

The BDNF Val⁶⁶Met polymorphism regulates vulnerability to chronic stress and phantom perception

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Abstract

Auditory phantom percepts, such as tinnitus, are a heterogeneous condition with great inter-individual variations regarding both the percept itself and its concomitants. Tinnitus causes a considerable amount of distress, with as many as 25% of affected people reporting that it interferes with their daily lives. Although previous research gives an idea about the neural correlates of tinnitus-related distress, it cannot explain why some tinnitus patients develop distress and while others are not bothered by their tinnitus. BDNF Val⁶⁶Met polymorphism (rs6265) is a known risk factor for affective disorders due to its common frequency and established functionality. To elucidate, we explore the neural activation pattern of tinnitus associated with the BDNF Val⁶⁶Met polymorphism using electrophysiological data to assess activity and connectivity changes. A total of 110 participants (55 tinnitus and 55 matched control subjects) were included. In this study, we validate that the BDNF Val⁶⁶Met polymorphism plays an important role in the susceptibility to the clinical manifestation of tinnitus-related distress. We demonstrate that Val/Met carriers have increased alpha power in the subgenual anterior cingulate cortex that correlates with distress levels. Furthermore, distress mediates the relationship between BDNF Val⁶⁶Met polymorphism and tinnitus loudness. In other words, for Val/Met carriers, the subgenual anterior cingulate cortex sends distress-related information to the parahippocampus, which likely integrates the loudness and distress of the tinnitus percept.

Keywords

Tinnitus, Distress, Subgenual anterior cingulate cortex, Parahippocampus, BDNF, Val⁶⁶Met, Polymorphism

1 Significance statement

With a prevalence of up to 43% worldwide, chronic tinnitus lacks both widely effective treatments and adequate understanding of its brain mechanisms. Tinnitus describes a very heterogeneous condition with great interindividual variations regarding both the percept itself and its concomitants. Consistent with this idea, our research shows that tinnitus-related distress is related to the underlying neurogenetic architecture. We establish, in a human study, that BDNF Val⁶⁶Met polymorphism plays an important role in the susceptibility to the clinical manifestation that affects the distress directly and tinnitus loudness indirectly.

2 Introduction

Tinnitus, the phantom perception of a sound in the absence of an external stimulus, is a widespread disorder with a prevalence varying between 5% and 43% worldwide (Axelsson and Ringdahl, 1989; Baguley et al., 2013). Tinnitus describes a very heterogeneous condition with great interindividual variations regarding both the percept itself and its concomitants (Jagoda et al., 2018). Tinnitus causes a considerable amount of distress, with as many as 25% of affected people reporting that it interferes with their lives (Heller, 2003). Distress can play an important role in the development of tinnitus, but not everyone who experiences tinnitus becomes chronically distressed (Andersson and Westin, 2008).

Research has demonstrated that tinnitus-related distress is linked to anterior cingulate activity (Chen et al., 2018; Golm et al., 2013, 2016; Vanneste and De Ridder, 2015), and the amount of distress was correlated to the amount of alpha activity in subgenual anterior cingulate cortex (Vanneste et al., 2010b). Highly distressed tinnitus patients exhibit functional connectivity between the subgenual anterior cingulate cortex and the left parahippocampus within the alpha frequency band (Vanneste et al., 2014). Further research provided evidence that tinnitus-related distress results from activity and connectivity in subgenual anterior cingulate cortex extending to the pregenual anterior cingulate cortex and the ventromedial prefrontal cortex, amygdala and parahippocampal area (Chen et al., 2017, 2018; De Ridder et al., 2011; Hullfish et al., 2018; Kandepan et al., 2019; Qu et al., 2019; Vanneste et al., 2014). Interestingly, this network overlaps with brain regions implicated in distress in patients suffering from pain (Moisset and Bouhassira, 2007; Price, 2000), dyspnea (von Leupoldt et al., 2009), functional somatic syndromes, and posttraumatic stress disorder (Vermetten et al., 2007).

Brain-derived neurotrophic factor (BDNF) (rs6265) has been widely studied as a susceptibility factor for both stress and affective dysregulation. The BDNF Val⁶⁶Met polymorphism—named after a Valine-Methionine substitution at codon 66 within the BDNF prodomain—is a known risk factor for affective disorders due to its common frequency and established functionality (Notaras et al., 2015). The heterozygous Val/Met genotype occurs in approximately 20–30% of the Caucasian population. The Val⁶⁶Met substitution results in the diminished activity-dependent release of BDNF (Egan et al., 2003), which has been associated with inefficient secretion compared to the valine variant. BDNF Met carriers have been associated with impairments in emotional processing, increased vulnerability to stress, and reduced resilience (Duman et al., 1999; Hasler et al., 2004; Yu et al., 2012). Furthermore, research has shown that BDNF Val⁶⁶Met plays a role in the adaptation to stress and noise (Nam et al., 2000; Uchida et al., 2011), in processing auditory information during an acoustic startle paradigm (Hajcak et al., 2009), during an auditory oddball task (Schofield et al., 2009), and for other auditory challenges (Juckel et al., 2010). A large body of evidence supports the influence of the BDNF Val⁶⁶Met polymorphism on the development, operation, and structure of brain regions and connections, including the subgenual cingulate (Gerritsen et al., 2012; Wei et al., 2012), the ventromedial prefrontal cortex (Forbes et al., 2012; Lotfipour et al., 2009), and the (para)hippocampus (Montag et al., 2009; Nestler et al., 2002).

Although previous research gives an idea about the neural correlates of tinnitus-related distress, it cannot explain why some tinnitus patients develop distress and others are not bothered by their tinnitus. To elucidate this question, we explored the neural activation pattern of tinnitus between *BDNF* Val/Met carriers and Val-homozygotes using electrophysiological data (i.e., electroencephalography) to assess activity and connectivity (both functional and effective) changes between participants with and without tinnitus-related distress. Based on previous findings, we hypothesize that Met carriers with tinnitus perceive more distress, that goes together with increased activity in the subgenual anterior cingulate cortex for the alpha frequency band, and have increased connectivity, between the subgenual anterior cingulate cortex and parahippocampus for the alpha frequency band, than Val-homozygotes.

3 Methods

3.1 Participants

A total of 55 tinnitus subjects (age: 54.49 ± 15.29 years; males: 38; female: 17) and 55 matched control subjects (age: 54.63 ± 12.71 years; males: 36; female: 19) were enrolled in this study. All participants were Caucasian, due to the common occurrence of the Val/Met genotype in that population. Informed consent was obtained from all participants in accordance with the protocols approved by the Institutional Review Board of the University of Texas at Dallas. Healthy controls were carefully

screened to match tinnitus patients for age, gender, and hearing loss. Individuals with pulsatile tinnitus, Ménière's disease, otosclerosis, chronic headache, neurological disorders such as brain tumors, traumatic brain injury or stroke, and individuals being treated for mental disorders were not included in the study in order to increase the sample homogeneity. There was no significant difference for gender ($\chi^2=0.17$, $P=0.68$) nor age ($t=1.01$, $P=0.32$).

All participants reported their tinnitus-related distress over the last 2 weeks using a visual analogue scale from 0 to 10; 0 meaning no stress and 10 indicating severely stressed. All subjects were screened for the extent of hearing loss (dB HL) using a pure tone audiometry using the British Society of Audiology procedures at 0.125, 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz (Audiology, 2008). For unilateral tinnitus, the hearing loss contralateral to where the patient perceived the tinnitus was considered, while for bilateral tinnitus patients we calculated the mean of hearing thresholds. For the loudness severity, participants were asked to rate the loudness of their tinnitus on a visual analogue scale from 0 to 10; 0 meaning no tinnitus and 10 indicating the loudest tinnitus that they can imagine. This estimation was performed for both ears (or documented as only occurring in one ear).

Tinnitus patients were further tested for the tinnitus pitch (frequency) by performing a tinnitus matching analysis. All tinnitus patients were first interviewed, as to the perceived location of the tinnitus (the left ear, in both ears, the right ear), as well as the tinnitus sound characteristics (pure tone-like tinnitus or noise-like tinnitus). In unilateral tinnitus patients, tinnitus matching was performed contralateral to the tinnitus ear. In bilateral tinnitus patients, tinnitus matching was performed contralateral to the worse tinnitus ear. First, a 1-kHz pure tone was presented contralateral to the (worse) 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 his/her tinnitus most (Meeus et al., 2010, 2011). Based on the tinnitus frequency, we calculated the hearing loss at the tinnitus frequency as obtained by tinnitus matching. In five tinnitus patients we were not able to obtain a tinnitus matching and pitch.

The Tinnitus Questionnaire (TQ) (Hiller and Goebel, 1992; McCombe et al., 2001) 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 and Goebel, 1992; McCombe 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 to 84) was computed according to standard criteria published in previous work (Hiller and Goebel, 1992; Hiller et al., 1994; Meeus et al., 2007). See Table 1 for demographics.

Table 1 Demographics.

	Control		Tinnitus		P-values
	Val homozygotes (n = 34)	Val/Met carriers (n = 21)	Val homozygotes (n = 26)	Val/Met carriers (n = 29)	
Age	53.41 (12.87)	56.62 (9.48)	51.81 (18.78)	56.90 (11.07)	0.71
Gender	M:27 /F:7	M:12/F:9	M:17/F:9	M:21/F:8	0.68
BDI	2.70 (2.17)	3.67 (2.22)	4.31 (3.11)	6.62 (3.84)	0.24
Tinnitus duration			3.42 (0.81)	2.52 (0.74)	0.65
Tinnitus type			N: 18/PT: 8	N: 20/PT: 9	0.98
Tinnitus lateralization			UNI: 9/BIL: 17	UNI: 14/BIL: 15	0.15
Tinnitus frequency (Hz)			4000 (2166.94)	4518.52 (2407.85)	0.43

M: male; F: Female; NRS: numeric rating scale; N: noise-like tinnitus; PT: pure tone like tinnitus; UNI: unilateral; BIL: bilateral. Hearing loss is measured based on a pure tone audiogram.

3.2 Genotyping

Genotyping of the SNP rs6265 was carried out at DNA Genotek in Ottawa (www.dnagenotek.com). DNA was extracted from 700 µL of 60/60 Oragene saliva samples. The average DNA yield was 7 µg (<1–24 µg) by PicoGreen measurement and 15 µg (<1–59 µg) by Nanodrop. Sample MBR023T had a 260/280 ratio outside the desired range of 1.6–2.5. This sample had a lower yield that may have contributed to the lower purity. An aliquot of all samples was normalized to approximately 3 ng/µL for genotyping using Taqman chemistry. All 60 samples were genotyped using Taqman chemistry for rs6265. All samples genotyped 100% on all markers. The TaqMan assay is an allele discrimination assay using PCR amplification and a pair of fluorescent dye detectors that target the SNP. One fluorescent dye is attached to the detector that is a perfect match to the first allele and a different fluorescent dye is attached to the detector that is a perfect match to the second allele. During PCR, the polymerase will release the fluorescent probe into solution where it is detected using endpoint analysis in a Life Technologies, Inc. (Foster City, CA) 7900HT Real-Time instrument. Primers and probes were obtained through Life Technologies design and manufacturing. Due to a low number of Met/Met carriers (n = 5), we did not include these participants in this study.

3.3 EEG 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 5 min. The EEG was sampled using a 64-electrode Neuroscan Quickcap, Neuroscan SynAmps2 amplifiers, and Scan 4.3.2. Impedances were checked to remain below $5\text{ k}\Omega$. Data were collected eyes-closed (sampling rate = 1000 Hz, band passed 0.15–200 Hz). Using EEGlab offline, data were resampled to 128 Hz, band-pass filtered in the range 2–44 Hz, plotted, and carefully inspected for manual artifact rejection. All episodic artifacts including eye blinks, eye movements, teeth clenching, body movement, or ECG artifacts were removed from the EEG stream. Average Fourier cross-spectral matrices was computed for the alpha frequency band (8–12 Hz).

3.4 Source localization

Standardized low-resolution brain electromagnetic tomography (sLORETA) (Pascual-Marqui, 2002) was used to estimate the intracerebral electrical sources. As a standard procedure, a common average reference transformation (Pascual-Marqui, 2002) is performed before applying the sLORETA algorithm. SLORETA computes electric neuronal activity as current density (A/m^2) 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 leadfield produced by Fuchs et al. (2002) applying the boundary element method on the MNI-152 (Montreal neurological institute, Canada). The sLORETA-key anatomical template divides and labels the neocortical (including hippocampus and anterior cingulated cortex) MNI-152 volume in 6239 voxels of dimension 5 mm^3 , based on probabilities returned by the Daemon Atlas (Lancaster et al., 2000). The co-registration makes use of the correct translation from the MNI-152 space into the Talairach and Tournoux space.

3.5 Statistical analyses on the whole brain

The methodology used is a non-parametric permutation test. It is based on estimating, via randomization, the empirical probability distribution for the max-statistic, under the null hypothesis comparisons (Nichols and Holmes, 2002). This methodology corrects for multiple testing (i.e., for the collection of tests performed for all voxels, and for all frequency bands). Due to the non-parametric nature of this method, its validity does not rely on any assumption of Gaussianity (Nichols and Holmes, 2002). The significance threshold for all tests was based on a permutation test with 5000 permutations. A comparison was made between high- and low-distress tinnitus patients (based on a median split for TQ), Val homozygotes,

and Val/Met carriers that have tinnitus, and Val/Met carriers that are tinnitus or control subjects. These comparisons were performed on a whole brain by sLORETA statistical contrast maps through multiple voxel-by-voxel comparisons in a logarithm of *t*-ratio.

3.6 Region of interest analysis

The log-transformed electric current density was averaged across all voxels belonging to the regions of interest. The regions of interest are the subgenual anterior cingulate cortex for the alpha frequency band (8–12 Hz). An ANOVA was performed with group (control vs. tinnitus subjects) and BDNF (Val homozygotes vs. Val/Met carriers) as independent variables and the current density for the subgenual anterior cingulate cortex for the alpha frequency band as the dependent variable. Pearson correlations were calculated between the region of interest and the tinnitus-related distress including Val homozygotes and Val/Met carriers. In addition, a mediation analysis was calculated to look at the mediation of distress mediate between BDNF Val¹⁶⁶Met polymorphism and tinnitus loudness. The selection of this region of interest was based on previous literature as introduced in the introduction (a priori) and confirmed by the comparison of activity between the tinnitus groups with Val homozygotes and Val/Met carriers (a posteriori).

3.7 Lagged phase coherence

Coherence and phase synchronization between time series corresponding to different spatial locations are usually interpreted as indicators of connectivity. However, any measure of dependence is highly contaminated with an instantaneous, non-physiological contribution due to volume conduction (Pascual-Marqui, 2007a). However, Pascual-Marqui (2007b) introduced new measures of coherence and phase synchronization taking into accounts only non-instantaneous (lagged) connectivity, effectively removing the confounding factor of volume conduction. 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 et al., 2010). Since the two components oscillate coherently with a phase lag, the crosstalk 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 at the Fourier frequencies (Bloomfield, 2013). 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 10Hz is 100ms. The sine is shifted a quarter of a cycle (25 ms) with the respect to the cosine. Then the lagged phase coherence at 10Hz indicates coherent oscillations with a 25 ms delay, while at 20Hz the delay is 12.5ms, etc. The threshold of significance for a given lagged phase coherence value according to asymptotic results can be found as described (Pascual-Marqui et al., 2011), where the definition of lagged phase coherence can be found as well. 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 non-negative and take the value zero only when there is independence and defined in alpha (8–12 Hz) the frequency domain. Based on this principle, lagged linear connectivity was calculated. Time-series of current density were extracted for different region of interests using sLORETA. Power in all 6239 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. The regions of interest selected were the subgenual anterior cingulate cortex and the left parahippocampus.

3.8 Statistical analyses for the lagged phase coherence

Lagged phase synchronization/coherence or functional connectivity contrast maps were calculated and correlated with the mean hearing loss, the range of the hearing loss and the hearing loss at the tinnitus frequency for the different frequency bands. The significance threshold was based on a permutation test with 5000 permutations. This methodology corrects for multiple testing (i.e., for the collection of tests performed for all voxels). A comparison was made between high and low distress tinnitus patients, Val homozygotes and Val/Met carriers that have tinnitus, and Val/Met carriers that are tinnitus or control subjects. To assign tinnitus subjects in the high and low distress group we applied a median split analysis.

3.9 Granger causality

Granger causality reflects the strength of effective connectivity (i.e., causal interactions, extract activity of one are of causal influences of one neural element over another) from one region to another by quantifying how much the signal in the seed region is able to predict the signal in the target region (Geweke, 1982; Granger, 1969). In other words, it can be considered as directional functional connectivity. Granger causality is defined as the log-ratio between the error variance of a reduced model, which predicts one time series based only on its own past values, and that of the full model, which in addition includes the past values of another time series. It is important to note that Granger causality does not imply anatomical connectivity between regions but directional functional connectivity between two sources. A comparison was made between control subjects and tinnitus patients that are Met carriers and Val/Val carriers on Granger causality outcome measure using a MANOVA. Pearson correlations were calculated between the Granger causality outcome measures and tinnitus-related distress for Val homozygotes and Met carriers. In addition, a mediation analysis was calculated to look at the mediation of Granger causality outcome measure ($\text{sgACC} \rightarrow \text{PHC}$; $\text{PHC} \rightarrow \text{sgACC}$) between BDNF Val⁶⁶ Met polymorphism and distress.

4 Results

4.1 Genetic characteristics

Hardy-Weinberg calculation showed no significant deviation from the equilibrium (whole group $P=0.17$; tinnitus group $P=0.16$; control group $P=0.57$). The distribution of the BDNF genotype frequencies for the total group were 54.5% Val/Val ($n=60$) and 45.5% for Val/Met ($n=50$). No participant had the genotype Met/Met. This distribution was 47.3% Val/Val ($n=26$), 52.7% and for Val/Met ($n=29$) for the tinnitus subjects and 61.8% Val/Val ($n=34$), 38.2% and for Val/Met ($n=21$) for the control group. When comparing both the control and tinnitus group and their frequency of being a Val/Val or Val/Met genotype, no significant effect was observed ($\chi^2(1)=2.35$, $P=0.13$), suggesting that the distributions for the tinnitus and healthy groups are similar. No difference in the distribution of the BDNF genotype frequencies and gender ($\chi^2(1)=2.20$, $P=0.14$). See also Table 1.

4.2 Behavioral results

Fig. 1 shows no significant difference for the audiogram between the groups (control vs. tinnitus subjects) \times BDNF (Val homozygotes vs. Val/Met carriers) ($F(16,84)=1.10$, $P=37$).

A comparison between control and tinnitus subjects that are Val homozygotes or Val/Met carriers for stress levels over the last 2 weeks showed that a main effect for

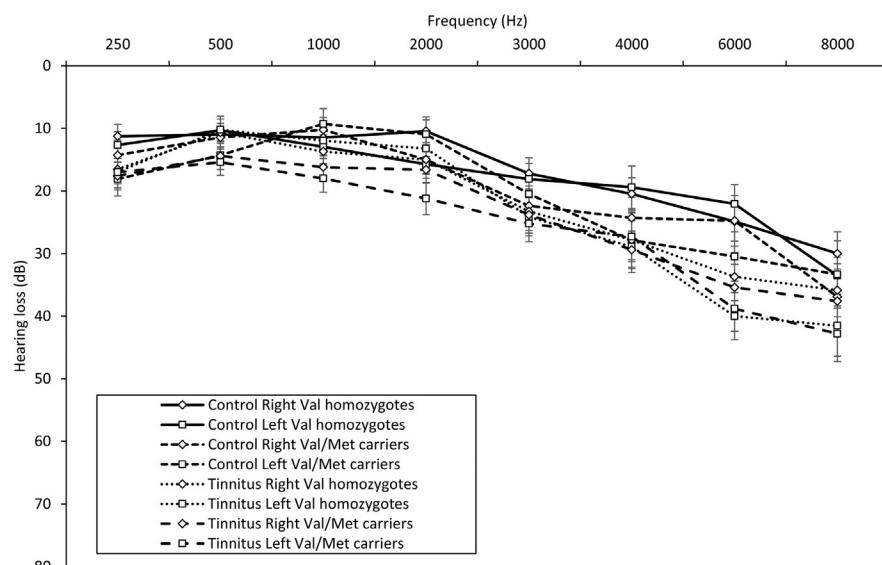
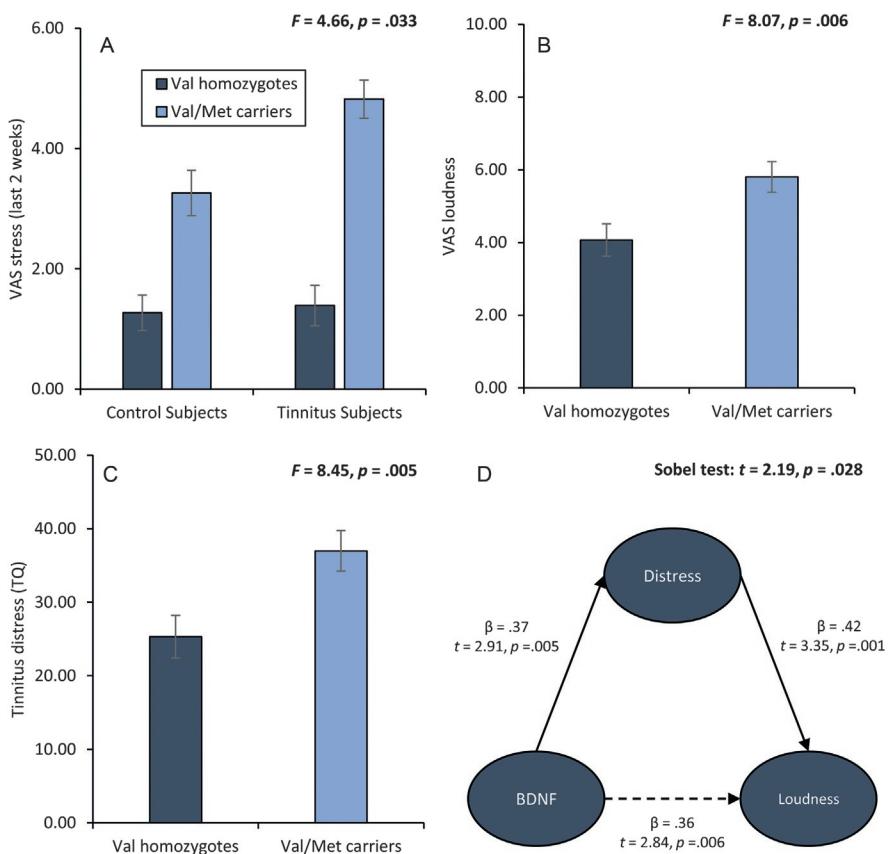


FIG. 1

Audiogram for the different groups.

**FIG. 2**

Behavioral assessments: (A) The left top panel shows a comparison between control and tinnitus subjects for the BDNF Val⁶⁶Met polymorphism for stress levels over the last 2 weeks. (B) The top right panel indicates a difference in tinnitus loudness as measure by a Visual Analogue Scale (VAS) between Val homozygotes and Val/Met carriers. (C) Bottom left panel demonstrates a difference in tinnitus-related distress as measured with Tinnitus Questionnaire between Val homozygotes and Val/Met carriers. (D) Bottom right panel reveals a mediation effect for distress between the BDNF Val⁶⁶Met polymorphism and tinnitus loudness (** $P < 0.001$; ** $P < 0.01$).

the BDNF demonstrated that subject that are Val/Met carriers ($M = 4.04, Sd = 1.83$) have a higher stress level in comparison to Val homozygotes ($M = 1.33, Sd = 1.83$) ($F(1,106) = 66.52, P < 0.001$; see Fig. 2). Furthermore, tinnitus subjects ($M = 3.11, Sd = 2.44$) have higher stress levels in comparison to control subjects ($M = 2.27, Sd = 1.95$) ($F(1,106) = 66.39, P = 0.013$). These effects moderated due to an interaction between BDNF (Val/Val vs. Val/Met) \times subjects (control vs. tinnitus) ($F(1,106) = 4.66, P = 0.033$). A simple contrast analysis showed that tinnitus

subjects that are Val/Met carriers ($M=4.82$, $Sd=1.77$) have higher stress levels in comparison to, tinnitus subject that are Val homozygotes ($M=1.39$, $Sd=1.69$; $F(1,106)=54.68$, $P<0.001$), control subject that are Val/Met carriers ($M=3.26$, $Sd=1.52$; $F(1,106)=10.03$, $P=0.002$), and control subject that are Val homozygotes ($M=1.27$, $Sd=1.80$; $F(1,106)=66.97$, $P<0.001$; see Fig. 2), respectively. Including gender as a covariate showed a similar effect for the BDNF \times subjects ($F(1,105)=5.84$, $P=0.017$). No effect is obtained for gender ($F(1,105)=1.69$, $P=0.20$).

No significant effect was obtained between Val homozygotes and Val/Met carriers for tinnitus pitch ($F(1,49)=0.65$, $P=0.43$). Furthermore, a comparison between Val homozygotes ($M=41.50$, $Sd=19.93$) and Val/Met carriers ($M=56.41$, $Sd=21.26$) for Loudness Match ($F(1,49)=6.65$, $P=0.013$) was obtained. In addition, no effect was obtained for tinnitus lateralization (unilateral vs. bilateral and Val homozygotes vs. Val/Met carriers) ($\chi^2=1.05$, $P=0.31$). For the tinnitus group, we further show that there is a significant effect between Val homozygotes ($M=4.07$, $Sd=2.45$; $M=37.00$, $Sd=16.67$) and Val/Met carriers ($M=5.80$, $Sd=2.09$; $M=25.31$, $Sd=12.61$) for tinnitus loudness ($F(1,53)=8.07$, $P=0.006$; see Fig. 2) and tinnitus-related distress ($F(1,53)=8.45$, $P=0.005$; see Fig. 2). Given group differences in tinnitus loudness and tinnitus-related distress between Val homozygotes and Val/Met carriers, we assessed whether tinnitus-related distress mediates the association between BDNF and tinnitus loudness. We found that tinnitus-related distress significantly mediates the effect of BDNF on tinnitus loudness (Sobel test $t=2.19$, $P=0.028$; see Fig. 2). Including gender as a covariate, we found a similar effect for the BDNF for tinnitus loudness ($F(1,52)=8.18$, $P=0.006$) and tinnitus distress ($F(1,52)=8.60$, $P=0.005$). No effect is obtained for gender on tinnitus loudness ($F(1,52)=0.36$, $P=0.55$) or tinnitus distress ($F(1,52)=0.42$, $P=0.52$).

5 Behavioral

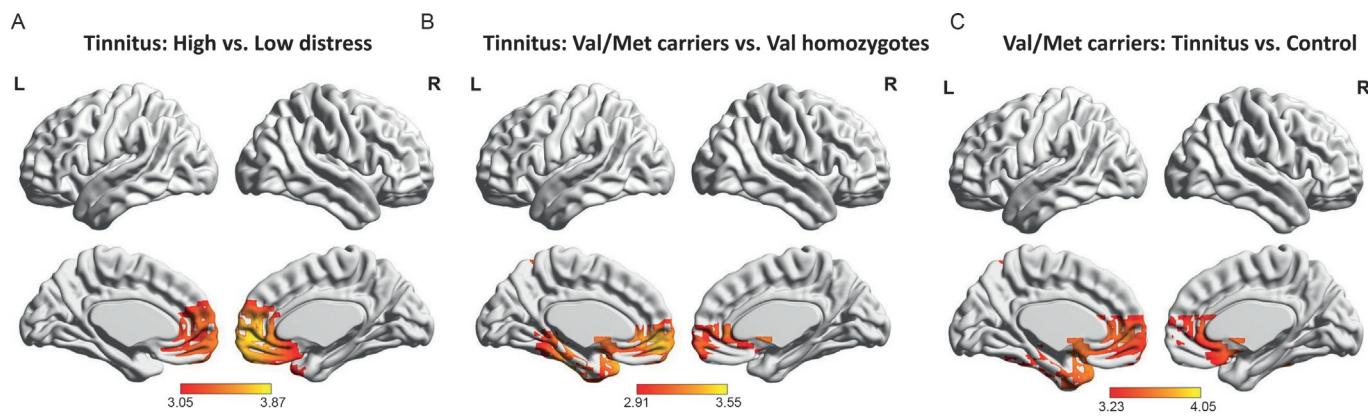
5.1 Whole brain analysis

1. Tinnitus vs. control subjects

For the alpha frequency band, a comparison between tinnitus and healthy control subjects that are Val homozygotes did not show a significant effect ($F=1.67$, $P=0.56$). A similar comparison for the Val/Met carriers showed a significant difference ($F=3.23$, $P<0.05$), demonstrating that tinnitus subjects have increased synchronized activity over the subgenual anterior cingulate cortex extending into the ventromedial prefrontal cortex as well as the left parahippocampal area (see Fig. 3).

2. Tinnitus patients: High vs. low distress

A comparison between tinnitus patients with high vs. low distress shows a significant effect for the alpha frequency band over the subgenual anterior

**FIG. 3**

Whole brain analysis: (A) For participants with tinnitus, a comparison between high and low distress tinnitus patients. (B) For participants with tinnitus, a comparison between Val/Met carriers and Met carriers. (C) Participants who are Val/Met carriers, a comparison between tinnitus vs. control subjects.

cingulate cortex extending into the ventromedial prefrontal cortex for highly distress tinnitus patients ($F = 3.05$, $P < 0.05$; see Fig. 3).

3. Tinnitus patients: Val/Met carriers vs. Val homozygotes

When comparing tinnitus patients that are Val/Met carriers with tinnitus patients that are Val homozygotes, a significant effect was demonstrated for the alpha frequency band for the subgenual anterior cingulate cortex extending into the ventromedial prefrontal cortex for Val/Met tinnitus carriers ($F = 3.05$, $P < 0.05$; see Fig. 3).

4. Region of interest analysis

A region of interest for the subgenual anterior cingulate cortex for the alpha frequency band between the control subjects and tinnitus subjects that are BDNF Val/Val carriers or Val/Met carriers showed a significant main effect for both subjects and BDNF as well as an interaction effect. For subjects (tinnitus vs. control), a main significant effect ($F(1,106) = 17.66$, $P < 0.001$) was revealed, indicating that tinnitus patients ($M = 2.44$, $Sd = 0.69$) have increased alpha power in the subgenual anterior cingulate cortex in comparison to control subjects ($M = 2.34$, $Sd = 0.53$). For BDNF, a main significant effect was obtained, revealing that Val/Met carriers ($M = 2.68$, $Sd = 0.75$) have increased alpha power over the subgenual anterior cingulate cortex in comparison to Val homozygotes ($M = 2.44$, $Sd = 0.53$) ($F(1,106) = 5.23$, $P = 0.024$). Furthermore, a significant interaction was obtained between group \times BDNF ($F(1,106) = 31.36$, $P < 0.001$; see Fig. 4). A simple contrast revealed that tinnitus subjects who are Val/Met carriers ($M = 3.19$, $Sd = 0.58$) have increased alpha power in the subgenual anterior cingulate cortex in comparison to tinnitus subjects whom are Val homozygotes ($M = 2.37$, $Sd = 0.55$; $F(1,106) = 31.98$, $P < 0.001$), control subjects who are Val/Met carriers ($M = 2.17$, $Sd = 0.52$; $F(1,106) = 43.88$, $P < 0.001$), and control subjects who are Val homozygotes ($M = 2.52$, $Sd = 0.50$; $F(1,106) = 24.2$, $P < 0.001$), respectively.

A Pearson correlation between the current density for the subgenual anterior cingulate cortex for the alpha frequency band and the tinnitus-related distress as measured by the TQ showed a positive correlation ($r = 0.55$, $P < 0.001$; see Fig. 4), indicating that the higher the TQ score, the higher the current density is for the subgenual anterior cingulate cortex.

Furthermore, given group differences in tinnitus-related distress and the current density for the subgenual anterior cingulate cortex in alpha between Val homozygotes and Val/Met carriers, we assessed whether current density for the subgenual anterior cingulate cortex in alpha mediates the association between BDNF and tinnitus-related distress. We found that current density for the subgenual anterior cingulate cortex in alpha significantly mediates the effect of BDNF on tinnitus-related distress (Sobel test $t = 3.59$, $P < 0.001$; see Fig. 4).

For the tinnitus group, our analyses did not reveal an effect of gender ($F(1,47) = 0.07$, $P = 0.66$) and tinnitus laterality ($F(1,47) = 0.028$, $P = 0.87$) on the subgenual anterior cingulate cortex in alpha. However the effect of BDNF

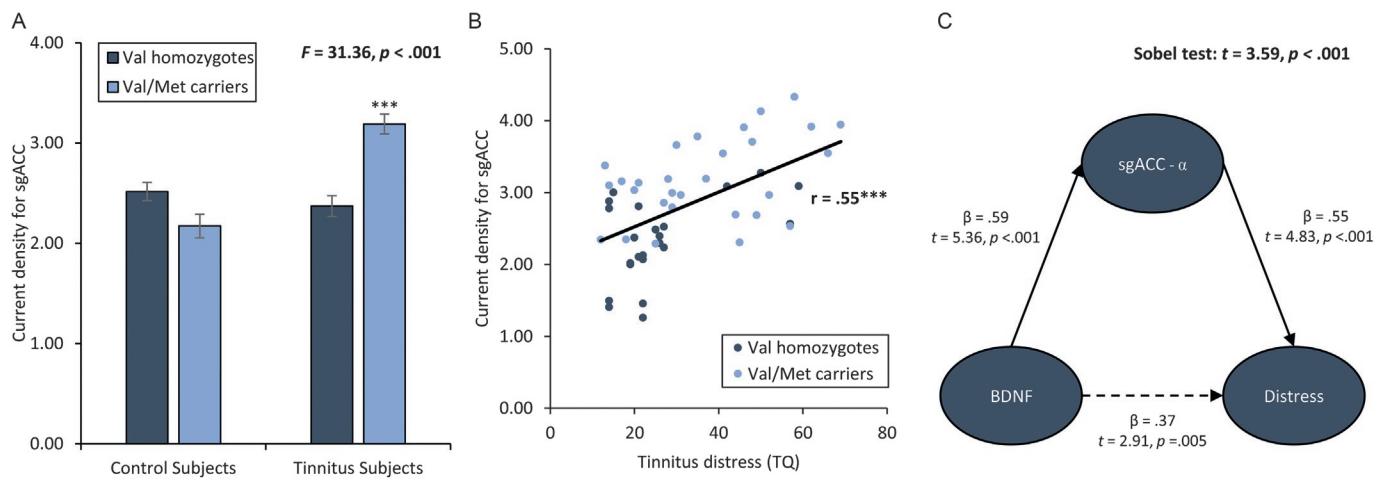


FIG. 4

Region of interest analysis: (A) A comparison between control and tinnitus subjects for the BDNF Val⁶⁶Met polymorphism for the current density for the subgenual anterior cingulate cortex for the alpha frequency band (left panel). (B) A Pearson correlation between tinnitus-related distress as measured by the tinnitus questionnaire (TQ) and the current density for the subgenual anterior cingulate cortex for the alpha frequency band for Val homozygotes and Val/Met carriers (middle panel). (C) A mediation effect the current density for the subgenual anterior cingulate cortex for the alpha frequency band between the BDNF Val⁶⁶Met polymorphism and tinnitus tinnitus-related distress as measured by the TQ (*** $P < 0.001$).

remained ($F(1,47)=28.85, P<0.001$). In addition, no interaction was obtained between gender and BDNF ($F(1,47)=2.19, P=0.15$), tinnitus laterality and BDNF ($F(1,47)=0.06, P=0.81$), gender and tinnitus laterality ($F(1,47)=0.97, P=0.33$), or the three-way interaction between gender, tinnitus laterality and BDNF ($F(1,47)=0.02, P=0.88$) on the subgenual anterior cingulate cortex for the alpha frequency band.

5.2 Functional connectivity: Lagged phase coherence

1. Tinnitus vs. control subjects

A comparison between tinnitus subjects and controls subjects revealed a significant difference in phase coherence for alpha frequency band revealing increased coherence between the subgenual anterior cingulate cortex and left parahippocampus for tinnitus subjects ($F=3.11, P<0.05$; see Fig. 5).

2. Tinnitus patients high vs. low distress for tinnitus patients

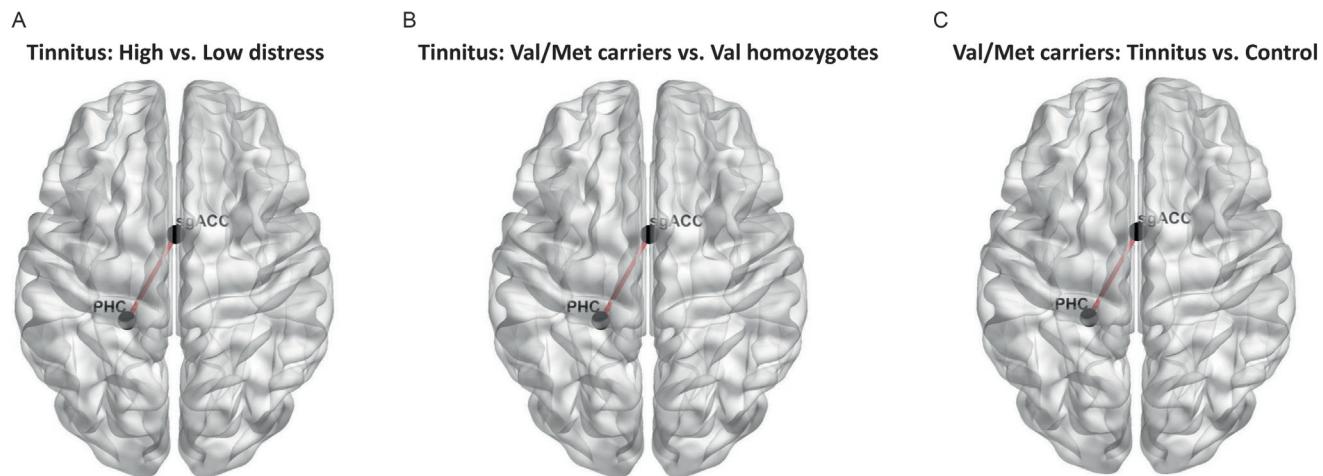
For the alpha frequency band, a significant effect was obtained when comparing high vs. low distress for tinnitus patients ($F=3.72, P<0.05$; see Fig. 5), indicating increased coherence between the subgenual anterior cingulate cortex and left parahippocampus for high distress tinnitus patients.

3. Tinnitus patients: Val/Met carriers vs. Val homozygotes

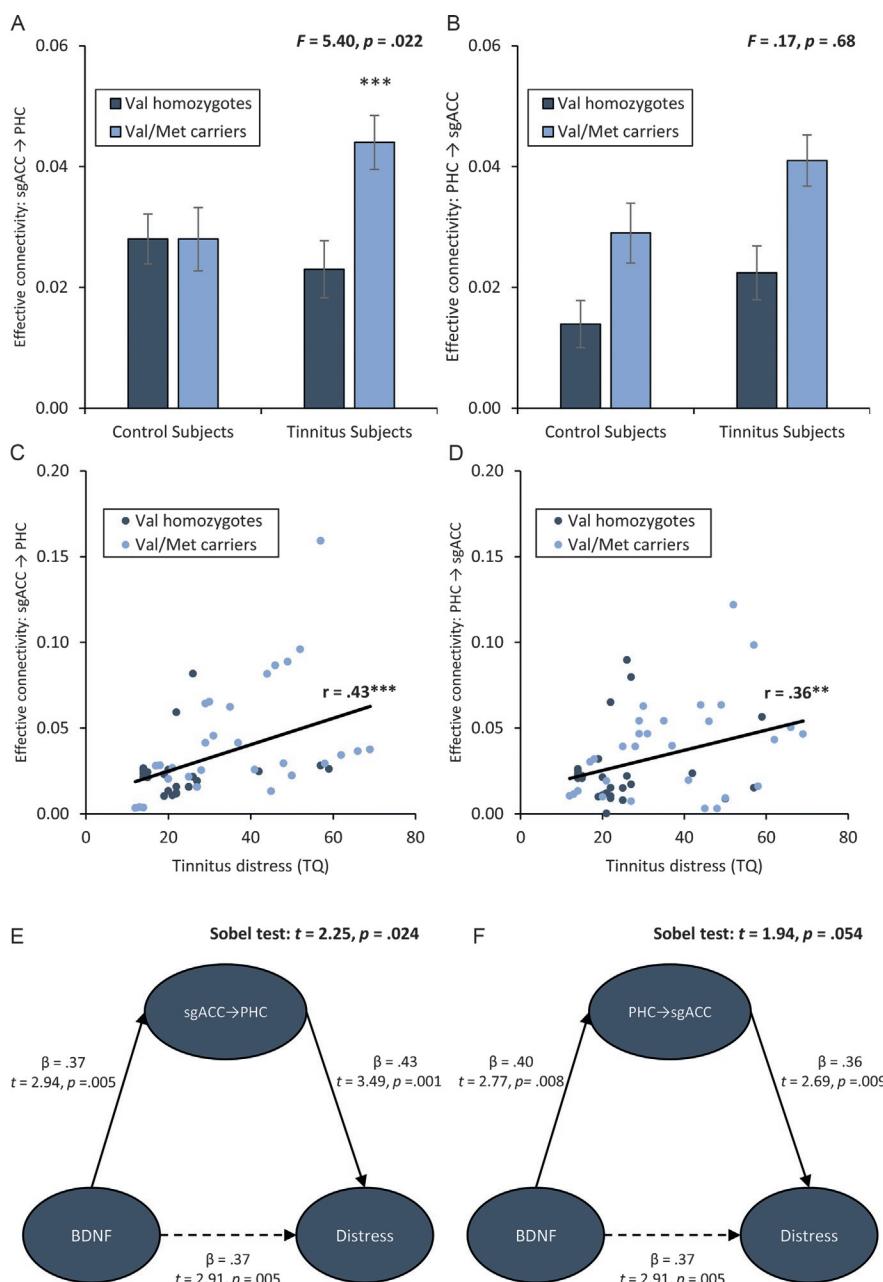
A comparison between the Val/Val genotype and Val/Met carriers showed a significant effect for the alpha frequency bands ($F=3.41, P<0.05$; see Fig. 5). Increased coherence was observed between the subgenual anterior cingulate cortex and left parahippocampus for Val/Met carriers.

5.3 Effective connectivity: Granger causality

A MANOVA was conducted with group (tinnitus vs. controls subjects) and BDNF (Met carriers vs. Val/Val genotype) as independent variables and the effective connectivity (subgenual anterior cingulate cortex (sgACC) → left parahippocampus (PHC) vs. left PHC → sgACC) for the alpha frequency band as the dependent variable. This analysis revealed a significant main effects for both group ($F(2,105)=3.28, P=0.042$) and BDNF ($F(2,105)=8.60, P<0.001$). For the main effect group, a significant effect was obtained for connection left PHC → sgACC ($F(1,106)=6.28, P=0.014$), indicating that tinnitus subjects ($M=0.032, Sd=0.026$) have an increased connectivity in comparison to control subjects ($M=0.021, Sd=0.019$). For the connection sgACC → left PHC, no effect was obtained for group ($F(1,106)=1.41, P=0.24$). For the main effect BDNF, a significant effect was demonstrated for both the connection sgACC → left PHC ($F(1,106)=5.14, P=0.025$) and left PHC → sgACC ($F(1,106)=17.07, P<0.001$), indicating for both connection that Met/Val carriers ($M=0.036, Sd=0.030$; $M=0.035, Sd=0.026$, respectively) have an increased connectivity in comparison to Val homozygotes ($M=0.025, Sd=0.018$; $M=0.018, Sd=0.017$, respectively). In addition, a significant interaction effect between group × BDNF was obtained ($F(2,105)=3.71, P=0.028$; see Fig. 6).

**FIG. 5**

Functional connectivity between subgenual anterior cingulate cortex and left parahippocampus was revealed for (A) for tinnitus subjects that high levels distress in comparison to low distress (left panel), (B) for tinnitus subjects that are Val/Met carriers in comparison to Val/Met carriers (middle panel), (C) for participants who are Val/Met carriers, a comparison between tinnitus vs. control subjects (right panel).

**FIG. 6**

Effective connectivity: (A) A comparison between control and tinnitus subjects for the BDNF Val⁶⁶Met polymorphism for the connection from the subgenual anterior cingulate cortex to the left parahippocampus for the alpha frequency band (left top panel). (B) A comparison between control and tinnitus subjects for the BDNF Val⁶⁶Met polymorphism for the connection from the left parahippocampus to the subgenual anterior cingulate cortex for the (Continued)

For the connection sgACC → left PHC, a significant effect was obtained ($F(1,106) = 5.40, P = 0.022$). A simple contrast analysis revealed that tinnitus subjects who are Val/Met carriers ($M = 0.044, Sd = 0.033$) have increased connectivity for the alpha frequency for the connection sgACC → left PHC in comparison to, tinnitus subjects who are Val homozygotes ($M = 0.023, Sd = 0.017; F(1,106) = 10.83, P = 0.001$), control subjects who are Val/Met carriers ($M = 0.028, Sd = 0.021; F(1,106) = 5.63, P = 0.020$), and control subjects who are Val homozygotes ($M = 0.028, Sd = 0.020; F(1,106) = 6.99, P = 0.009$), respectively. For the connection left PHC → sgACC, no significant effect was revealed ($F(1,106) = 0.17, P = 0.68$; see Fig. 6).

A Pearson correlation for the connection left sgACC → left PHC between the effective connectivity for the alpha frequency band and the tinnitus-related distress as measured by the TQ showed a positive correlation ($r = 0.43, P = 0.001$; see Fig. 6), indicating that the higher the TQ score, the higher is the effective connectivity for the connection sgACC → left PHC. A similar analysis for left PHC → sgACC showed also a positive correlation ($r = 0.36, P = 0.008$; see Fig. 6), indicating that the higher the TQ score the higher is the effective connectivity.

Given group differences in tinnitus-related distress and the effective connectivity for the subgenual anterior cingulate cortex and parahippocampus and vice versa in alpha between Val homozygotes and Val/Met carriers, we assessed the effective connectivity between BDNF and tinnitus-related distress. We found that the connection sgACC → left PHC significantly mediates the effect of BDNF on tinnitus loudness (Sobel test $t = 2.25, P = 0.024$; see Fig. 6), but not for the connection left PHC → sgACC (Sobel test $t = 1.94, P = 0.054$; see Fig. 6).

In the tinnitus group, including gender and tinnitus laterality in our analyses together with BDNF, we did not reveal an effect for effective connectivity for the subgenual anterior cingulate cortex and parahippocampus and vice versa for gender ($F(2,46) = 2.54, P = 0.09$) and tinnitus laterality ($F(2,46) = 0.94, P = 0.40$). However the effect of BDNF remained ($F(2,46) = 3.45, P = 0.04$). That is, for both the

FIG. 6—Cont'd

alpha frequency band (right top panel). (C) A Pearson correlation between tinnitus-related distress as measured by the tinnitus questionnaire (TQ) and for the connection from the subgenual anterior cingulate cortex to the left parahippocampus for the alpha frequency band for Val homozygotes and Val/Met carriers (middle left panel). (D) A Pearson correlation between tinnitus-related distress as measured by the TQ and for the connection from left parahippocampus to the subgenual anterior cingulate cortex for the alpha frequency band for Val homozygotes and Val/Met carriers (middle left panel). (E) A mediation effect for the connection from the subgenual anterior cingulate cortex to the left parahippocampus for the alpha frequency band between the BDNF Val⁶⁶Met polymorphism and tinnitus-related distress as measured by the TQ (bottom left panel). (F) A mediation effect for the connection from the left parahippocampus to the subgenual anterior cingulate cortex for the alpha frequency band between the BDNF Val⁶⁶Met polymorphism and tinnitus-related distress as measured by the TQ (bottom right panel). (**P < 0.001; **P < 0.01).

connection sgACC → left PHC ($F(1,47)=7.02, P=0.011$) and left PHC → sgACC ($F(1,47)=4.35, P=0.042$), indicating for both connection that Met/Val carriers ($M=0.043, Sd=0.033; M=0.041, Sd=0.029$, respectively) have an increased connectivity in comparison to Val homozygotes ($M=0.023, Sd=0.016; M=0.022, Sd=0.019$, respectively). In addition, no interaction was obtained between gender and BDNF ($F(2,46)=1.01, P=0.37$), tinnitus laterality and BDNF ($F(2,46)=0.98, P=0.38$), gender and tinnitus laterality ($F(2,46)=2.20, P=0.12$), or the three-way interaction between gender, tinnitus laterality and BDNF ($F(2,46)=1.66, P=0.20$) for the effective connectivity for the subgenual anterior cingulate cortex and parahippocampus and vice versa.

6 Discussion

Our data provide several new insights into stress responses to tinnitus and its association with the BDNF Val⁶⁶Met polymorphism. We show that BDNF Val⁶⁶Met polymorphism is a good predictor for tinnitus-related distress and indirectly predicts for the loudness of the tinnitus percept, i.e., via mediation by tinnitus-related distress. Met carriers have increased activity in the subgenual anterior cingulate cortex and increased connectivity between the subgenual anterior cingulate cortex and the left parahippocampus. In addition, the amount of distress seems to be mainly driven by increased communication from the subgenual anterior cingulate cortex to the left parahippocampus in tinnitus patients who are Met carriers. To our knowledge, this is the first study investigating the clinical and associated electrophysiological effects, both in activity and connectivity, of BDNF Val⁶⁶Met polymorphism in tinnitus.

Interestingly, our data confirm previous findings that distress is related to alpha power and coherence (De Ridder et al., 2011; Vanneste et al., 2010b, 2014). Previous research has linked alpha oscillations to inhibition of task-irrelevant sensory modalities. For example, alpha power in auditory cortex has been positively associated with both auditory attention (Anderson and Ding, 2011) and auditory oddball task performance (Bollimunta et al., 2008; Makeig and Inlow, 1993). These inhibitory alpha modulations appear to be driven by the activity of frontal control regions (Clayton et al., 2015). Studies using EEG in combination with fMRI (Liu et al., 2016) have shown that alpha power correlates significantly with the activity of medial prefrontal cortex. An increase in MEG-measured alpha power in the frontal regions has been associated with inhibition of undesired, stimulus-driven attention (Hwang et al., 2014). Specific to tinnitus, it is possible that alpha oscillations promote sustained attention to the tinnitus percept by suppressing distracting information. In addition, alpha power was found to mediate between BDNF Val⁶⁶Met polymorphism and mood (Zoon et al., 2013). This corroborates with our findings indicating that Val/Met carriers are more distressed and that alpha power in the subgenual anterior cingulate cortex mediates the effect between BDNF Val⁶⁶Met polymorphism and distress.

Previous research on the effect of BDNF Val⁶⁶Met polymorphism already suggests the relevance of this polymorphism for the activity of specific brain regions including the ventromedial prefrontal cortex, the subgenual anterior cingulate cortex and parahippocampus that are associated with mood, depression, high rumination in life stress, post-traumatic stress disorders and neuroticism (Drevets, 2001; Mayberg, 2009; Terracciano et al., 2010). This is not surprising because the BDNF Val⁶⁶Met polymorphism exacerbates monoamine deficiencies at the genetic level (Ren-Patterson et al., 2005) and enhances hyperactivity in the emotional system because of executive dysfunction at the neural level (Clasen et al., 2011). This corroborates with our present findings, indicating that tinnitus patients who are Val/Met Carriers have higher levels of distress in comparison to Val homozygotes. This goes together with increased activity in the subgenual anterior cingulate cortex, the ventromedial prefrontal cortex, and parahippocampus. The activity in the subgenual anterior cingulate cortex correlates with tinnitus-related distress. The parallel between what we see for BDNF Val⁶⁶Met polymorphism in tinnitus and other disorders including major depression, post-traumatic stress disorders, and pain is consistent with the idea of a general distress network that can become activated by different triggers.

Our research also shows that distressed tinnitus patients have increased connectivity strength between the subgenual anterior cingulate cortex and the left hippocampus, similar to what was demonstrated in previous research (Vanneste et al., 2014). Furthermore, this effect seems to be mainly driven by the BDNF Val⁶⁶Met polymorphism. Indeed, our data showed increased connectivity strength between the subgenual anterior cingulate cortex and the left hippocampus for Val/Met carriers. This communication goes mainly from the subgenual anterior cingulate cortex to the left hippocampus in Met carriers, which correlates with distress and mediates the effect between BDNF Val⁶⁶Met polymorphism and distress. Previous research has also shown greater connectivity in circuits important for emotional processing related to the BDNF Val⁶⁶Met polymorphism (Beste et al., 2010; Wei et al., 2012). The parahippocampal area is involved in different tinnitus characteristics such as lateralization, tinnitus type, and tinnitus loudness (Carpenter-Thompson et al., 2014; Sedley et al., 2015; Vanneste and De Ridder, 2016; Vanneste et al., 2019, 2010a, 2011a,b). The parahippocampal area is also associated with tinnitus-related distress (De Ridder et al., 2011; Vanneste et al., 2010b). It is therefore not surprising that the parahippocampal area, which has a sensory gating function for irrelevant or redundant auditory input (Boutros et al., 2008), and subgenual anterior cingulate cortex, which has an attentional gating function, were functionally coupled.

Interestingly, our data do not show an effect for tinnitus laterality or gender. Two large studies in Swedish populations have reported significant differences in heritability between unilateral and bilateral tinnitus (Cederroth et al., 2019; Maas et al., 2017). However, it is important to keep in mind that here we do not directly look at the effect of tinnitus, but at the tinnitus related distress. So far, no research has indicated that distress is modulated by tinnitus laterality. This is different for gender. Research indicates that the BDNF polymorphism interacts with gender to influence acute stress (Jiang et al., 2017); however, it is not known what the effect of gender is

on chronic stress. Specifically for tinnitus, previous research indicated that gender differences in tinnitus perception are mainly reflected by differences in scores on depression, rather than distress related to the tinnitus (Vanneste et al., 2012). Our data further indicates that gender does not moderate the effect of BDNF on distress, or on brain activity, or the subgenual anterior cingulate cortex, or connectivity between the subgenual anterior cingulate cortex and the parahippocampus for the alpha frequency band.

Progress in finding a treatment for tinnitus has been hampered by the fact that tinnitus represents a highly heterogeneous condition (Schecklmann et al., 2012, 2013). Hence, it was suggested that there might be different subtypes of tinnitus. Our research fits with this idea, showing that there might be different subtypes of tinnitus depending on, for example, the underlying BDNF genotype. Further studies should be performed evaluating these results with other functional imaging techniques as well as neuromodulation techniques to confirm this idea of subtyping. Some caveats warrant mention regarding the present work. First, although many important factors including age, race, sex, and past psychiatric illness were carefully controlled, other factors such as early life stress and the environment could interact with genes and influence the underlying neurobiology of the intermediate phenotype.

In conclusion, this study shows that the BDNF Val⁶⁶Met polymorphism plays an important role in the susceptibility to the clinical manifestation of tinnitus-related distress. We demonstrated Val/Met carriers have increased alpha power in the subgenual anterior cingulate cortex that correlate with the distress perceived. Furthermore, distress mediates the relationship between BDNF Val⁶⁶Met polymorphism and tinnitus loudness. For Val/Met carriers, the subgenual anterior cingulate cortex sends distress related information to the parahippocampus, which likely integrates the loudness and distress of the tinnitus percept.

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References

- Anderson, K.L., Ding, M., 2011. Attentional modulation of the somatosensory mu rhythm. *Neuroscience* 180, 165–180.
- Andersson, G., Westin, V., 2008. Understanding tinnitus distress: introducing the concepts of moderators and mediators. *Int. J. Audiol.* 47 (Suppl. 2), S106–S111.
- Audiology, B.S.o, 2008. Recommended Procedure:Pure Tone Air and Bone Conduction Threshold Audiometry With and Without Masking and Determination of Uncomfortable Loudness Levels.

- Axelsson, A., Ringdahl, A., 1989. Tinnitus—a study of its prevalence and characteristics. *Br. J. Audiol.* 23, 53–62.
- Baguley, D., McFerran, D., Hall, D., 2013. Tinnitus. *Lancet* 382, 1600–1607.
- Beste, C., Kolev, V., Yordanova, J., Domschke, K., Falkenstein, M., Baune, B.T., Konrad, C., 2010. The role of the BDNF Val66Met polymorphism for the synchronization of error-specific neural networks. *J. Neurosci.* 30, 10727–10733.
- Bloomfield, P., 2013. Fourier Analysis of Time Series: An Introduction, 2nd ed. Wiley-Interscience, New York.
- Bollimunta, A., Chen, Y., Schroeder, C.E., Ding, M., 2008. Neuronal mechanisms of cortical alpha oscillations in awake-behaving macaques. *J. Neurosci.* 28, 9976–9988.
- Boutros, N.N., Mears, R., Pflieger, M.E., Moxon, K.A., Ludowig, E., Rosburg, T., 2008. Sensory gating in the human hippocampal and rhinal regions: regional differences. *Hippocampus* 18, 310–316.
- Carpenter-Thompson, J.R., Akrofi, K., Schmidt, S.A., Dolcos, F., Husain, F.T., 2014. Alterations of the emotional processing system may underlie preserved rapid reaction time in tinnitus. *Brain Res.* 1567, 28–41.
- Cederroth, C.R., PirouziFard, M., Trpchevska, N., Idrizbegovic, E., Canlon, B., Sundquist, J., Sundquist, K., Zoller, B., 2019. Association of genetic vs environmental factors in Swedish adoptees with clinically significant tinnitus. *JAMA Otolaryngol. Head Neck Surg.* 145, 222–229.
- Chen, Y.C., Xia, W., Chen, H., Feng, Y., Xu, J.J., Gu, J.P., Salvi, R., Yin, X., 2017. Tinnitus distress is linked to enhanced resting-state functional connectivity from the limbic system to the auditory cortex. *Hum. Brain Mapp.* 38, 2384–2397.
- Chen, Y.C., Chen, H., Bo, F., Xu, J.J., Deng, Y., Lv, H., Cai, Y., Xia, W., Yin, X., Gu, J.P., Lu, G., 2018. Tinnitus distress is associated with enhanced resting-state functional connectivity within the default mode network. *Neuropsychiatr. Dis. Treat.* 14, 1919–1927.
- Clasen, P.C., Wells, T.T., Knopik, V.S., McGahey, J.E., Beevers, C.G., 2011. 5-HTTLPR and BDNF Val66Met polymorphisms moderate effects of stress on rumination. *Genes Brain Behav.* 10, 740–746.
- Clayton, M.S., Yeung, N., Cohen Kadosh, R., 2015. The roles of cortical oscillations in sustained attention. *Trends Cogn. Sci.* 19, 188–195.
- Congedo, M., John, R.E., 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.
- De Ridder, D., Vanneste, S., Congedo, M., 2011. The distressed brain: a group blind source separation analysis on tinnitus. *PLoS one* 6, e24273.
- Drevets, W.C., 2001. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. *Curr. Opin. Neurobiol.* 11, 240–249.
- Duman, R.S., Malberg, J., Thome, J., 1999. Neural plasticity to stress and antidepressant treatment. *Biol. Psychiatry* 46, 1181–1191.
- Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., Weinberger, D.R., 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112, 257–269.
- Forbes, C.E., Poore, J.C., Barbey, A.K., Krueger, F., Solomon, J., Lipsky, R.H., Hodgkinson, C.A., Goldman, D., Grafman, J., 2012. BDNF polymorphism-dependent OFC and DLPFC plasticity differentially moderates implicit and explicit bias. *Cereb. Cortex* 22, 2602–2609.

- Fuchs, M., Kastner, J., Wagner, M., Hawes, S., Ebersole, J.S., 2002. A standardized boundary element method volume conductor model. *Clin. Neurophysiol.* 113, 702–712.
- Gerritsen, L., Tendolkar, I., Franke, B., Vasquez, A.A., Kooijman, S., Buitelaar, J., Fernandez, G., Rijpkema, M., 2012. BDNF Val66Met genotype modulates the effect of childhood adversity on subgenual anterior cingulate cortex volume in healthy subjects. *Mol. Psychiatry* 17, 597–603.
- Geweke, J., 1982. Measurement of linear dependence and feedback between multiple time series. *J. Am. Stat. Assoc.* 77, 304.
- Golm, D., Schmidt-Samoa, C., Dechent, P., Kroner-Herwig, B., 2013. Neural correlates of tinnitus related distress: an fMRI-study. *Hear. Res.* 295, 87–99.
- Golm, D., Schmidt-Samoa, C., Dechent, P., Kroner-Herwig, B., 2016. Tinnitus-related distress: evidence from fMRI of an emotional stroop task. *BMC Ear Nose Throat Disord.* 16, 10.
- Granger, C.W.J., 1969. Investigating causal relations by econometrics models and cross-spectral methods. *Econometrica* 37, 424.
- Hajcak, G., Castille, C., Olvet, D.M., Dunning, J.P., Roohi, J., Hatchwell, E., 2009. Genetic variation in brain-derived neurotrophic factor and human fear conditioning. *Genes Brain Behav.* 8, 80–85.
- Hasler, G., Drevets, W.C., Manji, H.K., Charney, D.S., 2004. Discovering endophenotypes for major depression. *Neuropsychopharmacology* 29, 1765–1781.
- Heller, A.J., 2003. Classification and epidemiology of tinnitus. *Otolaryngol. Clin. North Am.* 36, 239–248.
- 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.
- Hullfish, J., Abenes, I., Kovacs, S., Sunaert, S., De Ridder, D., Vanneste, S., 2018. Functional connectivity analysis of fMRI data collected from human subjects with chronic tinnitus and varying levels of tinnitus-related distress. *Data Brief* 21, 779–789.
- Hwang, K., Ghuman, A.S., Manoach, D.S., Jones, S.R., Luna, B., 2014. Cortical neurodynamics of inhibitory control. *J. Neurosci.* 34, 9551–9561.
- Jagoda, L., Giroud, N., Neff, P., Kegel, A., Kleinjung, T., Meyer, M., 2018. Speech perception in tinnitus is related to individual distress level—a neurophysiological study. *Hear. Res.* 367, 48–58.
- Jiang, R., Babyak, M.A., Brummett, B.H., Siegler, I.C., Kuhn, C.M., Williams, R.B., 2017. Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism interacts with gender to influence cortisol responses to mental stress. *Psychoneuroendocrinology* 79, 13–19.
- Juckel, G., Schumacher, C., Giebling, I., Assion, H.J., Mavrogiorgou, P., Pogarell, O., Mulert, C., Hegerl, U., Norra, C., Rujescu, D., 2010. Serotonergic functioning as measured by the loudness dependence of auditory evoked potentials is related to a haplotype in the brain-derived neurotrophic factor (BDNF) gene. *J. Psychiatr. Res.* 44, 541–546.
- Jurcak, V., Tsuzuki, D., Dan, I., 2007. 10/20, 10/10, and 10/5 systems revisited: their validity as relative head-surface-based positioning systems. *Neuroimage* 34, 1600–1611.
- Kandeepan, S., Maudoux, A., Ribeiro de Paula, D., Zheng, J.Y., Cabay, J.E., Gomez, F., Chronik, B.A., Ridder, D., Vanneste, S., Soddu, A., 2019. Tinnitus distress: a paradoxical attention to the sound? *J. Neurol.* 266, 2197–2207.
- Lancaster, J.L., Woldorff, M.G., Parsons, L.M., Liotti, M., Freitas, C.S., Rainey, L., Kochunov, P.V., Nickerson, D., Mikiten, S.A., Fox, P.T., 2000. Automated Talairach atlas labels for functional brain mapping. *Hum. Brain Mapp.* 10, 120–131.

- Liu, Y., Bengson, J., Huang, H., Mangun, G.R., Ding, M., 2016. Top-down modulation of neural activity in anticipatory visual attention: control mechanisms revealed by simultaneous EEG-fMRI. *Cereb. Cortex* 26, 517–529.
- Lotfipour, S., Ferguson, E., Leonard, G., Perron, M., Pike, B., Richer, L., Seguin, J.R., Toro, R., Veillette, S., Pausova, Z., Paus, T., 2009. Orbitofrontal cortex and drug use during adolescence: role of prenatal exposure to maternal smoking and BDNF genotype. *Arch. Gen. Psychiatry* 66, 1244–1252.
- Maas, I.L., Bruggemann, P., Requena, T., Bulla, J., Edvall, N.K., Hjelmborg, J.V.B., Szczepk, A.J., Canlon, B., Mazurek, B., Lopez-Escamez, J.A., Cederroth, C.R., 2017. Genetic susceptibility to bilateral tinnitus in a Swedish twin cohort. *Genet. Med.* 19, 1007–1012.
- Makeig, S., Inlow, M., 1993. Lapses in alertness: coherence of fluctuations in performance and EEG spectrum. *Electroencephalogr. Clin. Neurophysiol.* 86, 23–35.
- Mayberg, H.S., 2009. Targeted electrode-based modulation of neural circuits for depression. *J. Clin. Invest.* 119, 717–725.
- 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., Heyndrickx, K., Lambrechts, P., De Ridder, D., Van de Heyning, P., 2010. Phase-shift treatment for tinnitus of cochlear origin. *Eur. Arch. Otorhinolaryngol.* 276 (6), 881–888.
- Meeus, O., De Ridder, D., Van de Heyning, P., 2011. Administration of the combination clonazepam-deanxit as treatment for tinnitus. *Otol. Neurotol.* 32, 701–709.
- Moisset, X., Bouhassira, D., 2007. Brain imaging of neuropathic pain. *Neuroimage* 37 (Suppl. 1), S80–S88.
- Montag, C., Weber, B., Fliessbach, K., Elger, C., Reuter, M., 2009. The BDNF Val66Met polymorphism impacts parahippocampal and amygdala volume in healthy humans: incremental support for a genetic risk factor for depression. *Psychol. Med.* 39, 1831–1839.
- Nam, Y.J., Stover, T., Hartman, S.S., Altschuler, R.A., 2000. Upregulation of glial cell line-derived neurotrophic factor (GDNF) in the rat cochlea following noise. *Hear. Res.* 146, 1–6.
- Nestler, E.J., Barrot, M., DiLeone, R.J., Eischt, A.J., Gold, S.J., Monteggia, L.M., 2002. Neurobiology of depression. *Neuron* 34, 13–25.
- Nichols, T.E., Holmes, A.P., 2002. Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum. Brain Mapp.* 15, 1–25.
- Notaras, M., Hill, R., van den Buuse, M., 2015. The BDNF gene Val66Met polymorphism as a modifier of psychiatric disorder susceptibility: progress and controversy. *Mol. Psychiatry* 20, 916–930.
- Pascual-Marqui, R.D., 2002. Standardized low-resolution brain electromagnetic tomography (sLORETA): technical details. *Methods Find Exp. Clin. Pharmacol.* 24 (Suppl. D), 5–12.
- Pascual-Marqui, R., 2007a. Instantaneous and Lagged Measurements of Linear and Nonlinear Dependence Between Groups of Multivariate Time Series: Frequency Decomposition [Online]. Available by <http://arxiv.org/abs/0711.1455>.
- Pascual-Marqui, R., 2007b. Discrete, 3D distributed, linear imaging methods of electric neuronal activity. Part 1: exact, zero error localization [online]. Available by <http://arxiv.org/abs/0710.3341>.

- Pascual-Marqui, R.D., Lehmann, D., Koukkou, M., Kochi, K., Anderer, P., Saletu, B., Tanaka, H., Hirata, K., John, E.R., Prichep, L., Biscay-Lirio, R., Kinoshita, T., 2011. Assessing interactions in the brain with exact low-resolution electromagnetic tomography. *Philos. Trans. A Math. Phys. Eng. Sci.* 369, 3768–3784.
- Price, D.D., 2000. Psychological and neural mechanisms of the affective dimension of pain. *Science* 288, 1769–1772.
- Qu, T., Qi, Y., Yu, S., Du, Z., Wei, W., Cai, A., Wang, J., Nie, B., Liu, K., Gong, S., 2019. Dynamic changes of functional neuronal activities between the auditory pathway and limbic systems contribute to noise-induced tinnitus with a normal audiogram. *Neuroscience* 408, 31–45.
- Ren-Patterson, R.F., Cochran, L.W., Holmes, A., Sherrill, S., Huang, S.J., Tolliver, T., Lesch, K.P., Lu, B., Murphy, D.L., 2005. Loss of brain-derived neurotrophic factor gene allele exacerbates brain monoamine deficiencies and increases stress abnormalities of serotonin transporter knockout mice. *J. Neurosci. Res.* 79, 756–771.
- Schecklmann, M., Lehner, A., Poepll, T.B., Kreuzer, P.M., Hajak, G., Landgrebe, M., Langguth, B., 2012. Cluster analysis for identifying sub-types of tinnitus: a positron emission tomography and voxel-based morphometry study. *Brain Res.* 1485, 3–9.
- Schecklmann, M., Lehner, A., Poepll, T.B., Kreuzer, P.M., Rupprecht, R., Rackl, J., Burger, J., Frank, E., Hajak, G., Langguth, B., Landgrebe, M., 2013. Auditory cortex is implicated in tinnitus distress: a voxel-based morphometry study. *Brain Struct. Funct.* 218, 1061–1070.
- Schofield, P.R., Williams, L.M., Paul, R.H., Gatt, J.M., Brown, K., Luty, A., Cooper, N., Grieve, S., Dobson-Stone, C., Morris, C., Kuan, S.A., Gordon, E., 2009. Disturbances in selective information processing associated with the BDNF Val66Met polymorphism: evidence from cognition, the P300 and fronto-hippocampal systems. *Biol. Psychol.* 80, 176–188.
- Sedley, W., Gander, P.E., Kumar, S., Oya, H., Kovach, C.K., Nourski, K.V., Kawasaki, H., Howard 3rd, M.A., Griffiths, T.D., 2015. Intracranial mapping of a cortical tinnitus system using residual inhibition. *Curr. Biol.* 25, 1208–1214.
- Terracciano, A., Tanaka, T., Sutin, A.R., Deiana, B., Balaci, L., Sanna, S., Olla, N., Maschio, A., Uda, M., Ferrucci, L., Schlessinger, D., Costa Jr., P.T., 2010. BDNF Val66-Met is associated with introversion and interacts with 5-HTTLPR to influence neuroticism. *Neuropsychopharmacology* 35, 1083–1089.
- Uchida, S., Hara, K., Kobayashi, A., Otsuki, K., Yamagata, H., Hobara, T., Suzuki, T., Miyata, N., Watanabe, Y., 2011. Epigenetic status of Gdnf in the ventral striatum determines susceptibility and adaptation to daily stressful events. *Neuron* 69, 359–372.
- Vanneste, S., De Ridder, D., 2015. Stress-related functional connectivity changes between auditory cortex and cingulate in tinnitus. *Brain Connect.* 5, 371–383.
- Vanneste, S., De Ridder, D., 2016. Deafferentation-based pathophysiological differences in phantom sound: tinnitus with and without hearing loss. *Neuroimage* 129, 80–94.
- Vanneste, S., Plazier, M., van der Loo, E., Van de Heyning, P., De Ridder, D., 2010a. The differences in brain activity between narrow band noise and pure tone tinnitus. *PLoS one* 5, e13618.
- Vanneste, S., Plazier, M., der Loo, E., de Heyning, P.V., Congedo, M., De Ridder, D., 2010b. The neural correlates of tinnitus-related distress. *Neuroimage* 52, 470–480.
- Vanneste, S., van de Heyning, P., De Ridder, D., 2011a. Contralateral parahippocampal gamma-band activity determines noise-like tinnitus laterality: a region of interest analysis. *Neuroscience* 199, 481–490.

- Vanneste, S., Plazier, M., van der Loo, E., Van de Heyning, P., De Ridder, D., 2011b. The difference between uni- and bilateral auditory phantom percept. *Clin. Neurophysiol.* 122, 578–587.
- Vanneste, S., Joos, K., De Ridder, D., 2012. Prefrontal cortex based sex differences in tinnitus perception: same tinnitus intensity, same tinnitus distress, different mood. *PLoS one* 7, e31182.
- Vanneste, S., Congedo, M., De Ridder, D., 2014. Pinpointing a highly specific pathophysiological functional connection that turns phantom sound into distress. *Cereb. Cortex* 24, 2268–2282.
- Vanneste, S., Alsalmán, O., De Ridder, D., 2019. Top-down and bottom-up regulated auditory phantom perception. *J. Neurosci.* 39, 364–378.
- Vermetten, E., Schmahl, C., Southwick, S.M., Bremner, J.D., 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.
- von Leupoldt, A., Sommer, T., Kegat, S., Baumann, H.J., Klose, H., Dahme, B., Buchel, C., 2009. Dyspnea and pain share emotion-related brain network. *Neuroimage* 48, 200–206.
- Wei, S.M., Eisenberg, D.P., Kohn, P.D., Kippenhan, J.S., Kolachana, B.S., Weinberger, D.R., Berman, K.F., 2012. Brain-derived neurotrophic factor Val(6)(6)Met polymorphism affects resting regional cerebral blood flow and functional connectivity differentially in women versus men. *J. Neurosci.* 32, 7074–7081.
- Yu, H., Wang, D.D., Wang, Y., Liu, T., Lee, F.S., Chen, Z.Y., 2012. Variant brain-derived neurotrophic factor Val66Met polymorphism alters vulnerability to stress and response to antidepressants. *J. Neurosci.* 32, 4092–4101.
- Zoon, H.F., Veth, C.P., Arns, M., Drinkenburg, W.H., Talloen, W., Peeters, P.J., Kenemans, J.L., 2013. EEG alpha power as an intermediate measure between brain-derived neurotrophic factor Val66Met and depression severity in patients with major depressive disorder. *J. Clin. Neurophysiol.* 30, 261–267.