Pinpointing a highly specific pathological functional connection that turns phantom sound into distress

Sven Vanneste, Marco Congedo, Dirk de Ridder

Pinpointing a highly specific pathological functional connection that turns phantom sound into distress

Sven Vanneste[1], Marco Congedo[2] & Dirk De Ridder[1,3]

1. Department of Translational Neuroscience, Faculty of Medicine, University of Antwerp, Belgium.
2. Vision and Brain Signal processing (ViBS) research group, GIPSA-lab, CNRS, Grenoble University, Grenoble, France.
3. Department of Surgical Sciences, Dunedin School of Medicine, University of Otago, New Zealand.

Short title: A connection that turns tinnitus into distress

Key Words: Independent component analysis, distress, loudness, auditory phantom percept, tinnitus

Acknowledgements: This work was supported by TRI (Tinnitus Research Initiative), Research Foundation Flanders (FWO), TOP financiering of University of Antwerp, and the Neurological Foundation of New Zealand

Correspondence to: Sven Vanneste, Brai²n, University Hospital Antwerp, Wilrijkstraat 10, 2650 Edegem, Belgium. Tel: +32 821 33 36; email: sven.vanneste@ua.ac.be; website: http://www.brai2n.org

Abstract

It has been suggested that an auditory phantom percept is the result of multiple, parallel but overlapping networks. One of those networks encodes tinnitus loudness and is electrophysiologically separable from a non-specific distress network. The present study investigates how these networks anatomically overlap, what networks are involved and how and when these networks interact. The EEG data of 317 tinnitus patients and 256 healthy subjects were analyzed, using independent component analysis. Results demonstrate that tinnitus is characterized by at least two major brain networks, each consisting of multiple independent components. One network reflects tinnitus distress, while another network reflects the loudness of the tinnitus. The component coherence analysis shows that the independent components that make up the distress and loudness networks communicate within their respective network at several discrete frequencies in parallel. The distress and loudness networks do not intercommunicate for patients without distress, but do when patients are distressed by their tinnitus. The obtained data demonstrate that the components that build up these two separable networks communicate at discrete frequencies within the network, and only between the distress and loudness networks in those patients in whom the symptoms are also clinically linked.

Introduction

Tinnitus is an auditory phantom percept with a tone or hissing in the absence of any objective physical sound source that is experienced by 5-15% in the population (Eggermont JJ and LE Roberts 2004). Up to 25% of the affected people report interference with their lives as tinnitus causes a considerable amount of distress (Heller AJ 2003). Distress can play an important role in the development of tinnitus, however it is not a necessity as not everyone who experiences tinnitus becomes chronically distressed (Andersson G and V Westin 2008).

It is known that the cerebral cortex is organized into parallel, segregated systems of brain areas that are specialized for processing distinct forms of information (Buckner RL et al. 2009). Based on previous findings it has been proposed that the unified percept of tinnitus could be considered as an emergent property of multiple parallel networks (De Ridder D, AB Elgoyhen, et al. 2011). That is, since there is not always a relationship between measures of loudness and distress, two separate networks in the brain might underpin these two aspects of tinnitus.

Research has shown that tinnitus is related to the reorganization (Muhlnickel W et al. 1998) and hyperactivity (Weisz N et al. 2007) of the auditory cortex. The loudness of auditory verbal hallucinations, a complex phantom sound, has further been associated with the anterior cingulate cortex and frontal gyrus, insula, and with strong activation of the inner speech processing networks (Vercammen A et al. 2011), that could be considered to be a ‘loudness network’.

On the other hand, it was revealed that in tinnitus a ‘distress network’ of functionally interconnected non-auditory brain areas including amygdala, anterior cingulate cortex, parahippocampus and insula are important (Vanneste S, M Plazier, E der Loo, et al. 2010). Using sLORETA source analysis of Fourier transformed data demonstrated that the distress was related to anterior cingulate beta activity, and the amount of distress was correlated to the

amount of alpha activity in the medial temporal lobe (amygdala, hippocampus, parahippocampus) as well as the subgenual anterior cingulate cortex, and insula (Vanneste S, M Plazier, E der Loo, *et al.* 2010). By using a blind source separation technique, namely independent component analysis, in a different group of patients with low and high distress, it was shown that tinnitus distress results from alpha and beta abnormal activity in subgenual anterior cingulate cortex extending to the pregenual and dorsal anterior cingulate cortex and the ventromedial and ventrolateral prefrontal cortex, insula, and parahippocampal area (De Ridder D, S Vanneste, et al. 2011). This network overlaps partially with brain areas implicated in distress in patients suffering from pain (Price DD 2000; Moisset X and D Bouhassira 2007), dyspnea (von Leupoldt A et al. 2009), functional somatic syndromes and posttraumatic stress disorder (Vermetten E et al. 2007), and might therefore represent an aspecific distress network.

A study of resting-state electroencephalography (EEG) in large databases of healthy subjects has pointed to the existence of multiple distributed independent components with partially overlapping brain areas, each with a specific spontaneous oscillatory pattern (Congedo et al., 2010a). The analyses of the lagged phase coherence between these components have suggested that these components at rest are organized in a small number of networks. That is, ensembles of components communicate within networks, but do not communicate between networks (Congedo M, RE John, D De Ridder, L Prichep, et al. 2010). The interaction between the components occurs at multiple discrete frequencies in parallel (Congedo M, RE John, D De Ridder, L Prichep, *et al.* 2010). Such narrowband communication is analogous to what has been described for animals (Fujisawa S and G Buzsaki 2011). The identification of specific oscillatory patterns and connectivity signatures within parallel networks for tinnitus might further explain the underlying neurophysiological

mechanism and as a result help in the identification of a treatment as to date no treatment exists for this auditory phantom phenomenon (Langguth B et al. 2009).

The present study used group blind source separation of resting state EEG to map the involvement of different brain networks in auditory phantom perception. Resting state networks can be identified using completely data-driven approaches, using EEG, MEG, but also fMRI techniques. The resting state networks are evident in the human brain during the awake resting state, as well as during task performance, sleep and anesthesia (Fox MD and ME Raichle 2007), or at different vigilance levels (Olbrich S et al. 2009). Meanwhile, emerging evidence shows that neurological or psychiatric diseases are associated with alterations in resting state activity (Fornito A and ET Bullmore 2010). Thus, the spontaneous activity of resting state network reflects a fundamental aspect of cerebral physiology and pathophysiology.

The blind source separation (BSS) approach, such as independent component analysis (ICA), is currently enjoying increasing popularity thanks to its complete data-driven nature (Scheeringa R et al. 2008). While the BSS analysis shows the relationship between different brain areas within a component (i.e. network), we also verify the lagged phase coherence (i.e. out of phase) between the different independent components (i.e. sources) by verifying the inter-component coherence (Congedo M, RE John, D De Ridder, L Prichep, et al. 2010). This latter method helps to understand how different networks can communicate with each other in an out of phase matter. We hypothesized that patients with an auditory phantom percept would be discernible from a healthy control group during resting activity and that different independent components might form two separable networks involved in the distress and loudness of the auditory phantom percept. We furthermore hypothesized that tinnitus related distress would be electrophysiologically characterized by the presence of inter-network functional connectivity.

Methods & Materials

Patients with an auditory phantom percept

317 patients (*M* = 50.24 years; *Sd* = 14.32; 184 males and 133 females) with continuous tinnitus were included in this study. Tinnitus was considered chronic if its onset dated back one year or more. Individuals with pulsatile tinnitus, Ménière disease, otosclerosis, chronic headache, neurological disorders such as brain tumors, and individuals being treated for mental disorders were excluded from the study in order to increase the sample homogeneity. All patients were interviewed as to their perceived location of the tinnitus (the left ear, in both ears, and centralized in the middle of the head (bilateral), the right ear) as well the tinnitus tone (pure tone like tinnitus or noise-like tinnitus). In addition all patients were screened for the extent of hearing loss using a pure tone audiometry using the British Society of Audiology procedures at .125 kHz, .25 kHz, .5 kHz, 1 kHz, 2 kHz, 3 kHz, 4 kHz, 6 kHz and 8 kHz (Audiology BSo 2008). Tinnitus patients were tested for the tinnitus frequency doing a tinnitus analysis. In unilateral tinnitus patients, the tinnitus analysis was performed contralateral to the tinnitus ear. In bilateral tinnitus patients, tinnitus analysis was performed contralateral to the worst tinnitus ear. The tinnitus analysis consisted of the assessment of the tinnitus pitch and loudness. First, a 1 kHz pure tone was presented contralateral to the (worst) tinnitus ear at 10 dB above the patient's hearing threshold in that ear. The pitch was adjusted until the patient judged the sound to resemble most to his/her tinnitus. The loudness of this tone was then adjusted in a similar way until it corresponded to the patient's specific tinnitus as well. The tinnitus loudness (dB SL) was computed by subtracting the absolute tinnitus loudness (dB HL) with the auditory threshold at that frequency (Meeus O et al. 2009; Meeus O et al. 2011). See Table 1 for an overview of the tinnitus characteristics.

A visual analogue scale for loudness ('How loud is your tinnitus?': 0 = no tinnitus and 10 = as loud as imaginable') was assessed as well as the Dutch translation of the Tinnitus Questionnaire (TQ) (Meeus O et al. 2007). This scale is comprised of 52 items and is a well-established measure for the assessment of a broad spectrum of tinnitus-related psychological complaints. The TQ measures emotional and cognitive distress, intrusiveness, auditory perceptual difficulties, sleep disturbances, and somatic complaints. As previously mentioned, the global TQ score can be computed to measure the general level of psychological and psychosomatic distress. In several studies, this measure has been shown to be a reliable and valid instrument in different countries (Hiller W and G Goebel 1992; McCombe A et al. 2001). A 3-point scale is given for all items, ranging from 'true' (2 points) to 'partly true' (1 point) and 'not true' (0 points). The total score (from 0-84) was computed according to standard criteria published in previous work (Hiller W and G Goebel 1992; Hiller W et al. 1994; Meeus O *et al.* 2007). Based on the total score on the TQ, patients can be assigned to a distress category: slight (0-30 points; grade 1), moderate (31-46; grade 2), severe (47-59; grade 3), and very severe (60-84; grade 4) distress. Goebel and Hiller stated that grade 4 tinnitus patients are psychologically decompensated, indicating that patients categorized into this group cannot cope with their tinnitus (Goebel G and W Hiller 1994). In contrast, patients that have a score lower than 60 on the TQ can cope with their tinnitus.

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

Healthy control group

EEG data of a healthy control group (N = 256; *M* = 49.514 years; *Sd* = 14.82; 154 males and 102 females) was collected. None of these subjects was known to suffer from tinnitus. Exclusion criteria were known psychiatric or neurological illness, psychiatric history or

drug/alcohol abuse, history of head injury (with loss of consciousness) or seizures, headache, or physical disability. For these healthy controls hearing assessment was not performed.

Data collection

EEG data were obtained as a standard procedure. Recordings were obtained in a fully lighted room with each participant sitting upright on a small but comfortable chair. The actual recording lasted approximately five min. The EEG was sampled using Mitsar-201 amplifiers (NovaTech http://www.novatecheeg.com/) with 19 electrodes placed according to the standard 10-20 International placement (Fp1, Fp2, F7, F3, Fz, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1, O2) referenced to digitally linked ears, analogous to what is was done in the normative group. Impedances were checked to remain below 5 kΩ. Data were collected eyes-closed (sampling rate = 500 Hz, band passed 0.15-200Hz). Off-line data were resampled to 128 Hz, band-pass filtered in the range 2-44 Hz and subsequently transposed into Eureka! software (Congedo M 2002), plotted and carefully inspected for manual artifact-rejection. All episodic artifacts including eye blinks, eye movements, teeth clenching, body movement, or ECG artifact were removed from the stream of the EEG.

Group Blind Source Separation

In the fMRI literature BSS approaches such as ICA are currently enjoying increasing popularity thanks to their complete data-driven nature (Greicius MD et al. 2004; Bluhm RL et al. 2008; Scheeringa R *et al.* 2008). In EEG also, BSS has recently been extended to group analyses of the resting state (Congedo M, RE John, D De Ridder and L Prichep 2010). As any other source separation method of this family, the BSS approach we use decomposes the whole EEG in a number of elementary statistically independent components. Each component

is characterised by its time course and spatial pattern, therein used as input to tomographic source localization by the sLORETA inverse solution (Pascual-Marqui RD 2002).

We employed the group blind source separation approach consisting in the approximate joint diagonalization of grand-average Fourier cospectral matrices on the tinnitus group (Congedo M, RE John, D De Ridder, L Prichep, *et al.* 2010). Such method can separate uncorrelated sources with non-proportional power spectra (Congedo M et al. 2008) and is analogous to the averaging group ICA approach described for fMRI (Schmithorst VJ and SK Holland 2004). The BSS method we employ measures the intra-component relationship between different brain areas. Only co-spectra in the range 2-44 Hz were diagonalized because in this band-pass region continuous EEG features the highest signal-to-noise ratio. This method finds a "group" mixing and demixing matrix. The demixing matrix was then used to extract the power of the components on both the tinnitus and healthy group, as described in detail in Congedo et al. (Congedo M, RE John, D De Ridder, L Prichep, *et al.* 2010). In order to estimate the number of components in the tinnitus group that can be found reliably we apply a bootstrap resampling test-retest strategy; each resample is obtained selecting 100 patients at random. For each resample the group BSS analysis is performed limiting the number of components to 2, 3, 4, 5, 6, 7 and 8 components. This process was conducted 50 times. Based on these analyses we conducted the group blind source separation on the total group estimating the six most energetic components, as these generated the most reliable and reproducible findings over the different resamplings.

Source localization

Standardized low-resolution brain electromagnetic tomography (sLORETA; Pascual-Marqui, 2002) was used to estimate the intracerebral electrical sources that generated the seven group BSS components. As a standard procedure a common average reference

transformation (Pascual-Marqui RD 2002) is performed before applying the sLORETA algorithm. sLORETA computes electric neuronal activity as current density (A/m2) without assuming a predefined number of active sources. The solution space used in this study and associated leadfield matrix are those implemented in the LORETA-Key software (freely available at http://www.uzh.ch/keyinst/loreta.htm). This software implements revisited realistic electrode coordinates (Jurcak et al. 2007) and the lead field produced by Fuchs et al. (2002) applying the boundary element method on the MNI-152 (Montreal neurological institute, Canada) template of Mazziotta et al. (2001). The sLORETA-key anatomical template divides and label the neocortical (including hippocampus and anterior cingulate cortex) MNI-152 volume in 6,239 voxels of dimension 5 mm^3, based on probabilities returned by the Demon Atlas (Lancaster et al. 2000). The co-registration makes use of the correct translation from the MNI-152 space into the Talaiach and Tournoux (1988) space (Brett et al. 2002).

Comparison between BSS component power of patients with auditory phantom percept group with a healthy control group

For each group BSS components relative power was computed with 1 Hz resolution with respect to the total energy across all components, on both the tinnitus and healthy group. Then the relative power for each frequency and each component was compared between the two groups. Multiple comparison student t-tests were performed separately for each component. The significance threshold was based on a permutation t-max test with 5000 permutations. The methodology used is non-parametric. It is based on estimating, via randomization, the empirical probability distribution for the max-statistic, under the null hypothesis (Nichols TE and AP Holmes 2002). This methodology corrects for multiple testing across frequencies and guarantees that the probability of falsely rejecting even only one hypothesis is less than the

chosen alpha level. To correct for multiple testing across components, the type II error rate for significance of the t-max tests was set to .05 components.

Correlation analysis

A correlation analysis was conducted between the relative power of the six tinnitus components and the scores of tinnitus distress as measured with the TQ and tinnitus loudness as measured with the VAS. The correlation analysis was performed in all 4 Hz spaced discrete Fourier frequencies in the range 2-44 Hz (2-4 Hz, 4-8 Hz, …, 42-44 Hz). Corrections were performed for multiple comparisons across the 10 frequency bands using a Bonferroni method. Each component was tested separately without correction.

Lagged phase coherence (out of phase coherence)

The BSS method we employed cancels the in-phase correlation at all frequencies between all sources taken pair-wise, however it does not interfere with their out of phase correlation. The residual out-of-phase correlation among sources can then be studied, for instance in the frequency domain (coherence). Such "lagged phase coherence" between two sources can be interpreted as the amount of cross-talk between the regions contributing to the source activity (Congedo M, RE John, D De Ridder, L Prichep, *et al.* 2010). Since the two components oscillate coherently with a phase lag, the cross-talk can be interpreted as information sharing by axonal transmission. More precisely, the discrete Fourier transform decomposes the signal in a finite series of cosine and sine waves (in-phase and out-of-phase carrier waves, forming the real and imaginary part of the Fourier decomposition) at the Fourier frequencies (Bloomfield 2000). The lag of the cosine waves with respect to their sine counterparts is inversely proportional to their frequency and amounts to a quarter of the period; for example, the period of a sinusoidal wave at 10 Hz is 100 ms. The sine is shifted a quarter of a cycle (25

ms) with the respect to the cosine. Then the lagged phase coherence at 10 Hz indicates coherent oscillations with a 25 ms delay, while at 20 Hz the delay is 12.5 ms, etc. The threshold of significance for a given lagged phase coherence value according to asymptotic results can be found as described by Pascual-Marqui (2007), where the definition of lagged phase coherence can be found as well. This analysis was corrected for the amount of pairwise comparisons using a Bonferroni correction.

In addition, time-series of current density were extracted for different region of interests using sLORETA. Power in all 6,239 voxels was normalized to a power of 1 and log transformed at each time point. Region of interest values thus reflect the log transformed fraction of total power across all voxels, separately for specific frequencies. Regions of interest were defined based upon all brain areas involved in component III and IV (see Figure 3) at specific frequencies (see Figure 7). We calculated the log transformed power for each brain area with the two different networks separately. A comparison was made between each tinnitus group (grade 1, grade 2, grade 3 and grade 4) and control subjects for the lagged phase coherence for the frequencies 10 Hz and 11.5 Hz respectively. We conducted this additional analysis to verify how exactly the two networks, i.e. the loudness network and the distress network, communicate with each other.

Results

Behavioral measurements

A significant positive correlation between the TQ and the tinnitus loudness was obtained, indicating that the higher the TQ the louder patients perceive their tinnitus (see Table 2). When dividing the tinnitus patients into different grades, going from slight to very severe distress, based on their TQ score, no significant correlations could be obtained between the TQ and the tinnitus loudness for each group separately (see Table 2).

Group Blind Source Separation on the Control Sample

Similar to Congedo et al. (Congedo M, RE John, D De Ridder, L Prichep, *et al.* 2010) we applied a blind source separation analysis extracting 7 components explaining 82.35% of the total variance. Components I, II, III, VII are located more posteriorly, while components IV, V and VI are located more anteriorly (see Figure 1). Component I shows activity in the dorsal anterior cingulate (BA24) extending into the subgenual anterior cingulate cortex/ventromedial prefrontal cortex (BA25), the insula (BA13) and the parahippocampal area (BA28). Component II demonstrates activity within the cuneus/precuneus (BA7 and BA31) extending into the posterior cingulate gyrus (BA23 and BA31) and the right superior parietal lobule (BA7), while component III shows activity in the cuneus/precuneus (BA31 and BA7), retrosplenial posterior cingulate (BA30) and visual areas (BA18 and BA19). Component IV revealed activity in the lingual gyrus, fusiform gyrus, middle and inferior occipital gyrus (occipital pole) (BA17, BA18 and BA19). Component V shows activity in the dorsal anterior cingulate (BA 24), the subgenual anterior cingulate cortex/ventromedial prefrontal cortex (BA25), the inferior frontal gyrus (BA 47) and the parahippocampal gyrus (BA28 and BA34). Component VI demonstrated activity within the subgenual anterior cingulate/ventromedial prefrontal cortex (BA25), the inferior frontal gyrus (BA 47), the parahippocampal gyrus (BA28 and BA34) and the insula (BA13). Component VII shows activity in the post-central gyrus (BA1, BA2 and BA3), the middle occipital gyrus (BA 18), the superior and middle temporal gyrus (BA39 and BA41) and the angular gyrus (BA39).

Group Blind Source Separation on the Tinnitus Sample

Based on the bootstrap analysis 6 components could repeatedly be obtained. Analysis on the total group reveals that these six components explain 62.07% of the total variance.

Components I, II and III are located posteriorly in the brain, while components IV, V and VI are positioned anteriorly in the brain (see Figure 2). Component I reveals activity within the posterior cingulate cortex (BA23, BA30 and BA31) and the precuneus (BA7) and component II demonstrates activity within the posterior cingulate cortex (BA30, BA30 and BA31) extending into the precuneus (BA7) and the inferior parietal cortex (BA40) as well as in the parahippocampal area (BA19, BA30, BA35 and BA36). The third component shows activity in the retrospenial posterior cingulate cortex (BA29, BA30, BA31), the anterior end of lingual gyrus (BA7, BA18) and the parahippocampal area (BA19, BA30). Component IV yielded activity within the pregenual anterior cingulate cortex (BA24 and BA32), subgenual anterior cingulate cortex/ventromedial prefrontal cortex (BA25), and left and right insula (B13). Component V demonstrates activity in the subgenual anterior cingulate cortex/ventromedial prefrontal cortex (BA25), hippocampal area (BA34), amygdala, and medial frontal gyrus (BA11) while component VI shows activity in the dorsal anterior cingulate cortex (BA24) supplementary motor area (BA6), subgenual anterior cingulate cortex/ventromedial prefrontal cortex (BA25) and medial frontal gyrus (BA11).

Component I is characterized by prominent alpha activity, while component II and III by beta activity (see Figure 3A). Component II can be categorized by low beta and component III low and medium beta activity. Component IV is characterized by high beta and gamma activity, while component V and VI are characterized by delta, theta and gamma activity (see Figure 3A).

A comparison between the power produced by the six independent components in the Tinnitus group and the healthy control group revealed several significant results. For component I the tinnitus group had less delta, theta, alpha and beta activity and more gamma activity (see Figure 3B). Component II revealed less beta activity and more gamma activity, while component III revealed less beta activity (see Figure 3B). Component IV and V yielded

more delta, theta, alpha, and beta activity for the tinnitus group in comparison to a healthy control group. Furthermore, Component IV showed additional increased gamma activity, while component V showed decreased gamma activity for the tinnitus group in comparison to the healthy control group (see Figure 3B). For the tinnitus group Component VI showed more beta and gamma activity in comparison to the control group (see Figure 3B).

Auditory cortex

Further analyses were conducted to verify the involvement of the auditory cortex. As no group BSS component involved the auditory cortex, a separate group BSS analysis was conducted for three separate subgroups: a group that presents right-sided tinnitus, left-sided tinnitus and bilateral tinnitus. The reason for this latter analysis is that previous research indicated that the activity in the auditory cortex can differ depending on the tinnitus lateralization.

Extracting seven components instead of six revealed that component VII was indeed an auditory component for both left and right-sided tinnitus patients (see Figure 4). For the bilateral tinnitus patients only after extracting eight components such an auditory component could be found (see Figure 4). The explained variance of the auditory component for the different groups was very low, 4.75% for left-sided tinnitus patients, 4.59% for the right-sided tinnitus patients and 4.75% for the bilateral tinnitus patients.

Lagged phase coherence on the Healthy Sample

The estimated lagged phase coherence was computed between all components in the healthy control subjects (see Figure 5). This analysis revealed no significant results indicating that all components are uncorrelated in an out of phase manner.

Lagged phase coherence on the Tinnitus Sample

The estimated lagged phase coherence was obtained between all components (see Figure 6A & B). The profile appears clearly non-random and seems to concentrate in discrete frequency regions of high-communication rate, interleaved with by regions of low communication rate. Our data show communication among component pairs interplaying at multiple frequencies (multiple time-lags) simultaneously, although most pairs demonstrate a narrow band communication window. Significant coherences are reported in figure 6C in the form of a connectivity graph. By definition (of ICA BSS) all components are uncorrelated in-phase, however here we look at the lagged 'out of phase' coherence. Since the different components oscillate coherently with a phase lag, the cross-talk can be interpreted as information sharing by cortico-cortical transmission. We see that the six components are organized in two independent networks (neither in-phase nor out of phase communication exists between networks), whereas significant out of phase crosstalk exists within each network. Within the two independent networks it seems that the first network, including components I, II, IV and VI are functionally connected at slow frequencies (i.e. 2.5-9 Hz), while the second network, which including components III and V are functionally connected at fast frequencies (i.e. 30.5 Hz).

Correlation analysis between brain activity and distress and loudness

Separate correlation analyses were conducted between tinnitus distress as measured by the TQ and the log-power of 4 Hz spaced discrete Fourier frequencies in the range 2-44 Hz (2-4 Hz, 4-8 Hz, …, 42-44 Hz) for the different components (component I, II, III, IV, V and VI). These correlation analyses demonstrated significant negative correlations between tinnitus distress and the log-power for component I at frequency range 8-12 Hz, 12-16 Hz and for component II at frequency range 8-12 Hz, 24-28 Hz, 29-32 Hz and 32-38 Hz (see Table 3). In

addition, a positive correlation was obtained between tinnitus distress and the log-power of component IV at the frequency range 8-12 Hz and the log power of Component VI at frequency range 24-28 z and 28-32 Hz (see Table 3). No significant results were obtained between tinnitus distress and the log-power of component III and component IV at the specific frequency ranges. Based on these findings we could claim that the combination of component I, II, IV and VI represents a distress network.

Separate correlation analyses were conducted between the tinnitus loudness as measured by a visual analogue scale and the log-power of 4 Hz spaced discrete Fourier frequencies in the range 2-44 Hz (2-4 Hz, 4-8 Hz, …, 42-44 Hz) for the different components (component I, II, III, IV, V and VI). This correlation analysis revealed a significant negative correlation between tinnitus loudness and the log-power of component III at frequency range 20-24 Hz, 24-28 and 28-32 Hz (see Table 3). For component V a positive correlation was obtained between tinnitus loudness and the log-power at the frequency 12-16 Hz (see Table 3). No significant results were obtained between tinnitus loudness and the log-power of component I, II, IV and VI at the specific frequency ranges. As such we could name this III-V network a loudness network.

Correlation analyses between the log-power of discrete frequencies in the range 2-44 Hz (2-4 Hz, 4-8 Hz, …, 42-44 Hz) of the different components (component I, II, III, IV, V and VI) and respectively hearing loss as measured by the loss in decibels (dB SL) at the tinnitus frequency, tinnitus frequency (Hz) and age obtained no significant results.

In addition a correlation analysis was conducted on the auditory component (component VII or VIII depending on the tinnitus lateralization) to verify whether tinnitus loudness correlated with this component for the respective tinnitus groups. No significant results could be obtained. A correlation analysis between tinnitus distress (TQ) and the auditory component for the respective tinnitus groups showed no significant effects.

Inter-relationship between the distress and loudness network

Our results suggest two independent networks exist, one related to distress and another related to tinnitus loudness. Based on the behavioral measurements a positive correlation was obtained between distress and loudness. Previous research also suggested that there might be a link between the distress and loudness network for specific groups of tinnitus patients (De Ridder D, AB Elgoyhen, *et al.* 2011). Hence we conducted a secondary lagged phase coherence analysis for respectively tinnitus patients with a grade 1 (slight distress), grade 2 (moderate distress), grade 3 (severe distress) or grade 4 (very severe distress) distress score.

We applied a BSS analysis on the different tinnitus groups separately to verify whether similar components could be obtained. This BSS analysis revealed similar six components for all four grades going from slight to very severe distress (see Figure 2As, Bs, Cs & Ds). A second step was to calculate the lagged phase coherence for the four grades (see Figure 2s). Figure 7A, B, C and D shows the estimated lagged phase coherence obtained between all pair-wise components for respectively grade 1, grade 2, grade 3 and grade 4. For grade 1 no significant communication was found between the different components (see Figure 7). However for grade 2, grade 3 and grade 4 a specific communication was obtained within the loudness and distress networks (see Figure 7A, B, C and D and Figure 2s). Again the profile appears clearly non-random and seems to concentrate in discrete frequency regions of high-communication rate, interleaved with by regions of low communication rate. In addition, we found significant coherences between the loudness and distress network (trough communication between component III and component IV) for grade 3 and grade 4 tinnitus patients, but not for grade 1 and grade 2 (see Figure 7C, D, E and F). No other significant lagged phase coherence could be obtained between the distress and loudness network. Our

data show a lagged phase coherence between component III and IV at 10 Hz for grade 3 (see Figure 7A, B & D), and at 11.5 Hz for grade 4 (see Figure 7A, B & E).

Brain specific connectivity between the distress and loudness network

To further explore the lagged phase coherence between the different tinnitus groups (i.e. grade 1 (slight distress), grade 2 (moderate distress), grade 3 (severe distress) or grade 4 (very severe distress)) and an age- and gender-matched healthy control group the current density was extracted on the raw EEG data including all the regions of component III (i.e. the posterior cingulate cortex, the anterior lingual gyrus and the parahippocampal area) and component V (i.e. the subgenual anterior cingulate cortex/ventromedial prefrontal cortex, dorsal anterior cingulate, left and right insula) specifically for 10 and 11.5 Hz. We opt for these latter frequencies as the above mentioned analyses clearly revealed that both frequencies are important in respectively grade 3 and grade 4 tinnitus patients.

A sLORETA connectivity comparison between the control group and the respective tinnitus groups (i.e. healthy control, grade1, grade 2, grade 3 and grade 4) demonstrated a significant difference at 10 Hz in lagged phase coherence between healthy subjects and respectively grade 3 and 4 patients (see Figure 8). A significant connection at 10 Hz between the parahippocampal area that was included in component III and the subgenual anterior cingulate cortex/ventromedial prefrontal cortex that was included in component IV between the grade 3 tinnitus patients and the healthy control subjects was obtained, revealing that grade 3 tinnitus patients had an increased lagged phase coherence. For grade 4 tinnitus patients a similar result was obtained between the parahippocampal area and the subgenual anterior cingulate cortex/ventromedial prefrontal cortex at 10 Hz as well as 11.5 Hz in comparison to the healthy subjects group. No significant effects were obtained between both grade 1 and grade 2 tinnitus patients and the healthy control group for the 10 Hz, as well as

for grade 1 tinnitus patients at 11.5 Hz in comparison to the healthy control group. A significant decrease in lagged phase coherence was however obtained in grade 2 tinnitus patients in comparison to healthy control group at 11.5 Hz between the parahippocampal area included in component III and the subgenual anterior cingulate cortex/ventromedial prefrontal cortex included in component IV.

Discussion

Recently, it was shown that spontaneous, temporally fast, electrophysiological activity as reflected in EEG, is correlated with the slower hemodynamic fluctuations of the BOLD signal in resting state fMRI (Britz J et al. 2010; Musso F et al. 2010). Results revealed that each of the BOLD resting state networks identified in the resting state fMRI data was characterized by a relatively specific electrophysiological signature involving a combination of several microstates (Yuan H et al. 2012). The concurrently acquired fMRI and EEG data thus reveals that complex spatial and temporal dynamics of neuronal activity are reflected by the interrelationships between neuroimaging measures obtained using modalities that vastly differ in their spatial and temporal properties (Yuan H *et al.* 2012). These findings support the potential of multimodal fMRI and EEG approaches to elucidate normal and pathological interactions between cerebral function and behavior, cognition or emotion (Britz J *et al.* 2010; Musso F *et al.* 2010; Yuan H *et al.* 2012).

The main goal of the study was to characterize the loudness and distress networks in patients with an auditory phantom percept to verify whether the loudness and distress are generated by two separable networks and to analyze how the components of these two networks communicate within the networks and between the networks by using a BSS method on electrophysiological data. Understanding how and when the loudness and distress interact at an electrophysiological level might lead in the future to a neurophysiologically

based, frequency-selective and anatomically restricted neuromodulation approach to functionally separate these networks, clinically leading to the continued perception of the phantom sound without the associated distress. The study reveals two separate networks, one reflecting the loudness and the other distress, with partially overlapping brain areas, each with a specific spontaneous oscillatory pattern and functional connectivity signature.

The independent components

An independent component analysis in the healthy control subjects revealed similar networks as obtained previously by Congedo et al. (Congedo M, RE John, D De Ridder, L Prichep, *et al.* 2010). A comparison between the components obtained for the healthy control subjects and the tinnitus patients showed similar components. However, component VII is different between the healthy control subjects and tinnitus patients. In healthy controls this component is more localized within the post-central gyrus, the middle occipital gyrus, the superior and middle temporal gyrus and the angular gyrus (BA39).

Only after a further analysis an additional auditory component (i.e. component VII/VIII) was obtained for patients with an auditory phantom percept. These results depended on the lateralization of the tinnitus. This component is specifically associated to the auditory cortex. Previous research already demonstrated that this brain area might be important in tinnitus. That is, previous research has demonstrated a reorganization (Muhlnickel W *et al.* 1998) and hyperactivity (Weisz N *et al.* 2007) of the auditory cortex in tinnitus patients in comparison to healthy control subjects and tinnitus loudness might be related to the amount of hyperactivity within the auditory cortex (van der Loo E et al. 2009). Our results showed that for the healthy controls no auditory component could be demonstrated, which is likely due to the fact that resting state electrical brain activity recording was performed in a sound attenuating room, precluding a constant auditory percept during recording.

The distress network

The distress network is characterized by increased alpha activity in the subgenual anterior/ventromedial prefrontal cortex and beta activity in the dorsal anterior cingulate cortex, correlating positively with distress, while for the posterior cingulate cortex distress correlates negatively with alpha and high beta activity. These findings are in agreement with previous research conducted on a separate group of tinnitus patients revealing that highly distressed tinnitus patients have increased alpha activity within the subgenual anterior cingulate cortex/ventromedial prefrontal cortex extending to ventromedial prefrontal cortex, insula, and parahippocampal area and decreased activity in the posterior cingulate cortex extending in the precuneus in comparison to tinnitus patients with low distress (Vanneste S, M Plazier, E der Loo, *et al.* 2010). In comparison to the healthy control group highly distressed tinnitus patients show more alpha and beta activity within the dorsal anterior cingulate cortex (Vanneste S, M Plazier, E der Loo, *et al.* 2010; De Ridder D, S Vanneste, *et al.* 2011). This network overlaps partially with brain areas implicated in distress in patients suffering from pain, dyspnea, functional somatic syndromes and posttraumatic stress disorder, and might therefore represent a non-specific distress network (Craig, 2003; Critchley, 2005; Phan et al., 2002; Peyron et al., 2000). This suggests that the distress associated with tinnitus perception might be related to activation of a general 'distress network'.

The loudness network

The loudness network is built up by two components, one located anteriorly and one posteriorly. This is different than the results obtained by an auditory cortex centered approach used in previous research, demonstrating that tinnitus loudness is correlated to decreased alpha (Lorenz I et al. 2009) and increased gamma band activity in the auditory cortex (Llinas

RR et al. 1999; Weisz N *et al.* 2007). An independent component, source localized to the auditory areas could only be retrieved in subgroups selected on perceived lateralization of the phantom sound. Based on this analysis it appears that the auditory cortex is less important in tinnitus than previously expected considering that the auditory component could only explain a small amount (4-5%) of the total variance. Furthermore, no correlation was found between the auditory component and tinnitus loudness. This finding seems in contrast with previous findings that showed that the auditory cortex is correlated to gamma activity (van der Loo E *et al.* 2009). However, the auditory components are spectrally constructed of multiple frequency bands, and the gamma band on continuously recorded EEG features a very low signal to noise ratio. Moreover, the electrical dipolar activity of the primary auditory cortex is mainly tangential to the cortical surface, resulting in little visibility by EEG. Similar investigations using magnetoencephalography, which is sensitive to tangential dipole orientation, may confirm this hypothesis. The poor sensitivity of EEG to electrical activity produced in the auditory cortex, especially in the gamma band, can explain the lack of correlation between the auditory component and subjectively perceived loudness.

Communication between the independent components within each network

Our results indicate that the independent components within the two networks communicate at different frequency bands. While the distress network (C1-C2-C4-C6) seems to communicate or is functionally connected at slow frequencies (i.e. 2.5-9 Hz), the loudness network (C3-C5) communicates or is functionally connected at fast frequencies (i.e. 30.5 Hz).

It has already been argued that both slow and fast rhythms have a different role in perception (Varela F et al. 2001). More precisely slow waves would constitute the ‘context’ and fast waves the ‘content’ of neuronal representations (Poppel E 1994; Llinas R et al. 1998). Applied to our findings it can be suggested that the distress network would function as

context, while the tinnitus loudness is important for the content of the auditory phantom percept. It has indeed been shown that highly distressed patients perceive the tinnitus louder (Henry JL and PH Wilson 1995), demonstrating the contextual modulation of the phantom auditory content.

Decreased posterior and increased anterior brain activity

The group BSS analysis resulted in six components of which three components are located more posteriorly, including the retrosplenial posterior cingulate cortex, the posterior cingulate cortex, the precuneus, the parahippocampal area and three components located more anteriorly including the subgenual anterior cingulate cortex/ventromedial prefrontal cortex, the pregenual anterior cingulate cortex, the left and right insula, the hippocampal area, the amygdala, the medial frontal gyrus and the dorsal anterior cingulate cortex. These six non-auditory components obtained in our study are in line with previous research on source localized independent component analysis on resting-state EEG conducted in healthy subjects (Congedo M, RE John, D De Ridder and L Prichep 2010) and in tinnitus distress (De Ridder D, S Vanneste, *et al.* 2011). Spectrally, the tinnitus group was characterized by decreased activity in the delta, theta, alpha and beta band for the first three posterior components (i.e. I, II, III) and increased activity was demonstrated within delta, theta, alpha, and beta band for the last three anterior components (i.e. IV, V, VI) in comparison to the control subjects. Tinnitus patients are thus characterized by decreased activity posteriorly and increased activity anteriorly in comparison to the healthy control group. These finding are in line with previous research using transcranial magnetic stimulation (TMS), suggesting that targeting the anterior cingulate with low-frequency TMS (supposedly decreasing the activity of the underlying cortex) can reduce the tinnitus perception, while targeting the posterior cingulate cortex with high frequency TMS (supposedly increasing the activity of the underlying cortex)

can decrease the tinnitus perception (Vanneste S, M Plazier, P Van de Heyning, et al. 2011; Vanneste S and D De Ridder 2012).

While the anterior cingulate has been implicated in emotional (Sinha R et al. 2004), attentional (Cohen RA et al. 1999), reward (Bush G et al. 2002) and executive (Vogt BA et al. 1992) processing, the posterior cingulate seems to be related more to cognitive and memory aspects of information processing (Vogt BA *et al.* 1992). The posteriorly based components found in the group BSS analysis might be related to cognitive and memory aspects of the tinnitus percept, as the posterior cingulate cortex is implicated in auditory memory (Grasby PM et al. 1993; Fletcher PC et al. 1995) and in cognitive aspects of auditory processing (Laufer I et al. 2009). Activity in the precuneus and adjacent retrosplenial posterior cingulate cortex and posterior cingulate cortex has indeed been linked to successful retrieval from auditory (and visual) memory (Shannon BJ and RL Buckner 2004; Sadaghiani S et al. 2009). The posterior cingulate cortex, precuneus component has been proposed to exert a salience based cognitive auditory comparator function (Laufer I *et al.* 2009).

Multiple networks with partially overlapping brain areas

Our results demonstrate that component I, II, IV and VI, composing the first network, a distress network, as well as component III and V, composing the second network, a loudness network, communicate with each other at specific frequency bands in parallel. However these two brain networks do not communicate between each other in the total tinnitus population. In addition it was shown that the first brain network (components I-II-IV-VI) correlates with the distress, while the second brain network (components III-V) correlates with loudness. These latter findings could maybe explain why previous clinical research could not find a clear relationship between loudness, as measured by psychophysical tinnitus matching testing, and distress in tinnitus (Goodwin PE and RM Johnson 1980; Andersson G 2003) and it confirms

our hypothesis that tinnitus distress is the result of a 'distress network' separable from a tinnitus loudness network (De Ridder D, AB Elgoyhen, *et al.* 2011).

That is, the 'distress network' and the 'loudness network' both include the subgenual anterior cingulate cortex and the posterior cingulate cortex. However, the activity of these structures correlates with distress and loudness scores at different frequencies; the subgenual cingulate cortex/ventromedial prefrontal cortex correlates with distress in the alpha band (as part of component IV) whereas with loudness in the beta band (as part of component V); the posterior cingulate cortex correlates with distress in the alpha band and low beta band (as part of component I) and with loudness in high beta activity (as part of component III). The results within the subgenual anterior cingulate cortex however need to be interpreted with care, as tinnitus distress and loudness may be related to separate midline frontal structures (Leaver AM et al. 2012). Using spatial location of sources in EEG is relatively limited, especially in deeper midline frontal cortex and cingulate cortex areas due to the inherent low resolution of the used methodology. Hence it is difficult to claim that the distress and loudness in the subgenual anterior cingulate cortex really overlap or are generated very closely but adjacently to each other.

The lagged phase coherence between the distress and loudness network

It has also been shown that in highly distressed patients the tinnitus is perceived louder (Henry JL and PH Wilson 1995). And indeed in highly distressed patients (grade 3 and 4) a functional connection exists between independent component III and IV, thus between a component of the distress network and the loudness network that is not present in patients with low distress, i.e. in patients in whom the tinnitus, whatever its loudness is not distressing. This functional connection occurs in the alpha range, i.e. at 10 Hz and 11.5 Hz in the distressed patients.

In order to find out which brain areas are critically involved in determining whether a patient is distressed by the phantom sound or not, the brain areas that are part of the independent components III and IV are used as regions of interest and the lagged phase synchronization between the regions of interest are computed specifically for 10 and 11.5 Hz both for tinnitus without distress (grade 1 and 2) and tinnitus patients with distress (grade 3 and 4) and statistically compared to a non-tinnitus control group. This demonstrates that in patients with tinnitus but without distress there is no functional connection between any of the regions of interest that is different from non-tinnitus patients, as is expected, but that in distressed patients there exists a pathological functional connection between the parahippocampal area and the subgenual anterior cingulate cortex/ventromedial prefrontal cortex at 10 and 11.5 Hz. The subgenual anterior cingulate cortex/ventromedial prefrontal cortex are also functionally connected to the insula in these patients at the same discrete frequencies.

Thus, whether a patient is distressed or not by his/her phantom sound might be critically dependent on a very specific subgenual anterior cingulate cortex/ventromedial prefrontal cortex-parahippocampal connection at a very narrow frequency band. Being able to pinpoint this highly selective pathological connection opens up the way for very selective modulation of this connection, e.g. by implanting an electrode that is capable of disrupting the 10 and 11.5 Hz communication between the parahippocampus and subgenual anterior cingulate cortex/ventromedial prefrontal cortex.

This pathological 10-11.5 Hz connection is not surprising in view of the known tinnitus pathophysiology. It has been proposed that the subgenual anterior cingulate cortex extending into the ventromedial prefontal cortex is involved in tinnitus loudness perception (Muhlau M et al. 2006; Rauschecker JP et al. 2010; Leaver AM et al. 2011). Structural deficits have been observed in the subgenual cingulate cortex/nucleus accumbens area. Based on these findings,

it has been postulated that tinnitus is the result of a deficient sensory attentional gating mechanism, originating in the subgenual cingulate cortex/nucleus accumbens area and acting on the reticular thalamic nucleus (Rauschecker JP *et al.* 2010). Not only is the loudness modulated by the subgenual anterior cingulate cortex. The amount of distress perceived by tinnitus patients is related to alpha activity in a network encompassing the subgenual anterior cingulate cortex and insula, extending to the amygdala-hippocampus and parahippocampus (Vanneste S, M Plazier, E der Loo, *et al.* 2010; De Ridder D, S Vanneste, *et al.* 2011). The 10 and 11.5 Hz functional connectivity between the subgenual anterior cingulate cortex and insula, coupling loudness to increased distress, also fits with the fact that the insula is associated with tinnitus related distress (van der Loo E et al. 2011) and interoceptive perception (Craig AD 2002). The parahippocampal area is involved in different tinnitus characteristics such as lateralization and tinnitus type (Vanneste S, M Plazier, E van der Loo, et al. 2010; Vanneste S, PV de Heyning, et al. 2011; Vanneste S, M Plazier, E van der Loo, et al. 2011). Furthermore, in chronification its functional connectivity (lagged phase synchronization) to the auditory cortex is increased (Vanneste S, P van de Heyning, et al. 2011). The parahippocampal area is also associated with tinnitus distress (Vanneste S, M Plazier, E der Loo, *et al.* 2010; De Ridder D, S Vanneste, *et al.* 2011). Thus it is not surprising that the parahippocampal area, which has a sensory gating function for irrelevant or redundant auditory input (Boutros NN et al. 2008) and subgenual anterior cingulate cortex, which has an attentional gating function, when functionally coupled, link phantom sound to distress.

Is this a universal mechanism?

It has been suggested before that the tinnitus distress network is actually a non-specific distress network, in view of the fact that the areas involved in tinnitus distress are similar to those in pain (Moisset X and D Bouhassira 2007), social rejection (Masten CL et al. 2009),

somatoform disorder (Landgrebe M et al. 2008) and asthmatic apnea (von Leupoldt A *et al.* 2009).

The connectivity between the parahippocampal area and subgenual anterior cingulate cortex/ventromedial prefrontal cortex is proposed to be part of a general aversive network, involving the cerebellum, parahippocampal area and hypothalamus, as it is activated both by pain and unpleasant visual images (Moulton EA et al. 2011). Unfortunately EEG cannot pick-up electrical activity from neither the cerebellum nor the hypothalamus.

Our results also showed that communication between the different components as well as between the distress and loudness network are within narrowband frequencies. Narrowband frequency communication has already been described for animals (Fujisawa S and G Buzsaki 2011). In humans, interactions between the independent components also appear to occur at multiple narrowband frequencies (Congedo M, RE John, D De Ridder, L Prichep, *et al.* 2010). The findings of this study add to these previous findings and suggest that pathological functional connectivity between specific networks at specific frequencies can lead to a problematic auditory phantom percept.

Whether or not the described mechanism in this paper is universal is impossible to say, but in view of the above arguments it is definitely worthwhile to further explore this possibility.

Limitations

One limitation of the current study is related to the fact that we did not control between the tinnitus group and healthy control group for hearing loss. Previous research on structural differences in tinnitus patients has shown that hearing loss can have an important impact on the results (Husain FT et al. 2011; Melcher JR et al. 2012). As our research showed that similar components were obtained in both the healthy control subjects and the tinnitus group, this suggested that hearing loss might have only a minor influence on the component analysis.

In addition it was shown that correlations with the different components did not correlate with the hearing loss in the tinnitus group. Nevertheless, this might be a factor to take into account in further research.

In conclusion

In summary, this study suggests that tinnitus is the result of disproportionate activity between anteriorly and posteriorly based components in comparison to healthy control subjects. Based on these results it can be proposed that auditory phantom percept can be separated in at least two independent brain networks with overlapping brain areas characterized by a specific spontaneous oscillatory pattern. One network is involved in the distress, while another network is involved in the loudness of the phantom percept. Within the distress and loudness network the different independent components that make up the network communicate at narrowband frequencies, but the distress and loudness network do not seem to directly intercommunicate in the total group of patients. However, in those patients who are severely distressed, the sound and distress are linked both clinically and electrophysiologically. A specific functional connection exists in the distressed patients between component III (loudness) and IV (distress) at 10 and 11.5 Hz. More specifically it can be pinpointed between the parahippocampal area and the subgenual anterior cingulate cortex/ventromedial prefrontal cortex. This is in accordance with a recently proposed model that states that tinnitus is generated by multiple dynamically active separable but overlapping networks, each characterizing a specific aspect of the unified tinnitus percept (De Ridder D, AB Elgoyhen, *et al.* 2011), but adds to this concept empirical findings characterizing the communication protocol within and between these two of multiple separable networks.

References

Andersson G. 2003. Tinnitus loudness matchings in relation to annoyance and grading of severity. Auris Nasus Larynx. 30:129-133.

Andersson G, Westin V. 2008. Understanding tinnitus distress: introducing the concepts of moderators and mediators. Int J Audiol. 47 Suppl 2:S106-111.

Audiology BSo. 2008. Recommended procedure:pure tone air and bone conduction threshold audiometry with and without masking and determination of uncomfortable loudness levels.

Bluhm RL, Osuch EA, Lanius RA, Boksman K, Neufeld RW, Theberge J, Williamson P. 2008. Default mode network connectivity: effects of age, sex, and analytic approach. Neuroreport. 19:887-891.

Boutros NN, Mears R, Pflieger ME, Moxon KA, Ludowig E, Rosburg T. 2008. Sensory gating in the human hippocampal and rhinal regions: regional differences. Hippocampus. 18:310-316.

Britz J, Van De Ville D, Michel CM. 2010. BOLD correlates of EEG topography reveal rapid resting-state network dynamics. Neuroimage. 52:1162-1170.

Buckner RL, Sepulcre J, Talukdar T, Krienen FM, Liu H, Hedden T, Andrews-Hanna JR, Sperling RA, Johnson KA. 2009. Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. J Neurosci. 29:1860-1873.

Bush G, Vogt BA, Holmes J, Dale AM, Greve D, Jenike MA, Rosen BR. 2002. Dorsal anterior cingulate cortex: a role in reward-based decision making. Proc Natl Acad Sci U S A. 99:523-528.

Cohen RA, Kaplan RF, Moser DJ, Jenkins MA, Wilkinson H. 1999. Impairments of attention after cingulotomy. Neurology. 53:819-824.

Congedo M. 2002. EureKa! (Version 3.0) [Computer Software]. Knoxville, TN: NovaTech EEG Inc. Freeware available at www.NovaTechEEG.

Congedo M, Gouy-Pailler C, Jutten C. 2008. On the blind source separation of human electroencephalogram by approximate joint diagonalization of second order statistics. Clin Neurophysiol. 119:2677-2686.

Congedo M, John RE, De Ridder D, Prichep L. 2010. Group independent component analysis of resting state EEG in large normative samples. Int J Psychophysiol.

Congedo M, John RE, De Ridder D, Prichep L, Isenhart R. 2010. On the "dependence" of "independent" group EEG sources; an EEG study on two large databases. Brain Topogr. 23:134-138.

Craig AD. 2002. How do you feel? Interoception: the sense of the physiological condition of the body. Nat Rev Neurosci. 3:655-666.

De Ridder D, Elgoyhen AB, Romo R, Langguth B. 2011. Phantom percepts: tinnitus and pain as persisting aversive memory networks. Proc Natl Acad Sci U S A. 108:8075-8080.

De Ridder D, Vanneste S, Congedo M. 2011. The distressed brain: a group blind source separation analysis on tinnitus. PLoS One. 6:e24273.

Eggermont JJ, Roberts LE. 2004. The neuroscience of tinnitus. Trends Neurosci. 27:676-682.

Fletcher PC, Frith CD, Grasby PM, Shallice T, Frackowiak RS, Dolan RJ. 1995. Brain systems for encoding and retrieval of auditory-verbal memory. An in vivo study in humans. Brain. 118 (Pt 2):401-416.

Fornito A, Bullmore ET. 2010. What can spontaneous fluctuations of the blood oxygenation-level-dependent signal tell us about psychiatric disorders? Curr Opin Psychiatry. 23:239-249.

Fox MD, Raichle ME. 2007. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci. 8:700-711.

Fujisawa S, Buzsaki G. 2011. A 4 Hz oscillation adaptively synchronizes prefrontal, VTA, and hippocampal activities. Neuron. 72:153-165.

Goebel G, Hiller W. 1994. [The tinnitus questionnaire. A standard instrument for grading the degree of tinnitus. Results of a multicenter study with the tinnitus questionnaire]. HNO. 42:166-172.

Goodwin PE, Johnson RM. 1980. The loudness of tinnitus. Acta Otolaryngol. 90:353-359.

Grasby PM, Frith CD, Friston KJ, Bench C, Frackowiak RS, Dolan RJ. 1993. Functional mapping of brain areas implicated in auditory--verbal memory function. Brain. 116 (Pt 1):1-20.

Greicius MD, Srivastava G, Reiss AL, Menon V. 2004. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc Natl Acad Sci U S A. 101:4637-4642.

Heller AJ. 2003. Classification and epidemiology of tinnitus. Otolaryngol Clin North Am. 36:239-248.

Henry JL, Wilson PH. 1995. Coping with Tinnitus: Two Studies of Psychological and Audiological Characteristics of Patients with High and Low Tinnitus-Related Distress. Int Tinnitus J. 1:85-92.

Hiller W, Goebel G. 1992. A psychometric study of complaints in chronic tinnitus. J Psychosom Res. 36:337-348.

Hiller W, Goebel G, Rief W. 1994. Reliability of self-rated tinnitus distress and association with psychological symptom patterns. Br J Clin Psychol. 33 (Pt 2):231-239.

Husain FT, Medina RE, Davis CW, Szymko-Bennett Y, Simonyan K, Pajor NM, Horwitz B. 2011. Neuroanatomical changes due to hearing loss and chronic tinnitus: a combined VBM and DTI study. Brain Res. 1369:74-88.

Landgrebe M, Barta W, Rosengarth K, Frick U, Hauser S, Langguth B, Rutschmann R, Greenlee MW, Hajak G, Eichhammer P. 2008. Neuronal correlates of symptom formation in functional somatic syndromes: A fMRI study. Neuroimage. 41:1336-1344.

Langguth B, Salvi R, Elgoyhen AB. 2009. Emerging pharmacotherapy of tinnitus. Expert Opin Emerg Drugs. 14:687-702.

Laufer I, Negishi M, Constable RT. 2009. Comparator and non-comparator mechanisms of change detection in the context of speech--an ERP study. Neuroimage. 44:546-562.

Leaver AM, Renier L, Chevillet MA, Morgan S, Kim HJ, Rauschecker JP. 2011. Dysregulation of limbic and auditory networks in tinnitus. Neuron. 69:33-43.

Leaver AM, Seydell-Greenwald A, Turesky TK, Morgan S, Kim HJ, Rauschecker JP. 2012. Cortico-limbic morphology separates tinnitus from tinnitus distress. Front Syst Neurosci. 6:21.

Llinas R, Ribary U, Contreras D, Pedroarena C. 1998. The neuronal basis for consciousness. Philos Trans R Soc Lond B Biol Sci. 353:1841-1849.

Llinas RR, Ribary U, Jeanmonod D, Kronberg E, Mitra PP. 1999. Thalamocortical dysrhythmia: A neurological and neuropsychiatric syndrome characterized by magnetoencephalography. Proc Natl Acad Sci U S A. 96:15222-15227.

Lorenz I, Muller N, Schlee W, Hartmann T, Weisz N. 2009. Loss of alpha power is related to increased gamma synchronization-A marker of reduced inhibition in tinnitus? Neurosci Lett. 453:225-228.

Masten CL, Eisenberger NI, Borofsky LA, Pfeifer JH, McNealy K, Mazziotta JC, Dapretto M. 2009. Neural correlates of social exclusion during adolescence: understanding the distress of peer rejection. Soc Cogn Affect Neurosci. 4:143-157.

McCombe A, Baguley D, Coles R, McKenna L, McKinney C, Windle-Taylor P. 2001. Guidelines for the grading of tinnitus severity: the results of a working group

commissioned by the British Association of Otolaryngologists, Head and Neck Surgeons, 1999. Clin Otolaryngol Allied Sci. 26:388-393.

Meeus O, Blaivie C, Van de Heyning P. 2007. Validation of the Dutch and the French version of the Tinnitus Questionnaire. B-ENT. 3 Suppl 7:11-17.

Meeus O, De Ridder D, Van de Heyning P. 2011. Administration of the Combination Clonazepam-Deanxit as Treatment for Tinnitus. Otol Neurotol.

Meeus O, Heyndrickx K, Lambrechts P, De Ridder D, Van de Heyning P. 2009. Phase-shift treatment for tinnitus of cochlear origin. Eur Arch Otorhinolaryngol.

Melcher JR, Knudson IM, Levine RA. 2012. Subcallosal brain structure: Correlation with hearing threshold at supra-clinical frequencies (>8 kHz), but not with tinnitus. Hear Res.

Moisset X, Bouhassira D. 2007. Brain imaging of neuropathic pain. Neuroimage. 37 Suppl 1:S80-88.

Moulton EA, Elman I, Pendse G, Schmahmann J, Becerra L, Borsook D. 2011. Aversion-related circuitry in the cerebellum: responses to noxious heat and unpleasant images. J Neurosci. 31:3795-3804.

Muhlau M, Rauschecker JP, Oestreicher E, Gaser C, Rottinger M, Wohlschlager AM, Simon F, Etgen T, Conrad B, Sander D. 2006. Structural brain changes in tinnitus. Cereb Cortex. 16:1283-1288.

Muhlnickel W, Elbert T, Taub E, Flor H. 1998. Reorganization of auditory cortex in tinnitus. Proc Natl Acad Sci U S A. 95:10340-10343.

Musso F, Brinkmeyer J, Mobascher A, Warbrick T, Winterer G. 2010. Spontaneous brain activity and EEG microstates. A novel EEG/fMRI analysis approach to explore resting-state networks. Neuroimage. 52:1149-1161.

Nichols TE, Holmes AP. 2002. Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum Brain Mapp. 15:1-25.

Olbrich S, Mulert C, Karch S, Trenner M, Leicht G, Pogarell O, Hegerl U. 2009. EEG-vigilance and BOLD effect during simultaneous EEG/fMRI measurement. Neuroimage. 45:319-332.

Pascual-Marqui RD. 2002. Standardized low-resolution brain electromagnetic tomography (sLORETA): technical details. Methods Find Exp Clin Pharmacol. 24 Suppl D:5-12.

Poppel E. 1994. Temporal mechanisms in perception. Int Rev Neurobiol. 37:185-202; discussion 203-187.

Price DD. 2000. Psychological and neural mechanisms of the affective dimension of pain. Science. 288:1769-1772.

Rauschecker JP, leaver AM, Muhlau M. 2010. Tuning Out the Noise: Limbic-Auditory Interactions in Tinnitus. Neuron. 66:819-826.

Sadaghiani S, Hesselmann G, Kleinschmidt A. 2009. Distributed and antagonistic contributions of ongoing activity fluctuations to auditory stimulus detection. J Neurosci. 29:13410-13417.

Scheeringa R, Bastiaansen MC, Petersson KM, Oostenveld R, Norris DG, Hagoort P. 2008. Frontal theta EEG activity correlates negatively with the default mode network in resting state. Int J Psychophysiol. 67:242-251.

Schmithorst VJ, Holland SK. 2004. Comparison of three methods for generating group statistical inferences from independent component analysis of functional magnetic resonance imaging data. J Magn Reson Imaging. 19:365-368.

Shannon BJ, Buckner RL. 2004. Functional-anatomic correlates of memory retrieval that suggest nontraditional processing roles for multiple distinct regions within posterior parietal cortex. J Neurosci. 24:10084-10092.

Sinha R, Lacadie C, Skudlarski P, Wexler BE. 2004. Neural circuits underlying emotional distress in humans. Ann N Y Acad Sci. 1032:254-257.

van der Loo E, Congedo M, Vanneste S, De Heyning PV, De Ridder D. 2011. Insular lateralization in tinnitus distress. Auton Neurosci. 165:191-194.

van der Loo E, Gais S, Congedo M, Vanneste S, Plazier M, Menovsky T, Van de Heyning P, De Ridder D. 2009. Tinnitus intensity dependent gamma oscillations of the contralateral auditory cortex. PLoS One. 4:e7396.

Vanneste S, de Heyning PV, Ridder DD. 2011. Contralateral parahippocampal gamma-band activity determines noise-like tinnitus laterality: a region of interest analysis. Neuroscience.

Vanneste S, De Ridder D. 2012. Differences between a single session and repeated sessions of 1 Hz TMS by double-cone coil prefrontal stimulation for the improvement of tinnitus. Brain Stimul.

Vanneste S, Plazier M, der Loo E, de Heyning PV, Congedo M, De Ridder D. 2010. The neural correlates of tinnitus-related distress. Neuroimage. 52:470-480.

Vanneste S, Plazier M, Van de Heyning P, De Ridder D. 2011. Repetitive transcranial magnetic stimulation frequency dependent tinnitus improvement by double cone coil prefrontal stimulation. J Neurol Neurosurg Psychiatry. 82:1160-1164.

Vanneste S, Plazier M, van der Loo E, Van de Heyning P, De Ridder D. 2010. The Differences in Brain Activity between Narrow Band Noise and Pure Tone Tinnitus. PLoS One. 5:e13618.

Vanneste S, Plazier M, van der Loo E, Van de Heyning P, De Ridder D. 2011. The difference between uni- and bilateral auditory phantom percept. Clin Neurophysiol. 122:578-587.

Vanneste S, van de Heyning P, De Ridder D. 2011. The neural network of phantom sound changes over time: a comparison between recent-onset and chronic tinnitus patients. Eur J Neurosci. 34:718-731.

Varela F, Lachaux JP, Rodriguez E, Martinerie J. 2001. The brainweb: phase synchronization and large-scale integration. Nat Rev Neurosci. 2:229-239.

Vercammen A, Knegtering H, Bruggeman R, Aleman A. 2011. Subjective loudness and reality of auditory verbal hallucinations and activation of the inner speech processing network. Schizophr Bull. 37:1009-1016.

Vermetten E, Schmahl C, Southwick SM, Bremner JD. 2007. Positron tomographic emission study of olfactory induced emotional recall in veterans with and without combat-related posttraumatic stress disorder. Psychopharmacol Bull. 40:8-30.

Vogt BA, Finch DM, Olson CR. 1992. Functional heterogeneity in cingulate cortex: the anterior executive and posterior evaluative regions. Cereb Cortex. 2:435-443.

von Leupoldt A, Sommer T, Kegat S, Baumann HJ, Klose H, Dahme B, Buchel C. 2009. Dyspnea and pain share emotion-related brain network. Neuroimage. 48:200-206.

Weisz N, Muller S, Schlee W, Dohrmann K, Hartmann T, Elbert T. 2007. The neural code of auditory phantom perception. J Neurosci. 27:1479-1484.

Yuan H, Zotev V, Phillips R, Drevets WC, Bodurka J. 2012. Spatiotemporal dynamics of the brain at rest--exploring EEG microstates as electrophysiological signatures of BOLD resting state networks. Neuroimage. 60:2062-2072.

Table 1. Tinnitus characteristics

Ear		
	Left	61
	Right	49
	Bilateral	207
Tone		
	Pure tone	138
	Noise Like	179
Tinnitus Frequency (Hz)		
	Mean	4905.51
	Sd	3257.72
Hearing loss at the Tinnitus Frequency (dB SL)		
	Mean	7.60
	Sd	8.58

Table 2. The means and standard deviations of TQ (Distress) and VAS (loudness) for the total patient group and patients with a grade 1 (slight distress), grade 2 (moderate distress), grade 3 (severe distress) or grade 4 (very severe distress) separately as well as the Pearson correlations (r) between the TQ and VAS for the different groups.

	TQ (Distress) *M* (*Sd*)	**VAS (Loudness)** *M* (*Sd*)	**r**	**N**
Total	35.88 (16.28)	5.11 (2.44)	.45***	317
Grade 1	20.12 (6.79)	3.74 (2.29)	.05	53
Grade 2	37.75 (4.93)	5.58 (1.98)	.01	104
Grade 3	52.03 (4.29)	6.12 (1.82)	.18	84
Grade 4	66.07 (4.63)	7.51 (2.05)	.12	76

* $p < .05$; ** $p < .01$; *** $p < .01$

Table 3. Significant Pearson correlations (r) between TQ (distress) and the VAS (loudness) with the log-power of the six BSS components. Corrections were performed for multiple comparisons across the 10 frequency bands using a Bonferroni method. Each component was tested separately without correction.

		Frequencies	r
Component I	*Distress*	8-12	-.24**
		12-16	-.28**
		16-20	-.29**
	Loudness	-	-
Component II	*Distress*	8-12	-.23**
		24-28	-.18*
		28-32	-.18*
		32-38	-.17*
	Loudness	-	-
Component III	*Distress*	-	-
	Loudness	20-24	-.23**
		24-28	-.18*
		28-32	-.25**
Component IV	*Distress*	8-12	.18*
	Loudness	-	-
Component V	*Distress*	-	-
	Loudness	12-16	.21**
Component VI	*Distress*	24-28	.21**
		28-32	.18*
	Loudness	-	-

* $p < .05$; ** $p < .01$

Figure legends

Figure 1. Overview of the obtained BSS components for the healthy control group. Component I: dorsal anterior cingulate (BA24) extending into the subgenual anterior cingulate cortex/ventromedial prefrontal cortex (BA25), insula (BA13) and parahippocampal area (BA28). Component II: cuneus/precuneus (BA7 and BA31) extending into posterior cingulate gyrus (BA23 and BA31), right superior parietal lobule (BA7). Component III: cuneus/precuneus (BA31 and BA7), retrosplenial posterior cingulate (BA30), parahippocampal gyrus (BA18 and BA19). Component IV: lingual gyrus, fusiform gyrus, middle and inferior occipital gyrus (occipital pole) (BA17, BA18 and BA19). Component V: dorsal anterior cingulate (BA 24), subgenual anterior cingulate cortex/ventromedial prefrontal cortex (BA25), inferior frontal gyrus (BA 47), parahippocampal gyrus (BA28 and BA34). Component VI: subgenual anterior cingulate/ventromedial prefrontal cortex (BA25), inferior frontal gyrus (BA 47), parahippocampal gyrus (BA28 and BA34), insula (BA13). Component VII: post-central gyrus (BA1, BA2 and BA3), middle occipital gyrus (BA 18), superior and middle temporal gyrus (BA39 and BA41), angular gyrus (BA39).

Figure 2. Overview of the obtained BSS components for the patients with an auditory phantom percept. Component I: posterior cingulate cortex (BA23, BA30 and BA31) extending into precuneus (BA7). Component II: posterior cingulate cortex (BA30, BA30 and BA31) extending into the precuneus (BA7) and inferior parietal cortex (BA40) and parahippocampal area (BA19, BA30, BA35 and BA36). Component III: Posterior cingulate cortex (BA29, BA30, BA31), extending into anterior end of the lingual gyrus (BA7, BA18) and parahippocampal area (BA19, BA30). Component IV: subgenual anterior cingulate cortex and dorsal anterior cingulate cortex (BA, 25, 24 and BA32), extending into left and right insula (B13). Component V: subgenual anterior cingulate

cortex (BA25), hippocampal area (BA34), amygdala, and orbitofrontal gyrus (BA11). Component VI: subgenual anterior cingulate cortex and dorsal anterior cingulate cortex (BA25 and 24) supplementary motor area (BA6), orbitofrontal gyrus (BA11).

Figure 3. (A) Relative spectral power (Y-Axis) for patients with an auditory phantom percept in the 2–44 Hz (X-axis) range. CI: component I, CII: component II, CIII: component III, CIV: component IV, CV: component V, CVI: component VI. (B) The differences between patients with an auditory phantom percept and the healthy control subjects. Pink disks flag a statistically higher power ($p < 0.05$, uncorrected) in the normal power of the healthy subjects as compared to the patients with an auditory phantom percept. Green disks flag a statistically lower power ($p < 0.05$, uncorrected) in the normal power of the healthy subjects as compared to the patients with an auditory phantom percept.

Figure 4. Overview of the obtained auditory BSS components for the patients with an auditory phantom percept in respectively left-sided, right-sided and bilateral tinnitus.

Figure 5. (A & B) Lagged phase coherence (Y-Axis) in the 2–44 Hz (X-axis) range for all pair-wise couples of the seven components for healthy control subjects (i.e. 1-2: is coupling between Component I and Component II). No significant results could be obtained.

Figure 6. (A & B) Lagged phase coherence (Y-Axis) in the 2–44 Hz (X-axis) range for all pair-wise couples of the six components for patients with an auditory phantom percept (i.e. 1-2: is coupling between Component I and Component II). The horizontal black line indicates the threshold of significance ($p < .05$) In (A) the coherence profiles of component pairs exceeding the threshold for at least one frequency are drawn using a thick line. (C) Significant lagged phase coherence as a connectivity graph. Blue: Loudness network, Purple: Distress network. Significant connections are represented by lines connecting the components. The six components organize in two fully independent modules. CI:

component I, CII: component II, CIII: component III, CIV: component IV, CV: component V, CVI: component VI. This analysis was corrected for the amount of pairwise comparisons.

Figure 7. (A)-(D): Lagged phase coherence connectivity graph for grade 1 (slight distress) (A), grade 2 (moderate distress) (B), grade 3 (severe distress) (C) and grade 4 (very severe distress) (D) Blue: Loudness network, Purple: Distress network. (E) Healthy control subjects show no significant connections. Significant connections are represented by lines connecting the components. (F & G): Lagged phase coherence (Y-Axis) in the 2–44 Hz (X-axis) range for coupling between Component III and Component IV for patients with an auditory phantom percept. This analysis was corrected for the amount of pairwise comparisons.

Figure 8. A comparison for the lagged phase coherence in healthy controls and group grade 1 (slight distress), grade 2 (moderate distress), grade 3 (severe distress), grade 4 (very severe distress) tinnitus patients and matched healthy control groups at 10 Hz and 11.5 Hz. Blue lines show significantly decreased lagged phase coherence in the specific tinnitus group in comparison to the healthy control subjects, while red lines show significantly increased lagged phase coherence in the specific tinnitus group in comparison to the control group. This analysis was corrected for the frequency bands, but not for the amount of comparisons.