



Research Paper

COMT and the neurogenetic architecture of hearing loss induced tinnitus

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ABSTRACT

As the COMT polymorphism is especially prominent in the prefrontal cortex and has been associated with auditory gating, we hypothesize that tinnitus patients with this polymorphism have altered activity in the ventromedial prefrontal/anterior cingulate areas that modulates the tinnitus percept. To test this, we recruited a total of 40 tinnitus subjects and 20 healthy controls for an EEG study. A comparison between tinnitus subjects and healthy controls and their frequency of being Val/Val genotype or Met carriers (including Val/Met and Met/Met genotype) shows no significant effect, suggesting that the distributions for the tinnitus and healthy groups are similar. Our results show that an interaction between the amount of hearing loss and the COMT Val¹⁵⁸Met polymorphism can increase susceptibility to the clinical manifestation of tinnitus. We further demonstrate that the parahippocampus becomes involved in tinnitus in patients with hearing loss that are Met carriers. In these patients, the parahippocampus sends more tinnitus information to the pregenual anterior cingulate cortex and auditory cortex that is specifically related with increased loudness. At the same time, the pregenual anterior cingulate cortex, which normally functions as a gatekeeper, is not cancelling this auditory information, ultimately leading to increased tinnitus loudness.

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1. Introduction

The main function of the enzyme catechol-O-methyltransferase (COMT) is to inactivate dopamine, norepinephrine, and epinephrine neurotransmitters in the mammalian brain (Tunbridge et al., 2006). A single-nucleotide polymorphism (SNP) of the gene for COMT results in a valine-to-methionine mutation at position 158. The homozygous Val variant metabolizes dopamine up to four times the rate of its methionine (Gogos et al., 1998; Mattay et al., 2003; Slifstein et al., 2008). The influence of the COMT polymorphism is especially prominent in the prefrontal cortex (PFC) due to the lack of dopamine transporter in this region (Mattay et al., 2003; Slifstein et al., 2008). Due to these increased synaptic dopamine levels, Met carriers feature more stress reactivity and pain sensitivity as well as schizophrenia (Meyer-Lindenberg, 2010;

Zubieta et al., 2003). Other research has also shown that the COMT Val¹⁵⁸Met polymorphism is significantly associated with changes in brain connectivity (Zhang et al., 2015) and that it interacted with both parental warmth and stressful life events to influence affective decision-making (He et al., 2012).

‘Sensory gating’ describes a filter mechanism protecting the central nervous system from sensory overload by inhibiting behaviorally irrelevant input (Boutros et al., 2004, 2008). This permits adaptation to a changing contextual environment (Fruhstorfer et al., 1970; Grunwald et al., 2003). Auditory gating has been anatomically linked to the auditory cortex (AUD), hippocampus, parahippocampus (PHC), and cingulate cortices (Boutros et al., 2008; Grunwald et al., 2003; Majic et al., 2011); prefrontal cortex (PFC) dopamine is also involved in auditory gating (Grunwald et al., 2003). A direct relationship was recently shown between the COMT polymorphism and poor auditory gating via a PFC–AUD mechanism (Majic et al., 2011). Indeed, this study shows that COMT Met carrying is associated with a poor sensory gating of the N100 component - a mid-latency component of auditory evoked potentials with a peak between 80 and 120 ms after the presentation of an acoustic stimulus, suggesting that a high prefrontal processing

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capacity allows a pronounced afferent input of sensory information from the AUD as reflected by poor sensory gating (Majic et al., 2011).

Tinnitus is the perception of simple sound (pure tones and/or noise) in the absence of a corresponding external sound source, and is considered an auditory phantom percept analogous to phantom pain (Eggermont et al., 2004; Jastreboff, 1990). Tinnitus is proposed to be an emergent property of network activity (De Ridder et al., 2014b) most commonly related to auditory deafferentation with or without hearing loss (Vanneste et al., 2016). The deafferentation is commonly due to noise trauma, presbycusis, or other causes of auditory deprivation (Hesse et al., 2016). It has been postulated that tinnitus, based on the pain literature, can be the result of a deficient auditory gating mechanism (Leaver et al., 2011; Rauschecker et al., 2010). One line of research, although not consistently replicated, shows that structural deficits and functional changes in the ventromedial PFC, pregenual anterior cingulate cortex (pgACC), and the nucleus accumbens are associated with a deficient frontostriatal auditory gating mechanism (Leaver et al., 2011; Rauschecker et al., 2010, 2015). This mechanism is central gatekeeper that evaluates the relevance and affective meaning of sensory stimuli and modulates information via descending inhibitory pathways to the thalamic reticular nucleus which modulates the information flow between the thalamus and the AUD by inhibiting specific thalamic neurons in a highly selective and frequency-specific manner (Rauschecker et al., 2015; Yu et al., 2009).

Some preliminary evidence from pharmacological interventions in humans have demonstrated that a decrease in dopamine activity could reduce tinnitus perception (Lopez-Gonzalez et al., 2007; Meeus et al., 2011). As the COMT polymorphism is especially prominent in PFC and has been associated with auditory gating, we hypothesize that tinnitus patients with this polymorphism have altered activity in the ventromedial PFC/anterior cingulate cortex that modulate the tinnitus percept.

2. Methods

2.1. Participants

A total of 40 subjects with chronic subjective and constant tinnitus (age: 45.97 years \pm 14.19; males: 28; females: 12) and 20 healthy controls (age: 45.60 years \pm 16.27; males: 13; females: 7) were recruited for this study. Both tinnitus and control subjects were recruited from the Dallas area and were screened in a similar way. Informed consent was obtained from all participants in accordance with the protocols approved by the Institutional Review Boards of the University of Texas at Dallas. All subjects were carefully screened both to match tinnitus subjects with controls for age, gender, and hearing loss as well as to ensure that no subject had a history of neurological or psychiatric illness. Due to this matching, we had to exclude six control participants. There was no significant difference between the tinnitus subjects and healthy controls for gender ($\chi^2 = .15$, $p = .70$) or for age ($t = 1.26$, $p = .21$).

All tinnitus patients were interviewed to determine the perceived location of their tinnitus (i.e. the left ear, in both ears, the right ear) as well as the characteristics of the tinnitus percept (i.e. pure tone-like or noise-like tinnitus). All subjects were additionally screened for the extent of hearing loss (in dB HL) using a pure tone audiometry using the British Society of Audiology procedures at .125, .25, .5, 1, 2, 3, 4, 6, and 8 kHz (Audiology, 2008).

Tinnitus patients were further tested for the tinnitus pitch (frequency) by performing a tinnitus-matching analysis. In unilateral tinnitus patients, tinnitus matching was performed contralaterally to the tinnitus ear. In bilateral tinnitus patients, tinnitus matching was performed contralaterally to the worse tinnitus ear.

First, a 1-kHz pure tone was presented contralaterally 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 the most (Meeus et al., 2009, 2011). We calculated the hearing loss at the tinnitus frequency as obtained by tinnitus matching. For unilateral tinnitus, the hearing loss in the ear contralateral to where the patient perceived tinnitus was considered, while for bilateral tinnitus patients we calculated the mean hearing threshold across both ears.

Participants were further asked to rate the loudness of their tinnitus on visual analogue scales (VAS) from 0 to 10, with 0 indicating 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).

The Tinnitus Handicap Inventory (THI) tries to identify, quantify, and evaluate the difficulties that a patient experiences because of tinnitus. (Newman et al., 1996). The THI is a 25-item self-administered questionnaire that aims to quantify the impact of tinnitus on daily life. Respondents are asked to answer the questions with 'Yes' (4 points), 'Sometimes' (2 points), or 'No' (0 points). A higher THI score is indicative of a greater tinnitus handicap, up to a maximum score of 100.

The Beck Depression Inventory II (BDI) was also collected to evaluate the severity of depressive mood states. The BDI scores severity of components such as feelings of hopelessness and guilt in addition to fatigue and other physical symptoms (Richter et al., 1998). It consists of 21 questions, each rated between 0 (no symptom impact) and 3 (maximum symptom impact), with a maximum score of 63.

2.2. Genotyping

Genotyping of the single nucleotide polymorphism (SNP) rs4680 was carried out at DNA Genotek in Ottawa (www.dnagenotek.com). DNA was extracted from 700 μ L of 60/60 Ora-gene saliva samples. The average DNA yield was 7 μ g (<1–24 μ g) by PicoGreen measurement and 15 μ g (<1–59 μ g) by Nanodrop. This sample had a lower yield in comparison to the Genotek database that may have contributed to the lower purity. An aliquot of all samples was normalized to approximately 3 ng/ μ L for genotyping using Taqman chemistry (Taqman Assay: C 25746809 50). All 60 samples were genotyped using Taqman chemistry for rs4680. 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 (e.g. an "A" nucleotide) and a different fluorescent dye is attached to the detector that is a perfect match to the second allele (e.g. a "C" nucleotide). 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.

2.3. EEG data collection

Recordings were obtained in a fully-lit room with each participant sitting upright in a small but comfortable chair and was identical for the tinnitus and control subjects. 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 k Ω . Data were collected eyes-closed (sampling rate = 1000 Hz, band-passed 0.15–200 Hz). Using EEGLab, off-line 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, and ECG artifacts were removed from the stream of the EEG. Average Fourier cross-spectral matrices were computed for frequency bands delta (2–3.5 Hz), theta (4–7.5 Hz), alpha1 (8–10 Hz), alpha2 (10–12 Hz), beta1 (13–18 Hz), beta2 (18.5–21 Hz), beta3 (21.5–30 Hz) and gamma (30.5–44 Hz). These frequency bands are based on previous research in tinnitus (Vanneste et al., 2010, 2011a, 2011b, 2011c).

2.4. 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) was 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 lead-field 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 standard brain (Montreal Neurological Institute, Canada). The sLORETA-Key anatomical template divides and labels the neocortical (including hippocampus and anterior cingulate cortex) MNI-152 vol into 6239 voxels of dimension 5 mm isotropic, 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.

2.5. Region of interest analysis

The log-transformed electric current density was averaged across all voxels belonging to the regions of interest: pgACC, left AUD, and left PHC for delta (2–3.5 Hz), theta (4–7.5 Hz), alpha1 (8–10 Hz), alpha2 (10–12 Hz), beta1 (13–18 Hz), beta2 (18.5–21 Hz), beta3 (21.5–30 Hz) and gamma (30.5–44 Hz).

2.6. Lagged phase coherence

Coherence and phase synchronization between time series corresponding to different spatial locations are usually interpreted as indicators of connectivity between those locations. However, any measure of dependence is highly contaminated with an instantaneous, non-physiological contribution due to volume conduction (Pascual-Marqui, 2007a). However, Pascual-Marqui introduced new measures of coherence and phase synchronization taking into account only non-instantaneous (lagged) connectivity, effectively removing the confounding factor of volume conduction (Pascual-Marqui, 2007). This lagged phase coherence between two sources can be interpreted as the amount of crosstalk 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 into a finite series of cosine and sine waves 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 lagged phase coherence is defined. 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 non-negative, and take the value zero only when there is independence. They are defined in the frequency domain 2–44 Hz in discrete bands: delta, theta, alpha1, alpha2, beta1, beta2, beta3, and gamma. These measures were used to calculate lagged linear connectivity. The time-series of current density were extracted for different regions of interest 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, calculated separately for specific frequencies. The regions of interest selected were the left AUD, left PHC, pgACC, and dorsal anterior cingulate cortex (dACC). The selection of these regions of interest was based on the sensory gating mechanism (De Ridder et al., 2011; Rauschecker et al., 2015). These areas are also associated with the tinnitus percept (Vanneste et al., 2016) as confirmed by the changes observed when comparing tinnitus subjects with healthy controls as well as Met carriers with Val homozygotes.

2.7. Granger causality

Granger causality reflects the strength of effective connectivity (i.e. causal interactions) between two brain regions 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 a directional measure of 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 additionally includes the past values of another time series. It is important to note that Granger causality does not imply anatomical connectivity between regions but instead the directional functional connectivity between two sources.

2.8. Statistics

2.8.1. 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 et al., 2002). This methodology corrects for multiple comparisons (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 et al., 2002). The significance threshold for all tests was based on a permutation test with 5000 permutations. Comparisons were made between the healthy controls and the tinnitus group for both Met carriers and Val/Val genotype together as well as separately. These comparisons were performed on the whole brain by sLORETA statistical contrast maps through multiple voxel-wise comparisons in a logarithm of *t*-ratio for each comparison separately.

2.8.2. Statistical analysis for region of interest analysis

Pearson correlations were calculated between each region of interest (pgACC, left AUD, and left PHC) and tinnitus loudness separately for the tinnitus patients, the tinnitus patients that are Met carriers, and those that are Val/Val genotype for all frequency

bands (delta (2–3.5 Hz), theta (4–7.5 Hz), alpha1 (8–10 Hz), alpha2 (10–12 Hz), beta1 (13–18 Hz), beta2 (18.5–21 Hz), beta3 (21.5–30 Hz) and gamma (30.5–44 Hz)). We corrected for multiple comparisons using the false discovery rate (FDR) correction to correct for region of interest and frequency bands (Benjamini et al., 1995).

2.8.3. 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 comparisons (i.e. for the collection of tests performed for all voxels, and for all frequency bands). In addition, Pearson correlations were calculated between tinnitus loudness and the connectivity strength between pgACC and AUD and between pgACC and PHC, respectively. We corrected for multiple comparisons using the FDR correction per the Benjamini–Hochberg procedure for each comparison separately (Benjamini et al., 1995).

2.8.4. Statistical analyses for granger causality

A comparison was made between tinnitus patients that are Met carriers and Val/Val genotype on the Granger causality outcome measure using a repeated-measures ANOVA. A simple contrast analysis was applied to compare specific conditions within the repeated-measures ANOVA.

Pearson correlations were calculated between the Granger causality outcome measures per patient and tinnitus loudness for the tinnitus that are Met carriers as well as Val/Val genotype for the theta frequency band for pgACC→AUD, pgACC→PHC, PHC→pgACC and AUD→pgACC. The theta frequency band based on the functional connectivity results. We corrected for regions of interest using the FDR correction per the Benjamini–Hochberg procedure.

We further calculate the balance between pgACC→PHC connectivity relative to the PHC→pgACC connectivity and correlate this with the loudness for the tinnitus patients that are Met carriers as well as Val/Val genotype. In order to compare the correlation coefficients between tinnitus patients that are Met carriers as well as Val/Val genotype, we rely on a formula found in Cohen and Cohen (Cohen et al., 1983). The formula yields a *t*-statistic with *n* – 3 degrees of freedom.

3. Results

3.1. Genetic characteristics

The distribution of the COMT genotype frequencies for the total group were 30.0% Val/Val (*n* = 18), 56.7% for Val/Met (*n* = 34), and 13.3% for Met/Met (*n* = 8). This distribution was 27.5% Val/Val (*n* = 11), 57.5% for Val/Met (*n* = 23), and 15.0% for Met/Met (*n* = 6) for the tinnitus subjects and 35.0% Val/Val (*n* = 7), 55.0% for Val/Met (*n* = 11), and 10.0% (*n* = 2) for Met/Met for the control group. No significant deviation from the Hardy-Weinberg equilibrium was observed (whole group *p* = .19; tinnitus group *p* = .29; control group *p* = .44).

Due to the low 'N' of Met/Met individuals and we are interested in the effect of Met carriers in comparison to Val/Val genotype, we merged the Val/Met and Met/Met group together. When comparing both the control and tinnitus group and their frequency of being a Val/Val genotype or Met carrier, no significant effect was observed ($\chi^2 = .36, p = .55$), suggesting that the distributions for the tinnitus and healthy groups are similar (see Table 1).

Table 1

The carrier distribution for healthy controls and tinnitus subjects.

	Val/Val carriers	Met carriers	Total
Healthy Controls	7 (11.7%)	13 (27.7%)	20 (33.3%)
Tinnitus Subjects	11 (18.3%)	29 (48.3%)	40 (66.7%)
Total	18 (30.0%)	42 (70.0%)	

3.2. Behavioral results

A comparison between the audiograms for healthy controls and tinnitus patients revealed no significant differences overall or for the individual frequencies (see Fig. 1). A univariate ANOVA with group (tinnitus vs. control) as independent variables and mean hearing loss as the dependent variable was conducted demonstrating no significant effect for group ($F = 3.43, p = .08$). A similar analysis for the COMT gene (Val/Val genotype vs. Met carriers) as independent variables and hearing loss as dependent variable showed no significant effect for the COMT gene ($F = .49, p = .49$).

A regression analysis including the interaction between the COMT polymorphism and mean hearing loss shows a significant effect on VAS Loudness ($\beta = .33, F = 4.654, p = .037$). To further explore this data, we divide the hearing loss group into mild (≤ 25 dB HL) and more severe (> 25 dB HL) hearing loss subgroups. A univariate ANOVA with COMT polymorphism (Val/Val genotype and Met carriers) and mean hearing loss (mild vs. more severe) as independent variables and tinnitus loudness as the dependent variable demonstrated no significant main effect for COMT polymorphism ($F = .50, p = .48$) and mean hearing loss ($F = 1.74, p = .20$). The interaction between COMT polymorphism and mean hearing loss revealed a significant effect on the tinnitus loudness ($F = 4.99, p = .032$). A simple contrast analysis showed that subjects that are Met carriers with more severe hearing loss perceive tinnitus more loudly in comparison to the Val/Val genotype ($F = 7.45, p = .01$). A comparison for the mild hearing loss group between the Val/Val genotype and Met carriers did not show a significant effect ($F = .82, p = .37$) in tinnitus loudness (see Fig. 2). No significant effect was observed for the COMT gene for Pitch Match ($F = .06, p < .82$) and the Tinnitus Matching ($F = .04, p < .84$).

An ANOVA with independent variable the COMT gene (Val/Val vs. Met carriers) on the THI ($F = 1.59, p = .22$), no significant was observed between the Val/Val genotype and Met carriers. In addition, no significant effect ($F = .59, p = .45$) was observed for the BDI between the Val/Val genotype and the Met carriers in tinnitus patients. See Table 2 for an overview for other tinnitus characteristics.

3.3. Whole brain analysis

3.3.1. Tinnitus vs. control subjects

A comparison between tinnitus and healthy control subjects revealed a significant effect for the theta and gamma frequency bands ($F = 2.58, p < .05$), demonstrating that tinnitus subjects have increased synchronized activity over the pgACC for the theta frequency and increased synchronized activity over the left AUD, extending into the temporoparietal junction for the gamma frequency band as well as the parahippocampal area (see Fig. 3). No significant effects were observed for the delta, alpha1, alpha2, beta1, beta2, and beta3 frequency bands.

An additional comparison between tinnitus and healthy control subjects including only the Val/Val genotype revealed only increased synchronized activity over the left ventrolateral prefrontal cortex for the gamma frequency band ($F = 3.54, p < .05$) for the tinnitus subjects (see Fig. 3). No significant effects were

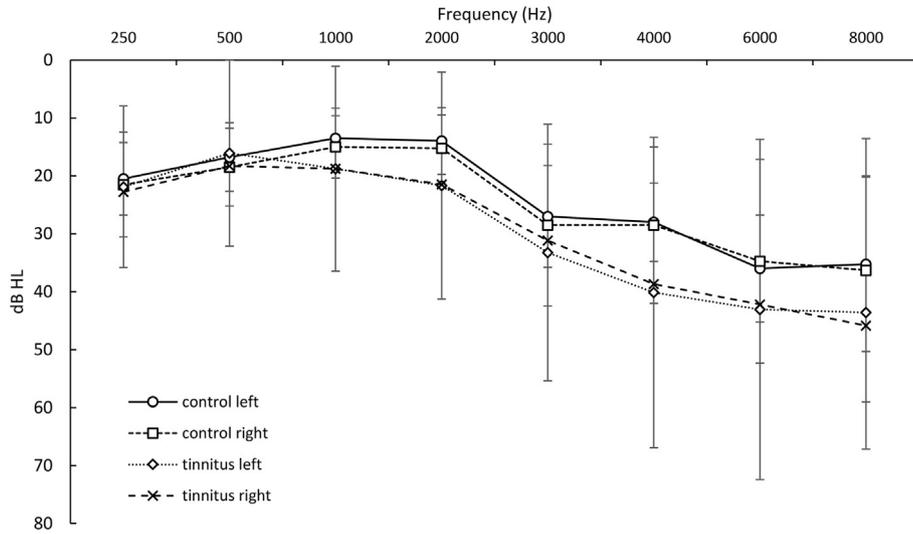


Fig. 1. A comparison between the audiogram for healthy controls and tinnitus patients revealed that no significant difference can be obtained by the two groups.

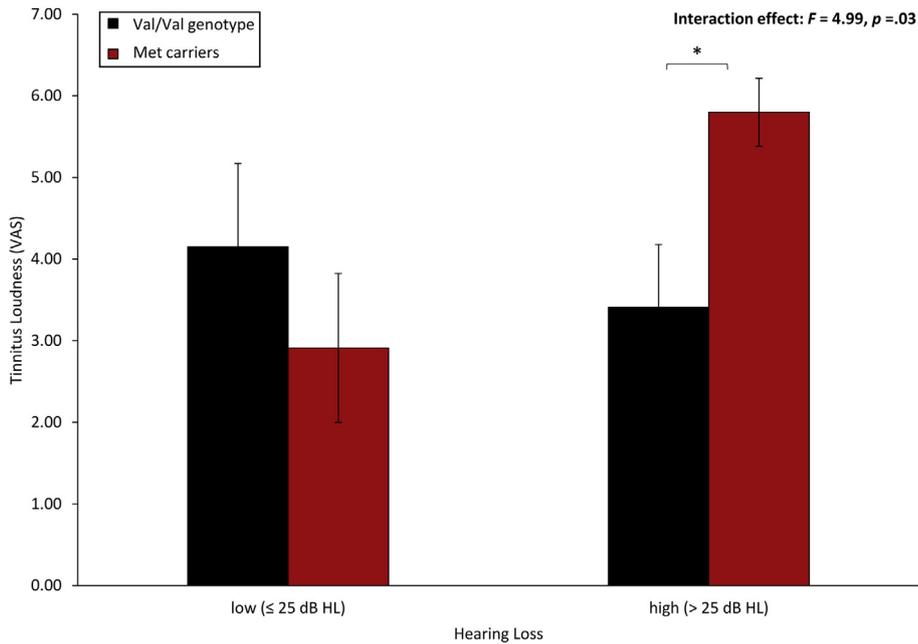


Fig. 2. The effect of COMT polymorphism (Val/Val genotype and Met carriers) and mean hearing loss (mild vs more severe) on tinnitus loudness demonstrated and interaction effect showing that subjects that are Met carriers with a more severe hearing loss perceive tinnitus louder in comparison to Val/Val genotype ($F = 7.45, p = .01$). A comparison for the mild hearing loss group between Val/Val genotype and Met carriers did not show a significant effect on the perceived tinnitus loudness.

Table 2
Tinnitus Characteristics: Average, Sd (between parenthesis) and percentages.

	Val/Val genotype	Met Carriers	Statistics
BDI	6.91 (6.88)	9.24 (7.64)	$F = .78, p = .38$
VAS	3.67 (2.08)	5.30 (2.25)	$F = 3.45, p = .12$
THI	23.81 (15.37)	33.79 (24.36)	$F = 1.59, p = .22$
Tinnitus onset	2.63 years (.78)	2.44 years (sd = .78)	$F = .49, p = .48$
Tinnitus lateralization	Uni: 36.2%; Bi: 63.6%	Uni: 44.8%; Bi: 55.2%	$\chi^2 = 1.20, p = .55$
Tinnitus type	PT: 72.7%; NBN: 27.3%	PT: 75.9%; NBN: 24.1%	$\chi^2 = .42, p = .83$

BDI: Beck Depression Inventory; Uni: unilateral; Bi: Bilateral; PT: Pure Tone; NBN: Narrow Band Noise.

observed for the delta, theta, alpha1, alpha2, beta1, beta2, and beta3 frequency bands.

A comparison between tinnitus subjects and healthy control

subjects including only the Met genotype revealed only increased synchronized activity over the pgACC for the theta frequency band for tinnitus subjects ($F = 3.11, p < .05$; see Fig. 3). No significant

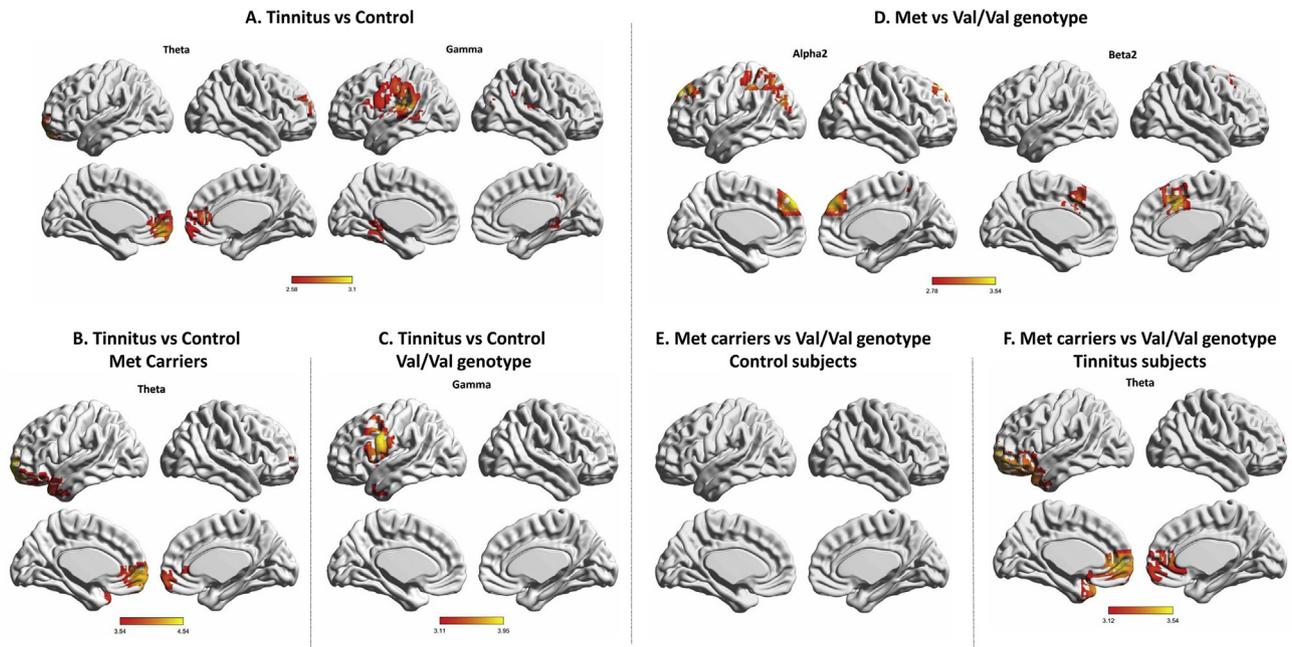


Fig. 3. (A) A comparison between tinnitus and healthy control subjects revealed a significant effect for the theta and gamma frequency band over respectively the pregenual anterior cingulate cortex and the left auditory cortex. (B) A comparison between tinnitus and healthy control subjects including only the Val/Val genotype revealed only increased synchronized activity over the left auditory cortex for the gamma frequency band for the tinnitus subjects. (C) A comparison between tinnitus subjects and healthy control subjects including only the Met carriers revealed only increased synchronized activity over the pregenual anterior cingulate cortex for the theta frequency band for tinnitus subjects. (D). A comparison between Val/Val genotype and Met carriers revealed increased significant activity for the alpha2 and beta2 frequency band over the dorsal anterior cingulate cortex. (E) A comparison only including the control subjects comparing the Val/Val genotype and Met carriers revealed no significant effects. (F). A comparison comparing between the Val/Val genotype and Met carriers including only the tinnitus patients shows significant increased synchronized activity in the pregenual anterior cingulate cortex for the Met carriers.

effects were observed for the delta, alpha1, alpha2, beta1, beta2, beta3, and gamma frequency bands.

3.3.2. Val/Val genotype versus met carriers

A comparison between the Val/Val genotype and Met carriers revealed a significant effect for the alpha2 and beta2 frequency bands ($F = 2.78$, $p < .05$). For the alpha2 frequency band, we revealed a significant increase over the rostral/dorsal ACC extending into the dorsolateral PFC and the superior parietal cortex for the Met carriers. For the beta2 frequency, we found increased synchronized activity in the dACC for the Met carriers (Fig. 3). No significant effects were observed for the delta, theta, alpha1, beta1, beta3, and gamma frequency bands.

A comparison between the Val/Val genotype and Met carriers in healthy control subjects revealed no significant effects for the delta, theta, alpha1, alpha2, beta1, beta2, beta3, and gamma frequency bands.

A comparison between the Val/Val genotype and Met carriers in the tinnitus patients showed significant increased synchronized activity in the pgACC for the Met carriers for the theta frequency band ($F = 3.12$, $p < .05$; see Fig. 3). No significant effects were observed for the delta, alpha1, beta1, beta2, beta3, and gamma frequency bands.

3.3.3. Mild vs. more severe hearing loss

For the Val/Val genotype, a comparison for tinnitus subjects with mild hearing loss with tinnitus subjects with more severe hearing loss did not show a significant effect for delta, theta, alpha1, alpha2, beta1, beta2, beta3, and gamma frequency bands.

For Met carriers, comparing tinnitus subjects with mild hearing loss to tinnitus subjects with more severe hearing loss revealed a significant increase for the theta frequency band at pgACC together with an increase in the gamma frequency at PHC and inferior

parietal cortex for tinnitus patients with more severe hearing loss ($F = 2.95$, $p < .05$; see Fig. 4). No significant effects were observed for the delta, alpha1, alpha2, beta1, beta2, and beta3 frequency bands.

3.3.4. Correlations between loudness and regions of interest

Looking at the regions of interest (pgACC, AUD, and PHC), we observed a significant positive correlation for loudness and the log-transformed current density for the left PHC ($r = .44$, $p = .002$) as well as for the left AUD ($r = .31$, $p = .026$) at the gamma frequency band, showing that an increase in current density at the left PHC goes together with an increase in tinnitus loudness. No significant correlation was observed for the pgACC at the theta frequency ($r = -.06$, $p = .71$). For the left PHC, we see that the effect was mainly driven by Met carriers ($r = .44$, $p = .009$) and not by the Val/Val genotype ($r = .30$, $p = .19$). For the left AUD, we see that mainly the Val/Val ($r = .51$, $p = .047$) genotype contribute the effect and not the Met carriers ($F = 2.01$, $p = .15$). For the pgACC at the theta frequency, no significant correlation was observed for the Met ($r = -.09$, $p = .66$) carriers and the Val/Val ($r = .30$, $p = .37$) genotype. See Fig. 5 for an overview. These effects survived correction for multiple comparisons.

3.4. Functional connectivity: lagged phase coherence

3.4.1. Tinnitus vs control subjects

A comparison between tinnitus subjects and healthy controls revealed a significant difference in phase coherence for the theta and alpha1 frequency band revealing for both frequency bands increased coherence between the left PHC and left AUD for tinnitus subjects ($F = 4.02$, $p < .05$; see Fig. 6). No significant effect was observed for the delta, alpha2, beta1, beta2, beta3 and gamma frequency band. When comparing between tinnitus subjects and

High vs Low hearing loss: Met carriers

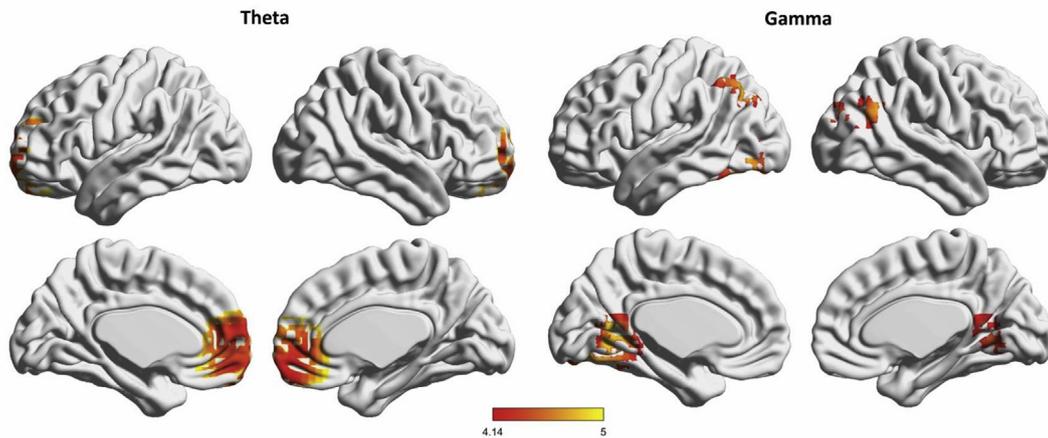


Fig. 4. A comparison for tinnitus subjects with mild hearing loss to tinnitus subjects with more severe hearing loss that are Met carriers shows a significant increase for theta frequency band at the pregenual anterior cingulate cortex together with an increase in the gamma frequency at the parahippocampus for tinnitus patients with more severe hearing loss (band).

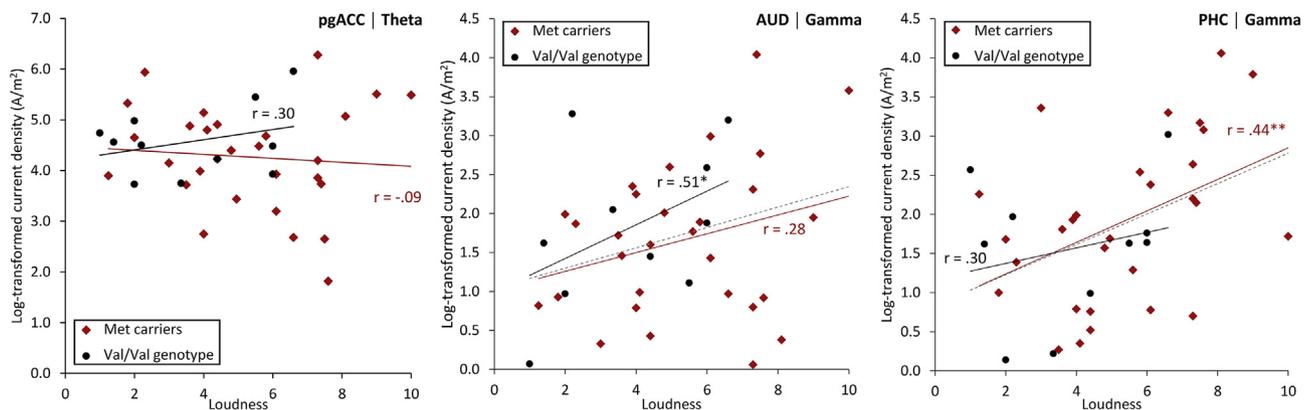


Fig. 5. Correlation analysis between tinnitus loudness and log-transformed current density for the pregenual anterior cingulate cortex, left auditory cortex and the left parahippocampus for respectively Val/Val genotype and Met carriers. (* $p < .05$; ** $p < .01$).

healthy controls that are Val/Val genotype, no significant effect was observed for the delta, theta, alpha1, alpha2, beta1, beta2, beta3 and gamma frequency band. For Met carriers, a comparison between tinnitus subjects and healthy control subjects yielded a significant effect for the theta frequency band revealing increased coherence between the left PHC and left AUD for the tinnitus subjects in comparison to healthy subjects ($F = 3.11$, $p < .05$; see Fig. 6). No significant effects were observed for the delta, theta, alpha1, alpha2, beta1, beta3, and gamma frequency bands.

3.4.2. Val/Val genotype versus met carriers

A comparison between the Val/Val genotype and Met carriers showed no significant effects for the delta, theta, alpha1, alpha2, beta1, beta2, beta3, and gamma frequency bands. For healthy subjects, a comparison between the Val/Val genotype and Met carriers identified no significant effects for the delta, theta, alpha1, alpha2, beta1, beta2, beta3, and gamma frequency bands. A comparison between the Val/Val genotype and Met carriers including only the tinnitus patients showed significant phase coherence changes for the theta frequency band ($F = 3.24$, $p < .05$; see Fig. 6). Increased coherence was observed between the left PHC and left AUD for the Met carriers. Furthermore, decreased coherence was observed between the pgACC and respectively the left PHC, and the

left AUD. No significant effects were observed for the delta, alpha1, alpha2, beta1, beta2, beta3, and gamma frequency bands.

3.4.3. Mild vs. more severe hearing loss

A comparison between tinnitus subjects with mild hearing loss and tinnitus subjects with more severe hearing loss revealed no significant effects for the delta, alpha1, alpha2, beta1, beta2, beta3, and gamma frequency bands. For Met carriers, comparing tinnitus subjects with mild hearing loss with tinnitus subjects with high hearing loss a significant effect was observed for the theta and gamma frequency band. ($F = 4.14$, $p < .05$; see Fig. 7). For both frequency bands, we observed increased phase coherence between the left PHC and left AUD for the tinnitus patients with more severe hearing loss as well decreased phase coherence between the pregenual anterior cingulate and the left PHC. No significant effects were observed for the delta, alpha1, alpha2, beta1, beta2, and beta3 frequency bands.

3.4.4. Correlations with loudness and connectivity strength based on phase coherence

To further confirm our findings, we looked at the correlation between tinnitus loudness and connectivity strength between pgACC and left AUD ($r = -.27$, $p = .043$) and between pgACC and the

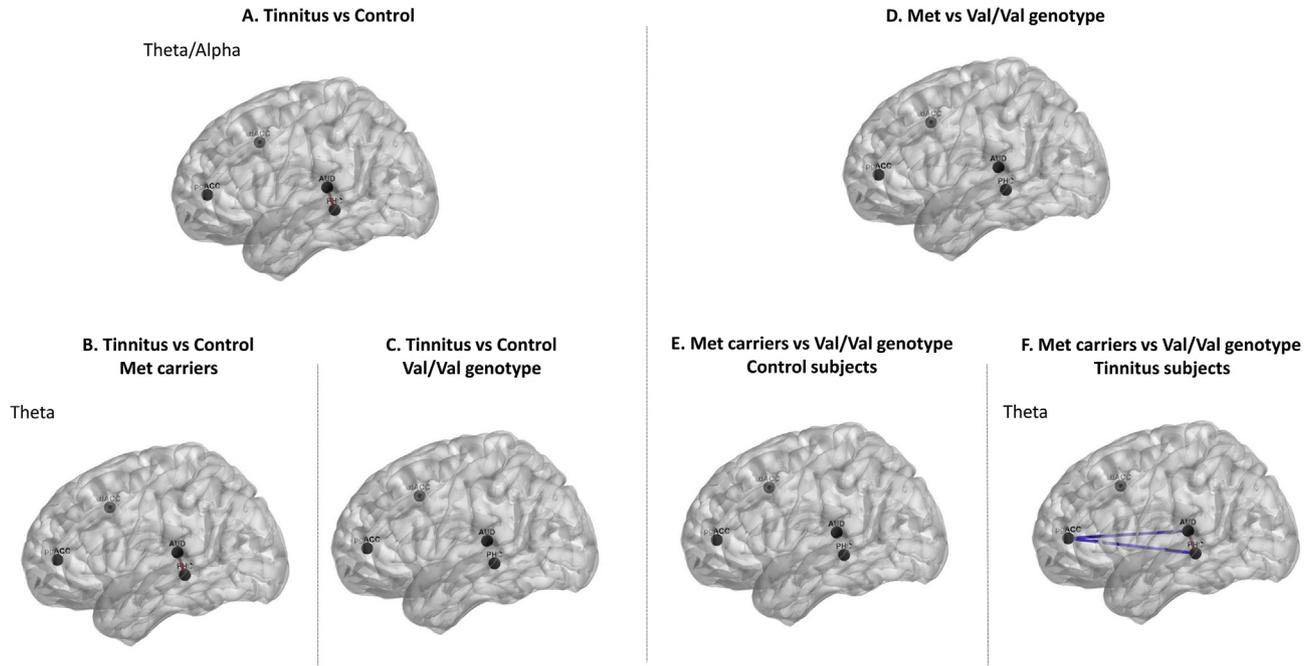


Fig. 6. (A) A comparison between tinnitus subjects and healthy controls revealed a significant difference in phase coherence for the theta and alpha1 frequency band between the left parahippocampus and left auditory cortex for tinnitus subjects. (B) A comparison between tinnitus subjects and healthy controls that are Val/Val genotype revealed no significant effects. (C) For Met carriers, a comparison between tinnitus subjects and healthy control subjects yielded a significant effect for the theta frequency band between the left parahippocampus and left auditory cortex for the tinnitus subjects. (D) A comparison between Val/Val genotype and Met carriers showed no significant effects. (E) For healthy subjects, a comparison between Val/Val genotype and Met carriers no significant effects. (F) A comparison comparing between the Val/Val genotype and Met carriers including only the tinnitus patients shows significant phase coherence changes were observed for the theta frequency band between the left parahippocampus and left auditory cortex for the Met carriers as well as decrease coherence was observed between the pregenual anterior cingulate and respectively the left parahippocampus, and the left auditory cortex.

High vs Low hearing loss: Met carriers

Theta/Gamma

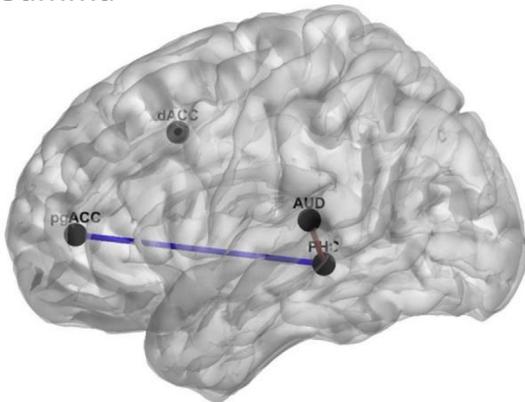


Fig. 7. A comparison between tinnitus subjects with mild hearing loss and tinnitus subjects with more severe hearing loss yielded a significant effect was observed for the theta and gamma frequency band. For both frequency bands, we observed increased phase coherence between the left parahippocampus and left auditory cortex for the tinnitus patients with more severe hearing loss, while decreased phase coherence between the pregenual anterior cingulate and the left parahippocampus.

left PHC ($r = -.51, p < .001$) for the tinnitus patients, including both Met carriers and the Val/Val genotype (see Fig. 8). We found a significant negative correlation for both connections, indicating that weaker pgACC and left AUD left and the pgACC and left PHC connectivity both correlate with increased tinnitus loudness. For the Met carriers, we found a negative correlation for pgACC and the

left PHC connectivity strength and loudness ($r = -.55, p < .001$), but not for pgACC and left AUD connectivity strength and loudness ($r = -.23, p = .11$). For the Val genotype, we did not find a significant correlation between pgACC and left AUD ($r = -.38, p = .12$) or pgACC and left PHC ($r = -.37, p = .13$) with loudness (see Fig. 8). These effects remained after correction for multiple comparisons.

3.5. Effective connectivity: granger causality

A repeated-measures ANOVA was conducted with group (Met carriers vs. Val/Val genotype) as the between-subjects variable and the effective connectivity (pgACC → left AUD vs. left AUD → pgACC) for the theta frequency band as the within-subjects variable for the tinnitus patients. This analysis revealed no significant main effects for group ($F = 1.57, p = .22$) or for effective connectivity ($F = 1.64, p = .21$). However, a significant interaction was observed between group x effective connectivity ($F = 4.14, p = .049$). A simple contrast shows that there was a significant difference ($F = 6.55, p = .015$) between Met carriers ($M = .016, Sd = .012$) in comparison to the Val/Val genotype ($M = .032, Sd = .029$) for the connection from the pgACC → left AUD. For the connection from the left AUD → pgACC no significant difference was observed between the Val/Val genotype and the Met carriers ($F = .026, p = .87$) (see Fig. 9).

A similar analysis for the effective connectivity (pgACC → left PHC vs. left PHC → pgACC) for the theta frequency band revealed no significant main effects for group ($F = 1.33, p = .26$) or effective connectivity ($F = .10, p = .75$). A significant interaction effect was observed between group x effective connectivity ($F = 4.50, p = .040$). A simple contrast shows that there was a significant difference ($F = 10.65, p = .002$) between Met carriers ($M = .005, Sd = .006$) in comparison to the Val/Val genotype ($M = .017, Sd = .017$) for the left pgACC → PHC connection. For the left

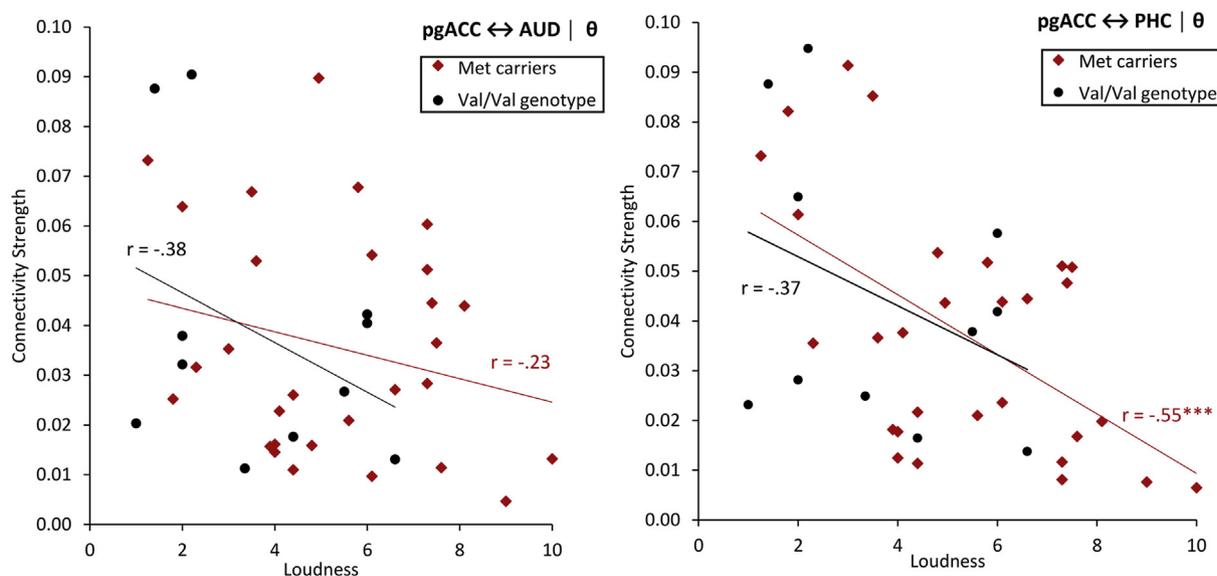


Fig. 8. Correlation between the loudness and connectivity strength between the pregenual anterior cingulate cortex and both the left auditory cortex and left parahippocampus (for the tinnitus patients that are Met carriers and Val/Val genotype for the theta frequency band. (* $p < .05$; ** $p < .01$; *** $p < .001$).

PHC→pgACC connection, no significant difference was observed between the Val/Val genotype and the Met carriers ($F = .39$, $p = .54$) (see Fig. 9).

To further explore the effect, we also compared Met carriers with tinnitus in comparison to healthy subjects that are Met carriers. A repeated-measures ANOVA with group (tinnitus vs. healthy subjects) as the between-subjects variable and effective connectivity (pgACC→left AUD vs. left AUD→pgACC) for the theta frequency band as within-subjects' variable for Met carriers revealed no significant effects for main effects for group ($F = .92$, $p = .34$), effective connectivity ($F = .16$, $p = .77$) or the interaction effect. ($F = 1.66$, $p = .21$). However, a similar analysis conducted for the effective connectivity (pgACC→left AUD vs. left AUD→pgACC) revealed a significant interaction effect between group x effective connectivity ($F = 4.90$, $p = .033$). A simple contrast shows that there was a significant difference ($F = 9.84$, $p = .003$) between tinnitus patients ($M = .005$, $Sd = .006$) in comparison to healthy subjects ($M = .01$, $Sd = .013$) for the connection pgACC→PHC. A simple contrast comparing the connection left PHC→pgACC revealed a significant difference ($F = 14.39$, $p = .001$) between tinnitus patients ($M = .014$, $Sd = .02$) in comparison to healthy subjects ($M = .005$, $Sd = .004$). No significant effect was observed for the main effects for group ($F = .64$, $p = .43$) or effective connectivity ($F = .66$, $p = .42$). See Fig. 10 for overview.

For the tinnitus participants, Pearson correlations between the tinnitus loudness and respectively the effective connectivity for pgACC→left AUD ($r = -.14$, $p = .20$) as well as for pgACC→left PHC ($r = -.14$, $p = .20$) for theta frequency band revealed no significant effects. A similar effect for tinnitus loudness and respectively the effective connectivity for left AUD→pgACC ($r = .34$, $p = .016$) as well as from the left PHC→pgACC ($r = .34$, $p = .016$) the theta frequency band revealed a significant positive correlation. These latter findings suggest that the stronger the connection from the AUD or PHC →pgACC, the louder tinnitus patients perceive their tinnitus. For the Met carriers, we found a negative significant correlation between the tinnitus loudness and respectively the effective connectivity from the pgACC→left AUD ($r = -.34$, $p = .035$) as well as for the pgACC→left PHC ($r = -.44$, $p = .009$) for theta frequency band. These findings suggest that the louder tinnitus was perceived the weaker the connections was from the pgACC→left AUD or the

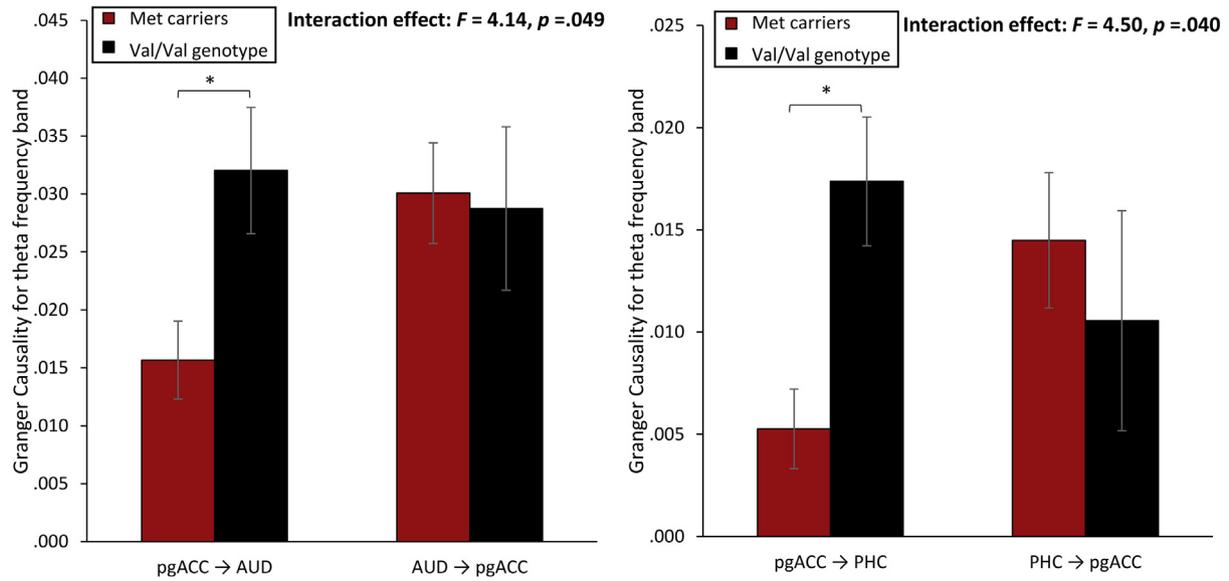
pgACC→left PHC. In addition, we found a positive correlation for the Met carriers for the effective connectivity from the AUD→pgACC ($r = .40$, $p = .017$) as well as for the left PHC→pgACC ($r = .45$, $p = .008$) for theta frequency band. These latter findings suggest that the louder tinnitus was perceived the stronger the connections was from the left AUD or PHC→pgACC. A similar analysis for the Val/Val genotype revealed no significant effects for loudness and respectively the pgACC→left AUD ($r = .40$, $p = .12$), the pgACC→left PHC ($r = .52$, $p = .051$), the left AUD→pgACC ($r = .24$, $p = .24$), and the left PHC→pgACC ($r = -.17$, $p = .31$) for theta frequency band. These effects remained after correction for multiple comparison. See Fig. 11 for an overview.

To further explore the effect of hearing loss, we looked at the balance between left AUD→pgACC, and the pgACC→left AUD as well as at the balance between left PHC→pgACC, and the pgACC→left PHC. A significant negative correlation between the balance for the left AUD and the loudness ($r = -.44$, $p = .016$) was observed. A similar analysis between the balance for the left PHC and the loudness ($r = -.81$, $p < .001$) showed a significant negative correlation. A comparison between the two correlations showed a significant stronger correlation for the balance between the left PHC and loudness in comparison to the balance for the left AUD and loudness ($t = -2.78$, $p = .006$). See Fig. 11 for an overview.

4. Discussion

The current study aimed to understand the influence of the COMT polymorphism and the neurogenetic architecture of hearing loss induced tinnitus. Our data identify that Met carriers in combination with severe hearing loss are a good predictor for the loudness of the tinnitus percept. This is mainly driven by increased activity in the pgACC and decreased pgACC–PHC functional connectivity in Met carriers. Furthermore, the connectivity is increased from the left PHC to the pgACC, while decreased from the pgACC to the left PHC in tinnitus patients who are Met carriers with severe hearing loss. The result indicates that the pgACC, which has been associated with sensory gating in tinnitus, is mediating the percept loudness (see Fig. 12). To our knowledge, this is the first study investigating the clinical and associated electrophysiological effects, both in activity and connectivity, of COMT Val¹⁵⁸Met

Tinnitus



Met carriers

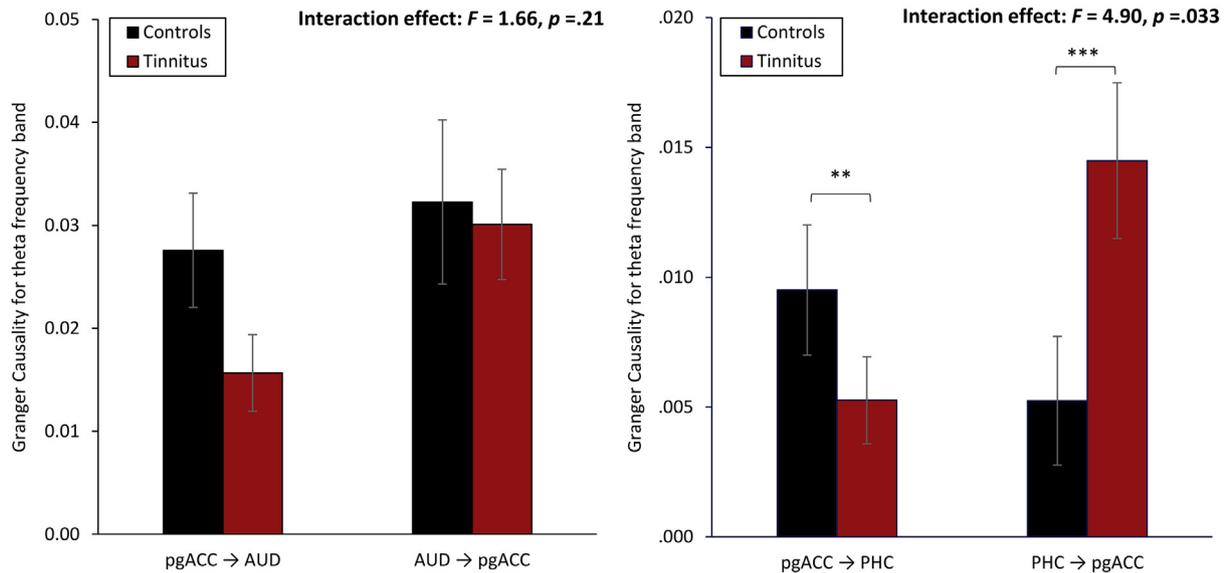


Fig. 9. The effect of Met carriers vs. Val/Val genotype tinnitus patients on the effective connectivity (from pgACC to AUD vs. from AUD to pgACC) for the theta frequency band revealed a significant interaction showing that Met carriers in comparison to Val/Val genotype showed a reduced connectivity from the pregenual anterior cingulate cortex to the auditory cortex. For the connection from the auditory cortex to the pregenual anterior cingulate cortex no significant difference was observed between the Val/Val genotype and the Met carriers. A similar analysis was conducted for the effective connectivity (from pgACC to PHC vs. from PHC to pgACC) for the theta frequency band in tinnitus patients revealed a significant interaction effect showing that there was a significant difference for the connection from the pregenual anterior cingulate cortex to the parahippocampus for Met carriers. For the connection from the parahippocampus to the pregenual anterior cingulate cortex, no significant difference was observed between the Val/Val genotype and the Met carriers. A comparison between Met carriers with tinnitus in comparison to healthy subjects for the theta frequency band as within-subjects' variable for Met carriers revealed no significant effects. A similar analysis conducted for the effective connectivity (from pgACC to PHC vs. from pgACC to PHC) revealed a significant interaction effect revealing a significant difference for the connection from the pregenual anterior cingulate cortex to the parahippocampus as well as connection from the parahippocampus to pregenual anterior cingulate cortex.

polymorphism in tinnitus.

A comparison between tinnitus and controls subjects showed increased activity in the left AUD and PHC for the gamma frequency band. The link between gamma band activity in the AUD and tinnitus has been identified using both EEG (van der Loo et al., 2009) and MEG (Weisz et al., 2005, 2007). This is supported by a positive correlation found in the present study between the tinnitus loudness and the activity in left AUD for the gamma frequency, confirming previous results (De Ridder et al., 2015; van der

Loo et al., 2009). Further research showed however that the tinnitus percept might have a different generating mechanism depending on the amount of hearing loss (Vanneste et al., 2016). That is, the PHC, which is involved in auditory memory, becomes involved in tinnitus with more severe hearing loss (De Ridder et al., 2006, 2011; Engelen et al., 2000). This is in accordance with the recently proposed Bayesian model for tinnitus that describes a putative multiphase compensation mechanism linking auditory deafferentation to tinnitus (De Ridder et al., 2014a; Vanneste et al.,

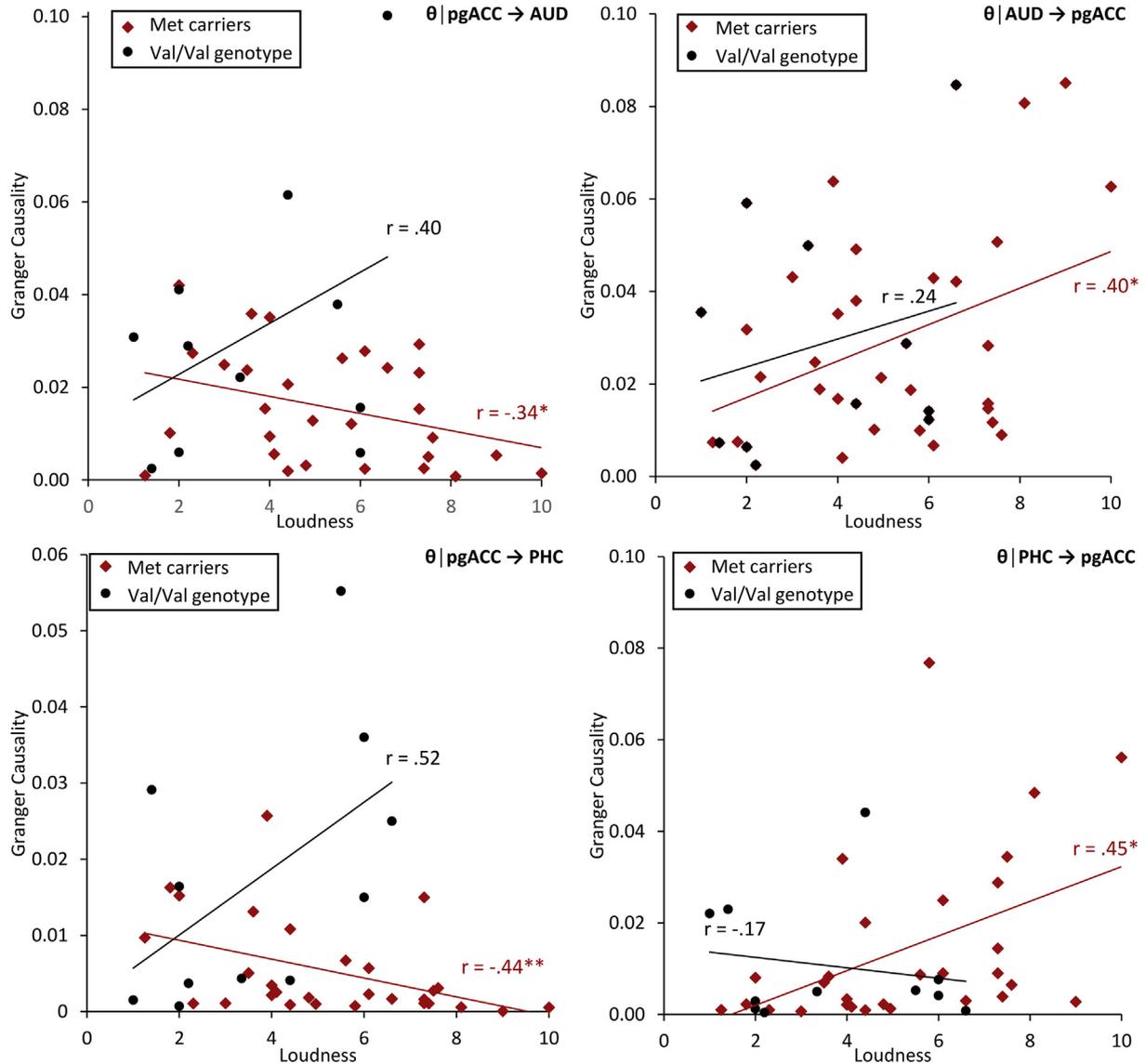


Fig. 10. A Pearson correlation between the tinnitus loudness and respectively the effective connectivity from the pregenual anterior cingulate cortex to the auditory cortex, Auditory cortex to pregenual anterior cingulate cortex, the pregenual anterior cingulate cortex to the parahippocampus as well as the parahippocampus to pregenual anterior cingulate cortex for theta frequency band revealed for both Met and Val/Val genotype. (* $p < .05$; ** $p < .01$; *** $p < .001$).

2016). Our data support this hypothesis in tinnitus patients with severe hearing loss by showing increased gamma activity in the PHC that correlates with tinnitus loudness (De Ridder et al., 2015).

Previous studies on the effect of COMT polymorphism have suggested the importance of PFC dopamine to sensory gating. Recent research shows that healthy subjects who are Met carriers have poorer gating compared to the Val/Val genotype (Majic et al., 2011). The poorer gating in Met carriers corroborates our findings. Tinnitus patients perceive their tinnitus as louder, which might be related to a poorer pgACC-associated gating mechanism. Indeed, Met carriers exhibit increased activity in the pgACC for the theta frequency band, and the COMT polymorphism is known to be a crucial component for determining pgACC activity (Larisch et al., 1999). However, it is also possible that these changes in Met carriers are due increased hearing loss. Furthermore, both the AUD and PHC send and receive input to and from other cortical areas including the pgACC (Drabant et al., 2006). This is in line with our findings demonstrating increased input from both the AUD and PHC to the pgACC in tinnitus patients who are Met carriers. In

addition, we find a strong association between activity in the AUD and PHC \rightarrow pgACC with the level of tinnitus loudness. It has already been suggested that the pgACC in Met carriers should be able to process a high amount of input from sensory cortices (i.e. AUD and PHC) due to the increased dopaminergic neurotransmission (Majic et al., 2011). This could explain why we see increased activity in the pgACC for the theta frequency band and fits with the idea that the pgACC may open the sensory gate filter to utilize the high processing capacity for sensory input in cases of high PFC dopamine flux driven by COMT genetic variants (Majic et al., 2011). Another possible explanation could be that there is increased input of auditory information in the dACC and that tinnitus is the result of an imbalance between tinnitus-provoking activity in the dACC and tinnitus-suppressing activity in the pgACC, analogous to what has been described for pain in fibromyalgia (De Ridder et al., 2017). The rationale behind this idea is that the pgACC attempts to counter-balance the increased tinnitus-provoking input by increasing its suppression, but not enough to eliminate the imbalance.

At the same time, we see decreased connectivity from the pgACC

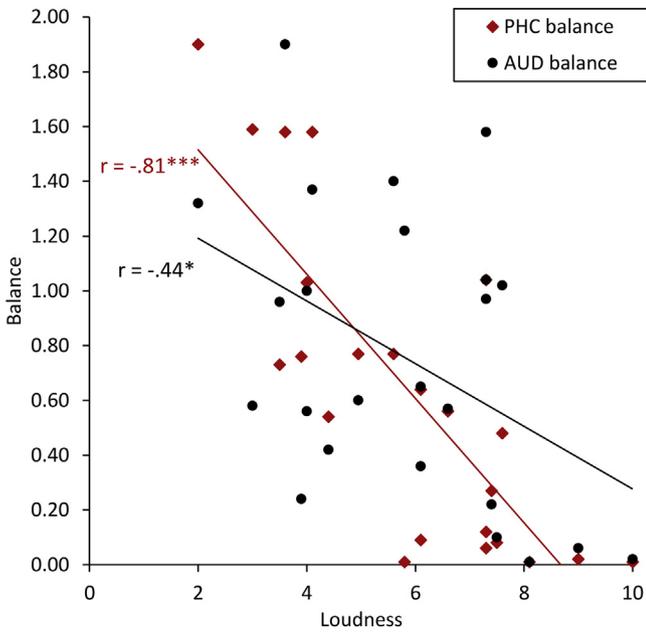


Fig. 11. The balance between auditory cortex to the pregenual anterior cingulate cortex and the pregenual anterior cingulate cortex to the auditory cortex as well as at the balance between parahippocampus to the pregenual anterior cingulate cortex, and the pregenual anterior cingulate cortex to the parahippocampus for the theta frequency band in tinnitus patients with severe hearing loss. (* $p < .05$; ** $p < .01$; *** $p < .001$).

to both the AUD and PHC in Met-carrier tinnitus individuals, which would be in keeping with the second proposal, i.e. that the pgACC is deficient in its tinnitus-suppressive effect. This is further supported by the fact that Met carriers demonstrate a negative correlation between the putative inhibitory information going from the pgACC to both the left AUD and the left PHC, in other words, the weaker the connectivity from pgACC to the AUD and PHC, the louder patients perceive their tinnitus. Mechanistically, COMT has been shown to play an important role in top-down modulation targeting the AUD and PHC (Gallinat et al., 2002; Winterer et al., 2006). Met carriers have decreased coupling between the anterior pgACC and PHC in comparison to the Val/Val genotype (Meyer et al., 2016; Tian et al., 2013; Tunbridge et al., 2013), which fits with the fact that the medial PFC and PHC are the regions identified as having the largest effect of COMT as demonstrated by fMRI (Drabant et al., 2006) and are known to show the most abundant expressions of COMT (Chen et al., 2004; Masuda et al., 2003; Matsumoto et al., 2003). This fits with the ‘noise-cancellation system’ hypothesis (Rauschecker et al., 2010, 2015). Due to a deficiency in the pgACC, the thalamic reticular nucleus is hypothesized not to inhibit the highly selective and frequency-specific information transmission from the auditory thalamus to the AUD (Rauschecker et al., 2015; Yu et al., 2009).

Tinnitus Met carriers with severe hearing loss perceive their tinnitus as being louder, which is associated with increased activity in the PHC, increased PHC→pgACC connectivity, as well as decreased pgACC→PHC connectivity. This indicates that Met carriers with severe hearing loss have increased tinnitus input as well as decreased tinnitus inhibition, as mentioned before. Previous studies have reported pgACC-mediated inhibition deficiencies in aggression (Buckholtz et al., 2008a), proneness to vertigo (Alsaman et al., 2016), and fibromyalgia (Jensen et al., 2013). That is, the pgACC sends an inhibitory projection to several other areas including the amygdala and PHC and thus might have a general stimulus-inhibition function (Buckholtz et al., 2008b; Vogt et al., 2001). This has been linked to negative feedback function in a Bayesian framework (De Ridder et al., 2016).

In tinnitus with severe hearing loss, we see the imbalance explained by the connection with the PHC rather with the AUD. Notably, several studies report an effect of COMT Val¹⁵⁸Met on the PHC as well as its connectivity to other brain areas (Bertolino et al., 2006; Meyer et al., 2016; Zhang et al., 2015). This further fits with the central concept of the Bayesian brain model for tinnitus that predicts that patients with severe hearing loss increasingly recruit auditory memory-related areas to fill in the missing information when local auditory cortical map plasticity cannot recruit it from the local cortical neighborhood (De Ridder et al., 2014a; Vanneste et al., 2016). This fits with the finding that in tinnitus with more severe hearing loss, the PHC becomes more involved as a tinnitus-generating mechanism, whereas in tinnitus without audiometric hearing loss the AUD should be more involved (Vanneste et al., 2016). The present study adds to this mechanistic explanation by identifying that severe hearing loss in combination with being a Met carrier makes the tinnitus percept louder due to a deficit in the pgACC-driven cancellation mechanism.

It is important to acknowledge a second COMT (COMT2), which is related to COMT1 that regulates dopamine levels in the brain, is widely expressed in inner and outer hair cells of the cochlea. A mutation to a COMT2 shows defects in cochlear function in both mice and humans that are related to hearing loss or deafness phenotype (Du et al., 2008). Hence, it is possible that COMT can also be attributed to a peripheral component that affects hearing and tinnitus. Due to this association, it is possible that COMT could have an impact on tinnitus due to a hearing impairment that is not measurable with a pure tone audiogram (i.e. “hidden hearing loss”) but that might contribute to our findings.

Furthermore, research suggests that the COMT Val¹⁵⁸Met polymorphism influences the human experience of pain and may underlie interindividual differences in the adaptation and responses to pain and other stressful stimuli (Zubieta et al., 2003). Previous research also revealed the existence of an alternative neuronal pathway from the cochlea to the brainstem that is activated by tissue-damaging noise, possibly by type-II cochlear afferents, representing auditory nociception (Flores et al., 2015). As Met carriers

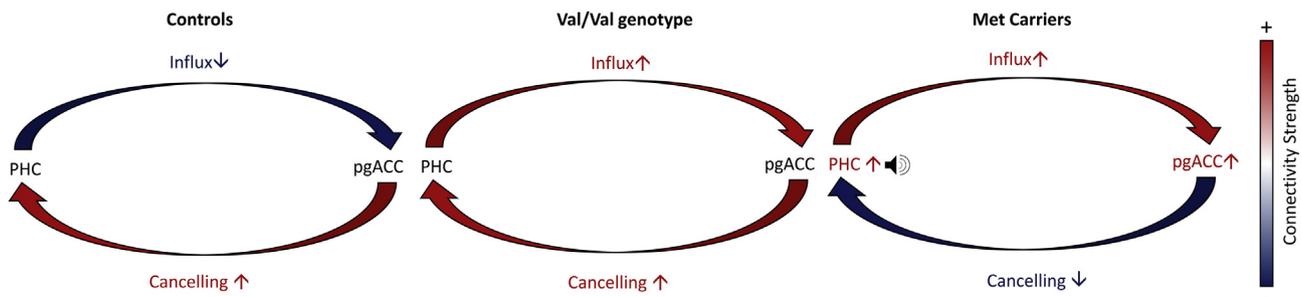


Fig. 12. Summary figure of the observed results.

are more sensitive to pain, it might be that these subjects respond in a different way to noise induced tinnitus in comparison to Val/Val homozygotes.

Progress in finding a treatment for tinnitus has been hampered by the fact that tinnitus represents a highly heterogeneous condition (Scheckmann 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 COMT 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. It has already been shown, for example, that the effect of tDCS on auditory hallucinations—which can be considered a complex form of tinnitus, analogous to hallucinosis (Vanneste et al., 2013)—depends on the COMT polymorphism (Wiegand et al., 2016).

Although the control group and tinnitus group do not significantly differ based on age, gender or hearing loss, a limitation of this study is that the control group is only group-matched, and not individually matched. Furthermore, we only tested hearing acuity in tinnitus patients via standard pure tone audiometry, i.e. limited to 8000 Hz. Recent research has shown that tinnitus can occur in relationship with hearing loss at supra-clinical frequencies (i.e. above 8000 Hz) (Melcher et al., 2013). Future research should also include a high-frequency audiogram as well as audiometric data for healthy subjects. Another limitation is that we did not use a MRI to map the source. In theory, there is no problem in doing EEG source reconstruction, but the way it is computed, is more convoluted and opens up for more potential errors. The major difference is that to construct a lead field for EEG, a volume conductor model that models several compartments in the head—the inner skull, the skull, and the outer skin—is needed. Three layers means more room for errors. However, the areas obtained in this study were already confirmed using structural and functional MRI (Leaver et al., 2011) as well intracranial EEG recordings (electrocorticography, ECoG) (Sedley et al., 2015). In addition, we merged the Val/Met carriers and Met/Met carriers due to a low sample size of Met/Met carriers. Further research in a larger sample could also look at the difference between Val/Met and Met/Met carriers. It is possible that there is a genetic dosage or gradual effect. That is, an effect in the phenotype observed in the heterozygous genotype (Val/Met) that becomes more pronounced in the homozygous (Met/Met).

In conclusion, our results show that the interaction between the level of hearing loss and the COMT Val¹⁵⁸Met polymorphism can increase the susceptibility to the clinical manifestation of tinnitus. We demonstrated that the PHC becomes involved in tinnitus in patients with hearing loss that are Met carriers. The PHC sends more tinnitus related information to the pgACC and AUD that is related with increased loudness perception, but at the same time the pgACC, which normally functions as a gatekeeper, is not cancelling this auditory information, leading to increased tinnitus loudness.

Conflicts of interest

None.

Significance statement

Permanently affecting one in seven adults, chronic tinnitus lacks both widely effective treatments and adequate understanding of its brain mechanisms. This is largely due to the fact that tinnitus represents a highly heterogeneous condition. Consistent with this idea, our research shows that tinnitus indeed has different subtypes related to the underlying neurogenetic architecture of hearing loss

induced tinnitus. We establish, in a human study tightly controlled for hearing loss, that the amount of hearing loss and the COMT Val¹⁵⁸Met polymorphism can increase the susceptibility to the clinical manifestation of tinnitus that goes together with not cancelling auditory information, leading to increased tinnitus loudness.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.heares.2018.05.020>.

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