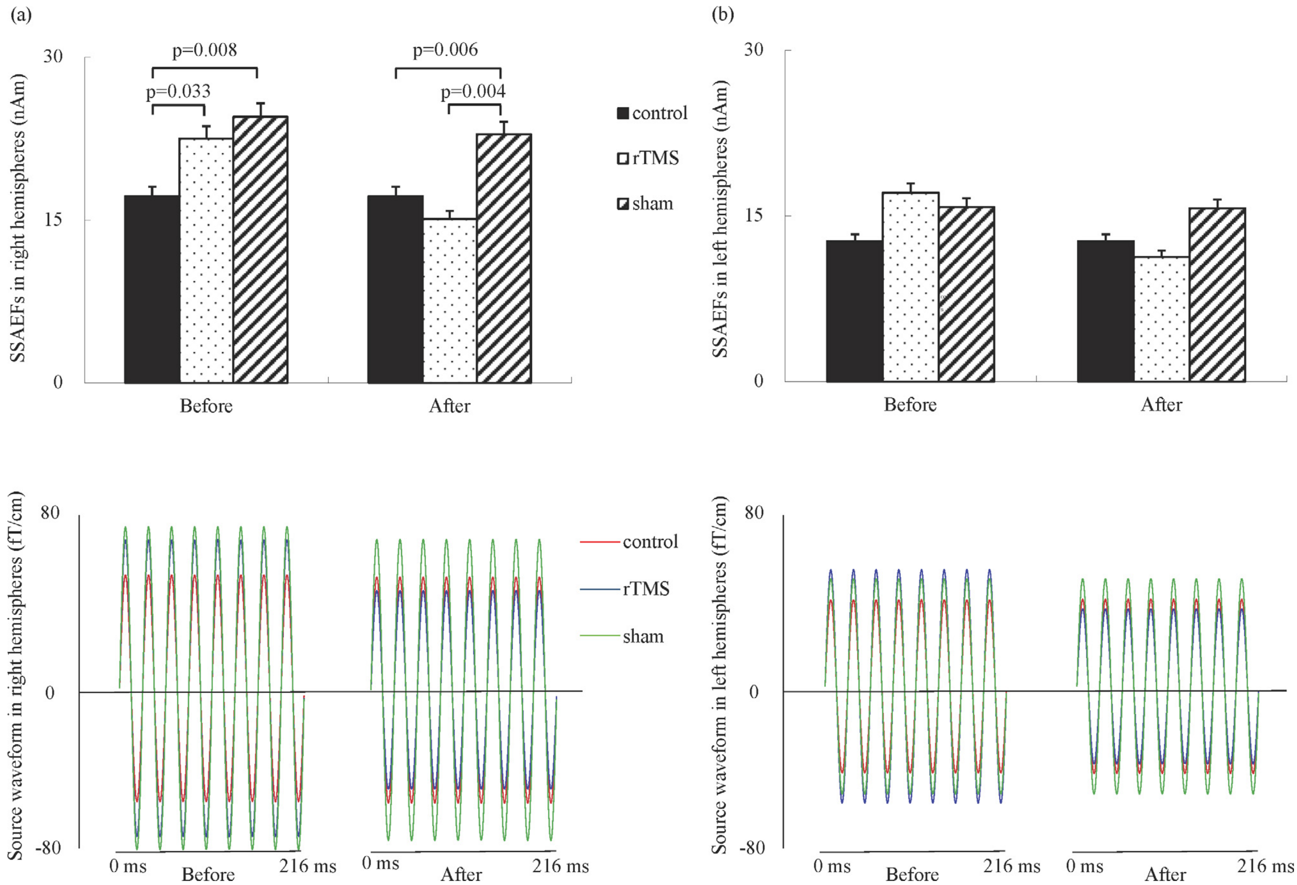

Fig. 3. Among-group differences (controls, rTMS group, and sham group) in the strength of SSAEFs for (a) right and (b) left hemispheres. (a) Before rTMS/sham treatment, there were significant differences in SSAEFs among the controls and patients with tinnitus over the right hemispheres: SSAEFs for the patients with tinnitus in both the rTMS and sham groups were significantly stronger than those in the controls. After rTMS/sham treatment, there were also significant differences in SSAEFs among the controls and patients with tinnitus over the right hemispheres: SSAEFs for the patients with tinnitus after sham treatment were significantly stronger than those for both the controls and patients with tinnitus after rTMS. (b) Over the left hemisphere, there was no significant difference in SSAEFs among the controls and patients with tinnitus both before and after rTMS/sham treatment. Statistical significance was thresholded at $p < 0.05$.

be done once (one month later). Since the instant effect of rTMS on decreasing SSAEFs in patients with tinnitus has been confirmed in previous studies, the short-term follow-up (i.e. immediately or in days after the rTMS intervention) was not performed to avoid redundancy. In addition, the effect of rTMS in terms of other stimulation protocol was not discussed. Further studies with long-term follow-up are warranted to investigate these issues.

5. Conclusion

In conclusion, this study verified again that SSAEFs are right-side dominant, and that SSAEFs in patients with tinnitus are enhanced in contrast to those in controls. In addition, the increased strength of the SSAEFs in the patients with tinnitus remained decreased 1 month after rTMS treatment. This phenomenon was not found in the sham group. Although immediate changes of SSAEFs following rTMS in patients with tinnitus have been reported, evidence of the persistent consequence for rTMS regarding evoked signals observed by modalities of functional brain imaging in humans was lacking. Therefore, the results of this study provide an objective clue in terms of SSAEFs for the long-term effect of rTMS on tinnitus as noted by the results of questionnaires in previous studies. Further longitudinal studies are warranted to investigate the development of an objective index based on SSAEFs for the subjective severity of tinnitus.

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Declaration of Competing Interest

The authors did not have any relevant conflicts of interest that could bias the conduct of the reported work.

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