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RESEARCH ARTICLE

Different resting state brain activity and functional connectivity in patients who respond and not respond to bifrontal tDCS for tinnitus suppression

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Abstract Tinnitus is an ongoing phantom percept. It has been demonstrated that bifrontal transcranial direct current stimulation (tDCS) can reduce tinnitus. In this study, one group of patients reported a substantial improvement in their tinnitus perception, whereas another group described minor or no beneficial effect at all. The objective was to verify whether the activity and connectivity of the resting brain is different for people who will respond to bifrontal tDCS for tinnitus in comparison with non-responders. Higher gamma band activity was demonstrated in right primary and secondary auditory cortex and right parahippocampus for responders. It has been shown that gamma band activity in the auditory cortex is correlated with tinnitus loudness and that the anterior cingulate is involved in tinnitus distress. People who were going to respond to bifrontal tDCS also demonstrated an increased functional connectivity in the gamma band between the right dorsolateral prefrontal cortex (DLPFC) and the right parahippocampus as well as the right DLPFC and subgenual anterior cingulate cortex (sgACC). An analysis revealed that responders to bifrontal tDCS also experienced a larger suppression effect on TMS placed over the right temporal cortex (i.e. auditory cortex) than non-responders. Responders to bifrontal tDCS seem to differ in resting brain activity compared to non-responders in the right auditory cortex and parahippocampal area. They also have a different functional connectivity between DLPFC and, respectively, the sgACC and parahippocampal area. These connectivities might explain the suppression effect for both tinnitus loudness and tinnitus-related distress.

Keywords Bifrontal · Transcranial direct current stimulation · tDCS · EEG · sLORETA · Functional connectivity · Gamma

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Introduction

Non-pulsatile tinnitus is considered to be an auditory phantom percept and can be described as an intrinsic sound sensation that cannot be attributed to an external sound source. Tinnitus is a common disorder experienced by 10–15% of the adult population (Eggermont and Roberts 2004), and it interferes severely with the quality of life in 2–4% of the total population (Axelsson and Ringdahl 1989; Heller 2003).

Based on animal studies and neurobiological research, it is generally accepted that most forms of subjective tinnitus are attributable to the central nervous system reorganization due to damage to the auditory system (Muhlnickel et al. 1998; Weisz et al. 2007a). A discrepancy between excitatory and inhibitory transmitter systems leads to maladaptive plasticity, altering the structural and functional organization of the entire auditory system (Kaltenbach 2000). Recent research indicates that non-auditory brain

areas are also involved in subjective tinnitus. Using a voxel-based morphometry, structural differences between tinnitus sufferers and controls in gray matter in non-auditory structures, namely subcallosal region including the nucleus accumbens and the subgenual anterior cingulate cortex (sgACC), were visualized (Muhlau et al. 2006). In a PET study, increased neural activity for tinnitus sufferers was demonstrated in the right hemisphere, on the middle frontal and middle temporal regions as well as in lateral mesial posterior sites (Mirz et al. 1999). The dorsolateral prefrontal cortex (DLPFC) seems to play a specific role as well in auditory processing. Studies indicate that the DLPFC has a bilateral facilitatory effect on auditory memory storage and contains auditory memory cells (Bodner et al. 1996). The DLPFC also exerts early inhibitory modulation of input to primary auditory cortex in humans (Knight et al. 1989) and has been found to be associated with auditory attention (Alain et al. 1998; Lewis et al. 2000; Voisin et al. 2006) resulting in top-down modulation of auditory processing (Mitchell et al. 2005). This was further confirmed by electrophysiological data, indicating that tinnitus might occur as the result of a dysfunction in the top-down inhibitory processes (Norena et al. 1999).

Transcranial direct current stimulation (tDCS) is a non-invasive procedure of cortical stimulation. When tDCS is applied in humans, a relatively weak constant current (between 0.5 and 2 mA) is passed through the cerebral cortex via scalp electrodes. Depending on the polarity of the stimulation, tDCS can increase or decrease cortical excitability in the brain regions to which it is applied (Miranda et al. 2006). Currently, tDCS is usually applied through two surface electrodes: one serving as the anode and the other as the cathode. Some of the applied current is shunted through scalp tissue and only a part of the applied current passes through the brain. Anodal tDCS typically has an excitatory effect on the underlying cerebral cortex by depolarizing neurons, while the opposite occurs under the cathode due to induced hyperpolarization. This effect of tDCS typically outlasts the stimulation by an hour or longer after a single treatment session of sufficiently long stimulation duration (Nitsche and Paulus 2000, 2001; Nitsche et al. 2003; Antal et al. 2004a).

Several tDCS studies targeting the DLPFC demonstrated clinically beneficial results in treating major depression (Fregni et al. 2006a, b), as well as reducing impulsiveness (Beeli et al. 2008) and increasing pain threshold (Boggio et al. 2008, 2009). In a recent paper, it was demonstrated that bifrontal tDCS, placing the anodal electrode on the right DLPFC and the cathodal electrode on left tDCS, could also suppress tinnitus (Vanneste et al. 2010b). As the DLPFC is involved in attention-mediated top-down control of auditory processing and possibly tinnitus as well, tDCS can modulate both the tinnitus perception and the tinnitus-related distress. In this study, one group of patients reported a substantial improvement in their tinnitus sensation reflected by a significant reduction in the tinnitus loudness, whereas another group described minor treatment effect or no effect at all. These results are similar to tDCS studies for treating major depression and pain (Fregni et al. 2006b; Boggio et al. 2008, 2009) and the treatment of tinnitus by transcranial magnetic stimulation (TMS). Hence, in this study, we examine the potential neurobiological factor explaining why some tinnitus patients benefit from bifrontal tDCS and others do not.

Study 1

In a first study, the objective is to verify whether brain activity and/or functional connectivity of the resting brain differ between patients who will respond to bifrontal tDCS for the treatment of tinnitus in comparison with people who will not respond. We therefore compare source-localized EEGs recorded before tDCS was applied of the responders and non-responders to bifrontal tDCS for both spectral and functional connectivity differences within a group of selected ROIs. The ROIs were chosen based on areas involved in tinnitus according to the PET, SPECT, MEG, EEG, and VBM tinnitus literature.

Methods and materials

Participants

Forty-five tinnitus patients (30 women and 15 men) with a mean age of 50.07 (SD = 12.40 years) were selected from the multidisciplinary Tinnitus Research Initiative (TRI) Clinic of the University Hospital of Antwerp, Belgium. Individuals with pulsatile tinnitus, Ménière disease, otosclerosis, chronic headache, neurological disorders such as brain tumors, and individuals being treated for mental disorders were not included in the study in order to obtain a homogeneous sample.

All patients were investigated for the extent of hearing loss using audiograms. Tinnitus matching was performed looking for tinnitus pitch (frequency) and tinnitus loudness. Participants were requested to refrain from alcohol consumption 24 h prior to recording and from caffeinated beverages on the day of recording. See Table 1 for overview of patient population. This study was approved by the local ethical committee (Antwerp University Hospital) and was in accordance with the declaration of Helsinki. Patients gave an oral informed consent before the procedure. The tDCS was performed as a diagnostic test in a screening for potential treatment.

Table 1 Patients' characteristics

	tDCS		
	Responders	Non-responders	*t* test
Gender			
Male	8	7	
Female	13	17	
Age (years)			
M	52.75	48.27	$p = .34$
SD	16.06	9.32	
Tinnitus duration (years)			
M	3.49	4.86	$p = .43$
SD	6.40	4.50	
Tinnitus type			
Pure tone	6	11	
Narrow band noise	15	13	
Tinnitus laterality			
Unilateral			
Left	5	4	
Right	6	4	
Bilateral	10	16	
VAS loudness[a]			
M	6.00	6.46	$p = .48$
SD	2.38	2.36	
VAS distress[a]			
M	6.03	6.61	$p = .38$
SD	2.32	2.48	
Tinnitus frequency (Hz)			
M	4,816.67	4,864.58	$p = .97$
SD	2,768.72	3,038.68	
Hearing loss[b](dB HL)			
M	28.57	29.17	$p = .82$
SD	13.88	18.56	

No differences were found between responders and non-responders for gender, age, tinnitus duration, tinnitus type, tinnitus laterality, VAS loudness, and VAS distress

[a] VAS loudness and VAS distress were both assessed before tDCS

[b] Mean HL at the tinnitus frequency

tDCS

Direct current was transmitted by a saline-soaked pair of surface sponge (35 cm^2) and delivered by specially developed, battery-driven, constant current stimulator with a maximum output of 10 mA (Eldith©; http://www.eldith.de). For each patient receiving tDCS, the cathodal electrode was placed over the left DLPFC and anode was placed on the right DLPFC as determined by the International 10/20 Electroencephalogram System corresponding to F3 and F4, respectively. A constant current of 1.5 mA intensity was applied for 20 min.

A visual analog scale (VAS) for tinnitus loudness ('How loud is your tinnitus? 0 = no tinnitus, 10 = as loud as imaginable') and tinnitus distress ('How stressful is your tinnitus? 0 = no distress, 10 = suicidally distressed') was asked before (pre) and immediately after (post) tDCS stimulation.

Responders were defined as patients who respond to tDCS treatment both to tinnitus loudness and tinnitus distress (VAS pre–VAS post > 0), while non-responders are defined as patients who do not respond to tDCS treatment (VAS pre–VAS post $\leq$ 0) (Vanneste et al. 2010b).

EEG data collection

EEGs (Mitsar, Nova Tech EEG, Inc, Mesa) were obtained in a fully lighted room with each participants sitting upright in a comfortable chair. The EEG was sampled with 19 electrodes (Fp1, Fp2, F7, F3, Fz, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1 O2) in the standard 10–20 International placement referenced to linked lobes, and impedances were checked to remain below 5 kΩ. Data were collected for 100 2-s epochs eyes closed, sampling rate = 1024 Hz, and band passed 0.15–200 Hz. Data were resampled to 128 Hz and band-pass filtered (fast Fourier transform filter) to 2–44 Hz. These data were transposed into Eureka! Software (Congedo 2002), plotted, and carefully inspected for manual for artifact. All episodic artifacts including eye blinks, eye movements, teeth clenching, body movement, or ECG artifacts were removed from the stream of the EEG. In addition, an independent component analysis (ICA) was conducted to further verify whether all artifacts were excluded. To investigate the effect of possible ICA component rejection, we compared the power spectra in two approaches: (1) after visual artifact rejection only (before ICA) and (2) after additional ICA component rejection (after ICA). To test for significant differences between the two approaches, we performed a repeated-measure ANOVA, considering mean band power as within-subject variables. The mean power in delta (2–3.5 Hz), theta (4–7.5 Hz), alpha1 (8–10 Hz), alpha2 (10–12 Hz), beta1 (13–18 Hz), beta2 (18.5–21 Hz), beta3 (21.5–30 Hz), and gamma (30.5–45 Hz) did not show a statistically significant difference between the two approaches. Therefore, we continue by reporting the results of ICA-corrected data.

Source localization

Standardized low-resolution brain electromagnetic tomography (sLORETA) was used to estimate the intracerebral electrical sources that generated the scalp-recorded activity in each of the eight frequency bands (Pascual-Marqui 2002). sLORETA computes electrical neuronal activity as current density (A/m^2) without assuming a predefined number of active sources. The sLORETA solution space

consists of 6,239 voxels (voxel size: 5 × 5 × 5 mm) and is restricted to cortical gray matter and hippocampi, as defined by digitized MNI152 template (Fuchs et al. 2002). Scalp electrode coordinates on the MNI brain are derived from the international 5% system (Jurcak et al. 2007).

Connectivity

Coherence and phase synchronization between time series corresponding to different spatial locations are usually interpreted as indicators of the "functional connectivity". However, any measure of dependence is highly contaminated with an instantaneous, non-physiological contribution due to volume conduction and low spatial resolution (Pascual-Marqui 2007a). Therefore, Pascual-Marqui (2007b) introduced a new technique (i.e. Hermitian covariance matrices) that removes this confounding factor considerably. As such, this measure of dependence can be applied to any number of brain areas jointly, i.e. distributed cortical networks, whose activity can be estimated with sLORETA. Measures of linear dependence (coherence) between the multivariate time series are defined. The measures are expressed as the sum of lagged dependence and instantaneous dependence. The measures are non-negative and take the value zero only when there is independence of the pertinent type and are defined in the frequency domain: delta (2–3.5 Hz), theta (4–7.5 Hz), alpha1 (8–10 Hz), alpha2 (10–12.5 Hz), beta1 (13–18 Hz), beta2 (18.5–21 Hz), beta3 (21.5–30 Hz), and gamma (30.5–45 Hz). Based on this principle, lagged linear connectivity was calculated. Regions of interest were defined based on previous brain research on tinnitus (see Table 2 for overview).

Table 2 Regions of interest

	Authors
Amygdala	De Ridder et al. (2006) Landgrebe et al. (2009)
Anterior cingulate cortex	
Dorsal	Plewnia et al. (2007) Vanneste et al. (2010a) Schlee et al. (2009)
Subgenual	Muhlau et al. (2006) Vanneste et al. (2010a)
Auditory cortex	Muhlnickel et al. (1998) Schneider et al. (2009) Smits et al. (2007) Weisz et al. (2007a, b)
Dorsal lateral prefrontal cortex	Mirz et al. (2000)
Insula	Smits et al. (2007) Vanneste et al. (2010a)
Parahippocampus	Landgrebe et al. (2009)

Statistical analyses

Statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA). To verify whether there were differences in age, tinnitus duration, and VAS loudness before tDCS and VAS distress before tDCS between responders and non-responders to bifrontal tDCS, an independent-sample *t* test was performed. To confirm that there was no distinction between responder and non-responder groups for tinnitus type and tinnitus laterality, we conducted χ^2-test, respectively.

To compare pre- and post-tDCS VAS score, a repeated-measure ANOVA is conducted for VAS loudness, pre- and post-tDCS, and VAS distress pre- and post-tDCS, for both responders and non-responders to bifrontal tDCS in one model to correct for multiple comparisons. In addition, a paired two-sample *t* test is computed for VAS loudness pre- and post-tDCS and VAS distress pre- and post-tDCS, respectively, for both responders and non-responders to bifrontal tDCS, respectively.

In order to identify potential differences in brain electrical activity between responders and non-responders, sLORETA was then used to perform voxel-by-voxel between-condition comparisons of the current density distribution. Nonparametric statistical analyses of functional sLORETA images (statistical nonparametric mapping; SnPM) were performed for each contrast employing a *t* statistic for unpaired groups and corrected for multiple comparisons ($P < 0.05$). As explained by Nichols and Holmes, the SnPM methodology does not require any assumption of Gaussianity and corrects for all multiple comparisons (Nichols and Holmes 2002). We performed one voxel-by-voxel test (comprising 6,239 voxels each) for the different frequency bands.

Results

Patient population

No significant differences were found for age, tinnitus duration, VAS loudness, and VAS distress between responders and non-responders (see Table 1).

tDCS results

The repeated-measure ANOVA revealed a significant effect for both VAS loudness ($F = 34.63$, $P < .001$) and VAS distress ($F = 49.55$, $P < .001$) as well as an interaction effect between response (responders vs non-responders) and VAS loudness ($F = 4.23$, $P < .05$) and an interaction effect between response and VAS distress ($F = 7.47$, $P < .01$). That is, twenty-one patients responded to tDCS, indicating they had a significant suppression effect for tinnitus perception

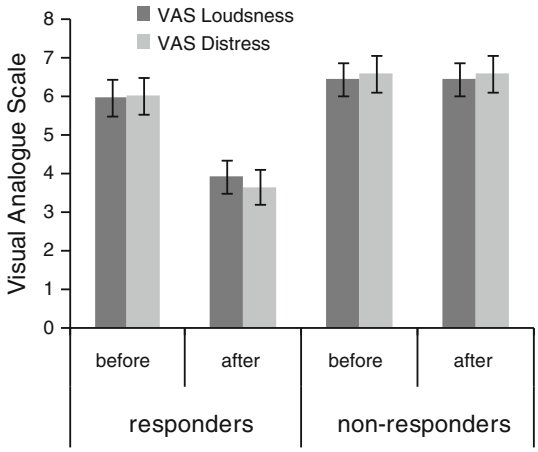

Fig. 1 Visual analog scale for loudness and distress for responders and non-responders before and after tDCS treatment ($P < .05$) with *error bars*

and tinnitus-related distress when comparing baseline (Mean = 6.00 and Mean = 6.03; SD = 2.38 and SD = 2.32, respectively), with post-tDCS (Mean = 3.92 and Mean = 3.65; $SD = 2.20$ and $SD = 2.15$, respectively), ($t = 5.78$, $P < .001$ and $t = 6.37$, $P < .001$). Twenty-four patients did not respond to tDCS revealing that they had no significant suppression effect for tinnitus perception and tinnitus-related distress when comparing baseline (Mean = 6.46 and Mean = 6.61; SD = 2.36 and SD = 2.48, respectively) with post-tDCS (Mean = 6.46 and Mean = 6.61; SD = 2.36 and SD = 2.48, respectively), ($t = 0$, *n.s.* and $t = 0$, *n.s.*). See Fig. 1 for an overview.

Correlations

No significant results were obtained for the correlation between the amount of tinnitus decrease and the different frequency bands.

Neural activity: responders versus non-responders

The sLORETA showed significant differences between responders and non-responders on tDCS treatment for gamma frequency only. Increased gamma band activity could be found in the right primary auditory cortex (BA41 and BA42), right secondary auditory cortex (BA21 and BA22), and the parahippocampal areas (BA19, BA20, BA37) for responders in comparison with non-responders (see Fig. 2; $P < 0.05$). No significant differences could be retrieved in delta, theta, alpha1, alpha2, beta1, beta2, and beta3 frequencies.

No significant differences were found for hearing loss between responders and non-responders, as measured by the loss in decibels (dB SPL) at the tinnitus frequency.

Neural connectivity: responders versus non-responders

Connectivity analysis of the prestimulation obtained EEGs in resting state yielded a significant difference between tinnitus patients who were going to respond versus those who were not going to respond to bifrontal tDCS treatment (see Fig. 3). Increased lagged phase synchronization (functional connectivity) for gamma band activity could be found for responders in comparison with non-responders between the right dorsolateral prefrontal cortex (DLPFC; BA9 and BA46) and the right parahippocampal area (BA37) on the one hand and between right dorsolateral prefrontal cortex (DLPFC; BA9 and BA46) and subgenual anterior cingulate cortex (sgACC; BA25) on the other hand. Also, increased gamma functional connectivity could be found between the right secondary auditory cortex (sAC; BA 21 and BA22) and the left parahippocampal area (BA37) for responders in comparison with non-responders. No significant differences could be retrieved in delta, theta, alpha1, alpha2, beta1, beta2, and beta3 connectivity.

Study 2

If indeed increased gamma activity at the right auditory cortex is related to future tDCS response, this would imply that these patients with increased gamma would also have a larger suppression effect on right-sided TMS on the temporal lobe in comparison with non-responders to tDCS. Previous studies already revealed that TMS can indeed alter abnormal activity in the auditory cortex and can suppress tinnitus transiently (Langguth et al. 2003; De Ridder et al. 2004, 2005; Londero et al. 2006). TMS is a non-invasive tool provoking a strong impulse of magnetic field that induces an electrical current that can alter the neural activity at the applied area. This makes it possible to selectively and safely stimulate specific regions of the human brain.

Hence, the second study applies TMS on the right temporal lobe and hypothesizes that responders to tDCS would also have a larger suppression effect on tinnitus than non-responders to tDCS, as this first group has an increased gamma activity in the right auditory cortex and TMS can alter this activity (Langguth et al. 2006b; Londero et al. 2006; De Ridder et al. 2007b; Meeus et al. 2009).

This study was approved by the local ethical committee (Antwerp University Hospital) and was in accordance with the declaration of Helsinki.

Methods and materials

Participants

We studied the effect of TMS in 19 individuals (9 men and 10 women) with bilateral tinnitus (mean age was

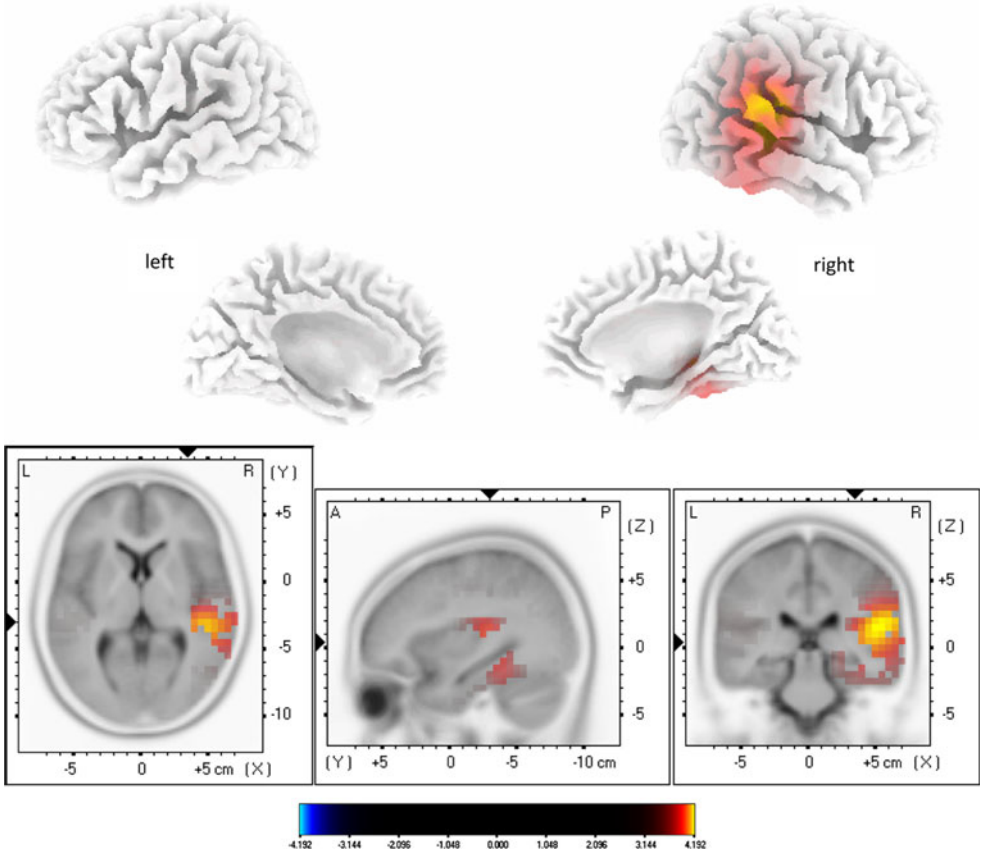

Fig. 2 sLORETA contrast analysis between responders and non-responders ($P < 0.05$). Increased neural synchronization for responders in comparison with non-responders within gamma band (30.5–45 Hz) was found for, respectively, right primary auditory cortex (BA41 and BA42), right secondary auditory cortex (BA21 and BA22), and parahippocampal areas (BA19, BA20, BA37)

51.03 years, SD = 12.73, mean tinnitus duration was 4.12) who were involved in the first study. Eight had pure tone tinnitus, and 11 had narrow band noise tinnitus. We performed TMS on the right auditory cortex (7 tDCS responders and 12 tDCS non-responders). The presence of placebo effect is tested by placing the coil perpendicular to the auditory cortex at the frequencies that yield maximal tinnitus suppression rates for TMS. The criterion for a placebo effect was having a suppression effect > 10% during the sham stimulation. Visual analog scale (VAS) was used to assess tinnitus loudness. TMS is performed using a super rapid stimulator (Magstim Inc, Wales, UK) with a figure-eight coil placed over the right auditory cortex.

Before the TMS session, patients grade their tinnitus on a numeric rating scale from 0–10.

The motor threshold to TMS is first determined by placing the coil over the motor cortex. The coil was positioned tangentially to the scalp and oriented so that the induced electrical currents would flow approximately perpendicular to the central sulcus, at 45° angle from the mid-sagittal line. The intensity of the magnetic stimulation is slowly increased until a clear contraction is observed in the contralateral thenar muscle. Then, the optimal position for eliciting this muscle contraction is determined. The stimulator output is reduced to the stimulation intensity at which still a visible muscle contraction can be elicited in 4 out of 8 trials. This method has been shown to be reliable for determining motor threshold (Pridmore et al. 1998). The coil is then moved to a location over the right auditory cortex (5–6 cm above the entrance of external auditory meatus on straight line to the vertex). The presence of placebo effect is tested by placing the coil perpendicular to the auditory cortex. The maximal tinnitus suppression is determined for sham, 1, 5, 10, and 20 Hz stimulation. The order of the stimulation sessions was random over the participants. When tinnitus suppression is noted, the patient is asked to estimate the decrease in tinnitus in percentage using a numeric rating scale. Each stimulation session consisted of 200 pulses. When tinnitus suppression is induced by TMS, the patient is asked to notify when tinnitus has returned back to baseline, i.e. when the tinnitus loudness is back to its initial VAS before the next TMS frequency is applied.

Statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA). To compare pre- and post-TMS VAS score, a paired *t* test is conducted for tinnitus loudness pre and post for both responders and non-responders to bifrontal tDCS. In addition, we also conducted a paired *t* test comparing the amount of suppression between tDCS responders and non-responders on TMS. Analyses were obtained for the different stimulation sessions as well as for each patient the maximal tinnitus suppression was included similar to previous studies. (De Ridder et al. 2005, 2007b).

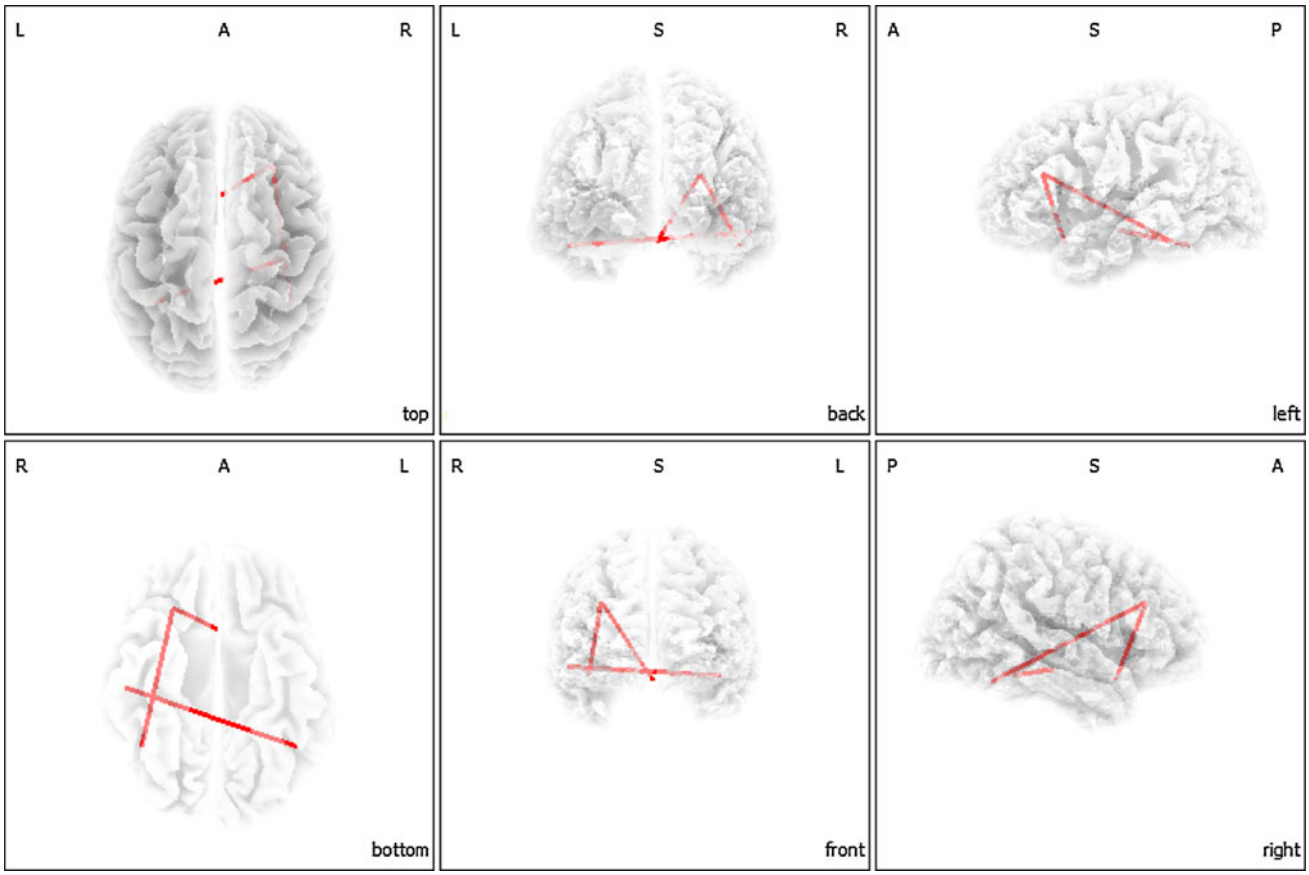

Fig. 3 Connectivity contrast analysis between responders and non-responders ($P < 0.05$). Increased functional connectivity for responders in comparison with non-responders within gamma (30–45 Hz) band between the dorsal lateral prefrontal cortex and parahippocampal areas

Results

One non-responder to tDCS was placebo positive having a suppression of 29% and was excluded. tDCS responders had a significant suppression effect of 31% when comparing baseline with 1-Hz TMS (Mean = 5.40; SD = 2.31 versus post-TMS Mean = 3.71 *SD* = 2.06) ($t = 3.07$, $P < .05$). However, for patients who did not respond to tDCS, only a suppression effect of 5% was demonstrated, indicating that there was no significant effect obtained when comparing baseline (Mean = 6.97; SD = 2.36) with post-TMS (Mean = 6.62; SD = 2.63) ($t = .58$, *n.s.*) for 1 Hz. When comparing the amount of tinnitus loudness suppression by TMS for responders and non-responders to tDCS, a significance difference was obtained ($t = 3.98$, $P < .01$): responders to tDCS (Mean = 31%; SD = 22.94) had a significantly larger suppression effect for tinnitus loudness with TMS than non-responders to tDCS (Mean = 5%; SD = 12.45). No significant results were obtained for 5, 10, and 20 Hz.

Based on the maximal suppression, the analysis yielded a significant difference for TMS on the right temporal lobe between tDCS responders and non-responders ($t = 3.52$, $P < .01$): responders to tDCS (Mean = 44.44%; SD = 34.09) demonstrated a significantly larger suppression effect for tinnitus loudness with TMS than non-responders to tDCS (Mean = 5.00%; SD = 12.45). That is tDCS responders had a significant suppression effect for VAS loudness when comparing baseline (Mean = 5.40; SD = 2.31) with post-TMS (Mean = 3.00 *SD* = 2.15) ($t = 2.93$, $P < .05$). For patients who did not respond to tDCS, no significant suppression effect was obtained for tinnitus loudness when comparing baseline (Mean = 6.97; SD = 2.36) with post-TMS (Mean = 6.62; SD = 2.63) ($t = .58$, *n.s.*).

Discussion

Not all patients respond to tDCS, and the question arises whether the functional state of the brain determines who will and who will not respond to bifrontal tDCS. Differences in resting state electrical brain activity were demonstrated in right primary and secondary auditory cortex and the right parahippocampal area, associated with a difference in the functional gamma connectivity between the right DLPFC and the right parahippocampal area on the one hand and right DLPFC and sgACC on the other hand for

bifrontal tDCS responders. As the gamma band in the auditory cortex is related to the tinnitus percept (Llinas et al. 1999; Weisz et al. 2007b), and more specifically to the perceived tinnitus loudness (van der Loo et al. 2009), TMS was performed targeting the gamma band activity in the right auditory cortex to verify whether the tinnitus suppressing effect of bifrontal tDCS is related to rTMS over the right auditory cortex. Indeed, responders to tDCS also experience a larger suppression effect on TMS (1 Hz and on the maximal suppression) placed over the right temporal cortex (i.e. auditory cortex) than non-responders, suggesting that the gamma band activity is indeed involved in responsiveness to bifrontal tDCS.

Compared to other studies, the placebo response rate in this study is very low. One reason might be that for the sham conditions, the coil only mimics the sound of active TMS but lacks the somatosensory sensation, which might not be an optimal control condition. Nevertheless, it has already been shown that TMS effect on tinnitus is not mediated by the somatosensory stimulation (Langguth et al. 2006a; Londero et al. 2006; Rossi et al. 2007). This might be due to the fact that most patients were not naïve for TMS as they already had a previous experience with TMS.

Previous research already revealed that tDCS modulates predominantly beta and gamma frequency powers (Antal et al. 2004b). That is, anodal tDCS increases beta and gamma, while cathodal tDCS decreases beta and gamma (Antal et al. 2004b). Our study revealed that responders to bifrontal tDCS had more gamma activity and gamma functional connectivity than non-responders.

The question arises how frontal cortex stimulation modulates the gamma band activity in the auditory cortex. It is known that the DLPFC exerts early inhibitory modulation of input to the primary auditory cortex in humans (Knight et al. 1989) and has been found to be associated with auditory attention (Alain et al. 1998; Lewis et al. 2000; Voisin et al. 2006), resulting in top-down modulation of auditory processing (Mitchell et al. 2005). As bifrontal tDCS modulates the DLPFC directly, it might modulate auditory attention reallocating attention away from the tinnitus for responders.

However, our results indicate increased gamma connectivity between the right DLPFC and the parahippocampal area and increased gamma activity in the right parahippocampal area in patients who respond to bifrontal tDCS. Interestingly, both animal and human invasive electrophysiological recordings in the parahippocampus and hippocampus demonstrated that auditory sensory gating is mediated by a network, which includes the auditory cortex, prefrontal cortex, and the parahippocampus (Grunwald et al. 2003; Boutros et al. 2005; Korzyukov et al. 2007; Boutros et al. 2008). Sensory gating involves suppression of redundant or irrelevant auditory information, and the parahippocampus is considered the entry to the auditory hippocampus (Tulving and Markowitsch 1997). It has been hypothesized that the hippocampus could be constantly updating the tinnitus that is being generated in the thalamocortical system (De Ridder et al. 2006) preventing habituation. Bifrontal tDCS might also modulate the parahippocampal area as there is direct and increased gamma connectivity between both areas. Clinical evidence already revealed that selectively injecting amobarbital in the anterior choroidal artery that supplies the hippocampus can suppress the pure tone component of tinnitus (De Ridder et al. 2006). Electrical stimulation as applied by bifrontal tDCS might have an effect on the parahippocampal area, modulating sensory gating, and hereby suppressing the irrelevant tinnitus percept. This parahippocampal gating mechanism possibly involves a hippocampal influence, preventing the tinnitus percept to be updated or to be pulled from hippocampal memory as previously proposed (De Ridder et al. 2006).

In addition, increased gamma connectivity between right DLPFC and sgACC for responders to bifrontal tDCS was also found. Previous research already revealed that the sgACC is responsible in processing aversive sounds (Zald and Pardo 2002) and unpleasant music (Blood et al. 1999) as well as tinnitus (Muhlau et al. 2006). This area has been implicated as the key component of social distress (Masten et al. 2009). It is therefore possible that direct DLPFC modulation exerts an indirect modulatory effect on the sgACC, suppressing tinnitus-related distress. This could explain why tDCS of the DLPFC not only alters tinnitus loudness but also tinnitus-related distress. The exact mechanism of how the subgenual cingulate area suppresses tinnitus is unknown, but a recent hypothesis (Rauschecker et al. 2010) suggests that this might involve the connections to the reticular nucleus of the thalamus, thereby interfering with tinnitus associated thalamocortical dysrhythmia (Llinas et al. 1999) responsible for the tinnitus (Rauschecker et al. 2010).

Studies analyzing resting state fMRI have shown functional connectivity between the sgACC and parahippocampus, (Margulies et al. 2007; Kahn et al. 2008). We also found an increased lagged phase synchronization of gamma band activity between the right DLPFC and, respectively, the sgACC (Margulies et al. 2007) and the parahippocampus. Taking these results together, it is conceivable that there is a triangular network between these three areas. This triangular network–DLPFC–sgACC–parahippocampus—might be related to the increased gamma band activity in the right auditory cortex. As already demonstrated, auditory sensory gating is mediated by a network including the auditory cortex, and the parahippocampus (Grunwald et al. 2003; Boutros et al. 2005; Korzyukov et al. 2007; Boutros et al. 2008). Because there

is a direct connection between the parahippocampus and the auditory cortex, increased gamma connectivity in the triangular network might consequently increase gamma activity in the right auditory cortex. This hypothesis is supported by our second study as responders to bifrontal tDCS had also a larger suppression effect on right auditory cortex TMS stimulation.

Our results demonstrate that responders and non-responders to bifrontal tDCS seem to have differences in resting brain activity and functional connectivity that might explain the suppression effect for both tinnitus loudness and tinnitus-related distress. The fact that auditory cortex TMS resulted in tinnitus suppression in bifrontal tDCS responders also suggest that it might not be so important where exactly the distributed tinnitus network is modulated, as long as it occurs in a part of the network. These results corroborate with the findings of Kleinjung et al. (2008) showing that combined TMS of the temporal cortex preceding TMS on the DLPFC had better long-lasting results for tinnitus-related distress than TMS on the temporal cortex, suggesting that modulating more than one area of the distributed network is better than limiting the modulating to one area of the distributed tinnitus network.

Although a clear differentiation could be made between responders and non-responders for tDCS, which was confirmed by TMS, this does not mean that non-responders will not respond to other neuromodulation techniques such as transcutaneous electrical nerve stimulation (Vanneste et al. 2010b) or cortical implantations (De Ridder et al. 2007a, 2010). It is possible that non-responders have a typical different resting brain activity and functional connectivity where different targets need to be modulated. Future research is required to further explore this hypothesis and to verify whether other regions of interest are important. Future research is also needed to verify what the differences are comparing pre- and post-tDCS with EEG to draw more firm conclusions in the specific effect of tDCS for responders and non-responders.

Conclusion

In summary, responders to bifrontal tDCS seem to differ in resting state electrical brain activity compared to non-responders in the right auditory cortex and parahippocampal area. They also have a different functional connectivity between DLPFC and, respectively, the sgACC and parahippocampal area. The connectivity might explain the suppression effect for both tinnitus loudness and tinnitus-related distress.

Conflict of interest None.

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