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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 Val^{158}Met 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.

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 Val^{158}Met 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 form 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, presbyacusis, 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 no 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 ± 14.19; males: 28; females: 12) and 20 healthy controls (age: 45.60 years ± 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 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. 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/μl 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 kΩ. 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