Aus dem Tinnituszentrum der Klinik für Hals-Nasen-Ohrenheilkunde und der Medizinischen Klinik mit Schwerpunkt Psychosomatik der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Comorbidities and stress-related hair biomarkers in bothersome tinnitus

Komorbiditäten und stressbezogene Haar-Biomarker bei belastendem Tinnitus

zur Erlangung des akademischen Grades
Doctor of Philosophy (PhD)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

Laura Basso
aus Bern, Schweiz

Datum der Promotion: 30.11.2023

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

ARs	Adjusted Residuals
BDNF	Brain-Derived Neurotrophic Factor
BL	Baseline
BT	Bothersome Tinnitus
CBT	Cognitive Behavioral Therapy
CI	Confidence Interval
ELISA	Enzyme-Linked Immunosorbent Assay
FU	Follow-up
HADS	Hospital Anxiety and Depression Scale
HPA	Hypothalamus–Pituitary–Adrenal Axis
MCS	Mental Component Summary
Non-BT	Non-Bothersome Tinnitus
OR	Odds Ratio
PBS	Phosphate-Buffered Saline
PCS	Physical Component Summary
PDS	Posttraumatic Diagnostic Scale
PSQ-20	Perceived Stress Questionnaire (20 item version)
PTA	Pure-Tone Audiometry
REML	Restricted Maximum Likelihood
RMSE	Root Mean Square Error
SF-12	Short Form-12 Health Survey
SOMS	Screening of Somatoform Disorders
STAI	State-Trait Anxiety Inventory
TE	Treatment End
TQ	Tinnitus Questionnaire
VI	Variable Importance

ABSTRACT

Stress-related psychological and physiological processes might be important for the manifestation and/or maintenance of bothersome tinnitus (BT). This thesis aims to 1) investigate the influence of stress-related mental comorbidities on BT and their mediation effects for hearing-related factors and physical comorbidities[1], 2) explore associations of tinnitus-related and psychological factors with two stress-related biological markers, namely cortisol and brain-derived neurotrophic factor (BDNF) measured in hair[2], and 3) explore the potential of these hair-biomarkers as therapeutic efficacy markers in BT[3].

For the first research aim, existing survey data from the Swedish general population were analyzed[1]. In the subsample of individuals with self-reported tinnitus (N=7615), logistic regression analyses were used to identify mental and physical comorbidities as well as hearing-related factors associated with BT (N=697), and logistic mediation analyses were used to identify dependencies between these influencing factors[1]. Results showed a) specific hearing-related, physical, and mental influences on BT as well as mediating effects of b) hearing-related factors on the influence of mental comorbidities on BT, and c) mental comorbidities on the influences of physical comorbidities and hearing-related factors on BT[1]. This suggests that mental symptoms and hearing-related factors are important treatment targets due to their direct and indirect effects on BT[1].

For the other two research aims, a longitudinal study was conducted with 91 chronic tinnitus inpatients who were assessed before, directly after, and three months after a multimodal tinnitus-specific cognitive-behavioral therapy program[2,3]. Data collection included audiometry, psychometric questionnaires, and hair sampling[2,3]. Results showed a) a negative effect of tinnitus-related distress on hair-BDNF levels, b) a positive effect of tinnitus loudness on hair-cortisol levels, and c) a negative effect of tinnitus loudness on hair-BDNF levels[2]. In addition, d) decreases in tinnitus-related distress and perceived stress levels after treatment were present but no changes in hair-cortisol or hair-BDNF levels[3]. The effects of tinnitus loudness were surprising, may have been influenced by imputation, and thus require further research[2]. Yet the results tentatively suggest that tinnitus-related distress may negatively affect hair-BDNF levels[2]. However, the magnitude of the observed treatment-related decrease in tinnitus-related distress may have been too limited to induce changes in hair-BDNF levels[3]. Further studies investigating changes in hair-BDNF levels after longer-lasting interventions with systematic control of medical confounders are recommended[3].

Overall, the results suggest that the interconnectedness between stress-related mental symptoms, hearing-related factors, and other physical symptoms as well as stress-related changes in BDNF levels may play important roles in BT.

ZUSAMMENFASSUNG

Stressbedingte mentale und physiologische Prozesse könnten für die Manifestation und/oder Aufrechterhaltung von belastendem Tinnitus (BT) bedeutsam sein. Diese Arbeit untersucht 1) den Einfluss von stressbezogenen mentalen Komorbiditäten auf BT und deren Mediationseffekte für hörbezogene Faktoren und physische Komorbiditäten[1], 2) Assoziationen von tinnitusbezogenen und psychologischen Faktoren mit zwei im Haar gemessenen stressbezogenen Biomarkern, nämlich Cortisol und Brain-Derived Neurotrophic Factor (BDNF)[2], und 3) deren Potenzial als therapeutische Wirksamkeitsmarker bei BT[3].

Für das erste Forschungsziel wurden bestehende Umfragedaten aus der schwedischen Allgemeinbevölkerung analysiert[1]. In der Teilstichprobe von Personen mit selbstberichtetem Tinnitus (N=7615) wurden logistische Regressionsanalysen verwendet, um mentale und physische Komorbiditäten sowie hörbezogene Faktoren zu identifizieren, die mit BT (N=697) assoziiert sind, sowie logistische Mediationsanalysen, um Abhängigkeiten zwischen diesen Einflussfaktoren zu ermitteln[1]. Die Ergebnisse zeigten a) spezifische hörbezogene, physische und mentale Einflüsse auf BT sowie Mediationseffekte von b) hörbezogenen Faktoren auf den Einfluss mentaler Komorbiditäten auf BT und c) mentalen Komorbiditäten auf die Einflüsse physischer Komorbiditäten und hörbezogener Faktoren auf BT[1]. Dies legt nahe, dass mentale Symptome und hörbezogene Faktoren aufgrund ihrer direkten und indirekten Auswirkungen auf BT wichtige Behandlungsziele darstellen[1].

Für die zwei weiteren Forschungsziele wurde eine Längsschnittstudie mit 91 stationären chronischen Tinnituspatienten/-innen durchgeführt, die vor, direkt und drei Monate nach einem multimodalen tinnitusspezifischen kognitiven Verhaltenstherapieprogramm mittels Audiometrie, psychometrischen Fragebögen und Haarproben untersucht wurden[2,3]. Die Ergebnisse zeigten Effekte a) der Tinnitusbelastung auf Haar-BDNF (negativ) sowie b) der Tinnituslautstärke auf Haar-Cortisol (positiv) und c) Haar-BDNF (negativ)[2]. Zudem zeigte sich d) eine Abnahme der Tinnitusbelastung und des Stresserlebens nach der Behandlung, aber keine Veränderungen der Haar-Biomarker-Werte[3]. Die Effekte der Tinnituslautstärke waren überraschend, könnten durch Imputation beeinflusst worden sein und erfordern daher weitere Forschung[2]. Die Ergebnisse legen jedoch nahe, dass sich die Tinnitusbelastung negativ auf den Haar-BDNF-Spiegel auswirken könnte[2]. Das Ausmaß des behandlungsbedingten Rückgangs der Tinnitusbelastung könnte aber zu gering gewesen sein, um Veränderungen im Haar-BDNF-Spiegel zu bewirken[3]. Weitere Studien zu Veränderungen der Haar-BDNF-Werte nach länger andauernden Interventionen mit systematischer Kontrolle medizinischer Störfaktoren werden empfohlen[3].

Insgesamt legen die Ergebnisse nahe, dass die Verflechtung von stressbezogenen mentalen, hörbezogenen und anderen physischen Symptomen sowie stressbezogene Veränderungen des Haar-BDNF-Spiegels eine wichtige Rolle bei BT spielen könnten.

1. INTRODUCTION

1.1. Tinnitus and bothersome tinnitus

Tinnitus is an auditory phantom perception without a corresponding external acoustic stimulus[4–6]. Tinnitus is heterogeneous with regard to its sound characteristics, associated conditions, and pathological causes[4–6]. An important distinction is the psychological impact of tinnitus, i.e., whether the tinnitus sound is associated with distress[4–6]. A recent proposition in the field states that tinnitus should be considered a disorder "*when associated with emotional distress, cognitive dysfunction, and/or autonomic arousal, leading to behavioural changes and functional disability*"[6(p8)]. This thesis is focused on individuals to whom the latter applies, but the term bothersome tinnitus (BT) is used. Emotional and cognitive distress associated with BT can include depressed mood (frustration, despair), anxiety symptoms (fear, worry), sleep disturbance, irritation/annoyance, and concentration difficulties or other cognitive problems[5,7,8].

Epidemiological studies found 12-month prevalence rates of any tinnitus (lasting for more than five minutes) ranging between 6%-15%[9] indicating that it affects a substantial part of the population. Prevalence estimates of BT vary strongly between studies[10], likely due to methodological differences. In this regard, it is important to distinguish between the prevalence of BT in the population and among individuals with tinnitus, as they cannot be directly compared. In the adult population, the prevalence rate of BT has been reported to be 1.2%[11] or 1.3%[12]; and 3% in the elderly population[13]. Regarding the rate of BT in tinnitus sufferers, a study based on the Korea National Health and Nutrition Examination Survey showed that while 69.2% of affected individuals reported no discomfort, 27.9% reported moderate, and 3% severe tinnitus annoyance[1,14]. Similarly, a nationally representative study from the United States found that while 31% of affected individuals were not bothered by it, 41.6% perceived tinnitus as a small, 20.2% as a moderate, and 7.2% as a big or a very big problem[15]. In sum, these studies indicate that tinnitus is accompanied by moderate or severe distress in around one-third of affected individuals.

1.2. Causes and risk factors

The pathophysiology of tinnitus is complex and multifactorial[4,16–18]. Tinnitus generation seems to result from "*a complex interaction between peripheral and central mechanisms within the auditory pathway*"[4(p4)]. In addition to auditory structures, nonauditory brain

networks are involved in the conscious perception of tinnitus, its maintenance, and associated distress[4,17,19–22]. Tinnitus can be caused by auditory deprivation, most commonly due to sensorineural hearing loss (age-related or noise-induced), and by pathologies affecting the auditory nerve, such as vestibular schwannomas[17,18,23]. Other factors that can lead to tinnitus include head injury/trauma[16,18,24], infections/inflammation in different parts of the ear[16,18,24,25], and ototoxic and other medications[16,26–28]. Tinnitus is also a symptom of Meniere's disease[16,18,24,29]. Furthermore, in a subtype of tinnitus, its perception can be generated or influenced by the somatosensory system[30,31].

The most clearly identified risk factor for tinnitus is hearing loss[26,27], and some studies indicate relationships with lifestyle factors, yet not conclusively[9]. Tinnitus prevalence seems to increase with age, while no clear association with sex has been identified[9]. For BT, sociodemographic factors such as age and education[11,14,15,32,33], physical symptoms such as hearing loss and somatic complaints[14,32–37], and mental/emotional factors[14,32,34–38] appear to influence the level of tinnitus-related distress.

1.3. Bothersome tinnitus and comorbidities

Different physical and mental conditions can constitute risk factors or relevant associated comorbidities for tinnitus[1,12–14,26,39]. For BT, mental comorbidities appear to be of primary importance, with anxiety[40] and depression[41–43] being the most common[1]. The lifetime prevalence of anxiety disorders in tinnitus at 45% seems to be markedly higher than in the general population[40]. For comorbid depression in tinnitus, a median prevalence rate of 33% was observed across 28 studies, which is also increased compared to the general population[43]. Different associations between mental conditions and BT are possible. Comorbid anxiety and depression can increase tinnitus severity[40,41,44] and tinnitus may predispose to anxiety and depression[40,42,44,45]. There is a known overlap in the involved brain networks between the conditions[40,42], and a shared (stress-related) vulnerability for anxiety, depression, and BT might exist[40,42,44].

While the presence of mental comorbidities in BT has long been known, the presence of physical comorbidities has been less frequently studied, except for the association between hearing loss and BT[14,34,36,46,47]. Other observations include associations of hypertension and coronary heart disease[37], a history of cardiovascular disease[47,48] or hyperlipidemia[14] with BT. More broadly, somatization tendencies and somatic complaints have also been frequently associated with higher tinnitus-related distress[32,33,35,36]. Overall,

hearing impairment and comorbid mental and other physical symptoms may influence tinnitus severity[1]. As these factors may be interacting and mutually reinforcing[37], research on their interplay could advance the understanding of BT.

1.4. Treatment: cognitive-behavioral therapy

While tinnitus cannot currently be eliminated by treatment, tinnitus-related distress and associated impairment in quality of life can be effectively improved by cognitive behavioral therapy (CBT)[3,49–51]. CBT is a psychotherapeutic approach with demonstrated high efficacy for improving various psychological problems[3,52]. CBT for tinnitus[50] is focused on addressing maladaptive tinnitus-related emotional reactions like tinnitus-related fear[53–55], cognitions like dysfunctional beliefs, catastrophizing interpretations, and worry[53,56,57], and behavior like maladaptive coping strategies[58–60], which can negatively affect the quality of life of the affected individuals.

While evidence-based recommendations for CBT are strongest, a multidisciplinary CBT-based approach that includes audiological diagnostics, education, and counseling is advisable for clinical care[49,61]. A multimodal treatment approach for chronic tinnitus with audiological and psychological elements of the Tinnitus Center of *Charité – Universitätsmedizin Berlin* was found to show positive effects on tinnitus-related distress and comorbid mental symptoms[3,62–65]. The most current version of this treatment is a compact multimodal treatment (usually lasting five days) based on tinnitus-specific CBT[3]. It includes audiological elements, namely ENT diagnostics, ENT education, and auditory attention training; psychological elements, namely psychological education, counseling, tinnitus-specific CBT (individual and group) sessions, and relaxation exercises; and somatic elements, namely internal medicine diagnostics and physiotherapeutic sessions[3]. This treatment approach thus attempts to counteract tinnitus-related distress and associated impairments via multiple pathways.

1.5. Bothersome tinnitus and stress

Stress is a complex and multidimensional construct[66]. Different conceptualizations of stress locate it in environmental stimuli, in their psychological appraisal, or in associated responses[67]. Here, the focus is on psychological and physiological stress responses and their possible links to BT.

Physiologically, stimuli that are appraised as stressful/threatening lead to the activation of the sympathetic–adrenal–medullary (SAM) system and the hypothalamic–pituitary–adrenal (HPA) axis and affect the metabolic and immune system[67,68]. These physiological changes are an adaptive response to environmental demands[67–70]. However, repeated or prolonged stressful experiences can result in dysregulation of the physiological stress response[67–70]. In the long-term, chronic stress levels can lead to harmful physiological imbalances, e.g., increased levels of glucocorticoids, elevated blood pressure, suppression of the thyroid axis and immune function, and neuroplasticity changes, increasing the risk for various diseases[67–71]. Stress-induced physiological processes may also play a pathophysiological role in the development of tinnitus[72–74]. Potential mechanisms might include the involvement of the HPA axis, SAM axis, and immune system in the generation of tinnitus via alterations in the auditory system, but these mechanisms are not clearly understood and require further evidence[72].

From a psychological perspective, it is important to note that subjectively experienced stress levels are associated with tinnitus severity[2,73–76]. Currently experienced stress levels were found to partially mediate the relationship between tinnitus loudness and tinnitus-related distress (in addition to mediating effects of emotional valence and arousal)[2,77]. Moreover, chronic stress can lead to emotional exhaustion and it has been observed that emotional exhaustion explains the relationship between hearing loss and tinnitus severity[2,76,78]. These and other findings[14,47,79,80] highlight the importance of psychological stress responses for BT. In addition, vulnerability-stress interactions also appear important, as tinnitus-related distress likely results from interactions between pre-existing psychological vulnerability and stressful experiences[83,84]. Furthermore, stress experiences and associated physiological alterations are known to be involved in the development of depression and anxiety disorders[2,69,81,82]. Potentially, comorbid depression or anxiety, emotional stress, and tinnitus severity can mutually reinforce each other and establish a vicious circle[76].

1.6. Stress-related biomarkers in tinnitus/bothersome tinnitus

Cortisol is the main stress hormone in humans, secreted by the adrenal glands as part of the HPA-axis stress response[67,71]. The HPA axis has an integrated inhibitory feedback loop, by which high cortisol levels normally downregulate HPA axis activity and terminate the stress response[67,71]. Chronic stress can result in hyperactivity or hypoactivity of the

HPA axis[67,71]. In depression, hyperactivity has been observed with increased cortisol levels and impaired feedback regulation of the HPA axis[82,85]. Similarly, anxiety disorders also appear to be characterized by HPA axis hyperactivity[86].

Some studies also report HPA axis dysregulation in tinnitus patients (see also Basso et al[2]): compared to controls, the salivary cortisol response to an acute psychosocial stressor was found to be blunted[87], overall salivary cortisol levels were found to be lower[88], and the HPA feedback response was found to be hypersensitive (stronger and longer-lasting cortisol suppression after dexamethasone challenge)[89]. Moreover, another study found negative associations between cortisol levels and tinnitus indices (loudness and frequency)[90].

Regarding BT specifically, participants with high tinnitus-related distress were found to show chronically increased salivary cortisol levels compared to participants with low tinnitus-related distress and healthy controls[91]. In addition, tinnitus-related distress and psychological symptoms as well as serum cortisol levels were found to decrease after CBT[92]. Moreover, tinnitus patients with high distress levels were found to show flatter cortisol awakening curves[93]. However, other studies observed no relationship between cortisol and tinnitus-related distress[94] or daily satisfaction levels[95].

Overall, these findings indicate blunted HPA axis reactivity in tinnitus, but suggest that HPA axis dysregulation may be different in BT, with potentially increased cortisol levels in highly distressed patients. However, only a few studies have been conducted so far and more evidence is needed.

Other stress-related physiological changes may also occur in tinnitus, including alterations in immunological/inflammatory, metabolic, neurological, or oxidative parameters[96]. Brain-derived neurotrophic factor (BDNF) is a neurological parameter that might be particularly relevant for hearing and tinnitus[96–98]. BDNF is an important nerve growth factor involved in developmental processes, neurogenesis/-protection, and synaptic plasticity[96,99,100]. BDNF expression is reduced in various neurodegenerative disorders and stress-related disorders such as depression[2,99,101–103].

To date, few studies have studied BDNF levels in tinnitus (see also Basso et al[2]). Lower serum BDNF levels were observed in tinnitus subjects compared to controls[104]. Goto et al[105] found higher plasma BDNF levels in tinnitus patients with low distress levels (mild handicap) compared to patients with severe distress (severe handicap) and healthy controls. However, BDNF levels were also higher in patients with low compared to high depression levels in this study, and the association between tinnitus-related distress and

plasma BDNF was no longer significant after adjustment for depressive symptoms[105]. This suggests that potential reductions in BDNF levels in BT might be linked to comorbid depressive symptomatology. In a therapeutic study by Xiong et al[106], plasma BDNF levels were increased in tinnitus patients compared to controls; and they decreased in patients with severe tinnitus after treatment. However, in this study, no direct association was found between plasma BDNF levels and tinnitus severity[106]. Similarly, in another study[107], serum BDNF and tinnitus-related distress were not related. However, these studies were conducted in small sample sizes, particularly concerning the subgroup of patients with severe tinnitus (Goto et al[105]: N=18 and Xiong et al[106]: N=14), raising issues regarding their generalizability. Thus, the association of BDNF with tinnitus-related distress requires further investigation.

In summary, cortisol and BDNF are two stress mediators which might be relevant for BT, but the literature is not conclusive. Previous studies investigating these two biomarkers in tinnitus patients used saliva or blood sampling for their quantification[2]. However, both cortisol and BDNF can also be measured in hair[2,108,109]. An advantage of quantification in hair is that it provides retrospective, cumulative long-term concentrations[2,109–112]. Hair sampling allows the direct assessment of long-term concentrations; e.g., concentrations that have accumulated over the past month can be quantified in the most recently grown 1-cm hair segment[2,109–112]. Other advantages include non-invasiveness and less situational confounding than in saliva/blood sampling[2,109,111,112]. Thus, investigating cortisol and BDNF levels in hair might provide new insights into their relationship with tinnitus-related distress and chronic stress-related physiological changes in BT.

1.7. Research aims

The literature indicates that stress-related psychological and physiological processes might be important factors for the manifestation and/or maintenance of BT. This thesis aims to 1) investigate the impact of stress-related mental comorbidities on BT and their mediation effects for hearing-related factors and physical comorbidities[1], 2) explore associations of tinnitus-related distress with two chronic stress-related biological markers, namely cortisol and BDNF measured in hair[2], and 3) explore the potential of cortisol and BDNF measured in hair as therapeutic efficacy markers[3]. These research aims are addressed in three publications. Publication 1 is focused on the identification of mental and physical comorbidities that are associated with BT and investigating dependencies

between mental, hearing-related and other physical factors in their influences on BT[1]. The underlying hypothesis of this study was that stress-related mental comorbidities are strongly associated with tinnitus-related distress and partially explain the effects of hearing impairment and -related difficulties and other physical comorbidities on BT[1]. Publication 2 examines associations of the stress-related hair-biomarkers cortisol and BDNF with tinnitus characteristics and psychological factors in chronic tinnitus patients[2]. Due to the known connection between stress and tinnitus severity, a positive association of tinnitus-related distress with hair-cortisol concentrations as well as a negative association with hair-BDNF concentrations were expected[2]. Lastly, publication 3 investigates treatment effects in these stress-related hair-biomarkers following multimodal tinnitus-specific CBT, to test the assumption that psychological treatment effects are paralleled by physiological changes in stress-related systems[3]. Specifically, a reduction in hair-cortisol and an increase in hair-BDNF levels (i.e., normalization) were expected after treatment[3].

2. METHODS

2.2. Publication 1

2.2.1. LifeGene study

"LifeGene" is a prospective national cohort study in a randomly recruited sample from the Swedish general population[1,113,114]. In publication 1[1], epidemiological data previously collected as part of the LifeGene study were analyzed[113,114]. Of the available baseline data from the web-based LifeGene survey on health-related themes[113,114] collected between 2009 and 2016 in 31926 participants, the subsample of 7615 participants (23.9%) with self-reported tinnitus was analyzed[1]. Of these participants, 6918 reported intermittent/non-BT and 697 (9.2%) constant/BT[1] (here referred to as non-BT and BT for simplicity). The project was approved by the local ethics committee of Karolinska Institute, Stockholm (2015/2129-31/1)[1]. All participants provided informed consent; parental consent was obtained for participants under 18 years of age[1]. The same sample was additionally analyzed by our research group with regard to gender differences[115].

2.2.2. Sample characteristics

Fifty-six percent of the sample were women (N=4301), and the mean age of the sample was 35.80 years (SD=12.44), ranging from 11 to 84 years[1]; see Figure 1. More sociodemographic information can be found in Basso et al[1,115].

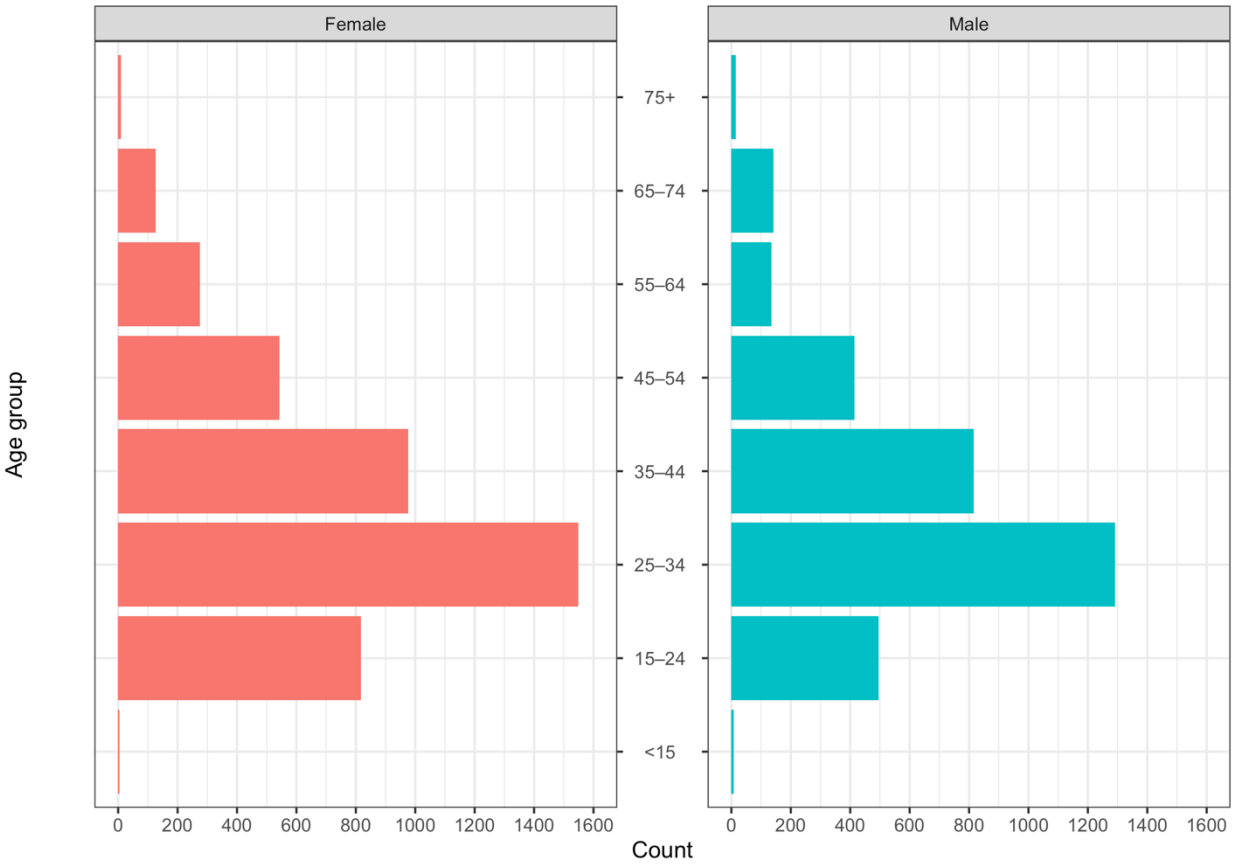

Figure 1. Age distribution of study participants (N=7615) separated by gender. All participants were sampled from the general Swedish population and reported having tinnitus. Newly created figure by the author based on the sample analyzed in Basso et al[1].

2.2.3. Data preparation

The distinction between BT and non-BT was selected as the outcome variable[1]. Predictor variables were selected based on three groups: (current or previous) physical comorbidities, mental comorbidities, and hearing-related factors[1]. The term "comorbidities" was chosen because BT was conceptualized as a stand-alone disorder (see Introduction). Ménière's disease is a condition in which tinnitus can occur and therefore was included in the analysis, despite not constituting a "comorbidity"[1]. Hearing-related factors included subjective hearing ability ratings categorized as "good" or (somewhat or very) "reduced"[1(p56)], and a mean score across five items asking about hearing-related difficulties in social situations, which was converted to the presence or absence of such difficulties[1].

2.2.4. Statistical analysis

Statistical analyses are described in detail in Basso et al[1]. IBM SPSS Statistics (version 25)[116] was used for data analysis[1]. For all analyses, *p*-values <0.05 were considered

significant[1]. First, descriptive analyses were performed comparing the frequencies of selected variables between BT and non-BT using Pearson's χ^2 test[1]. Differences in frequencies were assessed using adjusted residuals (ARs); ARs of ≥1.96 or ≤-1.96 were considered significant[1]. Second, three logistic regression models adjusted for age and sex were calculated for the prediction of BT by physical comorbidities, mental comorbidities, and hearing-related factors[1]. For all effect estimates, odds ratios (ORs) and 95% confidence intervals (CIs) were computed[1]. Third, logistic mediation models[117,118] were calculated; see Figure 2 for the conceptual models[1]. Mediation analysis evaluates how much the total effect of a predictor *X* on an outcome *Y* (coefficient c) is reduced (coefficient c') when a mediator variable *M* is taken into account (coefficients a x b)[1]; see Figure 2. Dividing the mediation effect (a x b) by the total effect (c) yields the percentage of the effect being mediated[1,117,118]. The significance of mediation effects was assessed using the Sobel test (Aroian version)[1,119].

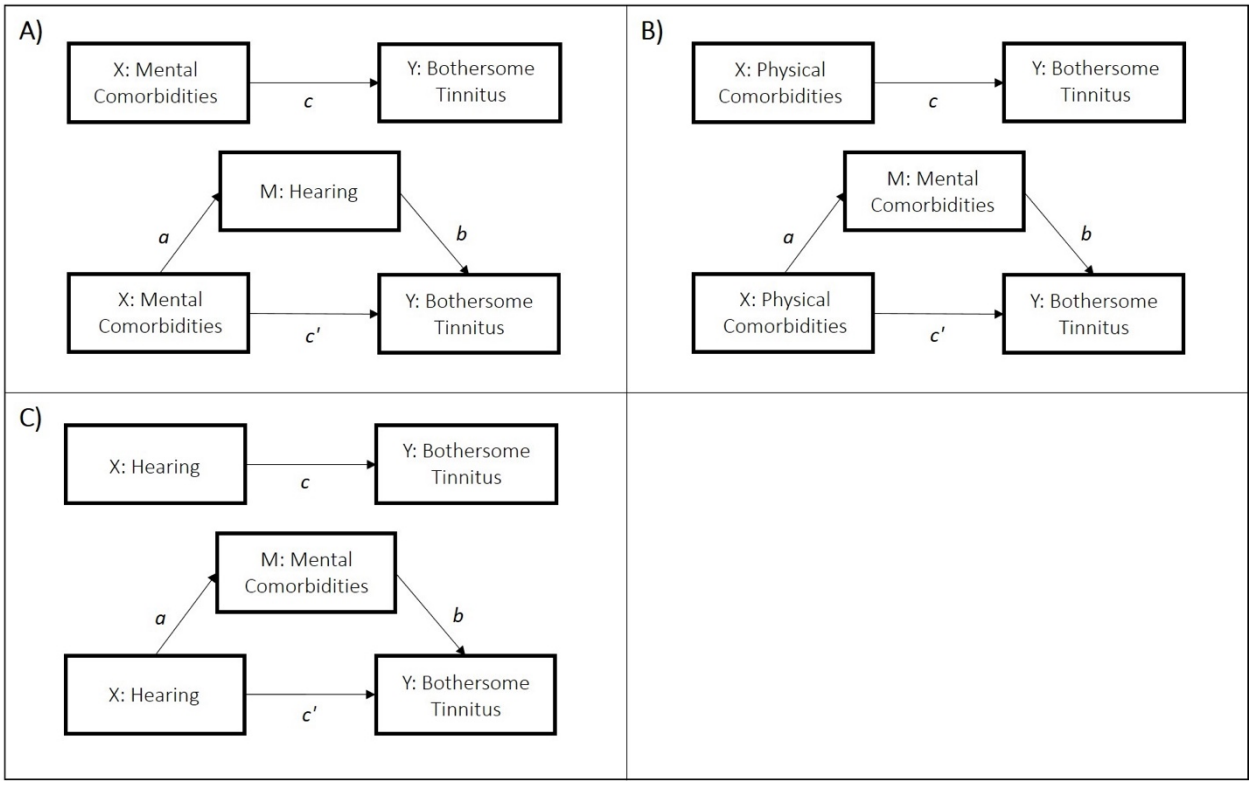

Figure 2. "Conceptual models of the performed mediation analyses. X=predictor, M=mediator, Y=outcome. Coefficient *c* designates the total effect of the respective X on Y (ignoring M); coefficient *a* is the effect of X on M, coefficient *b* is the effect of M on Y (controlling for X); coefficient *c'* designates the direct effect of X on Y when M is controlled; and *a x b* reflects the indirect or mediation effect." Figure reprinted from Basso et al[1(p58)], with permission from Elsevier.

2.3. Publications 2 and 3

2.3.1. Study design

The second and third publication[2,3] are based on a longitudinal clinical study conducted between December 2018 and June 2020 in a sample of 94 inpatients with chronic tinnitus (ICD-10 code H93.1). All participants attended the treatment program for chronic tinnitus offered at the Tinnitus Center of Charité – Universitätsmedizin Berlin[3] (see Introduction). The mean treatment duration was 4.78 days (SD=1.10), ranging from four to nine days[3]. All inpatients treated at the Tinnitus Center during the recruitment period of this study were eligible for participation; inclusion and exclusion criteria are summarized in Table 1. Approximately 16% of the treated inpatients voluntarily participated in this study[2]. In March 2020, recruitment of new participants was discontinued due to the onset of the COVID-19 pandemic[2]. The study was carried out in accordance with the Declaration of Helsinki and approved by the ethics committee of Charité – Universitätsmedizin Berlin (No. EA1/035/16)[2,3]. Written informed consent was provided by all participants[2,3].

Table 1. Inclusion and exclusion criteria for study participation

Inclusion criteria	Exclusion criteria
"Diagnosis of chronic subjective tinnitus"	*"Inability to consent due to serious mental or physical impairments"*
"Age ≥ 18 years"	*"Simultaneous participation in other research studies"*
"Written informed consent"	*"Any chemical hair treatment within 1 month prior to sampling (dying, bleaching, perming, or else)"*
	"Hair length < 3 cm"
	"Hair washing or the use of hair products (hair mousse, hair gel, hair wax, hair spray) within 3 days prior to sampling"
	"Hair combing on the day of sampling"

Note. Table reprinted from Basso et al[2(p2)] (CC BY 4.0).

The study included a baseline (BL) measurement session before treatment, a second session directly after treatment (TE), and a 3-month follow-up session (FU)[3]. Data collection included hair sampling to determine biomarkers (BL and FU), psychometric questionnaires to assess psychological symptoms (all measurements), and audiometric tests to determine hearing ability and psychoacoustic tinnitus characteristics (BL). However, audiometric testing was not performed specifically for this study, but audiometric data were retrieved from outpatient records[2,3]. These outpatient measurements preceded the BL

session by a mean of M=70.14 days (SD=57.62)[2]. Sociodemographic, hair-related, and health-related information was additionally collected to detect potential confounding influences (BL and FU)[3]. An overview of the collected data is shown in Figure 3.

		Baseline	Treatment end (+5 days)	Follow-up (+3 months)
Biomarkers	• Hair-cortisol	X		X
	• Hair-BDNF	X		X
Psychometric questionnaires	• TQ: tinnitus-related distress	X	X	X
	• PSQ-20: perceived stress	X	X	X
	• HADS: anxiety and depression	X	X	X
	• SOMS: somatization	X		X
	• STAI: state anxiety	X		X
	• PDS: traumatic experiences	X		
	• SF-12: physical and mental health-related quality of life	X	X	X
Tinnitus and hearing	• Tinnitus frequency and loudness (matching data)	X		
	• Hearing threshold (audiogram)	X		
	• Tinnitus/hearing-related characteristics	X		
Covariates	• Sociodemographic information	X		X
	• Season and time of sampling	X		X
	• Hair-related criteria/hair care	X		X
	• Health-related factors/behavior	X		X

Figure 3. "Overview of all collected study variables across measurements (baseline, treatment end, and follow-up)." BDNF=Brain-Derived Neurotrophic Factor; HADS=Hospital Anxiety and Depression Scale; PDS=Posttraumatic Diagnostic Scale; PSQ-20=Perceived Stress Questionnaire (20 item version); SF-12=Short Form-12 Health Survey; SOMS=Screening of Somatoform Disorders; STAI=State-Trait Anxiety Inventory (State-Anxiety); TQ=Tinnitus Questionnaire. Figure reprinted from Basso et al[3(p3)] (CC BY 4.0).

2.3.2. Sample characteristics

Ninety-four participants were recruited for this study[2,3]. For cross-sectional analysis, 91 participants were included for the prediction of hair-cortisol and 87 for hair-BDNF, due to missing questionnaire data (N=1), violation of hair-related criteria (N=2), and missing hair-BDNF values (N=4)[2]. For longitudinal analysis, complete data were available of 80 participants, due to dropouts (N=4), violation of hair-related criteria at FU (N=1), and missing biomarker values (N=6)[3]. Sixty-six percent of participants were female (N=60) and the mean age of the sample was M=51.5 years (SD=12; range: 19–80 years)[2]; see Figure 4. Most participants had normal hearing ability (62.6%, N=57)[2]. All participants suffered from chronic tinnitus (i.e., for at least three months) and sought clinical help at the Tinnitus Center, indicating a certain level of suffering. Therefore, the sample is conceptualized here as BT, but psychometrically measured levels of tinnitus-related distress varied among participants: 39.6% (N=36) reported mild, 37.4% (N=34) moderate, 12.1% (N=11) severe, and 11.0% (N=10) very severe distress[2]. See Basso et al[2] for more information.

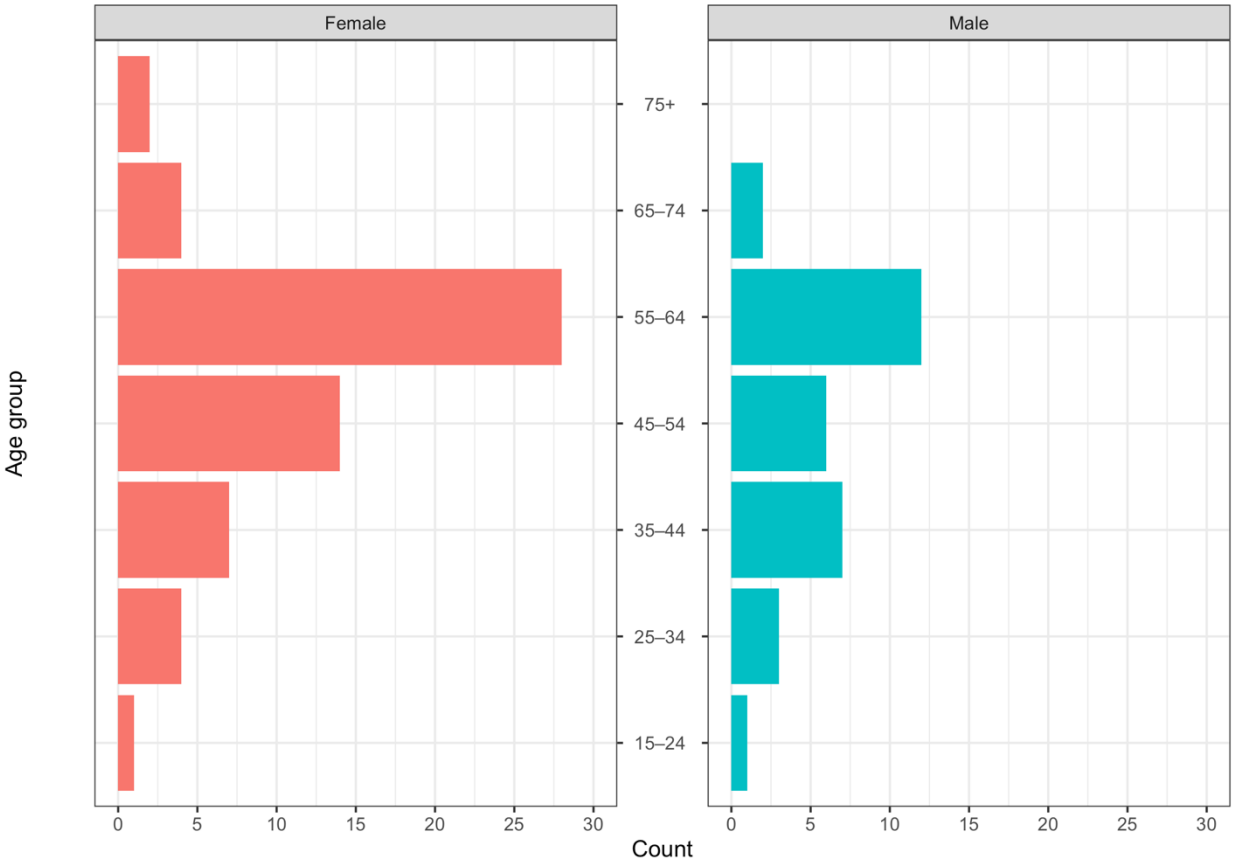

Figure 4. Age distribution of study participants (N=91) separated by gender. All participants had chronic tinnitus and sought clinical help at the Tinnitus Center. Newly created figure by the author based on the sample analyzed in Basso et al[2].

2.3.3. Psychometric questionnaires

The following validated psychometric questionnaires were used[2,3].

2.3.3.1. Tinnitus Questionnaire (TQ)

The German version of the Tinnitus Questionnaire (TQ)[120] was used to assess tinnitus-related distress[2,3]. This instrument consists of 52 items measuring six domains of tinnitus-related distress, namely emotional distress, cognitive distress, tinnitus intrusiveness, auditory perceptual difficulties, sleep disturbances, and somatic complaints[120]. Only the total score was used, which is the sum of (partially recoded) 40 items, with two items added twice[120].

2.3.3.2. Perceived Stress Questionnaire (PSQ-20)

The German version of the Perceived-Stress-Questionnaire (PSQ-20)[121,122] was used to assess perceived stress levels[2,3]. It consists of 20 items measuring four domains of subjectively experienced stress, namely worries, tension, (lack of) joy, and demands[121,122]. Only the total score was used, which is the linearly transformed mean (with recoded joy items)[121,122].

2.3.3.3. Hospital Anxiety and Depression Scale (HADS)

The German version of the Hospital Anxiety and Depression Scale (HADS)[123] was used to assess anxiety and depression levels[2,3]. It consists of 14 items assessing anxiety and depression symptoms during the past week[123,124]. Both the anxiety and depression subscales were used, i.e., the sum scores across seven (partially recoded) items each[123,124].

2.3.3.4. State-Trait Anxiety Inventory (STAI)

The State-Trait Anxiety Inventory (STAI) is a questionnaire assessing state anxiety (varying emotional state) and trait anxiety (personality trait)[125,126]. Here, the German version of the STAI[126] was used to measure state anxiety[2,3]. The state anxiety score is calculated as the sum over all (partially recoded) 20 items[126].

2.3.3.5. Screening of Somatoform Disorders (SOMS)

The German “Screening für Somatoforme Störungen” / ”Screening of Somatoform Disorders” (SOMS)[127], was used to assess somatization tendencies[2,3]. This instrument consists of 52 items for women and 48 for men representing somatoform symptoms, defined

as physical symptoms that remain medically unexplained and cause emotional distress[127]. The seven days version was used, which captures the presence of somatoform symptoms and the degree of associated suffering in the past week[127]. Only the number of complaints was used.

2.3.3.6. Posttraumatic Diagnostic Scale (PDS)

The event list of the German version of the Posttraumatic Diagnostic Scale (PDS)[128] was used to assess the number of experienced traumatic events[2,3]. It consists of 12 items representing relevant traumatic events[128]. Responses indicate whether the particular event was experienced (personally or as a witness) at some point in the personal history, with one item representing an open response option [128]. The number of reported traumatic experiences was used.

2.3.3.7. Short Form-12 Health Survey (SF-12)

The German version of the Short Form-12 Health Survey (SF-12) version 2[129,130] was used to assess health-related quality of life[2,3]. This questionnaire consists of 12 items measuring physical or mental health-related quality of life (during the past week)[129]. It includes a physical component summary (PCS) and a mental component summary (MCS) based on eight scales (general health perception, physical functioning, physical role functioning, emotional role functioning, pain, mental health, vitality, and social functioning)[129]. The two summary scores were calculated as T-standardized scale scores based on normative data[130].

2.3.4. Audiometric tests

Outpatient records were used to obtain audiometric data (closest recordings to BL were selected)[2]. All tests had been performed in the audiological department of the clinic in soundproof booths. They included pure-tone audiometry (PTA) and tinnitus pitch and loudness matching[2]. PTA included the following frequencies: 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz[2]. Hearing thresholds for each frequency were recorded for both ears in 5-dB intervals[2]. These values were averaged to obtain a single parameter for the mean hearing ability[2]. The tinnitus matching procedure determines psychoacoustic approximation measures of tinnitus pitch/frequency (Hz) and loudness (dB) and is described in detail in Basso et al[2]. Subjects were asked to compare their tinnitus to sequentially played pure tones or narrow-band noise until a match was reached (and confirmed twice) in terms of

frequency and loudness[2]. Because hearing thresholds were recorded to the nearest 5 dB, whereas tinnitus loudness was determined at a 1 dB level, the accuracy of the sensation level (i.e., tinnitus loudness corrected for the hearing threshold in the tinnitus frequency) was reduced[2]. Thus, the uncorrected "absolute" tinnitus loudness was used instead[2]. However, because it is not independent of the hearing threshold, the latter was controlled for in all analyses[2,3]. Tinnitus matching data were missing for 25 participants[2].

2.3.5. Hair sample collection

Hair samples were collected mostly in the morning; median sampling times were 09:55 a.m. at BL[2] and 10:15 a.m. at FU[3]. To obtain the samples, they were "*cut with scissors from the region of the posterior vertex, as close to the scalp as possible*"[2,3(p5)], due to lower intra-individual variation in cortisol concentrations in this region[131], and then placed in aluminum foil in plastic bags[2,3]; see Figure 5. To avoid contamination, gloves were worn and all materials were cleaned (using 70% isopropyl alcohol) between sampling[2]. For storage, the samples were put in a dark container and kept at room temperature until further analysis in summer/autumn 2020[2,3].

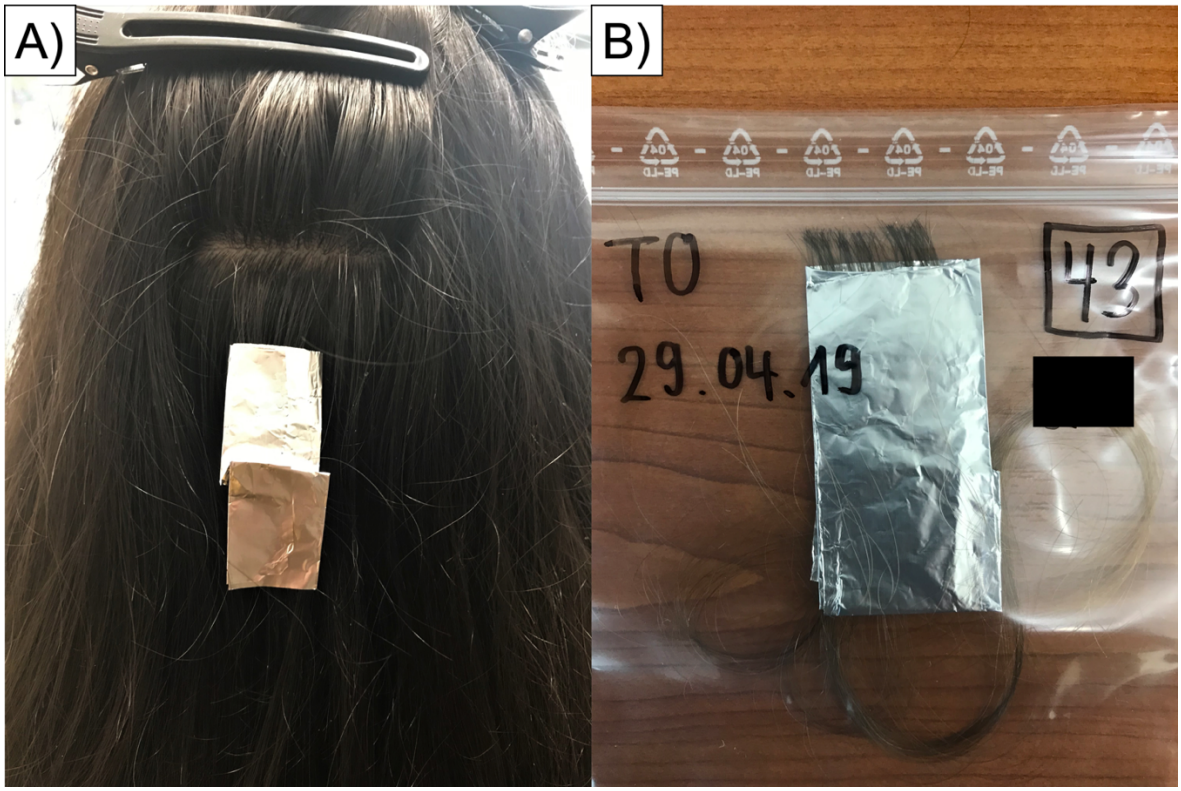

Figure 5. Illustration of hair sample collection[2,3]. A) Hair samples were collected from the region of the posterior vertex, cut as close to the scalp as possible, and B) stored in aluminum foil in a dark container[2,3]. Photographs were taken by the author.

2.3.6. Biomarker quantification

Samples were analyzed at the Psychoneuroimmunology Laboratory of the Justus-Liebig University Giessen (samples were kept at room temperature during relocation), approx. 1.75 to 0.5 years after collection[2]. Samples were processed according to established laboratory protocols[2,3,108]. Due to the average hair growth rate of approx. 1 cm per month[132], the most recent 1-cm hair segment (proximal to the scalp) was analyzed to determine cumulative concentrations of cortisol and BDNF over the past month[2,3]. Sample preparation included cutting the most proximal 1-cm segment, weighing (5–20 mg), freezing in liquid nitrogen for 2 min, grinding in a ball mill for 2–3 min with 7-mm-diameter metal balls at 25 Hz[108], and subsequent extraction procedures for cortisol and BDNF[2,108]. Cortisol and BDNF extraction procedures are described in Harb et al[108]. After sample preparation, dry hair extracts were resuspended using 100 µl of phosphate-buffered saline (PBS) for 10 mg pulverized hair (for cortisol, a 1:10 dilution was used; for BDNF, a 1:1000 dilution)[3,108] and quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits[2,3,108]. For cortisol ELISA (IBL International®, Hamburg, Germany)[108], a sensitivity of 0.005 µg/dl is reported (standard range: 0.15–30 ng/ml)[3], and intra- and inter-assay coefficients of variation of +4.3% and +13.2%[2,3,108]; in our study (all samples), these were +1.91% and 7.49 ± 2.81, respectively[2]. For BDNF ELISA (Emax® ImmunoAssay System, Promega, Madison, WI, USA)[108], a sensitivity of 15.6 pg/ml is reported (standard range: 0–1000 pg/ml)[3], and intra- and inter-assay coefficients of variation of +3.7% and +8.5%[2,3,108]; in our study (all samples), these were +2.73% and 5.31 ± 3.35, respectively[2]. All cortisol values could be detected; BDNF detection was not possible in seven cases[3].

2.3.7. Statistical analysis

Cross-sectional analyses[2] and longitudinal analyses[3] were performed in R (version 4.0.0)[133]. For all analyses, *p*-values <0.05 were considered significant[3].

The main cross-sectional analysis[2] consisted of elastic net regression which "*is a penalized linear regression method […] that performs shrinkage of correlated predictors and automatic variable selection*"[2(p6)]. Two elastic net regression models (using 'caret' and 'glmnet')[134,135] with n-fold cross-validation were calculated for the prediction of hair-cortisol and hair-BDNF (35 predictors each)[2]. The data were divided into a training dataset (70%) and a test dataset (30%)[2]. Models were created using the training datasets

(cortisol: N=66; BDNF: N=63) and evaluated using the test datasets (cortisol: N=25; BDNF: N=24) to validate the model performance on new data[2]. Hair-cortisol values were log-transformed before analysis due to non-normal distribution, and all numerical variables (outcome and predictor) were standardized[2]. The standardized effect estimates were ranked (0-100) by their absolute magnitude (variable importance). In addition, missing values on tinnitus matching data (27.5%) and SF-12 scores (2.2%)[2] were imputed with k-nearest neighbor imputation (using 'RANN')[136].

The main longitudinal analyses[3] consisted of linear mixed-effects models for the prediction of TQ, PSQ-20, hair-cortisol, and hair-BDNF (using 'lme4')[137], which were reduced by backward elimination (using 'lmerTest')[138]. Model fit was based on restricted maximum likelihood (REML), the significance of fixed effects estimates was assessed using z-tests (with 'multcomp')[139], and Holm's correction[140] was applied to adjust for multiple testing[3]. The predictors included in the full and reduced linear mixed-effects models for each outcome can be found in Basso et al[3]. All numerical predictors were standardized[3]. Missing values on tinnitus matching data (26.3%), hair color (6.3%), SF-12 scores (3.8%), and hearing aid use (1.3%)[3] were imputed with k-nearest neighbor imputation (using 'DMwR2')[141].

3. RESULTS

Results are described in detail in the respective publications[1,2,3]. Here, only key findings are summarized.

3.2. Publication 1

3.2.1. Comorbidities with associations to bothersome tinnitus (compared to non-bothersome tinnitus) identified in frequency and logistic regression analyses

First, frequencies of reported comorbidities/problems were compared between participants with BT (N=697) and non-BT (N=6918)[1]. Second, these factors were analyzed in multivariate logistic regression models (adjusted for sex and age), separately for hearing-related factors, physical comorbidities, and mental comorbidities[1]. All significant results are detailed in Table 2.

Participants with BT (compared to non-BT) reported higher frequencies of reduced subjective hearing ability and hearing-related difficulties in social situations[1]. Both factors showed increased ORs in predicting BT (numbers in square brackets indicate 95%-CIs): reduced subjective hearing ability: OR=2.65 [2.15, 3.26], and hearing-related difficulties in social situations: OR=1.61 [1.28. 2.04][1].

Regarding physical comorbidities, participants with BT (compared to non-BT) reported higher frequencies of chronic shoulder pain, osteoarthritis, fibromyalgia, Ménière's disease, hypertension, hyperlipidemia, cardiovascular disease, and thyroid disease[1]. Of these, chronic shoulder pain: OR=1.88 [1.43, 2.47], cardiovascular disease: OR=1.49 [1.08, 2.04], thyroid disease: OR=1.48 [1.06, 2.08], and Ménière's disease: OR=3.42 [1.29, 9.05] showed increased ORs in predicting BT[1].

Regarding mental comorbidities, participants with BT (compared to non-BT) reported higher frequencies of depression, burnout, panic disorder, generalized anxiety syndrome, social anxiety, and posttraumatic stress disorder[1]. Of these, depression: OR=1.25 [1.00, 1.56], generalized anxiety syndrome: OR=1.38 [1.04, 1. 83], and social anxiety: OR=1.57 [1.08, 2.30] showed increased ORs in predicting BT[1].

Table 2. Frequencies of hearing-related factors, physical comorbidities, and mental comorbidities with significant differences between bothersome tinnitus and non-bothersome tinnitus (left) and significant results of logistic regression models predicting bothersome tinnitus (right).

	Frequency comparison							Logistic regression analysis			
	Bothersome tinnitus (BT)		Non-bothersome tinnitus (non-BT)								
Variable	%	N/N_{BT}	%	$N/N_{non\text{-}BT}$	ARs	Pearson's χ^2	p	β	SE β	Wald's χ^2	p
Reduced subjective hearing ability	63.9	432/676	31.5	2125/6754	16.9	285.11	<0.001	0.97	0.11	83.44	<0.001
Hearing-related difficulties in social situations	78.8	502/637	55.5	3185/5742	11.3	127.09	<0.001	0.48	0.12	16.25	<0.001
Chronic shoulder pain	11.5	80/695	5.3	367/6882	6.6	42.30	<0.001	0.63	0.14	20.80	<0.001
Osteoarthritis	9.4	65/695	4.7	323/6882	5.3	27.25	<0.001				
Fibromyalgia	2.3	16/695	0.8	54/6882	4.0	14.27	<0.001				
Ménière's disease	1.0	7/695	0.2	13/6882	4.0	13.10	<0.001	1.23	0.50	6.14	0.013
Hypertension	9.5	66/695	5.8	399/6886	3.9	14.39	<0.001				
Hyperlipidemia	6.3	44/695	3.1	211/6886	4.6	19.73	<0.001				
Cardiovascular disease	7.8	54/695	4.0	277/6886	4.6	20.34	<0.001	0.40	0.16	6.07	0.014
Thyroid disease	6.8	47/695	3.6	251/6886	4.0	15.43	<0.001	0.39	0.17	5.19	0.023
Depression	26.8	186/695	20.4	1405/6882	3.9	14.95	<0.001	0.22	0.22	3.91	0.048
Burnout	15.1	105/695	10.1	697/6882	4.1	16.02	<0.001				
Panic disorder	14.0	97/695	11.1	763/6882	2.3	4.89	0.027				
Generalized anxiety	14.4	100/695	10.0	688/6882	3.6	12.60	<0.001	0.32	0.14	5.04	0.025
Social anxiety	6.3	44/695	3.4	231/6882	4.0	15.13	<0.001	0.45	0.19	5.50	0.019
Posttraumatic stress disorder	2.7	19/695	1.6	107/6882	2.3	4.67	0.031				

Note. Left: Frequency comparison of problems/comorbidities between bothersome and non-bothersome tinnitus using Pearson's χ^2 tests (with continuity correction where applicable)[1]. Only problems/comorbidities with significant differences are shown. Right: Significant results of age- and gender-adjusted logistic regression models for the prediction of bothersome tinnitus calculated separately for hearing-related factors, physical comorbidities, and mental comorbidities[1]. ARs=Adjusted residuals. Table adapted from Basso et al[1(pp60-62)], with permission from Elsevier.

3.2.2. Mediating effects between hearing-related factors, physical and mental comorbidities in their association with bothersome tinnitus

Logistic mediation models were calculated to investigate mediating effects between the identified factors in their association with BT[1]. These models investigated A) mediation by hearing-related factors for the association between mental comorbidities and BT, B) mediation by mental comorbidities for the association between physical comorbidities and BT, and C) mediation by mental comorbidities for the association between hearing-related factors and BT[1] (see Figure 2 for conceptual models). Overall, 15 significant mediation effects were observed: detailed results are depicted in Figure 6. For A), the strongest effect was found for hearing-related difficulties in social situations partially mediating the effect of depression on BT[1]; for B), the strongest effect was found for depression partially mediating the effect of chronic shoulder pain on BT[1]; for C), the strongest effect was found for social anxiety partially mediating the effect of hearing-related difficulties in social situations on BT[1].

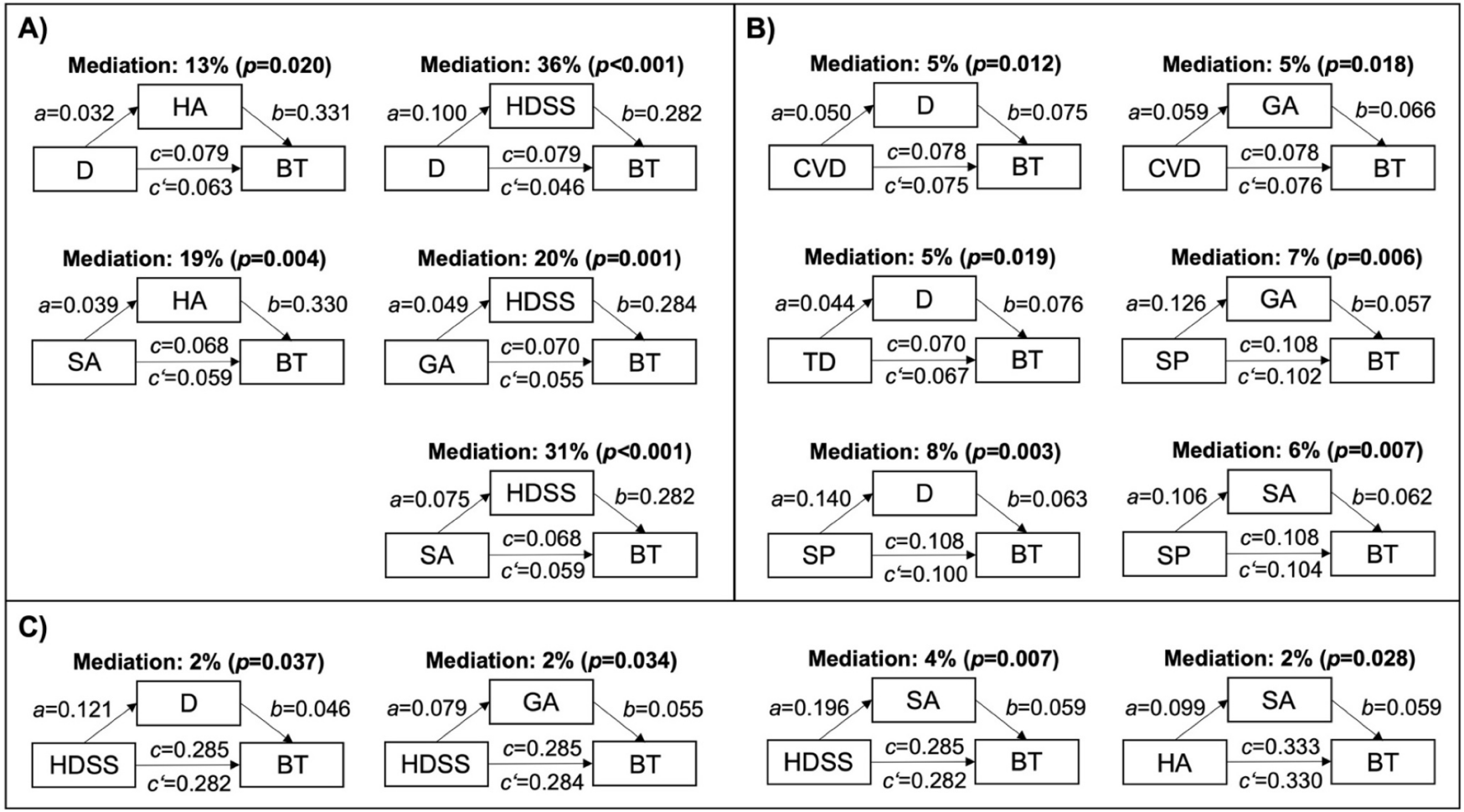

Figure 6. Significant mediation effects: A) hearing-related factors partially mediate between mental comorbidities and BT; B) mental comorbidities partially mediate between physical comorbidities and BT; C) mental comorbidities partially mediate between hearing-related factors and BT. BT=bothersome tinnitus; CVD=cardiovascular disease; D=depression; GA=generalized anxiety syndrome; HA=hearing ability (self-report); HDSS=hearing-related difficulties in social situations; SA=social anxiety; SP=shoulder pain (chronic); TD=thyroid disease. Newly created figure by the author. Data from Basso et al[1(pp62, 63)] with permission from Elsevier.

3.3. Publication 2

3.3.1. Elastic net regression for the prediction of hair-cortisol levels

For hair-cortisol, 10% of the variance could be explained by the elastic net regression model in the test data (N=25; RMSE=1.11), and 6% of the variance in the training data (N=66; RMSE=0.91)[2]. The most relevant finding based on the research question was the positive effect of tinnitus loudness on hair-cortisol levels, β=0.089, variable importance (VI)=55.91[2]; see Figure 7.

3.3.2. Elastic net regression for the prediction of hair-BDNF levels

For hair-BDNF, 28% of the variance could be explained by the elastic net regression model in the test data (N=24; RMSE=0.98), and 25% of the variance in the training data (N=63; RMSE=0.85)[2]. The most relevant findings based on the research question were the negative effects of tinnitus loudness, β=-0.247, VI=92.22, and tinnitus-related distress, β=-0.171, VI=63.80, on hair-BDNF levels[2]; see Figure 8.

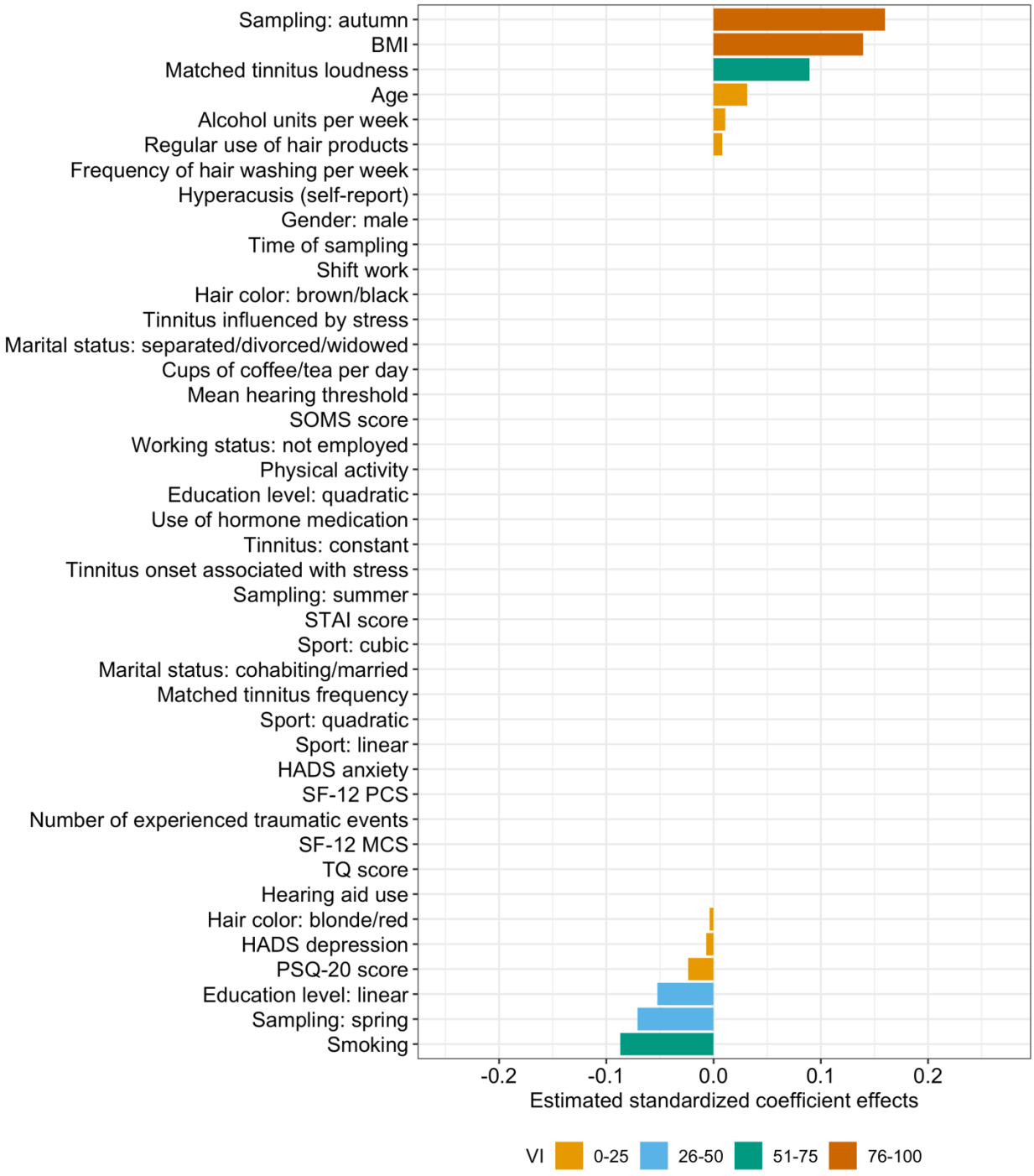

Figure 7. "Estimated standardized coefficient effects by elastic net regression with n-fold cross-validation for the prediction of hair-cortisol in chronic tinnitus patients (training data: N=66)." BMI=Body-Mass-Index; HADS=Hospital Anxiety and Depression Scale; MCS=Mental Component Summary; PCS=Physical Component Summary; PSQ-20=Perceived Stress Questionnaire (20 item version); SF-12=Short Form-12 Health Survey; SOMS=Screening of Somatoform Disorders; STAI=State-Trait Anxiety Inventory (State Anxiety); TQ=Tinnitus Questionnaire; VI=Variable Importance. Figure adapted from Basso et al[2(p8)] (CC BY 4.0).

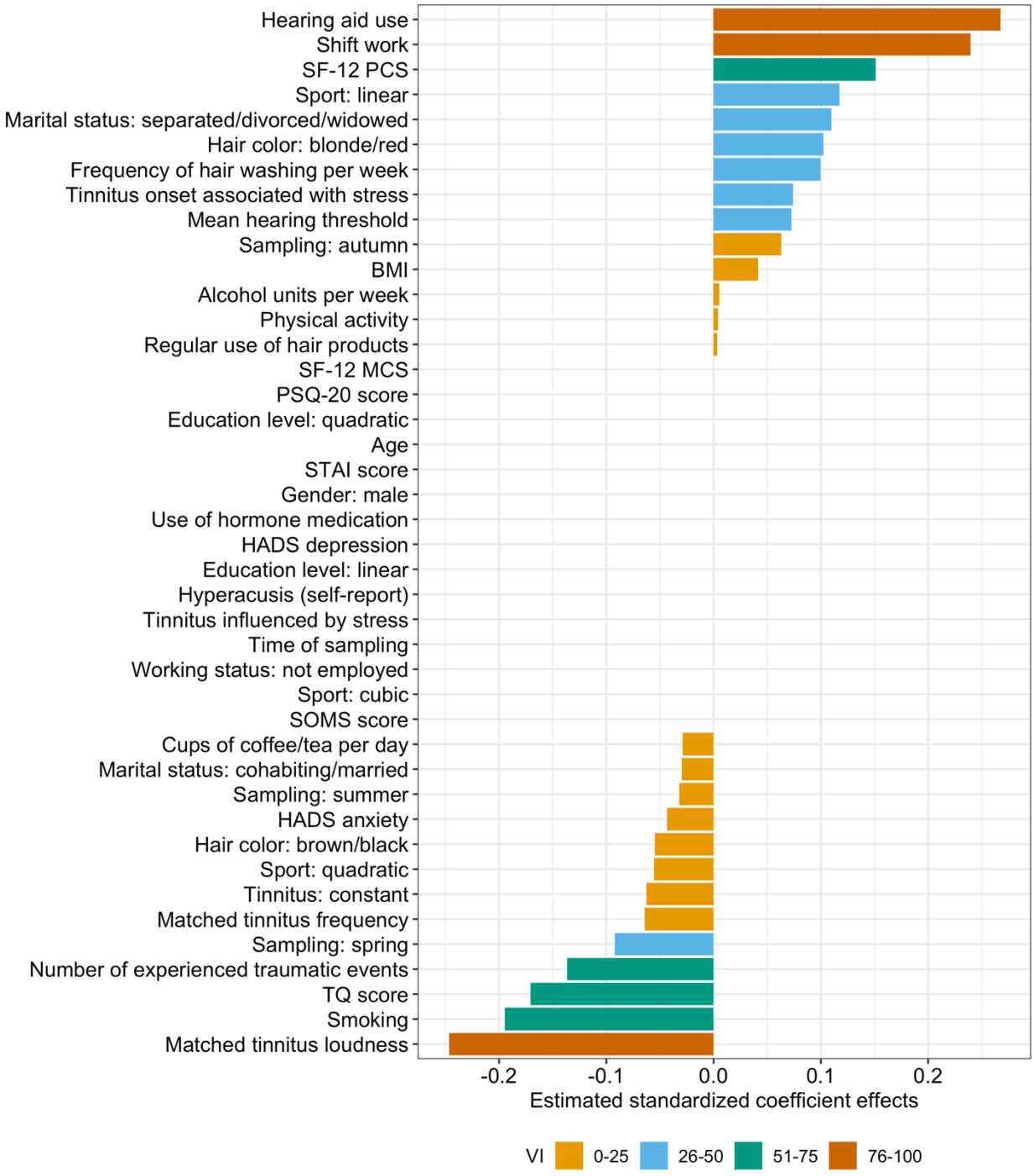

Figure 8. "Estimated coefficient effects by elastic net regression with n-fold cross-validation for the prediction of hair-BDNF in chronic tinnitus patients (training data: N=63)". BMI=Body-Mass-Index; HADS=Hospital Anxiety and Depression Scale; MCS=Mental Component Summary; PCS=Physical Component Summary; PSQ-20=Perceived Stress Questionnaire (20 item version); SF-12=Short Form-12 Health Survey; SOMS=Screening of Somatoform Disorders; STAI=State-Trait Anxiety Inventory (State Anxiety); TQ=Tinnitus Questionnaire; VI=Variable Importance. Figure adapted from Basso et al[2(p9)] (CC BY 4.0).

3.4. Publication 3

3.4.1. Descriptive analysis

The distribution of the outcome variables TQ, PSQ-20, hair-cortisol, and hair-BDNF from baseline to follow-up (N=80) is depicted in Figure 9.

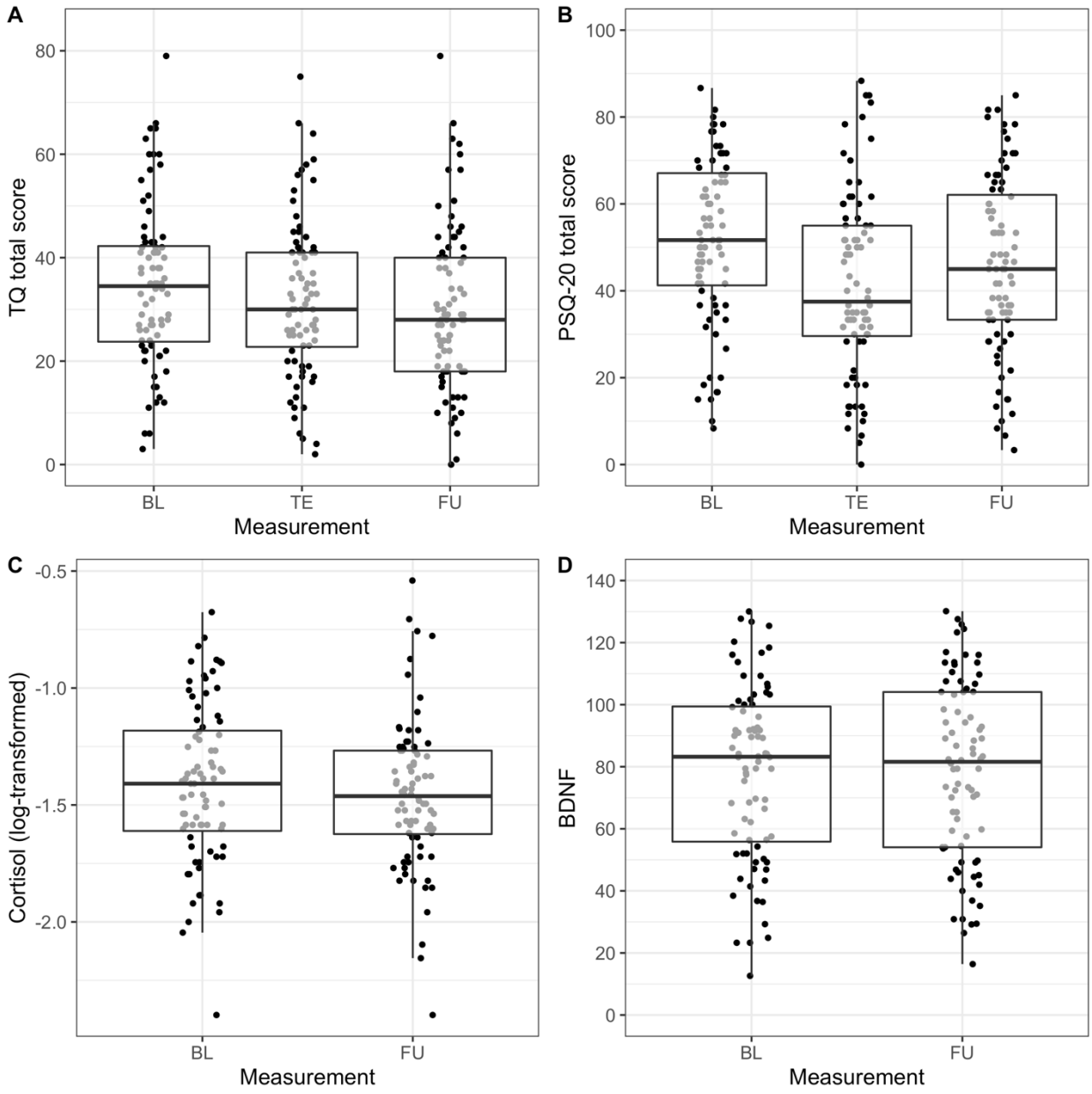

Figure 9. Distribution of the outcome variables: A) TQ total score, B) PSQ-20 total score, C) hair-cortisol levels (log-transformed), and D) hair-BDNF levels across measurements (N=80). BDNF=Brain-Derived Neurotrophic Factor; BL=Baseline; FU=Follow-Up; PSQ-20=Perceived Stress Questionnaire-20; TE=Treatment End; TQ=Tinnitus Questionnaire. Newly created figure by the author based on the sample analyzed in Basso et al[3].

3.4.2. Treatment effects identified by backward reduced mixed-effects models

To investigate treatment effects, linear mixed-effects models including all selected potentially relevant predictors were calculated for TQ, PSQ-20, hair-cortisol, and hair-BDNF and reduced by stepwise backward elimination[3]. Significant fixed effects observed in the backward reduced models after adjustment for multiple testing using Holm's method are summarized in Table 3. Significant negative effects of "measurement" (BL, TE, FU) on TQ and PSQ-20 scores were observed, indicating a reduction in tinnitus-related distress and perceived stress levels across time[3]. No treatment effects were observed for hair-cortisol and hair-BDNF[3]. Moreover, some general associations were observed across all measurement sessions[3]. Separation from a partner, divorce, or widowhood and lower SF-12 PCS values at BL (i.e., lower physical health-related quality of life) were associated with higher TQ scores across all measurement sessions[3]. Higher HADS anxiety scores (i.e., more anxiety symptoms) and lower SF-12 MCS scores (i.e., lower mental health-related quality of life) at BL were associated with higher PSQ-20 scores across all measurement sessions[3]. Higher hearing thresholds (i.e., reduced hearing ability), lower perceived tinnitus loudness, and lower TQ scores at BL were associated with higher hair-BDNF levels across all measurement sessions[3]; see Table 3.

Table 3. Significant fixed effects identified by backward reduced mixed-effect models after p-value adjustment with Holm's method (N=80).

Predictor	β	95%-CI	z	$p_{unadjusted}$	$p_{adjusted}$
Tinnitus Questionnaire (TQ)					
Measurement	-2.31	-3.31, -1.31	-4.53	<0.001	<0.001
Marital status: separated/divorced/widowed (vs. single)	12.12	4.58, 19.67	3.15	0.002	0.033
SF-12 PCS baseline	-4.48	-7.42, -1.53	-2.98	0.003	0.049
Perceived Stress Questionnaire (PSQ-20)					
Measurement	-2.97	-4.90, -1.04	-3.02	0.003	0.045
HADS anxiety baseline	5.03	1.95, 8.11	3.20	0.001	0.028
SF-12 MCS baseline	-7.29	-10.83, -3.75	-4.03	<0.001	0.001
Hair-BDNF					
Mean hearing threshold	10.79	3.64, 17.93	2.96	0.003	0.049
Tinnitus loudness	-11.59	-18.98, -4.19	-3.07	0.002	0.040
TQ baseline	-9.58	-14.21, -4.96	-4.06	<0.001	0.001

Note. Only significant effects after Holm correction are reported[3]. BDNF=Brain-Derived Neurotrophic Factor; HADS=Hospital Anxiety and Depression Scale; MCS=Mental Component Summary; PCS=Physical Component Summary; PSQ-20=Perceived Stress Questionnaire-20; SF-12=Short Form-12 Health Survey; TQ=Tinnitus Questionnaire. Table adapted from Basso et al[3(pp9, 11, 15)] (CC BY 4.0).

4. DISCUSSION

4.1. Summary of results

Associations with BT (compared to non-BT) were identified for reduced subjective hearing ability, hearing-related difficulties in social situations, chronic shoulder pain, cardiovascular disease, thyroid disease, Ménière's disease, depression, generalized anxiety syndrome, and social anxiety[1]. Moreover, subjective hearing ability and hearing-related difficulties in social situations partially mediated the association of mental comorbidities with BT, and mental comorbidities partially mediated the associations of physical comorbidities and hearing-related factors with BT[1]. Furthermore, a positive effect of tinnitus loudness on hair-cortisol levels was identified as well as negative effects of tinnitus loudness and tinnitus-related distress on hair-BDNF levels[2]. In addition, tinnitus-related distress and perceived stress levels decreased after short-term tinnitus-specific CBT, whereas hair-cortisol or hair-BDNF levels were unaffected by the treatment[3].

4.2. Hearing-related factors, physical and mental comorbidities in bothersome tinnitus

The associations between anxiety/depression and BT[40,43] as well as hearing loss and BT[14,34,36,46] are well-known[1]. Here, additional specific effects of hearing-related difficulties in social situations were observed, suggesting that the psychological impact of hearing impairment may be particularly relevant for BT[1]. Regarding physical comorbidities, cardiovascular diseases have been previously associated with BT[1,37,47,48]. The observed association of Ménière's disease with BT is consistent with previous findings on high tinnitus severity in Ménière's disease[1,142–144]. Associations with tinnitus have previously been observed for chronic shoulder pain[1,145] and thyroid disease[1,14,39] but not specifically with BT. In addition, the effects of physical comorbidities and hearing-related factors on BT were partially dependent on mental comorbidities (depression and anxiety), whereas the effects of mental comorbidities on BT were partially dependent on subjective hearing impairment and hearing-related difficulties in social situations[1]. This is in line with our hypothesis that stress-related mental comorbidities play an important role in BT and partially explain the effects of hearing impairment and -related difficulties and other physical comorbidities on BT[1]. These results indicate an important interplay between hearing-related factors and mental symptoms in BT[1].

Hearing impairment can be a risk factor for psychological distress[146] and can lead to social isolation and loneliness[147], which in turn are related to depression[148]. Similarly, reduced social functioning[149] and a weak sense of community belonging[150] seem relevant for BT[1]. Therefore, the strong mediation (36%) by hearing-related difficulties in social situations for the effect of depression on BT could be related to social isolation/loneliness. Given the observed interdependencies between influences of mental symptoms and hearing-related factors, our findings highlight the importance of addressing reduced hearing performance in addition to psychosocial functioning to alleviate distress in BT[1].

In shoulder pain patients, catastrophic thinking and reduced self-efficacy were found to be associated with higher levels of shoulder pain and disability[151], suggesting an important role of psychological distress in these patients[1,151]. Mental conditions can constitute risk factors for cardiovascular disease and seem linked to worse cardiovascular outcomes[1,152–154]. Similarly, associations between thyroid disease and mental conditions are known[1,155–157]. These mental-physical connections support our observation that mental symptoms are an important link between different physical symptoms/problems and their influences on tinnitus-related distress[1]. In summary, the observed dependencies between the influences of hearing-related factors, physical comorbidities, and mental comorbidities indicate that BT is a complex phenomenon characterized by psychosomatic interactions[1]. Further research is required for a more detailed understanding of the interplay between these factors and potential underlying mechanisms[1].

4.3. Hair-cortisol and hair-BDNF and their association with tinnitus loudness and distress

4.3.1. Negative effect of tinnitus-related distress on hair-BDNF levels

The observed negative effect of tinnitus-related distress on hair-BDNF levels was in line with our hypothesis[2]. It suggests that similar chronic stress-related changes might be present in BT as in depression, for which reduced BDNF expression is known[2,103]. The previously observed association between tinnitus-related distress and plasma BDNF in the study by Goto et al[105] was accounted for by depressive symptoms[105]. In our analyses, on the other hand, the effect of tinnitus-related distress on hair-BDNF was independent of depressive symptoms, for which no effect was found[2]. However, depressive symptom levels were low in our sample, so the effect of depression might be underestimated[2]. Therefore, it is unclear whether tinnitus-related distress and depressive symptoms truly

have independent influences on hair-BDNF levels. Studies assessing hair-BDNF levels in tinnitus patients with varying levels of tinnitus-related distress and depressive symptom levels as well as healthy controls are needed to obtain a more complete picture of these relationships.

For depression, it has been hypothesized that reduced BDNF expression might be connected to (stress-related) reductions in neuroplasticity and volume reductions of the hippocampus[2,102,103]. Consequently, similar stress-induced changes in hippocampal neurogenesis might be present in BT[2,42]. However, findings on structural changes of the hippocampus in tinnitus are mixed[2,158–161]. Further research on hippocampal volume changes and the influence of comorbid depression is needed for an improved understanding of stress-related physiological changes in BT.

4.3.2. No effect of tinnitus-related distress on hair-cortisol levels

For hair-cortisol, the expected positive relationship with tinnitus-related distress was not present in our data[2]. Regarding psychological factors, only small negative effects of perceived stress and depressive symptoms on hair-cortisol levels were observed, but these were below the applied threshold for variable importance[2]. Similarly, in longitudinal analysis, no effect on hair-cortisol levels remained significant after correction for multiple testing[3]. Overall, it appears that hair-cortisol levels in our study were largely unaffected by psychological symptoms[2]. However, mean perceived stress and depression/anxiety levels were low in our sample[2] and higher severity of these factors might be required to detect respective associations. Possibly, low modulatory influences on hair-cortisol levels in our sample could be connected to previous observations of reduced HPA axis responsiveness in tinnitus[2,87–89,93]. However, some previous findings suggest a positive association between tinnitus-related distress and cortisol levels[91,92] but not all[94,95] (see Basso et al[2]). Furthermore, a meta-analysis on determinants of hair-cortisol found differences in hair-cortisol levels between chronically stressed groups compared to controls (elevated levels by 22%), but no significant associations between hair-cortisol levels and self-report measures of perceived stress or depressive symptoms were identified[2,109]. Possible explanations for this discrepancy include limitations of subjective self-report measures and low stress levels in the study samples the respective effect sizes were primarily obtained from[109]. Thus, further research should include tinnitus patients with mild and severe tinnitus-related distress/perceived stress levels (as well as controls) to investigate the relationship between distress and hair-cortisol levels in tinnitus.

4.3.3. Positive effect of tinnitus loudness on hair-cortisol levels and negative effect on hair-BDNF levels

Tinnitus loudness was found to be positively related to hair-cortisol and negatively to hair-BDNF levels[2]. This was surprising because the direction of these effects is consistent with the expected effects for tinnitus-related distress. Yet, in general, tinnitus loudness is only moderately correlated with tinnitus distress[162], and there was no correlation between these two factors in our sample[2]. However, tinnitus loudness can increase with stress[75,163] and 79.1% (N=72) of our sample reported that stress influences their tinnitus perception[2]. Nevertheless, the effects of tinnitus loudness cannot be fully attributed to stress-related mechanisms because they were observed in addition to the effects of tinnitus-related distress[2]. However, tinnitus loudness was positively correlated with somatization in our sample[2]. In accordance with the dependencies between influencing factors on BT[1] described above, this could indicate that stress-related psychosomatic aggravation of tinnitus loudness may have been present in some patients.

Another possible explanation is confounding by hearing, because the absolute tinnitus loudness was used, which does not account for the hearing threshold in the tinnitus frequency[2]. However, the analyses were corrected for the mean hearing threshold; thus, the effects of tinnitus loudness were observed independent of it[2]. For hair-BDNF, the negative effect of tinnitus loudness was found in addition to a small opposite effect of the mean hearing threshold[2]. Furthermore, the correlation between tinnitus loudness and hair-cortisol levels remained significant when controlling for the mean hearing threshold[a]. Therefore, it seems unlikely that the observed effects can be attributed to hearing impairment.

Another explanation could be that the effects of tinnitus loudness, especially on hair-BDNF levels, result from tinnitus-specific mechanisms. Studies have shown that tinnitus is related to pathophysiological changes in the auditory system and limbic structures, as well as altered connectivity between these areas[164–166]. Tinnitus is thought to be related to hyperactivity in auditory structures[165,167] and a complex interplay of different brain networks seems involved in its perception, severity, and persistence[17,20]. The observed negative effect of tinnitus loudness on hair-BDNF levels could potentially be related to hyperactivity-induced grey matter decreases in auditory structures[165,166,168,169] and/or volume

[a] Spearman correlation: r=0.26, p=0.037, n=65 (data not shown in publication).

decreases of the subcallosal area in tinnitus[170,171]. However, findings regarding these volume changes are not consistent[158–160,172] and can be confounded by age and hearing loss[160,169].

For the positive effect of tinnitus loudness on hair-cortisol, tinnitus-specific mechanisms might also be possible. In some agreement with this assumption, both tinnitus intensity and salivary cortisol levels were found to increase after noise exposure in highly distressed patients (N=10)[2,88]. However, this study did not directly examine the relationship between tinnitus intensity and salivary cortisol, and the noise-induced increases may have been caused by independent mechanisms. Another study (N=28) found an effect of blood-cortisol on tinnitus loudness and frequency in a joint prediction model of these factors but in the opposite direction[2,90]. However, the correlation analysis of this study only showed a significant negative relationship between cortisol and tinnitus frequency but not loudness[90]. Thus, clear evidence on the relationship between cortisol and tinnitus loudness is lacking.

Another explanation might be that the effects of tinnitus loudness were overestimated in our analysis due to the imputation of missing values (N=25)[2]. The effects of tinnitus loudness on hair-cortisol and hair-BDNF were reduced when only data without imputed values were analyzed; with variable importance values below the applied threshold[2]. Therefore, overestimation due to imputation seems possible (see Limitations)[2]. Thus, the observed effects of tinnitus loudness on hair-cortisol and hair-BDNF require replication.

4.4. Treatment effects in bothersome tinnitus

Based on the cross-sectional results, an increase in hair-BDNF levels after treatment was expected[3]. No changes in hair-cortisol or hair-BDNF were observed[3]. The initial reduction in perceived stress levels during treatment (by 19.9%) was greater than the reduction three months later (by 11.5%), indicating a relative increase after treatment ended[3]. This may indicate that the observed psychological changes induced by the multimodal tinnitus-specific CBT were too small or too short-lived to be associated with changes in hair-BDNF levels[3]. The literature indicates that in depression, serum BDNF levels increase after (pharmacological) antidepressant treatment[3,103,173,174]. Such interventions usually last several weeks or months, and antidepressant treatment duration appears to correlate with changes in BDNF levels[3,173]. Similarly, peripheral BDNF levels increased in various populations after exercise- or meditation-based mindfulness interventions lasting

between 5 and 24 weeks (with one or more weekly sessions)[3,175]. In contrast, the mean treatment duration here was 4.78 days[3]. Potentially, a longer treatment duration of several weeks or an extension by weekly refresher sessions might be needed to measurably influence hair-BDNF levels[3].

A recent systematic review[176] investigated the effect of psychotherapy on blood BDNF levels across different mental conditions. Only three out of nine studies showed an increase in BDNF levels after psychotherapy (lasting 2–24 weeks)[176]. Therefore, no conclusion can be drawn regarding the effect of psychotherapy on BDNF levels[176]. The factors contributing to this inconsistency — age, sex, physical activity, meditation, pharmacotherapy, nutrition, and comorbidity[176] — may also have been relevant to our result. In our sample, patients taking antidepressants (N=11) showed no differences in hair-BDNF levels, and no participant suffered from a comorbid neurodegenerative disease[3]. Yet neither influences of medication nor comorbidities on hair-BDNF levels were systematically investigated because of the high number of predictors and the large clinical heterogeneity in our sample. Effects of age, gender, and physical activity were controlled for in the analyses[3], but effects of meditation and nutrition/supplements were not examined. Therefore, confounding influences on our results cannot be excluded[3].

Overall, it remains unanswered whether hair-BDNF could serve as a therapeutic efficacy marker in BT[3]. To clarify this question, larger studies are recommended that investigate the effect of longer-lasting treatment interventions on hair-BDNF levels in BT, include a control group, and systematically control for all above-mentioned medical confounders.

4.5. Limitations

Several limitations need to be considered. In publication 1, all variables studied were assessed by self-report[1]. Self-reported occurrence of diseases may be biased; however, no information on validated medical diagnoses was available[1]. Similarly, psychometric questionnaires to measure tinnitus-related distress and PTA to assess hearing impairment would have been more accurate sources of information but were not available for the LifeGene cohort[1]. A replication of this study using medical diagnoses, psychometric questionnaires, and PTA would be needed to investigate the possible influence of self-report bias. Furthermore, the use of a large sample (N=7615) recruited from the general population suggests reasonable generalizability[1]. However, the findings may not generalize to other cultural contexts[1].

The main limitation of publication 2 is the possible distortion due to the imputation of tinnitus matching data[2]. These data were missing for different reasons related to tinnitus characteristics[2]. To retain as much information as possible, participants with missing tinnitus matching data were not excluded from the analysis. Moreover, matching data were not missing completely at random[177], as the probability, e.g., was presumably higher for intermittent than constant tinnitus. Due to these non-random aspects, excluding participants with missing matching data could have introduced bias[177]. Imputation by the k-nearest neighbor algorithm replaces missing values with predicted values based on similarity to other observations in the dataset; for numeric variables, missing values are replaced by the mean value of the respective variable among identified similar observations[177,178]. Since tinnitus loudness is related to the hearing threshold, the latter should have provided relevant information for k-nearest-neighbor imputation. As expected, imputation did not affect the correlation between tinnitus loudness and mean hearing threshold[3], and the distribution of tinnitus loudness values was only slightly affected[b]. Nevertheless, the same models without imputed matching data revealed markedly lower influences of tinnitus loudness on both estimates[2]. Yet because of the exclusion of participants with missing values for these additional analyses, the sample sizes were reduced by 27.5%, which could limit their power. Overall, it appears possible that the effects of tinnitus loudness on hair-cortisol and hair-BDNF were overestimated due to imputation, and replication of these effects is necessary. Regarding generalizability, the probability of overfitting in our analyses appears to be low because both models were trained on a different portion of the data than that on which their performance was evaluated; and for both outcomes, model performance was similar in the training and test datasets[2]. However, it must be noted that our sample mostly consisted of individuals with normal/mild perceived stress and psychological symptom levels[2]. Therefore, the obtained findings might not extend to tinnitus patients with higher levels of psychological suffering. Moreover, it is unclear whether hair-cortisol and hair-BDNF levels differ between tinnitus patients and healthy subjects as no control group was included[2] which should be investigated further.

The lack of a control group is also an important limitation for publication 3 because the study design did not allow discrimination between treatment effects and other time-related

[b] Without imputation (N=66): Min.=5.00, 1st quartile=23.5, median=34.75, mean=39.07, 3rd quartile=54.00, max=79.00. With imputation (N=91): Min.=5.00, 1st quartile=28.55, median=39.00, mean=39.97, 3rd quartile=51.50, max=79.00 (data not shown in publication).

effects[3]. Therefore, it cannot be excluded that the observed changes took place due to spontaneous tinnitus habituation[3]. However, the decrease in tinnitus-related distress by 13.3% at the 3-month follow-up was greater than the average decrease of 3% to 8% observed over 6–12 weeks in waiting-list control groups[179]. Moreover, low statistical power could potentially explain the observed null effects for changes in hair-biomarkers[3]. However, for hair-BDNF, the 95%-CI of the null effect indicates reasonable accuracy in the estimation[3]. Nevertheless, studies with larger sample sizes are recommended. In addition, confounding influences by medical factors such as medication and comorbidities might have been present[3]. The observed trend for a temporal relationship between tinnitus-related distress and hair-BDNF levels was not statistically significant[3] and thus should not be interpreted. Overall, caution is needed for the interpretation of the obtained findings which require independent replication. Moreover, aspects of the study design such as the follow-up period could limit the generalizability of the results[3].

4.6. Implications for clinical practice

Given the observed direct and indirect influences of hearing-related factors, physical symptoms, and mental symptoms on BT, these factors should be equally considered in treatment[1]. Therefore, treatment approaches for BT should be multimodal, offered in an interdisciplinary setting, and include psychotherapy, treatment of physical symptoms, and the provision of hearing aids/hearing therapy (if needed)[1]. Furthermore, the multimodal CBT-based treatment intervention studied here reduced the levels of tinnitus-related distress and perceived stress[3]. However, there was a relative increase in perceived stress levels after completion of treatment until follow-up, suggesting that (at least for some patients) a longer treatment period or refresher sessions could be advisable[3]. It seems likely that such prolonged CBT-based multimodal treatment interventions could lead to more sustained psychological and possibly physiological effects[3]. Moreover, if the negative association between tinnitus-related distress and hair-BDNF levels is replicated by further research, this may suggest that additional interventions could be useful to counteract reduced BDNF levels in BT, such as regular physical exercise[180], nutritional interventions[181], or mindfulness-based interventions[175]. Further research is required to establish whether the therapeutic efficacy of multimodal CBT-based treatment can be increased by longer treatment duration/refresher sessions and additional lifestyle-oriented interventions targeting BDNF levels.

5. CONCLUSION

The aims of this thesis were to 1) investigate the influence of stress-related mental comorbidities on BT and their mediation effects for hearing-related factors and physical comorbidities, 2) explore associations of tinnitus-related and psychological factors with hair-cortisol and hair-BDNF, and 3) explore the potential of hair-cortisol and hair-BDNF as therapeutic efficacy markers in BT.

Publication 1 revealed dependencies of mental comorbidities, hearing-related factors, and physical comorbidities in their associations with BT[1]. The results show that anxiety and depression have mediating influences on hearing-related factors and other physical symptoms, whereas hearing-related factors have mediating influences on mental symptoms in BT[1]. Overall, this indicates the presence of interactions between psychological, hearing-related, musculoskeletal, cardiovascular, and endocrine factors in BT that should be investigated in more detail. Ideally, further research on this topic should aim to reduce the risk of self-report bias by drawing on other information sources, like medical records and audiometric testing. Clinically, the results emphasize that mental symptoms are relevant treatment targets in BT due to their direct and indirect effects[1]. However, they should be considered in the broader context of the medical status of the affected individual, including hearing impairment and other physical symptoms, as well as interactions between these factors[1]. Therefore, treatment for BT should simultaneously address hearing-related problems and comorbid physical and mental symptoms[1].

The main finding of publication 2 was a negative relationship between tinnitus-related distress and hair-BDNF levels[2]. This is the first report of this association, as hair-BDNF levels have not been previously investigated in tinnitus patients[2]. It suggests that a reduction in BDNF levels due to chronic stress, as in depression, may also be present in BT. Further studies are needed to confirm this finding. Subsequently, the interplay between tinnitus-related distress and depressive symptoms in their influences on hair-BDNF levels in BT as well as underlying mechanisms should be investigated further. In addition, effects of tinnitus loudness on hair-cortisol and hair-BDNF levels were observed; however, these findings were limited by possible distortions due to imputation[2]. Ideally, future studies investigating the relationship between tinnitus-related distress/tinnitus loudness and hair-biomarkers should include larger tinnitus samples with mild and severe distress levels and control subjects.

Publication 3 was the first to examine the effects of multimodal CBT-based treatment on hair-cortisol and hair-BDNF levels in tinnitus patients[3]. The results indicate that the treatment was effective in reducing tinnitus-related distress and perceived stress levels but had no effect on the investigated hair-biomarkers[3]. The comparatively short treatment duration of approx. five days could be a possible explanation for the lack of hair-biomarker changes[3]. For further research on this topic, it seems advisable to take measures to control for confounding medical factors such as medication and comorbidity, to include a control group, and use a larger sample to examine treatment-related changes in hair-biomarkers in BT. Despite its exploratory nature, this study raises some interesting clinical questions for future research, namely whether providing CBT-based multimodal interventions over several weeks or extending it by refresher sessions, or including additional lifestyle-oriented interventions (targeting exercise, diet, or mindfulness) could lead to more sustained psychological treatment effects and counteract potentially decreased hair-BDNF levels in BT.

Taken together, these results indicate that influences of stress-related mental symptoms, hearing-related factors, and physical symptoms on BT appear interconnected, and tentatively suggest that BT may be characterized by stress-related reductions in hair-BDNF levels. The investigated factors and their assumed associations are summarized in Figure 10. Overall, these findings reinforce the idea that BT can be characterized as a stress-related psychosomatic disorder and emphasize the importance of a holistic perspective as well as a focus on stress-related processes in treatment.

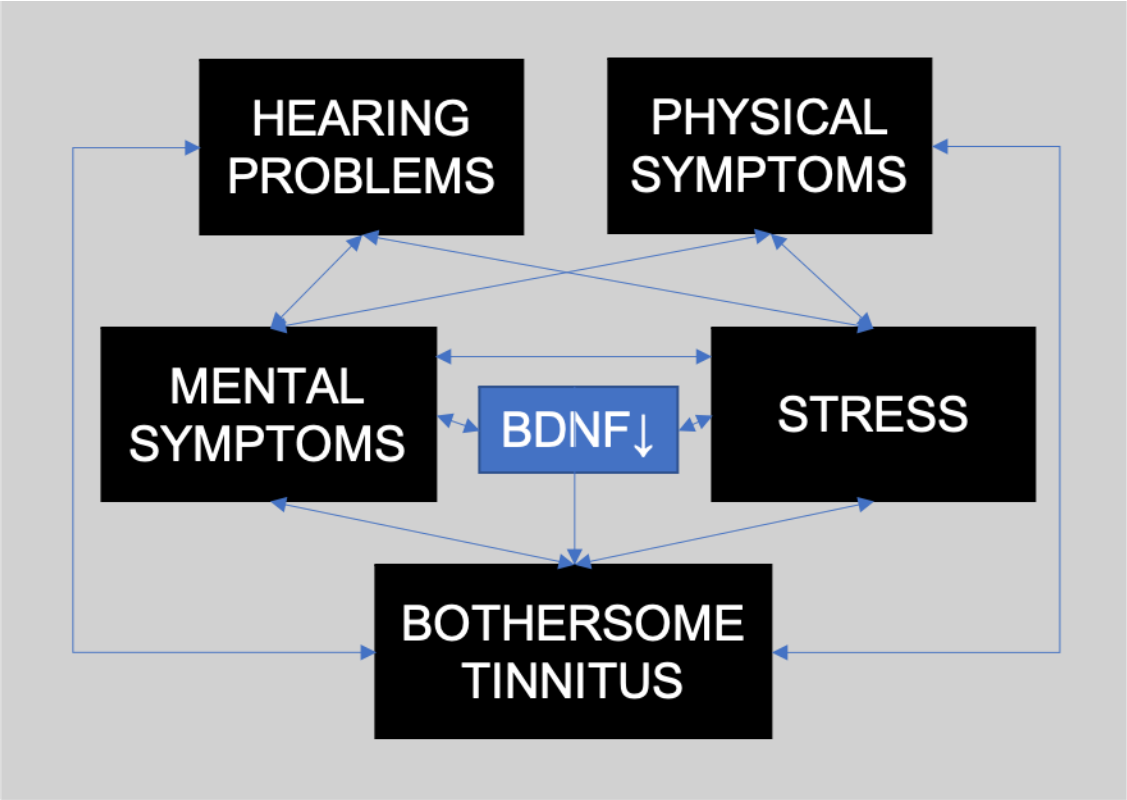

Figure 10. Illustration of the assumed interplay between hearing-related problems, physical symptoms, mental symptoms, (chronic) stress, and reduced BDNF levels in bothersome tinnitus. Newly created figure by the author.

REFERENCES

1. Basso L, Boecking B, Brueggemann P, Pedersen NL, Canlon B, Cederroth CR, Mazurek B. Subjective hearing ability, physical and mental comorbidities in individuals with bothersome tinnitus in a Swedish population sample. In: Schlee W, Langguth B, Kleinjung T, Vanneste S, De Ridder D, eds. *Progress in Brain Research*. Vol 260. Elsevier; 2021:51-78. doi:10.1016/bs.pbr.2020.10.001
2. Basso L, Boecking B, Neff P, Brueggemann P, Peters EMJ, Mazurek B. Hair-cortisol and hair-BDNF as biomarkers of tinnitus loudness and distress in chronic tinnitus. *Sci Rep*. 2022;12(1):1934. doi:10.1038/s41598-022-04811-0
3. Basso L, Boecking B, Neff P, Brueggemann P, Mazurek B, Peters EMJ. Psychological treatment effects unrelated to hair-cortisol and hair-BDNF levels in chronic tinnitus. *Front Psychiatry*. 2022;13:764368. doi:10.3389/fpsyt.2022.764368
4. Haider HF, Bojić T, Ribeiro SF, Paço J, Hall DA, Szczepek AJ. Pathophysiology of Subjective Tinnitus: Triggers and Maintenance. *Front Neurosci*. 2018;12. doi:10.3389/fnins.2018.00866
5. Langguth B. A review of tinnitus symptoms beyond 'ringing in the ears': a call to action. *Curr Med Res Opin*. 2011;27(8):1635-1643. doi:10.1185/03007995.2011.595781
6. De Ridder D, Schlee W, Vanneste S, Londero A, Weisz N, Kleinjung T, Shekhawat GS, Elgoyhen AB, Song JJ, Andersson G, Adhia D, de Azevedo AA, Baguley DM, Biesinger E, Binetti AC, Del Bo L, Cederroth CR, Cima R, Eggermont JJ, Figueiredo R, Fuller TE, Gallus S, Gilles A, Hall DA, Van de Heyning P, Hoare DJ, Khedr EM, Kikidis D, Kleinstaeuber M, Kreuzer PM, Lai JT, Lainez JM, Landgrebe M, Li LPH, Lim HH, Liu TC, Lopez-Escamez JA, Mazurek B, Moller AR, Neff P, Pantev C, Park SN, Piccirillo JF, Poeppl TB, Rauschecker JP, Salvi R, Sanchez TG, Schecklmann M, Schiller A, Searchfield GD, Tyler R, Vielsmeier V, Vlaeyen JWS, Zhang J, Zheng Y, de Nora M, Langguth B. Tinnitus and tinnitus disorder: Theoretical and operational definitions (an international multidisciplinary proposal). In: *Progress in Brain Research*. Vol 260. Elsevier; 2021:1-25. doi:10.1016/bs.pbr.2020.12.002
7. Henry JA, Reavis KM, Griest SE, Thielman EJ, Theodoroff SM, Grush LD, Carlson KF. Tinnitus: An Epidemiologic Perspective. *Otolaryngol Clin North Am*. 2020;53(4):481-499. doi:10.1016/j.otc.2020.03.002
8. Tyler RS, Baker LJ. Difficulties Experienced by Tinnitus Sufferers. *J Speech Hear Disord*. 1983;48(2):150-154. doi:10.1044/jshd.4802.150
9. Biswas R, Hall DA. Prevalence, Incidence, and Risk Factors for Tinnitus. In: Searchfield GD, Zhang J, eds. *The Behavioral Neuroscience of Tinnitus*. Current Topics in Behavioral Neurosciences. Vol 51. Springer International Publishing; 2020:3-28. doi:10.1007/7854_2020_154
10. McCormack A, Edmondson-Jones M, Somerset S, Hall D. A systematic review of the reporting of tinnitus prevalence and severity. *Hear Res*. 2016;337:70-79. doi:10.1016/j.heares.2016.05.009
11. Gallus S, Lugo A, Garavello W, Bosetti C, Santoro E, Colombo P, Perin P, La Vecchia C, Langguth B. Prevalence and Determinants of Tinnitus in the Italian Adult Population. *Neuroepidemiology*. 2015;45(1):12-19. doi:10.1159/000431376
12. Nondahl DM, Cruickshanks KJ, Huang GH, Klein BEK, Klein R, Nieto FJ, Tweed TS. Tinnitus and its risk factors in the Beaver Dam offspring study. *Int J Audiol*. 2011;50(5):313-320. doi:10.3109/14992027.2010.551220
13. Michikawa T, Nishiwaki Y, Kikuchi Y, Saito H, Mizutari K, Okamoto M, Takebayashi T.

Prevalence and Factors Associated with Tinnitus: A Community-Based Study of Japanese Elders. *J Epidemiol*. 2010;20(4):271-276. doi:10.2188/jea.JE20090121

14. Kim HJ, Lee HJ, An SY, Sim S, Park B, Kim SW, Lee JS, Hong SK, Choi HG. Analysis of the Prevalence and Associated Risk Factors of Tinnitus in Adults. Chen L, ed. *PLOS ONE*. 2015;10(5):e0127578. doi:10.1371/journal.pone.0127578

15. Bhatt JM, Lin HW, Bhattacharyya N. Prevalence, Severity, Exposures, and Treatment Patterns of Tinnitus in the United States. *JAMA Otolaryngol Neck Surg*. 2016;142(10):959. doi:10.1001/jamaoto.2016.1700

16. Han BI, Lee HW, Kim TY, Lim JS, Shin KS. Tinnitus: Characteristics, Causes, Mechanisms, and Treatments. *J Clin Neurol*. 2009;5(1):11-19. doi:10.3988/jcn.2009.5.1.11

17. Langguth B, Kreuzer PM, Kleinjung T, De Ridder D. Tinnitus: causes and clinical management. *Lancet Neurol*. 2013;12(9):920-930. doi:10.1016/S1474-4422(13)70160-1

18. Møller AR. Pathology of the Auditory System that Can Cause Tinnitus. In: Møller AR, Langguth B, De Ridder D, Kleinjung T, eds. *Textbook of Tinnitus*. Springer New York; 2011:77-93. doi:10.1007/978-1-60761-145-5_10

19. De Ridder D. A Heuristic Pathophysiological Model of Tinnitus. In: Møller AR, Langguth B, De Ridder D, Kleinjung T, eds. *Textbook of Tinnitus*. Springer New York; 2011:171-197. doi:10.1007/978-1-60761-145-5_21

20. De Ridder D, Vanneste S, Weisz N, Londero A, Schlee W, Elgoyhen AB, Langguth B. An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci Biobehav Rev*. 2014;44:16-32. doi:10.1016/j.neubiorev.2013.03.021

21. Schlee W, Lorenz I, Hartmann T, Müller N, Schulz H, Weisz N. A Global Brain Model of Tinnitus. In: Møller AR, Langguth B, De Ridder D, Kleinjung T, eds. *Textbook of Tinnitus*. Springer New York; 2011:161-169. doi:10.1007/978-1-60761-145-5_20

22. Elgoyhen AB, Langguth B, De Ridder D, Vanneste S. Tinnitus: perspectives from human neuroimaging. *Nat Rev Neurosci*. 2015;16(10):632-642. doi:10.1038/nrn4003

23. Kleinjung T, De Ridder D. Introduction. In: Møller AR, Langguth B, De Ridder D, Kleinjung T, eds. *Textbook of Tinnitus*. Springer New York; 2011:277-277. doi:10.1007/978-1-60761-145-5_33

24. Baracca G, Del Bo L, Ambrosetti U. Tinnitus and Hearing Loss. In: Møller AR, Langguth B, De Ridder D, Kleinjung T, eds. *Textbook of Tinnitus*. Springer New York; 2011:285-291. doi:10.1007/978-1-60761-145-5_35

25. Kleinjung T. Conductive and Cochlear Hearing Loss. In: Møller AR, Langguth B, De Ridder D, Kleinjung T, eds. *Textbook of Tinnitus*. Springer New York; 2011:279-284. doi:10.1007/978-1-60761-145-5_34

26. Baguley D, McFerran D, Hall D. Tinnitus. *The Lancet*. 2013;382(9904):1600-1607. doi:10.1016/S0140-6736(13)60142-7

27. Henry JA, Dennis KC, Schechter MA. General Review of Tinnitus. *J Speech Lang Hear Res*. 2005;48(5):1204-1235. doi:10.1044/1092-4388(2005/084)

28. Enrico P, Goodey R. Complications to Medical Treatment. In: Møller AR, Langguth B, De Ridder D, Kleinjung T, eds. *Textbook of Tinnitus*. Springer New York; 2011:343-361. doi:10.1007/978-1-60761-145-5_42

29. Ying YLM, Arriaga MA. Tinnitus and Ménière's Disease. In: Møller AR, Langguth B, De Ridder D, Kleinjung T, eds. *Textbook of Tinnitus*. Springer New York; 2011:311-316. doi:10.1007/978-1-60761-145-5_38

30. Michiels S, Ganz Sanchez T, Oron Y, Gilles A, Haider HF, Erlandsson S, Bechter K,

Vielsmeier V, Biesinger E, Nam EC, Oiticica J, de Medeiros ÍRT, Bezerra Rocha C, Langguth B, Van de Heyning P, De Hertogh W, Hall DA. Diagnostic Criteria for Somatosensory Tinnitus: A Delphi Process and Face-to-Face Meeting to Establish Consensus. *Trends Hear*. 2018;22:2331216518796403. doi:10.1177/2331216518796403

31. Sanchez TG, Rocha CB. Tinnitus Caused and Influenced by the Somatosensory System. In: Møller AR, Langguth B, De Ridder D, Kleinjung T, eds. *Textbook of Tinnitus*. Springer New York; 2011:363-368. doi:10.1007/978-1-60761-145-5_43

32. Brueggemann P, Mebus W, Boecking B, Amarjargal N, Niemann U, Spiliopoulou M, Dobel C, Rose M, Mazurek B. Dimensions of Tinnitus-Related Distress. *Brain Sci*. 2022;12(2):275. doi:10.3390/brainsci12020275

33. Hoekstra CEL, Wesdorp FM, van Zanten GA. Socio-Demographic, Health, and Tinnitus Related Variables Affecting Tinnitus Severity. *Ear Hear*. 2014;35(5):544-554. doi:10.1097/AUD.0000000000000045

34. Beukes EW, Manchaiah V, Allen PM, Andersson G, Baguley DM. Exploring tinnitus heterogeneity. In: *Progress in Brain Research*. Vol 260. Elsevier; 2021:79-99. doi:10.1016/bs.pbr.2020.05.022

35. Kleinstäuber M, Weise C. Psychosocial Variables That Predict Chronic and Disabling Tinnitus: A Systematic Review. In: Searchfield GD, Zhang J, eds. *The Behavioral Neuroscience of Tinnitus*. Current Topics in Behavioral Neurosciences. Vol 51. Springer International Publishing; 2021:361-380. doi:10.1007/7854_2020_213

36. Brueggemann P, Szczepek A, Rose M, McKenna L, Olze H, Mazurek B. Impact of multiple factors on the degree of tinnitus distress. *Front Hum Neurosci*. 2016;10. doi:10.3389/fnhum.2016.00341

37. Stobik C, Weber RK, Münte TF, Walter M, Frommer J. Evidence of psychosomatic influences in compensated and decompensated tinnitus: evidencia de influencias psicosomáticas en acúfenos compensados y descompensados. *Int J Audiol*. 2005;44(6):370-378. doi:10.1080/14992020500147557

38. Sun H, Feng G, Yu H, Su Y, Zhang F, Feng J, Gao Z. Risk factors of decompensated tinnitus and the interaction effect of anxiety and poor sleep on decompensated tinnitus: a multicenter study. *Acta Otolaryngol (Stockh)*. 2021;141(12):1049-1054. doi:10.1080/00016489.2021.1922754

39. Schubert NMA, Rosmalen JGM, van Dijk P, Pyott SJ. A retrospective cross-sectional study on tinnitus prevalence and disease associations in the Dutch population-based cohort Lifelines. *Hear Res*. 2021;411:108355. doi:10.1016/j.heares.2021.108355

40. Pattyn T, Van Den Eede F, Vanneste S, Cassiers L, Veltman DJ, Van De Heyning P, Sabbe BCG. Tinnitus and anxiety disorders: A review. *Hear Res*. 2016;333:255-265. doi:10.1016/j.heares.2015.08.014

41. Geocze L, Mucci S, Abranches DC, Marco MA de, Penido N de O. Systematic review on the evidences of an association between tinnitus and depression. *Braz J Otorhinolaryngol*. 2013;79(1):106-111. doi:10.5935/1808-8694.20130018

42. Langguth B, Landgrebe M, Kleinjung T, Sand GP, Hajak G. Tinnitus and depression. *World J Biol Psychiatry*. 2011;12(7):489-500. doi:10.3109/15622975.2011.575178

43. Salazar JW, Meisel K, Smith ER, Quiggle A, McCoy DB, Amans MR. Depression in Patients with Tinnitus: A Systematic Review. *Otolaryngol Head Neck Surg*. 2019;161(1):28-35. doi:10.1177/0194599819835178

44. Pinto PCL, Marcelos CM, Mezzasalma MA, Osterne FJV, de Melo Tavares de Lima MA, Nardi AE. Tinnitus and its association with psychiatric disorders: Systematic review. *J Laryngol Otol*. 2014;128(08):660-664. doi:10.1017/S0022215114001030

45. Malakouti S, Mahmoudian M, Alifattahi N, Salehi M. Comorbidity of chronic tinnitus and mental disorders. *Int Tinnitus J*. 2011;16(2):118-122.

46. Holgers KM, Erlandsson SI, Barrenäs ML. Predictive factors for the severity of tinnitus. *Audiol Off Organ Int Soc Audiol*. 2000;39(5):284-291. doi:10.3109/00206090009073093

47. Park KH, Lee SH, Koo JW, Park HY, Lee KY, Choi YS, Oh KW, Lee A, Yang JE, Woo SY, Kim SW, Cho YS. Prevalence and Associated Factors of Tinnitus: Data From the Korean National Health and Nutrition Examination Survey 2009–2011. *J Epidemiol*. 2014;24(5):417-426. doi:10.2188/jea.JE20140024

48. Nondahl DM, Cruickshanks KJ, Wiley TL, Klein R, Klein BEK, Tweed TS. Prevalence and 5-year incidence of tinnitus among older adults: the epidemiology of hearing loss study. *J Am Acad Audiol*. 2002;13(6):323-331.

49. Cima RFF, Mazurek B, Haider H, Kikidis D, Lapira A, Noreña A, Hoare DJ. A multidisciplinary European guideline for tinnitus: diagnostics, assessment, and treatment. *HNO*. 2019;67(1):10-42. doi:10.1007/s00106-019-0633-7

50. Fuller T, Cima R, Langguth B, Mazurek B, Vlaeyen JW, Hoare DJ. Cognitive behavioural therapy for tinnitus. *Cochrane Database Syst Rev*. 2020;2020(1):CD012614. doi:10.1002/14651858.CD012614.pub2

51. Martinez-Devesa P, Perera R, Theodoulou M, Waddell A. Cognitive behavioural therapy for tinnitus. *Cochrane Database Syst Rev*. 2010;(9). doi:10.1002/14651858.CD005233.pub3

52. Hofmann SG, Asnaani A, Vonk IJJ, Sawyer AT, Fang A. The Efficacy of Cognitive Behavioral Therapy: A Review of Meta-analyses. *Cogn Ther Res*. 2012;36(5):427-440. doi:10.1007/s10608-012-9476-1

53. Caldirola D, Teggi R, Daccò S, Sangiorgio E, Bussi M, Perna G. Role of worry in patients with chronic tinnitus and sensorineural hearing loss: a preliminary study. *Eur Arch Otorhinolaryngol*. 2016;273(12):4145-4151. doi:10.1007/s00405-016-4100-8

54. Kleinstäuber M, Jasper K, Schweda I, Hiller W, Andersson G, Weise C. The Role of Fear-Avoidance Cognitions and Behaviors in Patients with Chronic Tinnitus. *Cogn Behav Ther*. 2013;42(2):84-99. doi:10.1080/16506073.2012.717301

55. Cima RFF, van Breukelen G, Vlaeyen JWS. Tinnitus-related fear: Mediating the effects of a cognitive behavioural specialised tinnitus treatment. *Hear Res*. 2018;358:86-97. doi:10.1016/j.heares.2017.10.003

56. Lee SY, Kim JH, Hong SH, Lee DS. Roles of Cognitive Characteristics in Tinnitus Patients. *J Korean Med Sci*. 2004;19(6):864. doi:10.3346/jkms.2004.19.6.864

57. Weise C, Hesser H, Andersson G, Nyenhuis N, Zastrutzki S, Kröner-Herwig B, Jäger B. The role of catastrophizing in recent onset tinnitus: Its nature and association with tinnitus distress and medical utilization. *Int J Audiol*. 2013;52(3):177-188. doi:10.3109/14992027.2012.752111

58. Beukes EW, Manchaiah V, Andersson G, Allen PM, Terlizzi PM, Baguley DM. Situationally influenced tinnitus coping strategies: a mixed methods approach. *Disabil Rehabil*. 2018;40(24):2884-2894. doi:10.1080/09638288.2017.1362708

59. Meijers SM, Lieftink AF, Stegeman I, Smit AL. Coping in Chronic Tinnitus Patients. *Front Neurol*. 2020;11:570989. doi:10.3389/fneur.2020.570989

60. Bartels H, Middel BL, van der Laan BF a. M, Staal MJ, Albers FWJ. The additive effect of co-occurring anxiety and depression on health status, quality of life and coping strategies in help-seeking tinnitus sufferers. *Ear Hear*. 2008;29(6):947-956. doi:10.1097/AUD.0b013e3181888f83

61. Cima RFF, Andersson G, Schmidt CJ, Henry JA. Cognitive-Behavioral Treatments for Tinnitus: A Review of the Literature. *J Am Acad Audiol*. 2014;25(1):29-61. doi:10.3766/jaaa.25.1.4

62. Brueggemann P, Otto J, Lorenz N, Schorsch S, Szczepek AJ, Boecking B, Mazurek B. Long-term changes in multimodal intensive tinnitus therapy: A 5-year follow-up. *HNO*. 2018;66(S1):34-38. doi:10.1007/s00106-017-0463-4

63. Brueggemann P, Seydel C, Schaefer C, Szczepek AJ, Amarjargal N, Boecking B, Rose M, Mazurek B. ICD-10 Symptom Rating questionnaire for assessment of psychological comorbidities in patients with chronic tinnitus. *HNO*. 2019;67(3):178-183. doi:10.1007/s00106-019-0625-7

64. Seydel C, Haupt H, Szczepek AJ, Klapp BF, Mazurek B. Long-Term Improvement in Tinnitus after Modified Tinnitus Retraining Therapy Enhanced by a Variety of Psychological Approaches. *Audiol Neurotol*. 2010;15(2):69-80. doi:10.1159/000231632

65. Seydel C, Haupt H, Szczepek AJ, Hartmann A, Rose M, Mazurek B. Three Years Later: Report on the State of Well-Being of Patients with Chronic Tinnitus Who Underwent Modified Tinnitus Retraining Therapy. *Audiol Neurotol*. 2015;20(1):26-38. doi:10.1159/000363728

66. Nater UM. The multidimensionality of stress and its assessment. *Brain Behav Immun*. 2018;73:159-160. doi:10.1016/j.bbi.2018.07.018

67. O'Connor DB, Thayer JF, Vedhara K. Stress and Health: A Review of Psychobiological Processes. *Annu Rev Psychol*. 2021;72:663-688. doi:10.1146/annurev-psych-062520-122331

68. McEwen BS, Bowles NP, Gray JD, Hill MN, Hunter RG, Karatsoreos IN, Nasca C. Mechanisms of stress in the brain. *Nat Neurosci*. 2015;18(10):1353-1363. doi:10.1038/nn.4086

69. McEwen BS. Protection and Damage from Acute and Chronic Stress: Allostasis and Allostatic Overload and Relevance to the Pathophysiology of Psychiatric Disorders. *Ann N Y Acad Sci*. 2004;1032(1):1-7. doi:10.1196/annals.1314.001

70. McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med*. 1998;338(3):171-179. doi:10.1056/NEJM199801153380307

71. Tsigos C, Kyrou I, Kassi E, Chrousos GP. Stress: Endocrine Physiology and Pathophysiology. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, Dungan K, Hershman JM, Hofland J, Kalra S, Kaltsas G, Koch C, Kopp P, Korbonits M, Kovacs CS, Kuohung W, Laferrère B, Levy M, McGee EA, McLachlan R, Morley JE, New M, Purnell J, Sahay R, Singer F, Sperling MA, Stratakis CA, Trence DL, Wilson DP, eds. *Endotext*. MDText.com, Inc.; 2000. Accessed March 2, 2022. https://web.archive.org/web/20220317222837/http://www.ncbi.nlm.nih.gov/books/NBK278995/

72. Szczepek AJ, Mazurek B. Neurobiology of Stress-Induced Tinnitus. In: Searchfield GD, Zhang J, eds. *The Behavioral Neuroscience of Tinnitus*. Current Topics in Behavioral Neurosciences. Vol 51. Springer International Publishing; 2021:327-347. doi:10.1007/7854_2020_215

73. Mazurek B, Szczepek AJ, Hebert S. Stress and tinnitus. *HNO*. 2015;63(4):258-265. doi:10.1007/s00106-014-2973-7

74. Mazurek B, Boecking B, Brueggemann P. Association Between Stress and Tinnitus—New Aspects. *Otol Neurotol*. 2019;40(4):e467-e473. doi:10.1097/MAO.0000000000002180

75. Elarbed A, Fackrell K, Baguley DM, Hoare DJ. Tinnitus and stress in adults: a scoping review. *Int J Audiol*. 2021;60(3):171-182. doi:10.1080/14992027.2020.1827306

76. Hébert S, Mazurek B, Szczepek AJ. Stress-Related Psychological Disorders and Tinnitus. In: Szczepek AJ, Mazurek B, eds. *Tinnitus and Stress*. Springer International Publishing; 2017:37-51. doi:10.1007/978-3-319-58397-6_3

77. Probst T, Pryss R, Langguth B, Schlee W. Emotional states as mediators between tinnitus loudness and tinnitus distress in daily life: Results from the “TrackYourTinnitus” application. *Sci Rep*. 2016;6(1). doi:10.1038/srep20382

78. Hébert S, Canlon B, Hasson D. Emotional Exhaustion as a Predictor of Tinnitus. *Psychother Psychosom*. 2012;81(5):324-326. doi:10.1159/000335043

79. Ciminelli P, Machado S, Palmeira M, Carta MG, Beirith SC, Nigri ML, Mezzasalma MA, Nardi AE. Tinnitus: The Sound of Stress? *Clin Pract Epidemiol Ment Health*. 2018;14(1):264-269. doi:10.2174/1745017901814010264

80. Baigi A, Oden A, Almlid-Larsen V, Barrenäs ML, Holgers KM. Tinnitus in the General Population With a Focus on Noise and Stress: A Public Health Study: *Ear Hear*. 2011;32(6):787-789. doi:10.1097/AUD.0b013e31822229bd

81. Craske MG, Stein MB, Eley TC, Milad MR, Holmes A, Rapee RM, Wittchen HU. Anxiety disorders. *Nat Rev Dis Primer*. 2017;3(1):1-19. doi:10.1038/nrdp.2017.24

82. Otte C, Gold SM, Penninx BW, Pariante CM, Etkin A, Fava M, Mohr DC, Schatzberg AF. Major depressive disorder. *Nat Rev Dis Primer*. 2016;2(1):16065. doi:10.1038/nrdp.2016.65

83. Biehl R, Boecking B, Brueggemann P, Grosse R, Mazurek B. Personality Traits, Perceived Stress, and Tinnitus-Related Distress in Patients With Chronic Tinnitus: Support for a Vulnerability-Stress Model. *Front Psychol*. 2020;10:3093. doi:10.3389/fpsyg.2019.03093

84. Boecking B, Brueggemann P, Mazurek B. Tinnitus: psychosomatische Aspekte. *HNO*. 2019;67(2):137-152. doi:10.1007/s00106-019-0609-7

85. Dean J, Keshavan M. The neurobiology of depression: An integrated view. *Asian J Psychiatry*. 2017;27:101-111. doi:10.1016/j.ajp.2017.01.025

86. Juruena MF, Eror F, Cleare AJ, Young AH. The Role of Early Life Stress in HPA Axis and Anxiety. In: Kim YK, ed. *Anxiety Disorders.* Advances in Experimental Medicine and Biology. Springer; 2020:141-153. doi:10.1007/978-981-32-9705-0_9

87. Hébert S, Lupien SJ. The sound of stress: Blunted cortisol reactivity to psychosocial stress in tinnitus sufferers. *Neurosci Lett*. 2007;411(2):138-142. doi:10.1016/j.neulet.2006.10.028

88. Hébert S, Lupien SJ. Salivary cortisol levels, subjective stress, and tinnitus intensity in tinnitus sufferers during noise exposure in the laboratory. *Int J Hyg Environ Health*. 2009;212(1):37-44. doi:10.1016/j.ijheh.2007.11.005

89. Simoens VL, Hébert S. Cortisol suppression and hearing thresholds in tinnitus after low-dose dexamethasone challenge. *BMC Ear Nose Throat Disord*. 2012;12(1):4. doi:10.1186/1472-6815-12-4

90. Chrbolka P, Palúch Z, Hill M, Alušík Š. Circulating steroids negatively correlate with tinnitus. *Steroids*. 2017;123:37-42. doi:10.1016/j.steroids.2017.04.004

91. Hébert S, Paiement P, Lupien SJ. A physiological correlate for the intolerance to both internal and external sounds. *Hear Res*. 2004;190(1-2):1-9. doi:10.1016/S0378-5955(04)00021-8

92. Li J, Jin J, Xi S, Zhu Q, Chen Y, Huang M, He C. Clinical efficacy of cognitive behavioral therapy for chronic subjective tinnitus. *Am J Otolaryngol*. 2019;40(2):253-256. doi:10.1016/j.amjoto.2018.10.017

93. Jackson JG. The Cortisol Awakening Response: A Feasibility Study Investigating the Use of the Area Under the Curve With Respect to Increase as an Effective Objective Measure of Tinnitus Distress. *Am J Audiol*. 2019;28(3):583-596. doi:10.1044/2019_AJA-18-0174

94. Kim DK, Chung DY, Bae SC, Park KH, Yeo SW, Park SN. Diagnostic value and clinical significance of stress hormones in patients with tinnitus. *Eur Arch Otorhinolaryngol*. 2014;271(11):2915-2921. doi:10.1007/s00405-013-2785-5

95. Savastano M, Aita M, Barlani F. Psychological, Neural, Endocrine, and Immune Study of Stress in Tinnitus Patients: Any Correlation between Psychometric and Biochemical Measures? *Ann Otol Rhinol Laryngol*. 2007;116(2):100-106. doi:10.1177/000348940711600204

96. Kang DW, Kim SS, Park DC, Kim SH, Yeo SG. Objective and Measurable Biomarkers in Chronic Subjective Tinnitus. *Int J Mol Sci*. 2021;22(12):6619. doi:10.3390/ijms22126619

97. Mazurek B. Tinnitus: from basic principles to therapy. *HNO*. 2015;63(4):253-257. doi:10.1007/s00106-014-2974-6

98. Szczepek AJ, Mazurek B. Stress-Related Blood Biomarkers. In: Szczepek AJ, Mazurek B, eds. *Tinnitus and Stress: An Interdisciplinary Companion for Healthcare Professionals*. Springer International Publishing; 2017:95-116. doi:10.1007/978-3-319-58397-6_6

99. Binder DK, Scharfman HE. Brain-derived Neurotrophic Factor. *Growth Factors*. 2004;22(3):123-131. doi:10.1080/08977190410001723308

100. Kowiański P, Lietzau G, Czuba E, Waśkow M, Steliga A, Moryś J. BDNF: A Key Factor with Multipotent Impact on Brain Signaling and Synaptic Plasticity. *Cell Mol Neurobiol*. 2018;38(3):579-593. doi:10.1007/s10571-017-0510-4

101. Zuccato C, Cattaneo E. Brain-derived neurotrophic factor in neurodegenerative diseases. *Nat Rev Neurol*. 2009;5(6):311-322. doi:10.1038/nrneurol.2009.54

102. Castrén E, Rantamäki T. The role of BDNF and its receptors in depression and antidepressant drug action: Reactivation of developmental plasticity. *Dev Neurobiol*. 2010;70(5):289-297. doi:10.1002/dneu.20758

103. Sen S, Duman R, Sanacora G. Serum BDNF, Depression and Anti-Depressant Medications: Meta-Analyses and Implications. *Biol Psychiatry*. 2008;64(6):527-532. doi:10.1016/j.biopsych.2008.05.005

104. Coskunoglu A, Orenay-Boyacioglu S, Deveci A, Bayam M, Onur E, Onan A, Cam F. Evidence of associations between brain-derived neurotrophic factor (BDNF) serum levels and gene polymorphisms with tinnitus. *Noise Health*. 2017;19(88):140. doi:10.4103/nah.NAH_74_16

105. Goto F, Saruta J, Kanzaki S, To M, Tsutsumi T, Tsukinoki K, Ogawa K. Various levels of plasma brain-derived neurotrophic factor in patients with tinnitus. *Neurosci Lett*. 2012;510(2):73-77. doi:10.1016/j.neulet.2012.01.001

106. Xiong H, Yang H, Liang M, Ou Y, Huang X, Cai Y, Lai L, Pang J, Zheng Y. Plasma brain-derived neurotrophic factor levels are increased in patients with tinnitus and correlated with therapeutic effects. *Neurosci Lett*. 2016;622:15-18. doi:10.1016/j.neulet.2016.04.032

107. Szczepek AJ, Haupt H, Klapp BF, Olze H, Mazurek B. Biological correlates of tinnitus-related distress: An exploratory study. *Hear Res*. 2014;318:23-30. doi:10.1016/j.heares.2014.10.007

108. Harb H, González-de-la-Vara M, Thalheimer L, Klein U, Renz H, Rose M, Kruse J, Potaczek DP, Peters EMJ. Assessment of Brain Derived Neurotrophic Factor in hair to study stress responses: A pilot investigation. *Psychoneuroendocrinology*. 2017;86:134-

143. doi:10.1016/j.psyneuen.2017.09.007

109. Stalder T, Steudte-Schmiedgen S, Alexander N, Klucken T, Vater A, Wichmann S, Kirschbaum C, Miller R. Stress-related and basic determinants of hair cortisol in humans: A meta-analysis. *Psychoneuroendocrinology*. 2017;77:261-274. doi:10.1016/j.psyneuen.2016.12.017

110. Russell E, Koren G, Rieder M, Van Uum S. Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions. *Psychoneuroendocrinology*. 2012;37(5):589-601. doi:10.1016/j.psyneuen.2011.09.009

111. Stalder T, Kirschbaum C. Analysis of cortisol in hair – State of the art and future directions. *Brain Behav Immun*. 2012;26(7):1019-1029. doi:10.1016/j.bbi.2012.02.002

112. Wosu AC, Valdimarsdóttir U, Shields AE, Williams DR, Williams MA. Correlates of cortisol in human hair: implications for epidemiologic studies on health effects of chronic stress. *Ann Epidemiol*. 2013;23(12):797-811.e2. doi:10.1016/j.annepidem.2013.09.006

113. Almqvist C, Adami HO, Franks PW, Groop L, Ingelsson E, Kere J, Lissner L, Litton JE, Maeurer M, Michaëlsson K, Palmgren J, Pershagen G, Ploner A, Sullivan PF, Tybring G, Pedersen NL. LifeGene—a large prospective population-based study of global relevance. *Eur J Epidemiol*. 2011;26(1):67-77. doi:10.1007/s10654-010-9521-x

114. LifeGene. Description of the LifeGene Resource. Published online February 3, 2017. Accessed March 27, 2020. https://web.archive.org/web/20200923003953/https://lifegene.se/wp-content/uploads/1LifeGeneresource20170203version24.pdf

115. Basso L, Boecking B, Brueggemann P, Pedersen NL, Canlon B, Cederroth CR, Mazurek B. Gender-Specific Risk Factors and Comorbidities of Bothersome Tinnitus. *Front Neurosci*. 2020;14:706. doi:10.3389/fnins.2020.00706

116. IBM Corp. *IBM SPSS Statistics for Windows*. IBM Corp.; 2017.

117. Herr NR. Mediation with Dichotomous Outcomes. Published February 13, 2006. Accessed February 2, 2020. http://web.archive.org/web/20200202182013/http://nrhpsych.com/mediation/logmed.html

118. Mackinnon DP, Dwyer JH. Estimating Mediated Effects in Prevention Studies. *Eval Rev*. 1993;17(2):144-158. doi:10.1177/0193841X9301700202

119. Aroian LA. The Probability Function of the Product of Two Normally Distributed Variables. *Ann Math Stat*. 1947;18(2):265-271. doi:10.1214/aoms/1177730442

120. Goebel G, Hiller W. *Tinnitus-Fragebogen (TF): Ein Instrument zur Erfassung von Belastung und Schweregrad bei Tinnitus*. Hogrefe; 1998.

121. Fliege H, Rose M, Arck P, Walter OB, Kocalevent RD, Weber C, Klapp BF. The Perceived Stress Questionnaire (PSQ) Reconsidered: Validation and Reference Values From Different Clinical and Healthy Adult Samples: *Psychosom Med*. 2005;67(1):78-88. doi:10.1097/01.psy.0000151491.80178.78

122. Fliege H, Rose M, Arck P, Levenstein S, Klapp BF. Validation of the “Perceived Stress Questionnaire” (PSQ) in a German sample. *Diagnostica*. 2001;47(3):142-152. doi:10.1026//0012-1924.47.3.142

123. Herrmann-Lingen C, Buss U, Snaith RP. *Hospital Anxiety and Depression Scale - Deutsche Version*. Huber; 2011.

124. Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. *Acta Psychiatr Scand*. 1983;67(6):361-370. doi:10.1111/j.1600-0447.1983.tb09716.x

125. Spielberger C, Gorsuch RI, Lushene R, Vagg P, Jacobs G. Manual for the state-trait inventory. *Consult Psychol Palo Alto Calif*. 1970.

126. Laux L, Glanzmann P, Schaffner P, Spielberger CD. *Das State-Trait-Angstinventar (STAI)*. Beltz; 1981.

127. Rief W, Hiller W, Heuser J. *SOMS—Das Screening Für Somatoforme Störungen. Manual Zum Fragebogen.* Huber-Verlag; 1997.

128. Ehlers A, Steil R, Winter H, Foa E. *Deutsche Übersetzung Der Posttraumatic Diagnostic Scale (PDS)*. Oxford University, Warneford Hospital, Department of Psychiatry; 1996.

129. Wirtz MA, Morfeld M, Glaesmer H, Brähler E. Konfirmatorische Prüfung der Skalenstruktur des SF-12 Version 2.0 in einer deutschen bevölkerungs-repräsentativen Stichprobe. *Diagnostica*. 2018;64(2):84-96. doi:10.1026/0012-1924/a000194

130. Wirtz MA, Morfeld M, Glaesmer H, Brähler E. Normierung des SF-12 Version 2.0 zur Messung der gesundheitsbezogenen Lebensqualität in einer deutschen bevölkerungsrepräsentativen Stichprobe. *Diagnostica*. 2018;64(4):215-226. doi:10.1026/0012-1924/a000205

131. Sauvé B, Koren G, Walsh G, Tokmakejian S, Van Uum SHM. Measurement of cortisol in human hair as a biomarker of systemic exposure. *Clin Investig Med Med Clin Exp*. 2007;30(5):E183-191.

132. Wennig R. Potential problems with the interpretation of hair analysis results. *Forensic Sci Int*. 2000;107(1-3):5-12. doi:10.1016/S0379-0738(99)00146-2

133. R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing; 2020. Accessed November 9, 2021. https://www.R-project.org

134. Friedman J, Hastie T, Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent. *J Stat Softw*. 2010;33(1). doi:10.18637/jss.v033.i01

135. Kuhn M. caret: Classification and Regression Training. R package version 6.0-90. 2021. Accessed February 9, 2022. https://CRAN.R-project.org/package=caret

136. Arya S, Mount D, Kemp SE, Jefferis G. RANN: Fast Nearest Neighbour Search (Wraps ANN Library) Using L2 Metric. R package version 2.6.1. 2019. Accessed February 9, 2022. https://CRAN.R-project.org/package=RANN

137. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. *J Stat Softw*. 2015;67:1-48. doi:10.18637/jss.v067.i01

138. Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest Package: Tests in Linear Mixed Effects Models. *J Stat Softw*. 2017;82:1-26. doi:10.18637/jss.v082.i13

139. Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models. *Biom J*. 2008;50(3):346-363. doi:10.1002/bimj.200810425

140. Holm S. A simple sequentially rejective multiple test procedure. *Scand J Stat*. 1979;6(No. 2):65-70.

141. Torgo L. *Data Mining with R, Learning with Case Studies*. 2nd edition. Chapman and Hall/CRC; 2016. Accessed February 9, 2022. http://ltorgo.github.io/DMwR2

142. Yoshida T, Stephens D, Kentala E, Levo H, Auramo Y, Poe D, Pyykkö I. Tinnitus complaint behaviour in long-standing Menière's disorder: its association with the other cardinal symptoms: Tinnitus in Menière's disease. *Clin Otolaryngol*. 2011;36(5):461-467. doi:10.1111/j.1749-4486.2011.02381.x

143. Romero Sánchez I, Pérez Garrigues H, Rodríguez Rivera V. Clinical characteristics of tinnitus in Ménière's disease. *Acta Otorrinolaringol Engl Ed*. 2010;61(5):327-331. doi:10.1016/S2173-5735(10)70060-0

144. Stouffer JL, Tyler RS. Characterization of tinnitus by tinnitus patients. *J Speech Hear Disord*. 1990;55(3):439-453. doi:10.1044/jshd.5503.439

145. Kuttila S, Kuttila M, Bell YL, Alanen P, Suonpää J. Recurrent tinnitus and associated ear symptoms in adults. *Int J Audiol*. 2005;44(3):164-170. doi:10.1080/14992020500057608

146. Bigelow RT, Reed NS, Brewster KK, Huang A, Rebok G, Rutherford BR, Lin FR. Association of Hearing Loss With Psychological Distress and Utilization of Mental Health Services Among Adults in the United States. *JAMA Netw Open*. 2020;3(7):e2010986. doi:10.1001/jamanetworkopen.2020.10986

147. Rutherford BR, Brewster K, Golub JS, Kim AH, Roose SP. Sensation and Psychiatry: Linking Age-Related Hearing Loss to Late-Life Depression and Cognitive Decline. *Am J Psychiatry*. 2018;175(3):215-224. doi:10.1176/appi.ajp.2017.17040423

148. Ge L, Yap CW, Ong R, Heng BH. Social isolation, loneliness and their relationships with depressive symptoms: A population-based study. *PloS One*. 2017;12(8):e0182145. doi:10.1371/journal.pone.0182145

149. Haider HF, Ribeiro SF, Hoare DJ, Fialho G, Hall DA, Antunes M, Caria H, Paço J. Quality of Life and Psychological Distress in Portuguese Older Individuals with Tinnitus. *Brain Sci*. 2021;11(7):953. doi:10.3390/brainsci11070953

150. Ramage-Morin PL, Banks R, Pineault D, Atrach M. Tinnitus in Canada. *Health Rep*. 2019;30(3):3-11. doi:10.25318/82-003-x201900300001-eng

151. Menendez ME, Baker DK, Oladeji LO, Fryberger CT, McGwin G, Ponce BA. Psychological Distress Is Associated with Greater Perceived Disability and Pain in Patients Presenting to a Shoulder Clinic. *JBJS*. 2015;97(24):1999-2003. doi:10.2106/JBJS.O.00387

152. Cohen BE, Edmondson D, Kronish IM. State of the Art Review: Depression, Stress, Anxiety, and Cardiovascular Disease. *Am J Hypertens*. 2015;28(11):1295-1302. doi:10.1093/ajh/hpv047

153. Seligman F, Nemeroff CB. The interface of depression and cardiovascular disease: therapeutic implications. *Ann N Y Acad Sci*. 2015;1345(1):25-35. doi:10.1111/nyas.12738

154. Tully PJ, Harrison NJ, Cheung P, Cosh S. Anxiety and Cardiovascular Disease Risk: a Review. *Curr Cardiol Rep*. 2016;18(12):120. doi:10.1007/s11886-016-0800-3

155. Hage MP, Azar ST. The Link between Thyroid Function and Depression. *J Thyroid Res*. 2012;2012:1-8. doi:10.1155/2012/590648

156. Zader SJ, Williams E, Buryk MA. Mental Health Conditions and Hyperthyroidism. *Pediatrics*. 2019;144(5):e20182874. doi:10.1542/peds.2018-2874

157. Bode H, Ivens B, Bschor T, Schwarzer G, Henssler J, Baethge C. Association of Hypothyroidism and Clinical Depression: A Systematic Review and Meta-analysis. *JAMA Psychiatry*. 2021;78(12):1375-1383. doi:10.1001/jamapsychiatry.2021.2506

158. Boyen K, Langers DRM, de Kleine E, van Dijk P. Gray matter in the brain: Differences associated with tinnitus and hearing loss. *Hear Res*. 2013;295:67-78. doi:10.1016/j.heares.2012.02.010

159. Landgrebe M, Langguth B, Rosengarth K, Braun S, Koch A, Kleinjung T, May A, de Ridder D, Hajak G. Structural brain changes in tinnitus: Grey matter decrease in auditory and non-auditory brain areas. *NeuroImage*. 2009;46(1):213-218. doi:10.1016/j.neuroimage.2009.01.069

160. Profant O, Škoch A, Tintěra J, Svobodová V, Kuchárová D, Svobodová Burianová J, Syka J. The Influence of Aging, Hearing, and Tinnitus on the Morphology of Cortical Gray Matter, Amygdala, and Hippocampus. *Front Aging Neurosci*. 2020;12. doi:10.3389/fnagi.2020.553461

161. Tae WS, Yakunina N, Lee WH, Ryu YJ, Ham HK, Pyun SB, Nam EC. Changes in the regional shape and volume of subcortical nuclei in patients with tinnitus comorbid with

mild hearing loss. *Neuroradiology*. 2018;60(11):1203-1211. doi:10.1007/s00234-018-2093-2

162. Hiller W, Goebel G. When Tinnitus Loudness and Annoyance Are Discrepant: Audiological Characteristics and Psychological Profile. *Audiol Neurotol*. 2007;12(6):391-400. doi:10.1159/000106482

163. Probst T, Pryss RC, Langguth B, Rauschecker JP, Schobel J, Reichert M, Spiliopoulou M, Schlee W, Zimmermann J. Does Tinnitus Depend on Time-of-Day? An Ecological Momentary Assessment Study with the "TrackYourTinnitus" Application. *Front Aging Neurosci*. 2017;9:253. doi:10.3389/fnagi.2017.00253

164. Minen MT, Camprodon J, Nehme R, Chemali Z. The neuropsychiatry of tinnitus: a circuit-based approach to the causes and treatments available. *J Neurol Neurosurg Psychiatry*. 2014;85(10):1138-1144. doi:10.1136/jnnp-2013-307339

165. Leaver AM, Seydell-Greenwald A, Rauschecker JP. Auditory–limbic interactions in chronic tinnitus: Challenges for neuroimaging research. *Hear Res*. 2016;334:49-57. doi:10.1016/j.heares.2015.08.005

166. Aldhafeeri FM, Mackenzie I, Kay T, Alghamdi J, Sluming V. Neuroanatomical correlates of tinnitus revealed by cortical thickness analysis and diffusion tensor imaging. *Neuroradiology*. 2012;54(8):883-892. doi:10.1007/s00234-012-1044-6

167. Shore SE, Roberts LE, Langguth B. Maladaptive plasticity in tinnitus-triggers, mechanisms and treatment. *Nat Rev Neurol*. 2016;12(3):150-160. doi:10.1038/nrneurol.2016.12

168. Schneider P, Andermann M, Wengenroth M, Goebel R, Flor H, Rupp A, Diesch E. Reduced volume of Heschl's gyrus in tinnitus. *NeuroImage*. 2009;45(3):927-939. doi:10.1016/j.neuroimage.2008.12.045

169. Allan TW, Besle J, Langers DRM, Davies J, Hall DA, Palmer AR, Adjamian P. Neuroanatomical Alterations in Tinnitus Assessed with Magnetic Resonance Imaging. *Front Aging Neurosci*. 2016;8. doi:10.3389/fnagi.2016.00221

170. Leaver AM, Renier L, Chevillet MA, Morgan S, Kim HJ, Rauschecker JP. Dysregulation of Limbic and Auditory Networks in Tinnitus. *Neuron*. 2011;69(1):33-43. doi:10.1016/j.neuron.2010.12.002

171. Mühlau M, Rauschecker JP, Oestreicher E, Gaser C, Röttinger M, Wohlschläger AM, Simon F, Etgen T, Conrad B, Sander D. Structural Brain Changes in Tinnitus. *Cereb Cortex*. 2006;16(9):1283-1288. doi:10.1093/cercor/bhj070

172. Melcher JR, Knudson IM, Levine RA. Subcallosal brain structure: Correlation with hearing threshold at supra-clinical frequencies (>8 kHz), but not with tinnitus. *Hear Res*. 2013;295:79-86. doi:10.1016/j.heares.2012.03.013

173. Brunoni AR, Lopes M, Fregni F. A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *Int J Neuropsychopharmacol*. 2008;11(8):1169-1180. doi:10.1017/S1461145708009309

174. Shi SS, Shao SH, Yuan BP, Pan F, Li ZL. Acute Stress and Chronic Stress Change Brain-Derived Neurotrophic Factor (BDNF) and Tyrosine Kinase-Coupled Receptor (TrkB) Expression in Both Young and Aged Rat Hippocampus. *Yonsei Med J*. 2010;51(5):661. doi:10.3349/ymj.2010.51.5.661

175. Gomutbutra P, Yingchankul N, Chattipakorn N, Chattipakorn S, Srisurapanont M. The Effect of Mindfulness-Based Intervention on Brain-Derived Neurotrophic Factor (BDNF): A Systematic Review and Meta-Analysis of Controlled Trials. *Front Psychol*. 2020;11:2209. doi:10.3389/fpsyg.2020.02209

176. Piotrkowicz M, Janoska-Jazdzik M, Koweszko T, Szulc A. The Influence of Psychotherapy on Peripheral Brain-Derived Neurotrophic Factor Concentration Levels and Gene Methylation Status: A Systematic Review. *J Clin Med*. 2021;10(19):4424. doi:10.3390/jcm10194424

177. Batista GEAPA, Monard MC. An analysis of four missing data treatment methods for supervised learning. *Appl Artif Intell*. 2003;17(5-6):519-533. doi:10.1080/713827181

178. Fouad KM, Ismail MM, Azar AT, Arafa MM. Advanced methods for missing values imputation based on similarity learning. *PeerJ Comput Sci*. 2021;7:e619. doi:10.7717/peerj-cs.619

179. Hesser H, Weise C, Rief W, Andersson G. The effect of waiting: A meta-analysis of wait-list control groups in trials for tinnitus distress. *J Psychosom Res*. 2011;70(4):378-384. doi:10.1016/j.jpsychores.2010.12.006

180. Szuhany KL, Bugatti M, Otto MW. A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. *J Psychiatr Res*. 2015;60:56-64. doi:10.1016/j.jpsychires.2014.10.003

181. Gravesteijn E, Mensink RP, Plat J. Effects of nutritional interventions on BDNF concentrations in humans: a systematic review. *Nutr Neurosci*. 2021;0(0):1-12. doi:10.1080/1028415X.2020.1865758

EIDESSTATTLICHE VERSICHERUNG

„Ich, Laura Basso, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „*Comorbidities and stress-related hair-biomarkers in bothersome tinnitus“* – „*Komorbiditäten und stressbezogene Haar-Biomarker bei belastendem Tinnitus“* selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.
Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.
Ich versichere ferner, dass ich die in Zusammenarbeit mit anderen Personen generierten Daten, Datenauswertungen und Schlussfolgerungen korrekt gekennzeichnet und meinen eigenen Beitrag sowie die Beiträge anderer Personen korrekt kenntlich gemacht habe (siehe Anteilserklärung). Texte oder Textteile, die gemeinsam mit anderen erstellt oder verwendet wurden, habe ich korrekt kenntlich gemacht.
Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Erstbetreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass ich mich zur Einhaltung der Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis verpflichte.
Weiterhin versichere ich, dass ich diese Dissertation weder in gleicher noch in ähnlicher Form bereits an einer anderen Fakultät eingereicht habe.
Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Datum Unterschrift

ANTEILSERKLÄRUNG / CONTRIBUTION STATEMENT

Laura Basso had the following share in the following publications:

Publication 1: Basso L, Boecking B, Brueggemann P, Pedersen NL, Canlon B, Cederroth CR, Mazurek B. Subjective hearing ability, physical and mental comorbidities in individuals with bothersome tinnitus in a Swedish population sample. In: Schlee W, Langguth B, Kleinjung T, Vanneste S, De Ridder D, eds. *Progress in Brain Research*. Vol 260. Elsevier; 2021:51-78. doi:10.1016/bs.pbr.2020.10.001

Contribution in detail:

- Conception of the research question in combination with BM, BB, PB
- Development of the statistical analysis strategy in cooperation with BB
- Data preparation
- Conducting the statistical analysis including the creation of all figures and tables
- Interpretation of the results
- Writing of the original draft
- Revising the original draft based on feedback from all co-authors and in the peer-review process

Publication 2: Basso L, Boecking B, Neff P, Brueggemann P, Peters EMJ, Mazurek B. Hair-cortisol and hair-BDNF as biomarkers of tinnitus loudness and distress in chronic tinnitus. *Scientific Reports.* 2022;12(1):1934. doi:10.1038/s41598-022-04811-0

Contribution in detail:

- Planning of the study in cooperation with BB, BM and EMJP
- Organization, coordination, and documentation of data collection
- Study enrollment of participants, collection of hair samples and questionnaire data
- Assisting with sample preparation for biomarker quantification in the laboratory of EMJP
- Conception of the research question
- Development of the statistical analysis strategy in combination with PN

- Conducting the statistical analysis including the creation of all figures and tables
- Interpretation of the results
- Writing of the original draft
- Revising the original draft based on feedback from all co-authors and in the peer-review process

Publication 3: Basso L, Boecking B, Neff P, Brueggemann P, Mazurek B, Peters EMJ. Psychological treatment effects unrelated to hair-cortisol and hair-BDNF levels in chronic tinnitus. *Frontiers in Psychiatry.* 2022;13:764368. doi:10.3389/fpsyt.2022.764368

Contribution in detail:

- Planning of the study in cooperation with BB, BM and EMJP
- Organization, coordination, and documentation of data collection
- Study enrollment of participants, collection of hair samples and questionnaire data
- Assisting with sample preparation for biomarker quantification in the laboratory of EMJP
- Conception of the research question
- Development of the statistical analysis strategy in combination with PN
- Conducting the statistical analysis including the creation of all figures and tables
- Interpretation of the results
- Writing of the original draft
- Revising the original draft based on feedback from all co-authors and in the peer-review process

_____

Signature of the doctoral candidate

EXTRACTS FROM THE JOURNAL SUMMARY LIST AND PRINT COPIES OF THE PUBLICATIONS

Publication 1: Basso L, Boecking B, Brueggemann P, Pedersen NL, Canlon B, Cederroth CR, Mazurek B. Subjective hearing ability, physical and mental comorbidities in individuals with bothersome tinnitus in a Swedish population sample. In: Schlee W, Langguth B, Kleinjung T, Vanneste S, De Ridder D, eds. *Progress in Brain Research*. Vol 260. Elsevier; 2021:51-78. doi:10.1016/bs.pbr.2020.10.001

- Article (book chapter) submitted March 30, 2020; published February 4, 2021 in *Progress in Brain Research* (Elsevier)
- Impact Factor 2018: 2.961
- Excerpt from Journal Summary List from 2018, Category “NEUROSCIENCES”: Rank 129 of 267 journals

Journal Data Filtered By: **Selected JCR Year: 2018** Selected Editions: SCIE,SSCI
Selected Categories: **"NEUROSCIENCES"** Selected Category Scheme: WoS
Gesamtanzahl: 267 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	NATURE REVIEWS NEUROSCIENCE	43,107	33.162	0.068480
2	NATURE NEUROSCIENCE	63,390	21.126	0.164700
3	ACTA NEUROPATHOLOGICA	20,206	18.174	0.041660
4	BEHAVIORAL AND BRAIN SCIENCES	9,377	17.194	0.010240
5	TRENDS IN COGNITIVE SCIENCES	27,095	16.173	0.040040
6	JOURNAL OF PINEAL RESEARCH	10,695	15.221	0.010560
7	NEURON	95,348	14.403	0.218680
8	TRENDS IN NEUROSCIENCES	20,163	12.314	0.024480
9	Annual Review of Neuroscience	14,042	12.043	0.015020
10	MOLECULAR PSYCHIATRY	20,353	11.973	0.049290
11	BRAIN	52,970	11.814	0.074030
12	BIOLOGICAL PSYCHIATRY	43,122	11.501	0.053320
13	PROGRESS IN NEUROBIOLOGY	12,929	10.658	0.013230
14	Nature Human Behaviour	1,230	10.575	0.006550
15	SLEEP MEDICINE REVIEWS	6,920	10.517	0.010920
16	ANNALS OF NEUROLOGY	37,336	9.496	0.048630
17	Molecular Neurodegeneration	4,248	8.274	0.011350
18	NEUROSCIENCE AND BIOBEHAVIORAL REVIEWS	26,724	8.002	0.051580
19	FRONTIERS IN NEUROENDOCRINOLOGY	4,196	7.852	0.005490
20	Neurology-Neuroimmunology & Neuroinflammation	1,996	7.353	0.008220
21	NEUROPSYCHOPHARMACOLOGY	25,672	7.160	0.039090

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
22	Brain Stimulation	5,457	6.919	0.014470
23	NEUROPATHOLOGY AND APPLIED NEUROBIOLOGY	3,876	6.878	0.006420
24	NEUROENDOCRINOLOGY	5,046	6.804	0.005690
25	NEUROSCIENTIST	4,986	6.791	0.008520
26	BRAIN BEHAVIOR AND IMMUNITY	14,533	6.170	0.025700
27	BRAIN PATHOLOGY	5,263	6.155	0.007880
28	Alzheimers Research & Therapy	3,160	6.142	0.010700
29	JOURNAL OF NEUROSCIENCE	175,046	6.074	0.233460
30	JOURNAL OF CEREBRAL BLOOD FLOW AND METABOLISM	19,766	6.040	0.028050
31	PAIN	38,312	6.029	0.039070
32	CURRENT OPINION IN NEUROBIOLOGY	15,090	6.014	0.033650
33	Acta Neuropathologica Communications	3,063	5.883	0.014190
34	Translational Stroke Research	1,955	5.847	0.004330
35	GLIA	14,003	5.829	0.018760
36	NEUROIMAGE	99,720	5.812	0.132720
37	NEURAL NETWORKS	13,063	5.785	0.016060
38	NEUROPSYCHOLOGY REVIEW	2,971	5.739	0.003940
39	Molecular Autism	2,107	5.712	0.008000
40	Journal of Neuroinflammation	11,767	5.700	0.023240
41	Multiple Sclerosis Journal	11,501	5.649	0.022750
42	Annual Review of Vision Science	458	5.622	0.003300
43	Neurotherapeutics	4,475	5.552	0.009060
44	Translational Neurodegeneration	810	5.534	0.002420

Selected JCR Year: 2018; Selected Categories: “NEUROSCIENCES”

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
45	CEREBRAL CORTEX	30,675	5.437	0.059570
46	JOURNAL OF PAIN	10,405	5.424	0.018280
47	NEUROBIOLOGY OF DISEASE	16,363	5.160	0.026710
48	NEUROINFORMATICS	1,277	5.127	0.002920
49	JOURNAL OF PHYSIOLOGY-LONDON	52,037	4.950	0.041100
50	BIPOLAR DISORDERS	5,143	4.936	0.006760
51	Developmental Cognitive Neuroscience	2,470	4.920	0.009240
52	JOURNAL OF PSYCHIATRY & NEUROSCIENCE	3,293	4.899	0.004540
53	JOURNAL OF NEUROCHEMISTRY	35,902	4.870	0.026140
54	Dialogues in Clinical Neuroscience	3,384	4.867	0.004730
55	Annals of Clinical and Translational Neurology	1,858	4.656	0.008750
56	CURRENT OPINION IN NEUROLOGY	5,290	4.647	0.009650
57	MOLECULAR NEUROBIOLOGY	12,806	4.586	0.027560
58	SLEEP	21,434	4.571	0.024240
59	Current Neuropharmacology	3,508	4.568	0.005650
60	EXPERIMENTAL NEUROLOGY	20,500	4.562	0.023440
61	HUMAN BRAIN MAPPING	22,040	4.554	0.043230
62	Journal of Neural Engineering	7,336	4.551	0.012190
63	EUROPEAN NEUROPSYCHOPHARMACOLOGY	7,488	4.468	0.015500
64	CEPHALALGIA	9,983	4.438	0.014480
65	NEUROBIOLOGY OF AGING	22,409	4.398	0.037090
66	EUROPEAN JOURNAL OF NEUROLOGY	10,488	4.387	0.016970

Selected JCR Year: 2018; Selected Categories: "NEUROSCIENCES"

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
67	NEUROPHARMACOLOGY	20,604	4.367	0.034460
68	PROGRESS IN NEURO-PSYCHOPHARMACOLOGY & BIOLOGICAL PSYCHIATRY	10,674	4.315	0.012400
69	Cognitive Computation	1,578	4.287	0.002230
70	CORTEX	10,302	4.275	0.024590
71	Neuroscience Bulletin	2,027	4.246	0.004070
72	JOURNAL OF PSYCHOPHARMACOLOGY	6,460	4.221	0.010120
73	INTERNATIONAL JOURNAL OF NEUROPSYCHOPHARMACOLOGY	6,551	4.207	0.012320
74	JOURNAL OF NEUROSCIENCE RESEARCH	12,976	4.139	0.010060
75	Molecular Brain	2,467	4.051	0.007180
76	PSYCHONEUROENDOCRINOLOGY	16,809	4.013	0.028150
77	NEUROCHEMISTRY INTERNATIONAL	8,775	3.994	0.009020
78	NUTRITIONAL NEUROSCIENCE	1,778	3.950	0.002260
79	Frontiers in Systems Neuroscience	4,801	3.928	0.015360
80	JOURNAL OF HEADACHE AND PAIN	3,308	3.918	0.007210
81	Frontiers in Cellular Neuroscience	9,711	3.900	0.035870
82	Journal of Neuroimmune Pharmacology	2,486	3.870	0.004750
83	ACS Chemical Neuroscience	5,238	3.861	0.013320
84	CELLULAR AND MOLECULAR NEUROBIOLOGY	4,488	3.811	0.005740
85	NEUROGASTROENTEROLOGY AND MOTILITY	8,314	3.803	0.014510
86	JOURNAL OF NEUROTRAUMA	14,754	3.754	0.019770
87	Fluids and Barriers of the CNS	1,127	3.727	0.002650
88	Frontiers in Molecular Neuroscience	4,752	3.720	0.014230

Selected JCR Year: 2018; Selected Categories: “NEUROSCIENCES”

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
89	Journal of Parkinsons Disease	1,768	3.698	0.006340
90	CLINICAL NEUROPHYSIOLOGY	19,574	3.675	0.021420
91	Social Cognitive and Affective Neuroscience	6,966	3.662	0.020880
92	Frontiers in Neuroscience	13,198	3.648	0.043000
93	Frontiers in Aging Neuroscience	6,791	3.633	0.020910
94	Brain Structure & Function	6,077	3.622	0.019520
95	NEURAL PLASTICITY	3,691	3.591	0.010510
96	Journal of Neurodevelopmental Disorders	1,253	3.590	0.003420
97	Journal of NeuroEngineering and Rehabilitation	4,974	3.582	0.008800
98	Neurophotonics	809	3.581	0.002760
99	JOURNAL OF ALZHEIMERS DISEASE	20,383	3.517	0.041470
100	PSYCHIATRY AND CLINICAL NEUROSCIENCES	3,720	3.489	0.004230
101	JOURNAL OF NEUROPATHOLOGY AND EXPERIMENTAL NEUROLOGY	9,205	3.460	0.007510
102	JOURNAL OF SLEEP RESEARCH	5,432	3.432	0.007450
103	PSYCHOPHARMACOLOGY	23,565	3.424	0.022260
104	Current Opinion in Behavioral Sciences	1,763	3.422	0.009020
105	CEREBELLUM	2,785	3.413	0.005970
106	Current Neurology and Neuroscience Reports	3,004	3.400	0.007210
107	CNS Neuroscience & Therapeutics	2,993	3.394	0.005990
108	PSYCHOPHYSIOLOGY	14,275	3.378	0.012150
109	Cognitive Neuroscience	570	3.361	0.001630
110	NEUROTOXICITY RESEARCH	3,067	3.311	0.003750

Selected JCR Year: 2018; Selected Categories: "NEUROSCIENCES"

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
111	HIPPOCAMPUS	8,733	3.267	0.013090
112	NEUROTOXICOLOGY	7,180	3.263	0.007100
113	NEUROSCIENCE	45,939	3.244	0.050820
114	JOURNAL OF COMPARATIVE NEUROLOGY	30,418	3.239	0.017320
115	Current Alzheimer Research	4,026	3.211	0.005930
116	EUROPEAN JOURNAL OF PAIN	7,263	3.188	0.011070
117	GENES BRAIN AND BEHAVIOR	3,670	3.157	0.005300
118	BRAIN TOPOGRAPHY	2,629	3.104	0.004920
119	BRAIN RESEARCH BULLETIN	9,445	3.103	0.006570
120	Frontiers in Neural Circuits	3,107	3.101	0.014190
121	JOURNAL OF THE INTERNATIONAL NEUROPSYCHOLOGICAL SOCIETY	6,773	3.098	0.007380
122	Nature and Science of Sleep	520	3.054	0.001290
123	JOURNAL OF NEUROENDOCRINOLOGY	5,826	3.040	0.005430
124	Purinergic Signalling	1,617	3.038	0.002390
125	JOURNAL OF COGNITIVE NEUROSCIENCE	16,898	3.029	0.017960
126	Cognitive Neurodynamics	914	3.021	0.001650
127	NEUROBIOLOGY OF LEARNING AND MEMORY	6,836	3.010	0.013440
128	Frontiers in Neurorobotics	609	3.000	0.001370
129	Progress in Brain Research	8,018	2.961	0.006860
130	HEARING RESEARCH	9,237	2.952	0.010490
131	BRAIN RESEARCH	53,805	2.929	0.031770
132	Frontiers in Neuroanatomy	2,971	2.923	0.010280

Selected JCR Year: 2018; Selected Categories: “NEUROSCIENCES”

CHAPTER

3

Subjective hearing ability, physical and mental comorbidities in individuals with bothersome tinnitus in a Swedish population sample

Laura Basso[a], Benjamin Boecking[a], Petra Brueggemann[a], Nancy L. Pedersen[b], Barbara Canlon[c], Christopher R. Cederroth[c,d,e], and Birgit Mazurek[a,*]

[a]*Tinnitus Center, Charité—Universitätsmedizin Berlin, Berlin, Germany*
[b]*Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden*
[c]*Laboratory of Experimental Audiology, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden*
[d]*National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom*
[e]*Hearing Sciences, Division of Clinical Neuroscience, School of Medicine, University of Nottingham, Nottingham, United Kingdom*
**Corresponding author: Tel.: +49-30-450-555-061, e-mail address: birgit.mazurek@charite.de*

Abstract

Objective: This study investigates associations of subjective hearing ability, physical comorbidities, and mental comorbidities with bothersome (vs. non-bothersome) tinnitus and mediating effects between these influences.

Methods: The Swedish LifeGene cohort was used to sample cross-sectional survey data (collected 2009–2016) of 7615 participants with tinnitus, 697 (9.2%) of whom rated their tinnitus as bothersome. Associations between bothersome tinnitus and subjective hearing ability, physical and mental comorbidities were investigated by separate age- and gender-adjusted multiple logistic regression models. Interrelationships between these associations were investigated by logistic mediation models.

Results: Compared to non-bothersome tinnitus, bothersome tinnitus was associated with higher age, reduced subjective hearing ability, hearing-related difficulties in social situations, cardiovascular disease, chronic shoulder pain, thyroid disease, Ménière's disease, depression, anxiety

Progress in Brain Research, Volume 260, ISSN 0079-6123, https://doi.org/10.1016/bs.pbr.2020.10.001

syndrome, and social anxiety. Subjective hearing impairment or hearing-related difficulties mediated 13–36% of the effects of mental comorbidities on bothersome tinnitus. Depression or anxiety syndrome mediated 5–8% of most relationships between physical comorbidities and bothersome tinnitus. Depression, anxiety syndrome, or social anxiety mediated 2–4% of the effects of subjective hearing impairment or hearing-related difficulties on bothersome tinnitus.

Conclusion: Psychological factors, subjective hearing impairment, and hearing-related difficulties in social situations play key roles in predicting bothersome (vs. non-bothersome) tinnitus in a large population sample. Psychological factors contribute to explaining the impact of physical comorbidities and hearing-related effects on bothersome tinnitus. This highlights their transdiagnostic importance for aggravating varied physical symptom clusters. Interventions to improve or prevent high tinnitus burden should be interdisciplinary/multimodal and target auditory, physical, and psychological factors.

Keywords

Bothersome tinnitus, Physical comorbidity, Mental comorbidity, Hearing ability, Mediation analysis

1 Introduction

Tinnitus, commonly defined as the sensation of sound without a corresponding external acoustic source, can lead to considerable distress (Tyler and Baker, 1983) and an increased risk for suicide attempts (Lugo et al., 2019; Seo et al., 2016). Most individuals who are affected by tinnitus, however, report not to be bothered by it; e.g., in a study by Kim et al. (2015) in the South Korean population, 69.2% of subjects with tinnitus reported no tinnitus-related annoyance, 27.9% slight annoyance, and 3.0% severe annoyance. Regarding factors that distinguish between low levels of tinnitus-related distress (non-bothersome tinnitus) and high levels of tinnitus-related distress (bothersome tinnitus), influences of psychological factors such as maladaptive coping styles (Beukes et al., 2018; Budd and Pugh, 1996), cognitive factors (Caldirola et al., 2016; Lee et al., 2004; Weise et al., 2013), and stress (Baigi et al., 2011; Ciminelli et al., 2018; Kim et al., 2015) have been identified. Moreover, rates of mental comorbidities are high among individuals with tinnitus and they seem to correlate with tinnitus severity (Pinto et al., 2014). Anxiety disorders (45% lifetime prevalence; Pattyn et al., 2016) and depressive disorders (33% median prevalence; Salazar et al., 2019) are most predominant. It is also known that certain physical conditions are associated with tinnitus; see Table 1 for an overview of physical and mental comorbidities with associations to tinnitus (and potentially bothersome tinnitus) which are included in the present study.

The presence of physical symptoms can lead to psychosocial distress, and previous studies report associations between somatic complaints and tinnitus-related distress (Brueggemann et al., 2016; Hoekstra et al., 2014; Sahin et al., 2016; Stobik et al., 2005). Furthermore, there is strong evidence from research on chronic pain disorders that psychological processes can play a major role in the perception and chronification of physical symptoms (Borsook et al., 2018; Nees and Becker, 2018), and not surprisingly, tinnitus and chronic pain share many neurological similarities (Rauschecker et al., 2015).

Table 1 Selected physical and mental comorbidities with associations to tinnitus.

Physical comorbidities	References	Mental comorbidities	References
Hypertension	Figueiredo et al. (2015), Yang et al. (2015)	Burnout	Hasson et al. (2011), Hébert et al. (2012), Herr et al. (2016), Peterson et al. (2008)
Hyperlipidemia	Coelho et al. (2020), Kim et al. (2015), Lin et al. (2018), Martines et al. (2015), Shargorodsky et al. (2010)	Depression	Geocze et al. (2013), Langguth et al. (2011), Salazar et al. (2019)
Cardiovasculardisease[a]	Borghi et al. (2011), Fujii et al. (2011), Lin et al. (2018), Michikawa et al. (2010), Nondahl et al. (2002), Park et al. (2014), Stobik et al. (2005)	Bipolar disease	Malakouti et al. (2011), Ramage-Morin et al. (2019)
Asthma	Fujii et al. (2011), Kim et al. (2015)	Generalized anxiety syndrome	Andersson et al. (2004), Belli et al. (2008), Holgers et al. (2005), Mathias et al. (2011), Pattyn et al. (2016), Shargorodsky et al. (2010)
Diabetes	Coelho et al. (2020), Gibrin et al. (2013), Lin et al. (2018), Martines et al. (2015), Shargorodsky et al. (2010), Shih et al. (2017), Spankovich et al. (2018)	Panic	Andersson et al. (2004), Holgers et al. (2005), Mathias et al. (2011), Pattyn et al. (2016, 2018)
Thyroiddisease	Coelho et al. (2020), Kim et al. (2015), Mahafzah et al. (2018), Malik et al. (2002), Singh et al. (2019)	Agoraphobia	Andersson et al. (2004), Mathias et al. (2011), Pattyn et al. (2016)
Chronicshoulder pain	Bjorne and Agerberg (1996), Kuttila et al. (2005), Ren and Isberg (1995)	Social anxiety/ phobia	Andersson et al. (2004), Belli et al. (2008), Holgers et al. (2005), Mathias et al. (2011), Pattyn et al. (2016)
Arthritis (osteoarthritis/ rheumatoid arthritis)[b]	Coelho et al. (2020), Kim et al. (2015), Nondahl et al. (2011), Ramage-Morin et al. (2019), Spankovich et al. (2018)	Obsessive-compulsive disorder	Andersson et al. (2004), Brueggemann et al. (2019), Folmer et al. (2008), Holgers et al. (2005), Mathias et al. (2011)
Systemiclupuserythematosus	Coelho et al. (2020), Di Stadio and Ralli (2017)	Posttraumatic stress disorder	Fagelson, (2007), Hinton et al. (2006), Kreuzer et al. (2014)
Migraine/Headache	Guichard et al. (2016), Hwang et al. (2018), Kostev et al. (2019), Langguth et al. (2015), Lugo et al. (2020), Rhee et al. (2020), Sindhusake et al. (2003), Stohler et al. (2019)		
Ménière's disease[c]	Figueiredo et al. (2017), Kostev et al. (2019), Lin et al. (2018)		
Epilepsy	Coebergh et al. (2019), Hamed and Oseilly (2018)		
Multiple sclerosis	Coelho et al. (2020), Daugherty et al. (1983), Fischer et al. (1985), Rodriguez-Casero et al. (2005)		
Fibromyalgia	Cil et al. (2020), Iikuni et al. (2013), Stohler et al. (2019), Waylonis and Heck (1992)		

[a]*In the present study, angina, myocardial infarction, and cardiac arrhythmia were grouped as cardiovascular diseases.*
[b]*In the present study, osteoarthritis and rheumatoid arthritis were differentiated, which has not been done consistently in the literature.*
[c]*Ménière's disease is not a comorbidity but was included as tinnitus can be a symptom of Ménière's disease.*

Bothersome tinnitus is likely to be influenced by auditory and other physical factors as well as psychological factors. Previous studies that have investigated factors which can influence tinnitus-related distress have either looked at single factors (Ciminelli et al., 2018; Sahin et al., 2016) or multiple factors, e.g., in multivariate regression approaches (Brueggemann et al., 2016; Hoekstra et al., 2014; Kim et al., 2015), but how influences of different factors might affect each other has rarely been explored.

The current study aims not only to investigate the contributions of subjective hearing ability, physical symptoms, and mental symptoms in the prediction of bothersome (vs. non-bothersome) tinnitus in a large Swedish population sample, but also possible interrelationships between these factors by using mediation analysis. Mediation analysis is a method to assess whether the relationship between an independent variable (e.g., migraine) and a dependent variable (e.g., bothersome tinnitus) is mediated, i.e., fully or partly explained by another variable (e.g., depression). Any variable that is related to the dependent and affected by the independent variable can be a potential mediator. In mediation analysis, it is assessed via three regression equations whether the relationship between the independent and the dependent variable changes when the mediator is controlled: if the relationship is reduced, the mediator partially accounts for the relationship; if the relationship is no longer present, the mediator fully accounts for it (Baron and Kenny, 1986). For example, Probst et al. (2016) found that the relationship between tinnitus loudness and tinnitus distress is partially mediated by stress level and emotional state.

The main objective of this study is to identify physical and mental comorbidities that are related to bothersome tinnitus (compared with non-bothersome tinnitus) and to investigate mediating effects by mental comorbidities. In addition, we also include subjective hearing ability in our analyses, as hearing impairment is a well-known risk factor for tinnitus (Henry et al., 2005; Shore et al., 2016) which might potentially mediate the effects of certain comorbidities on bothersome tinnitus. On the other hand, the influence of subjective hearing ability might also be mediated by the presence of mental comorbidities. Moreover, since the risk of tinnitus seems to increase with age, and conflicting findings have been made regarding tinnitus severity and gender (McCormack et al., 2016), we include these factors as covariates in our analyses. We hypothesize that the presence of mental comorbidities is strongly linked to bothersome tinnitus and partly explains the effects of physical comorbidities and subjective hearing impairment on bothersome tinnitus.

2 Method

2.1 Study design and sample

This study used cross-sectional survey data from the LifeGene cohort, a random sample from the Swedish general population (Almqvist et al., 2011; LifeGene, 2017). Recruitment of participants for LifeGene took place via invitation letters

to randomly selected households (subjects aged 18–50 years), spontaneous online registration (for subjects aged ≥18 years), or invitation by other participants (with the possibility for parents to invite their children; Almqvist et al., 2011; LifeGene, 2017). Other than age (invitation letters) and living in Sweden, no exclusion criteria were applied.

For this study, retrospective data of the LifeGene baseline survey (collected between 2009 and 2016) were used, which is a web-based epidemiological survey spanning different health-related themes (LifeGene, 2017). Of the $N = 31{,}926$ participants who completed the survey, participants without tinnitus were excluded, leading to the final sample of $N = 7615$ (23.9%) of participants who reported to have tinnitus ("Is there a constant ringing in the ears or do you have any other bothersome sound in the ears [tinnitus]?"). The dependent variable for all analyses was the rating of the tinnitus as bothersome ($N = 697$; 9.2%) or non-bothersome ($N = 6918$; 90.8%). The same sample was used in Basso et al. (2020). The onset of the tinnitus and the percentage of study participants in clinical care due to their tinnitus are not known from the data.

On average, participants were 35.80 years old ($SD = 12.44$, range: 11–84 years), and 56.5% ($N = 4301$) were female. Forty-three participants (0.6%) were younger than 18 years. Sample characteristics regarding marital status, education level, and employment status can be found in Table 2. Informed consent was obtained from all participants (for participants <18 years, consent was provided by the parents). In addition, the local ethics committee "*Regionala etikprövningsnämnden*" in Stockholm approved the project (2015/2129-31/1).

2.2 Variables

The LifeGene survey consists of various modules (LifeGene, 2017). All data used in this study were taken from the medical history module of the LifeGene survey (self-reported data).

2.2.1 Outcome variable

All participants who gave affirmative responses to the survey question on tinnitus ("Is there a constant ringing in the ears or do you have any other bothersome sound in the ears [tinnitus]?") were included in the study. Response options distinguished between "sometimes, but the sound doesn't bother me" and "all the time, the sound is very bothersome" which were classified as non-bothersome tinnitus and bothersome tinnitus, respectively.

2.2.2 Predictors

Predictors included physical and mental comorbidities and subjective hearing (subjective hearing ability and hearing-related difficulties in social situations). Physical and mental comorbidities were assessed by the question: "Which of the following diseases do you currently have or have you had?". All comorbidities included in this study can be found in Table 1. Angina, myocardial infarction, and cardiac arrhythmia

Table 2 Sample characteristics: marital status, education level, and employment status.

Variable	Percentage (%)	*N*
Marital status		7381
Cohabiting	33.4	2544
Married	25.0	1904
Single	24.0	1824
Living apart	9.5	722
Separated/divorced	4.7	359
Widowed	0.3	25
Same-sex marriage	0.04	3
Education level		7438
University	61.7	4696
Secondary school	24.9	1896
Primary school (9 years)	2.7	205
Other	8.4	641
Employment status		6726
Employed	53.7	4090
Student	15.0	1140
Running owned or part-owned company	7.8	592
Age pension	3.5	264
Unemployed	3.0	225
Parental leave (for 2 months or longer)	2.2	165
Sick leave (for 2 months or longer)	0.8	63
Early retirement due to illness/disability	0.7	57
On leave	0.1	10
Housewife/-man	0.1	10
Other	1.4	110

were combined into cardiovascular diseases. Ménière's disease was included even though it is not strictly a comorbidity of tinnitus, but a disease which tinnitus can be part of. Regarding arthritis, the survey differentiated between osteoarthritis and rheumatoid arthritis, which has not been done consistently in the literature. In total, 15 physical and 9 mental comorbidities were analyzed.

Subjective hearing ability ("How is your hearing?") could either be rated as "good," "somewhat reduced" or "very reduced," but for our analyses, the latter two categories were combined into "reduced hearing ability". For the assessment of hearing-related difficulties in social situations, we calculated a mean score across the following items: "Do you have difficulties hearing when speaking to one person in a silent room?", "Do you have difficulties hearing when speaking to multiple people at the same time?", "Do you have difficulties hearing when

speaking to someone in city traffic?", "Do you have difficulties hearing where different sounds come from, e.g., cars in traffic?" and "Do you have problems with your hearing and are therefore avoiding meeting people?" with the response options "yes, very difficult" (3), "sometimes, a little difficult" (2), and "no, not at all" (1). The mean score was then dichotomized into the presence or absence of hearing-related difficulties.

2.3 Statistical analysis

Statistical analyses comprised descriptive analyses, logistic regression models, and logistic mediation models and were computed using IBM SPSS Statistics (v. 25) for Windows 7. The significance level was set to $\alpha = 0.05$.

2.3.1 Descriptive analyses

Pearson's Chi-Square tests (with continuity correction where applicable) and adjusted residuals (*ARs*) were used to assess frequency differences between bothersome and non-bothersome tinnitus. Significant differences in category frequencies are present if $ARs \geq 1.96$ or ≤ -1.96. Age was not normally distributed, but moderately right-skewed (skewness = 0.949, SE = 0.028) and heavy-tailed (kurtosis = 0.494, SE = 0.056), Kolmogorov-Smirnov test: D(7615) = 0.11, $P < 0.001$. Therefore, the non-parametric Mann-Whitney-*U* test was used for its comparison between non-bothersome and bothersome tinnitus.

2.3.2 Logistic regression models

Associations with bothersome (vs. non-bothersome) tinnitus were identified using separate age- and gender-adjusted multiple logistic regression models for (1) subjective hearing (subjective hearing ability and hearing-related difficulties in social situations), (2) physical comorbidities (see Table 1), and (3) mental comorbidities (see Table 1), respectively. Odds ratios (ORs) with 95%-CIs were calculated for all predictors, and Nagelkerke R^2 and effect size *f* (Cohen, 1992, 1988) were used for model comparison. Regarding the assumptions of logistic regression, all variance inflation factor (VIF) values were ≤ 1.4 (no multicollinearity among predictors), and the predictor age was linearly related to the log odds (Box-Tidwell approach). Concerning outliers, no cases with studentized residuals greater than 3 were present; cases with studentized residuals greater than 2 were not excluded ($N = 267$ in model 1; $N = 490$ in model 2; $N = 490$ in model 3).

2.3.3 Logistic mediation models

Interrelationships between factors that significantly predicted bothersome (vs. non-bothersome) tinnitus in regression analyses were further analyzed in logistic mediation models, as described by Herr (2006), based on equations from Mackinnon and Dwyer (1993). Logistic mediation models analyzed: (A) if subjective hearing ability mediated the relationship between mental comorbidities and bothersome tinnitus; (B) if mental

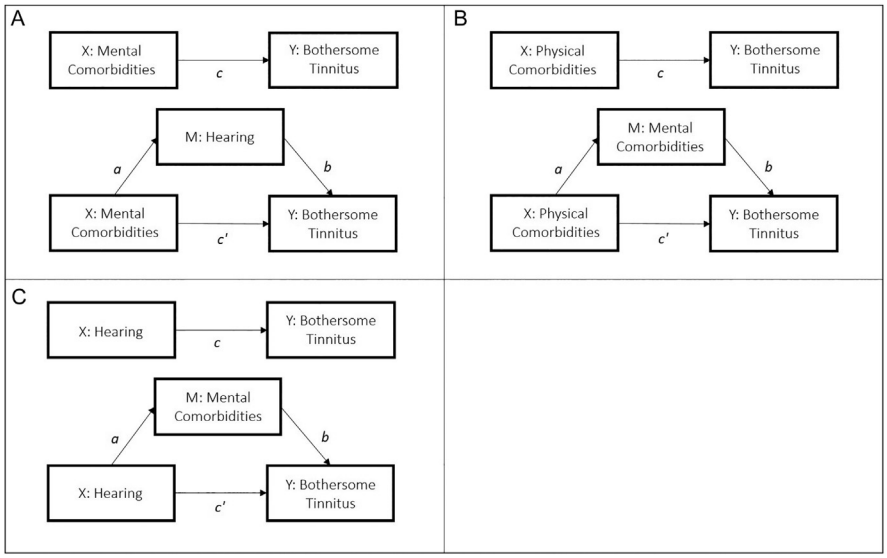

FIG. 1

Conceptual models of the performed mediation analyses. X = predictor, M = mediator, Y = outcome. Coefficient *c* designates the total effect of the respective X on Y (ignoring M); coefficient *a* is the effect of X on M, coefficient *b* is the effect of M on Y (controlling for X); coefficient *c*′ designates the direct effect of X on Y when M is controlled; and $a \times b$ reflects the indirect or mediation effect.

comorbidities mediated the relationship between physical comorbidities and bothersome tinnitus; and (C) if mental comorbidities mediated the relationship between subjective hearing ability and bothersome tinnitus (see Fig. 1). The following standardized coefficients were calculated: coefficient *c* designates the *total effect* of the predictor variable on the outcome (ignoring the mediator); coefficient *a* is the effect of the predictor on the mediator; coefficient *b* is the effect of the mediator on the outcome (controlling for the predictor); and coefficient *c'* reflects the *direct effect* of the predictor on the outcome when the mediator is controlled for; see Fig. 1. The product of the coefficients *a* and *b* reflects the *mediation effect* (or *indirect effect* of the predictor on the outcome), which was divided by the total effect *c* to calculate the percentage of the total effect being mediated (Baron and Kenny, 1986; Herr, 2006). The Aroian version of the Sobel test (Aroian, 1947) was used to assess significance, as suggested by Baron and Kenny (1986).

Response rates were high; 99.6% (6 variables) or 99.5% (18 variables) for physical and mental comorbidities, 97.6% for subjective hearing ability, and 83.8% for hearing-related difficulties in social situations (and complete data for age and gender). Overall, 1.0% of values were missing.

3 Results

3.1 Descriptive analyses

The proportion of female participants did not differ between participants with bothersome (56.4%) and non-bothersome tinnitus (56.5%). On average, participants with non-bothersome tinnitus were 35.26 years old ($SD = 12.07$, median = 32), and participants with bothersome tinnitus were 41.16 years old ($SD = 14.58$, median = 40), $U = 1{,}847{,}821$, $P < 0.001$. Compared to participants with non-bothersome tinnitus, participants with bothersome tinnitus more often reported reduced subjective hearing ability ($ARs = 16.9$), more hearing-related difficulties in social situations ($ARs = 11.3$), higher frequencies of chronic shoulder pain ($ARs = 6.6$), hypertension ($AR = 3.9$), osteoarthritis ($ARs = 5.3$), cardiovascular disease ($ARs = 4.6$), thyroid disease ($ARs = 4.0$), hyperlipidemia ($ARs = 4.6$), fibromyalgia ($ARs = 4.0$), Ménière's disease ($ARs = 4.0$), as well as higher frequencies of depression ($ARs = 3.9$), burnout ($ARs = 4.1$), panic ($ARs = 2.3$), anxiety syndrome ($ARs = 3.6$), social anxiety ($ARs = 4.0$), and posttraumatic stress disorder ($ARs = 2.3$); see Table 3.

3.2 Logistic regression models

The control variable age had significant influences in all three regression models (individuals with higher age had increased odds of reporting bothersome tinnitus), while gender showed no influence for the prediction of bothersome (vs. non-bothersome) tinnitus.

3.2.1 Subjective hearing

Both subjective hearing ability and hearing-related difficulties in social situations significantly predicted bothersome tinnitus (vs. non-bothersome tinnitus), $X^2(4) = 309.11$, $P < 0.001$, Nagelkerke $R^2 = 0.101$, $f = 0.34$, see Table 4, model 1.

3.2.2 Physical comorbidities

Of the investigated physical comorbidities, chronic shoulder pain, cardiovascular disease, thyroid disease, and Ménière's disease significantly predicted bothersome tinnitus (vs. non-bothersome tinnitus), $X^2(17) = 182.12$, $P < 0.001$, Nagelkerke $R^2 = 0.052$, $f = 0.23$, see Table 4, model 2.

3.2.3 Mental comorbidities

Of the investigated mental comorbidities, depression, anxiety syndrome, and social anxiety significantly predicted bothersome tinnitus (vs. non-bothersome tinnitus), $X^2(11) = 166.26$, $P < 0.001$, Nagelkerke $R^2 = 0.047$, $f = 0.22$, see Table 4, model 3.

3.3 Logistic mediation models

Standardized coefficients and standard errors of all significant mediation models can be found in Table 5.

Table 3 Frequencies of subjective hearing ability and hearing-related difficulties in social situations, physical and mental comorbidities in participants with non-bothersome and bothersome tinnitus.

Variable	Non-bothersome tinnitus	Bothersome tinnitus	X^2	*P*
Hearing				
Subjective hearing ability* **	*N* = 6754	*N* = 676	285.11	<0.001
Good	68.5% (4629)	36.1% (244)		
Reduced	31.5% (2125)	63.9% (432)		
Hearing-related difficulties in social situations* **	*N* = 5742	*N* = 637	127.09	<0.001
No	44.5% (2557)	21.2% (135)		
Yes	55.5% (3185)	78.8% (502)		
Physical comorbidities	*N* = 6882	*N* = 695		
Migraine	14.8% (1016)	16.7% (116)	1.70	0.193
Chronic shoulder pain* **	5.3% (367)	11.5% (80)	42.30	<0.001
Osteoarthritis* **	4.7% (323)	9.4% (65)	27.25	<0.001
Fibromyalgia* **	0.8% (54)	2.3% (16)	14.27	<0.001
Epilepsy	0.7% (47)	1.3% (9)	2.44	0.118
Rheumatoid arthritis	0.6% (39)	0.7% (5)	0.06	0.808
Ménière's disease* **	0.2% (13)	1.0% (7)	13.10	<0.001
Systemic lupus erythematosus	0.1% (5)	0.0% (0)	<0.00	1
Multiple sclerosis	0.1% (10)	0.0% (0)	0.21	0.647
	N = 6886	*N* = 695		
Asthma	11.2% (774)	11.9% (83)	0.24	0.621
Hypertension* **	5.8% (399)	9.5% (66)	14.39	<0.001
Hyperlipidemia* **	3.1% (211)	6.3% (44)	19.73	<0.001
Cardiovascular disease* **	4.0% (277)	7.8% (54)	20.34	<0.001
Diabetes	0.6% (41)	0.6% (4)	<0.00	1
Thyroid disease* **	3.6% (251)	6.8% (47)	15.43	<0.001
Mental comorbidities	*N* = 6882	*N* = 695		
Depression* **	20.4% (1405)	26.8% (186)	14.95	<0.001
Burnout* **	10.1% (697)	15.1% (105)	16.02	<0.001
Panic*	11.1% (763)	14.0% (97)	4.89	0.027
Anxiety syndrome* **	10.0% (688)	14.4% (100)	12.60	<0.001
Social anxiety* **	3.4% (231)	6.3% (44)	15.13	<0.001
Obsessive-compulsive disorder	2.0% (140)	2.7% (19)	1.18	0.277
Posttraumatic stress disorder*	1.6% (107)	2.7% (19)	4.67	0.031
Bipolar disease	0.8% (52)	1.0% (7)	0.24	0.622
Agoraphobia	0.6% (38)	1.0% (7)	1.51	0.219

Note. *Pearson X^2 tests with continuity correction. Bold factors indicate significant differences in frequencies.*
*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Table 4 Logistic regression models for the prediction of bothersome tinnitus (vs. non-bothersome tinnitus): subjective hearing (model 1), mental comorbidities (model 2), and physical comorbidities (model 3).

Variable	β	SE β	Wald's X^2	P	OR	95%-CI	
Model 1 (N = 6250)							
Constant	*−3.96*	*0.15*	*668.25*	*<0.001*	*0.02*		
*Age****	*0.02*	*<0.01*	*55.41*	*<0.001*	*1.02*	*1.02*	*1.03*
Gender							
Subjective hearing ability***	0.97	0.11	83.44	<0.001	2.65	2.15	3.26
Hearing-related difficulties in social situations***	0.48	0.12	16.25	<0.001	1.61	1.28	2.04
Model 2 (N = 7577)							
Constant	*−3.49*	*0.14*	*609.14*	*<0.001*	*0.03*		
*Age****	*0.03*	*<0.01*	*78.29*	*<0.001*	*1.03*	*1.02*	*1.04*
Gender							
Migraine							
Asthma							
Hypertension							
Chronic shoulder pain***	0.63	0.14	20.80	<0.001	1.88	1.43	2.47
Osteoarthritis							
Cardiovascular disease*	0.40	0.16	6.07	0.014	1.49	1.08	2.04
Thyroid disease*	0.39	0.17	5.19	0.023	1.48	1.06	2.08
Hyperlipidemia							
Fibromyalgia							
Epilepsy							
Rheumatoid arthritis							
Diabetes							
Ménière's disease*	1.23	0.50	6.14	0.013	3.42	1.29	9.05
Systemic lupus erythematosus							
Multiple sclerosis							
Model 3 (N = 7577)							
Constant	*−3.75*	*0.14*	*727.39*	*<0.001*	*0.02*		
*Age****	*0.04*	*<0.01*	*141.57*	*<0.001*	*1.04*	*1.03*	*1.04*
Gender							
Depression*	0.22	0.11	3.91	0.048	1.25	1.00	1.56
Burnout							
Panic							

Continued

Table 4 Logistic regression models for the prediction of bothersome tinnitus (vs. non-bothersome tinnitus): subjective hearing (model 1), mental comorbidities (model 2), and physical comorbidities (model 3).—cont'd

Variable	β	SE β	Wald's X^2	P	OR	95%-CI	
Anxiety syndrome*	0.32	0.14	5.04	0.025	1.38	1.04	1.83
Social anxiety*	0.45	0.19	5.50	0.019	1.57	1.08	2.30
Obsessive-compulsive disorder							
Posttraumatic stress disorder							
Bipolar disease							
Agoraphobia							

Note. *Only significant results are displayed. OR = Odds ratio.*
*** P < *0.001,* ** P < *0.01,* * P < *0.05.*

3.3.1 X: Mental comorbidities, M: Subjective hearing, Y: Bothersome tinnitus (vs. non-bothersome tinnitus)

Subjective hearing ability mediated 13% of the effect of depression on bothersome tinnitus, $P = 0.020$, and 19% of the effect of social anxiety on bothersome tinnitus, $P = 0.004$. Hearing-related difficulties in social situations mediated 36% of the effect of depression, $P < 0.001$, 20% of the effect of anxiety syndrome, $P = 0.001$, and 31% of the effect of social anxiety, $P < 0.001$, on bothersome tinnitus.

3.3.2 X: Physical comorbidities, M: Mental comorbidities, Y: Bothersome tinnitus (vs. non-bothersome tinnitus)

Depression mediated 5% of the effect of cardiovascular disease, $P = 0.012$, and 5% of the effect of thyroid disease, $P = 0.019$, and 8% of the effect of chronic shoulder pain, $P = 0.003$, on bothersome tinnitus. Anxiety syndrome mediated 5% of the effect of cardiovascular disease, $P = 0.018$, and 7% of the effect of chronic shoulder pain, $P = 0.006$, on bothersome tinnitus. Social anxiety mediated 6% of the effect of chronic shoulder pain, $P = 0.007$, on bothersome tinnitus.

3.3.3 X: Subjective hearing, M: Mental comorbidities, Y: Bothersome tinnitus (vs. non-bothersome tinnitus)

Depression mediated 2% of the effect of hearing-related difficulties in social situations, $P = 0.037$, on bothersome tinnitus. Anxiety syndrome mediated 2% of the effect of hearing-related difficulties in social situations, $P = 0.034$, on bothersome tinnitus. Social anxiety mediated 2% of the effect of subjective hearing ability, $P = 0.028$, and 4% of the effect of hearing-related difficulties in social situations, $P = 0.007$, on bothersome tinnitus.

Table 5 Standardized coefficients and standard errors of significant mediation effects.

Predictor (X)	Mediator (M)	Effect of X on M		Effect of M on Y controlled for X		Effect of X on Y		Effect of X on Y controlled for M	
		a	*SE*	*b*	*SE*	*c*	*SE*	*c′*	*SE*
Depression	Subjective hearing ability	0.032	0.013	0.331	0.021	0.079	0.020	0.063	0.020
Social anxiety	Subjective hearing ability	0.039	0.013	0.330	0.021	0.068	0.017	0.059	0.017
Depression	Hearing-related difficulties in social situations	0.100	0.014	0.282	0.026	0.079	0.020	0.046	0.021
Anxiety syndrome	Hearing-related difficulties in social situations	0.049	0.014	0.284	0.026	0.070	0.019	0.055	0.020
Social anxiety	Hearing-related difficulties in social situations	0.075	0.016	0.282	0.026	0.068	0.017	0.059	0.018
Cardiovascular disease	Depression	0.050	0.014	0.075	0.020	0.078	0.017	0.075	0.017
Thyroid disease	Depression	0.044	0.014	0.076	0.020	0.070	0.018	0.067	0.018
Chronic shoulder pain	Depression	0.140	0.013	0.063	0.021	0.108	0.017	0.100	0.017
Cardiovascular disease	Anxiety syndrome	0.059	0.017	0.066	0.019	0.078	0.017	0.076	0.017
Chronic shoulder pain	Anxiety syndrome	0.126	0.016	0.057	0.020	0.108	0.017	0.102	0.017
Chronic shoulder pain	Social anxiety	0.106	0.025	0.062	0.017	0.108	0.017	0.104	0.017
Hearing-related difficulties in social situations	Depression	0.121	0.017	0.046	0.021	0.285	0.026	0.282	0.026
Hearing-related difficulties in social situations	Anxiety syndrome	0.079	0.023	0.055	0.020	0.285	0.026	0.284	0.026
Hearing-related difficulties in social situations	Social anxiety	0.196	0.040	0.059	0.018	0.285	0.026	0.282	0.026
Subjective hearing ability	Social anxiety	0.099	0.033	0.059	0.017	0.333	0.021	0.330	0.021

Note. *X = predictor, M = mediator, Y = outcome (bothersome tinnitus).*

4 Discussion

4.1 Prevalence

The prevalence of bothersome tinnitus ($N = 697$) was 2.2% in the total population sample and 9.2% in the tinnitus sample. Many studies found similar prevalence rates (1.2–3%) of bothersome tinnitus in the population (Gallus et al., 2015; Michikawa et al., 2010; Nondahl et al., 2011), while others report higher rates of 5.8–7% (Park et al., 2014; Ramage-Morin et al., 2019). This variance might result from the different study populations as well as from the varying definitions of "bothersome" tinnitus: tinnitus posing a big or very big problem (Gallus et al., 2015), tinnitus interfering with concentration or sleep (Michikawa et al., 2010), tinnitus in its worst form being severe (Nondahl et al., 2011), tinnitus in daily life being annoying (irritating) or severely annoying and causing sleep problems (Park et al., 2014), or tinnitus being bothering by affecting sleep, concentration or mood (Ramage-Morin et al., 2019).

4.2 Age and gender

The prevalence of bothersome tinnitus did not differ between genders. This is in accordance with other studies that report equal rates of bothersome or frequent tinnitus in both genders (Axelsson and Ringdahl, 1989; Park et al., 2014; Shargorodsky et al., 2010). However, conflicting findings exist as well (McCormack et al., 2016). The relationship between older age and bothersome tinnitus is consistent with several other findings (Gallus et al., 2015; Kim et al., 2015; Park et al., 2014; Shargorodsky et al., 2010), but not all (Jalessi et al., 2013). In addition, we found distinct effects of both higher age and reduced subjective hearing ability on bothersome tinnitus in the regression analysis. Higher age might therefore increase the risk of bothersome tinnitus independently of age-related hearing loss—for example via age-related life changes that can negatively affect the quality of life (e.g., functional loss), which may in turn increase tinnitus-related distress (Henry et al., 2005).

4.3 Subjective hearing

Reduced hearing ability (OR = 2.65 [2.15, 3.26]) was associated with bothersome tinnitus, in accordance with other cross-sectional population studies (Kim et al., 2015; Park et al., 2014; Shargorodsky et al., 2010). In addition, we found an effect of hearing-related difficulties in social situations (OR = 1.61 [1.28, 2.04]). Of the three regression models, subjective hearing (model 1) showed the highest goodness-of-fit (Nagelkerke $R^2 = 0.101$), with a medium effect size ($f = 0.34$), in the prediction of bothersome vs. non-bothersome tinnitus. Hearing impairment may exert direct influences on tinnitus-related distress as well as indirect ones via increased psychological distress in social situations, possibly leading to impaired social functioning.

Previous research found that 45% of individuals with bothersome tinnitus report a weak sense of community belonging (Ramage-Morin et al., 2019), highlighting the importance to address social functioning in treatment interventions.

The effects of hearing-related difficulties in social situations on bothersome tinnitus were partially mediated by depression (2%), anxiety syndrome (2%), and social anxiety (4%). The latter also partially mediated the effects of subjective hearing impairment (2%). On the other hand, subjective hearing ability partially mediated the effects of depression (13%) and social anxiety (19%) on bothersome tinnitus. Moreover, hearing-related difficulties in social situations mediated the effects of depression (36%), anxiety syndrome (20%), and social anxiety (31%) on bothersome tinnitus by a large degree. These results suggest that impaired subjective hearing ability and hearing-related difficulties in social situations exert indirect effects on bothersome tinnitus through their impact on emotional factors. At the same time, mental comorbidities seem to exert indirect effects on bothersome tinnitus through their impact on subjective hearing ability and hearing-related difficulties in social situations. Hearing and emotional factors hence appear highly interconnected.

These results implicate the need for thorough distinctions between subjective and objective hearing ability. With objective hearing loss, hearing aid provision in tinnitus patients may reduce tinnitus-related distress not only through direct effects of improved hearing but also through minimizing the negative effects of reduced hearing ability on emotional wellbeing (e.g., due to social withdrawal). In contrast, subjective hearing impairment might represent a coping strategy under depressogenic strain. Moreover, emotional factors can influence the way hearing impairment is dealt with by the affected individual and may, for example, underlie the disinclination to wear hearing aids. Given the strong interrelationships between subjective hearing and mental symptoms, measures to restore hearing and psychological interventions should ideally be combined to stimulate mutual transfer effects.

4.4 Physical comorbidities

Cardiovascular disease (OR = 1.49 [1.08, 2.04]), chronic shoulder pain (OR = 1.88 [1.43, 2.47]), thyroid disease (OR = 1.48 [1.06, 2.08]), and Ménière's disease (OR = 3.42 [1.29, 9.05]) were associated with the presence of bothersome (vs. non-bothersome) tinnitus. For the physical comorbidities model (model 2), the effect size was small ($f = 0.23$).

Associations between cardiovascular diseases and tinnitus have been reported in the literature, e.g., for congestive heart failure in elderly patients (Borghi et al., 2011), or coronary artery disease in different study populations (Fujii et al., 2011; Lin et al., 2018; Michikawa et al., 2010). In line with our result, some studies found specific relationships of cardiovascular diseases with bothersome tinnitus. In a cross-sectional study, Park et al. (2014) found a strong effect of a history of cardiovascular disease for the prediction of annoying tinnitus after multivariable adjustment. Nondahl

et al. (2002) found an association between a history of cardiovascular disease and the prevalence of "significant" tinnitus (at least moderately severe and/or causing sleep problems), as well as a predictive association between higher cholesterol levels (a cardiovascular risk factor) and the 5-year incidence of "significant" tinnitus. Stobik et al. (2005) found higher rates of cardiovascular diseases among patients with severe (decompensated) tinnitus than those with mild (compensated) tinnitus. Moreover, cardiovascular disease and depression are interrelated, and evidence exists for biological and behavioral mechanisms linking both conditions (Seligman and Nemeroff, 2015). In the present study, the effects of cardiovascular disease on bothersome tinnitus were partially mediated by depression (5%) and anxiety syndrome (5%), highlighting the importance of considering psychological factors in somatic conditions.

In line with our findings, Kuttila et al. (2005) found that shoulder pain is predictive of recurrent tinnitus. In their general population sample, 53% of individuals with recurrent tinnitus reported shoulder ache at least twice a month. Two other (relatively old) studies that report findings on shoulder pain and tinnitus cannot be interpreted clearly because of confounding issues. Bjorne and Agerberg (1996) found that patients with Ménière's disease more often report neck or shoulder pain than control subjects, yet this difference might be attributable to other symptoms in the patient group than tinnitus. Ren and Isberg (1995) found higher frequencies of back or shoulder pain in patients with tinnitus and internal derangement of the temporomandibular joint than a control group, but in their sample, this difference might be explained by age. As we controlled for age in our analyses, our findings suggest an age-independent effect of shoulder pain. However, the presence of temporomandibular joint dysfunction was not assessed in our sample. Moreover, research on neck and shoulder pain has identified psychosocial risk factors, e.g., psychological distress (Menendez et al., 2015; Siivola et al., 2004; Skov et al., 1996), and evidence suggests positive effects of psychosocial interventions for the management of musculoskeletal pain (Babatunde et al., 2017). Thus, links between bothersome tinnitus and shoulder pain are likely to be influenced by psychological factors and our result supports this notion, as effects of chronic shoulder pain on bothersome tinnitus were partially mediated by depression (8%), anxiety syndrome (7%), and social anxiety (6%).

Previous research found an association between tinnitus and thyroid diseases (Kim et al., 2015). Furthermore, causal relationships between hypothyroidism and hearing loss are known (Anand et al., 1989; Coelho et al., 2020; Mahafzah et al., 2018; Malik et al., 2002; Sharlin et al., 2018; Uziel et al., 1985). Tinnitus was found to improve in 57% (Malik et al., 2002) or 62% (Singh et al., 2019) of patients with hypothyroidism after thyroxine substitution therapy. Moreover, thyroid function and depression are related; both hypothyroidism and hyperthyroidism can lead to depressive symptoms, and depression can also be associated with subclinical thyroid abnormalities (Hage and Azar, 2012). Consistent with these connections, we found that the effects of thyroid disease on bothersome tinnitus were partially mediated by depression (5%).

Furthermore, our findings suggest that tinnitus in individuals with Ménière's disease might be perceived as particularly bothersome. The effect of Ménière's disease was the strongest of all predictors with an OR of 3.42—indicating that the risk of bothersome compared to non-bothersome tinnitus is three times higher in individuals with Ménière's disease than in individuals without the disease. This effect is in line with a previous report of more severe tinnitus in patients with Ménière's disease compared to patients with tinnitus and noise-induced or age-related hearing loss (Stouffer and Tyler, 1990). Moreover, in a sample of patients with long-standing Ménière's disease, tinnitus was rated by 19% as their most severe symptom, and 10% reported a severe or very severe impact of tinnitus on their life (Yoshida et al., 2011). In a cross-sectional study, higher tinnitus severity was associated with advanced stages of Ménière's disease/higher levels of hearing loss (Romero Sánchez et al., 2010). The impact of tinnitus also seems to be influenced by other symptoms of Ménière's disease such as aural pressure and gait problems (Yoshida et al., 2011). Our analysis revealed no mediating effects of psychological symptoms on the relationship between Ménière's disease and bothersome tinnitus. This might suggest that the presence of other symptoms of Ménière's disease is more relevant for tinnitus severity in these patients than psychological symptoms.

In sum, cardiovascular disease, chronic shoulder pain, and thyroid disease seem not only to exert direct influences on bothersome tinnitus but also indirect ones through their associations with emotional factors.

4.5 Mental comorbidities

Depression (OR = 1.25 [1.00, 1.56]), anxiety syndrome (OR = 1.38 [1.04, 1.83]), and social anxiety (OR = 1.57 [1.08, 2.30]) were associated with the presence of bothersome (vs. non-bothersome) tinnitus. For the mental comorbidities model (model 3), the effect size was small ($f = 0.22$).

These results are consistent with a systematic review by Pinto et al. (2014) who concluded that the comorbid presence of anxiety or depression is associated with higher tinnitus severity and annoyance. The relationship between mental illness and tinnitus is bidirectional, as mental conditions may impair the stress tolerance and thus lead to higher distress in tinnitus patients; tinnitus-related distress on the other hand can lead to psychological symptoms or increase the severity of pre-existing ones (Pinto et al., 2014; Ziai et al., 2017).

Our results suggest that depression and anxiety can *aggravate* negative hearing-related effects and negative effects of physical symptoms on bothersome (vs. non-bothersome) tinnitus. These findings implicate that states of emotional distress are important treatment targets in individuals with bothersome tinnitus. The improvement of affective and anxiety symptoms by psychological treatment interventions like cognitive-behavioral therapy (CBT) is likely to exert not only direct effects on tinnitus-related distress but also indirect ones by reducing negative influences of physical symptoms, subjective hearing impairment, or hearing-related difficulties in social situations.

4.6 Clinical implications

Our findings point to the issue that the distinction between physical and mental conditions is not as clear as suggested by diagnostic classification systems, since many conditions share both physical and psychological aspects. Generally, three different relationships between chronic physical diseases and mental conditions are possible (Turner and Kelly, 2000): (1) Chronic physical diseases can lead to the manifestation of mental conditions, often depression or anxiety. (2) In individuals with pre-existing mental conditions, the development of a chronic physical disease can *aggravate* their symptoms. (3) If physical symptoms in individuals with chronic diseases worsen or new ones develop, this can constitute an expression of emotional distress (Turner and Kelly, 2000). In the clinical care of chronic tinnitus patients, these possible connections between tinnitus and mental health need to be addressed.

Furthermore, recent literature has begun to address the limitations of traditional diagnostic classification systems for mental disorders which classify psychopathology in distinct categories that are not based on evidence (Hofmann, 2014; Kotov et al., 2017). New approaches include empirically-based frameworks such as structural approaches using dimensional classification (Kotov et al., 2017), theory-based cognitive behavior classifications (Hofmann, 2014), or network approaches (Fried et al., 2017).

Dimensional classification approaches are based on the assumption that psychopathology lies on a continuum and can be described by different dimensions in a systematic hierarchy (Kotov et al., 2017; Lahey et al., 2017). It has been proposed that a hierarchical taxonomy consisting of a general psychopathology factor encompassing several dimensions/spectra (internalizing, thought disorder, disinhibited externalizing, antagonistic externalizing, detachment, and somatoform) comprised of different syndromes is suitable to characterize the majority of psychopathology (Kotov et al., 2017). In line with this approach, Ivansic et al. (2019) found that mental health in tinnitus patients can best be described by a general psychopathology factor and a somatization factor. They found that the expression of the general psychopathology factor was as high in severe tinnitus as in depressed patients, but more pronounced in mild tinnitus than in healthy controls. The somatization factor, on the other hand, was higher in both mild and severe tinnitus than in depressed patients or healthy controls (Ivansic et al., 2019).

The cognitive-behavioral approach, on which CBT is built, looks at psychopathology as complex causal networks (Hofmann, 2014). In this framework, certain triggers (moderated by attentional processes and trait cognitions) can activate maladaptive cognitive processes, which in turn lead to psychological distress manifesting as a specific interplay of subjective experiences, physiological symptoms, and behavioral responses (Hofmann, 2014). The focus of this approach lies on cognitive processes and their consequences for emotion regulation, which have proven to be important—and modifiable by CBT—for many different mental conditions (Hofmann et al., 2012; Hofmann, 2014). CBT also is known to have a positive effect on tinnitus management (Martinez-Devesa et al., 2010).

In a similar approach, the network perspective conceptualizes psychopathology as complex dynamic networks of mutually interacting symptoms (Fried et al., 2017). In this conceptualization, comorbidity between different mental conditions is thought to be explained by interactions between symptoms, in that the presence of a specific disorder can lead to the manifestation of another disorder via bridge symptoms (Fried et al., 2017). With this approach, the high comorbidity among severe tinnitus and mental disorders could potentially be explained by shared bridge symptoms (e.g., insomnia, concentration problems). Moreover, network approaches have the potential to predict transitions from a healthy network state to a disease state (Fried et al., 2017; van de Leemput et al., 2014), e.g., from mild to severe tinnitus-related distress, which has high clinical relevance.

In sum, all of these approaches appear suitable to better conceptualize tinnitus-related distress (emotions, cognitions, reactions), comorbid mental and physical symptoms, and their interrelationships than current diagnostic classification systems. In line with Stobik et al. (2005), we argue that bothersome tinnitus should be understood as a complex psychosomatic phenomenon including somatic, auditory, and psychosocial aspects, which can mutually reinforce each other. Consistent with this view, our results implicate the need for multimodal psychosomatic treatment for bothersome tinnitus in an interdisciplinary setting. Treatment-induced reductions of affective or anxiety symptoms by CBT can directly improve tinnitus-related distress as well as reduce negative effects of comorbid physical symptoms and hearing-related effects, whereas measures to restore hearing impairment have the potential to decrease *aggravate*d negative effects of mental symptoms. Thus, multimodal treatment approaches combining psychological interventions, hearing aid provision, and medical treatment of comorbid physical symptoms appear to have the highest clinical potential to alleviate tinnitus-related distress.

4.7 Limitations

Limitations of this study include its cross-sectional design and the fact that all variables were measured via self-report and single-item questions. Validated information on medical diagnoses, objective data from audiometric testing, and standardized assessment of tinnitus burden via psychometric questionnaires would constitute preferable sources in terms of reliability and validity. Moreover, other psychological factors known to be related to bothersome tinnitus, e.g., coping styles or cognitive factors, could not be investigated in this study as they were not assessed by the survey. However, we expect that the inclusion of such factors would have improved the prediction of bothersome (vs. non-bothersome tinnitus), rather than changing the nature of our results. As the sample was large, heterogeneous, and, for some part, randomly recruited from the general population, selection biases do not seem likely. However, distorting influences based on self-selection by spontaneous online registration cannot be excluded. Moreover, results might not extend to other cultural contexts. Overall, the magnitude of the effects was rather small (the effect sizes of the regression models were small or medium, and the highest percentage of an effect being mediated was 36%).

4.8 Conclusion

Psychological factors and hearing-related difficulties play key roles in predicting bothersome tinnitus (vs. non-bothersome tinnitus) in a large population sample. As hypothesized, our results suggest that psychological factors partially contribute to explaining the impact of physical comorbidities and hearing-related effects on bothersome tinnitus. This highlights their transdiagnostic importance for aggravating varied physical symptom clusters and offers useful targets for psychological treatment strategies. Subjective hearing impairment and hearing-related difficulties in social situations, on the other hand, seem to partially explain the impact of mental comorbidities on bothersome tinnitus. Overall, these findings implicate the need for interdisciplinary multimodal treatment approaches for patients with bothersome tinnitus, combining psychological interventions, the provision of hearing aids, and medical treatment of comorbid physical symptoms in order to achieve the highest clinical efficacy.

Acknowledgments

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (Grant agreement no 764604).

Conflict of interest

CC is supported by the UK National Institute for Health Research (NIHR) Biomedical Research Centre but the views expressed herein are his own and do not represent those of NIHR nor the UK Department of Health and Social Care. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement

Requests to access the datasets should be directed to Nancy Pedersen: nancy.pedersen@ki.se. Restrictions are based on the Swedish Act (2013:794) requiring that a valid ethical approval is obtained in Sweden.

References

Almqvist, C., Adami, H.-O., Franks, P.W., Groop, L., Ingelsson, E., Kere, J., Lissner, L., Litton, J.-E., Maeurer, M., Michaëlsson, K., Palmgren, J., Pershagen, G., Ploner, A., Sullivan, P.F., Tybring, G., Pedersen, N.L., 2011. LifeGene—a large prospective population-based study of global relevance. Eur. J. Epidemiol. 26, 67–77. https://doi.org/10.1007/s10654-010-9521-x.

Anand, V.T., Mann, S.B., Dash, R.J., Mehra, Y.N., 1989. Auditory investigations in hypothyroidism. Acta Otolaryngol. 108, 83–87. https://doi.org/10.3109/00016488909107396.

Andersson, G., Carlbring, P., Kaldo, V., Ström, L., 2004. Screening of psychiatric disorders via the internet. A pilot study with tinnitus patients. Nord. J. Psychiatry 58, 287–291. https://doi.org/10.1080/08039480410005792.

Aroian, L.A., 1947. The probability function of the product of two normally distributed variables. Ann. Math. Stat. 18, 265–271. https://doi.org/10.1214/aoms/1177730442.

Axelsson, A., Ringdahl, A., 1989. Tinnitus—a study of its prevalence and characteristics. Br. J. Audiol. 23, 53–62. https://doi.org/10.3109/03005368909077819.

Babatunde, O.O., Jordan, J.L., Van der Windt, D.A., Hill, J.C., Foster, N.E., Protheroe, J., 2017. Effective treatment options for musculoskeletal pain in primary care: a systematic overview of current evidence. PLoS One 12, 1–30, e0178621. https://doi.org/10.1371/journal.pone.0178621.

Baigi, A., Oden, A., Almlid-Larsen, V., Barrenäs, M.-L., Holgers, K.-M., 2011. Tinnitus in the general population with a focus on noise and stress: a public health study. Ear Hear. 32, 787–789. https://doi.org/10.1097/AUD.0b013e31822229bd.

Baron, R.M., Kenny, D.A., 1986. The moderator–mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J. Pers. Soc. Psychol. 51, 1173–1182. https://doi.org/10.1037/0022-3514.51.6.1173.

Basso, L., Boecking, B., Brueggemann, P., Pedersen, N.L., Canlon, B., Cederroth, C.R., Mazurek, B., 2020. Gender-specific risk factors and comorbidities of bothersome tinnitus. Front. Neurosci. 14, 1–15, Article No. 706. https://doi.org/10.3389/fnins.2020.00706.

Belli, S., Belli, H., Bahcebasi, T., Ozcetin, A., Alpay, E., Ertem, U., 2008. Assessment of psychopathological aspects and psychiatric comorbidities in patients affected by tinnitus. Eur. Arch. Otorhinolaryngol. 265, 279–285. https://doi.org/10.1007/s00405-007-0440-8.

Beukes, E.W., Manchaiah, V., Andersson, G., Allen, P.M., Terlizzi, P.M., Baguley, D.M., 2018. Situationally influenced tinnitus coping strategies: a mixed methods approach. Disabil. Rehabil. 40, 2884–2894. https://doi.org/10.1080/09638288.2017.1362708.

Bjorne, A., Agerberg, G., 1996. Craniomandibular disorders in patients with Menière's disease: a controlled study. J. Orofac. Pain 10, 28–37.

Borghi, C., Cosentino, E.R., Rinaldi, E.R., Brandolini, C., Rimondi, M.C., Veronesi, M., et al., 2011. Tinnitus in elderly patients and prognosis of mild-to-moderate congestive heart failure: a cross-sectional study with a long-term extension of the clinical follow-up. BMC Med. 9, 1–8, Article No. 80. https://doi.org/10.1186/1741-7015-9-80.

Borsook, D., Youssef, A.M., Simons, L., Elman, I., Eccleston, C., 2018. When pain gets stuck: the evolution of pain chronification and treatment resistance. Pain 159, 2421–2436. https://doi.org/10.1097/j.pain.0000000000001401.

Brueggemann, P., Szczepek, A., Rose, M., McKenna, L., Olze, H., Mazurek, B., 2016. Impact of multiple factors on the degree of tinnitus distress. Front. Hum. Neurosci. 10, 1–11, Article No. 341. https://doi.org/10.3389/fnhum.2016.00341.

Brueggemann, P., Seydel, C., Schaefer, C., Szczepek, A.J., Amarjargal, N., Boecking, B., et al., 2019. ICD-10 symptom rating questionnaire for assessment of psychological comorbidities in patients with chronic tinnitus. HNO 67, 178–183. https://doi.org/10.1007/s00106-019-0625-7.

Budd, R.J., Pugh, R., 1996. Tinnitus coping style and its relationship to tinnitus severity and emotional distress. J. Psychosom. Res. 41, 327–335. https://doi.org/10.1016/S0022-3999(96)00171-7.

Caldirola, D., Teggi, R., Daccò, S., Sangiorgio, E., Bussi, M., Perna, G., 2016. Role of worry in patients with chronic tinnitus and sensorineural hearing loss: a preliminary study. Eur. Arch. Otorhinolaryngol. 273, 4145–4151. https://doi.org/10.1007/s00405-016-4100-8.

Cil, Ö.Ç., Zateri, C., Güçlü, O., Oymak, S., Tezcan, E., 2020. The effect of fibromyalgia treatment on tinnitus. Am. J. Otolaryngol. 41, 102390. https://doi.org/10.1016/j.amjoto.2020.102390.

Ciminelli, P., Machado, S., Palmeira, M., Carta, M.G., Beirith, S.C., Nigri, M.L., et al., 2018. Tinnitus: the sound of stress? Clin. Pract. Epidemiol. Ment. Health 14, 264–269. https://doi.org/10.2174/1745017901814010264.

Coebergh, J.A.F., Lauw, R.F., Sommer, I.E.C., Blom, J.D., 2019. Musical hallucinations and their relation with epilepsy. J. Neurol. 266, 1501–1515. https://doi.org/10.1007/s00415-019-09289-x.

Coelho, C.B., Santos, R., Campara, K.F., Tyler, R., 2020. Classification of tinnitus. Otolaryngol. Clin. North Am. 53, 515–529. https://doi.org/10.1016/j.otc.2020.03.015.

Cohen, J., 1992. A power primer. Psychol. Bull. 112, 155–159. https://doi.org/10.1037/0033-2909.112.1.155.

Cohen, J., 1988. Statistical Power Analysis for the Behavioral Sciences. L. Erlbaum Associates, Hillsdale, NJ.

Daugherty, W.T., Lederman, R.J., Nodar, R.H., Conomy, J.P., 1983. Hearing loss in multiple sclerosis. Arch. Neurol. 40, 33–35. https://doi.org/10.1001/archneur.1983.04050010053013.

Di Stadio, A., Ralli, M., 2017. Systemic lupus erythematosus and hearing disorders: literature review and meta-analysis of clinical and temporal bone findings. J. Int. Med. Res. 45, 1470–1480. https://doi.org/10.1177/0300060516688600.

Fagelson, M.A., 2007. The association between tinnitus and posttraumatic stress disorder. Am. J. Audiol. 16, 107–117. https://doi.org/10.1044/1059-0889(2007/015).

Figueiredo, R.R., de Azevedo, A.A., Penido, N.O., 2015. Tinnitus and arterial hypertension: a systematic review. Eur. Arch. Otorhinolaryngol 272, 3089–3094. https://doi.org/10.1007/s00405-014-3277-y.

Figueiredo, R.R., de Azevedo, A.A., Penido, N.O., 2017. Ménière's disease and tinnitus. In: Fayez, B. Jr. (Ed.), Up to Date on Meniere's Disease. https://doi.org/10.5772/66390.

Fischer, C., Mauguière, F., Ibanez, V., Confavreux, C., Chazot, G., 1985. The acute deafness of definite multiple sclerosis: Baep patterns. Electroencephalogr. Clin. Neurophysiol. 61, 7–15. https://doi.org/10.1016/0013-4694(85)91066-1.

Folmer, R.L., Griest, S.E., Martin, W.H., 2008. Obsessive-compulsiveness in a population of tinnitus patients. Int. Tinnitus J. 14, 127–130.

Fried, E.I., van Borkulo, C.D., Cramer, A.O.J., Boschloo, L., Schoevers, R.A., Borsboom, D., 2017. Mental disorders as networks of problems: a review of recent insights. Soc. Psychiatry Psychiatr. Epidemiol. 52, 1–10. https://doi.org/10.1007/s00127-016-1319-z.

Fujii, K., Nagata, C., Nakamura, K., Kawachi, T., Takatsuka, N., Oba, S., et al., 2011. Prevalence of tinnitus in community-dwelling Japanese adults. J. Epidemiol. 21, 299–304. https://doi.org/10.2188/jea.JE20100124.

Gallus, S., Lugo, A., Garavello, W., Bosetti, C., Santoro, E., Colombo, P., Perin, P., La Vecchia, C., Langguth, B., 2015. Prevalence and determinants of tinnitus in the Italian adult population. Neuroepidemiology 45, 12–19. https://doi.org/10.1159/000431376.

Geocze, L., Mucci, S., Abranches, D.C., de Marco, M.A., de Penido, N.O., 2013. Systematic review on the evidences of an association between tinnitus and depression. Braz. J. Otorhinolaryngol. 79, 106–111. https://doi.org/10.5935/1808-8694.20130018.

Gibrin, P.C.D., Melo, J.J., Marchiori, L.L.d.M., 2013. Prevalence of tinnitus complaints and probable association with hearing loss, diabetes mellitus and hypertension in elderly. CoDAS 25, 176–180. https://doi.org/10.1590/S2317-17822013000200014.

Guichard, E., Montagni, I., Tzourio, C., Kurth, T., 2016. Association between headaches and tinnitus in young adults: cross-sectional study. Headache 56, 987–994. https://doi.org/10.1111/head.12845.

Hage, M.P., Azar, S.T., 2012. The link between thyroid function and depression. J. Thyroid Res. 2012, 1–8. https://doi.org/10.1155/2012/590648.

Hamed, S.A., Oseilly, A.M., 2018. Cochlear function in adults with epilepsy and treated with carbamazepine. Audiol. Neurootol. 23, 63–72. https://doi.org/10.1159/000490232.

Hasson, D., Theorell, T., Wallén, M.B., Leineweber, C., Canlon, B., 2011. Stress and prevalence of hearing problems in the Swedish working population. BMC Public Health 11 (1), 130. https://doi.org/10.1186/1471-2458-11-130.

Hébert, S., Canlon, B., Hasson, D., 2012. Emotional exhaustion as a predictor of tinnitus. Psychother. Psychosom. 81, 324–326. https://doi.org/10.1159/000335043.

Henry, J.A., Dennis, K.C., Schechter, M.A., 2005. General review of tinnitus: prevalence, mechanisms, effects, and management. J. Speech Lang. Hear. Res. 48, 1204–1235. https://doi.org/10.1044/1092-4388(2005/084).

Herr, N.R., 2006. Mediation with Dichotomous Outcomes. viewed 25 February 2020. http://web.archive.org/web/20200202182013/http://nrhpsych.com/mediation/logmed.html.

Herr, R.M., Loerbroks, A., Bosch, J.A., Seegel, M., Schneider, M., Schmidt, B., 2016. Associations of organizational justice with tinnitus and the mediating role of depressive symptoms and burnout—findings from a cross-sectional study. Int. J. Behav. Med. 23, 190–197. https://doi.org/10.1007/s12529-015-9505-z.

Hinton, D.E., Chhean, D., Pich, V., Hofmann, S.G., Barlow, D.H., 2006. Tinnitus among Cambodian refugees: relationship to PTSD severity. J. Trauma. Stress 19, 541–546. https://doi.org/10.1002/jts.20138.

Hoekstra, C.E.L., Wesdorp, F.M., van Zanten, G.A., 2014. Socio-demographic, health, and tinnitus related variables affecting tinnitus severity. Ear Hear. 35, 544–554. https://doi.org/10.1097/AUD.0000000000000045.

Hofmann, S.G., 2014. Toward a cognitive-behavioral classification system for mental disorders. Behav. Ther. 45, 576–587. https://doi.org/10.1016/j.beth.2014.03.001.

Hofmann, S.G., Asnaani, A., Vonk, I.J.J., Sawyer, A.T., Fang, A., 2012. The efficacy of cognitive behavioral therapy: a review of meta-analyses. Cogn. Ther. Res. 36, 427–440. https://doi.org/10.1007/s10608-012-9476-1.

Holgers, K.-M., Zöger, S., Svedlund, K., 2005. Predictive factors for development of severe tinnitus suffering-further characterization. Int. J. Audiol. 44, 584–592. https://doi.org/10.1080/14992020500190235.

Hwang, J.H., Tsai, S.J., Liu, T.C., Chen, Y.C., Lai, J.T., 2018. Association of tinnitus and other cochlear disorders with a history of migraines. JAMA Otolaryngol. Head Neck Surg 144, 712–717. https://doi.org/10.1001/jamaoto.2018.0939.

Iikuni, F., Nomura, Y., Goto, F., Murakami, M., Shigihara, S., Ikeda, M., 2013. Why do patients with fibromyalgia complain of ear-related symptoms? Ear-related symptoms and otological findings in patients with fibromyalgia. Clin. Rheumatol. 32, 1437–1441. https://doi.org/10.1007/s10067-013-2287-2.

Ivansic, D., Besteher, B., Gantner, J., Guntinas-Lichius, O., Pantev, C., Nenadic, I., et al., 2019. Psychometric assessment of mental health in tinnitus patients, depressive and healthy controls. Psychiatry Res. 281, 112582. https://doi.org/10.1016/j.psychres.2019.112582.

Jalessi, M., Farhadi, M., Asghari, A., Kamrava, S.K., Amintehran, E., Ghalehbaghi, S., et al., 2013. Tinnitus: an epidemiologic study in Iranian population. Acta Med. Iran. 51, 886–891.

Kim, H.-J., Lee, H.-J., An, S.-Y., Sim, S., Park, B., Kim, S.W., Lee, J.S., Hong, S.K., Choi, H.G., 2015. Analysis of the prevalence and associated risk factors of tinnitus in adults. PLoS One 10, e0127578. https://doi.org/10.1371/journal.pone.0127578.

Kostev, K., Alymova, S., Kössl, M., Jacob, L., 2019. Risk factors for tinnitus in 37,692 patients followed in general practices in Germany. Otol. Neurotol. 40, 436–440. https://doi.org/10.1097/MAO.0000000000002161.

Kotov, R., Krueger, R.F., Watson, D., Achenbach, T.M., Althoff, R.R., Bagby, R.M., Brown, T.A., Carpenter, W.T., Caspi, A., Clark, L.A., Eaton, N.R., Forbes, M.K., Forbush, K.T., Goldberg, D., Hasin, D., Hyman, S.E., Ivanova, M.Y., Lynam, D.R., Markon, K., et al., 2017. The hierarchical taxonomy of psychopathology (HiTOP): a dimensional alternative to traditional nosologies. J. Abnorm. Psychol. 126, 454–477. https://doi.org/10.1037/abn0000258.

Kreuzer, P.M., Landgrebe, M., Vielsmeier, V., Kleinjung, T., De Ridder, D., Langguth, B., 2014. Trauma-associated tinnitus. J. Head Trauma Rehabil. 29, 432–442. https://doi.org/10.1097/HTR.0b013e31829d3129.

Kuttila, S., Kuttila, M., Bell, Y.L., Alanen, P., Suonpää, J., 2005. Recurrent tinnitus and associated ear symptoms in adults. Int. J. Audiol. 44, 164–170. https://doi.org/10.1080/14992020500057608.

Lahey, B.B., Krueger, R.F., Rathouz, P.J., Waldman, I.D., Zald, D.H., 2017. A hierarchical causal taxonomy of psychopathology across the life span. Psychol. Bull. 143, 142–186. https://doi.org/10.1037/bul0000069.

Langguth, B., Landgrebe, M., Kleinjung, T., Sand, G.P., Hajak, G., 2011. Tinnitus and depression. World J. Biol. Psychiatry 12, 489–500. https://doi.org/10.3109/15622975.2011.575178.

Langguth, B., Hund, V., Busch, V., Jürgens, T.P., Lainez, J.-M., Landgrebe, M., Schecklmann, M., 2015. Tinnitus and headache. Biomed. Res. Int. 2015, 1–7. https://doi.org/10.1155/2015/797416.

Lee, S.-Y., Kim, J.-H., Hong, S.-H., Lee, D.-S., 2004. Roles of cognitive characteristics in tinnitus patients. J. Korean Med. Sci. 19, 864. https://doi.org/10.3346/jkms.2004.19.6.864.

LifeGene, 2017. Description of the LifeGene Resource. viewed 27 March 2020, https://lifegene.se/wp-content/uploads/1LifeGeneresource20170203version24.pdf.

Lin, C.-E., Chen, L.-F., Chou, P.-H., Chung, C.-H., 2018. Increased prevalence and risk of anxiety disorders in adults with tinnitus: a population-based study in Taiwan. Gen. Hosp. Psychiatry 50, 131–136. https://doi.org/10.1016/j.genhosppsych.2017.11.006.

Lugo, A., Trpchevska, N., Liu, X., Biswas, R., Magnusson, C., Gallus, S., Cederroth, C.R., 2019. Sex-specific association of tinnitus with suicide attempts. JAMA Otolaryngol. Neck Surg. 145, 685. https://doi.org/10.1001/jamaoto.2019.0566.

Lugo, A., Edvall, N.K., Lazar, A., Mehraei, G., Lopez-Escamez, J.-A., Bulla, J., et al., 2020. Relationship between headaches and tinnitus in a Swedish study. Sci. Rep. 10, 8494. https://doi.org/10.1038/s41598-020-65395-1.

Mackinnon, D.P., Dwyer, J.H., 1993. Estimating mediated effects in prevention studies. Eval. Rev. 17, 144–158. https://doi.org/10.1177/0193841X9301700202.

Mahafzah, M.T., Mahafza, T., Omari, H., Al Hawari, H.H., 2018. Investigating the possible audiological effects of hypothyroidism. J. Phon. Audiol. 4. https://doi.org/10.4172/2471-9455.1000137.

Malakouti, S., Mahmoudian, M., Alifattahi, N., Salehi, M., 2011. Comorbidity of chronic tinnitus and mental disorders. Int. Tinnitus J. 16, 118–122.

Malik, V., Shukla, G.K., Bhatia, N., 2002. Hearing profile in hypothyroidism. Indian J. Otolaryngol. Head Neck Surg. 54, 285–290. https://doi.org/10.1007/BF02993744.

Martines, F., Sireci, F., Cannizzaro, E., Costanzo, R., Martines, E., Mucia, M., Plescia, F., Salvago, P., 2015. Clinical observations and risk factors for tinnitus in a Sicilian cohort. Eur. Arch. Otorhinolaryngol. 272, 2719–2729. https://doi.org/10.1007/s00405-014-3275-0.

Martinez-Devesa, P., Perera, R., Theodoulou, M., Waddell, A., 2010. Cognitive behavioural therapy for tinnitus. Cochrane Database Syst. Rev. (9), Article No. CD005233. https://doi.org/10.1002/14651858.CD005233.pub3.

Mathias, K.d.V., Mezzasalma, M.A., Nardi, A.E., 2011. Prevalência de transtorno de pânico em pacientes com zumbidos [the prevalence of panic disorder in patients with tinnitus]. Arch. Clin. Psychiatry 38, 139–142. https://doi.org/10.1590/S0101-60832011000400005.

McCormack, A., Edmondson-Jones, M., Somerset, S., Hall, D., 2016. A systematic review of the reporting of tinnitus prevalence and severity. Hear. Res. 337, 70–79. https://doi.org/10.1016/j.heares.2016.05.009.

Menendez, M.E., Baker, D.K., Oladeji, L.O., Fryberger, C.T., McGwin, G., Ponce, B.A., 2015. Psychological distress is associated with greater perceived disability and pain in patients presenting to a shoulder clinic. J. Bone Joint Surg. Am. 97, 1999–2003. https://doi.org/10.2106/JBJS.O.00387.

Michikawa, T., Nishiwaki, Y., Kikuchi, Y., Saito, H., Mizutari, K., Okamoto, M., Takebayashi, T., 2010. Prevalence and factors associated with tinnitus: a community-based study of Japanese elders. J. Epidemiol. 20, 271–276. https://doi.org/10.2188/jea.JE20090121.

Nees, F., Becker, S., 2018. Psychological processes in chronic pain: influences of reward and fear learning as key mechanisms—behavioral evidence, neural circuits, and maladaptive changes. Neuroscience 387, 72–84. https://doi.org/10.1016/j.neuroscience.2017.08.051.

Nondahl, D.M., Cruickshanks, K.J., Wiley, T.L., Klein, R., Klein, B.E.K., Tweed, T.S., 2002. Prevalence and 5-year incidence of tinnitus among older adults: the epidemiology of hearing loss study. J. Am. Acad. Audiol. 13, 323–331.

Nondahl, D.M., Cruickshanks, K.J., Huang, G.-H., Klein, B.E.K., Klein, R., Javier Nieto, F., Tweed, T.S., 2011. Tinnitus and its risk factors in the beaver dam offspring study. Int. J. Audiol. 50, 313–320. https://doi.org/10.3109/14992027.2010.551220.

Park, K.H., Lee, S.H., Koo, J.-W., Park, H.Y., Lee, K.Y., Choi, Y.S., Oh, K.W., Lee, A., Yang, J.-E., Woo, S.-Y., Kim, S.W., Cho, Y.-S., 2014. Prevalence and associated factors of tinnitus: data from the Korean National Health and Nutrition Examination Survey 2009–2011. J. Epidemiol. 24, 417–426. https://doi.org/10.2188/jea.JE20140024.

Pattyn, T., Van Den Eede, F., Vanneste, S., Cassiers, L., Veltman, D.J., Van De Heyning, P., et al., 2016. Tinnitus and anxiety disorders: a review. Hear. Res. 333, 255–265. https://doi.org/10.1016/j.heares.2015.08.014.

Pattyn, T., Vanneste, S., De Ridder, D., Van Rompaey, V., Veltman, D.J., Van de Heyning, P., et al., 2018. Differential electrophysiological correlates of panic disorder in non-pulsatile tinnitus. J. Psychosom. Res. 109, 57–62. https://doi.org/10.1016/j.jpsychores.2018.03.168.

Peterson, U., Demerouti, E., Bergström, G., Samuelsson, M., Åsberg, M., Nygren, Å., 2008. Burnout and physical and mental health among Swedish healthcare workers. J. Adv. Nurs. 62, 84–95. https://doi.org/10.1111/j.1365-2648.2007.04580.x.

Pinto, P.C.L., Marcelos, C.M., Mezzasalma, M.A., Osterne, F.J.V., de Melo Tavares de Lima, M.A., Nardi, A.E., 2014. Tinnitus and its association with psychiatric disorders: systematic review. J. Laryngol. Otol. 128, 660–664. https://doi.org/10.1017/S0022215114001030.

Probst, T., Pryss, R., Langguth, B., Schlee, W., 2016. Emotional states as mediators between tinnitus loudness and tinnitus distress in daily life: results from the "TrackYourTinnitus" application. Sci. Rep. 6, 1–8, Article No. 20382. https://doi.org/10.1038/srep20382.

Ramage-Morin, P.L., Banks, R., Pineault, D., Atrach, M., 2019. Tinnitus in Canada. Health Rep. 30, 3–11. https://doi.org/10.25318/82-003-x201900300001-eng.

Rauschecker, J.P., May, E.S., Maudoux, A., Ploner, M., 2015. Frontostriatal gating of tinnitus and chronic pain. Trends Cogn. Sci. 19, 567–578. https://doi.org/10.1016/j.tics.2015.08.002.

Ren, Y.-F., Isberg, A., 1995. Tinnitus in patients with temporomandibular joint internal derangement. Cranio 13, 75–80. https://doi.org/10.1080/08869634.1995.11678048.

Rhee, J., Lee, D., Suh, M.W., Lee, J.H., Hong, Y.-C., Oh, S.H., et al., 2020. Prevalence, associated factors, and comorbidities of tinnitus in adolescents. PLoS One 15, e0236723. https://doi.org/10.1371/journal.pone.0236723.

Rodriguez-Casero, M.V., Mandelstam, S., Kornberg, A.J., Berkowitz, R.G., 2005. Acute tinnitus and hearing loss as the initial symptom of multiple sclerosis in a child. Int. J. Pediatr. Otorhinolaryngol. 69, 123–126. https://doi.org/10.1016/j.ijporl.2004.08.006.

Romero Sánchez, I., Pérez Garrigues, H., Rodríguez Rivera, V., 2010. Clinical characteristics of tinnitus in Ménière's disease. Acta Otorrinolaringol. Esp. 61, 327–331. https://doi.org/10.1016/S2173-5735(10)70060-0.

Sahin, C., Aras, H.İ., Yilmaz, M.S., 2016. Somatoform disorders in patients with chronic subjective tinnitus. Eur. Arch. Otorhinolaryngol. 273, 3603–3607. https://doi.org/10.1007/s00405-016-3968-7.

Salazar, J.W., Meisel, K., Smith, E.R., Quiggle, A., McCoy, D.B., Amans, M.R., 2019. Depression in patients with tinnitus: a systematic review. Otolaryngol. Head Neck Surg. 161, 28–35. https://doi.org/10.1177/0194599819835178.

Seligman, F., Nemeroff, C.B., 2015. The interface of depression and cardiovascular disease: therapeutic implications. Ann. N. Y. Acad. Sci. 1345, 25–35. https://doi.org/10.1111/nyas.12738.

Seo, J.H., Kang, J.M., Hwang, S.H., Han, K.D., Joo, Y.H., 2016. Relationship between tinnitus and suicidal behaviour in Korean men and women: a cross-sectional study. Clin. Otolaryngol. 41, 222–227. https://doi.org/10.1111/coa.12500.

Shargorodsky, J., Curhan, G.C., Farwell, W.R., 2010. Prevalence and characteristics of tinnitus among US adults. Am. J. Med. 123, 711–718. https://doi.org/10.1016/j.amjmed.2010.02.015.

Sharlin, D.S., Ng, L., Verrey, F., Visser, T.J., Liu, Y., Olszewski, R.T., Hoa, M., Heuer, H., Forrest, D., 2018. Deafness and loss of cochlear hair cells in the absence of thyroid hormone transporters Slc16a2 (Mct8) and Slc16a10 (Mct10). Sci. Rep. 8, 4403. https://doi.org/10.1038/s41598-018-22553-w.

Shih, C.-P., Lin, H.-C., Chung, C.-H., Hsiao, P.-J., Wang, C.-H., Lee, J.-C., et al., 2017. Increased risk of tinnitus in patients with chronic kidney disease: a nationwide, population-based cohort study. PLoS One 12, e0183192. https://doi.org/10.1371/journal.pone.0183192.

Shore, S.E., Roberts, L.E., Langguth, B., 2016. Maladaptive plasticity in tinnitus-triggers, mechanisms and treatment. Nat. Rev. Neurol. 12, 150–160. https://doi.org/10.1038/nrneurol.2016.12.

Siivola, S.M., Levoska, S., Latvala, K., Hoskio, E., Vanharanta, H., Keinänen-Kiukaanniemi, S., 2004. Predictive factors for neck and shoulder pain: a longitudinal study in young adults. Spine 29, 1662–1669. https://doi.org/10.1097/01.BRS.0000133644.29390.43.

Sindhusake, D., Golding, M., Newall, P., Rubin, G., Jakobsen, K., Mitchell, P., 2003. Risk factors for tinnitus in a population of older adults: the Blue Mountains hearing study. Ear Hear. 24, 501–507. https://doi.org/10.1097/01.AUD.0000100204.08771.3D.

Singh, R., Aftab, M., Jain, S., Kumar, D., 2019. Audiological evaluation in hypothyroid patients and effect of thyroxine replacement therapy. Indian J. Otolaryngol. Head Neck Surg. 71, 548–552. https://doi.org/10.1007/s12070-018-1395-3.

Skov, T., Borg, V., Orhede, E., 1996. Psychosocial and physical risk factors for musculoskeletal disorders of the neck, shoulders, and lower back in salespeople. Occup. Environ. Med. 53, 351–356.

Spankovich, C., Gonzalez, V.B., Su, D., Bishop, C.E., 2018. Self reported hearing difficulty, tinnitus, and normal audiometric thresholds, the National Health and Nutrition Examination Survey 1999-2002. Hear. Res. 358, 30–36. https://doi.org/10.1016/j.heares.2017.12.001.

Stobik, C., Weber, R.K., Münte, T.F., Walter, M., Frommer, J., 2005. Evidence of psychosomatic influences in compensated and decompensated tinnitus. Int. J. Audiol. 44, 370–378. https://doi.org/10.1080/14992020500147557.

Stohler, N.A., Reinau, D., Jick, S.S., Bodmer, D., Meier, C.R., 2019. A study on the epidemiology of tinnitus in the United Kingdom. Clin. Epidemiol. 11, 855–871. https://doi.org/10.2147/CLEP.S213136.

Stouffer, J.L., Tyler, R.S., 1990. Characterization of tinnitus by tinnitus patients. J. Speech Hear. Disord. 55, 439–453. https://doi.org/10.1044/jshd.5503.439.

Turner, J., Kelly, B., 2000. Emotional dimensions of chronic disease. West. J. Med. 172, 124–128. https://doi.org/10.1136/ewjm.172.2.124.

Tyler, R.S., Baker, L.J., 1983. Difficulties experienced by tinnitus sufferers. J. Speech Hear. Disord. 48, 150–154. https://doi.org/10.1044/jshd.4802.150.

Uziel, A., Legrand, C., Rabie, A., 1985. Corrective effects of thyroxine on cochlear abnormalities induced by congenital hypothyroidism in the rat. I. Morphological study. Dev. Brain Res. 19, 111–122. https://doi.org/10.1016/0165-3806(85)90236-6.

van de Leemput, I.A., Wichers, M., Cramer, A.O.J., Borsboom, D., Tuerlinckx, F., Kuppens, P., et al., 2014. Critical slowing down as early warning for the onset and termination of depression. Proc. Natl. Acad. Sci. 111, 87–92. https://doi.org/10.1073/pnas.1312114110.

Waylonis, G.W., Heck, W., 1992. Fibromyalgia syndrome: new associations. Am. J. Phys. Med. Rehabil. 71, 343–348. https://doi.org/10.1097/00002060-199212000-00006.

Weise, C., Hesser, H., Andersson, G., Nyenhuis, N., Zastrutzki, S., Kröner-Herwig, B., Jäger, B., 2013. The role of catastrophizing in recent onset tinnitus: its nature and association with tinnitus distress and medical utilization. Int. J. Audiol. 52, 177–188. https://doi.org/10.3109/14992027.2012.752111.

Yang, P., Ma, W., Zheng, Y., Yang, H., Lin, H., 2015. A systematic review and meta-analysis on the association between hypertension and tinnitus. Int. J. Hypertens. 2015, 1–7. https://doi.org/10.1155/2015/583493.

Yoshida, T., Stephens, D., Kentala, E., Levo, H., Auramo, Y., Poe, D., Pyykkö, I., 2011. Tinnitus complaint behaviour in long-standing Menière's disorder: its association with the other cardinal symptoms. Clin. Otolaryngol. 36, 461–467. https://doi.org/10.1111/j.1749-4486.2011.02381.x.

Ziai, K., Moshtaghi, O., Mahboubi, H., Djalilian, H.R., 2017. Tinnitus patients suffering from anxiety and depression: a review. Int. Tinnitus J. 21, https://doi.org/10.5935/0946-5448.20170013.

Publication 2: Basso L, Boecking B, Neff P, Brueggemann P, Peters EMJ, Mazurek B. Hair-cortisol and hair-BDNF as biomarkers of tinnitus loudness and distress in chronic tinnitus. *Scientific Reports.* 2022;12(1):1934. doi:10.1038/s41598-022-04811-0

- Article submitted September 6, 2021; published February 4, 2022 in *Scientific Reports*
- Impact Factor 2020: 4.379
- Excerpt from Journal Summary List from 2020, Category “MULTIDISCIPLINARY SCIENCES”: Rank 17 of 73 journals

Journal Data Filtered By: **Selected JCR Year: 2020** Selected Editions: SCIE,SSCI Selected Categories: **"MULTIDISCIPLINARY SCIENCES"** Selected Category Scheme: WoS

Gesamtanzahl: 73 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	NATURE	915,925	49.962	1.089400
2	SCIENCE	814,971	47.728	0.895760
3	National Science Review	5,889	17.275	0.011400
4	Nature Communications	453,215	14.919	1.238540
5	Science Advances	65,205	14.136	0.218640
6	Nature Human Behaviour	5,549	13.663	0.023120
7	Science Bulletin	8,832	11.780	0.016400
8	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA	799,058	11.205	0.806620
9	Journal of Advanced Research	5,927	10.479	0.006800
10	GigaScience	5,876	6.524	0.018630
11	Scientific Data	10,617	6.444	0.034470
12	Frontiers in Bioengineering and Biotechnology	7,470	5.890	0.011340
13	ANNALS OF THE NEW YORK ACADEMY OF SCIENCES	52,619	5.691	0.021430
14	iScience	5,235	5.458	0.012300
15	Research Synthesis Methods	3,926	5.273	0.007520
16	NPJ Microgravity	594	4.415	0.001790
17	Scientific Reports	541,615	4.379	1.232500
18	PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A-MATHEMATICAL PHYSICAL AND ENGINEERING SCIENCES	24,950	4.226	0.025400

www.nature.com/scientificreports

scientific reports

Check for updates

OPEN

Hair-cortisol and hair-BDNF as biomarkers of tinnitus loudness and distress in chronic tinnitus

Laura Basso[1], Benjamin Boecking[1], Patrick Neff[2,3,4], Petra Brueggemann[1], Eva M. J. Peters[5,6] & Birgit Mazurek[1]✉

The role of stress and its neuroendocrine mediators in tinnitus is unclear. In this study, we measure cortisol as an indicator of hypothalamus–pituitary–adrenal (HPA) axis alterations and brain-derived neurotrophic factor (BDNF) as a marker of adaptive neuroplasticity in hair of chronic tinnitus patients to investigate relationships with tinnitus-related and psychological factors. Cross-sectional data from chronic tinnitus inpatients were analyzed. Data collection included hair sampling, pure tone audiometry, tinnitus pitch and loudness matching, and psychometric questionnaires. Elastic net regressions with n-fold cross-validation were performed for cortisol (N = 91) and BDNF (N = 87). For hair-cortisol (R^2 = 0.10), the strongest effects were sampling in autumn and body-mass index (BMI) (positive), followed by tinnitus loudness (positive) and smoking (negative). For hair-BDNF (R^2 = 0.28), the strongest effects were hearing aid use, shift work (positive), and tinnitus loudness (negative), followed by smoking, tinnitus-related distress (Tinnitus Questionnaire), number of experienced traumatic events (negative), and physical health-related quality of life (Short Form-12 Health Survey) (positive). These findings suggest that in chronic tinnitus patients, higher perceived tinnitus loudness is associated with higher hair-cortisol and lower hair-BDNF, and higher tinnitus-related distress with lower hair-BDNF. Regarding hair-BDNF, traumatic experiences appear to have additional stress-related effects, whereas hearing aid use and high physical health-related quality of life appear beneficial. Implications include the potential use of hair-cortisol and hair-BDNF as biomarkers of tinnitus loudness or distress and the need for intensive future research into chronic stress-related HPA axis and neuroplasticity alterations in chronic tinnitus.

The pathogenic mechanisms linking tinnitus and stress are still not fully understood[1–3]. Stress can be related to the onset of tinnitus, and higher stress levels seem associated with higher perceived tinnitus severity[1,2]. Recently, the need to distinguish between tinnitus perception (symptom) and tinnitus associated with suffering (tinnitus disorder) has been highlighted[4]. Moreover, perceived tinnitus loudness and tinnitus-related distress are two distinct phenomena[5] that appear linked by psychological factors like tinnitus acceptance[6] and subjective stress level[7].

The stress response refers to an organism's reactive or anticipatory response to acute challenges[8] and, in the short term, is an adaptive process to maintain homeostasis[9]. Chronic stress, however, can lead to maladaptation with long-term pathophysiological effects, often described by the concept of allostatic load/overload[9]. Emotional exhaustion, resulting from chronic stress, was found to mediate the relationship between hearing loss and tinnitus severity[10,11]. Moreover, common psychological conditions in tinnitus patients, such as anxiety[12] and depression[13], are known to be chronic stress-related[11,14]. Overall, chronic stress is an important factor in tinnitus patients seeking clinical help.

The hypothalamus–pituitary–adrenal (HPA) axis is a primary neuroendocrine stress response system. Chronic stress can lead to a dysregulation of the HPA axis, which can manifest in altered stress response profiles to acute challenges[8]. Previous studies on HPA axis function measuring salivary cortisol in tinnitus patients reported lower overall cortisol levels[15], a blunted cortisol response to an acute experimental psychosocial stressor[16], increased

[1]Tinnitus Center, Charité – Universitätsmedizin Berlin, Berlin, Germany. [2]Department of Psychiatry and Psychotherapy, University of Regensburg, Regensburg, Germany. [3]University Research Priority Program 'Dynamics of Healthy Aging', University of Zurich, Zurich, Switzerland. [4]Department of Psychology, Centre for Cognitive Neuroscience, University of Salzburg, Salzburg, Austria. [5]Psychoneuroimmunology Laboratory, Department of Psychosomatic Medicine and Psychotherapy, Justus-Liebig University Giessen, Giessen, Germany. [6]Psychosomatics and Psychotherapy, Charité Center 12 Internal Medicine and Dermatology, Charité – Universitätsmedizin Berlin, Berlin, Germany. ✉email: birgit.mazurek@charite.de

Inclusion criteria	Exclusion criteria
Diagnosis of chronic subjective tinnitus	Inability to consent due to serious mental or physical impairments
Age ≥ 18 years	Simultaneous participation in other research studies
Written informed consent	Hair length < 3 cm
	Any chemical hair treatment within 1 month prior to sampling (dying, bleaching, perming, or else)
	Hair washing or the use of hair products (hair mousse, hair gel, hair wax, hair spray) within 3 days prior to sampling
	Hair combing on the day of sampling

Table 1. Inclusion and exclusion criteria.

sensitivity of the HPA axis negative feedback response found with the dexamethasone suppression test[17], and a flattened cortisol awakening response in tinnitus patients with high distress[18]. While these studies on salivary cortisol indicate reduced responsiveness of the HPA axis in tinnitus, findings on blood-cortisol levels are conflicting, reporting no association[19], negative associations with tinnitus frequency and loudness[20], or treatment-related decreases[21]. Overall, HPA axis alterations in tinnitus and their relationship with tinnitus-related distress remain unclear.

Saliva and blood sampling are methods for the measurement of short-term cortisol release boosts, which can be influenced by situational factors[22,23]. By contrast, the measurement of cortisol in hair provides a reliable long-term measure of cumulative cortisol secretion, reflecting integrated HPA axis activity[24–26]. Given the average growth rate of hair by 1 cm per month[27], analysis of the 1 cm hair segment most proximal to the scalp allows a retrospective estimate of cumulative cortisol production over the past month. A meta-analysis aggregating data from 66 studies found that different groups exposed to chronic stress (e.g., caregiving stress, unemployment, natural disasters) showed overall elevated hair-cortisol levels by 22% compared to controls[22].

Brain-derived neurotrophic factor (BDNF) is another important stress-related biomarker and a crucial factor for neuroprotection and synaptic plasticity[28]. Several neurodegenerative and psychiatric disorders are associated with reduced BDNF levels in both the blood and the brain[29,30]. It is thought that acute stress increases BDNF levels, whereas chronic stress leads to a downregulation of BDNF[31,32]. The mixed results of previous studies on BDNF levels in tinnitus patients[33–36] might be related to situational influences on BDNF measurement in blood. BDNF concentrations can also be measured in hair, which was shown in a pilot study[37]. Measurement of hair-BDNF may offer a new approach to clarify the long-term neuroendocrine changes in chronic tinnitus and associations of BDNF with tinnitus-related distress.

In addition to the sampling material, another important issue is the handling of confounders, as both stress-related biomarkers and tinnitus characteristics can be influenced by a multiplicity of factors. The presence of many and correlated variables is associated with variable selection problems for multivariable regression modeling, which can cause selection bias, overfitting, and replication issues[38]. Elastic net regression is a modern penalized regression procedure that addresses these issues. It counteracts collinearity and overfitting by introducing a penalty term and selection bias by performing automatic variable selection[38,39].

To date, hair-cortisol and hair-BDNF have not been studied in tinnitus. This study investigates hair-cortisol and hair-BDNF in chronic tinnitus patients and their associations with tinnitus-related and psychological factors while adequately controlling for confounding influences by elastic net regression. The main aim of the present study is to identify relationships of these biomarkers with tinnitus-related distress, as this might provide instructive new insights into their potential use as therapeutic efficacy measures. Biomarker measurement in hair and the use of this state-of-the-art methodological approach represent the strengths that set our study apart from previous research. Based on assumed long-term stress-related effects in chronic tinnitus patients, our hypotheses are that increased hair-cortisol levels and decreased hair-BDNF levels are associated with higher tinnitus-related distress.

Methods

In total, 94 chronic tinnitus patients volunteered to participate in this study (approx. 16% of treated inpatients) between December 2018 and March 2020 (data collection was stopped due to the COVID-19 pandemic). Inclusion/exclusion criteria are shown in Table 1. Of the recruited patients, one was excluded due to missing data on all questionnaires (hair sample not analyzed), two patients were excluded due to the hair-related criteria, and four patients were excluded due to missing BDNF values. Thus, the final sample consisted of N = 91 for cortisol and N = 87 for BDNF analyses. All participants were European, around two-thirds of the sample were female (65.9%) and participants' age ranged from 19 to 80 years (M = 51.5, SD = 12). All participants provided written informed consent. The study was approved by the local ethic commission of the Charité – Universitätsmedizin Berlin (No. EA1/035/16) and was carried out in accordance with the Declaration of Helsinki.

Cross-sectional measurements included the collection of hair samples and the completion of psychometric questionnaires on the same day and were performed at the Tinnitus Center. In addition, audiometric data were obtained from outpatient audiometric records. On average, audiometric testing was performed 70.14 days (SD = 57.62) prior to the other measurements.

Hair sampling. *Sample collection.* The median sampling time was 09:55 a.m. Samples were cut from the region of the posterior vertex as close to the scalp as possible. Scissors and other materials were cleaned with 70%

isopropyl alcohol between samples. Samples were wrapped in aluminum foil and stored at room temperature in a dark container. All samples were analyzed in summer/autumn 2020 (around 1.75 to 0.5 years after collection).

Cortisol and BDNF extraction and detection. Hair sample analyses for the detection of cortisol and BDNF in the 1-cm hair segment closest to the scalp followed the previously published laboratory protocol detailed in[37]. Briefly summarized, it included the following steps: (1) segmentation and weighting (5–20 mg), (2) pulverization using a ball mill, (3) extraction procedures for cortisol (1 ml methanol per 10 mg pulverized hair, incubation, centrifugation, drying) and BDNF (220 µl citric acid per 10 mg pulverized hair, centrifugation, lyophilization), (4) quantification using ELISA following manufacturer instructions. According to the manufacturer, the intra- and inter-assay coefficients of variation are + 3.7% and + 8.5% for BDNF ELISA; and + 4.3% and + 13.2% for cortisol ELISA, respectively. In our study, the intra- and inter-assay coefficients of variation were + 2.73% and 5.31 ± 3.35 for BDNF; and + 1.91% and 7.49 ± 2.81 for cortisol. All but four BDNF values were inside the detection range.

Psychometric questionnaires. German versions of the following psychometric questionnaires were used.

Tinnitus questionnaire (TQ). The Tinnitus Questionnaire[40] is an instrument assessing tinnitus-related distress. It consists of 52 questions rated on a three-point Likert scale. Only the total score (sum over 40 items, with two items added twice) was used; for sample description, it was categorized based on clinical cut-off scores[40]. Cronbach's alpha = 0.94.

Perceived stress questionnaire (PSQ-20; 20 item version). The Perceived Stress Questionnaire[41,42] measures subjectively experienced stress and consists of 20 items rated on a four-point Likert scale. In the present study, the period surveyed has been changed from last month to last week. Only the total score (linear transformed mean over all items) was used; for sample description, it was categorized based on clinical norms from healthy adults[42]. Cronbach's alpha = 0.93.

Hospital anxiety and depression scale (HADS). The Hospital Anxiety and Depression Scale[43,44] measures levels of anxiety and depression in the past week and consists of 14 items rated on a four-point Likert scale. The sum scores for anxiety and depression were used (comprising 7 items each); for sample description, they were categorized based on clinical cut-off scores[43]. Cronbach's alpha: anxiety = 0.79; depression = 0.81.

Screening of somatoform disorders (SOMS; 7 days version). The Screening of Somatoform Disorders ("Screening für somatoforme Störungen")[45] is an instrument for recording somatoform symptoms, i.e., medically unexplained physical symptoms affecting the subject's well-being. The questionnaire consists of a list of symptoms (52 symptoms for women; 48 for men) and respondents are asked to indicate whether they have suffered from these symptoms in the last 7 days and rate the degree of associated impairment. The number of reported symptoms was used.

State-trait anxiety inventory (STAI): state anxiety. The scale of the State-Trait Anxiety Inventory (STAI)[46] which measures the current state anxiety (form X1) was used. The scale consists of 20 items on a four-point Likert scale, which were summed to form the total score. Cronbach's alpha = 0.93.

Posttraumatic diagnostic scale (PDS): event list. The traumatic event list of the Posttraumatic Diagnostic Scale (PSD)[47] was used to assess whether respondents had experienced relevant traumatic events in their past. It consists of 12 items reflecting highly stressful or traumatic experiences. For each event, respondents indicate whether they have experienced it (personally or as a witness). The number of experienced traumatic events was used.

Short form-12 health survey (SF-12). The Short Form-12 Health Survey ("Fragebogen zum Gesundheitszustand")[48,49], version 2, was used for the assessment of health-related quality of life. It consists of 12 items on a three- or five-point Likert scale. T-standardized scale values were calculated for the physical component summary and mental component summary using normative data for scoring[49]; for sample description, they were dichotomously categorized (using 1 SD below average as cut-off value). Cronbach's alpha: physical component summary = 0.89; mental component summary = 0.87.

Tinnitus and hearing. *Pure tone audiogram.* Pure tone audiometry data were collected from outpatient audiometric records. Hearing thresholds had been measured for the frequencies from 0.25 to 8 kHz and were collected in 5-decibel (dB) intervals for each ear. The average hearing threshold (dB) at all frequencies (0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz) was calculated for each ear, and further averaged across both sides (if possible). For sample description (Table 2), the mean hearing threshold was categorized by severity[50].

Tinnitus pitch and loudness matching. Along with hearing thresholds, tinnitus matching data were collected from outpatient audiometric records. Prior to tinnitus matching, patients had been asked to indicate whether the tinnitus was currently audible, its location (left, right, bilateral), sound quality (more alike to pure tone or narrow-band noise), and approximate frequency range (high, medium, low). For the matching procedure, patients had been asked to indicate when a tone corresponded to their tinnitus, first in terms of frequency and then in

Variable	Mean (SD)/percentage (N)
Biomarkers	
Hair-cortisol (µg/dl)	0.054 (0.047)
Hair-BDNF (ng/ml); N = 87	77.81 (27.56)
Sociodemographic information	
Sex	Female: 65.9% (60)
Age	51.5 (12.0)
Marital status	Single: 28.6% (26) Cohabiting or married: 53.8% (49) Separated or divorced or widowed: 17.6% (16)
Education level[a]	Low: 16.5% (15) Medium: 35.2% (32) High: 48.4% (44)
Employment	Yes: 74.7% (68)
Psychometric questionnaires	
TQ total score: tinnitus-related distress	36 (16)
	Mild (0–30): 39.6% (36) Moderate (31–46): 37.4% (34) Severe (47–59): 12.1% (11) Very severe (60–84): 11.0% (10)
PSQ-20 total score: perceived stress	51.2 (18.8)
	Normal (≤ 50): 49.5% (45) Mild (51–66): 27.5% (25) Moderate (67–83): 22.0% (20) Severe (≥ 84): 1.1% (1)
HADS: anxiety	8 (4.1)
	Normal (0–7): 47.3% (43) Mild (8–10): 20.9% (19) Moderate (11–14): 28.6% (26) Severe (15–21): 3.3% (3)
HADS: depression	6.1 (3.8)
	Normal (0–7): 60.4% (55) Mild (8–10): 27.5% (25) Moderate (11–14): 12.1% (11) Severe (15–21): 0% (0)
SOMS: somatization	9.6 (7.1)
STAI total score: state anxiety	45.2 (11.3)
PDS: number of traumatic experiences	1.7 (1.4)
SF-12: physical component summary	41.9 (10.1)
	Normal/average (≥ 40): 60.4% (55) Impairments (< 40): 37.4% (34) Missing: 2.2% (2)
SF-12: mental component summary	37.5 (10.1)
	Normal/average (≥ 40): 41.8% (38) Impairments (< 40): 56.0% (51) Missing: 2.2% (2)
Tinnitus and hearing	
Matched tinnitus frequency (Hz)	5386.4 (2424.3) Missing: 27.5% (25)
Matched tinnitus loudness (dB)	39.1 (19.8) Missing: 27.5% (25)
Tinnitus: course?	Intermittent: 58.2% (53) Constant: 41.8% (38)
Tinnitus: onset associated with stress?	Yes: 49.5% (45)
Tinnitus: influenced by stress?	Yes: 79.1% (72)
Hyperacusis (self-report)	Yes: 80.2% (73)
Mean hearing threshold (dB)[b]	22.7 (13.0)
	No impairment (≤ 25 dB): 62.6% (57) Mild/slight impairment (26–40 dB): 30.8% (28) Moderate impairment (41–60 dB): 4.4% (4) Severe impairment (61–80 dB): 2.2% (2) Profound impairment (≥ 81 dB): 0% (0)
Use of hearing aids	Yes: 17.6% (16)
Covariates	
Season of sample collection	Winter: 48.4% (44) Spring: 16.5% (15) Summer: 24.2% (22) Autumn: 11.0% (10)
Continued	

Variable	Mean (SD)/percentage (N)
Time of sample collection	10:06 a.m. (51 min)
Frequency of hair washing per week	2.8 (1.6)
Regular use of hair products	Yes: 39.6% (36)
Hair color	Grey/white: 19.8% (17) Blonde/red: 34.9% (30) Brown/black: 45.3% (39) I do not know/missing: 5.5% (5)
Smoking	Yes: 12.1% (11)
Alcohol units per week[c]	2.1 (4)
Medications: hormone supplements	Yes: 9.9% (9)
BMI[d]	25.8 (4.6)
	Underweight (< 18.50): 2.2% (2) Normal (18.50 – 24.99): 41.8% (38) Overweight (25 – 29.99): 39.6% (36) Obese (≥ 30): 16.5% (15)
Shift work	Yes: 16.5% (15)
Physical activity score[e]	6.3 (6.6)
Sport	Less than 1 h a week: 35.2% (32) Regularly, 1–2 h a week: 44.0% (40) Regularly, 3–4 h a week: 15.4% (14) Regularly, more than 4 h a week: 5.5% (5)
Cups of coffee/tea per day	2.8 (1.9)

Table 2. Sample characteristics (N = 91). *BMI* Body-Mass-Index; *HADS* Hospital Anxiety and Depression Scale; *PDS* Posttraumatic Diagnostic Scale; *PSQ-20* Perceived Stress Questionnaire (20 item version); *SF-12* Short Form-12 Health Survey; *SOMS* Screening of Somatoform Disorders; *STAI* State-Trait Anxiety Inventory (State Anxiety); *TQ* Tinnitus Questionnaire. [a]Education levels: low = elementary, secondary, or middle school; medium = high school or completed apprenticeship; high = university. [b]Mean hearing threshold across all measured frequencies. Grading of hearing thresholds:[50]. [c]Alcohol units consumed per week: one unit = 0.3 l beer or 0.2 l wine or shot glass of spirits. [d]BMI classification:[52]. [e]Physical activity score: number of days per week on which participants are physically active times the duration of the physical activity (1 = less than 10 min, 2 = 10–30 min, 3 = 30–60 min, 4 = more than 60 min).

terms of loudness. Depending on the specified frequency range (low, medium, high), three different frequencies had been initially played for comparison and were narrowed down to two different frequencies after a positive response; the final match had to be confirmed twice by the patients. Once the frequency had been identified, the loudness was adjusted in 1-dB steps starting at the hearing threshold; the final match had to be confirmed twice by the patients. Matched frequency (Hz) and loudness (dB) were averaged across both sides for bilateral tinnitus if measurements from both sides were available. The absolute matched tinnitus loudness was used as opposed to the relative sensation level (loudness above hearing threshold), as the hearing threshold was collected in 5-dB steps and tinnitus loudness in 1-dB steps, which limited the accuracy of the sensation level measure. However, the average hearing threshold was included in the analyses to control for the effect of hearing impairment on the absolute tinnitus loudness. Moreover, matched tinnitus loudness was chosen instead of subjective loudness ratings because the latter appeared to be more influenced by distress levels in our sample. For N = 25 patients, tinnitus matching was not possible either because the tinnitus was not audible at the time of measurement, its sound quality could not be captured by pure tones or narrow-band noise, or because its frequency exceeded the measurement range (> 10 kHz).

Additional tinnitus or hearing-related information. Additional information on tinnitus or hearing-related aspects were assessed by self-report: intermittent vs. constant tinnitus, tinnitus onset associated with stress, tinnitus influenced by stress, hyperacusis, and hearing aid use.

Covariates. Covariates assessed by self-report included sociodemographic information (sex, age, marital status, education level, employment), hair care (frequency of hair washing per week, regular use of hair products, natural hair color), and health-related behavior (smoking, alcohol units consumed per week, hormone medications, cortisone medications, body-mass index (BMI), shift work, physical activity, sports, and cups of coffee/tea consumed per day). The use of cortisone medication was excluded as a predictor because only N = 4 gave an affirmative answer. Other recorded covariates were season and time of sample collection.

Statistical analysis. Statistical analyses and plotting were performed using R (version 4.0.0)[51]. For data preparation, the package "tidyverse" was used; for correlations "Hmisc" and "corrplot"; for elastic net regression, "caret" and "glmnet"; and "RANN" for k-nearest neighbor imputation. Hair-cortisol concentrations were log-transformed to establish normal distribution.

Descriptive analyses and correlations. For sample description (Table 2), absolute numbers and frequencies are reported for categorical variables and mean values and standard deviations (SD) for numerical variables, psychometric questionnaires are categorized using the respective cut-offs, and hearing threshold and BMI values are categorized using defined WHO cut-offs[50,52]. For data exploration, Spearman correlations were calculated for continuous variables (missing values were deleted pairwise) and depicted in a correlation plot sorted by hierarchical clustering, see Supplementary Fig. S1.

Elastic net regression. Elastic net regression is a penalized linear regression method (a generalization of ridge and lasso regression) that performs shrinkage of correlated predictors and automatic variable selection[38,39]. Elastic net regression uses two tuning parameters: alpha (mixing parameter), ranging from 0 = ridge regression to 1 = lasso regression, and lambda (regularization parameter), which determines the overall strength of shrinkage/penalization (see glmnet[53] vignette).

Two elastic net regression models with hair-cortisol and hair-BDNF levels as outcome variables were calculated. Predictors included psychometric questionnaire scores, matched tinnitus loudness and frequency, hearing threshold (audiometry), and other covariates; a total of 35 predictors for each model; see Table 2. Both outcome variables and predictors were standardized for better comparability of the results. For both outcomes, normality of residuals was met (visual check and Kolmogorov–Smirnov Test) and no predictors had zero or near-zero variance. Among predictors, one correlation of $r > 0.75$ (Spearman) was present: between tinnitus loudness and mean hearing threshold, $r = 0.79$, $p < 0.001$, N = 66. For both elastic net regression models, the dataset was randomly divided into a training dataset consisting of 70% of the data (N = 66 for cortisol; N = 63 for BDNF) on which the models were trained and a test dataset consisting of 30% of the data (N = 25 for cortisol; N = 24 for BDNF) on which the accuracy of the model predictions was tested. The data splitting ensured similar distributions of the outcome variables in the training and test datasets. The 70% to 30% split was chosen to obtain a sample size of N > 20 for the test data.

N-fold cross-validation was used to tune each elastic net model across 10 different lambda values and 10 different alpha values. The optimal model for each outcome was selected by minimizing the root mean square error (RMSE). Optimal regularization parameters for the prediction of cortisol were alpha = 0.4 and lambda = 0.28671; and for BDNF, alpha = 0.2, lambda = 0.14914; see Supplementary Fig. S2 and S3. Performance metrics (RMSE and R^2) of the optimal models on the training and test datasets and estimated standardized coefficient effects are reported as results. In addition, variable importance (VI), a scaled ranking from zero to 100 based on the coefficient estimates, was used to group the effects by magnitude (in VI quartiles). Only effects with VI ≥ 50 were considered as main findings.

Imputation of missing values. Missing values on numeric predictors (27.5% on tinnitus matching data and 2.2% on SF-12) were imputed using k-nearest neighbor imputation. This method was chosen because it can handle different types of missing data. The remaining missing values on categorical predictors were 5.5% for natural hair color. Analyses were repeated in the subsample with complete tinnitus matching data (cortisol: N = 66, BDNF: N = 63) to assess possible influences of imputation.

Results

The variables examined in this study are summarized in Fig. 1. All measurements (biomarker sampling, psychometric questionnaires, tinnitus pitch and loudness matching, pure tone audiometry, and collection of other information) were performed on the entire sample; missing values for tinnitus matching data and SF-12 were imputed (see "Methods"). Because the main aim of this study was to investigate associations of hair-cortisol and hair-BDNF with tinnitus-related and psychological factors while controlling for confounding influences, the two biomarkers were investigated as outcome variables using elastic net regression, whereas all other assessed variables were used as predictors. These analyses included N = 91 for hair-cortisol and N = 87 for hair-BDNF; results are reported below and shown in Figs. 2 and 3. In addition, the analyses were repeated in the subsample with complete tinnitus matching data (N = 66) to assess the influences of imputation.

Sample description. The characteristics of the sample (N = 91) in terms of the assessed biomarkers, sociodemographic information, psychometric questionnaires, tinnitus/hearing as well as covariates are listed in Table 2. Approximately two-thirds of the sample were female (65.9%); on average, participants were middle-aged (M = 51.5, SD = 12); and most participants were cohabiting or married (53.8%), were employed (74.7%), and had medium (35.2%) to high (48.4%) levels of education; see Table 2.

Prediction of hair-cortisol. For the prediction of hair-cortisol, the strongest predictive effects (VI > 75) were found for *sampling in autumn* and *BMI* (positive), followed by effects (VI > 50) for *matched tinnitus loudness* (positive) and *smoking* (negative). The elastic net regression model explained 6% of the variance in hair-cortisol in the training data (RMSE = 0.91, $R^2 = 0.06$), and 10% of the variance in the test data (RMSE = 1.11, $R^2 = 0.10$).

All estimated standardized coefficient effects and their grouping by VI are displayed in Fig. 2. In detail, positive associations with hair-cortisol levels were found for *sampling in autumn* (β = 0.160, VI = 100), *BMI* (β = 0.140, VI = 87.24), *matched tinnitus loudness* (β = 0.089, VI = 55.91), *age* (β = 0.031, VI = 19.52), *consumed alcohol units per week* (β = 0.011, VI = 6.66), and *regular use of hair products* (β = 0.008, VI = 5.00). Negative associations with hair-cortisol levels were found for *smoking* (β = −0.087, VI = 54.38), *sampling in spring* (β = −0.071, VI = 44.28), *education level (linear relationship)* (β = −0.052, VI = 32.71), *PSQ-20* (β = −0.024, VI = 14.86), *HADS depression* (β = −0.007, VI = 4.29), and *hair color: blonde/red* (β = −0.004, VI = 2.36). Note: The interpretation of coefficient

Biomarkers	• Hair-Cortisol • Hair-BDNF
Psychometric Questionnaires	• Tinnitus-related distress: Tinnitus Questionnaire (TQ[40]) • Perceived stress: Perceived Stress Questionnaire (PSQ-20[41,42]) • Anxiety/Depression: Hospital Anxiety and Depression Scale (HADS[43,44]) • Somatization: Screening of Somatoform Disorders (SOMS[45]) • State anxiety: State-Trait Anxiety Inventory (STAI[46]) • Traumatic experiences: Posttraumatic Diagnostic Scale, Event List (PDS[47]) • Physical/mental health-related quality of life: Short Form-12 Health Survey (SF-12[48,49])
Tinnitus and Hearing	• Tinnitus: Pitch and loudness matching • Hearing threshold: Pure tone audiogram • Intermittent vs. constant tinnitus, stress-related tinnitus, hyperacusis, hearing aid use
Other factors	• Sociodemographic information: Sex, age, marital status, education level, employment • Season and time of hair sampling • Hair care: Frequency of hair washing per week, use of hair products, natural hair color • Health-related factors/behavior: Smoking, alcohol, hormone/cortisone medications, BMI, shift work, physical activity, sports, caffeine

Figure 1. Overview of included study variables. Biomarkers (cortisol and BDNF measured in hair) were investigated as outcome variables while all other variables (psychometric questionnaires, tinnitus and hearing, covariates) were used as predictors.

effects obtained from elastic net regression is not different from ordinary least square multiple regression models. As an advantage, predictor effects can be directly compared in magnitude due to standardization.

Prediction of hair-BDNF. For the prediction of hair-BDNF, the strongest effects (VI > 75) were found for *hearing aid use*, *shift work* (positive), and *matched tinnitus loudness* (negative); followed by (VI > 50) *smoking*, *TQ score*, *number of experienced traumatic events* (negative), and *SF-12 physical component summary* (positive). The elastic net regression model explained 25% of the variance in hair-BDNF in the training data (RMSE = 0.85, $R^2 = 0.25$), and 28% of the variance in the test data (RMSE = 0.98, $R^2 = 0.28$).

All estimated standardized coefficient effects and their grouping by VI are displayed in Fig. 3. In detail, positive associations with hair-BDNF levels were found for: *hearing aid use* (β = 0.267, VI = 100), *shift work* (β = 0.240, VI = 89.60), *SF-12 physical component summary* (β = 0.151, VI = 56.48), *sport (linear relationship)* (β = 0.117, VI = 43.89), *marital status: separated/divorced/widowed* (β = 0.110, VI = 41.05), *hair color: blonde/red* (β = 0.102, VI = 38.28), *frequency of hair washing per week* (β = 0.100, VI = 37.24), *tinnitus onset associated with stress* (β = 0.074, VI = 27.71), *mean hearing threshold* (β = 0.073, VI = 27.14), *sampling in autumn* (β = 0.063, VI = 23.64), *BMI* (β = 0.042, VI = 15.59), *alcohol units consumed per week* (β = 0.005, VI = 1.94), *physical activity* (β = 0.004, VI = 1.55), and *regular use of hair products* (β = 0.003, VI = 1.22). Negative associations with hair-BDNF levels were found for: *matched tinnitus loudness* (β = −0.247, VI = 92.22), *smoking* (β = −0.195, VI = 72.83), *TQ score* (β = −0.171, VI = 63.80), *number of experienced traumatic events (PDS)* (β = −0.136, VI = 51.02), *sampling in spring* (β = −0.092, VI = 34.49), *matched tinnitus frequency* (β = −0.064, VI = 24.07), *constant tinnitus* (β = −0.063, VI = 23.42), *sport (quadratic relationship)* (β = −0.056, VI = 20.81), *hair color: brown/black* (β = −0.055, VI = 20.56), *HADS anxiety* (β = −0.043, VI = 16.20), *sampling in summer* (β = −0.032, VI = 11.97), *marital status: cohabiting/married* (β = −0.029, VI = 11.02), and *cups of coffee/tea per day* (β = −0.029, VI = 10.86).

Models without imputation of tinnitus matching data. Additional analyses in the subsample with complete tinnitus matching data (N = 66), showed that without imputed matching data, tinnitus loudness was

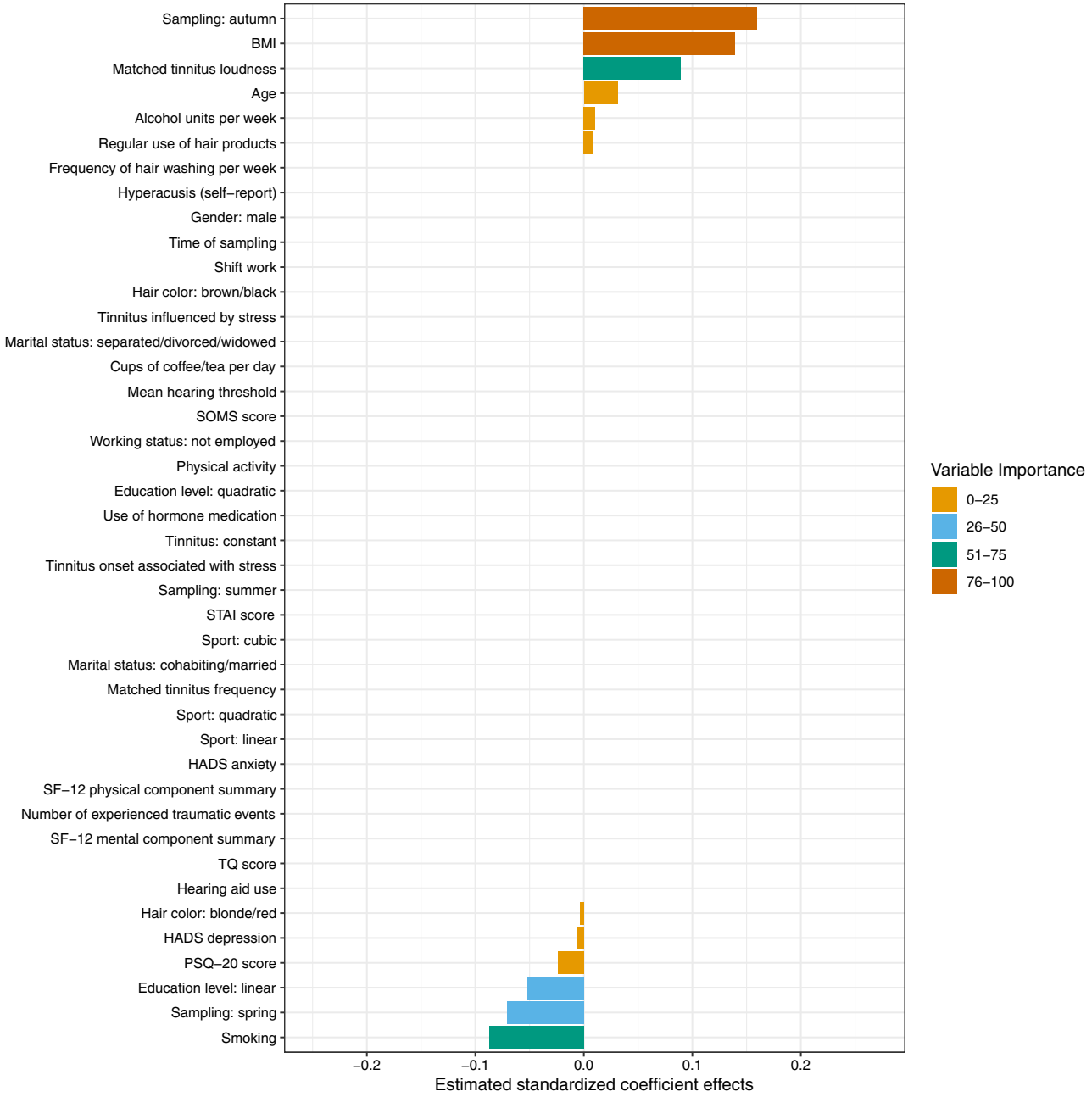

Figure 2. Estimated standardized coefficient effects by elastic net regression with n-fold cross-validation for the prediction of hair-cortisol in chronic tinnitus patients (training data: N = 66). *BMI* Body-Mass-Index; *HADS* Hospital Anxiety and Depression Scale; *PSQ-20* Perceived Stress Questionnaire (20 item version); *SF-12* Short Form-12 Health Survey; *SOMS* Screening of Somatoform Disorders; *STAI* State-Trait Anxiety Inventory (State Anxiety); *TQ* Tinnitus Questionnaire.

still identified as a predictor of hair-cortisol (β = 0.041, VI = 26.18) as well as of hair-BDNF (β = −0.005, VI = 3.11), but with lower variable importance.

Discussion

This study was the first to analyze hair-cortisol and hair-BDNF levels in chronic tinnitus patients. We assessed their associations with tinnitus matching data, tinnitus-related distress, psychometric measures, and hearing threshold while controlling for potential confounders by using elastic net regression. Our results show that in chronic tinnitus patients, tinnitus loudness is associated with both increased hair-cortisol and decreased hair-BDNF levels, and tinnitus-related distress is associated with decreased hair-BDNF levels. This suggests that loud and distressing chronic tinnitus is linked to substantial long-term alterations of HPA axis function and adaptive neuroplasticity. Additional findings for hair-cortisol were positive effects of sampling in autumn and BMI, and

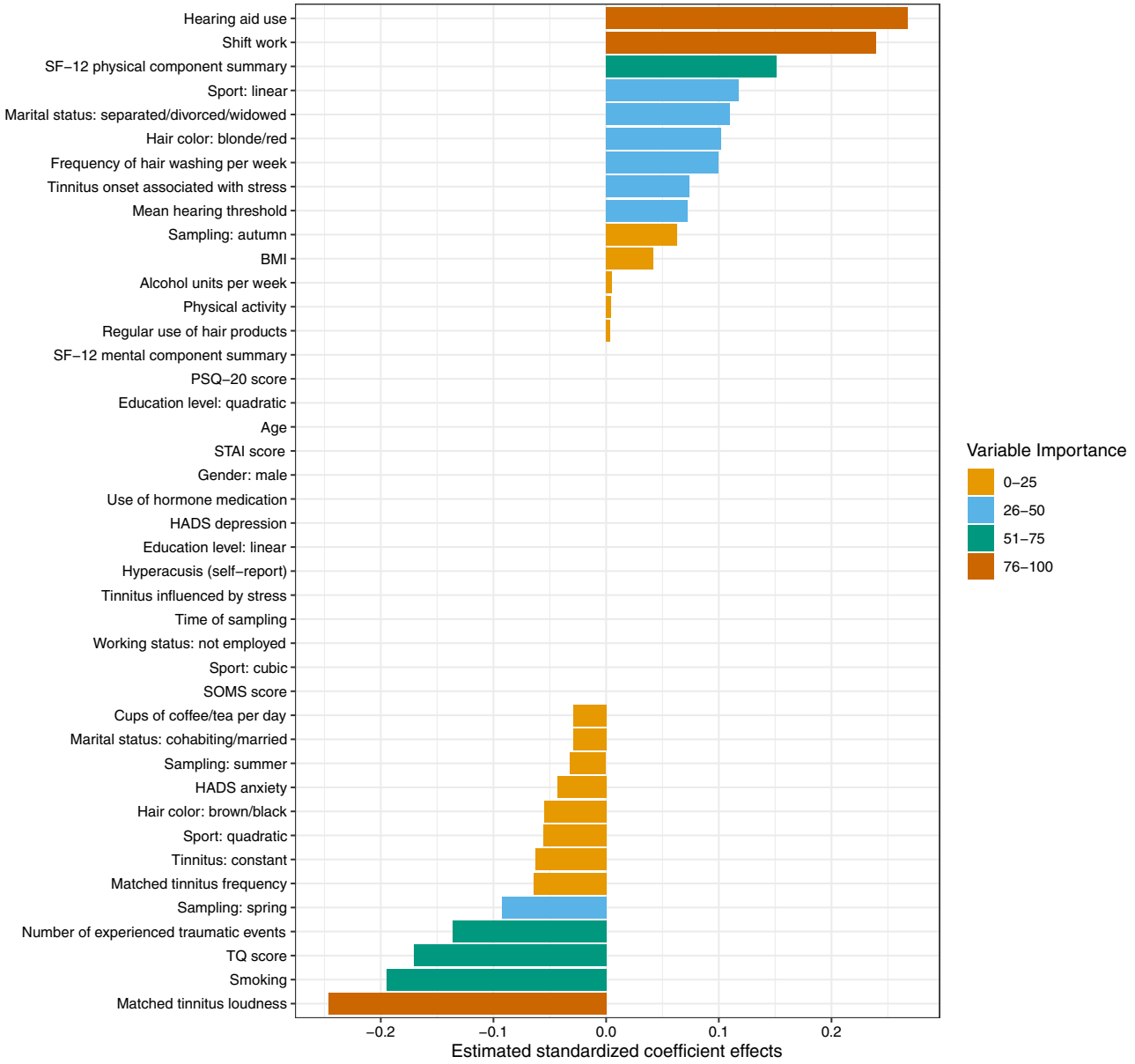

Figure 3. Estimated coefficient effects by elastic net regression with n-fold cross-validation for the prediction of hair-BDNF in chronic tinnitus patients (training data: N = 63). *BMI* Body-Mass-Index; *HADS* Hospital Anxiety and Depression Scale; *PSQ-20* Perceived Stress Questionnaire (20 item version); *SF-12* Short Form-12 Health Survey; *SOMS* Screening of Somatoform Disorders, *STAI* State-Trait Anxiety Inventory (State Anxiety); *TQ* Tinnitus Questionnaire.

a negative effect of smoking; for hair-BDNF, positive effects of hearing aid use, shift work, and higher physical health-related quality of life, and negative effects of smoking and previous traumatic experiences.

Hair-cortisol: effect of tinnitus loudness. The finding that patients with higher perceived tinnitus loudness have higher hair-cortisol levels suggests that these individuals may show substantial HPA axis dysregulation, comparable to long-term effects reported in other chronic stress-exposed groups[22]. This complements previous studies analyzing salivary cortisol in tinnitus patients, which indicate a tendency for decreased HPA axis responsiveness[16–18]. In addition to increases in salivary cortisol and subjective stress levels after noise exposure, [15] found that patients with high tinnitus-related distress show an increase in tinnitus intensity, indirectly providing support for our finding. Regarding the conflicting findings on blood-cortisol levels in tinnitus, our result is consistent with the negative association of loudness reported by[20], although in contrast, we did not observe any effect of tinnitus frequency. However, an important difference is that our result reflects long-term cortisol accumulation. Furthermore, no effect of the hearing threshold was identified, suggesting a hearing-independent effect of tinnitus loudness, apart from the limiting fact of generally normal hearing in our sample (63%).

Moreover, we did not find the expected relationship between tinnitus-related distress and hair-cortisol levels. This lack of association is consistent with findings on plasma cortisol in tinnitus by[19]. However, overall evidence regarding the relationship between hair-cortisol levels and subjective measures of perceived stress is inconsistent[22]. Mostly normal or mild perceived stress levels (77%) as well as predominantly normal depression (60%) and anxiety levels (47%) in our sample might explain the absence of psychological associations. Explained variance of hair-cortisol levels in the test data was comparatively low (10%), which may further indicate low modulatory influences on the HPA axis in our sample. Further indication for this assumption is the lack of an association between hair-cortisol and hair-BDNF levels, contrary to the observed negative association in the pilot hair-BDNF study in healthy stressed academics[37].

Hair-cortisol: effect of season, BMI, and smoking. Many confounding factors of hair-cortisol levels have been identified in the literature[54,55]. In our study, samples collected in autumn showed higher, and samples collected in spring showed lower hair-cortisol levels than samples collected in winter (reference category). This indicates seasonal variations in hair-cortisol concentrations, in line with previous studies[54–56], yet the sample size was relatively small for both seasons (autumn: N = 10; spring: N = 15). In line with our result, a positive correlation between hair-cortisol and BMI is known from the literature[22,23] and consistent with HPA axis dysregulation in obesity[57]. Two review articles concluded the absence of an association between smoking and hair-cortisol levels[22,23], contrary to our negative result. The small smoking subsample size (N = 11) may have influenced this result. Overall, these findings highlight the importance to include confounding factors in hair-cortisol analysis.

Hair-BDNF: effect of tinnitus-related distress and traumatic experiences. As expected, higher tinnitus-related distress was related to lower hair-BDNF, consistent with findings by[34], where highly distressed tinnitus patients had lower serum BDNF levels than patients with mild distress. However, they found no difference between the high distress and the control group. Other previous studies measuring blood-BDNF in tinnitus did not find an association between tinnitus distress and BDNF levels in plasma[36] or serum[33,35]. Our finding, however, should be more reflective of long-term effects than results from previous blood-BDNF measurements and is consistent with the expected negative effect of chronic stress on BDNF expression. This suggests hair-BDNF might be a useful biomarker to assess the clinical efficacy of treatments targeting tinnitus-related distress.

BDNF measured in hair may originate from blood circulation and from follicular epithelial cells[37]. However, the relative contribution of these mechanisms is unclear. For cortisol, the main mechanism is considered to be incorporation into growing hair cells by diffusion from the bloodstream[24,26], and a recent study demonstrated, in agreement, that hair-cortisol represents circulating cortisol[58]. BDNF is widely expressed in the brain, especially in the hippocampus[30], and 70–80% of circulating peripheral BDNF was found to originate from the brain[59]. Moreover, blood BDNF levels are positively correlated with hippocampal BDNF levels in animals[60].

Further, animal studies showed that chronic stress leads to decreased BDNF (mRNA or protein) expression in the hippocampus[61–63] as well as reduced volume of the hippocampus[64–66]. Depression is likewise associated with reduced BDNF in the periphery (serum:[67,68]) and reduced hippocampal volume[69]. Additionally, postmortem brain-tissue analysis showed reduced BDNF levels in the hippocampus of suicide subjects[70,71].

Regarding tinnitus, both volume reductions[72,73] and increases[74] of the hippocampus have been reported, which were, however, unrelated to tinnitus duration and severity[73,74]. Conversely, [75] found a negative correlation between tinnitus distress and the left hippocampal surface. For a better understanding of neurobiological changes in chronic tinnitus, further studies on the associations of neuroplasticity changes, especially of the hippocampus, with tinnitus-related distress and hair-BDNF levels are needed. Findings on severity-dependent short-term memory and learning performance reductions in tinnitus[76] are in line with the hypothesis of stress-related hippocampal neuroplasticity impairment in severe tinnitus.

BDNF has a complex role within the fear response circuitry[77]. Initially elevated serum BDNF levels in PTSD patients might be followed by long-term reduction[78], consistent with our observed negative effect of traumatic experiences. Evidence further suggests that the BDNF Val66Met polymorphism, associated with deficient activity-dependent BDNF release[79], might modulate the sensitivity to stress and trauma[77]. A recent study found that in tinnitus patients, the BDNF Val66Met polymorphism is associated with higher stress levels, higher levels of tinnitus-related distress, and activation/connectivity changes within a general distress network[80]. Whether our observed effects of tinnitus-related distress and traumatic experiences on hair-BDNF levels are influenced by the Val66Met polymorphism could be an interesting future research question.

Hair-BDNF: effect of tinnitus loudness and hearing aid use. In contrast to the negative effect found for tinnitus loudness on hair-BDNF, previous blood-BDNF studies in tinnitus patients found no association between tinnitus loudness (tinnitus matching or visual analog scale) and serum or plasma BDNF levels[33,35,36]. We assume this discrepancy can be attributed to long-term effects only captured by BDNF measurement in hair. However, imputation of tinnitus matching data may potentially have led to an overestimation of the effect, which was considerably smaller in the subsample with complete matching data.

Evidence indicates that hearing aid use leads to neuroplasticity changes in the brain[81]. Thus, our finding might potentially suggest that hearing aid use counteracts detrimental chronic stress-related neuroplasticity effects in severe chronic tinnitus. However, as the number of hearing aid users was relatively small (N = 16) and we additionally observed a small positive effect of the mean hearing threshold, no conclusions can be drawn and further investigation in a larger-scale study is clearly needed.

Hair-BDNF: effects of self-reported physical health-related quality of life, shift work, and smoking. Results of a meta-analysis indicate that regular exercise leads to subtle increases in peripheral

BDNF levels[82]. In agreement, we found a positive effect of self-reported physical health-related quality of life and a small positive effect of sports activity on hair-BDNF levels, supporting the association between BDNF and physical health. However, despite a negative correlation between hair-BDNF and somatization, consistent with[37], there was no effect of somatization when controlling for other influencing factors in the elastic net regression model. Other findings included effects of smoking and shift work but given the small number of smokers (N = 11) and shift workers (N = 15) and respective reliability issues, further research on these relationships is needed.

Limitations. This study has some limitations. First, model performance for predicting hair-BDNF levels was higher ($R^2 = 0.28$) than for hair-cortisol ($R^2 = 0.10$). Since the models were tested on a different part of the data than on which they were built, performance estimates should be relatively robust. Therefore, it may be that hair-cortisol levels were generally more stable than hair-BDNF levels or influenced by other, unmeasured factors. However, generally normal/mild perceived stress and psychological symptom levels in our sample might have influenced this result. Second, some findings were based on small subsample sizes (seasonal effects, smoking, hearing aid use, shift work) and thus might have limited validity. Third, tinnitus matching data were collected from audiometric records, with an average time difference of 1.83 months (SD = 1.85) to the other measurements, which might have influenced the results. In addition, for N = 25 (27.5%) tinnitus matching could not be performed, and these missing values were imputed using k-nearest neighbor imputation. In models without imputation of tinnitus matching data, tinnitus loudness was still identified as a predictor for both hair-cortisol and hair-BDNF, but the effects were smaller, especially for hair-BDNF. Together with the lack of a correlation between hair-BDNF and tinnitus loudness, the validity of the effect of tinnitus loudness on hair-BDNF may be limited. Fourth, we did not include a control group in the present study as it did not reflect the research aim; therefore, no information is available on whether hair-cortisol and hair-BDNF levels were altered in our chronic tinnitus sample compared to healthy individuals. Based on our results, we expect higher hair-cortisol and lower hair-BDNF levels in patients with loud and distressing chronic tinnitus than healthy controls, but this assumption remains to be tested. Lastly, the investigated clinical sample was heterogeneous, and we observed a small predictive effect of constant vs. intermittent tinnitus on hair-BDNF levels. Accordingly, the observed effects may be particularly relevant for the subgroup of individuals with constant tinnitus, and future research might aim to investigate respective differences.

Conclusions

In summary, we found that in chronic tinnitus patients, higher tinnitus loudness is associated with higher hair-cortisol and lower hair-BDNF levels, whereas higher levels of tinnitus-related distress are additionally associated with lower hair-BDNF levels. Effects were stronger for hair-BDNF than for hair-cortisol. Chronic tinnitus might be related to long-term changes in cortisol and BDNF expression, the strength of which may be moderated by perceived tinnitus loudness. High tinnitus-related distress and traumatic experiences appear to have additional detrimental effects on BDNF expression, whereas hearing aid use and high physical health-related quality of life appear beneficial. Results further highlight the importance of assessing confounders, like season, BMI, smoking, or shift work. The main implications of our findings are that cortisol levels measured in hair could serve as a biomarker of tinnitus loudness, whereas hair-BDNF levels might function as a presumably more sensitive biomarker of psychological or psychosomatic tinnitus-related distress in chronic tinnitus patients which could potentially be used to assess clinical treatment efficacy.

Data availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Received: 6 September 2021; Accepted: 21 December 2021
Published online: 04 February 2022

References

1. Mazurek, B., Boecking, B. & Brueggemann, P. Association between stress and tinnitus—New aspects. *Otol. Neurotol.* **40**, e467–e473. https://doi.org/10.1097/MAO.0000000000002180 (2019).
2. Elarbed, A., Fackrell, K., Baguley, D. M. & Hoare, D. J. Tinnitus and stress in adults: A scoping review. *Int. J. Audiol.* **60**, 171–182. https://doi.org/10.1080/14992027.2020.1827306 (2021).
3. Szczepek, A. J. & Mazurek, B. *Neurobiology of Stress-Induced Tinnitus*. https://doi.org/10.1007/7854_2020_215. (Springer, 2021).
4. De Ridder, D. *et al.* Tinnitus and tinnitus disorder: Theoretical and operational definitions (an international multidisciplinary proposal). in *Progress in Brain Research*. Vol. 260. 1–25. https://doi.org/10.1016/bs.pbr.2020.12.002 (Elsevier, 2021).
5. Szczepek, A. J. & Mazurek, B. Introduction. in *Tinnitus and Stress: An Interdisciplinary Companion for Healthcare Professionals* (eds. Szczepek, A. & Mazurek, B.). 1–6. https://doi.org/10.1007/978-3-319-58397-6_1 (Springer, 2017).
6. Weise, C., Kleinstäuber, M., Hesser, H., Westin, V. Z. & Andersson, G. Acceptance of tinnitus: Validation of the tinnitus acceptance questionnaire. *Cogn. Behav. Ther.* **42**, 100–115. https://doi.org/10.1080/16506073.2013.781670 (2013).
7. Probst, T., Pryss, R., Langguth, B. & Schlee, W. Emotional states as mediators between tinnitus loudness and tinnitus distress in daily life: Results from the "TrackYourTinnitus" application. *Sci. Rep.* **6**, 20382. https://doi.org/10.1038/srep20382 (2016).
8. Herman, J. P. *et al.* Regulation of the hypothalamic-pituitary-adrenocortical stress response. in *Comprehensive Physiology* (ed. Terjung, R.). 603–621. https://doi.org/10.1002/cphy.c150015. (Wiley, 2016).
9. McEwen, B. S. Neurobiological and systemic effects of chronic stress. *Chronic Stress* **1**, 1–11. https://doi.org/10.1177/2470547017692328 (2017).
10. Hébert, S., Canlon, B. & Hasson, D. Emotional exhaustion as a predictor of tinnitus. *Psychother. Psychosom.* **81**, 324–326. https://doi.org/10.1159/000335043 (2012).

11. Hébert, S., Mazurek, B. & Szczepek, A. J. Stress-related psychological disorders and tinnitus. in *Tinnitus and Stress* (eds. Szczepek, A. J. & Mazurek, B.). 37–51. https://doi.org/10.1007/978-3-319-58397-6_3. (Springer, 2017).
12. Pattyn, T. *et al.* Tinnitus and anxiety disorders: A review. *Hear. Res.* **333**, 255–265. https://doi.org/10.1016/j.heares.2015.08.014 (2016).
13. Salazar, J. W. *et al.* Depression in patients with tinnitus: A systematic review. *Otolaryngology–Head and Neck Surgery* **161**, 28–35. https://doi.org/10.1177/0194599819835178 (2019).
14. Packard, A. E. B., Egan, A. E. & Ulrich-Lai, Y. M. HPA axis interactions with behavioral systems. in *Comprehensive Physiology* (ed. Terjung, R.). 1897–1934. https://doi.org/10.1002/cphy.c150042. (Wiley, 2016).
15. Hébert, S. & Lupien, S. J. Salivary cortisol levels, subjective stress, and tinnitus intensity in tinnitus sufferers during noise exposure in the laboratory. *Int. J. Hyg. Environ. Health* **212**, 37–44. https://doi.org/10.1016/j.ijheh.2007.11.005 (2009).
16. Hébert, S. & Lupien, S. J. The sound of stress: Blunted cortisol reactivity to psychosocial stress in tinnitus sufferers. *Neurosci. Lett.* **411**, 138–142. https://doi.org/10.1016/j.neulet.2006.10.028 (2007).
17. Simoens, V. L. & Hébert, S. Cortisol suppression and hearing thresholds in tinnitus after low-dose dexamethasone challenge. *BMC Ear Nose Throat Disord.* **12**, 4. https://doi.org/10.1186/1472-6815-12-4 (2012).
18. Jackson, J. G. The cortisol awakening response: A feasibility study investigating the use of the area under the curve with respect to increase as an effective objective measure of tinnitus distress. *Am. J. Audiol.* **28**, 583–596. https://doi.org/10.1044/2019_AJA-18-0174 (2019).
19. Kim, D.-K. *et al.* Diagnostic value and clinical significance of stress hormones in patients with tinnitus. *Eur. Arch. Otorhinolaryngol.* **271**, 2915–2921. https://doi.org/10.1007/s00405-013-2785-5 (2014).
20. Chrbolka, P., Palúch, Z., Hill, M. & Alušík, Š. Circulating steroids negatively correlate with tinnitus. *Steroids* **123**, 37–42. https://doi.org/10.1016/j.steroids.2017.04.004 (2017).
21. Li, J. *et al.* Clinical efficacy of cognitive behavioral therapy for chronic subjective tinnitus. *Am. J. Otolaryngol.* **40**, 253–256. https://doi.org/10.1016/j.amjoto.2018.10.017 (2018).
22. Stalder, T. *et al.* Stress-related and basic determinants of hair cortisol in humans: A meta-analysis. *Psychoneuroendocrinology* **77**, 261–274. https://doi.org/10.1016/j.psyneuen.2016.12.017 (2017).
23. Wosu, A. C., Valdimarsdóttir, U., Shields, A. E., Williams, D. R. & Williams, M. A. Correlates of cortisol in human hair: Implications for epidemiologic studies on health effects of chronic stress. *Ann. Epidemiol.* **23**, 797-811.e2. https://doi.org/10.1016/j.annepidem.2013.09.006 (2013).
24. Russell, E., Koren, G., Rieder, M. & Van Uum, S. Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions. *Psychoneuroendocrinology* **37**, 589–601. https://doi.org/10.1016/j.psyneuen.2011.09.009 (2012).
25. Short, S. J. *et al.* Correspondence between hair cortisol concentrations and 30-day integrated daily salivary and weekly urinary cortisol measures. *Psychoneuroendocrinology* **71**, 12–18. https://doi.org/10.1016/j.psyneuen.2016.05.007 (2016).
26. Stalder, T. & Kirschbaum, C. Analysis of cortisol in hair—State of the art and future directions. *Brain. Behav. Immun.* **26**, 1019–1029. https://doi.org/10.1016/j.bbi.2012.02.002 (2012).
27. Wennig, R. Potential problems with the interpretation of hair analysis results. *Forensic Sci. Int.* **107**, 5–12. https://doi.org/10.1016/S0379-0738(99)00146-2 (2000).
28. Kowiański, P. *et al.* BDNF: A key factor with multipotent impact on brain signaling and synaptic plasticity. *Cell. Mol. Neurobiol.* **38**, 579–593. https://doi.org/10.1007/s10571-017-0510-4 (2018).
29. Lima Giacobbo, B. *et al.* Brain-derived neurotrophic factor in brain disorders: Focus on neuroinflammation. *Mol. Neurobiol.* **56**, 3295–3312. https://doi.org/10.1007/s12035-018-1283-6 (2019).
30. Miranda, M., Morici, J. F., Zanoni, M. B. & Bekinschtein, P. Brain-derived neurotrophic factor: A key molecule for memory in the healthy and the pathological brain. *Front. Cell. Neurosci.* **13**, 363. https://doi.org/10.3389/fncel.2019.00363 (2019).
31. Schmitt, K., Holsboer-Trachsler, E. & Eckert, A. BDNF in sleep, insomnia, and sleep deprivation. *Ann. Med.* **48**, 42–51. https://doi.org/10.3109/07853890.2015.1131327 (2016).
32. Szczepek, A. J. & Mazurek, B. Stress-related blood biomarkers. in *Tinnitus and Stress: An Interdisciplinary Companion for Healthcare Professionals* (eds. Szczepek, A. J. & Mazurek, B.). 95–116. https://doi.org/10.1007/978-3-319-58397-6_6. (Springer, 2017).
33. Coskunoglu, A. *et al.* Evidence of associations between brain-derived neurotrophic factor (BDNF) serum levels and gene polymorphisms with tinnitus. *Noise Health* **19**, 140–148. https://doi.org/10.4103/nah.NAH_74_16 (2017).
34. Goto, F. *et al.* Various levels of plasma brain-derived neurotrophic factor in patients with tinnitus. *Neurosci. Lett.* **510**, 73–77. https://doi.org/10.1016/j.neulet.2012.01.001 (2012).
35. Szczepek, A. J., Haupt, H., Klapp, B. F., Olze, H. & Mazurek, B. Biological correlates of tinnitus-related distress: An exploratory study. *Hear. Res.* **318**, 23–30. https://doi.org/10.1016/j.heares.2014.10.007 (2014).
36. Xiong, H. *et al.* Plasma brain-derived neurotrophic factor levels are increased in patients with tinnitus and correlated with therapeutic effects. *Neurosci. Lett.* **622**, 15–18. https://doi.org/10.1016/j.neulet.2016.04.032 (2016).
37. Harb, H. *et al.* Assessment of brain derived neurotrophic factor in hair to study stress responses: A pilot investigation. *Psychoneuroendocrinology* **86**, 134–143. https://doi.org/10.1016/j.psyneuen.2017.09.007 (2017).
38. Sauerbrei, W. *et al.* State of the art in selection of variables and functional forms in multivariable analysis—Outstanding issues. *Diagn. Progn. Res.* **4**, 3. https://doi.org/10.1186/s41512-020-00074-3 (2020).
39. Zou, H. & Hastie, T. Regularization and variable selection via the elastic net. *J. R. Stat. Soc. Ser. B Stat. Methodol.* **67**, 301–320. https://doi.org/10.1111/j.1467-9868.2005.00503.x (2005).
40. Goebel, G. & Hiller, W. *Tinnitus-Fragebogen (TF): Ein Instrument zur Erfassung von Belastung und Schweregrad bei Tinnitus*. (Hogrefe, 1998).
41. Fliege, H., Rose, M., Arck, P., Levenstein, S. & Klapp, B. F. Validation of the "perceived stress questionnaire" (PSQ) in a German sample. *Diagnostica* **47**, 142–152. https://doi.org/10.1026//0012-1924.47.3.142 (2001).
42. Fliege, H. *et al.* The perceived stress questionnaire (PSQ) reconsidered: Validation and reference values from different clinical and healthy adult samples. *Psychosom. Med.* **67**, 78–88. https://doi.org/10.1097/01.psy.0000151491.80178.78 (2005).
43. Herrmann-Lingen, C., Buss, U. & Snaith, R. P. *Hospital Anxiety and Depression Scale—Deutsche Version*. (Huber, 2011).
44. Zigmond, A. S. & Snaith, R. P. The hospital anxiety and depression scale. *Acta Psychiatr. Scand.* **67**, 361–370. https://doi.org/10.1111/j.1600-0447.1983.tb09716.x (1983).
45. Rief, W., Hiller, W. & Heuser, J. *SOMS—Das Screening für Somatoforme Störungen. Manual zum Fragebogen.* (Huber, 1997).
46. Laux, L., Glanzmann, P., Schaffner, P. & Spielberger, C. D. *Das State-Trait-Angstinventar (STAI)*. (Beltz, 1981).
47. Ehlers, A., Steil, R., Winter, H. & Foa, E. *Deutsche Übersetzung der Posttraumatic Diagnostic Scale (PDS).* (Oxford University, 1996).
48. Wirtz, M. A., Morfeld, M., Glaesmer, H. & Brähler, E. Confirmatory analysis of the SF-12 version 2.0 scale structure in a representative German sample. *Diagnostica* **64**, 84–96. https://doi.org/10.1026/0012-1924/a000194 (2018).
49. Wirtz, M. A., Morfeld, M., Glaesmer, H. & Brähler, E. Standardization of the SF-12 version 2.0 assessing health-related quality of life in a representative German sample. *Diagnostica* **64**, 215–226. https://doi.org/10.1026/0012-1924/a000205 (2018).
50. World Health Organization. *Report of the Informal Working Group on Prevention of Deafness and Hearing Impairment Programme Planning, Geneva, 18–21 June 1991*. (World Health Organization, 1991).
51. R Core Team. *R: A Language and Environment for Statistical Computing*. https://www.R-project.org/. (R Foundation for Statistical Computing, 2020).

52. World Health Organization. *Obesity: Preventing and Managing the Global Epidemic: Report of a WHO Consultation*. (World Health Organization, 2000).
53. Friedman, J., Hastie, T. & Tibshirani, R. Regularization paths for generalized linear models via coordinate descent. *J. Statistical Software* **33**, 1–22 (2010).
54. Abell, J. G. *et al.* Assessing cortisol from hair samples in a large observational cohort: The Whitehall II study. *Psychoneuroendocrinology* **73**, 148–156. https://doi.org/10.1016/j.psyneuen.2016.07.214 (2016).
55. Staufenbiel, S. M., Penninx, B. W. J. H., de Rijke, Y. B., van den Akker, E. L. T. & van Rossum, E. F. C. Determinants of hair cortisol and hair cortisone concentrations in adults. *Psychoneuroendocrinology* **60**, 182–194. https://doi.org/10.1016/j.psyneuen.2015.06.011 (2015).
56. Braig, S. *et al.* Determinants of maternal hair cortisol concentrations at delivery reflecting the last trimester of pregnancy. *Psychoneuroendocrinology* **52**, 289–296. https://doi.org/10.1016/j.psyneuen.2014.12.006 (2015).
57. Incollingo Rodriguez, A. C. *et al.* Hypothalamic-pituitary-adrenal axis dysregulation and cortisol activity in obesity: A systematic review. *Psychoneuroendocrinology* **62**, 301–318. https://doi.org/10.1016/j.psyneuen.2015.08.014 (2015).
58. Kapoor, A., Schultz-Darken, N. & Ziegler, T. E. Radiolabel validation of cortisol in the hair of rhesus monkeys. *Psychoneuroendocrinology* **97**, 190–195. https://doi.org/10.1016/j.psyneuen.2018.07.022 (2018).
59. Rasmussen, P. *et al.* Evidence for a release of brain-derived neurotrophic factor from the brain during exercise: Brain-derived neurotrophic factor release during exercise. *Exp. Physiol.* **94**, 1062–1069. https://doi.org/10.1113/expphysiol.2009.048512 (2009).
60. Klein, A. B. *et al.* Blood BDNF concentrations reflect brain-tissue BDNF levels across species. *Int. J. Neuropsychopharmacol.* **14**, 347–353. https://doi.org/10.1017/S1461145710000738 (2011).
61. Macedo, I. C. *et al.* Chronic stress associated with hypercaloric diet changes the hippocampal BDNF levels in male Wistar rats. *Neuropeptides* **51**, 75–81. https://doi.org/10.1016/j.npep.2015.01.002 (2015).
62. Murakami, S., Imbe, H., Morikawa, Y., Kubo, C. & Senba, E. Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. *Neurosci. Res.* **53**, 129–139. https://doi.org/10.1016/j.neures.2005.06.008 (2005).
63. Shi, S.-S., Shao, S., Yuan, B., Pan, F. & Li, Z.-L. Acute stress and chronic stress change brain-derived neurotrophic factor (BDNF) and tyrosine kinase-coupled receptor (TrkB) expression in both young and aged rat hippocampus. *Yonsei Med. J.* **51**, 661–671. https://doi.org/10.3349/ymj.2010.51.5.661 (2010).
64. Algamal, M. *et al.* Chronic hippocampal abnormalities and blunted HPA axis in an animal model of repeated unpredictable stress. *Front. Behav. Neurosci.* **12**, 150. https://doi.org/10.3389/fnbeh.2018.00150 (2018).
65. Lee, T., Jarome, T., Li, S.-J., Kim, J. J. & Helmstetter, F. J. Chronic stress selectively reduces hippocampal volume in rats: A longitudinal magnetic resonance imaging study. *NeuroReport* **20**, 1554–1558. https://doi.org/10.1097/WNR.0b013e328332bb09 (2009).
66. Schoenfeld, T. J., McCausland, H. C., Morris, H. D., Padmanaban, V. & Cameron, H. A. Stress and loss of adult neurogenesis differentially reduce hippocampal volume. *Biol. Psychiatry* **82**, 914–923. https://doi.org/10.1016/j.biopsych.2017.05.013 (2017).
67. Molendijk, M. L. *et al.* Serum BDNF concentrations as peripheral manifestations of depression: evidence from a systematic review and meta-analyses on 179 associations (N=9484). *Mol. Psychiatry* **19**, 791–800. https://doi.org/10.1038/mp.2013.105 (2014).
68. Polyakova, M. *et al.* BDNF as a biomarker for successful treatment of mood disorders: A systematic & quantitative meta-analysis. *J. Affect. Disord.* **174**, 432–440. https://doi.org/10.1016/j.jad.2014.11.044 (2015).
69. Levy, M. J. F. *et al.* Neurotrophic factors and neuroplasticity pathways in the pathophysiology and treatment of depression. *Psychopharmacology* **235**, 2195–2220. https://doi.org/10.1007/s00213-018-4950-4 (2018).
70. Dwivedi, Y. *et al.* Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. *Arch. Gen. Psychiatry* **60**, 804–815. https://doi.org/10.1001/archpsyc.60.8.804 (2003).
71. Karege, F., Vaudan, G., Schwald, M., Perroud, N. & La Harpe, R. Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. *Mol. Brain Res.* **136**, 29–37. https://doi.org/10.1016/j.molbrainres.2004.12.020 (2005).
72. Boyen, K., Langers, D. R. M., de Kleine, E. & van Dijk, P. Gray matter in the brain: Differences associated with tinnitus and hearing loss. *Hear. Res.* **295**, 67–78. https://doi.org/10.1016/j.heares.2012.02.010 (2013).
73. Landgrebe, M. *et al.* Structural brain changes in tinnitus: Grey matter decrease in auditory and non-auditory brain areas. *Neuroimage* **46**, 213–218. https://doi.org/10.1016/j.neuroimage.2009.01.069 (2009).
74. Profant, O. *et al.* The influence of aging, hearing, and tinnitus on the morphology of cortical gray matter, amygdala, and hippocampus. *Front. Aging Neurosci.* **12**, 449. https://doi.org/10.3389/fnagi.2020.553461 (2020).
75. Tae, W.-S. *et al.* Changes in the regional shape and volume of subcortical nuclei in patients with tinnitus comorbid with mild hearing loss. *Neuroradiology* **60**, 1203–1211. https://doi.org/10.1007/s00234-018-2093-2 (2018).
76. Clarke, N. A., Henshaw, H., Akeroyd, M. A., Adams, B. & Hoare, D. J. Associations between subjective tinnitus and cognitive performance: Systematic review and meta-analyses. *Trends Hear.* **24**, 1–23. https://doi.org/10.1177/2331216520918416 (2020).
77. Notaras, M. & van den Buuse, M. Neurobiology of BDNF in fear memory, sensitivity to stress, and stress-related disorders. *Mol. Psychiatry* **25**, 2251–2274. https://doi.org/10.1038/s41380-019-0639-2 (2020).
78. Hauck, S. *et al.* Serum brain-derived neurotrophic factor in patients with trauma psychopathology. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **34**, 459–462. https://doi.org/10.1016/j.pnpbp.2010.01.010 (2010).
79. Egan, M. F. *et al.* The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* **112**, 257–269. https://doi.org/10.1016/S0092-8674(03)00035-7 (2003).
80. Vanneste, S., Mohan, A., De Ridder, D. & To, W. T. The BDNF Val66Met polymorphism regulates vulnerability to chronic stress and phantom perception. in *Progress in Brain Research*. Vol. 260. 301–326. https://doi.org/10.1016/bs.pbr.2020.08.005 (Elsevier, 2021).
81. Pereira-Jorge, M. R. *et al.* Anatomical and functional MRI changes after one year of auditory rehabilitation with hearing aids. *Neural Plast.* **2018**, 1–13. https://doi.org/10.1155/2018/9303674 (2018).
82. Szuhany, K. L., Bugatti, M. & Otto, M. W. A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. *J. Psychiatr. Res.* **60**, 56–64. https://doi.org/10.1016/j.jpsychires.2014.10.003 (2015).

Acknowledgements

We would like to cordially thank all patients who volunteered to participate in this study. We gratefully acknowledge the laboratory work of Susanne Tumala and Marie Dippel, Psychoneuroimmunology Laboratory Giessen, and thank Raphael Biehl, Tinnitus Center, Charité – Universitätsmedizin Berlin, for his help with data collection. This project forms part of the European consortium TIN-ACT (Tinnitus Assessment Causes Treatments), and we acknowledge the Heinz und Heide Dürr Stiftung for the support of this study.

Author contributions

L.B.: Project administration; Investigation; Formal analysis; Visualization; Writing–Original draft preparation. B.B.: Supervision; Writing–Reviewing and Editing. P.N.: Methodology; Supervision; Writing–Reviewing and Editing. P.B.: Writing–Reviewing and Editing. E.M.J.P.: Conceptualization; Resources; Project administration;

Writing–Reviewing and Editing. B.M.: Funding acquisition; Conceptualization; Resources; Project administration; Supervision; Writing–Reviewing and Editing.

Funding

Open Access funding enabled and organized by Projekt DEAL. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement [No 764604]; and the Heinz und Heide Dürr Stiftung. The funding sources had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-04811-0.

Correspondence and requests for materials should be addressed to B.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Publication 3: Basso L, Boecking B, Neff P, Brueggemann P, Mazurek B, Peters EMJ. Psychological treatment effects unrelated to hair-cortisol and hair-BDNF levels in chronic tinnitus. Frontiers in Psychiatry. 2022;13:764368. doi:10.3389/fpsyt.2022.764368

- Article submitted August 25, 2021; published February 18, 2022 in *Frontiers in Psychiatry*
- Impact Factor 2020: 4.157
- Excerpt from Journal Summary List from 2020, Category “PSYCHIATRY”: Rank 56 of 156 journals

Journal Data Filtered By: **Selected JCR Year: 2020** Selected Editions: SCIE,
Selected Categories: **"PSYCHIATRY"** Selected Category
Scheme: WoS

Gesamtanzahl: 156 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	World Psychiatry	9,619	49.548	0.020030
2	Lancet Psychiatry	14,839	27.083	0.036240
3	JAMA Psychiatry	19,105	21.596	0.052990
4	AMERICAN JOURNAL OF PSYCHIATRY	48,206	18.112	0.031970
5	PSYCHOTHERAPY AND PSYCHOSOMATICS	6,123	17.659	0.006750
6	MOLECULAR PSYCHIATRY	28,622	15.992	0.046220
7	BIOLOGICAL PSYCHIATRY	50,155	13.382	0.045540
8	JOURNAL OF NEUROLOGY NEUROSURGERY AND PSYCHIATRY	37,094	10.154	0.026380
9	BRITISH JOURNAL OF PSYCHIATRY	30,003	9.319	0.019160
10	SCHIZOPHRENIA BULLETIN	21,642	9.306	0.023290
11	JOURNAL OF CHILD PSYCHOLOGY AND PSYCHIATRY	25,273	8.982	0.021190
12	JOURNAL OF THE AMERICAN ACADEMY OF CHILD AND ADOLESCENT PSYCHIATRY	25,046	8.829	0.017190
13	Evidence-Based Mental Health	1,201	8.141	0.003220
14	NEUROPSYCHOPHARMACOLOGY	30,856	7.853	0.034600
15	PSYCHOLOGICAL MEDICINE	34,876	7.723	0.038850
16	BRAIN BEHAVIOR AND IMMUNITY	24,161	7.217	0.026930
17	Clinical Psychological Science	3,811	7.169	0.010420
18	Epidemiology and Psychiatric Sciences	2,571	6.892	0.005580
19	Journal of Behavioral Addictions	4,024	6.756	0.008100
20	BIPOLAR DISORDERS	6,185	6.744	0.007510

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
21	ADDICTION	23,843	6.526	0.025580
22	DEPRESSION AND ANXIETY	12,440	6.505	0.013220
23	ACTA PSYCHIATRICA SCANDINAVICA	16,412	6.392	0.011290
24	Translational Psychiatry	13,269	6.222	0.030670
25	JOURNAL OF PSYCHIATRY & NEUROSCIENCE	4,100	6.186	0.004200
26	PHARMACOPSYCHIATRY	2,099	5.788	0.001500
27	CNS DRUGS	5,948	5.749	0.007070
28	AUSTRALIAN AND NEW ZEALAND JOURNAL OF PSYCHIATRY	8,920	5.744	0.008520
29	EUROPEAN PSYCHIATRY	7,865	5.361	0.010160
30	Current Psychiatry Reports	7,165	5.285	0.012870
31	EUROPEAN ARCHIVES OF PSYCHIATRY AND CLINICAL NEUROSCIENCE	5,451	5.270	0.005150
32	npj Schizophrenia	830	5.200	0.002760
33	PSYCHIATRY AND CLINICAL NEUROSCIENCES	5,454	5.188	0.004700
34	INTERNATIONAL JOURNAL OF NEUROPSYCHOPHARMACOLOGY	7,865	5.176	0.008440
35	PROGRESS IN NEURO-PSYCHOPHARMACOLOGY & BIOLOGICAL PSYCHIATRY	13,777	5.067	0.013440
36	Therapeutic Advances in Psychopharmacology	961	5.000	0.001570
37	SCHIZOPHRENIA RESEARCH	26,508	4.939	0.027790
38	PSYCHONEUROENDOCRINOLOGY	22,335	4.905	0.025020
39	INTERNATIONAL JOURNAL OF EATING DISORDERS	12,593	4.861	0.011620
40	JOURNAL OF AFFECTIVE DISORDERS	46,992	4.839	0.062720
41	JOURNAL OF PSYCHIATRIC RESEARCH	20,371	4.791	0.020030
42	EUROPEAN CHILD & ADOLESCENT PSYCHIATRY	7,765	4.785	0.010300

Selected JCR Year: 2020; Selected Categories: "PSYCHIATRY"

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
43	CURRENT OPINION IN PSYCHIATRY	5,634	4.741	0.006910
44	Eating and Weight Disorders-Studies on Anorexia Bulimia and Obesity	3,458	4.652	0.003750
45	EUROPEAN NEUROPSYCHOPHARMACOLOGY	8,999	4.600	0.011190
46	World Journal of Psychiatry	1,184	4.571	0.002650
47	PSYCHOPHARMACOLOGY	26,451	4.530	0.017630
48	DRUG AND ALCOHOL DEPENDENCE	25,688	4.492	0.038510
49	JMIR Mental Health	2,188	4.388	0.005240
50	JOURNAL OF CLINICAL PSYCHIATRY	21,978	4.384	0.015000
51	CANADIAN JOURNAL OF PSYCHIATRY-REVUE CANADIENNE DE PSYCHIATRIE	8,554	4.356	0.008550
52	International Journal of Bipolar Disorders	730	4.340	0.001590
53	Internet Interventions-The Application of Information Technology in Mental and Behavioural Health	1,658	4.333	0.003310
54	SOCIAL PSYCHIATRY AND PSYCHIATRIC EPIDEMIOLOGY	11,913	4.328	0.013200
55	PSYCHOSOMATIC MEDICINE	14,749	4.312	0.008850
56	Frontiers in Psychiatry	13,383	4.157	0.027120
57	JOURNAL OF PSYCHOPHARMACOLOGY	8,158	4.153	0.010010
58	WORLD JOURNAL OF BIOLOGICAL PSYCHIATRY	3,122	4.132	0.003160
59	AMERICAN JOURNAL OF GERIATRIC PSYCHIATRY	9,399	4.105	0.008920
60	Journal of Eating Disorders	1,364	4.049	0.002940
61	INTERNATIONAL JOURNAL OF METHODS IN PSYCHIATRIC RESEARCH	4,339	4.035	0.003080
62	PSYCHOLOGY AND PSYCHOTHERAPY-THEORY RESEARCH AND PRACTICE	1,904	3.915	0.001770
63	INTERNATIONAL PSYCHOGERIATRICS	9,732	3.878	0.010150
64	International Journal of Mental Health and Addiction	3,487	3.836	0.003280

Selected JCR Year: 2020; Selected Categories: "PSYCHIATRY"

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 18 February 2022
doi: 10.3389/fpsyt.2022.764368

Psychological Treatment Effects Unrelated to Hair-Cortisol and Hair-BDNF Levels in Chronic Tinnitus

Laura Basso[1], *Benjamin Boecking*[1], *Patrick Neff*[2,3,4], *Petra Brueggemann*[1], *Birgit Mazurek*[1]* *and Eva M. J. Peters*[5,6]

[1] Tinnitus Center, Charité – Universitätsmedizin Berlin, Berlin, Germany, [2] Department of Psychiatry and Psychotherapy, University of Regensburg, Regensburg, Germany, [3] University Research Priority Program "Dynamics of Healthy Aging", University of Zurich, Zurich, Switzerland, [4] Centre for Cognitive Neuroscience and Department of Psychology, University of Salzburg, Salzburg, Austria, [5] Psychoneuroimmunology Laboratory, Department of Psychosomatic Medicine and Psychotherapy, Justus-Liebig University Giessen, Giessen, Germany, [6] Psychosomatics and Psychotherapy, Charité Center 12 Internal Medicine and Dermatology, Charité – Universitätsmedizin Berlin, Berlin, Germany

Background: Currently, there are no objective markers to measure treatment efficacy in chronic (distressing) tinnitus. This study explores whether stress-related biomarkers cortisol and brain-derived neurotrophic factor (BDNF) measured in hair samples of chronic tinnitus patients change after compact multimodal tinnitus-specific cognitive behavioral therapy.

Methods: In this longitudinal study, hair-cortisol and hair-BDNF levels, self-reported tinnitus-related distress (Tinnitus Questionnaire; TQ), and perceived stress (Perceived Stress Questionnaire; PSQ-20) were assessed before and 3 months after 5 days of treatment in $N = 80$ chronic tinnitus patients. Linear mixed-effects models with backward elimination were used to assess treatment-induced changes, and a cross-lagged panel model (structural equation model) was used for additional exploratory analysis of the temporal associations between TQ and hair-BDNF.

Results: At follow-up, a reduction in TQ ($p < 0.001$) and PSQ-20 scores ($p = 0.045$) was observed, which was not influenced by baseline hair-cortisol or hair-BDNF levels. No changes in biomarker levels were observed after treatment. The exploratory analysis tentatively suggests that a directional effect of baseline TQ scores on hair-BDNF levels at follow-up (trend; $p = 0.070$) was more likely than the opposite directional effect of baseline hair-BDNF levels on TQ scores at follow-up (n.s.).

Discussion: While the treatment effectively reduced tinnitus-related distress and perceived stress in chronic tinnitus patients, this effect was not mirrored in biological changes. However, the lack of changes in hair-cortisol and hair-BDNF levels might have been influenced by the treatment duration, follow-up interval, or confounding medical factors, and therefore must be interpreted with caution. The relationship between tinnitus-related distress and hair-BDNF levels should be explored further to obtain a better understanding of stress-related effects in chronic tinnitus.

Keywords: chronic tinnitus, stress, treatment, cognitive behavioral therapy (CBT), biomarker, cortisol, brain-derived neurotrophic factor (BDNF)

OPEN ACCESS

Edited by:
Michael Noll-Hussong, Saarland University Hospital, Germany

Reviewed by:
Karl Bechter, University of Ulm, Germany
Nadia Cattane, San Giovanni di Dio Fatebenefratelli Center (IRCCS), Italy

***Correspondence:**
Birgit Mazurek
birgit.mazurek@charite.de

Specialty section:
This article was submitted to Psychosomatic Medicine, a section of the journal Frontiers in Psychiatry

Received: *25 August 2021*
Accepted: *04 January 2022*
Published: *18 February 2022*

Citation:
Basso L, Boecking B, Neff P, Brueggemann P, Mazurek B and Peters EMJ (2022) Psychological Treatment Effects Unrelated to Hair-Cortisol and Hair-BDNF Levels in Chronic Tinnitus. Front. Psychiatry 13:764368. doi: 10.3389/fpsyt.2022.764368

INTRODUCTION

Tinnitus is the subjective perception of a sound in absence of an external source. Chronic tinnitus is a frequent phenomenon with prevalence estimates in adults ranging up to 15% (1). In many affected individuals, tinnitus leads to considerable distress; constituting a big or very big problem for 7% and a moderate problem for 20% (2). Tinnitus associated with suffering can be conceptualized as "tinnitus disorder" (3) and is known to be influenced by personal vulnerability-stress interactions (4).

Currently, no existing treatment option can eliminate the tinnitus percept. However, the negative impact of tinnitus on the quality of life (QoL) in tinnitus patients can be reduced by cognitive behavioral therapy (5, 6). Cognitive behavioral therapy is a widely studied, evidence-based therapeutic approach that can be used for the treatment of various mental health problems (7). In the clinical care of tinnitus patients, cognitive behavioral therapy is focused on addressing dysfunctional cognitions, behaviors, and emotions related to tinnitus (which negatively affect the QoL) through cognitive restructuring and behavioral modification (5, 8). Because of the complex and multifactorial etiology and maintenance of chronic tinnitus, cognitive behavioral therapy-based multidisciplinary treatment approaches are recommended (9, 10). Multidisciplinary interventions for chronic tinnitus with cognitive behavioral therapy elements were found to be effective and have stable long-term effects (11–14).

At present, treatment efficacy can only be assessed by subjective measures; commonly, psychometric questionnaires are used (15, 16). Objective measures of treatment efficacy, e.g., biomarkers that are sensitive to distress-related treatment responses in individuals suffering from chronic tinnitus, would be highly useful, as they could provide objective criteria for the evaluation and comparison of treatment approaches.

Stress-related biomarkers such as cortisol are traditionally mainly quantified in biological fluids (saliva, blood, or urine) but can also be measured in hair. Hair sampling has the advantage of being non-invasive, less influenced by situational factors, and allowing direct measurement of long-term concentrations (cumulative concentrations over one or several months) without requiring repeated sampling (17).

Hair-cortisol is an established stress-related measure of cumulative cortisol secretion (18). However, the results of individual studies on its association with self-reported levels of perceived stress are not always conclusive (18).

Brain-derived neurotrophic factor (BDNF) is another stress-related marker that can be measured in hair (19). Among the important functions of BDNF is its involvement in neuroprotection and synaptic plasticity (20). Animal research has shown that BDNF expression is strongly affected by stress (21, 22). Moreover, peripheral BDNF levels appear to be decreased in stress-related mood disorders (23–25) and reduced BDNF expression may be involved in their pathogenesis (22). Peripheral BDNF levels have been shown to increase after antidepressant treatment in patients with major depressive disorder (23, 24, 26) and after mindfulness-based interventions in different study populations (27).

We previously investigated cross-sectional relationships between tinnitus loudness and distress with hair-cortisol and hair-BDNF in a sample of chronic tinnitus patients and observed a negative association between tinnitus-related distress and hair-BDNF (28), suggesting that hair-BDNF might be treatment-sensitive to psychological interventions in chronic tinnitus. The objective of the present longitudinal analysis of the same sample is to investigate treatment-induced changes in hair-cortisol and hair-BDNF levels to explore, for the first time, their potential as biomarkers of treatment efficacy.

This study has four research questions. (1) Whether tinnitus-related distress and perceived stress are reduced after compact multimodal tinnitus-specific cognitive behavioral therapy; which we expect to find based on previous studies that used a similar treatment approach (11–14). (2) Whether hair-cortisol or hair-BDNF levels show measurable and meaningful changes after the intervention. Based on our previous cross-sectional findings (28), suggesting that hair-cortisol is relatively independent of psychological factors in chronic tinnitus, no directional hypothesis was specified for hair-cortisol. However, based on the observed association with tinnitus-related distress, we expect hair-BDNF levels to increase in parallel with treatment-induced reductions in tinnitus-related distress. (3) Furthermore, we aim to identify which factors (sociodemographic, psychological, biological, tinnitus-/hearing-related, lifestyle, or hair-related) influence the outcome variables and respective treatment effects (questions 1 and 2). Linear mixed-effects models with backward elimination for each outcome will be used to address these research questions. (4) Based on the obtained results, an additional exploratory research question is to further investigate the temporal relationships between identified associated psychological and biological factors. Cross-lagged panel analysis will be used to assess such temporal relationships, accounting for the stability of the investigated factors over time.

MATERIALS AND METHODS

Study Design and Sample

Between December 2018 and March 2020, 94 chronic tinnitus inpatients volunteered to participate in this study, which consisted of three measurements: (1) before and (2) directly after 5 days of compact multimodal tinnitus-specific cognitive behavioral therapy, which is the current standard clinical treatment for chronic tinnitus offered at the Tinnitus Center of Charité – Universitätsmedizin Berlin, and (3) a 3-month follow-up measurement (lasting until June 2020). Baseline data of the present study ($N = 91$ for hair-cortisol, $N = 87$ for hair-BDNF) have been previously analyzed in cross-section (28).

The baseline measurement included the collection of hair samples and psychometric questionnaires (day of treatment begin); additionally, pure tone audiograms and tinnitus matching data were collected from audiometric records (most recent measurement before treatment begin; M = 73.8 days prior, SD = 59.41). The second measurement, performed approx. 5 days later (directly after treatment end), only included

		Baseline	Treatment end (+5 days)	Follow-up (+3 months)
Biomarkers	• Hair-cortisol	X		X
	• Hair-BDNF	X		X
Psychometric questionnaires	• TQ: tinnitus-related distress	X	X	X
	• PSQ-20: perceived stress	X	X	X
	• HADS: anxiety and depression	X	X	X
	• SOMS: somatization	X		X
	• STAI: state anxiety	X		X
	• PDS: traumatic experiences	X		
	• SF-12: physical and mental health-related quality of life	X	X	X
Tinnitus and hearing	• Tinnitus frequency and loudness (matching data)	X		
	• Hearing threshold (audiogram)	X		
	• Tinnitus/hearing-related characteristics	X		
Covariates	• Sociodemographic information	X		X
	• Season and time of sampling	X		X
	• Hair-related criteria/hair care	X		X
	• Health-related factors/behavior	X		X

FIGURE 1 | Overview of all collected study variables across measurements (baseline, treatment end, and follow-up). BDNF, Brain-Derived Neurotrophic Factor; HADS, Hospital Anxiety and Depression Scale; PDS, Posttraumatic Diagnostic Scale; PSQ-20, Perceived Stress Questionnaire (20 item version); SF-12, Short Form-12 Health Survey; SOMS, Screening of Somatoform Disorders; STAI, State-Trait Anxiety Inventory (State Anxiety); TQ, Tinnitus Questionnaire.

psychometric questionnaires. The third measurement performed approx. 3 months later (M = 93.81 days, SD = 11.94), included hair sample collection and psychometric questionnaires. All collected variables are summarized in **Figure 1**. Primary outcomes were tinnitus-related distress, perceived stress, hair-cortisol, and hair-BDNF.

Inclusion criteria were "diagnosis of chronic subjective tinnitus", "age $\geq$ 18 years", and "written informed consent" (28). Exclusion criteria were "inability to consent due to serious mental or physical impairments", "simultaneous participation in other research studies", "hair length $<$ 3 cm", "any chemical hair treatment within 1 month prior to sampling (dying, bleaching, perming, or else)", "hair washing or the use of hair products (hair mousse, hair gel, hair wax, hair spray) within 3 days prior to sampling", and "hair combing on the day of sampling" (28).

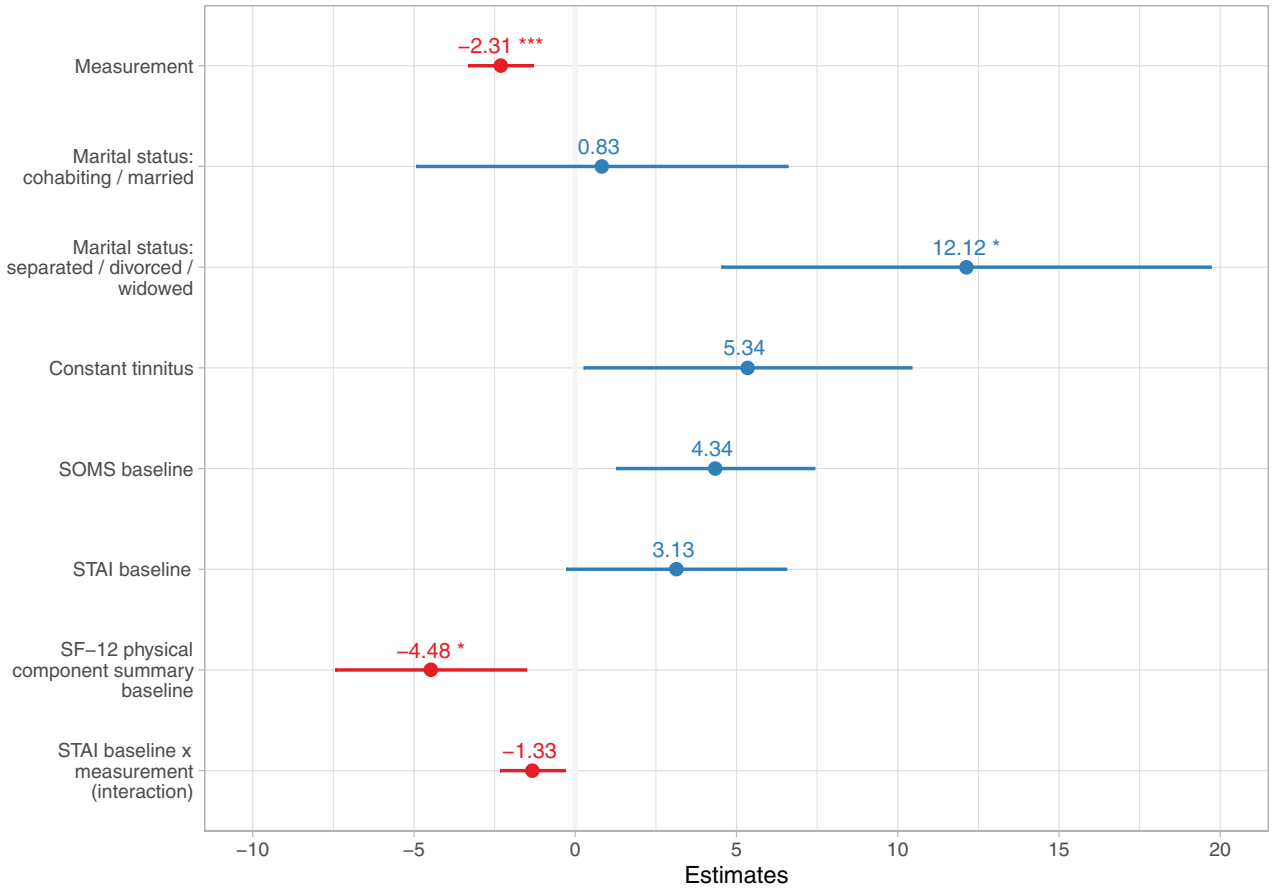

FIGURE 2 | Reduced linear mixed-effects model with stepwise backward elimination for change in Tinnitus Questionnaire (TQ) scores across baseline, treatment end, and follow-up ($N = 80$). Numbers indicate estimated coefficient effects and lines depict 95% confidence intervals. Significance levels are displayed after adjustment for multiple testing with Holm's method. SF-12, Short Form-12 Health Survey; SOMS, Screening of Somatoform Disorders; STAI, State-Trait Anxiety Inventory (State Anxiety). $^{*}p < 0.05$; $^{***}p < 0.001$.

One patient was excluded due to missing questionnaire data at baseline, three patients were excluded due to hair-related criteria (at baseline or follow-up), four patients did not complete the follow-up measurement (due to the associated effort), and six patients were excluded due to incomplete biomarker measures. The final sample size was $N = 80$. The remaining missing values, mostly of tinnitus matching data, were imputed (see Section Linear Mixed-Effects Models).

All participants were European; on average, 50.96 years old (SD = 11.72), and 66.25% ($N = 53$) were female. The study was approved by the local ethic commission of Charité – Universitätsmedizin Berlin (No. EA1/035/16) and all participants provided written informed consent.

Compact Multimodal Tinnitus-Specific Cognitive Behavioral Therapy

The treatment took place over 4.78 days on average (SD = 1.10, range: 4–9), had a tinnitus-specific cognitive behavioral therapy focus (individual and group treatment sessions), and included the following other modalities: education, counseling, otorhinolaryngological and general medical diagnostics, auditory attention training, relaxation, and physiotherapeutic sessions.

Psychometric Questionnaires and Covariates

The following psychometric questionnaires were used (German versions):

- *Tinnitus Questionnaire* (TQ) (29).
- *Perceived Stress Questionnaire* (PSQ-20; 20 item version) (30, 31).
- *Hospital Anxiety and Depression Scale* (HADS) (32, 33).
- *Screening of Somatoform Disorders* (SOMS; 7 days version) (34).
- *State-Trait Anxiety Inventory (STAI) – State Anxiety* (35).
- *Posttraumatic Diagnostic Scale (PDS) – Event List* (36).
- *Short Form-12 Health Survey* (SF-12; version 2) (37, 38).

Covariates included sociodemographic information, information on hair care, and health-related behavior [see (28)].

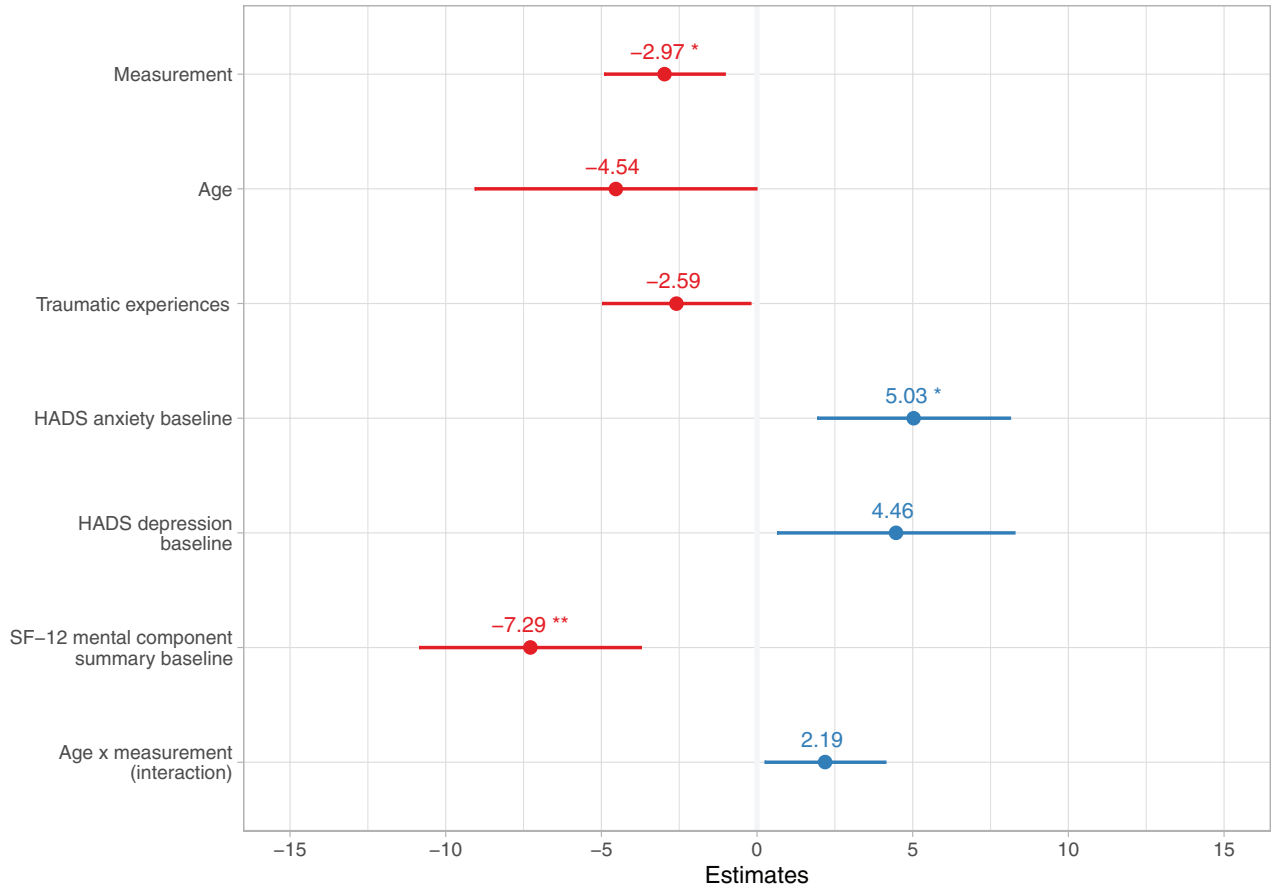

FIGURE 3 | Reduced linear mixed-effects model with stepwise backward elimination for change in Perceived Stress Questionnaire (PSQ-20) scores across baseline, treatment end, and follow-up ($N = 80$). Numbers indicate estimated coefficient effects and lines depict 95% confidence intervals. Significance levels are displayed after adjustment for multiple testing with Holm's method. HADS, Hospital Anxiety and Depression Scale, SF-12, Short Form-12 Health Survey. *$p < 0.05$; **$p < 0.01$.

Audiometry (Hearing Threshold and Tinnitus Pitch and Loudness Matching)

The mean hearing threshold at the frequencies 0.25, 0.5, 1, 2, 3, 4, 6, and 8 kHz measured by pure tone audiogram was calculated and averaged across ears if possible. The matched tinnitus frequency (Hz) and absolute loudness (dB) were averaged for bilateral tinnitus. Tinnitus pitch and loudness matching could not be performed in 21 patients [see (28)].

Hair Sampling

Hair samples were cut with scissors from the region of the posterior vertex, as close to the scalp as possible. The median sampling time was 09:55 a.m. at baseline and 10:15 a.m. at follow-up. Samples were stored (in a dark container at room temperature) until analysis in summer/autumn 2020. The most proximal 1-cm hair segment was analyzed, one month prior to sampling. Cortisol and BDNF quantification was performed using commercial kits and followed the previously described laboratory protocol (19). According to the manufacturer, the sensitivity of the cortisol ELISA is 0.005 μg/dl (standard range 0.15–30 ng/ml) and of the BDNF ELISA 15.6 pg/ml (standard range 0–1000 pg/ml; BDNF measurements were performed in a dilution of 1:1000). The intra- and inter-assay coefficients of variation as stated by the manufacturer are +4.3 and +13.2% for cortisol ELISA, and +3.7 and +8.5% for BDNF ELISA, respectively. In our study, the intra- and inter-assay coefficients of variation were +1.91% and 7.49 ± 2.81 for cortisol, and +2.73% and 5.31 ± 3.35 for BDNF. All but seven BDNF values were within the detection range.

Statistical Analysis

Analyses were performed using R (version 4.0.0) (39). Hair-cortisol values were log-transformed to establish normal distribution. For descriptive analyses, biomarker values between participants using antidepressant medication and the rest of the sample were compared using two-sample *t*-tests. To address research questions 1–3, linear mixed-effects models were calculated for TQ, PSQ-20, hair-cortisol, and hair-BDNF as outcome variables, and these models were reduced by backward elimination to identify relevant predictors. For research question

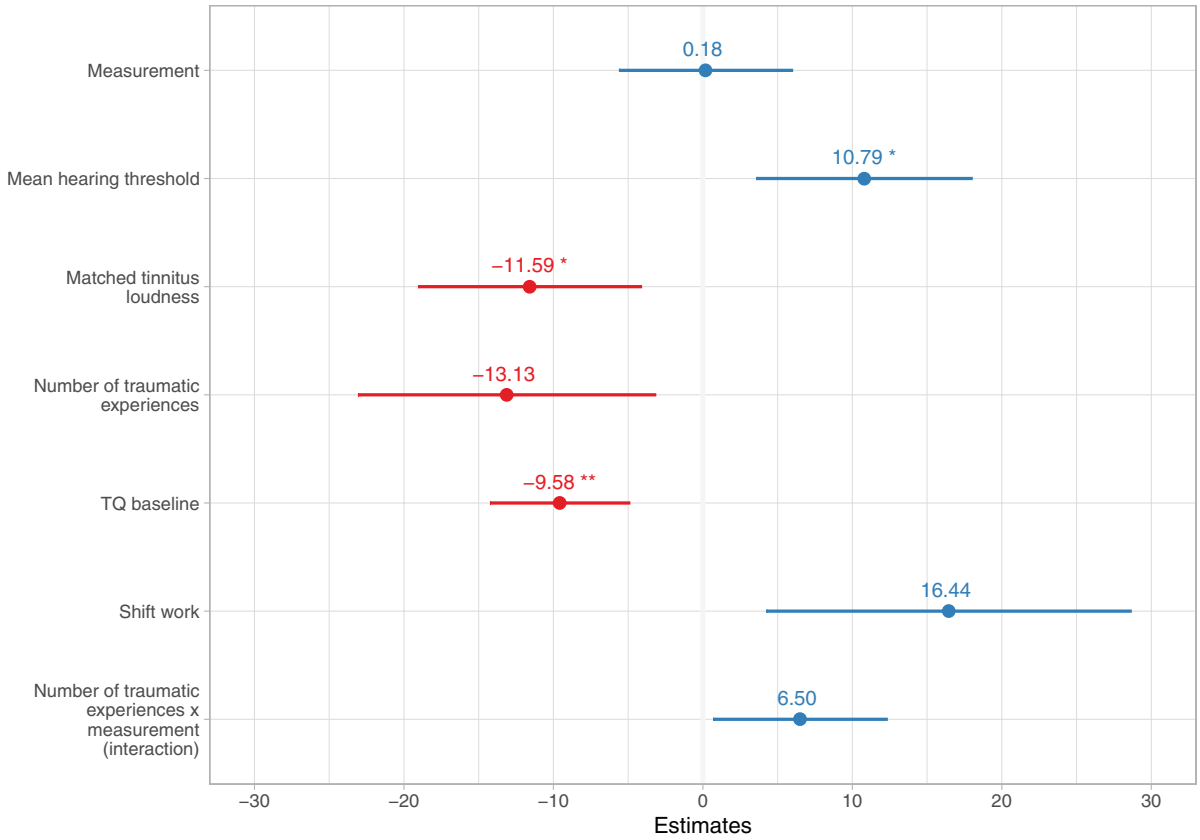

FIGURE 4 | Reduced linear mixed-effects model with stepwise backward elimination ($N = 80$) for change in hair-BDNF levels across baseline and follow-up ($N = 80$). Numbers indicate estimated coefficient effects and lines depict 95% confidence intervals. Significance levels are displayed after adjustment for multiple testing with Holm's method. TQ, Tinnitus Questionnaire. *$p < 0.05$; **$p < 0.01$.

4, cross-lagged panel analysis was used. The following packages were used for linear mixed-effects models: "lme4" for model building; "lmerTest" for backward elimination; "multcomp" for significance testing; "MuMIn" for estimates of marginal and conditional R^2, "sjPlot" for fixed effects plots; "glmmTMB" for diagnostic plots. For imputation of missing values "DMwR2" was used and for the cross-lagged panel analysis the packages "lavaan" and "semPlot". The significance threshold was set to $p < 0.05$.

Linear Mixed-Effects Models

Numeric predictors were centered and scaled. Missing values were imputed with k-nearest neighbor imputation (see below).

Model Building and Selection

First, the "full" model was estimated including all predictors of interest and their respective interaction terms with "measurement" (baseline, treatment end, and follow-up for psychometric questionnaires; baseline and follow-up for biomarkers) as fixed effects. For TQ and PSQ-20, selected predictors included sociodemographic factors, tinnitus- and hearing-related factors, psychometric factors, and biomarker scores at baseline, as well as interaction terms of all these baseline factors with the measurement variable. For hair-cortisol and hair-BDNF, selected predictors included sociodemographic factors, tinnitus- and hearing-related factors, tinnitus matching (loudness/frequency), psychometric factors and covariates, either at baseline or both measurements, as well as interaction terms of all baseline factors with the measurement variable; for time-varying covariates, no interaction terms were included. Covariates for the biomarker models were selected based on cross-sectional results (28).

Second, the random-effects structure was determined by comparing random intercept models with random intercept and slope models. For the prediction of TQ scores, no significant difference was present between the random intercept and random intercept and slope models, $\chi^2_{(2)} = 1.20$, $p = 0.549$. For the other outcomes, the comparison was not possible due to singular fit (PSQ-20) or an insufficient number of observations (hair-cortisol and hair-BDNF) for estimation of the respective random intercept and slope models. Consequently, for all outcomes, the more parsimonious random intercept model was chosen. Lastly, automated backward elimination was performed to obtain the final reduced model.

Models were fitted using restricted maximum likelihood (REML) (40). For significance testing, z-tests were used

TABLE 1 | Summary statistics of numeric variables ($N = 80$).

	Baseline				Treatment end				Follow-up			
Variable	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
TQ total score: tinnitus-related distress	34.70	15.61	3	79	31.88	15.11	2	75	30.08	15.74	0	79
PSQ-20 total score: perceived stress	51.79	19.10	8.33	86.67	41.48	21.07	0	88.33	45.85	20.42	3.33	85
Cortisol μg/dl	0.052	0.042	0.004	0.211					0.046	0.045	0.004	0.288
BDNF ng/ml	78.35	28.08	12.62	130.03					78.53	28.89	16.40	130.13
Age	50.96	11.72	19	75								
Mean hearing threshold (dB)	21.64	12.77	4.69	71.56								
Matched tinnitus frequency (Hz) ($N = 59$)	5,491.53	2,422.49	250	10,000								
Matched tinnitus loudness (dB) ($N = 59$)	37.99	20.06	5	79								
HADS: anxiety	7.90	4.13	0	18	7.19	4.07	0	17	6.85	3.78	0	16
HADS: depression	5.97	3.94	0	14	5.42	3.87	0	13	6.16	4.19	0	16
SF-12: physical component summary ($N = 79/78/80$)	42.08	10.24	16.05	59.08	44.51	9.56	18.76	59.08	43.20	9.58	18.75	59.08
SF-12: mental component summary ($N = 79/78/80$)	37.75	10.63	16.05	57.53	43.70	10.03	19.16	59.22	39.57	9.85	17.63	57.53
SOMS: somatization	9.22	7.06	0	29					9.20	6.44	0	24
STAI total score: state anxiety	44.42	11.25	26	75					41.23	11.30	23	70
PDS: number of traumatic experiences	1.68	1.34	0	5								
Frequency of hair washing per week	2.91	1.66	1	8								
Alcohol units per week[a]	2.12	4.02	0	21					2.65	3.24	0	18
BMI	25.75	4.55	17.62	41.38					25.42	4.18	17.93	37.13
Physical activity score[b]	6.04	5.95	0	24					6.26	5.52	0	28
Cups of coffee/tea per day	2.84	1.90	0	9					2.60	1.86	0	8

[a] *Alcohol units consumed per week: one unit = 0.3 l beer or 0.2 l wine or shot glass of spirits.* [b] *Physical activity score: number of days per week on which participants are physically active times the duration of the physical activity (1 = less than 10 min., 2 = 10–30 min., 3 = 30–60 min., 4 = more than 60 min). BDNF, Brain-Derived Neurotrophic Factor; BMI, Body Mass Index; HADS, Hospital Anxiety and Depression Scale; PDS, Posttraumatic Diagnostic Scale; PSQ-20, Perceived Stress Questionnaire (20 item version); SF-12, Short Form-12 Health Survey; SOMS, Screening of Somatoform Disorders; STAI, State-Trait Anxiety Inventory (State Anxiety); TQ, Tinnitus Questionnaire.*

(41); *p*-values were adjusted for multiple testing (see below). Model equations, model fit, fixed effects estimates with 95% confidence intervals, and random effect variance of the full and reduced models for each outcome can be found in **Tables 3–6**. Fixed effects estimates with 95% confidence intervals of the reduced models are displayed in **Figures 2–4** and test statistics of significant effects after adjustment are reported. Diagnostic plots for each outcome can be found in the **Supplementary Figures 1–4**.

Imputation

Imputation of missing values was performed before model building using k-nearest neighbor imputation. The following missing values were imputed: $N = 21$ for tinnitus loudness and frequency, $N = 5$ for hair color, $N = 3$ for SF-12, and $N = 1$ for hearing aid use. The high correlation of tinnitus loudness with mean hearing threshold was preserved after imputation (without imputation: Spearman $r = 0.798$, $p < 0.001$, with imputation: $r = 0.803$, $p < 0.001$), see **Supplementary Figure 5**.

Adjustment for Multiple Testing

All *p*-values of the fixed effects of all four reduced models (28 effects in total) were adjusted for multiple testing using Holm's method (42) (using "p.adjust"), as this method is more powerful than Bonferroni correction (43).

Exploratory Analysis: Cross-Lagged Panel Model

As an exploratory analysis (research question 4) based on the obtained results, a cross-lagged panel model was calculated to investigate temporal relations between tinnitus-related distress and hair-BDNF levels in structural equation modeling framework using maximum likelihood estimation with robust standard errors. Previously identified influencing factors on TQ and BDNF levels from the reduced linear mixed-effects models were included as control variables. Standardized estimates (based on latent variable variance), standard errors, and *p*-values are reported. Due to the exploratory nature of this analysis, *p*-values were not adjusted.

RESULTS

Sample Description

Sample characteristics across measurements are summarized in **Table 1** (numeric variables) and **Table 2** (categorical variables). Musculoskeletal symptoms like muscular imbalance ($N = 46$, 58.23%), segmental joint dysfunction ($N = 46$, 58.23%), chronic cervical syndrome ($N = 44$, 55.70%), craniomandibular/temporomandibular dysfunction ($N = 31$, 39.24%), and bruxism ($N = 35$, 44.30%), were common comorbidities in the sample ($N = 79$). None of the participants suffered from endocrine conditions with altered cortisol production (Cushing syndrome or Addison's disease) or from neurodegenerative diseases associated with changes in cortisol and BDNF levels like Alzheimer's disease, Parkinson's disease, or Huntington's disease (44, 45). Past substance abuse was reported by $N = 2$ participants (2.53%). Eleven patients (14.10%) were using antidepressants; their baseline hair-BDNF (M = 69.01, SD = 27.93 vs. M = 79.61, SD = 28.40; $t_{(76)} = -1.15$, $p = 0.254$) and (log-transformed) hair-cortisol values (M = −1.23, SD = 0.27 vs. M = −1.44, SD = 0.35; $t_{(76)} = 1.93$, $p = 0.057$) did not significantly differ from the rest of the sample ($N = 67$).

TABLE 2 | Summary statistics of categorical variables ($N = 80$).

	Baseline		Follow-up	
Variable	**N**	**%**	**N**	**%**
Sex: female	53	66.25		
Education level[a]				
Low	13	16.25		
Medium	29	36.25		
High	38	47.50		
Marital status				
Single	22	27.50	20	25
Cohabiting / married	44	55	45	56.25
Separated / divorced / widowed	14	17.50	15	18.75
Employment: yes	62	77.50	58	72.50
Tinnitus type				
Intermittent	49	61.25	43	53.75
Constant	31	38.75	37	46.25
Tinnitus onset associated with stress: yes	43	53.75		
Tinnitus influenced by stress: yes	64	80	77	96.25
Hyperacusis (self-report): yes	62	77.50	68	85
Use of hearing aids: yes	14	17.50	18	22.50
Missing			1	1.25
Season of sample collection				
Winter	40	50	11	13.75
Spring	12	15	40	50
Summer	18	22.50	15	18.75
Autumn	10	12.50	14	17.50
Regular use of hair products: yes	28	35		
Hair color				
Gray / white	15	18.75		
Blonde / red	27	33.75		
Brown / black	33	41.25		
I don't know / missing	5	6.25		
Smoking: yes	10	12.50	11	13.75
Shift work: yes	14	17.50		
Sport				
Less than 1 h a week	29	36.25	24	30
Regularly, 1–2 h a week	34	42.50	30	37.50
Regularly, 3–4 h a week	14	17.50	20	25
Regularly, more than 4 h a week	3	3.75	6	7.50

[a]*Education levels: low = elementary, secondary or middle school; medium = high school or completed apprenticeship; high = university.*

Linear Mixed-Effects Models

Tinnitus Questionnaire (TQ): Reduction Across Baseline, Treatment End, and Follow-Up

To investigate the change in tinnitus-related distress as measured by the TQ (research question 1) and relevant modulating

TABLE 3 | Full and backward reduced linear mixed-effects models for change in Tinnitus Questionnaire (TQ) scores across baseline, treatment end, and follow-up ($N = 80$).

	Full model	Backward reduced model
	Fixed effects estimates (95% confidence intervals)	
Measurement	−2.58 (−6.85, 1.68)	**−2.31*** (−3.31, −1.31)**
Sex: male (vs. female)	−1.17 (−9.30, 6.96)	
Age	−0.07 (−5.28, 5.14)	
Cohabiting / married (vs. single)	0.59 (−9.76, 10.95)	0.83 (−4.90, 6.56)
Separated / divorced / widowed (vs. single)	9.77 (−3.48, 23.02)	**12.12* (4.58, 19.67)**
Education: linear	−1.80 (−9.72, 6.13)	
Education: quadratic	−1.06 (−7.81, 5.69)	
Employment: no (vs. yes)	5.36 (−4.29, 15.02)	
Mean hearing threshold	−1.29 (−6.81, 4.23)	
Tinnitus onset associated with stress: yes (vs. no)	3.90 (−4.01, 11.80)	
Constant tinnitus (vs. intermittent)	7.11 (−0.64, 14.87)	5.34 (0.29, 10.40)
Tinnitus influenced by stress: yes (vs. no)	−1.50 (−11.83, 8.84)	
Hearing aids: yes (vs. no)	1.36 (−11.53, 14.24)	
Hyperacusis: yes (vs. no)	−6.08 (−16.48, 4.33)	
Number of traumatic experiences	−1.42 (−5.65, 2.82)	
SOMS baseline	1.05 (−4.17, 6.27)	4.34 (1.29, 7.40)
STAI baseline	2.19 (−3.86, 8.24)	3.13 (−0.26, 6.53)
PSQ-20 baseline	1.66 (−5.89, 9.21)	
HADS anxiety baseline	0.64 (−5.71, 7.00)	
HADS depression baseline	3.90 (−3.00, 10.79)	
SF-12 physical component summary baseline	−4.88 (−10.79, 1.03)	**−4.48* (−7.42, −1.53)**
SF-12 mental component summary baseline	2.99 (−4.78, 10.75)	
Hair-cortisol baseline	0.86 (−2.96, 4.69)	
Hair-BDNF baseline	−3.26 (−7.43, 0.91)	
Measurement × sex	0.20 (−2.39, 2.78)	
Measurement × age	0.79 (−0.87, 2.45)	
Measurement × cohabiting / married	−0.23 (−3.53, 3.06)	
Measurement × separated / divorced / widowed	0.25 (−3.96, 4.47)	
Measurement × education (linear)	−0.41 (−2.93, 2.11)	
Measurement × education (quadratic)	0.25 (−1.90, 2.39)	
Measurement × no employment	−1.28 (−4.35, 1.80)	
Measurement × mean hearing threshold	0.44 (−1.32, 2.19)	
Measurement × tinnitus onset associated with stress	−0.76 (−3.28, 1.75)	
Measurement × constant tinnitus	−0.83 (−3.30, 1.64)	
Measurement × tinnitus influenced by stress	−0.73 (−4.02, 2.56)	
Measurement × hearing aids	0.05 (−4.05, 4.15)	
Measurement × hyperacusis	2.55 (−0.76, 5.86)	
Measurement × number of traumatic experiences	0.36 (−0.99, 1.71)	
Measurement × SOMS baseline	1.05 (−0.61, 2.71)	
Measurement × STAI baseline	−1.46 (−3.39, 0.46)	−1.33 (−2.33, −0.32)
Measurement × PSQ-20 baseline	0.03 (−2.38, 2.43)	
Measurement × HADS anxiety baseline	−0.45 (−2.47, 1.58)	
Measurement × HADS depression baseline	−0.70 (−2.90, 1.49)	
Measurement × SF-12 physical component summary baseline	1.28 (−0.60, 3.16)	
Measurement × SF-12 mental component summary baseline	−1.49 (−3.96, 0.98)	
Measurement × hair-cortisol baseline	0.08 (−1.13, 1.30)	
Measurement × hair-BDNF Baseline	0.43 (−0.90, 1.75)	
Constant	35.17*** (21.77, 48.58)	32.19*** (27.06, 37.33)
	Random effects variance (SD)	
Subject (random intercept)	117.06 (10.82)	104.28 (10.21)

(Continued)

TABLE 3 | Continued

	Full model	Backward reduced model
	Model fit	
Log-likelihood	−797.65	−858.22
Aikake information criterion	1,695.31	1,738.44
Bayesian information criterion	1,869.34	1,776.73
Marginal R^2	0.43	0.42
Conditional R^2	0.84	0.83

Linear mixed model fit by REML; z-tests were used to test fixed effects estimates; significance levels are displayed after adjustment for multiple testing with Holm's method; significant effects in the reduced model are printed in bold. Observations = 240. ***Model equations****: Full model: "TQ ∼ measurement + sex × measurement + age × measurement + marital status × measurement + education × measurement + employment × measurement + mean hearing threshold × measurement + tinnitus onset associated with stress × measurement + tinnitus type × measurement + tinnitus influenced by stress × measurement + hearing aids × measurement + hyperacusis × measurement + number of traumatic experiences × measurement + SOMS baseline × measurement + STAI baseline × measurement + PSQ-20 baseline × measurement + HADS anxiety baseline × measurement + HADS depression baseline × measurement + SF-12 physical component summary baseline × measurement + SF-12 mental component summary baseline × measurement + hair-cortisol baseline × measurement + hair-BDNF baseline × measurement + (1 | subject)". Reduced model: "TQ ∼ measurement + marital status + tinnitus type + SOMS baseline + STAI baseline + SF-12 physical component summary baseline + STAI baseline × measurement + (1 | subject)." BDNF, Brain-Derived Neurotrophic Factor; HADS, Hospital Anxiety and Depression Scale; PDS, Posttraumatic Diagnostic Scale; PSQ-20, Perceived Stress Questionnaire (20 item version); SF-12, Short Form-12 Health Survey; SOMS, Screening of Somatoform Disorders; STAI, State-Trait Anxiety Inventory (State Anxiety); TQ, Tinnitus Questionnaire. *p < 0.05; ***p < 0.001.*

influences (research question 3), two linear mixed-effects models were calculated, the first including all potentially relevant factors (full model) and the second after excluding non-significant factors by backward elimination (reduced model); the results of both models can be found in **Table 3**. The following significant fixed effects estimates were identified in the reduced model after adjustment for multiple testing (see **Figure 2**): A reduction in TQ scores across measurements, $\beta = -2.31$ [−3.31, −1.31], $z = -4.53$, $p_{\text{unadjusted}} < 0.001$, $p_{\text{adjusted}} < 0.001$, generally higher TQ scores in separated, divorced, or widowed patients, $\beta = 12.12$ [4.58, 19.67], $z = 3.15$, $p_{\text{unadjusted}} = 0.002$, $p_{\text{adjusted}} = 0.033$, and generally lower TQ scores in patients with higher SF-12 physical component summary baseline scores, i.e., higher physical health-related QoL, $\beta = -4.48$ [−7.42, −1.53], $z = -2.98$, $p_{\text{unadjusted}} = 0.003$, $p_{\text{adjusted}} = 0.049$.

Perceived Stress Questionnaire (PSQ-20): Reduction Across Baseline, Treatment End, and Follow-Up

To investigate the change in perceived stress levels as measured by the PSQ-20 (research question 1) and relevant modulating influences (research question 3), two linear mixed-effects models were calculated, the first including all potentially relevant factors (full model) and the second after excluding non-significant factors by backward elimination (reduced model); the results of both models can be found in **Table 4**. The following significant fixed effects estimates were identified in the reduced model after adjustment for multiple testing (see **Figure 3**): A reduction in PSQ scores across measurements, $\beta = -2.97$ [−4.90, −1.04], $z = -3.02$, $p_{\text{unadjusted}} = 0.003$, $p_{\text{adjusted}} = 0.045$, generally higher PSQ scores in patients with higher HADS anxiety baseline scores, $\beta = 5.03$ [1.95, 8.11], $z = 3.20$, $p_{\text{unadjusted}} = 0.001$, $p_{\text{adjusted}} = 0.028$, and generally lower PSQ scores in patients with higher SF-12 mental component summary baseline scores, i.e., higher mental health-related QoL, $\beta = -7.29$ [−10.83, −3.75], $z = -4.03$, $p_{\text{unadjusted}} < 0.001$, $p_{\text{adjusted}} = 0.001$.

Hair-Cortisol: No Change Across Baseline and Follow-Up

To investigate the change in hair-cortisol levels (research question 2) and relevant modulating influences (research question 3), two linear mixed-effects models were calculated, the first including all potentially relevant factors (full model) and the second after excluding non-significant factors by backward elimination (reduced model); the results of both models can be found in **Table 5**. After adjustment for multiple testing, no effect in the reduced model remained significant.

Hair-BDNF: No Change Across Baseline and Follow-Up

To investigate the change in hair-BDNF levels (research question 2) and relevant modulating influences (research question 3), two linear mixed-effects models were calculated, the first including all potentially relevant factors (full model) and the second after excluding non-significant factors by backward elimination (reduced model); the results of both models can be found in **Table 6**. The following significant fixed effects estimates were identified in the reduced model after adjustment for multiple testing (see **Figure 4**): Generally higher hair-BDNF levels in patients with a higher mean hearing threshold, $\beta = 10.79$ [3.64, 17.93], $z = 2.96$, $p_{\text{unadjusted}} = 0.003$, $p_{\text{adjusted}} = 0.049$, generally lower hair-BDNF levels in patients with higher tinnitus loudness, $\beta = -11.59$ [−18.98, −4.19], $z = -3.07$, $p_{\text{unadjusted}} = 0.002$, $p_{\text{adjusted}} = 0.040$, and generally lower hair-BDNF levels in patients with higher TQ baseline scores, $\beta = -9.58$ [−14.21, −4.96], $z = -4.06$, $p_{\text{unadjusted}} < 0.001$, $p_{\text{adjusted}} = 0.001$. No significant change in hair-BDNF levels across measurements was present.

Exploratory Analysis: Cross-Lagged Panel Model

For research question 4, based on the linear mixed-effects model results indicating an effect of TQ baseline scores on hair-BDNF levels across measurements, a cross-lagged panel model in a

TABLE 4 | Full and backward reduced linear mixed-effects models for change in Perceived Stress Questionnaire (PSQ-20) scores across baseline, treatment end, and follow-up ($N = 80$).

	Full model	Backward reduced model
	Fixed effects estimates (95% confidence intervals)	
Measurement	−2.76 (−10.94, 5.42)	**−2.97* (−4.90, −1.04)**
Sex: male (vs. female)	−5.01 (−16.24, 6.23)	
Age	−3.93 (−11.28, 3.41)	−4.54 (−9.05, −0.04)
Cohabiting / married (vs. single)	1.29 (−13.25, 15.84)	
Separated / divorced / widowed (vs. single)	−2.25 (−21.79, 17.29)	
Education: linear	−1.81 (−13.02, 9.41)	
Education: quadratic	−2.05 (−11.63, 7.54)	
Employment: no (vs. yes)	−4.20 (−18.02, 9.62)	
Mean hearing threshold	0.97 (−6.86, 8.80)	
Tinnitus onset associated with stress: yes (vs. no)	0.02 (−11.26, 11.30)	
Constant tinnitus (vs. intermittent)	5.00 (−6.29, 16.29)	
Tinnitus influenced by stress: yes (vs. no)	2.59 (−12.08, 17.25)	
Hearing aids: yes (vs. no)	−5.46 (−23.62, 12.71)	
Hyperacusis: yes (vs. no)	−1.60 (−16.33, 13.14)	
Number of traumatic experiences	−2.77 (−8.68, 3.14)	−2.59 (−4.96, −0.22)
SOMS baseline	−4.44 (−11.59, 2.72)	
STAI baseline	0.90 (−7.66, 9.46)	
TQ baseline	0.74 (−6.23, 7.72)	
HADS anxiety baseline	5.41 (−3.02, 13.83)	**5.03* (1.95, 8.11)**
HADS depression baseline	8.41 (−0.82, 17.65)	4.46 (0.67, 8.25)
SF-12 physical component summary baseline	−1.70 (−10.20, 6.81)	
SF-12 mental component summary baseline	−7.76 (−17.87, 2.35)	**−7.29** (−10.83, −3.75)**
Hair-cortisol baseline	−0.34 (−5.67, 4.99)	
Hair-BDNF baseline	0.48 (−5.63, 6.58)	
Measurement × sex	0.67 (−4.15, 5.50)	
Measurement × age	1.64 (−1.51, 4.79)	2.19 (0.26, 4.12)
Measurement × cohabiting / married	0.34 (−5.91, 6.59)	
Measurement × separated / divorced / widowed	−0.34 (−8.73, 8.05)	
Measurement × education (linear)	1.93 (−2.88, 6.75)	
Measurement × education (quadratic)	0.86 (−3.26, 4.97)	
Measurement × no employment	1.99 (−3.95, 7.92)	
Measurement × mean hearing threshold	−0.43 (−3.79, 2.93)	
Measurement × tinnitus onset associated with stress	0.91 (−3.93, 5.76)	
Measurement × constant tinnitus	−2.64 (−7.49, 2.21)	
Measurement × tinnitus influenced by stress	−4.17 (−10.47, 2.13)	
Measurement × hearing aids	2.16 (−5.64, 9.96)	
Measurement × hyperacusis	2.69 (−3.64, 9.02)	
Measurement × number of traumatic experiences	−0.03 (−2.57, 2.50)	
Measurement × SOMS baseline	1.46 (−1.62, 4.53)	
Measurement × STAI baseline	0.56 (−3.11, 4.24)	
Measurement × TQ baseline	1.14 (−1.86, 4.13)	
Measurement × HADS anxiety baseline	−1.19 (−4.80, 2.43)	
Measurement × HADS depression baseline	−2.19 (−6.15, 1.78)	
Measurement × SF-12 physical component summary baseline	1.11 (−2.54, 4.76)	
Measurement × SF-12 mental component summary baseline	−0.06 (−4.41, 4.28)	
Measurement × hair-cortisol baseline	−0.34 (−2.63, 1.94)	
Measurement × hair-BDNF baseline	−0.10 (−2.72, 2.52)	
Constant	53.13*** (34.09, 72.18)	52.31*** (47.84, 56.78)
	Random effects variance (SD)	
Subject (random intercept)	62.31 (7.89)	55.26 (7.43)

(Continued)

TABLE 4 | Continued

	Full model	Backward reduced model
	Model fit	
Log-likelihood	−883.36	−961.61
Aikake information criterion	1,866.73	1,943.22
Bayesian information criterion	2,040.76	1,978.03
Marginal R^2	0.52	0.51
Conditional R^2	0.65	0.64

Linear mixed model fit by REML; z-tests were used to test fixed effects estimates; significance levels are displayed after adjustment for multiple testing with Holm's method; significant effects in the reduced model are printed in bold. Observations = 240. ***Model equations:*** *Full model: "PSQ-20 ∼ measurement + sex × measurement + age × measurement + marital status × measurement + education × measurement + employment × measurement + mean hearing threshold × measurement + tinnitus onset associated with stress × measurement + tinnitus type × measurement + tinnitus influenced by stress × measurement + hearing aids × measurement + hyperacusis × measurement + number of traumatic experiences × measurement + SOMS baseline × measurement + STAI baseline × measurement + TQ baseline × measurement + HADS anxiety baseline × measurement + HADS depression baseline × measurement + SF-12 physical component summary baseline × measurement + SF-12 mental component summary baseline × measurement + hair-cortisol baseline × measurement + hair-BDNF baseline × measurement + (1 | subject)". Reduced model: "PSQ-20 ∼ measurement + age + traumatic experiences + HADS anxiety baseline + HADS depression baseline + SF-12 mental component summary baseline + age × measurement + (1 | subject)". BDNF, Brain-Derived Neurotrophic Factor; HADS, Hospital Anxiety and Depression Scale; PDS, Posttraumatic Diagnostic Scale; PSQ-20, Perceived Stress Questionnaire (20 item version); SF-12, Short Form-12 Health Survey; SOMS, Screening of Somatoform Disorders; STAI, State-Trait Anxiety Inventory (State Anxiety); TQ, Tinnitus Questionnaire. *p < 0.05; **p < 0.01; ***p < 0.001.*

structural equation modeling framework was calculated. This model investigates the temporal relationships between TQ scores and hair-BDNF values while accounting for their stability over time and controlling for other identified influencing factors (see **Figure 5**).

Both TQ scores, $\beta = 0.716$, SE $= 0.074$, $p < 0.001$, and hair-BDNF values, $\beta = 0.431$, SE $= 0.119$, $p < 0.001$, were stable over the investigated 3-month period; with higher stability of TQ scores. The two measures showed significantly negative covariance at baseline, $\psi = -175.630$, SE $= 45.969$, $p < 0.001$, but not at follow-up, $\psi = 12.762$, SE $= 15.589$, $p = 0.413$. There was a trend toward statistical significance for the effect of TQ scores at baseline to predict hair-BDNF at follow-up, $\beta = -0.341$, SE $= 0.188$, $p = 0.070$, while the opposite cross-lagged path (of hair-BDNF at baseline to predict TQ scores at follow-up) was non-significant, $\beta = -0.015$, SE $= 0.037$, $p = 0.682$. Approximately 62% of the variance in TQ scores at follow-up ($R^2 = 0.621$), and approximately 36% of the variance in hair-BDNF values at follow-up ($R^2 = 0.355$), was accounted for by the model.

DISCUSSION

In summary, we found that the compact multimodal tinnitus-specific cognitive behavioral therapy effectively reduced tinnitus-related distress and perceived stress levels, in line with our hypothesis (research question 1). However, hair-cortisol and hair-BDNF levels did not reflect these improvements, contrary to our expectations (research question 2). Furthermore, the magnitude of the therapeutic effects was not influenced by the investigated factors (sociodemographic, tinnitus-/hearing-related, psychological, or biological) (research question 3), but some general associations (across all measurements) were identified. Separated, divorced, or widowed patients showed generally higher levels of tinnitus-related distress, which were, in turn, related to lower physical health-related quality of life (QoL). Higher perceived stress levels, on the other hand, were associated with higher anxiety and lower mental health-related QoL. Neither baseline hair-cortisol nor hair-BDNF levels were associated with psychological treatment outcomes, indicating that these biomarkers had no predictive clinical value in the present study. For hair-cortisol, no predictive influences were identified; for hair-BDNF, general associations with tinnitus-related distress, tinnitus loudness, and hearing threshold were found. The exploratory cross-lagged panel analysis (research question 4) tentatively suggests that the possibility of a time-lagged effect of tinnitus-related distress affecting hair-BDNF levels is more likely than the opposite effect. However, this effect was only observed as an uncorrected trend ($p = 0.070$).

A possible explanation for the absence of changes in hair-cortisol and hair-BDNF levels in the present study might be the relatively short treatment duration and follow-up period. The cognitive behavioral therapy-based multimodal treatment, which constitutes the current standard clinical treatment for chronic tinnitus offered at the Tinnitus Center (Charité – Universitätsmedizin Berlin), resulted in measurable reductions in tinnitus-related distress (−13.3%) and perceived stress (−11.5%) three months later. However, these reductions may not have been substantial enough to induce biological changes, or a longer period might have been needed to detect such changes. Regarding cortisol, Li et al. (46) examined the effects of a treatment intervention that combined cognitive behavioral therapy with masking therapy and sound treatment and lasted six months. In addition to a decrease in tinnitus-related distress, they found a decrease in serum cortisol levels in chronic tinnitus patients, suggesting that a longer treatment duration may be necessary to measurably affect the hypothalamic—pituitary—adrenal (HPA) axis function. Moreover, findings on the association between hair-cortisol and measures of perceived stress are inconsistent (18) and previous studies examining the effects of psychological interventions aimed at stress reduction on hair-cortisol levels in different highly stressed study populations made diverging findings. While similar to our results, some found decreases in

TABLE 5 | Full and backward reduced linear mixed-effects models for change in hair-cortisol levels across baseline and follow-up ($N = 80$).

	Full model	Backward reduced model
	Fixed effects estimates (95% confidence intervals)	
Measurement	−0.14 (−0.39, 0.10)	
Sex: male (vs. female)	0.02 (−0.31, 0.34)	
Age	0.13 (−0.06, 0.32)	
Cohabiting / married (vs. single)	−0.04 (−0.23, 0.16)	
Separated / divorced / widowed (vs. single)	−0.05 (−0.31, 0.20)	
Education: linear	−0.11 (−0.41, 0.20)	
Education: quadratic	−0.01 (−0.25, 0.24)	
Employment: no (vs. yes)	0.08 (−0.10, 0.26)	
Mean hearing threshold	−0.16 (−0.41, 0.09)	
Matched tinnitus frequency	0.01 (−0.15, 0.18)	
Matched tinnitus loudness	0.08 (−0.16, 0.32)	
Tinnitus onset associated with stress: yes (vs. no)	−0.16 (−0.46, 0.15)	
Constant tinnitus (vs. intermittent)	0.04 (−0.08, 0.16)	
Tinnitus influenced by stress: yes (vs. no)	0.14 (−0.03, 0.32)	
Hearing aids: yes (vs. no)	0.004 (−0.19, 0.19)	
Hyperacusis: yes (vs. no)	0.09 (−0.06, 0.24)	
Number of traumatic experiences	0.05 (−0.11, 0.21)	
SOMS baseline	−0.02 (−0.20, 0.17)	
STAI baseline	0.21 (−0.02, 0.44)	
TQ baseline	−0.06 (−0.24, 0.13)	
PSQ-20 baseline	−0.13 (−0.41, 0.15)	
HADS anxiety baseline	−0.11 (−0.34, 0.13)	
HADS depression baseline	−0.10 (−0.35, 0.15)	−0.12 (−0.21, −0.02)
SF-12 physical component summary baseline	−0.05 (−0.26, 0.16)	
SF-12 mental component summary baseline	−0.12 (−0.38, 0.14)	−0.11 (−0.20, −0.01)
Sampling: spring (vs. winter)	0.05 (−0.08, 0.18)	
Sampling: summer (vs. winter)	0.17 (0.02, 0.31)	
Sampling: autumn (vs. winter)	0.06 (−0.10, 0.23)	
BMI	0.03 (−0.04, 0.10)	
Alcohol units per week	−0.02 (−0.09, 0.05)	
Regular use of hair products	−0.03 (−0.32, 0.26)	
Smoking: yes (vs. no)	−0.28 (−0.54, −0.01)	
Hair color: blonde / red (vs. gray / white)	0.10 (−0.31, 0.52)	
Hair color: brown / black (vs. gray / white)	−0.09 (−0.50, 0.32)	
Measurement × sex	0.10 (−0.08, 0.28)	
Measurement × age	−0.08 (−0.19, 0.02)	
Measurement × education (linear)	−0.06 (−0.23, 0.11)	
Measurement × education (quadratic)	0.04 (−0.10, 0.17)	
Measurement × mean hearing threshold	0.02 (−0.11, 0.15)	
Measurement × matched tinnitus frequency	−0.01 (−0.10, 0.09)	
Measurement × matched tinnitus loudness	0.03 (−0.10, 0.17)	
Measurement × tinnitus onset associated with stress	0.06 (−0.11, 0.23)	
Measurement × number of traumatic experiences	−0.03 (−0.11, 0.06)	
Measurement × SOMS baseline	−0.03 (−0.13, 0.07)	
Measurement × STAI baseline	−0.10 (−0.22, 0.03)	
Measurement × TQ baseline	0.09 (−0.01, 0.18)	
Measurement × PSQ-20 baseline	0.04 (−0.11, 0.20)	
Measurement × HADS anxiety baseline	0.06 (−0.07, 0.19)	
Measurement × HADS depression baseline	−0.03 (−0.17, 0.11)	
Measurement × SF-12 physical component summary baseline	0.08 (−0.04, 0.19)	

(Continued)

TABLE 5 | Continued

	Full model	Backward reduced model
Measurement × SF-12 mental component summary baseline	−0.02 (−0.17, 0.12)	
Measurement × regular use of hair products	0.03 (−0.13, 0.19)	
Measurement × hair color: blonde / red (vs. gray / white)	−0.06 (−0.29, 0.16)	
Measurement × hair color: brown / black (vs. gray / white)	0.06 (−0.17, 0.28)	
Constant	−1.42*** (−1.89, −0.96)	−1.44*** (−1.50, −1.37)
	Random effects variance (SD)	
Subject (random intercept)	0.06 (0.25)	0.06 (0.24)
	Model fit	
Log-likelihood	−111.82	−36.20
Aikake information criterion	337.64	82.39
Bayesian information criterion	512.92	97.77
Marginal R^2	0.27	0.06
Conditional R^2	0.69	0.59

Linear mixed model fit by REML; z-tests were used to test fixed effects estimates; significance levels are displayed after adjustment for multiple testing with Holm's method. Observations = 160. ***Model equations****: Full model: "Hair-cortisol ~ measurement + sex × measurement + age × measurement + marital status + education × measurement + employment + mean hearing threshold × measurement + matched tinnitus frequency × measurement + matched tinnitus loudness × measurement + tinnitus onset associated with stress × measurement + tinnitus type + tinnitus influenced by stress + hearing aids + hyperacusis + number of traumatic experiences × measurement + SOMS baseline × measurement + STAI baseline × measurement + TQ baseline × measurement + PSQ-20 baseline × measurement + HADS anxiety baseline × measurement + HADS depression baseline × measurement + SF-12 physical component summary baseline × measurement + SF-12 mental component summary baseline × measurement + season of sample collection + BMI + alcohol units per week + regular use of hair products × measurement + smoking + hair color × measurement + (1 | subject)." Reduced model: "Hair-cortisol ~ HADS depression baseline + SF-12 mental component summary baseline + (1 | subject)". BDNF, Brain-Derived Neurotrophic Factor; BMI, Body Mass Index; HADS, Hospital Anxiety and Depression Scale; PDS, Posttraumatic Diagnostic Scale; PSQ-20, Perceived Stress Questionnaire (20 item version); SF-12, Short Form-12 Health Survey; SOMS, Screening of Somatoform Disorders; STAI, State-Trait Anxiety Inventory (State Anxiety); TQ, Tinnitus Questionnaire. ***p < 0.001.*

perceived stress levels that were not accompanied by changes in hair-cortisol levels (47, 48), others observed reductions in hair-cortisol levels following the treatment intervention (49, 50). More research is needed to explore the relationship of hair-cortisol with stress reduction by psychological treatment interventions in different highly stressed groups and to disentangle methodological and treatment-related influences.

Measurement of BDNF in hair is a relatively new method first used in a pilot study by Harb et al. (19). In this study, it was shown that BDNF can be measured in hair samples using a commercially available BDNF assay, that hair-BDNF negatively correlates with hair-cortisol, is associated with hair-biology measures indicative of stress-induced dyshomeostasis, and is a stable measure across independent samples. While immunohistology of human hair follicles confirms BDNF incorporation into hair (51), additional validation studies for the quantification of hair-BDNF are needed. However, the good intra- and inter-assay coefficients of variation observed here indicate a sound methodological approach.

Although we observed no treatment effect for hair-BDNF, general associations of baseline tinnitus-related distress, tinnitus loudness, and hearing threshold with hair-BDNF levels at both measurements were found, extending our cross-sectional findings (baseline measurements) in the same sample (28). Louder tinnitus was related to lower hair-BDNF and higher hearing thresholds to higher hair-BDNF levels at baseline and follow-up. However, the previously observed positive cross-sectional association between hearing aid use and hair-BDNF levels (at baseline) was not observed here, possibly due to the higher number of hair samples included in the present longitudinal analysis. While the negative effect of tinnitus loudness might reflect detrimental distress-related influences on neuroplasticity, the positive effect of mean hearing threshold was surprising. However, the relationship between hearing loss and neuroplasticity is complex. Neuroanatomical studies found that hearing loss in older adults is associated with volume decreases of the primary auditory cortex (52, 53). However, in middle-aged hearing-impaired subjects, volume increases in the auditory association cortex (Brodmann area 22) have been observed (54), as well as volume increases of the angular gyrus (55); both findings are likely indicative of compensatory mechanisms (54, 55). As most of our participants were middle-aged and had mostly no-to-mild hearing impairment, the observed association might potentially be related to compensatory neuroplasticity alterations in certain brain regions and associated increased BDNF levels. However, this explanation is entirely speculative and requires further investigation.

Regarding BDNF measured in blood, evidence shows that serum/plasma BDNF levels increase in response to antidepressant treatment in patients with major depressive disorder (24, 26, 56). The magnitude of the respective change in BDNF levels appears to be positively related to treatment duration (24). Similarly, peripheral BDNF levels were found to increase after several weeks of mindfulness-based interventions (27). Compared with the literature, it seems likely that the treatment duration of 5 days in the present study, even though leading to relevant psychological changes, may have been too short to induce BDNF changes. Moreover, in contrast to our results, Xiong et al. (57) observed a decrease in plasma BDNF

TABLE 6 | Full and backward reduced linear mixed-effects models for change in hair-BDNF levels across baseline and follow-up ($N = 80$).

	Full model	Backward reduced model
	Fixed effects estimates (95% confidence intervals)	
Measurement	2.60 (−19.37, 24.58)	0.18 (−5.57, 5.93)
Sex: male (vs. female)	−11.60 (−40.72, 17.53)	
Age	−0.59 (−17.57, 16.39)	
Cohabiting / married (vs. single)	−3.88 (−21.91, 14.16)	
Separated / divorced / widowed (vs. single)	4.24 (−19.59, 28.07)	
Education: linear	−5.05 (−32.09, 21.98)	
Education: quadratic	−6.17 (−29.04, 16.69)	
Employment: no (vs. yes)	6.89 (−8.63, 22.40)	
Mean hearing threshold	−11.28 (−33.08, 10.53)	**10.79* (3.64, 17.93)**
Matched tinnitus frequency	−8.16 (−23.02, 6.70)	
Matched tinnitus loudness	12.07 (−10.12, 34.26)	**−11.59* (−18.98, −4.19)**
Tinnitus onset associated with stress: yes (vs. no)	4.18 (−22.82, 31.19)	
Constant tinnitus (vs. intermittent)	−7.00 (−17.32, 3.31)	
Tinnitus influenced by stress: yes (vs. no)	−3.15 (−18.55, 12.26)	
Hearing aids: yes (vs. no)	11.74 (−5.05, 28.53)	
Hyperacusis: yes (vs. no)	−5.74 (−19.22, 7.74)	
Number of traumatic experiences	−18.61 (−32.45, −4.77)	−13.13 (−23.00, −3.26)
SOMS baseline	1.38 (−15.57, 18.33)	
STAI baseline	6.22 (−13.86, 26.29)	
TQ baseline	−12.20 (−28.20, 3.80)	**−9.58** (−14.21, −4.96)**
PSQ-20 baseline	9.44 (−15.02, 33.90)	
HADS anxiety baseline	−11.30 (−32.34, 9.74)	
HADS depression baseline	10.71 (−10.99, 32.41)	
SF-12 physical component summary baseline	−4.52 (−23.24, 14.19)	
SF-12 mental component summary baseline	13.84 (−9.17, 36.84)	
Shift work: yes (vs. no)	43.59 (6.29, 80.88)	16.44 (4.35, 28.53)
Sport: linear	9.22 (−8.38, 26.82)	
Sport: quadratic	7.51 (−4.26, 19.29)	
Sport: cubic	3.56 (−5.82, 12.94)	
Hair color: blonde / red (vs. gray / white)	−7.55 (−44.32, 29.23)	
Hair color: brown / black (vs. gray / white)	−5.39 (−42.42, 31.64)	
Frequency of hair washing per week	3.90 (−9.28, 17.09)	
Sampling: spring (vs. winter)	−4.76 (−16.33, 6.80)	
Sampling: summer (vs. winter)	−3.81 (−16.38, 8.76)	
Sampling: autumn (vs. winter)	−9.57 (−24.30, 5.17)	
BMI	−1.40 (−7.27, 4.47)	
Alcohol units per week	4.94 (−1.38, 11.26)	
Physical activity score	−0.73 (−6.61, 5.15)	
Regular use of hair products	12.83 (−12.41, 38.06)	
Smoking: yes (vs. no)	−13.27 (−36.00, 9.46)	
Cups of coffee / tea per day	−0.35 (−7.60, 6.90)	
Measurement × sex	4.48 (−11.84, 20.79)	
Measurement × age	−0.36 (−10.36, 9.63)	
Measurement × education (linear)	0.80 (−14.60, 16.19)	
Measurement × education (quadratic)	5.37 (−7.55, 18.30)	
Measurement × mean hearing threshold	11.18 (−0.60, 22.95)	
Measurement × matched tinnitus frequency	7.12 (−1.71, 15.96)	
Measurement × matched tinnitus loudness	−12.58 (−24.90, −0.27)	
Measurement × tinnitus onset associated with stress	1.23 (−14.11, 16.56)	
Measurement × number of traumatic experiences	10.53 (2.32, 18.73)	6.50 (0.73, 12.26)

(Continued)

TABLE 6 | Continued

	Full model	Backward reduced model
Measurement × SOMS baseline	−2.70 (−11.90, 6.50)	
Measurement × STAI baseline	−1.60 (−13.11, 9.91)	
Measurement × TQ baseline	4.55 (−4.26, 13.35)	
Measurement × PSQ-20 baseline	−6.99 (−21.06, 7.08)	
Measurement × HADS anxiety baseline	5.77 (−6.26, 17.80)	
Measurement × HADS depression baseline	−7.89 (−20.01, 4.22)	
Measurement × SF-12 physical component summary baseline	3.06 (−7.24, 13.35)	
Measurement × SF-12 mental component summary baseline	−8.19 (−21.45, 5.06)	
Measurement × shift work	−20.80 (−41.04, −0.57)	
Measurement × hair color: blonde / red (vs. gray / white)	5.10 (−15.58, 25.77)	
Measurement × hair color: brown / black (vs. gray / white)	1.29 (−19.00, 21.58)	
Measurement × frequency of hair washing per week	−2.76 (−10.09, 4.56)	
Measurement × regular use of hair products	−5.59 (−20.06, 8.88)	
Constant	87.56*** (45.29, 129.84)	75.29*** (65.34, 85.25)
	Random effects variance (SD)	
Subject (random intercept)	364.91 (19.10)	251.76 (15.87)
	Model fit	
Log-likelihood	−548.79	−710.35
Aikake information criterion	1,229.59	1,440.70
Bayesian information criterion	1,432.55	1,471.45
Marginal R^2	0.36	0.29
Conditional R^2	0.69	0.59

Linear mixed model fit by REML; z-tests were used to test fixed effects estimates; significance levels are displayed after adjustment for multiple testing with Holm's method; significant effects in the reduced model are printed in bold. Observations = 160. ***Model equations:*** *Full model: "BDNF ∼ measurement + sex × measurement + age × measurement + marital status + education × measurement + employment + mean hearing threshold × measurement + matched tinnitus frequency × measurement + matched tinnitus loudness × measurement + tinnitus onset associated with stress × measurement + tinnitus type + tinnitus influenced by stress + hearing aids + hyperacusis + number of traumatic experiences × measurement + SOMS baseline × measurement + STAI baseline × measurement + TQ baseline × measurement + PSQ-20 baseline × measurement + HADS anxiety baseline × measurement + HADS depression baseline × measurement + SF-12 physical component summary baseline × measurement + SF-12 mental component summary baseline × measurement + shift work × measurement + sport + hair color × measurement + hair washing frequency × measurement + season of sample collection + BMI + alcohol units per week + regular use of hair products × measurement + smoking + cups of coffee/tea per day + (1 | subject)." Reduced model: "BDNF ∼ measurement + mean hearing threshold + matched tinnitus loudness + number of traumatic experiences + TQ baseline + shift work + number of traumatic experiences × measurement + (1 | subject)". BDNF, Brain-Derived Neurotrophic Factor; BMI, Body Mass Index; HADS, Hospital Anxiety and Depression Scale; PDS, Posttraumatic Diagnostic Scale; PSQ-20, Perceived Stress Questionnaire (20 item version); SF-12, Short Form-12 Health Survey; SOMS, Screening of Somatoform Disorders; STAI, State-Trait Anxiety Inventory (State Anxiety); TQ, Tinnitus Questionnaire. *p < 0.05; **p < 0.01; ***p < 0.001.*

levels in patients with severe tinnitus three months after tinnitus retraining therapy (counseling and sound therapy). However, they found no correlation between plasma BDNF and tinnitus severity or loudness, which is also contrary to our results. Differences between Xiong et al. (57) and the present study include sample characteristics ($N = 14$ with severe tinnitus vs. $N = 80$ with predominantly moderate tinnitus), treatment approach (3-month tinnitus retraining therapy vs. 5-day compact multimodal tinnitus-specific cognitive behavioral therapy), sampling type (blood vs. hair), and methodological differences, all of which may have influenced the conflicting results.

Despite the absence of treatment-induced changes in hair-BDNF levels, our exploratory results tentatively suggest the possibility of a time-lagged effect of tinnitus-related distress (at baseline) affecting hair-BDNF levels (at follow-up). While this trend needs to be tested in larger-scale studies, it may further indicate that more substantial treatment-induced changes in tinnitus-related distress may be necessary to elicit measurable changes in hair-BDNF levels. Overall, further research is needed for a better understanding of the relationship between tinnitus-related distress and hair-BDNF levels.

In addition to treatment duration and follow-up period, other factors might have influenced the observed lack of changes in hair-cortisol and hair-BDNF values. Even though many covariates with potential associations to the investigated biomarkers were included (sociodemographic, psychological, tinnitus-/hearing-related, lifestyle, and hair-related), not all potentially confounding factors could be controlled for, e.g., medical comorbidities and medication. However, none of the participants suffered from endocrine diseases with altered cortisol production or neurodegenerative diseases (Alzheimer's, Parkinson's, or Huntington's disease) with known changes in cortisol and BDNF levels (44, 45). Moreover, confounding influences of antidepressant medication appear unlikely, as biomarker levels did not significantly differ between participants taking antidepressants and those not taking antidepressants (although there was a trend observed for hair-cortisol). However, influences of

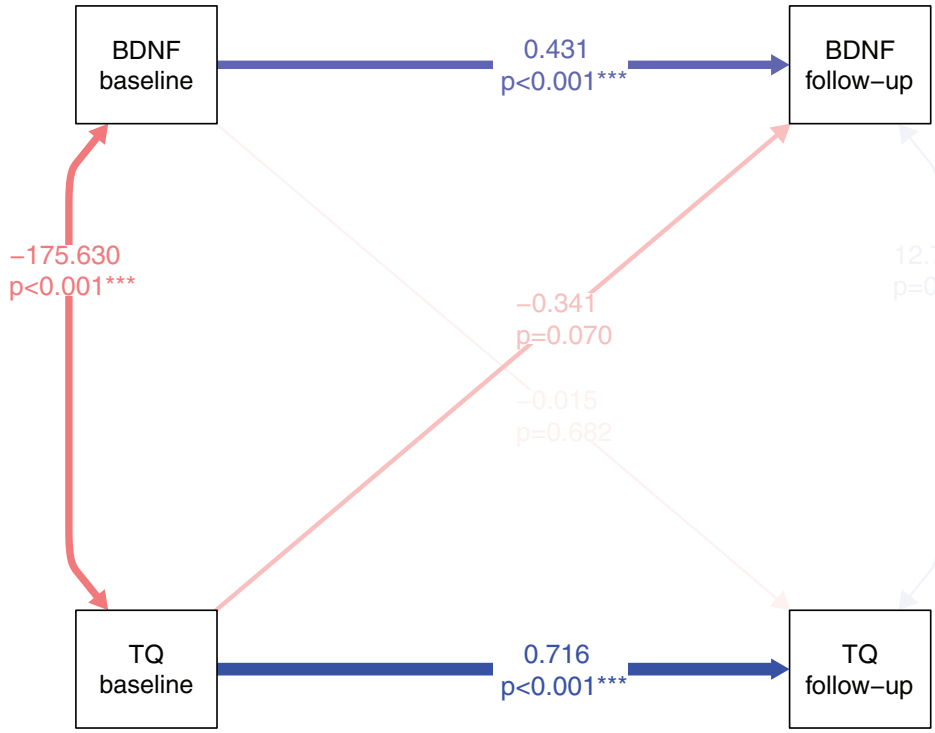

FIGURE 5 | Cross-lagged panel model (structural equation model) for the temporal relations between TQ scores and hair-BDNF values from baseline to follow-up. Blue lines indicate positive and red lines negative associations; line width indicates association strength. Numbers indicate standardized estimates and respective *p*-values. For the prediction of BDNF, mean hearing threshold and tinnitus loudness were included as control variables; for the prediction of TQ, concomitant SF-12 physical component summary scores and the dummy variable "marital status: separated / divorced / widowed" were included as control variables. To simplify the figure, control variables are not depicted. Model fit: $N = 80$, $\chi^2 = 36.807$, df = 12, $p < 0.001$; RMSEA = 0.144. BDNF, Brain-Derived Neurotrophic Factor; TQ, Tinnitus Questionnaire; SF-12, Short Form-12 Health Survey.

other medical comorbidities or medications might have been present.

Musculoskeletal symptoms (muscular imbalance, segmental joint dysfunction, chronic cervical syndrome, craniomandibular/temporomandibular dysfunction, and bruxism) were common in our sample (39–58%). While we did not specifically assess the presence of somatosensory tinnitus; i.e., tinnitus which is influenced by somatosensory afference from the cervical spine or temporomandibular area (58), the relatively high frequency of the reported musculoskeletal symptoms suggests that for a subgroup in our sample, somatosensory influences on tinnitus might have been present. Regarding somatosensory tinnitus, cervical muscle tension, particularly in upper posterior muscle groups, might in some cases have a pathophysiological role in tinnitus – likely in combination with stress (59).

While physical and mental symptoms appear generally interlinked in bothersome tinnitus (60), the interplay between stress, muscle tension, and tinnitus burden appears especially important for tinnitus with somatosensory influences. The multimodal treatment in this study also included physiotherapeutic elements. Therefore, beneficial treatment effects on the described musculoskeletal symptoms might have been present, although we did not investigate them. Consequently, in the subgroup of patients with somatosensory tinnitus, the treatment might have contributed to the improvement of tinnitus-related distress via reducing muscular tension. Overall, further research is needed for a better understanding of stress-related pathophysiological and therapeutic effects in chronic tinnitus.

Limitations

There are several limitations to this study. First, as no control group was included, the observed treatment effects cannot be clearly distinguished from other time effects, e.g.,

natural habituation over time. Moreover, no information was collected regarding more long-term effects after the 3-month follow-up measurement. In addition, the significance level was adjusted for multiple testing for the main analysis; however, the exploratory cross-lagged panel analysis faces potential validity limitations. Aspects of the treatment delivery and study design may have influenced the results and thus limit their generalizability. Insufficient power in our study might be an explanation for the lack of treatment effects in the assessed hair-biomarkers. However, the width of the confidence interval around the null effect of change in hair-BDNF levels was similar to that of the observed significant effects on hair-BDNF levels (reduced model), thus suggesting reasonable accuracy in the estimation. For hair-cortisol, on the other hand, the measurement variable was not selected to be included in the reduced model, and no significant effects were observed, which might indicate greater uncertainty in the estimation. Additional explanations for the lack of treatment effects might be potential confounding influences, e.g., by medical comorbidities or medication. Overall, the non-significant biomarker results need to be interpreted with caution. In addition, some follow-up measurements were performed during the beginning of the COVID-19 pandemic in Germany ($N = 9$ after March 2020), which might have affected the stress level of these participants.

CONCLUSION

Three months after compact multimodal tinnitus-specific cognitive behavioral therapy lasting for 5 days, reductions in tinnitus-related distress and perceived stress were observed. This suggests that the treatment (consisting of cognitive behavioral therapy, education, counseling, otorhinolaryngological and general medical diagnostics, auditory attention training, relaxation, and physiotherapeutic sessions) was successful in reducing tinnitus burden beyond the clinical setting in patients' daily lives. Generally, higher tinnitus-related distress was related to being separated, divorced, or widowed and to lower physical health-related QoL; higher perceived stress was related to higher anxiety levels and lower mental health-related QoL. No change occurred in hair-cortisol and hair-BDNF levels and no predictive influence of baseline biomarker scores on psychometric treatment outcomes was present. For hair-cortisol, no influencing factor could be identified; for hair-BDNF, relationships with hearing threshold, tinnitus loudness, and tinnitus-related distress appear relevant. In addition, the exploratory analysis provided tentative and limited evidence of a time-lagged effect of tinnitus-related distress (at baseline) on hair-BDNF levels (at follow-up). Possible explanations for the lack of treatment effects in hair-biomarkers are the short treatment duration (5 days) and follow-up interval (12 weeks) and potential confounding by medical factors. Further studies are needed to investigate treatment-induced changes in hair-biomarkers in chronic tinnitus, especially hair-BDNF, to obtain a better understanding of stress-related effects in chronic tinnitus.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because no consent of the participants to publish their data was obtained. Requests to access the datasets should be directed to Birgit Mazurek (birgit.mazurek@charite.de).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the local ethic commission of Charité – Universitätsmedizin Berlin (No. EA1/035/16). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LB: project administration, investigation, formal analysis, visualization, and writing—original draft preparation. BB: supervision and writing—reviewing and editing. PN: methodology, supervision, and writing—reviewing and editing. PB: writing—reviewing and editing. BM: conceptualization, funding acquisition, project administration, resources, supervision, and writing—reviewing and editing. EP: conceptualization, resources, project administration, and writing—reviewing and editing.

FUNDING

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Grant Agreement No 764604, and the Heinz und Heide Dürr Stiftung. We acknowledge support from the German Research Foundation (DFG) and the Open Access Publication Fund of Charité – Universitätsmedizin Berlin.

ACKNOWLEDGMENTS

We would like to cordially thank all patients who volunteered to participate in this study. We gratefully acknowledge the laboratory work of Susanne Tumala and Marie Dippel, Psychoneuroimmunology Laboratory Giessen, and thank Raphael Biehl, Tinnitus Center, Charité – Universitätsmedizin Berlin, for his help with data collection. This project forms part of the European consortium TIN-ACT (Tinnitus Assessment Causes Treatments), and we acknowledge the Heinz und Heide Dürr Stiftung for the support of this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.764368/full#supplementary-material

REFERENCES

1. Møller AR. Epidemiology of tinnitus in adults. In: *Textbook of Tinnitus*, eds. Møller, A. R., Langguth, B., De Ridder, D., Kleinjung, T. (New York, NY: Springer). (2011). p. 29–37. doi: 10.1007/978-1-60761-145-5_5
2. Bhatt JM, Lin HW, Bhattacharyya N. Prevalence, severity, exposures, and treatment patterns of tinnitus in the United States. *JAMA Otolaryngol Head Neck Surg.* (2016) 142:959. doi: 10.1001/jamaoto.2016.1700
3. De Ridder D, Schlee W, Vanneste S, Londero A, Weisz N, Kleinjung T, et al. Tinnitus and tinnitus disorder: Theoretical and operational definitions (an international multidisciplinary proposal). In: *Progress in Brain Research* (Elsevier). (2021). p. 1–25. doi: 10.1016/bs.pbr.2020.12.002
4. Biehl R, Boecking B, Brueggemann P, Grosse R, Mazurek B. Personality traits, perceived stress, and tinnitus-related distress in patients with chronic tinnitus: support for a vulnerability-stress model. *Front Psychol.* (2020) 10:3093. doi: 10.3389/fpsyg.2019.03093
5. Fuller T, Cima R, Langguth B, Mazurek B, Vlaeyen JW, Hoare DJ. Cognitive behavioural therapy for tinnitus. *Cochrane Database Syst Rev.* (2020) 2020:CD012614. doi: 10.1002/14651858.CD012614.pub2
6. Martinez-Devesa P, Perera R, Theodoulou M, Waddell A. Cognitive behavioural therapy for tinnitus. *Cochrane Library*. (2010) doi: 10.1002/14651858.CD005233.pub3
7. Hofmann SG, Asnaani A, Vonk IJJ, Sawyer AT, Fang A. The efficacy of cognitive behavioral therapy: a review of meta-analyses. *Cogn Ther Res.* (2012) 36:427–40. doi: 10.1007/s10608-012-9476-1
8. Langguth B. Treatment of tinnitus. *Curr Opin Otolaryngol Head Neck Surg.* (2015) 23:361–8. doi: 10.1097/MOO.0000000000000185
9. Cima RFF, Andersson G, Schmidt CJ, Henry JA. Cognitive-behavioral treatments for tinnitus: a review of the literature. *J Am Acad Audiol.* (2014) 25:29–61. doi: 10.3766/jaaa.25.1.4
10. Cima RFF, Mazurek B, Haider H, Kikidis D, Lapira A, Noreña A, et al. Multidisciplinary European guideline for tinnitus: diagnostics, assessment, and treatment. *HNO.* (2019) 67:10–42. doi: 10.1007/s00106-019-0633-7
11. Brueggemann P, Seydel C, Schaefer C, Szczepek AJ, Amarjargal N, Boecking B, et al. ICD-10 Symptom Rating questionnaire for assessment of psychological comorbidities in patients with chronic tinnitus. *HNO.* (2019) 67:178–83. doi: 10.1007/s00106-019-0618-6
12. Brueggemann P, Otto J, Lorenz N, Schorsch S, Szczepek AJ, Boecking B, et al. Long-term changes in multimodal intensive tinnitus therapy: a 5-year follow-up. *HNO.* (2018) 66:34–8. doi: 10.1007/s00106-017-0463-4
13. Seydel C, Haupt H, Szczepek AJ, Hartmann A, Rose M, Mazurek B. Three years later: report on the state of well-being of patients with chronic tinnitus who underwent modified tinnitus retraining therapy. *Audiol Neurotol.* (2015) 20:26–38. doi: 10.1159/000363728
14. Seydel C, Haupt H, Szczepek AJ, Klapp BF, Mazurek B. Long-term improvement in tinnitus after modified tinnitus retraining therapy enhanced by a variety of psychological approaches. *AUD.* (2010) 15:69–80. doi: 10.1159/000231632
15. McFerran DJ, Stockdale D, Holme R, Large CH, Baguley DM. Why is there no cure for tinnitus? *Front Neurosci.* (2019) 13:802. doi: 10.3389/fnins.2019.00802
16. Szczepek AJ, Mazurek B. Stress-Related Blood Biomarkers. In: *Tinnitus and Stress: An Interdisciplinary Companion for Healthcare Professionals*, eds. Szczepek, A. J., Mazurek, B. (Cham: Springer International Publishing). (2017). p. 95–116. doi: 10.1007/978-3-319-58397-6_6
17. Wosu AC, Valdimarsdóttir U, Shields AE, Williams DR, Williams MA. Correlates of cortisol in human hair: implications for epidemiologic studies on health effects of chronic stress. *Ann Epidemiol.* (2013) 23:797–811.e2. doi: 10.1016/j.annepidem.2013.09.006
18. Stalder T, Steudte-Schmiedgen S, Alexander N, Klucken T, Vater A, Wichmann S, et al. Stress-related and basic determinants of hair cortisol in humans: a meta-analysis. *Psychoneuroendocrinology.* (2017) 77:261–74. doi: 10.1016/j.psyneuen.2016.12.017
19. Harb H, González-de-la-Vara M, Thalheimer L, Klein U, Renz H, Rose M, et al. Assessment of Brain Derived Neurotrophic Factor in hair to study stress responses: a pilot investigation. *Psychoneuroendocrinology.* (2017) 86:134–43. doi: 10.1016/j.psyneuen.2017.09.007
20. Kowiański P, Lietzau G, Czuba E, Waśkow M, Steliga A, Moryś J. BDNF: A key factor with multipotent impact on brain signaling and synaptic plasticity. *Cell Mol Neurobiol.* (2018) 38:579–93. doi: 10.1007/s10571-017-0510-4
21. Bath KG, Schilit A, Lee FS. Stress effects on BDNF expression: effects of age, sex, and form of stress. *Neuroscience.* (2013) 239:149–56. doi: 10.1016/j.neuroscience.2013.01.074
22. Nowacka M, Obuchowicz E. BDNF and VEGF in the pathogenesis of stress-induced affective diseases: an insight from experimental studies. *Pharmacol Rep.* (2013) 65:535–46. doi: 10.1016/S1734-1140(13)71031-4
23. Polyakova M, Stuke K, Schuemberg K, Mueller K, Schoenknecht P, Schroeter ML. BDNF as a biomarker for successful treatment of mood disorders: a systematic & quantitative meta-analysis. *J Affect Disord.* (2015) 174:432–40. doi: 10.1016/j.jad.2014.11.044
24. Brunoni AR, Lopes M, Fregni F. A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *Int J Neuropsychopharmacol.* (2008) 11:1169–80. doi: 10.1017/S1461145708009309
25. Fernandes BS, Molendijk ML, Köhler CA, Soares JC, Leite CMGS, Machado-Vieira R, et al. Peripheral brain-derived neurotrophic factor (BDNF) as a biomarker in bipolar disorder: a meta-analysis of 52 studies. *BMC Med.* (2015) 13:289. doi: 10.1186/s12916-015-0529-7
26. Shi Y, Luan D, Song R, Zhang Z. Value of peripheral neurotrophin levels for the diagnosis of depression and response to treatment: a systematic review and meta-analysis. *Eur Neuropsychopharmacol.* (2020) 41:40–51. doi: 10.1016/j.euroneuro.2020.09.633
27. Gomutbutra P, Yingchankul N, Chattipakorn N, Chattipakorn S, Srisurapanont M. The effect of mindfulness-based intervention on brain-derived neurotrophic factor (BDNF): a systematic review and meta-analysis of controlled trials. *Front Psychol.* (2020) 11:2209. doi: 10.3389/fpsyg.2020.02209
28. Basso L, Boecking B, Neff P, Brueggemann P, Peters EMJ, Mazurek B. Hair-cortisol and hair-BDNF as biomarkers of tinnitus loudness and distress in chronic tinnitus. *Sci Rep.* (2022). doi: 10.1038/s41598-022-04811-0
29. Goebel G, Hiller W. *Tinnitus-Fragebogen (TF): Ein Instrument zur Erfassung von Belastung und Schweregrad bei Tinnitus.* Göttingen: Hogrefe. (1998).
30. Fliege H, Rose M, Arck P, Levenstein S, Klapp BF. Validation of the "Perceived Stress Questionnaire" (PSQ) in a German sample. *Diagnostica.* (2001) 47:142–52. doi: 10.1026//0012-1924.47.3.142
31. Fliege H, Rose M, Arck P, Walter OB, Kocalevent R-D, Weber C, Klapp BF. The perceived stress questionnaire (PSQ) reconsidered: validation and reference values from different clinical and healthy adult samples. *Psychosomatic Medicine.* (2005) 67:78–88. doi: 10.1097/01.psy.0000151491.80178.78
32. Herrmann-Lingen C, Buss U, Snaith RP. *Hospital Anxiety and Depression Scale - Deutsche Version*. Bern: Huber. (2011).
33. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand.* (1983) 67:361–70. doi: 10.1111/j.1600-0447.1983.tb09716.x
34. Rief W, Hiller W, Heuser J. *SOMS—Das Screening für Somatoforme Störungen.* Manual zum Fragebogen Bern: Huber-Verlag. (1997).
35. Laux L, Glanzmann P, Schaffner P, Spielberger CD. *Das State-Trait-Angstinventar (STAI)*. Weinheim: Beltz. (1981).
36. Ehlers A, Steil R, Winter H, Foa E. *Deutsche Übersetzung der Posttraumatic Diagnostic Scale (PDS)*. Oxford University, Warneford Hospital, Department of Psychiatry. (1996).
37. Wirtz MA, Morfeld M, Glaesmer H, Brähler E. Confirmatory analysis of the SF-12 version 2.0 scale structure in a representative German sample. *Diagnostica.* (2018) 64:84–96. doi: 10.1026/0012-1924/a000194
38. Wirtz MA, Morfeld M, Glaesmer H, Brähler E. Standardization of the SF-12 version 2.0 assessing health-related quality of life in a representative German sample. *Diagnostica.* (2018) 1–12. doi: 10.1026/0012-1924/a000205
39. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Available online at: https://www.R-project.org. (2020)
40. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. *J Stat Softw.* (2015) 67:1–48. doi: 10.18637/jss.v067.i01

41. Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models. *Biom J.* (2008) 50:346–63. doi: 10.1002/bimj.200810425
42. Holm S. A simple sequentially rejective multiple test procedure. *Scandinav J Statist.* (1979) 6:65–70.
43. Aickin M, Gensler H. Adjusting for multiple testing when reporting research results: the Bonferroni vs Holm methods. *Am J Public Health.* (1996) 86:726–8. doi: 10.2105/AJPH.86.5.726
44. Zuccato C, Cattaneo E. Brain-derived neurotrophic factor in neurodegenerative diseases. *Nat Rev Neurol.* (2009) 5:311–22. doi: 10.1038/nrneurol.2009.54
45. Newhouse A, Chemali Z. Neuroendocrine disturbances in neurodegenerative disorders: a scoping review. *Psychosomatics.* (2020) 61:105–15. doi: 10.1016/j.psym.2019.11.002
46. Li J, Jin J, Xi S, Zhu Q, Chen Y, Huang M, He C. Clinical efficacy of cognitive behavioral therapy for chronic subjective tinnitus. *Am J Otolaryngol.* (2018) 40:253–6. doi: 10.1016/j.amjoto.2018.10.017
47. Lamothe M, Rondeau É, Duval M, McDuff P, Pastore YD, Sultan S. Changes in hair cortisol and self-reported stress measures following mindfulness-based stress reduction (MBSR): a proof-of-concept study in pediatric hematology-oncology professionals. *Complement Ther Clin Pract.* (2020) 41:101249. doi: 10.1016/j.ctcp.2020.101249
48. Wynne B, McHugh L, Gao W, Keegan D, Byrne K, Rowan C, et al. Acceptance and commitment therapy reduces psychological stress in patients with inflammatory bowel diseases. *Gastroenterology.* (2019) 156:935–45.e1. doi: 10.1053/j.gastro.2018.11.030
49. Iglesias S, Jacobsen D, Gonzalez D, Azzara S, Repetto EM, Jamardo J, et al. Hair cortisol: a new tool for evaluating stress in programs of stress management. *Life Sci.* (2015) 141:188–92. doi: 10.1016/j.lfs.2015.10.006
50. Romero-Gonzalez B, Puertas-Gonzalez JA, Strivens-Vilchez H, Gonzalez-Perez R, Peralta-Ramirez MI. Effects of cognitive-behavioural therapy for stress management on stress and hair cortisol levels in pregnant women: a randomised controlled trial. *J Psychosom Res.* (2020) 135:110162. doi: 10.1016/j.jpsychores.2020.110162
51. Peters EMJ, Hansen MG, Overall RW, Nakamura M, Pertile P, Klapp BF, et al. Control of human hair growth by neurotrophins: brain-derived neurotrophic factor inhibits hair shaft elongation, induces catagen, and stimulates follicular transforming growth factor β2 expression. *J Invest Dermatol.* (2005) 124:675–85. doi: 10.1111/j.0022-202X.2005.23648.x
52. Eckert MA, Cute SL, Vaden KI, Kuchinsky SE, Dubno JR. Auditory cortex signs of age-related hearing loss. *JARO.* (2012) 13:703–13. doi: 10.1007/s10162-012-0332-5
53. Peelle JE, Troiani V, Grossman M, Wingfield A. Hearing loss in older adults affects neural systems supporting speech comprehension. *J Neurosci.* (2011) 31:12638–43. doi: 10.1523/JNEUROSCI.2559-11.2011
54. Boyen K, Langers DRM, de Kleine E, van Dijk P. Gray matter in the brain: differences associated with tinnitus and hearing loss. *Hear Res.* (2013) 295:67–78. doi: 10.1016/j.heares.2012.02.010
55. Alfandari D, Vriend C, Heslenfeld DJ, Versfeld NJ, Kramer SE, Zekveld AA. Brain volume differences associated with hearing impairment in adults. *Trends in Hearing.* (2018) 22:233121651876368. doi: 10.1177/2331216518763689
56. Lee B-H, Kim Y-K. The roles of BDNF in the pathophysiology of major depression and in antidepressant treatment. *Psychiatry Investig.* (2010) 7:231. doi: 10.4306/pi.2010.7.4.231
57. Xiong H, Yang H, Liang M, Ou Y, Huang X, Cai Y, et al. Plasma brain-derived neurotrophic factor levels are increased in patients with tinnitus and correlated with therapeutic effects. *Neurosci Lett.* (2016) 622:15–8. doi: 10.1016/j.neulet.2016.04.032
58. Michiels S, Ganz Sanchez T, Oron Y, Gilles A, Haider HF, Erlandsson S, et al. Diagnostic criteria for somatosensory tinnitus: a delphi process and face-to-face meeting to establish consensus. *Trends in Hearing.* (2018) 22:2331216518796403. doi: 10.1177/2331216518796403
59. Bechter K, Wieland M, Hamann GF. Chronic cervicogenic tinnitus rapidly resolved by intermittent use of cervical collar. *Front Psychiatry.* (2016) 7:43. doi: 10.3389/fpsyt.2016.00043
60. Basso L, Boecking B, Brueggemann P, Pedersen NL, Canlon B, Cederroth CR, et al. Subjective hearing ability, physical and mental comorbidities in individuals with bothersome tinnitus in a Swedish population sample. In: *Progress in Brain Research* (Elsevier). (2021). doi: 10.1016/bs.pbr.2020.10.001

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

This article is reprinted from *Frontiers in Psychiatry*, Volume 13:764368, Basso L., Boecking B., Neff P., Brueggemann P., Mazurek B., Peters E.M.J., Psychological treatment effects unrelated to hair-cortisol and hair-BDNF levels in chronic tinnitus, 2022 (doi:10.3389/fpsyt.2022.764368).

CURRICULUM VITAE

My curriculum vitae will not be published in the electronic version of my thesis for data protection reasons.

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

COMPLETE LIST OF PUBLICATIONS

2019

Aust S, Gärtner M, **Basso L**, Otte C, Wingenfeld K, Chae WR, Heuser-Collier I, Regen F, Cosma NC, van Hall F, Grimm S, Bajbouj M. Anxiety during ketamine infusions is associated with negative treatment responses in major depressive disorder. *European Neuropsychopharmacology*. 2019;29(4):529-538. doi:10.1016/j.euroneuro.2019.02.005

Submitted: June 2018, published: April 2019
Impact Factor 2016: 4.239 / 2017: 4.129 / 2018: 4.468 / 2019: 3.853

2020

Basso L, Bönke L, Aust S, Gärtner M, Heuser-Collier I, Otte C, Wingenfeld K, Bajbouj M, Grimm S. Antidepressant and neurocognitive effects of serial ketamine administration versus ECT in depressed patients. *Journal of Psychiatric Research.* 2020;123:1-8. doi:10.1016/j.jpsychires.2020.01.002

Submitted: September 2019, published: January 2020
Impact Factor 2018: 3.917 / 2019: 3.745 / 2020: 4.791

Basso L, Boecking B, Brueggemann P, Pedersen NL, Canlon B, Cederroth CR, Mazurek B. Gender-specific risk factors and comorbidities of bothersome tinnitus. *Frontiers in Neuroscience*. 2020;14:706. doi:10.3389/fnins.2020.00706

Submitted: April 2020, published: September 2020
Impact Factor 2018: 3.648 / 2019: 3.707 / 2020: 4.677

2021

Basso L, Boecking B, Brueggemann P, Pedersen NL, Canlon B, Cederroth CR, Mazurek B. Subjective hearing ability, physical and mental comorbidities in individuals with bothersome tinnitus in a Swedish population sample. In: Schlee W, Langguth B, Kleinjung T, Vanneste S, De Ridder D, eds. *Progress in Brain Research*. Vol 260. Elsevier; 2021:51-78. doi:10.1016/bs.pbr.2020.10.001.

Submitted: March 2020, published: February 2021
Impact Factor 2018: 2.961 / 2019: 1.746 / 2020: 2.453 / 2021: 2.624

2022

Basso L, Boecking B, Neff P, Brueggemann P, Peters EMJ, Mazurek B. Hair-cortisol and hair-BDNF as biomarkers of tinnitus loudness and distress in chronic tinnitus. *Scientific Reports*. 2022;12(1):1934. doi:10.1038/s41598-022-04811-0

Submitted: September 2021, published: February 2022
Impact Factor 2020: 4.379 / 2021: 4.996

Basso L, Boecking B, Neff P, Brueggemann P, Mazurek B, Peters EMJ. Psychological treatment effects unrelated to hair-cortisol and hair-BDNF levels in chronic tinnitus. *Frontiers in Psychiatry*. 2022;13:764368. doi:10.3389/fpsyt.2022.764368

Submitted: August 2021, published: February 2022
Impact Factor 2020: 4.157 / 2021: 5.435

Basso L, Boecking B, Neff P, Brueggemann P, El Ahmad L, Brasanac J, Rose M, Gold SM, Mazurek B. Negative associations of stress and anxiety levels with cytotoxic and regulatory natural killer cell frequency in chronic tinnitus. *Frontiers in Psychology.* 2022;13:871822. doi:10.3389/fpsyg.2022.871822

Submitted: February 2022, published: June 2022
Impact Factor 2020: 2.990 / 2021: 4.232

Basso L, Boecking B, Neff P, Brueggemann P, Cederroth CR, Rose M, Mazurek B. Sex differences in comorbidity combinations in the Swedish population. *Biomolecules*. 2022;12(7):949. doi:10.3390/biom12070949

Submitted: May 2022, published: July 2022
Impact Factor 2020: 4.879 / 2021: 6.064

DANKSAGUNG / ACKNOWLEDGEMENTS

An erster Stelle möchte ich mich bei meiner Erstbetreuerin Prof. Dr. med. Birgit Mazurek für die hervorragende Betreuung, Förderung und Unterstützung bei der Erstellung meiner Doktorarbeit bedanken. Besonderer Dank geht auch an Dr. phil. DClinPsychol. Dipl.-Psych. Benjamin Böcking für seine Unterstützung und sein großes Engagement. Auch bei Prof. Dr. med. Matthias Rose und Dr. phil. Dipl.-Psych. Petra Brüggemann bedanke ich mich herzlich für ihre unterstützenden Beiträge.

Großer Dank geht auch an die Kooperationspartner, ohne deren wertvolle Arbeit die Umsetzung meiner Forschungsprojekte nicht möglich gewesen wäre. Für die inspirierende Zusammenarbeit bei der Haar-Biomarker-Studie und viele wertvolle Inputs möchte ich mich bei Prof. Dr. med. Eva Peters herzlich bedanken, sowie auch bei Susanne Tumalla und Marie Dippel für den spannenden Einblick in die Laborarbeit. Für die Zusammenarbeit bei der Blut-Biomarker-Studie geht mein Dank an Herrn Prof. Dr. Stefan Gold und sein Team, insbesondere Eva Müller, Petra Moschansky, Linda El-Ahmad und Jelena Brasanac. Für die Mithilfe an diesen beiden Studien möchte ich auch allen beteiligten Mitarbeitern/-innen des Tinnituszentrums vielmals danken.

Außerdem möchte ich mich bei allen (inzwischen teilweise ehemaligen) Mitarbeiter/-innen des Tinnituszentrums bedanken, die mich in besonderer Weise unterstützt haben, insbesondere bei Astrid Bohne, Adrijana Aliu, Sandy Specht, Raphael Biehl, Christina Baniotopoulou, Yasmin Ramminger, Tabea Schiele und Sabine Stark – herzlichen Dank für die großzügige Unterstützung und viele hilfreiche Gespräche.

Besonderer Dank geht zudem an PD Dr. Patrick Neff – Merci für dein Vertrauen in mich, deine hilfreichen Ideen und Einordnungen sowie dein überzeugtes Coachen und Fördern.

Many thanks go to my cooperation partners, without whose valuable work the implementation of my research projects would not have been possible.
For their work on our publications based on the "LifeGene" dataset, I sincerely thank Prof. Christopher Cederroth for his support and valuable inputs as well as Prof. Nancy Pedersen and Prof. Barbara Canlon.

Furthermore, I would like to thank Prof. Pim van Dijk, Dr. Sonja Pyott and Dr. Elouise Koops for bringing TIN-ACT to life, all members of the TIN-ACT consortium for the stimulating scientific exchange, and of course the other TIN-ACT PhD students for the mutual support and many fun memories. A special and heartfelt thanks go to Elza Daoud, Vera Lanaia and Dora Persic for many helpful conversations.

Bei meinen Eltern Erika und Mario Basso bedanke ich mich herzlich für ihre Unterstützung während meiner gesamten Studienzeit und bei meinem Bruder Giuliano für seine hilfreichen Ratschläge.

Zu guter Letzt möchte ich mich von Herzen bei Arnim bedanken – vielen Dank für den Rückhalt, deine Geduld, dein Verständnis und deine Ermutigungen, ohne die ich diese Arbeit nicht hätte zu Ende bringen können.