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Identification of functional biomarkers for tinnitus and tinnitus+hyperacusis in humans

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To my beloved parents

List of abbreviations

A	anterior
ABR	auditory brainstem response
AC	commissure anterior
Amyg	amygdala
AN	auditory nerve
BA	Brodmann area
BB	broadband
BOLD	blood-oxygenation-level dependent
CN	cochlear nucleus
CSF	cerebrospinal fluid
dB	decibel
DL	dorso-lateral
DpIns	dorsal-posterior insula
FA	flip angle
FDR	false discovery rate
fMRI	functional magnetic resonance imaging
FOV	field of view
FWHM	full width half maximum
G-H-S	Goebel Hiller score – tinnitus questionnaire
GLM	general linear model
GM	grey matter
HF	high-frequency
HKI	Hyperacusis-Inventar – hyperacusis questionnaire
Hipp	hippocampus
HL	hearing level
Hz	Hertz
IC	inferior colliculus
IPL	inter-peak latency
L	left
LL	lemniscus lateralis
LF	low-frequency
M	medial
MGB	medial geniculate body
Mam.-Body	mammillary body
ml	milliliter
mm	millimeter
MNI	Montreal Neurological Institute
ms	milliseconds
nHL	normal hearing level
P	posterior
PC	commissure posterior
PCG	postcentral gyrus
PTA	pure tone audiometry
R	right
r-fcMRI	resting state functional connectivity magnetic resonance imaging
ROI	region of interest

SD	standard deviation
SOC	superior olivary complex
SPL	sound pressure level
SPM	statistical parametric mapping
TE	echo time
Tinnitus-CRF	tinnitus case report form
TR	repetition time
WM	white matter

1. Introduction

1.1 Epidemiology and etiology of tinnitus

Tinnitus is known as the sound people perceive without an external stimulation. Tinnitus appears quite common in the population by affecting around 15 – 20 % (Baguley, McFerran et al. 2013, Bauer 2018). Tinnitus can be classified in objective and subjective tinnitus. The mechanisms of objective tinnitus, which is not part of the present study, can be caused by a pulsatile, muscular or spontaneous origin (Lockwood, Salvi et al. 2002, Chan 2009).

Concerned people perceive tinnitus as realistic sound with a huge burden. Tinnitus often affects their communication, has negative influence to their social life and can lead in worst cases to social isolation, anxiety or forms of depressive symptoms (Langguth, Kleinjung et al. 2007, Langguth, Landgrebe et al. 2011).

The tinnitus sound appears in many ways, from pure tone- or thunder-like sounds, to ringing, or scratching in different frequencies (Stouffer and Tyler 1990), it is perceived with about 40 % in both ears or in the middle of the head, 40 % in the left and 20 % in the right ear (Pilgramm, Rychlik et al. 1999).

Subjective tinnitus can be caused by many factors, however, it is barely possible to diagnose the source, since some of the factors can also stay in an interaction (Tyler, Coelho et al. 2008, Wu, Stefanescu et al. 2016, Ralli, Greco et al. 2017). These factors can be classified as hearing problems (like noise-induced hearing loss, sudden hearing loss or blast trauma), neurological disorders (like multiple sclerosis or acoustic neuroma), infectious disease (like inflammation of the middle/inner ear, meningitis or syphilis), drug related factors (salicylates, indomethacin or antidepressants) or others (Lockwood, Salvi et al. 2002, Goebel and Büttner 2004). It is notable, that hearing loss seems to be one of the biggest risk factors (Knipper, Van Dijk et al. 2013). Therefore, most of the literature describes the tinnitus on-set to be associated with a damage of the peripheral hearing system (Axelsson and Ringdahl 1989, Sirimanna, Stephens et al. 1996, Demeester, van Wieringen et al. 2007). An increasing factor during the last decades is also stress, named by people in advance of the tinnitus on-set (Schmitt, Patak et al. 2000, Knipper, Van Dijk et al. 2013, Sedley, Friston et al. 2016, Stefanescu and Shore 2017).

A comorbidity which often occurs with tinnitus is hyperacusis. A sensitivity to moderate or even mild environmental sounds, which leads to a discomfort or even a painful perception. Hyperacusis is distinguished from other noise hypersensitivities like recruitment, which is caused by damaged outer hair cells. Recruitment causes linear rather than non-linear, compressive sound processing and leads to a loss of sensitivity, dynamic range and selectivity of the hearing in the damaged frequency range (Janssen 2000). Thus, small intensity changes due to sound stimuli causes large change in the cochlea-response and make them discomfortable or even painful. Furthermore, hyperacusis is also distinct from phonophobia, where certain sounds lead to a discomfort and rather has a psychological origin. Factors leading to hyperacusis are similar as those for tinnitus, pathological changes of the hearing system (like noise-induced hearing loss or stapedectomy), pathological changes of the central nervous system (like headache or depression), hormonal/infectious diseases (like Lyme disease or Addison's disease) or further factors are classified (Lockwood, Salvi et al. 2002, Goebel and Büttner 2004).
There are different numbers, but up to 50 % of the people with tinnitus have additionally hyperacusis (Hesse, Rienhoff et al. 1999), hyperacusis beside tinnitus also affects people more due to significant higher occurrence of psychological comorbidities like anxiety or depression, than people with a tinnitus percept only (Goebel and Floezinger 2008). Vice versa, about 86 % of people with a hyperacusis also have tinnitus (Anari, Axelsson et al. 1999). Hyperacusis occur as single symptom with ranges from 2 % (Sammeth, Preves et al. 2000) up to 15 % (Fabijanska, Rogowski et al. 1999), according to different study populations, which makes hyperacusis rare as a single symptom group. Hyperacusis alone is therefore not a topic of this study.

Until now, there is no single treatment to eliminate the tinnitus percept permanently (Zenner, Delb et al. 2017), which is also owed by the fact, that the pathophysiological mechanism is poorly understood and controversial debated. It is accepted that tinnitus can occur without hearing loss, defined by clinically measured hearing threshold (Shiomi, Tsuji et al. 1997, Lockwood, Salvi et al. 2002, Saunders 2007, Roberts, Eggermont et al. 2010, Geven, de Kleine et al. 2011, Langers, de Kleine et al. 2012, Tan, Lecluyse et al. 2013, Lanting, de Kleine et al. 2014). This was shown in different tinnitus models in animals, that did not show greater threshold shifts when compared to animals without

tinnitus (Bauer, Brozoski et al. 2007, Eggermont, Zeng et al. 2012, Knipper, Müller et al. 2012, Rüttiger, Singer et al. 2013, Singer, Zuccotti et al. 2013). Therefore, a damage of outer hair cells in the cochlea as cause for tinnitus is unlikely.

Instead, tinnitus seems to be caused by a maladaptive process due to loss of auditory nerve fibers, called synaptopathy (Weisz, Hartmann et al. 2006, Roberts, Eggermont et al. 2010, Kim, Kim et al. 2012, Gilles, Schlee et al. 2016), but how these maladaptive mechanisms work, is controversial discussed in the proposed tinnitus models.

1.2 Current tinnitus models

By now, it is generally accepted that tinnitus is related to enhanced spontaneous firing rates in auditory neurons due to deafferentation (synaptopathy) of auditory nerve fibers, shown in a number of publications during the last decades (Jastreboff 1995, Norena and Eggermont 2003, Weisz, Moratti et al. 2005, Weisz, Hartmann et al. 2006, Eggermont and Roberts 2012, Eggermont 2015, Shore, Roberts et al. 2016). But how this enhanced spontaneous firing rate is affecting the system and causes tinnitus, is discussed controversial in two models. In one model it is assumed that a compensation mechanism in central auditory specific brain regions due to the peripheral deafferentation (synaptopathy) increased neuronal gain and leads to enhanced spontaneous firing in the whole ascending auditory path up to the auditory cortex going along a cortical reorganization (Schaette and McAlpine 2011, Yang, Weiner et al. 2011, Schaette and Kempter 2012, Yang and Bao 2013, Norena 2015, Sedley, Friston et al. 2016).

The other tinnitus model assumes there is a loss of this central compensation mechanism and a loss of gain, showed in a tinnitus animal model (Rüttiger, Singer et al. 2013, Singer, Zuccotti et al. 2013, Möhrle, Hofmeier et al. 2019). Additionally, animals with a tinnitus percept caused due to acoustic trauma, which can be differenciated by a behavioural model into two groups – tinnitus percept only and the cooccurance of hyperacusis in those animals, showed different sound induced central responses. While the tinnitus group showed signs for reduced central auditory gain, animals with additional hyperacusis showed central gain (Möhrle, Hofmeier et al. 2019) especially for higher stimulus sound intensities.

This leads to the hypothesis that the widely observed central auditory gain in tinntus has another origin. This study attempts to examine this in more detail.

1.3 Study hypothesis and aim

Since hearing disorders, as hearing loss and tinnitus increases during the last decades[1], even in younger people due to changes in their lifestyle, it is necessary to understand the pathophysiological mechanism behind tinnitus, especially for any future therapeutic strategy. Therefore, it was mandatory to have a valid study design as basis to differentiate potential findings. To avoid effects influenced due to noise-induced hearing loss, all included participants were therefore recruited to have nearly the same hearing level, consequently we only included participants with a normal hearing to slight hearing loss in higher frequencies.

Since comorbidities, as sound sensitivity disorders like hyperacusis or psychological disorders like anxiety or depression can appear beside tinnitus and could influence data outcomes, we tried to select them. This is why beside different questionnaires, also cortisol as stress associated marker was used, to evaluate and analyze the burden of the disease pattern.

Auditory evoked potentials are electric changes caused by sound, which can be measured alongside the whole auditory system from the cochlea to the auditory cortex and used since decades to understand auditory perception and hearing disorders in human and animal models (Picton, Stapells et al. 1981). We use this principle to analyze neural response to acoustic stimulation in our participant groups with two different methods, on the one side as temporal resolution technique, auditory brainstem response (ABR) measurements which represent neural synchronicity (Johnson and Kiang 1976, Rüttiger, Zimmermann et al. 2017) for supra-threshold fine structure analysis at the brainstem and mid-brain level were used.

On the other side, as a spatial resolution technique, functional magnetic resonance imaging (fMRI) measurement to look for effects caused by sound stimulation in cortical and subcortical areas due to changes in the blood-oxygenation-level dependent (BOLD) signal was used (Logothetis and Pfeuffer 2004, Drew 2019). Beside task-evoked fMRI measurements, we also used resting state functional connectivity measurements by fMRI (r-fcMRI), to analyze spontaneous activity in anatomically separated brain areas, which

1. [1] Word Health Organization (WHO) www. euro.int.de, Word Health Organization (WHO). The global burden of disease. 2004 update. Geneva: 2004 (cited 2020-05-18) http://www. euro.int.de

have a dependency in temporal neural activity (Van Den Heuvel and Pol 2010, Shen 2015, Lv, Wang et al. 2018).

With this study, we aimed to analyze and understand the pathophysiological mechanism behind tinnitus and the often-occurring comorbidity of hyperacusis in a more specific way, to potentially support the monitoring of therapeutically approaches and leads to a better treatment-outcome for tinnitus.

2. Material and Methods

2.1 Ethical proposal

The presented study was approved by the ethics committee of University Clinic Tübingen and Tübingen University (Faculty of Medicine) with the initial approval-number 444/2014BO2 and the title 'Funktionelle MR-Tomographie zur Darstellung der Hirnaktivität und der Hörbahn bei Tinnitus-Patienten und Vergleichspersonen I' followed by two approved extension applications for the follow-up study in 2016-2017 with the title 'Funktionelle MR-Tomographie zur Darstellung der Hirnaktivität und der Hörbahn bei Tinnitus-Patienten und Vergleichspersonen II' under the approval-number 264/2016BO1 as well the follow-up study in 2019 with the title 'Funktionelle MR-Tomographie zur Darstellung der Hirnaktivität und der Hörbahn bei Tinnitus-Patienten und Vergleichspersonen III' (approval-number 391/2018BO2). Besides, the study was registered under DRKS0006332 at the German Clinical Trials Register.

All study participants were informed before examinations about risks, privacy protection and study process. Written consent from every participant was required (**Appendix A**). The participants received a compensation for their effort to participate.

All methods were used according to the 'Declaration of Helsinki' by the World Medical Association for human research ethics. Methods and inclusion/exclusion criteria were predefined.

2.2 Examination procedure

The examinations were split into two days due to the long-lasting measurements. During the first study day, the participants were invited in the afternoon, for the ear examination, pure tone audiometry (**PTA**) measurements, check for the inclusion/exclusion criteria (**Appendix D**) and elucidation about the study and data privacy (**Appendix A**). When included, the participants did the other examinations, containing auditory brainstem response (**ABR**) measurements, questionnaires, tympanometry, speech understanding test, blood (epigenetic analysis – not part of this work) and saliva collection. The whole first visit took about 2-2.5 h.

The second day contained the functional magnetic resonance imaging (**fMRI**) measurements and the handover of the home-taken saliva samples, which was performed in the Department of Diagnostic and Interventional Neuroradiology Tübingen. The

participants had to agree an additional information sheet for the fMRI by the medical-technical radiology assistant before the measurements started. The second visit took about 1.5 – 2h. All the procedures were listed in **Table 1** (Hofmeier, Wolpert et al. 2018). If the participants were excluded, they got a compensation of 10 €. When they are included, they got for the whole participation, including additional audiometry measurements questionnaires and the second examination day for the fMRI measurements, 50 €.

Table 1 Overview examination procedure

examination day 1 (duration 2 - 2.5 h)	examination day 2 (1.5 - 2 h)

• clarification/privacy policy
• check for inclusion/exclusion criteria
• ear examination
• hyperacusis questionnaire
• audiological diagnostic
  ○ pure tone audiometry
  ○ tympanometry
  ○ tinnitus localization (for tinnitus participants only)
  ○ auditory brainstem response measurement
• blood sample collection
• handover saliva samples for home-use
	• handover home-made saliva samples
	• MRI clarification and instructions
	• MRI measurements
	  ○ structural image acquisition
	  ○ task-evoked broadband chirp stimulus
	  ○ task-evoked high frequency chirp stimulus
	  ○ task-evoked low frequency chirp stimulus
	  ○ task-evoked music piece stimulus
	  ○ resting state

2.3 Recruitment of study participants

2.3.1 Advertising and recruitment of the study participants

Participants for the study were recruited by two ways, we used flyers in the Department of Otolaryngology Head & Neck Surgery Tübingen to search for participants with tinnitus (**Appendix B**), additionally, we used the e-mail newsletter feature from the University of Tübingen (**Appendix C**), to address a larger number of people. The recruitment of the participants was consecutively from the start of the study until the number (**Appendix A**) of participants was reached.

2.3.2 Inclusion and exclusion criteria of tinnitus patients with and without co-occurrence of hyperacusis

In the pilot study (Hofmeier, Wolpert et al. 2018), we recruited participants for two groups (control and tinnitus) with an age between 18 and 70, with a normal hearing to slight form of hearing loss according to the clinical definition. A normal hearing is

clinically defined as hearing thresholds not more than 20 dB for frequencies from 0.25 kHz to 4 kHz. The participants should spoke German and had an inconspicuous ear microscopy as well as no further diseases of the hearing system like morbus Meniere, blast trauma, deafness or hearing aid supply. Neurological disorders, pregnancy, contraindications for MRI measurements, intake of medications, drug and alcohol abuse are exclusion criteria. The whole list is in the appendix (**Appendix D**).

To get included in the tinnitus group, the participants should experience their tinnitus permanently for at least 4 weeks, they also should not be treated for their tinnitus during 6 months before the study took place and no decompensated tinnitus should be diagnosed. A decompensated tinnitus is often associated with psychiatric comorbidities like anxiety or forms of depression (Goebel, Biesinger et al. 2005), which were exclusion criteria. Vice versa, the participants for the control group should not experience tinnitus in a permanently way.

For the pilot study (Hofmeier, Wolpert et al. 2018) participants with the co-occurrence of a sensitivity for moderate to loud sounds, called hyperacusis were excluded. Due to the recruitment of a large sub-group of participants with tinnitus and the co-occurrence of hyperacusis, we included them in the follow-up study in 2019.

2.3.3 group classification

The groups were hereinafter referred as the **control group** for participants without tinnitus and/or hyperacusis, **tinnitus group** for participants with tinnitus and without co-occurrence of hyperacusis and the **tinnitus+hyperacusis group** with tinnitus percept with co-occurrence of hyperacusis.

2.4 Audiological diagnostic

2.4.1 Pure tone audiometry

For every participant the hearing thresholds was determined by performing a PTA by an audiologist from the Department of Otolaryngology Head & Neck Surgery Tübingen. An audiometer (AT900, Auritec, Medizindiagnostische Systeme GmbH, Hamburg, Germany) was used, applying pure tone for following frequencies to determine the air conducting hearing thresholds (0.125 kHz, 0.25 kHz, 0.5 kHz, 1 kHz, 2 kHz, 3 kHz, 4 kHz, 6 kHz, 8 kHz, and 10 kHz). This measurement was performed in a soundproof

chamber (Industrial Acoustics Company, Niederkrüchten, Germany), using over-ear headphones (Telephonics TDH 39p, Telephonics, Farmingdale, USA).
Beside the PTA a monosyllabically (noun) speech understanding test ('Freiburger') is used for speech audiometry (Hoth 2016).

2.4.2 Tympanometry

For the middle ear function, tympanometry and acoustic reflex measurements was performed using stimuli of 0.5, 1, 2, and 4 kHz at 80 to 100 dB sound pressure level (**SPL**) on a Madsen-Zodiac 901 (GN Otometrics, Münster, Germany).

2.4.3 Hyperacusis questionnaire

To separate the tinnitus groups for the comorbidity of hyperacusis, we used as hyperacusis questionnaire the so called 'Hyperakusis-Inventar' (**HKI**). This questionnaire combines selective items from the German speaking noise sensitivity questionnaire (Nelting and Finlayson 2004) and the French hyperacusis questionnaire (Khalfa, Dubal et al. 2002), what yields to a high sensitivity (correlation) for complaints caused by hyperacusis and low correlation with tinnitus and hearing threshold loss (Fischer 2013). The test consists of 9 questions with 4 possible answers (**Appendix E**), while the answers are weighted with 0 to 3 points, a total range from 0 to 27 points is possible. For the HKI, due to the provided sensitivity and specificity of the test, a cut-off value of > 11 points define the occurrence of a hyperacusis (Fischer 2013).

2.4.4 Tinnitus questionnaire

To classify the distress by tinnitus, we applied the German version of the clinically used tinnitus questionnaire, called 'Goebel-Hiller-Score' (**G-H-S**) (Goebel and Hiller 1994). The G-H-S consists of 52 questions with 3 possible answers (true, partially true, and not true, with 2, 1, or 0 points for the possible answers) with a total range from 0 to 84 points. For the severity the test is quartered as mild, moderate, severe, and very severe for 0-30, 31-46, 47-59, and 60-84 points respectively. A decompensated tinnitus, which is often associated with psychiatric comorbidities, is assumed for scores above 46 points (bellow 46 points tinnitus is classified as compensated) (Goebel, Biesinger et al. 2005).

Therefore, we decided to exclude participants with extreme high tinnitus questionnaire scores, to made sure possible differences in group comparisons with tinnitus participants were not affected due to those comorbidities. The questions are categorized for the sub-scores emotional distress, cognitive distress, intrusiveness, auditory perceptual difficulties, sleep disturbances, and somatic complaints.

Additionally, the clinic-internal 'tinnitus case report form' (**Tinnitus-CRF**) (**Appendix F**), to track the tinnitus intrusiveness during the last days before the first examination day was used. The Tinnitus-CRF contains a scale with 8 possible answers for the tinnitus intrusiveness during the last days before the first examination from 'no annoyance' to 'extreme'. Also, the laterality of the perceived tinnitus was surveyed with this questionnaire, with possible answers for the 'left ear', 'right ear' or 'in the middle of the head', with 6 possible answers each, from 'not audible' till 'very high' (**Appendix F**).

2.4.5 Tinnitus localization

Participants with a perception of tinnitus performed a tinnitus localization to identify the tone pitch and loudness of their individual tinnitus. Therefore, they had to identify the frequency and loudness which was nearest to their tinnitus tone. The audiologist started a pure tone by asking for a lower or higher frequency of the tinnitus, following an adapting process to fit the nearest frequency. The same was done for the loudness of the tinnitus, where the participant had to told if the sound was lower or higher, after the frequency was fitted. Additionally, a tinnitus masking/suppression, where their tinnitus is overlaid by a pure tone, over the same frequency range as for the PTA (0.125 kHz, 0.25 kHz, 0.5 kHz, 1 kHz, 2 kHz, 3 kHz, 4 kHz, 6 kHz, 8 kHz, and 10 kHz) was performed. The loudness of each pure tone was increased by the audiologist, until the participant could no longer hear the own tinnitus tone. This intensity defined the masking/suppression for this pure tone frequency according the individual tinnitus percept. The tinnitus masking/suppression is generally used to proof the quality of the tinnitus localization (especially the intensity).

2.4.6 Auditory brainstem response measurements

For the ABR measurements, to analyze the sound induced auditory processing on the brainstem-level, we used the commercial clinical system GSI Audera (Grason-Stadler, Eden Prairie, USA) in combination with the headphone model Telephonics TDH 39P

(Telephonics, Farmingdale, USA). This two-channel system was used with four Neuroline 720 electrodes (Ambu, Bad Nauheim, Germany), which were placed according to the International Electrode System 1020 standard. Two placed behind every ear on the mastoid, one as ground electrode between the eye brows and a reference electrode on the middle sagittal line near to the hair-line (**Figure 1**).

For the electrode placement, the skin was cleaned, defatted, prepared with abrasive paste (Nuprep Skin Prep Gel, Weaver and Company, Aurora, USA) to reach a low impedance (<5 kOhm) for the measurement. If this low impedance was too high, the procedure was repeated until an impedance <5 kOhm was reached.

The ABR was recorded by stimulation with a broadband click stimulus at 11.1 Hz with 2000 repetitions during a 10 ms time window. At stimulus intensities, starting at 75 dB normal Hearing Level (**nHL**) with 10 dB decreasing steps down to 25 dB nHL, the signal is bandpass filtered between 150 and 3000 Hz (Hofmeier, Wolpert et al. 2018).

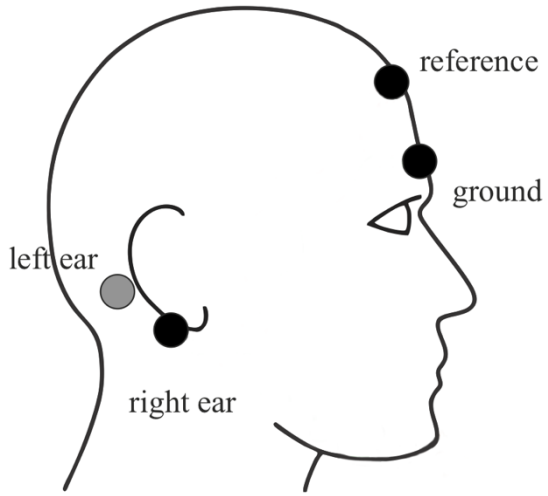

Figure 1 Schematic ABR electrode placement

Electrode placement for the ABR measurement. 4 electrodes were placed, the ground electrode between the eye brows, the reference electrode on the middle sagittal line near to the hair-line, and two electrodes behind every ear on the mastoid (in black for the visible side, grey for the hidden side). Figure modified from the GSI Audera manual (Grason-Stadler, Eden Prairie, USA).

2.4.7 Supra-threshold ABR analysis

ABR curves are counted according their deflection caused by sum action potential in auditory neuronal structures in the brainstem, following acoustic stimulation. ABR wave I is reflected with the distal part of the auditory nerve (**AN**), ABR wave III is represented

by projections of the superior olivary complex (**SOC**) but also from structures of the cochlear nerve (**CN**), ABR wave V reflects projections from the SOC into the inferior colliculus (**IC**) and the lemniscus lateralis (**LL**), and the ABR wave VI is represented by activity in the MGB (**Figure 2**) (Picton, Stapells et al. 1981, Møller, Jannetta et al. 1994, Melcher and Kiang 1996).

The responses in this auditory brainstem structures runs within a time window < 10 ms, therefore the recording window is set to 10 ms, where we define the stimulus start at 0 ms. During this time window we analyze the four waves I, III, V, and VI with a time interval of 1-2 ms, 3-4 ms, 5-6 ms, and 6-8 ms respectively according to data for 70 dB nHL click (11 Hz) ABR measurements (Campbell, Picton et al. 1981). The first positive peak (maxima) in this time interval for the wave defines the latency and is used to calculate the amplitude together with the following negative peak (minima).

Additionally, the interpeak-latencies (**IPL**) for the wave I-III, III-V and I-V are calculated for the analysis (Hofmeier, Wolpert et al. 2018).

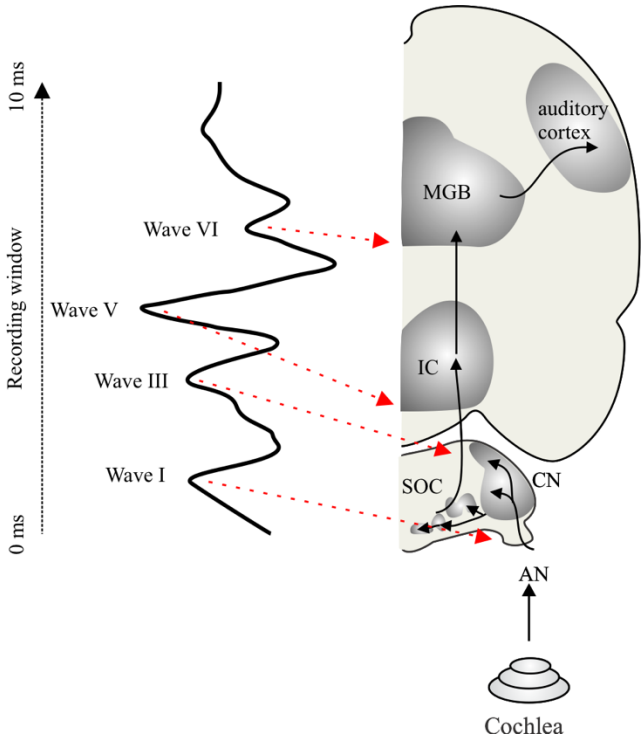

Figure 2 Anatomical representation of the ABR waves

ABR waves and their anatomical correlation. Wave I is equivalent to the CN, wave III is equivalent to the SOC, wave V is equivalent to the IC, and wave VI is equivalent to the MGB. Modified according to (Knipper, Van Dijk et al. 2013).

2.5 Functional magnetic resonance imaging

2.5.1 Stimuli selection and headphone calibration

We designed three so-called chirp sounds for the acoustic stimulation during the fMRI measurements with different frequency ranges according to (Fobel and Dau 2004). While a click stimulus is generally activating the whole basilar membrane in the cochlear, transducing sound waves into electrical signals, there is a small time delay during the stimulation, high frequency regions responds earlier than the low frequency regions (Picton, Stapells et al. 1981). Therefore, chirp sounds are used to maximize the temporal synchronization to enlarge the ABR. We use A-chirps with 20 Hz repetition frequency with a length of 10 ms including a cosines-square-ramp of 1 ms. To proof the influence of different frequency ranges for the chirps according to the audible frequency range of the study participants, the chirps were filtered in three spectra as low-frequency chirp (**LF**-chirp), broadband chirp (**BB**-chirp) and high-frequency chirp (**HF**-chirp) (**Figure 3**). Additionally, beside the chirp stimuli we chosen the clip of a modern pop-rock music piece (Leto 2010) for stimulation during the fMRI scan.

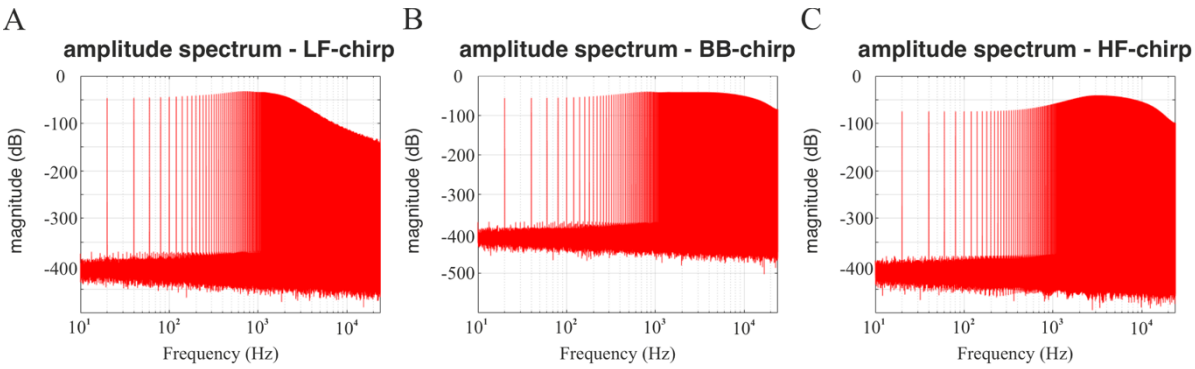

Figure 3 Amplitude spectrum for the chirp stimuli

A LF-chirp in the frequency range for 250 - 3000 Hz **B** BB-chirp in the frequency range for 300 - 25000 Hz **C** HF-chirp in the frequency range for 1200 - 20000. According to (Hofmeier, Wolpert et al. 2018).

To calibrate these sounds for the fMRI scan, an artificial ear (Brüel und Kjaer Type 4157 Nærum, Danmark) together with a measuring amplifier (Bruel & Kjaer measuring amplifier type 2610, Nærum, Dänemark) was used to adjusted the chirps to 85 dB SPL, as well as for the music piece as a maximum level of 85 dB SPL.

2.5.2 fMRI data acquisition

The fMRI measurements were performed on 3-Tesla scanners (Siemens Magnetom 3T and Siemens Prisma-Fit 3T, Siemens, Erlangen, Germany) in the Department Diagnostic and Interventional Neuroradiology Tübingen. To improve the image acquisition a 20-channel head coil was used. For transmitting the acoustic stimulation, we used a common stimulus presentation software (Neurobehavioral Systems Software, version 16.1, Neurobs, Berkeley, USA) in combination with MRI-applicable over-ear headphones (CONFON HP-SC03, MR Confon GmbH, Magdeburg, Germany) and an amplification system (Panasonic-SC-PMX5 Amplifier, Panasonic Marketing Europe GmbH, Hamburg, Germany).

2.5.3 Predefined region of interest analysis

To analyze sound induced BOLD response in cortical areas, predefined regions of interest (**ROI**) in different brain areas (particular auditory specific – **Table 2**, **Figure 4**, **and Figure 5** (Xia, Wang et al. 2013)) according to the so-called Montreal Neurological Institute (**MNI**) Space were defined. This is a 3-dimensional coordinate system based on an average of 152 co-registered T1-weighted structural brain scans (Ashburner and Friston 2005). Whereas we extracted the mean signals from these ROIs and did the group analysis (**2.5.7**).

Table 2 Predefined ROIs for task-evoked and resting state BOLD fMRI measurements
Predefined spherical ROIs with their anatomical term according the Brodmann areas (Kawamura 2017), the MNI coordinates and the radius, which is equivalent to 7 voxels according the image resolution. **A** For task-evoked BOLD fMRI. **B** For resting state BOLD fMRI. According to (Hofmeier, Wolpert et al. 2018)

A: ROIs for task-evoked BOLD fMRI					
Brain Region	**Anatomical term**	**MNI Coordinates (in mm)**			**Radius (mm)**
		X	Y	Z	
Subcortical Regions:					
CN-R/CN-L	cochlear nerve	10/-10	-39	-45	3
SOC-R/SOC-L	superior olivary complex	13/-13	-35	-41	3
IC-R/IC-L	inferior colliculus	6/-6	-33	-11	3
MGB-R/MGB-L	medial geniculate body	18/-18	-24	1	3
Primary Auditory Cortex Regions:					
BA41-R/BA41-L	transverse temporal gyrus (Heschl)	46/-46	-25	7	3
BA41A-R/BA41A-L	transverse temporal gyrus (Heschl)	51/-51	-16	11	3

BA41P-R/BA41P-L	transverse temporal gyrus (Heschl)	41/-41	-28	12	3
BA42-R/BA42-L	superior temporal gyrus	64/-64	-22	9	3
BA42A-R/BA42A-L	superior temporal gyrus	60/-60	-18	10	3
BA42P-R/BA42P-L	superior temporal gyrus	56/-56	-25	12	3
Sound Detection Regions:					
BA22A-R/BA22A-L	middle superior temporal gyrus	54/-54	-6	-6	3
BA21A-R/BA21A-L	middle temporal gyrus	66/-66	-13	-5	3
BA22P-R/BA22P-L	middle superior temporal gyrus	67/-67	-27	3	3
BA21P-R/BA21P-L	middle temporal gyrus	66/-66	-22	-5	3
Hipp-R/Hipp-L	hippocampus	28/-28	-35	18	3
BA13P-R/BA13P-L	insular cortex	35/-35	-21	16	3
Somatosensory/Pain Regions:					
BA1-R/BA1-L	intermediate postcentral gyrus	63/-63	-18	28	3
BA2-R/BA2-L	caudal postcentral gyrus	63/-63	-22	31	3
PCG1-R/PCG1-L	postcentral gyrus	59/-59	-23	25	3
PCG2-R/PCG2-L	postcentral gyrus	58/-58	-14	18	3
DpIns-R/DpIns-L	dorsal insular cortex	41/-41	-21	19	3
Mam.-Body-R/Mam.-Body-L	mammillary body	9/-9	-19	4	3

B: ROIs for resting state BOLD fMRI

Brain Region	**Anatomical term**	**MNI Coordinates (in mm)**			**Radius (mm)**
		X	Y	Z	
CN-R/CN-L	cochlear nerve	10/-10	-39	-45	3
SOC-R/SOC-L	superior olivary complex	13/-13	-35	-41	3
IC-R/IC-L	inferior colliculus	6/-6	-33	-11	3
MGB-R/MGB-L	medial geniculate body	18/-18	-24	1	3
BA41-R/BA41-L	transverse temporal gyrus (Heschl)	46/-46	-25	7	3
BA42-R/BA42-L	superior temporal gyrus	64/-64	-22	9	3
BA22A-R/BA22A-L	middle superior temporal gyrus	54/-54	-6	-6	3
BA21A-R/BA21A-L	middle temporal gyrus	66/-66	-13	-5	3
BA22P-R/BA22P-L	middle superior temporal gyrus	67/-67	-27	3	3
BA21P-R/BA21P-L	middle temporal gyrus	66/-66	-22	-5	3
Hipp-R/Hipp-L	hippocampus	28/-28	-35	18	3
BA13P-R/BA13P-L	insular cortex	35/-35	-21	16	3
BA13A-R/BA13A-L	insular cortex	36/-36	20	7	3
BA28-R/BA28-L	entorhinal area (parahippocampal gyrus)	18/-18	-13	-22	3
Amyg-R/Amyg-L	amygdala	22/-22	-5	-18	3
BA45-R/BA45-L	pars triangularis of inferior frontal gyrus	57/-57	26	14	3
BA46-R/BA46-L	middle precentral gyrus, rostral inferior frontal gyrus	46/-46	41	18	3
BA47-R/BA47-L	orbital inferior frontal gyrus	33/-33	32	-10	3
BA9M-R/BA9M-L	anterior superior frontal gyrus/middle frontal gyrus	7/-7	50	30	3

BA9DL-R/BA9DL-L	anterior superior frontal gyrus	50/-50	14	32	3

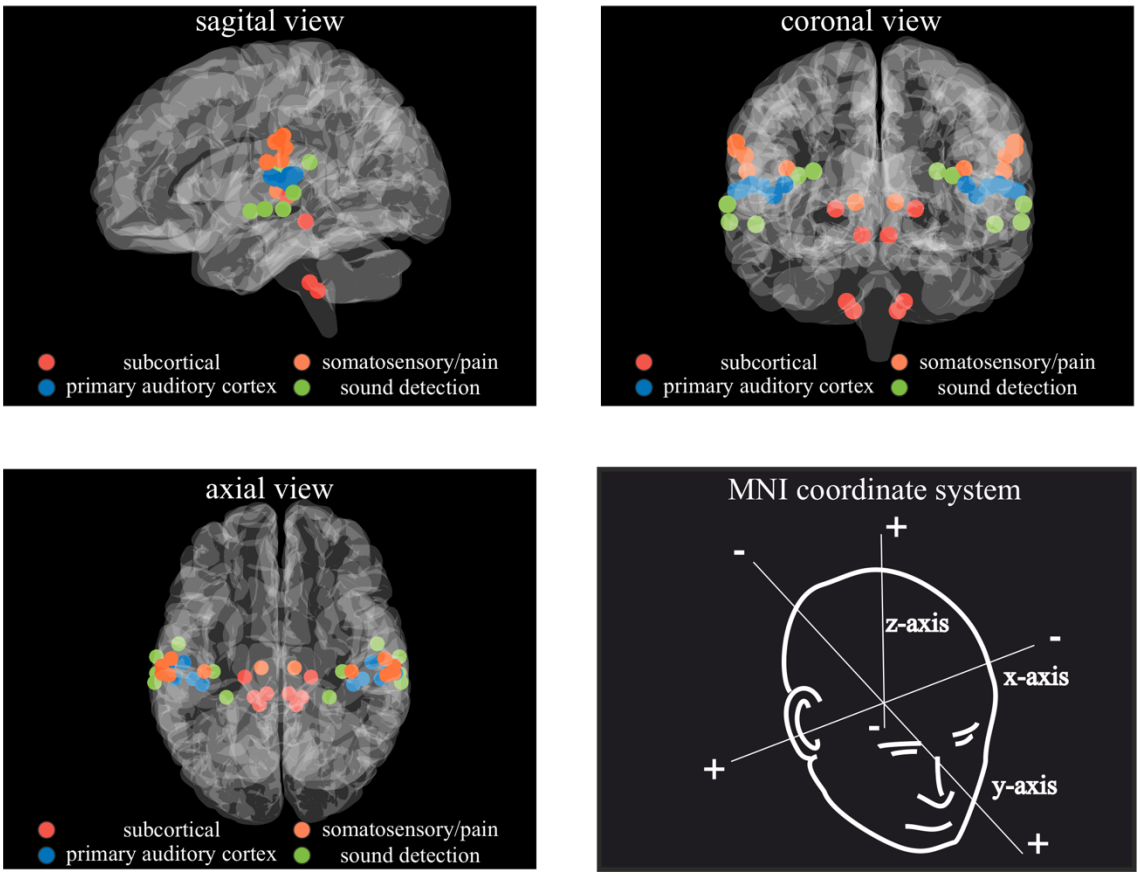

Figure 4 Coordinates in MNI space for task-evoked BOLD fMRI analysis

Predefined ROIs according to their coordinates in the MNI space in a normalized anatomical brain in three views (sagittal, coronal and axial). The ROI positions are clustered in four sections, according their anatomical region.

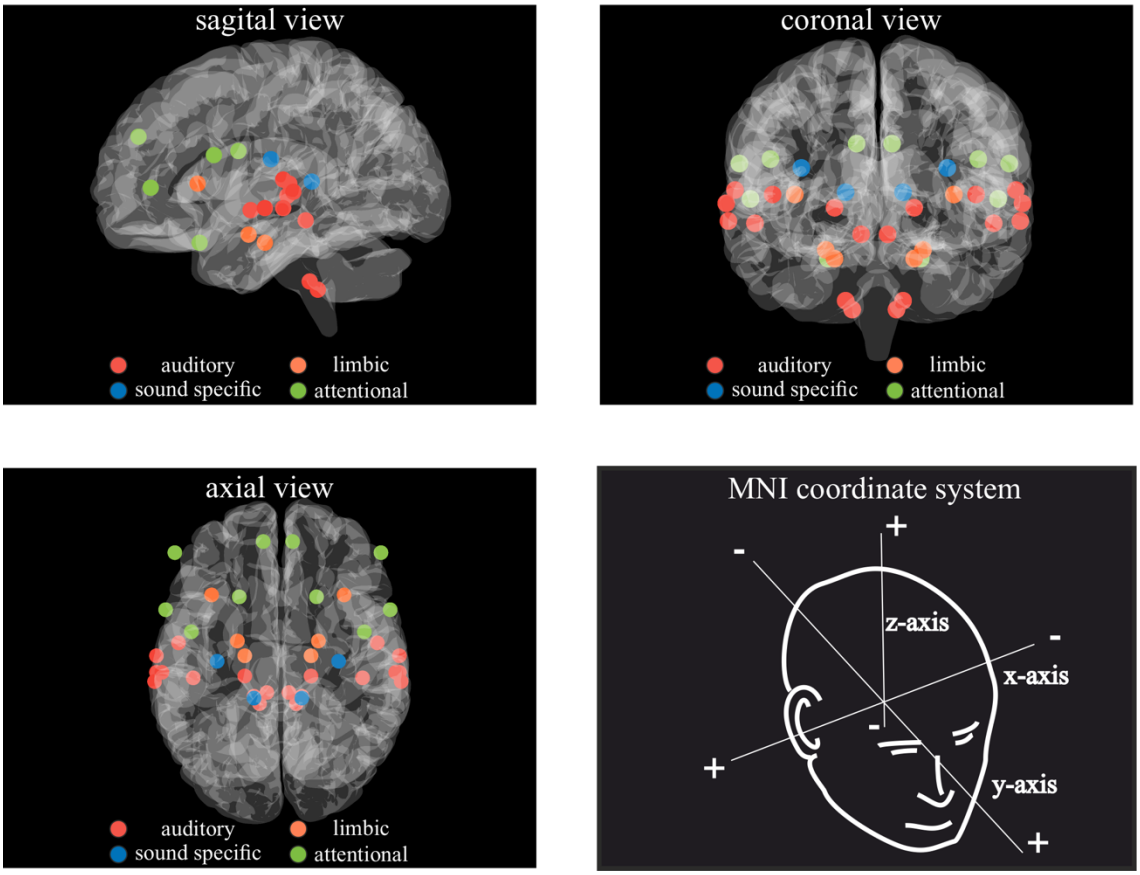

Figure 5 Coordinates in MNI space for resting state BOLD fMRI analysis

The figure shows the predefined ROIs according to their coordinates in the MNI space in a normalized anatomical brain in three views (sagittal, coronal and axial). The ROI position are clustered in four sections, according their main function.

2.5.4 Structural MRI image acquisition

Anatomical images of the participants brain were acquired in a first measurement. These images are necessary to align the functional fMRI images in a preprocessing step to the anatomical origin, since there is generally head movement during the measurements. We used a MPRAGE sequence for T1-weighted three-dimensional structural volumes, these consists of 192 slices for every dimension (coronal, sagittal, and axial view). The measurement parameters are listed in **Table 3**.

Table 3 MRI parameters for structural image acquisition

Parameters for structural image acquisition. According to (Hofmeier, Wolpert et al. 2018)

protocol	MPRAGE
repetition time (TR)	2300 ms
acquisition time (TA)	3.5 min
field of view (FOV)	240 mm
bandwidth	200 Hz/pixel
slice thickness	0.94 mm
inversion time (TI)	900 ms
slice distance	3.7 mm
echo time (TE)	2.1 ms

2.5.5 Task-evoked fMRI measurements - experimental design

For the task-evoked measurements, a T2* weighted echo-planar sequence was used in combination with cardiac gating image acquisition in line with an image-correction step. By the fact that especially the brainstem (beside the whole cortex) is moving quite large due to the heartbeat related blood flow, this step was necessary to detect also small blood-oxygenation-level dependent (**BOLD**) changes in auditory specific brainstem regions. A heart beat triggered image acquisition was used, which generally cause a non-constant repetition time (**TR**) leading to a large signal variance and a possible overlay of the BOLD-effect, which can produce a signal loss. The system was set up to achieve a TR between 2 and 3 s. Here the TR image-correction step takes place, where each image is calculated for a virtual TR of 2 s by an exponential fit, to reduce signal variance for the images (Guimaraes, Melcher et al. 1998).

To apply the cardiac gating, a pulse oximeter was used to detect the heart rate of the participants and synchronize it with the image acquisition. The TR was set between 2 and 3 s, depending on the heart rate. Cardiac gating is performed for all four measurements with acoustic stimulation.

Due to the cardiac gating and the resulting small time window between two heartbeat cycles, we limited the brain scan to 10 coronal slices covering the brainstem and the auditory cortex. Parameters for the task-evoked measurement are shown in **Table 4** (Hofmeier, Wolpert et al. 2018).

Table 4 Parameters task-evoked fMRI image acquisition

Parameters for sound induced BOLD fMRI measurements. According to (Hofmeier, Wolpert et al. 2018)

repetition time (TR)	between 2000 - 3000 ms (cardiac-gated)
flip angle (FA)	90°
field of view (FOV)	290 mm
matrix size	64 x 64
slices number	10 coronal slices
slice thickness	2.5 mm
slice gap	1.25 mm
images	150
slice distance	3.7 mm
echo time (TE)	35 ms

We used an alternating block design with a total of 150 images, containing eight off-blocks and seven on-blocks, 10 images each (**Figure 6**).

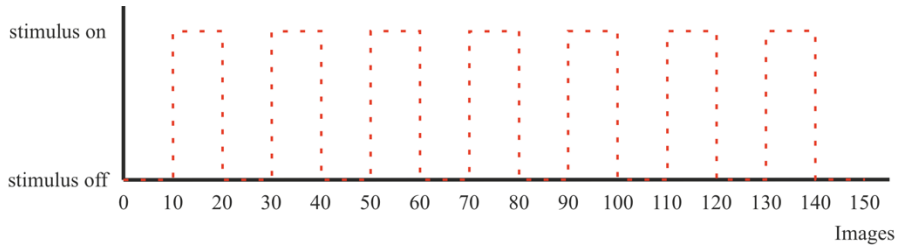

Figure 6 Scheme of image acquisition for fMRI task-evoked measurement

Scheme of block design for the task-evoked fMRI measurement, recording 150 functional images, using alternating blocks (each block containing 10 images) with and without acoustic stimulation (Hofmeier, Wolpert et al. 2018).

2.5.6 Resting State fMRI measurements - experimental design

For the resting state measurement, a whole brain scan including 300 images with a TR of 2 s was done, with a total of 10 minutes recording time. The field of view (**FOV**) block is adjusted to the commissure anterior-commissure posterior (**AC-PC**)-line by checking if the brainstem is covered to catch also the auditory specific brainstem regions for the SOC and CN. The measurement parameters are shown in **Table 5**. The participants were at a wakeful rest without external stimulation and had to close their eyes during the measurement. We did not use cardiac gating during the resting state measurement, which were performed directly after the task-evoked measurements (Hofmeier, Wolpert et al. 2018).

Table 5 Parameters task-evoked fMRI image acquisition

Parameters for resting state BOLD fMRI measurements. According to (Hofmeier, Wolpert et al. 2018)

repetition time (TR)	2000 ms
acquisition time (TA)	10 min
field of view (FOV)	290 mm
matrix size	64 x 64
images	300
slices number	30 coronal slices

2.5.7 fMRI data analysis for task-evoked measurements

All image preprocessing steps were performed in MATLAB (version R2014b and R2019b, MathWorks Inc., Natick, USA) using the Statistic Parametric Mapping (**SPM**) toolbox (version 8 and 12, Wellcome Trust Centre for Neuroimaging, UCL, London, GB). SPM is a toolbox to use statistic parametric mapping, a statistical method for analyzing differences in brain activity (Josephs, Turner et al. 1997). The single preprocessing steps are listed in **Table 6**. The structural images of every participant were aligned to the MNI coordinate system, which has its origin at the commissure anterior (**AC**) and the alignment to the y-axis through the commissure posterior (**PC**). The functional images are then aligned to the structural images to get the same origin and arrangement. After this first step, the functional images of the task-evoked measurement are TR-corrected as described before (**2.5.5**) for a virtual TR of 2 s.

For head motion correction, the functional images were realigned following a co-registration step, for every single measurement during the long-lasting scanning session according to the first recorded images, the structural images. The structural images were segmented into white matter (**WM**), grey matter (**GM**) and cerebrospinal fluid (**CSF**) structure. Afterwards a normalization step was performed to shrink the images to be aligned with the MNI template (equivalent to the MNI space). Additionally, a smoothing step with a Gaussian Kernel of 5 mm full width at half maximum (**FWHM**) was performed for the functional images. The previous segmented WM mask was used for a WM regression step, to avoid nuisance signals. Hereafter we defined the statistical model in SPM, according to the experimental block design, the general linear model (**GLM**) was created using the functional images for every single measurement after the smoothing step for a first, single subject analysis.

The model was estimated in the SPM toolbox and statistical parametric maps were produced. After proofing single subject measurements for stimulus induced BOLD

response, the group analysis was performed. All statistical parametric maps within a stimulus (BB-chirp, HF-chirp, LF-chirp, and Music) of every group participant were used to calculate group differences using a two-sample *t-test* in the SPM toolbox. After that group comparison mean t-scores for the predefined spherical ROIs (**Table 2**) were extracted, using t-scores, which is a technique for statistical analysis of fMRI data, by subtracting pixels from images in on-blocks (with stimulation) against images in off-block (without stimulation) (Clare 1997). We used the ROI-Signal-Extractor feature of the DPABI toolbox (Yan, Wang et al. 2016) to extracted the mean t-scores. Additionally, the p-values were false discovery rate (**FDR**) corrected with an alpha level of $\alpha = 0.05$ (Hofmeier, Wolpert et al. 2018).

Table 6 Preprocessing steps for task-evoked BOLD fMRI measurements

Preprocessing steps after image acquisition, until GLM is created for group comparison. According to (Hofmeier, Wolpert et al. 2018).

Job	Parameter/Setting
set origin/image reorientation	set origin to AC for structural images, y-axis to AC-PC line / reorient functional to structural images
TR-correction	linear fit to virtual TR of 2000 ms for the functional images
realignment	interpolation with 7th degree B-spline for functional images
segmentation	segmentation of the structural images into GM, WM, CSF
coregistration	coregistration of functional images to structural image
normalization	normalization to MNI Space by 7th degree B-spline interpolation for structural/functional images
smoothing	functional images FWHM with 5x5x5 mm
nuisance regression	regression of individual participant segmented WM and CSF mask

2.5.8 fMRI data analysis for resting state measurement

Preprocessing steps were performed in MATLAB and the DPARSF toolbox (Yan, Wang et al. 2016). The toolbox uses a graphical user interface to set all preprocessing parameters and performed the steps in one calculation. We set the parameters for slice timing correction, realignment, head motion corrections (including global mean subtraction), segmentation, co-registration, normalization, smoothing, and filtering for low fluctuation to exclude autonomous blood vessel fluctuation (Drew 2019) as described in **Table 7**. The preprocessing steps were the same as for the task-evoked measurements (**2.5.7**), except for the TR-correction step. After the preprocessing, the DPARSF toolbox extracted a matrix with correlation values between the defined ROI according to the

recorded time series (300 images) of spontaneous BOLD fluctuation for a single participant.

Then, the GraphVar toolbox for MATLAB (Kruschwitz, List et al. 2015) was used to analyze the correlations (functional connectivities) within a group. In the toolbox, we calculated the group Pearson correlation matrix from the single data sets of every participant within the groups and tested it against random networks as a non-parametric test for the correlation strength between the ROIs. We then FDR corrected the results with an alpha level of $\alpha = 0.05$. The network inspector of the GraphVar toolbox displayed the correlations as lines between the ROIs in dependence to plus/minus sign in red for significant positive correlations or blue for significant negative correlations. The strength of the lines defined the value of the correlation coefficient, the thicker the line was, the higher was the correlation coefficient (Hofmeier, Wolpert et al. 2018).

Table 7 Preprocessing steps for resting state BOLD fMRI measurement

Preprocessing steps after image acquisition, until ROIs time-course extraction for functional connectivity analysis. According to (Hofmeier, Wolpert et al. 2018).

Job	Parameter/Setting
set origin/image reorientation	set origin to AC for structural images, y-axis to AC-PC line / reorient functional to structural images
slice timing correction	middle slice as reference
realignment	interpolation with 7th degree B-spline for functional images
segmentation	segmentation of the structural images into GM, WM, CSF
coregistration	coregistration of functional images to structural image
nuisance regression	WM, CSF, Global Mean, head motion scrubbing for functional images
normalization	normalization to MNI Space by 7th degree B-spline interpolation for structural/functional images
smoothing	functional images FWHM with 5x5x5 mm
filtering	0.01 - 0.1 Hz

2.6 Body fluid diagnostic

2.6.1 Collecting body fluids

We collected saliva at three different time points during a day from each participant, at the day before they came for their second examination day. The sample are taken at 8:00 am, 4:00 pm, and 11:00 pm by using a cotton Salivette (Salivette no. 51.1534, Sarstedt, Nümbrecht, Germany). The unused samples were given to participants at the first examination day. An instruction for handling the samples was additionally given to the participants (**Appendix G**). An amount of approximately 1 ml was necessary, therefore

participants had to take the piece of cotton out of the Salivette and chew gently for about 30-60 s on it until they realized it was fully soaked with saliva. Afterwards they had to put it back into the Salivette.

The samples were pseudonymized and sent to an analytic lab (MVZ Labor Dr. Limbach, Heidelberg, Germany) (Hofmeier, Wolpert et al. 2018).

Additionally, a blood sample from every participant was taken for further epigenetic analysis, looking for tinnitus related markers, associated with stress or psychological disorders like anxiety or depression. These sample were stored frozen in a bio-database, the analysis is not part of this work.

2.6.2 Body-fluid analysis

The cortisol values were compared for the three different time points between the groups. Also, the time course within a group was analyzed for circadian changes (Hofmeier, Wolpert et al. 2018).

2.7 Statistical analysis

We tested statistical significance at the alpha-level of 5 %. The significance level indicated by a not significant (n.s.) = $p > 0.05$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. We used SPSS and MATLAB (for FDR correction of the fMRI data with an alpha-level of 5 %) for the statistical tests.

For the hyperacusis questionnaire (Figure 6A, 16A) Mann-Whitney U test was used to test for differences in questionnaire total score between the different groups (Hofmeier, Wolpert et al. 2018).

For the tinnitus questionnaire (Figure 6B, 16B) Mann-Whitney U test was used to test for differences in questionnaire total score between the different groups (Hofmeier, Wolpert et al. 2018).

For the pure tone audiometry (Figure 9, Figure 19) For data in Figure 9, three-way ANOVA with Tukey's multiple comparisons test was used to test for differences in hearing threshold between the different groups (Hofmeier, Wolpert et al. 2018). For data in Figure 19, Mann-Whitney U test was used to test for differences in hearing threshold between the different groups.

For the ABR wave measurement (Figure 10, Figure 20) Shapiro-Wilk test was used to test the normal distribution for amplitude and latency in each wave. For data in Figure 10, three-way ANOVA with Tukey's multiple comparisons test was used to test the wave amplitude and latency for significant differences between the groups (Hofmeier, Wolpert et al. 2018). For data in Figure 20, one-way ANOVA was used to test the wave amplitude and latency for significant differences between the groups.

For ROI analysis in task-evoked fMRI (Table 10, Figure 21, Figure 22, Figure 23) the second level specification independent two-sample *t*-test of the SPM toolbox was used for group analysis. Afterwards FDR correction was applied (Hofmeier, Wolpert et al. 2018).

For functional connectivity in predefined ROIs in resting-state fMRI (Figure 11, Figure 12, Figure 13) Pearson correlation coefficients within a group were calculated, afterwards FDR correction was applied (Hofmeier, Wolpert et al. 2018).

3. Results

In the first part of the results (**3.1** – **3.5**) data from the first study (Hofmeier, Wolpert et al. 2018) including the control group and the tinnitus group is presented. The second part of the results (**3.6** – **3.9**) showed data from the follow-up study in 2019, including beside the control group and the tinnitus group also the group of tinnitus+hyperacusis (Hofmeier, Wolpert et al. 2020, in preparation).

3.1 Recruitment of tinnitus patients without co-occurrence of hyperacusis

3.1.1 Identification of tinnitus and hyperacusis perception

To identify tinnitus-specific characteristics, two hearing matched groups with controls and tinnitus participants are necessary. According to the point that hyperacusis as idiopathic syndrome often occurs as comorbidity with around 60 % beside tinnitus (Hesse, Rienhoff et al. 1999), we excluded co-occurrence of hyperacusis beside tinnitus in the first study (Hofmeier, Wolpert et al. 2018), using the HKI questionnaire.

Study participants with and without tinnitus, who passed the ear examination, PTA and fulfilled the inclusion/exclusion criteria were included and performed the HKI questionnaire to classify a hyperacusis burden (**Appendix D**). Participants with an HKI score larger than 11 points were included in subgroups for the study of tinnitus+hyperacusis.

We include in a first approach 17 participants in the control group and 17 with tinnitus, none of them having a score larger than 11 points from the HKI. With a mean of 5.71 points (SD 2.42) the score for the tinnitus group was larger than the score of the control group (Mean 2.88 points, SD 2.80) but the difference was not statistically significant (**Figure 7A**).

Participants with a tinnitus percept also performed the tinnitus questionnaire to classify their tinnitus burden. Almost all of the tinnitus participants had a mild form of tinnitus with scores in the lowest quarter of the G-H-S classification (Goebel and Hiller 1994, Goebel and Büttner 2004), according to the G-H-S, with a mean of 14.82 points (SD 13.45), only two participants showed a moderate (second quarter of the G-H-S classification) tinnitus score (**Figure 7B**) (Hofmeier, Wolpert et al. 2018).

Summarized, we recruited a tinnitus group matched for hearing sensitivity and HKI score to the control group. With a homogenous tinnitus burden and the exclusion of the comorbidity of hyperacusis, due to a low HKI score.

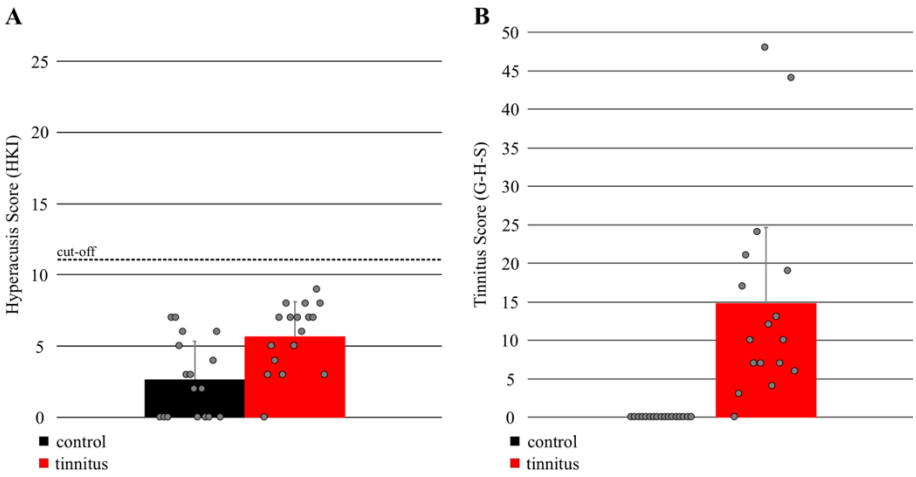

Figure 7 Hyperacusis and tinnitus classification

A Mean hyperacusis score for the control group (black, *n = 17*) and the tinnitus group (red, *n = 17*), included the single values in grey dots within each group bar. A cut-off line at 11 points differentiated between non-hyperacusis and hyperacusis (>11 points). **B** Mean tinnitus score for the control group (black, *n = 17*, due to no tinnitus percept, the group is just illustrated with 0 points) and the tinnitus group (red, *n = 17*), included the single values in grey dots within each group bar. Modified according to (Hofmeier, Wolpert et al. 2018).

3.1.2 Identification of middle and inner ear function

To proof a normal middle and inner ear function we performed tympanometry with stapedius reflex measurement to exclude participants with a disfunction of the middle ear. All participant in both groups showed a normal middle ear function and stapedius reflex. A selection of tympanograms from both groups is shown in **Figure 8** (Hofmeier, Wolpert et al. 2018).

Summarized, as part of the inclusion criteria all participants have a normal middle ear function.

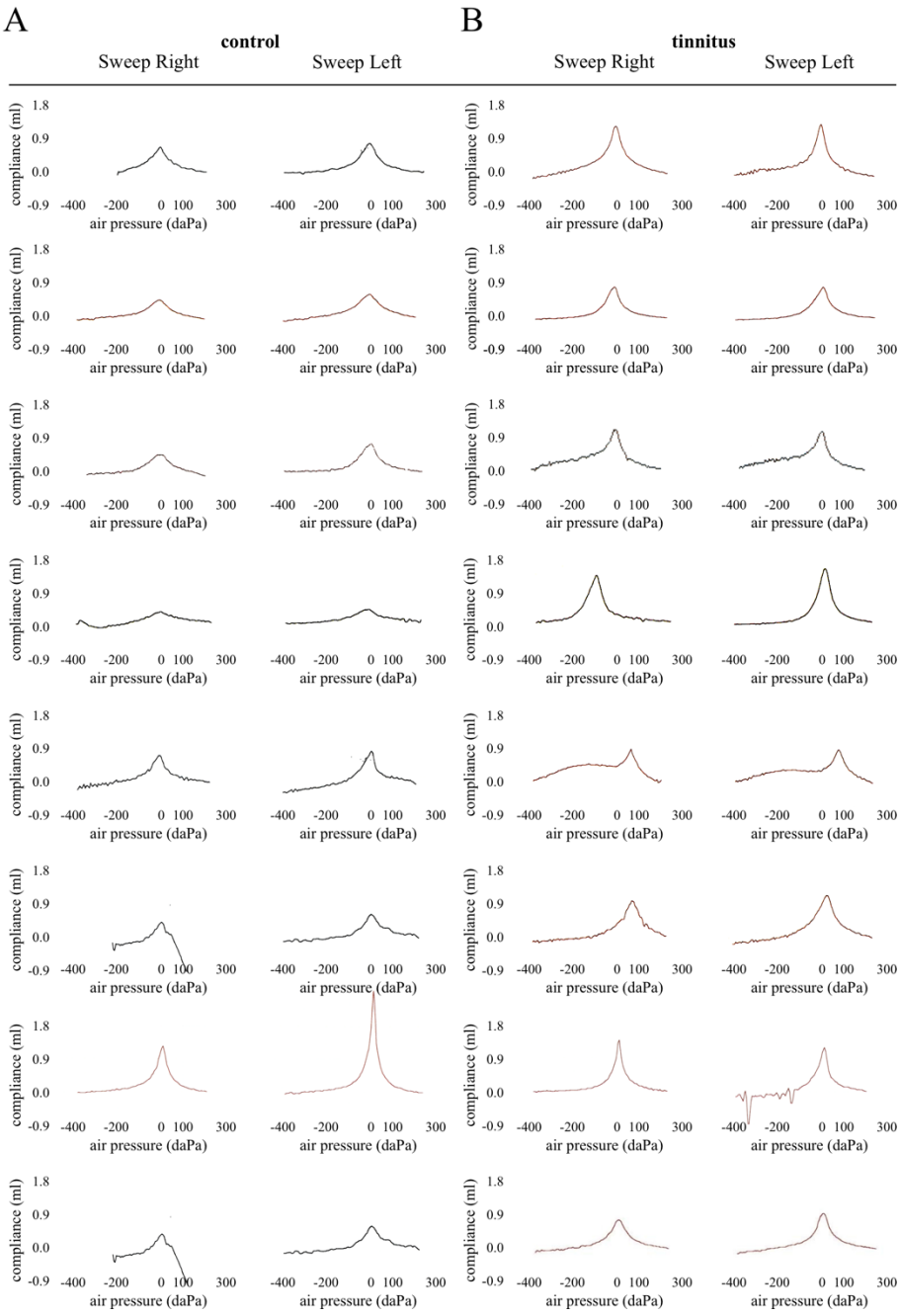

Figure 8 Tympanograms for the control and tinnitus group

Sample of single tympanograms **A** The control group. **B** The tinnitus group. Modified according to (Hofmeier, Wolpert et al. 2018).

3.1.3 Gender, age, handedness within the control and the tinnitus group

Table 8 showed age, sex and handedness. With an average age of 32.5 years (SD 13.3 years, range 18 - 53 years) for the controls and 36.5 years (SD 12.6 years, range 21 - 61 years) for the tinnitus group, the same number of men (*n = 11*) and women (*n = 6*) in each group, and almost the same handedness (controls *n = 15* for right handedness and

tinnitus $n = 13$ for right handedness), no significant differences existed in those datasets (Hofmeier, Wolpert et al. 2018).

Summarized, age, sex and handedness were matched between the groups and may therefore not cofound the study outcome.

Table 8 Non-audiological study participants data for the control and tinnitus group

Non-audiological data for sex and handedness. Modified according to (Hofmeier, Wolpert et al. 2018).

Group				Group			
Control	**Age**	**Sex**	**Handedness**	**Tinnitus**	**Age**	**Sex**	**Handedness**
K001	52	female	right	T001	36	male	left
K003	53	female	right	T002	21	male	right
K004	59	female	right	T003	31	male	right
K006	39	male	right	T006	45	female	right
KN01	21	female	right	T007	61	male	right
KN03	18	male	right	T009	34	male	right
KN05	41	female	left	TN01	26	male	right
KN09	19	male	right	TN02	31	female	left
KN11	24	female	right	TN03	34	male	left
KN15	27	male	right	TN05	33	male	right
KN16	27	male	left	TN07	49	male	right
KN19	53	male	right	TN08	27	male	right
KN20	27	male	right	TN10	25	female	right
KN21	28	male	right	TN11	25	female	right
KN22	26	male	right	TN16	26	male	left
KN23	22	male	right	TN18	61	female	right
KN24	28	male	right	TN26	56	female	right

3.2 Audiological evaluation for control and the tinnitus group

3.2.1 Analysis of hearing threshold differences between the control and tinnitus group

The PTA is measured to detected hearing loss, which is often causally related with acoustic trauma or long-lasting noise exposure. The PTA was used for the group matching to align the hearing of the participants to avoided effects in the ABR and fMRI measurements caused due to differences in the hearing threshold between the groups. We exclude participants (**Appendix C**), according to a hearing loss larger 40 dB at more than one frequency and a non-clinical normal hearing definition. In **Figure 9** we showed a sample of single pure tone audiograms for the control and tinnitus group. The red filled dots in the pure tone audiograms for the tinnitus group (right panels in **Figure 9**) showed

the tinnitus localization for the frequency and the intensity, which is described in the following chapter (**3.2.2**).

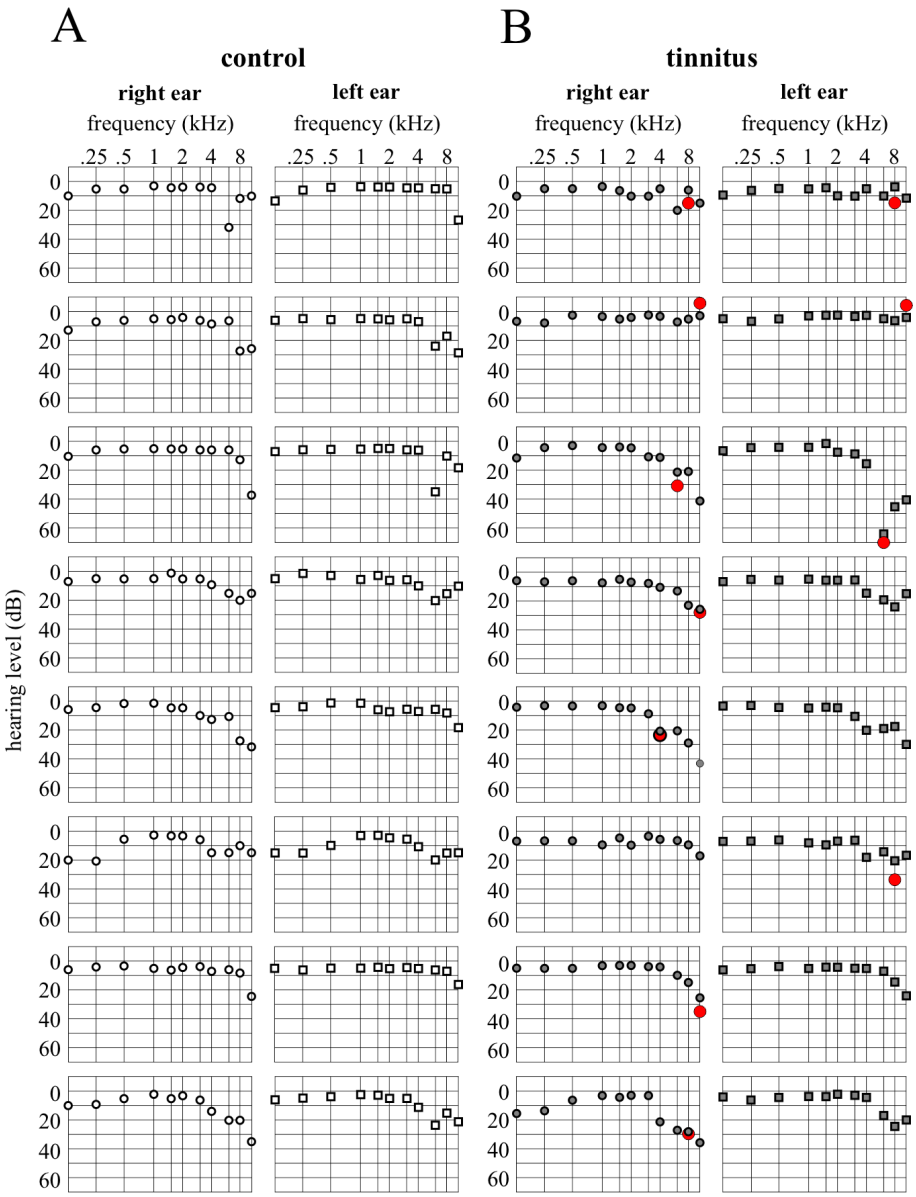

Figure 9 Pure tone audiograms for the control and tinnitus group

Sample of single pure tone audiograms for the right and left ear **A** Control group. **B** Tinnitus group, red dot in the audiogram defines the tinnitus localization for frequency and intensity. Modified according to (Hofmeier, Wolpert et al. 2018).

Both groups showed in higher frequencies (6 kHz and above) a hearing loss, which is in general similar between booth groups and was not significantly different (three-way ANOVA with Tukey's multiple comparisons test, group comparison $p = 0.56$; ear-side $p = 0.84$; frequency*ear-side $p = 0.95$; frequency*group*ear-side $p = 0.99$).

Only for the frequencies within the single groups, a significant difference was found for frequencies at 8 kHz and 10 kHz compared to low frequencies (three-way ANOVA with Tukey's multiple comparisons test, $p = 0.0001$).

Figure 10 showed the average PTA thresholds for the control and tinnitus group, illustrating similarity of hearing thresholds between both groups (Hofmeier, Wolpert et al. 2018).

Summarized, both groups showed nearly the same hearing threshold over all frequencies. Within the group, hearing thresholds were higher for 8 kHz and 10 kHz.

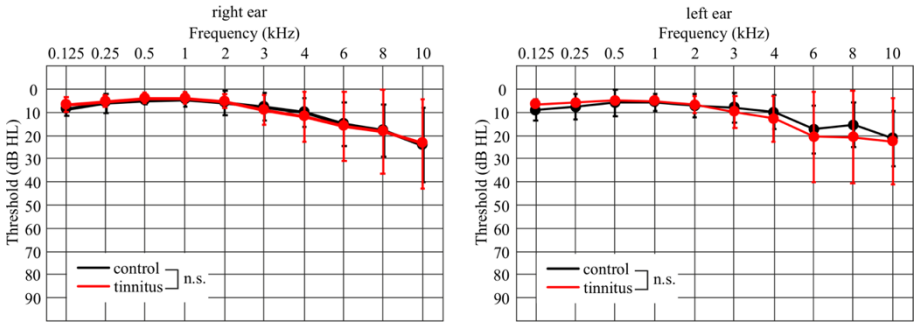

Figure 10 Pure Tone Audiometry measurement for the control group and tinnitus group

Averaged PTA measurements (mean value ± SD) for the control group (black, $n = 17$) and the tinnitus group (red, $n = 17$), separated for the right and left ear. Modified according to (Hofmeier, Wolpert et al. 2018).

3.2.2 Identification of individually perceived tinnitus frequency and loudness

To quantify the individual perceived tinnitus loudness in combination with the frequency of the tone, the so-called tinnitus localization was performed. **Table 9** summarized the results from the G-H-S (named as 'Tinnitus Score' in **Table 9**) and tinnitus localization (named as 'Tinnitus Frequency & Intensity' in **Table 9**) for the tinnitus group, together with the Tinnitus-CRF (named as 'Tinnitus laterality' for the tinnitus percept during the last days before the first examination day in **Table 9**). For the tinnitus localization, the tone is perceived with a mean frequency of 7.33 kHz for the right and 7.62 kHz for the left ear (SD 2.35 kHz and SD 2.14 kHz respectively), with an averaged loudness of 22.04 dB HL for the right and 24.62 dB HL for the left ear (SD 10.17 dB HL and SD 19.88 dB HL respectively). In general, the participants perceive their tinnitus bilateral (65 %, 23 % single-sided for the right and 12 % single-sided for the left ear), with nearly the same frequency for both sides. Whereas the tinnitus intensity differed for some participants with bilateral tinnitus within their ears in a larger range (Hofmeier, Wolpert et al. 2018).

Summarized, the tinnitus was perceived in higher frequencies (7.33 kHz and 7.62 kHz for the right and left ear respectively).

Table 9 Tinnitus specific characteristics for the tinnitus group

Individual tinnitus characteristics with total tinnitus score, tinnitus percepts over the last days (before examination day 1) and tinnitus localization. Modified according to (Hofmeier, Wolpert et al. 2018).

Group					Tinnitus Frequency and Intensity			
Tinnitus	Tinnitus score	Tinnitus laterality			Hz (right)	dB (right)	Hz (left)	dB (left)
T001	0	low	moderate	moderate	8000	15	8000	15
T002	3	low	low	---	10	6	10	4
T003	17	low	low	---	6000	30	6000	70
T006	21	moderate	moderate	---	6000	27	6000	27
T007	10	low	---	---	4000	23	---	---
T009	7	---	moderate	---	---	---	8000	34
TN01	24	moderate	---	---	10	35	---	---
TN02	7	low	---	---	8000	31	---	---
TN03	48	---	low	---	---	---	10	31
TN05	12	very low	inaudible	---	10	28	---	---
TN07	4	---	---	mild	10	47	10	48
TN08	13	inaudible	inaudible	very low	10	13	10	10
TN10	7	inaudible	inaudible	inaudible	4000	12	8000	7
TN11	10	very low	very low	very low	6000	14	8000	7
TN16	19	high	low	moderate	8000	15	6000	3
TN18	44	moderate	high	moderate	4000	41	3000	26
TN26	6	low	moderate	---	6000	19	6000	38

3.2.3 Identification of difference in early and late supra-threshold sound induced ABR

To analyze possible differences between the groups in the early auditory processing alongside the auditory brainstem we performed supra-threshold ABR measurements. The fine structure analysis of the ABR wave form allows a differential view for temporal processing according to the latency and amplitude.

The peaks of the amplitude of the ABR wave form are generated by synchronous firing of neurons from the cochlear nerve and the synchronicity of the summating potentials of the nerves along the auditory pathway (Rüttiger, Zimmermann et al. 2017).

To determine the waves within the recording window, we used a temporal criterion for wave I, III, V, and VI with a time window of 1-2 ms, 3-4 ms, 5-6 ms, and 6-8 ms respectively (Campbell, Picton et al. 1981).

For the ABR wave amplitude at a stimulus intensity of 75 dB nHL we analyzed for the wave V a significant reduction in amplitude for the tinnitus group, compared to the control group in both ears (three-way ANOVA with Tukey's multiple comparison test, for the right ear $p = 0.046$, for the left ear $p = 0.02$). But also, a prolonged wave V latency for 75 dB nHL in the tinnitus group in both ears (three-way ANOVA with Tukey's multiple comparison test, for the right ear $p = 0.034$, for the left ear $p = 0.016$) (**Figure 11**).

For the other waves (I, III, and VI) we observed no differences in latencies and amplitudes at 75 and 65 dB nHL.

We measured in 10 dB steps from 75 dB nHL down to 25 dB nHL. Due to the awake state of the participants during an ABR measurement, it is difficult to determine other waves than wave V near the hearing threshold (Lehnhardt 2009). Therefore, from 55 dB nHL to 25 dB nHL we only analyzed wave V. Here we observed significant prolonged wave V latency for the right ear at 25 and 35 dB nHL (three-way ANOVA with Tukey's multiple comparison test, for 25 dB nHL $p = 0.013$, for 35 dB nHL $p = 0.02$) and for the left ear at 35 dB (three-way ANOVA with Tukey's multiple comparison test, $p = 0.02$) (**Figure 11**). In general, for the other intensities, there was a prolonged latency, but this was not significant (Hofmeier, Wolpert et al. 2018).

Summarized, the results showed in the tinnitus group reduced and delayed responses to sound in late (central) brainstem regions (ABR wave V) compared to the control group. Also, for lower sound intensities there was a delayed late ABR response (wave V) – especially significant at 25 and 35 dB nHL for the right and at 35 dB nHL for the left ear.

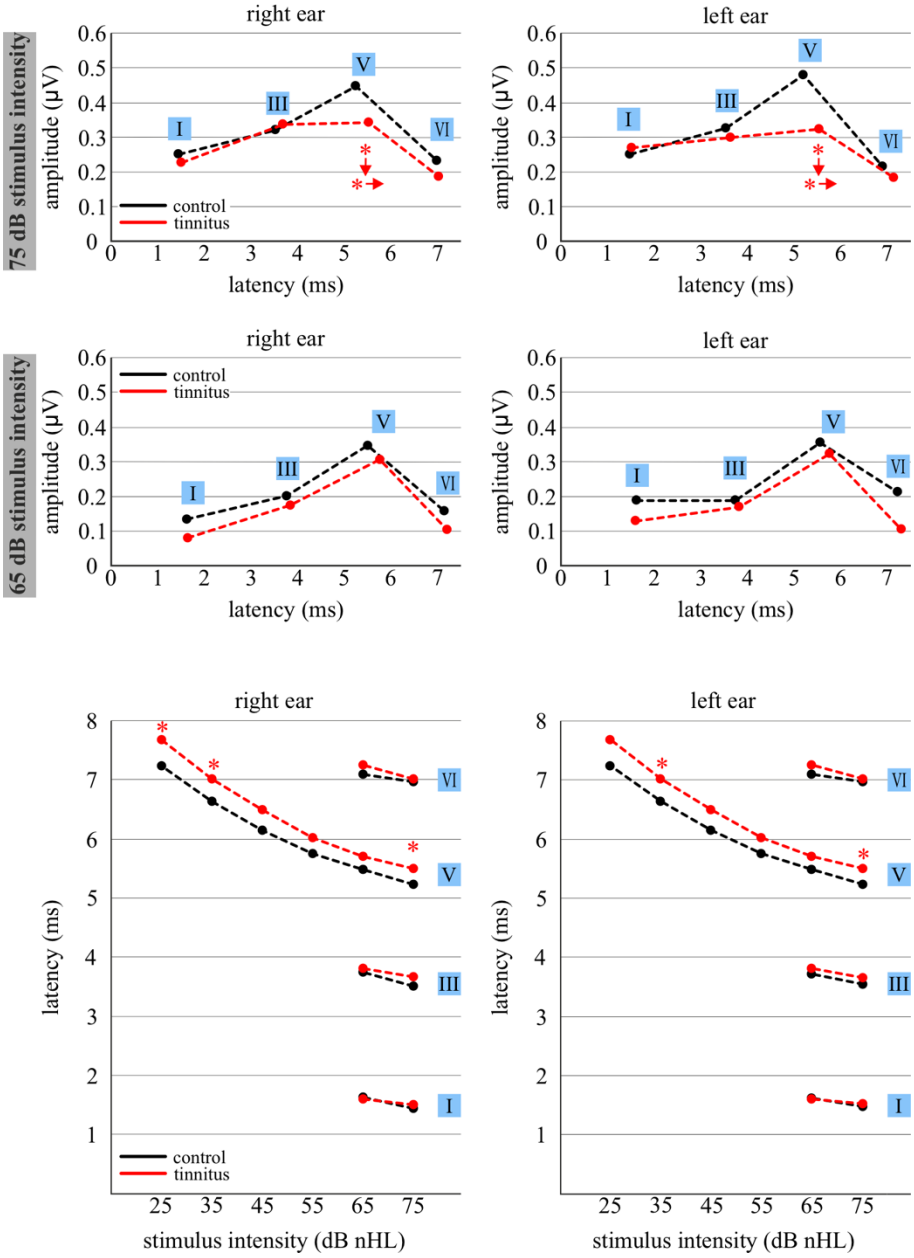

Figure 11 Supra-threshold Auditory Brainstem Response analysis for the control and tinnitus group

First row Averaged ABR wave I, III, V & VI as amplitude-latency plot at 75 dB nHL for the control group (black, *n = 17*) and the tinnitus group (red, *n = 17*) for right ear (left panel) and left ear (right panel). Amplitudes of the waves are connected via a doted-line. **Second raw** Averaged ABR wave I, III, V & VI latency-supra-threshold amplitude plot at 65 dB nHL, equivalent to first row. **Third row** Intensity-latency function curve for averaged ABR wave I, III, V, and VI latencies (at 75 and 65 dB nHL; Wave V also at 55, 45, 35 and 25 dB nHL) equivalent to first row. Modified according to (Hofmeier, Wolpert et al. 2018).

3.3 Analysis of task-evoked and resting state BOLD response

3.3.1 Identification of differences in sound induced BOLD response between the control and tinnitus group for different auditory stimuli

Beside the reduced late ABR response in the tinnitus group, we were curious if the response to sound in the fMRI measurements reflect these results. Therefore, we measured both groups with different acoustic stimuli in a 3-T MRI Scanner according to the described block design, using cardiac gating with a TR-correction procedure.

For the acoustic stimulation with the BB-chirp, we observed in the tinnitus group less activity compared to the control group for sound induced BOLD response in the left primary auditory cortex (BA41) the left and right Wernicke area (BA21, BA22) and in the left auditory thalamus (MGB), shown in **Table 10A**. Interestingly, lower BOLD response were also observed in regions of the left and right hippocampus and the left posterior insula (BA13P).

On the other side, we only observed enhanced activity for the BB-chirp stimuli in the tinnitus group, compared to the control group in one region, the right anterior insula (BA13A) (**Table 10B**).

For the HF-chirp measurement we also analyzed lower BOLD response in the tinnitus group, compared to the control group for the right primary and secondary auditory cortex (BA41, BA42) and also in the posterior insula (BA13P) and left and right hippocampus, shown in **Table 10A**. We did not find enhanced BOLD response in the predefined analyzed ROIs for the HF-chirp in the tinnitus group, compared to the control group.

The LF-chirp measurement showed less differences in lower BOLD response for the tinnitus group compared to the control group. But also, parts of the right primary auditory cortex (BA41) and the right posterior insula (BA13P) appeared to respond with less activity as we saw it for the BB- and HF-chirp (**Table 10A**). For enhanced activity in the tinnitus group compared to the control group, the left anterior insula (BA13A) appeared similar as it was for the BB-chirp. Interestingly, regions in the somatosensory cortex (BA1, BA2) responded to sound with elevated BOLD activity (**Table 10B**).

For the acoustic stimulation with music, we found lower activity in the tinnitus group in primary and secondary auditory cortex regions (BA41-L, BA42-R) and also for the Wernicke area (BA21-L, BA22-R) (**Table 10A**).

The tinnitus group showed enhanced activity for the music stimuli compared to the control group in the left anterior insula (BA13A), similar to what was found for the BB- and LF-chirp. In regions of the limbic system, as the right mamillary body (Mam.-Body), higher BOLD response to sound was observed (**Table 10B**) (Hofmeier, Wolpert et al. 2018).

Summarized, the tinnitus group showed generally lower BOLD responses to sound, compared to the control group in primary and secondary auditory cortex regions (BA41, BA42), in the multimodal regions in the lateral temporal lobe for complex sound and language processing (BA 21, BA22) (Mirz, Ovesen et al. 1999, Bernal, Altman et al. 2004) and in regions which are responsible for shaping auditory skills as the hippocampus or the posterior insula (BA13P) (Sadaghiani, Hesselmann et al. 2009, Kraus and White-Schwoch 2015, Weinberger 2015).

Enhanced activity as response to sound stimuli in the tinnitus group in comparison to the control group appeared in regions for distress, like the anterior insula (BA13A) and the mamillary body (Mam.-Body) as part of the limbic system (Wang, Rao et al. 2005, Vanneste, Plazier et al. 2010, Carpenter-Thompson, Akrofi et al. 2014, Bubb, Kinnavane et al. 2017).

Table 10 Results for task-evoked fMRI measurement

Significant *($p < 0.05$*, FDR corrected) activity differences (mean t-score value) in predefined ROIs (Table 2) for task-evoked fMRI in the tinnitus group (*$n = 17$*) compared to the control group (*$n = 17$*) for different types of stimuli (BB-chirp, HF-chirp, LF-chirp, and music piece). **A** For lower BOLD response in the tinnitus group (compared to the control group) **B** For higher BOLD response in the tinnitus group (compared to the control group). Modified according to (Hofmeier, Wolpert et al. 2018).

Stimuli	Brain Region	Lower BOLD response (as Δ t-score) in the tinnitus group (compared to the control group)
BB-chirp	BA41-L	-1.57
	BA21-L	-2.33
	BA22-L	-1.48
	BA21-R	-2.05
	BA22-R	-2.19
	MGB-L	-1.4
	Hippocampus-L	-1.51
	BA13P-L	-2.03
	Hippocampus-R	-1.8
HF-chirp	BA41-R	-1.76
	BA42-R	-2.33
	BA13P-R	-1.31

	Hippocampus-R	-1.78
	Hippocampus-L	-1.52
LF-chirp	BA41-R	-1.6
	BA13P-R	-1.42
Music piece	BA41-L	-1.4
	BA21-L	-2.37
	BA42-R	-1.99
	BA22-R	-2.24

Stimuli	**Brain Region**	**Higher BOLD response (as Δ t-score) in the tinnitus group (compared to the control group)**
BB-chirp	BA13A-R	2.04
LF-chirp	BA1-L	1.74
	BA2-L	1.96
	BA13A-L	2.08
Music piece	BA13A-L	2.44
	Mam.-Body-R	2.2

3.3.2 Functional connectivity analysis for resting state BOLD fluctuation between the control and tinnitus group

Previous studies, discovered relations between task-evoked BOLD activity and synchronous resting state activity (Haag, Heba et al. 2015). Therefore, we looked into resting state functional connectivity (**r-fcMRI**) measurements in our participant groups and screened for relations to our task-evoked fMRI measurement results. We analyzed whole brain resting state measurements, by looking for correlations between BOLD fluctuations for predefined auditory specific ROIs, stress and attentional associated ROIs in the prefrontal cortex and also ROIs in the limbic system.

We screened for correlations (functional connectivity) within ROIs in the auditory pathway (CN, SOC, IC, MGB, BA41, BA42, BA21, and BA22) and regions, not directly associated within the auditory cortex, which contains the posterior insula (BA13P) and the hippocampus as regions for shaping auditory skills (Sadaghiani, Hesselmann et al. 2009, Kraus and White-Schwoch 2015, Weinberger 2015) – named as auditory processing network (**Figure 12**).

Then for ROIs in the auditory pathway and regions in limbic system, containing ROIs in the amygdala, the entorhinal cortex (BA28), and the anterior insula (BA13A) as regions

associated with stress and distress (Husain 2016, Leaver, Turesky et al. 2016, Chen, Xia et al. 2017) – named as emotional distress network (**Figure 13**).

As last network – named as attentional network, for ROIs in the auditory pathway to regions the fronto-parietal and temporofrontal network (BA45, BA46, BA47, BA9), which were associated for attention (Cieslik, Mueller et al. 2015).

We analyzed the groups separately. We observed in the control group for the ROIs in the auditory pathway (CN, SOC, IC, MGB, BA41, BA42, BA21, and BA22) positive correlations between homologous right and left hemispheric regions (**Figure 12** left upper panel), while we did not see those correlations in the tinnitus group (**Figure 12** right upper panel). Also, positive correlations between lower auditory specific brainstem regions and the regions of the auditory processing network, the posterior insula (BA13P) and the hippocampus for the control group (**Figure 12** left upper panel), did not appear in the tinnitus group (**Figure 12** right upper panel).

We observed in the tinnitus group more negative correlations between regions in the auditory pathway, especially between lower auditory specific regions in the brainstem (CN, SOC, and IC) and the auditory thalamus (MGB) (**Figure 12** right lower panel), but also from the superior olivary complex (SOC) to the posterior insula (BA13P) and the hippocampus, negative correlation appeared.

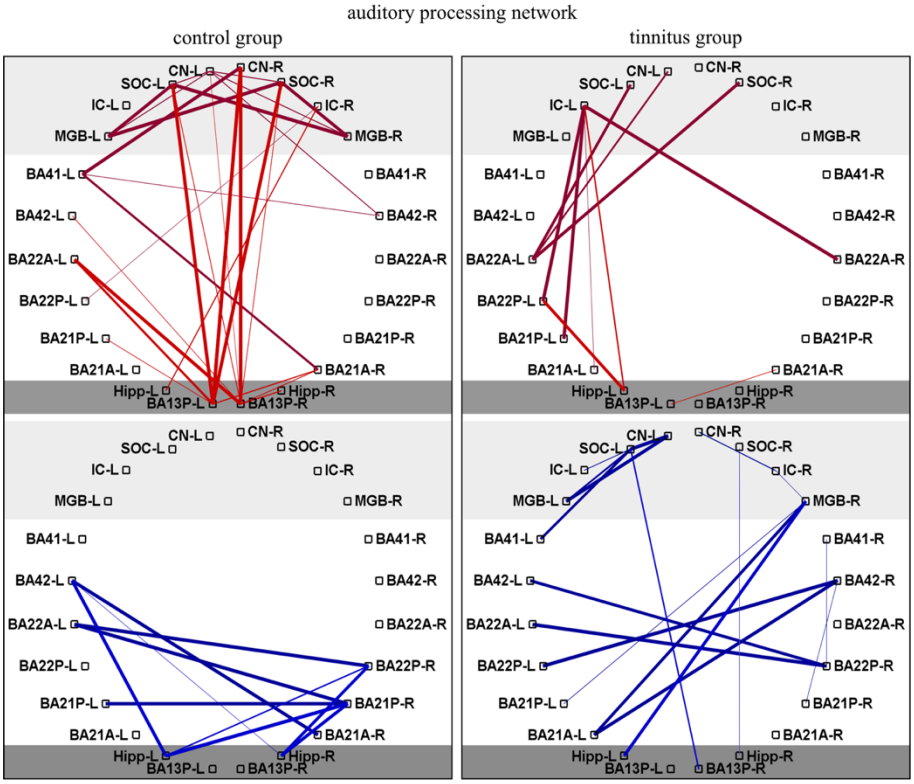

Figure 12 Functional connectivity pattern in the auditory processing network

Functional connectivity analysis for resting state measurements by Pearson correlation coefficient between different ROIs. Lines between the ROIs represent significant correlations ($p < 0.05$, FDR corrected), while the line thickness shows the strength of the correlation coefficient, the color stands for positive (red) or negative (blue) correlations. The left upper and lower panel shows the control group for positive and negative correlations separated, the right upper and lower panel shows the tinnitus group for positive and negative correlations. ROIs are separated in three areas, subcortical auditory regions (brighter grey background), primary and secondary auditory cortex regions (white background) and additional auditory processing areas (darker grey background). Modified according to (Hofmeier, Wolpert et al. 2018).

For the limbic network we found in the control group positive correlations between the cortical regions in the primary and secondary auditory cortex (BA41, BA42) to regions associated with distress and stress, as the amygdala, entorhinal cortex (BA28) and the anterior insula (BA13A) (**Figure 13** upper left panel), while those positive correlations did not appear in the tinnitus group (**Figure 13** upper right panel).

In contrast, the tinnitus group showed positive correlations between lower auditory pathway regions in the brainstem (CN, IC) and the regions of the limbic network (amygdala, BA28, BA13A) (**Figure 13** upper right panel).

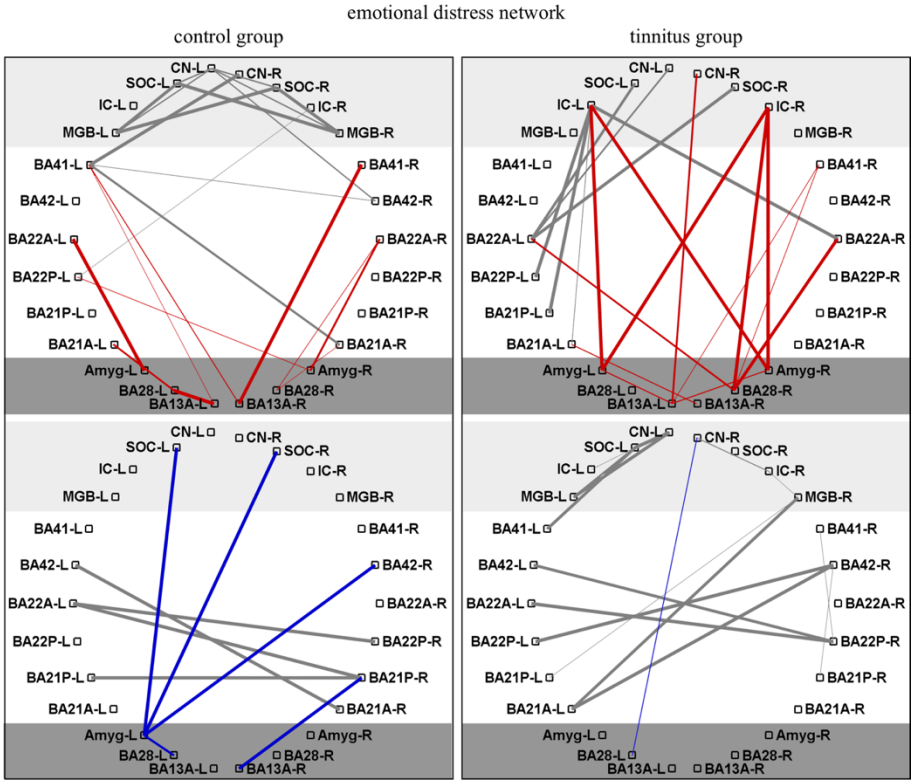

Figure 13 Functional connectivity pattern in the emotional distress network

Functional connectivity analysis for resting state measurements by Pearson correlation coefficient between different ROIs. Lines between the ROIs represent significant correlations ($p < 0.05$, FDR corrected), while the line thickness shows the strength of the correlation coefficient, the color stands for positive (red) or negative (blue) correlations. The left upper and lower panel shows the control group for positive and negative correlations separated, the right upper and lower panel shows the tinnitus group for positive and negative correlations. ROIs are separated in three areas, subcortical auditory regions (brighter grey background), primary and secondary auditory cortex regions (white background) and emotional distress regions (darker grey background). Modified according to (Hofmeier, Wolpert et al. 2018).

For the attentional network, ROIs in the fronto-parietal and temporofrontal network (BA45, BA46, BA47, BA9) in relation to ROIs from the auditory pathway were analyzed. We observed in the control group positive correlations between higher auditory specific regions in the primary and secondary auditory cortex to regions in the fronto-parietal and temporofrontal network (**Figure 14** left upper panel), which did not appear in the tinnitus group, instead we found here positive correlations from lower auditory specific regions in the brainstem (CN, SOC, and IC) and auditory thalamus (MGB) to regions in the fronto-parietal and temporofrontal network (**Figure 14** right upper panel).

For negative correlations we observed in the control group, a pattern like it appeared in the tinnitus group for positive correlations, from lower auditory specific regions in the brainstem (CN, SOC, and IC) and auditory thalamus (MGB) to regions in the fronto-

parietal and temporofrontal network (**Figure 14** compare left lower panel with right upper panel) (Hofmeier, Wolpert et al. 2018).

Summarized, we observed in the tinnitus group fewer positive correlations between ROIs in the auditory pathway, especially between lower auditory specific regions in the brainstem and for correlations from these regions to the auditory specific network (hippocampus and posterior insula) (**Figure 14** right upper panel compared to the left upper panel). While in the tinnitus group more negative correlations appeared in those lower brainstem regions, as we observe it in the control group (**Figure 12** right lower panel).

We observed additionally in the tinnitus group more positive correlations in the distress associated limbic network (amygdala, entorhinal cortex (BA28) and the anterior insula (BA13A), to ROIs from the auditory pathway (CN, SOC, IC, MGB, BA41, BA42, BA21, and BA22) (**Figure 13**).

For the attentional network, with ROIs in the fronto-parietal and temporofrontal cortex, we observed positive correlations in the tinnitus group from lower auditory specific ROIs to those attentional network regions, which were not observed in the control group. We found in the tinnitus group positive correlations from lower auditory pathway regions in the brainstem to the stress excitation associated region of the medial BA9 area, while the control group had more negative correlations for those connections.

Instead in the control group we analyzed positive correlations of higher auditory pathway regions in the multimodal lateral temporal lobe (BA22) with the stress inhibition associated and auditory-specific attentional regions within the dorso-lateral BA9.

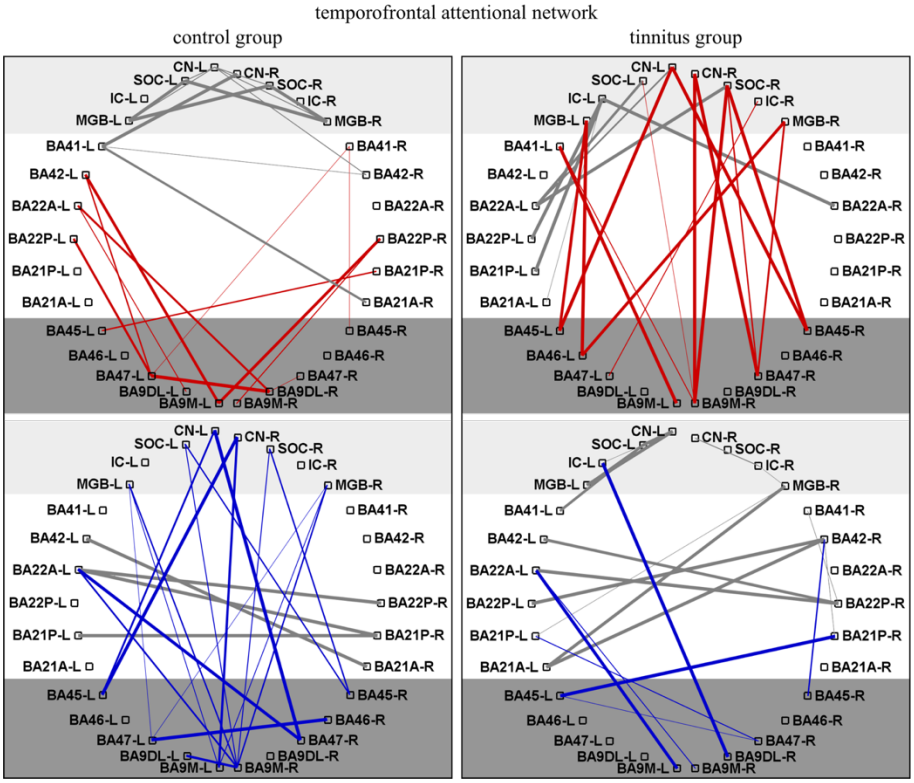

Figure 14 Functional connectivity pattern in the attentional network

Functional connectivity analysis for resting state measurements by Pearson correlation coefficient between different ROIs. Lines between the ROIs represent significant correlations ($p < 0.05$, FDR corrected), while the line thickness shows the strength of the correlation coefficient, the color stands for positive (red) or negative (blue) correlations. The left upper and lower panel shows the control group for positive and negative correlations separated, the right upper and lower panel shows the tinnitus group for positive and negative correlations. ROIs are separated in three areas, subcortical auditory regions (brighter grey background), primary and secondary auditory cortex regions (white background) and temporofrontal attentional areas (darker grey background). Modified according to (Hofmeier, Wolpert et al. 2018).

3.4 Analysis of cortisol saliva between the control and tinnitus group

Since tinnitus is associated with stress (Hébert, Canlon et al. 2012), we analyzed cortisol in saliva to detect possible differences between both groups.

We took three samples of saliva at defined time points (8:00 am, 4:00 pm, and 11:00 pm) and analyzed the time point between the groups and also within each group over the time points. While the tinnitus group had in general, a higher cortisol level for the single time points compared to the control group. These differences were statistically not significant (two-way ANOVA with repeated measurement, for the group comparison $p = 0.062$) (**Figure 15**). For both groups, the typical circadian rhythmic appeared over the sample time points, with higher cortisol values in the morning and dropping values during the day (Hofmeier, Wolpert et al. 2018).

Summarized, the tinnitus group showed a tendency of higher cortisol levels over all time points, with circadian rhythmic just as the controls.

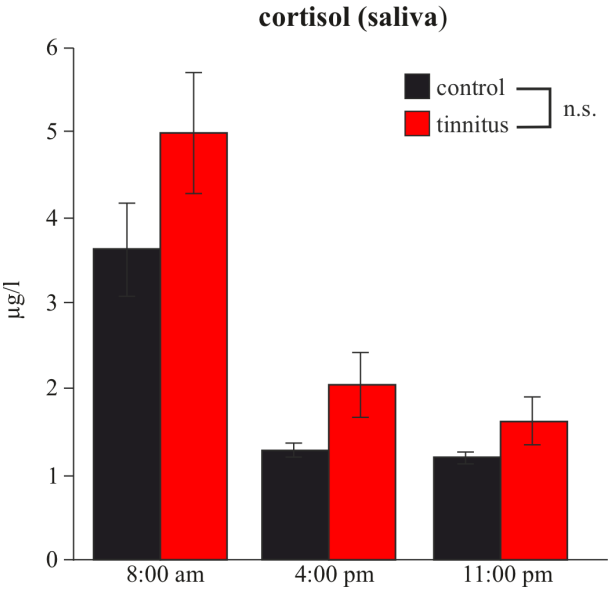

Figure 15 Cortisol in saliva for the control and tinnitus group

Cortisol level in saliva as mean for the control group (black, *n=17*) and tinnitus group (red, *n=17*) for three time points over the day at 8:00 am, 4:00 pm and 11:00 pm. Standard error is given for every value. Modified according to (Hofmeier, Wolpert et al. 2018).

3.5 Presentation of identified tinnitus-specific biomarkers

We identified in normal hearing to mild hearing-impaired matched participant with tinnitus, reduced and prolonged late brainstem responses, reduced task-evoked BOLD response together with reduced r-fcMRI in auditory specific regions compared to healthy controls. While these tinnitus participants showed increased r-fcMRI in attention and stress associated ROIs with a tendency of increased cortisol level in saliva, associated with higher stress response in comparison to the control group.

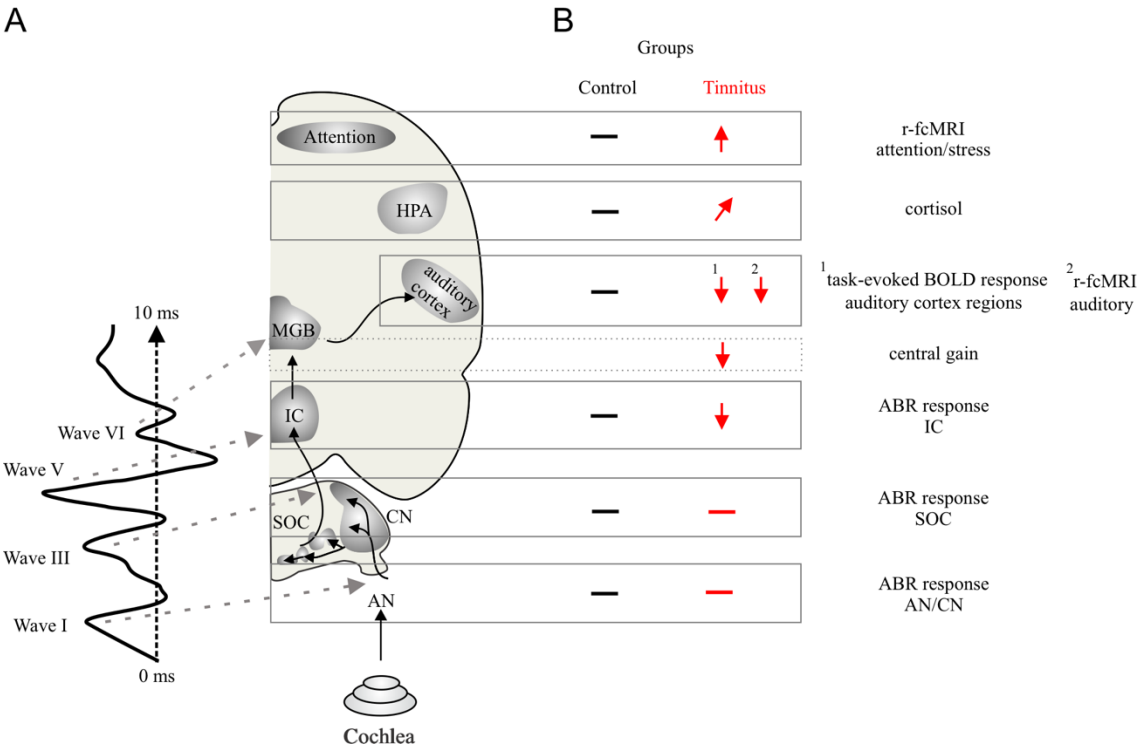

Figure 16 Summarized biomarker for tinnitus

Identified biomarkers in tinnitus participants for supra-threshold sound induced ABR, task-evoked BOLD fMRI/r-fcMRI and cortisol analysis. **A** Example of an auditory signal in the brainstem and a schematic drawing of the human ascending auditory pathway. **B** Alterations in the tinnitus group, compared to the control group for different level of analysis. Modified according to (Möhrle, Hofmeier et al. 2019)

3.6 Recruitment of tinnitus patients with co-occurrence of hyperacusis

3.6.1 Identification of tinnitus and hyperacusis perception

We separated participants with a tinnitus percept into two groups, one without the comorbidity of hyperacusis (named as tinnitus group) and a group including those with the comorbidity of hyperacusis (named as tinnitus+hyperacusis group) by using the HKI

questionnaire. During the recruitment in the follow-up study in 2019, we searched especially for tinnitus participants with co-occurrence of hyperacusis.

We included 43 participants in the control group, with an average HKI score of 4.10 points (SD 2.48 points), in the tinnitus group 30 participants with an average HKI score of 6.00 points (SD 2.91 points), and in the group of tinnitus+hyperacusis 20 participants with an average HKI score of 16.10 points (SD 3.60 points) (**Figure 17A**). Using Mann-Whitney-U test, significant differences were found between the control group and tinnitus+hyperacusis group ($p < 0.001$) and also between the tinnitus and tinnitus+hyperacusis group ($p < 0.001$).

The score from the G-H-S tinnitus questionnaire defined the intrusiveness of the perceived tinnitus. From the 30 participants included in the tinnitus group, 28 can be classified with a mild form of tinnitus (lowest quarter of the G-H-S classification), only two participants (6.66 %) showed a moderate (second quarter of the G-H-S classification) form of tinnitus. The tinnitus group had a mean of 14.21 points (SD 9.82 points), which was significant lower ($p < 0.001$, Mann-Whitney-U test) compared to the group of tinnitus+hyperacusis with a mean of 28.60 points (SD 12.03 points) (**Figure 17B**). In this group 7 of 20 participants (35 %) showed a moderate form of tinnitus (second quarter of the G-H-S classification).

Summarized, with the hyperacusis questionnaire we were able separated participants with a tinnitus percept and a tinnitus percept with the comorbidity of hyperacusis. The tinnitus group was not different compared to the control group, which also showed no affectedness by hyperacusis according the questionnaire score. Both groups with a tinnitus percept showed an averaged mild form of tinnitus, according to the severity classification of the tinnitus questionnaire, whereby the burden for the tinnitus+hyperacusis group is significantly higher compared to the tinnitus group.

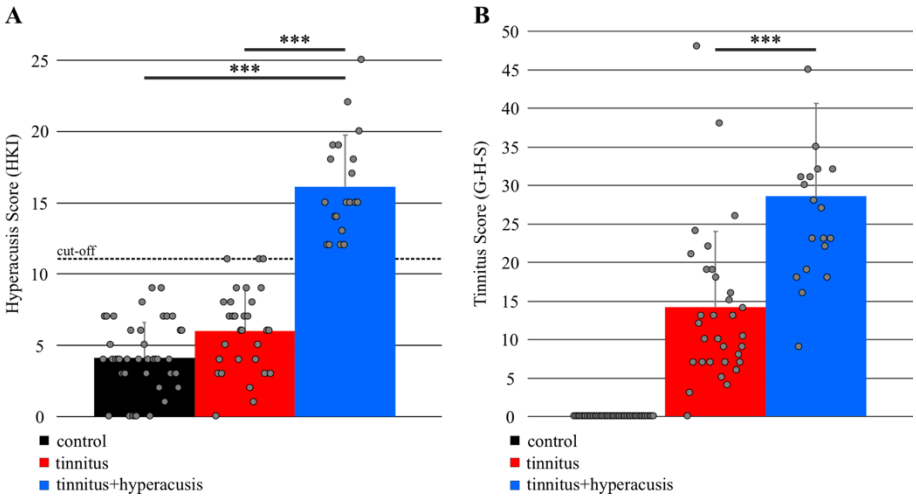

Figure 17 Hyperacusis and tinnitus classification

A Mean hyperacusis score for the control group (black, *n = 43*), the tinnitus group (red, *n = 30*) and the tinnitus+hyperacusis group (blue, *n = 20*), included the single values in grey dots within each group bar. A cut-off line at 11 points differentiated between non-hyperacusis and hyperacusis (>11 points). **B** Mean tinnitus score for the control group (black, *n = 43*, due to no tinnitus percept, the group is just illustrated with 0 points), the tinnitus group (red, *n = 30*) and the tinnitus+hyperacusis group (blue, *n = 20*), included the single values in grey dots within each group bar.

3.6.2 Identification of middle and inner ear function

We performed tympanometry with stapedius reflex measurements for the three groups. All participants showed a normal middle ear function and stapedius reflex. A sample of tympanograms of all three groups is shown in **Figure 18**.

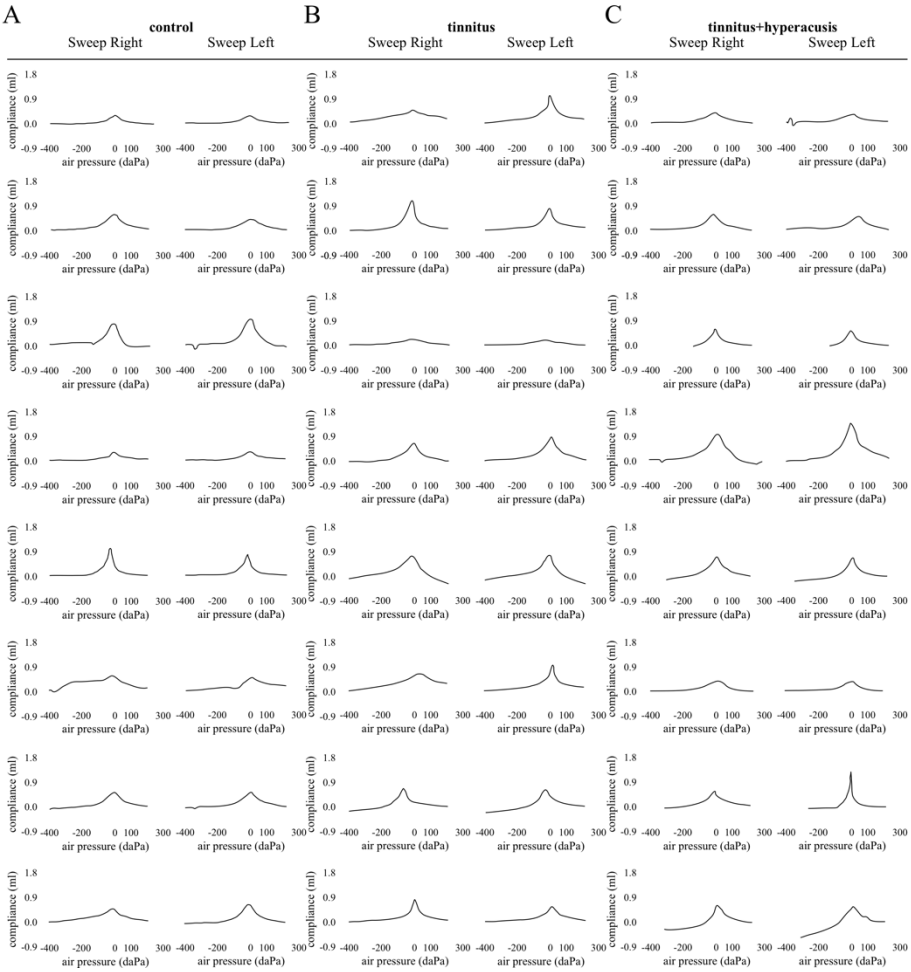

Figure 18 Tympanograms for the control, tinnitus and tinnitus+hyperacusis group

Sample of tympanograms **A** The control group. **B** The tinnitus group. **C** The tinnitus+hyperacusis group.

3.6.3 Gender, age, handedness within the control, tinnitus and tinnitus+hyperacusis group

The non-audiological characteristics between all three groups were listed in **Table 11**. The 43 included participants in the control group showed a similar averaged age (Mean 26.51 years, SD 5.83 years, range 18 - 45 years), compared to the tinnitus group (Mean 29.73 years, SD 7.86 years, range 20 - 50 years) and the tinnitus+hyperacusis group (Mean 26.95 years, SD 6.94 years, range 21 - 49 years). Also, the handedness did not differ significantly, with about 92 % are right handed in the control group (39 of 43 participants), 87 % in the tinnitus group (26 of 30 participants), and 85 % in the tinnitus+hyperacusis group (17 of 20 participants). We had also a homogenous matching with these non-audiological data.

For sex, we observe differences between the groups. The control group showed an equal distribution with 55 % included females (23 of 43 participants). The tinnitus group instead contained significant more men (21 of 30 participants), compared to the control group (Mann-Whitney-U test, $p = 0.048$). While the tinnitus+hyperacusis group had more recruited females (14 of 20 participants) compared to the control group but not statistically significant (Mann-Whitney-U test, $p = 0.22$). Between the tinnitus group and the tinnitus+hyperacusis group, the difference in sex was highly significant (Mann-Whitney-U test, $p = 0.006$).

Summarized, for non-audiological data, the groups showed, excepted sex, equal distribution of age and handedness.

Table 11 Non-audiological study participants data for the control, tinnitus and tinnitus+hyperacusis group

Non-audiological data for age, sex and handedness.

Group Control	Age	Sex	Handedness	Group Tinnitus	Age	Sex	Handedness	Group Tinnitus+ Hyperacusis	Age	Sex	Handedness
K002	27	female	right	T001	36	male	left	TN12	24	male	right
K006	39	male	right	T002	21	male	right	TN13	21	female	left
KN01	21	female	right	T006	45	female	right	TN17	26	female	right
KN02	26	female	right	T009	34	male	right	TN19	34	female	right
KN03	18	male	right	TN01	26	male	right	TN20	28	female	right
KN04	32	female	right	TN03	34	male	left	TN21	24	female	right
KN05	41	female	left	TN04	23	male	left	TN22	21	female	right
KN06	21	female	right	TN05	33	male	right	TN23	24	female	right
KN07	23	female	right	TN08	27	male	right	TN25	22	female	right
KN08	18	female	right	TN10	25	female	right	TN28	30	female	right
KN09	19	male	right	TN11	25	female	right	TS020	24	female	right
KN10	20	female	right	TN16	26	male	left	TS033	49	male	left
KN11	24	female	right	TS004	44	male	right	TS037	23	female	right
KN14	20	female	right	TS005	29	male	right	TS040	23	female	right
KN16	27	male	left	TS008	20	female	right	TS044	20	male	left
KN17	24	male	left	TS010	26	male	right	TS048	27	male	right
KN18	26	male	right	TS017	29	male	right	TS050	21	female	right
KN20	27	male	right	TS019	25	male	right	TS057	33	male	right
KN21	28	male	right	TS021	27	male	right	TS061	29	male	right
KN22	26	male	right	TS031	50	male	right	TS067	36	female	right
KN23	22	male	right	TS032	24	male	right				
KN25	22	female	right	TS036	27	female	right				
TS002	31	male	right	TS049	26	male	right				

TS003	30	female	right	TS053	29	male	right
TS012	19	male	right	TS054	21	female	right
TS014	26	male	right	TS059	35	female	right
TS015	30	female	right	TS062	36	male	right
TS016	26	female	right	TS068	25	male	right
TS024	27	male	right	TS070	20	female	right
TS025	27	male	right	TS073	44	female	right
TS027	24	female	right				
TS028	26	male	right				
TS029	45	female	right				
TS030	27	male	right				
TS039	26	female	right				
TS041	25	female	left				
TS042	31	male	right				
TS047	28	female	right				
TS056	22	male	right				
TS060	24	female	right				
TS063	35	female	right				
TS071	27	female	right				
TS072	33	male	right				

3.7 Audiological evaluation of control, tinnitus and the tinnitus+hyperacusis group

3.7.1 Analysis of hearing threshold differences between the control, tinnitus and tinnitus+hyperacusis group

The PTA is measured to detected hearing loss, which is often causally related with acoustic trauma or long-lasting noise exposure. The PTA was used for the group matching to align the hearing of the participants to avoided effects in the ABR and fMRI measurements caused due to differences in the hearing threshold between the groups. We exclude participants (**Appendix C**), according to a hearing loss larger 40 dB at more than one frequency and a non-clinical normal hearing definition.

In the three recruited groups, we observed in almost all participants even up to 10 kHz (highest measured frequency for the PTA) hearing thresholds not higher than 20 dB HL, which is the threshold to define a normal clinical hearing for the frequency range of 0.25 kHz to 4 kHz.

Figure 19 showed a sample of single pure tone audiograms for the different groups. For participants with a tinnitus percept, the red filled dot showed the tinnitus localization for the frequency and intensity.

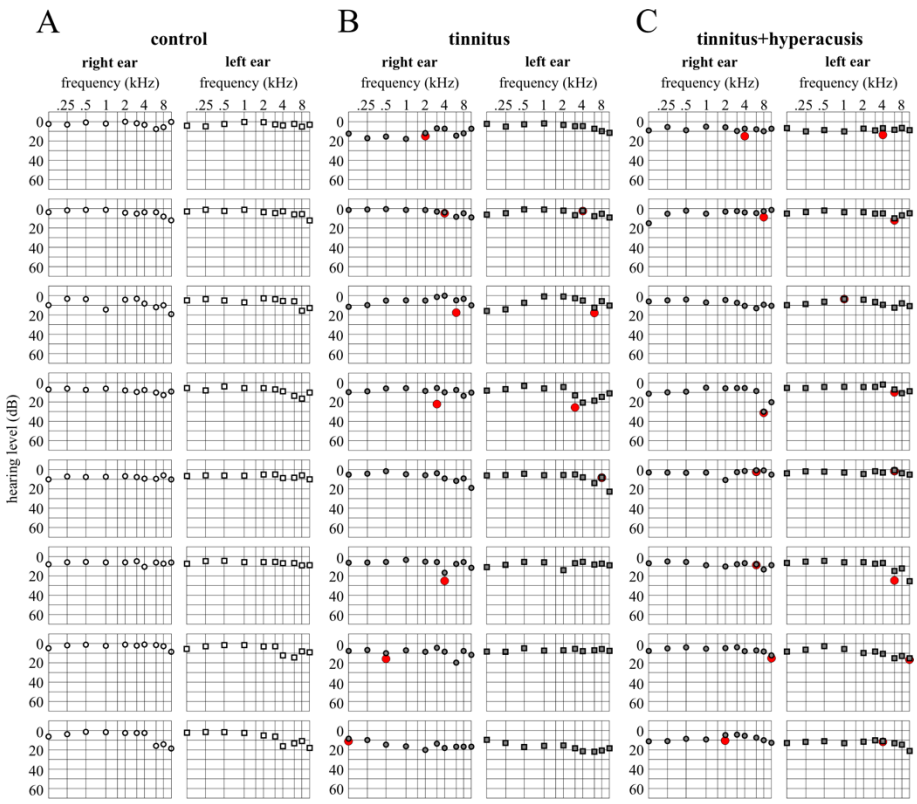

Figure 19 Pure tone audiograms for the control, tinnitus, and tinnitus+hyperacusis group

Samples of single pure tone audiograms for the right and left ear **A** Control group. **B** Tinnitus group, red dot in the audiogram defines the tinnitus localization for frequency and intensity. **C** Tinnitus+hyperacusis group, red dot in the audiogram defines the tinnitus localization for frequency and intensity.

The averaged PTA (**Figure 20**) results between the three groups were not significant different ($p > 0.05$), neither for the control group to the tinnitus group, nor for the control group to the tinnitus+hyperacusis group, nor between the tinnitus and tinnitus+hyperacusis group, using Mann-Whitney-U test ($p > 0.05$).

Summarized, all three groups had the same hearing threshold. There was also no difference between lower and higher frequencies within the groups.

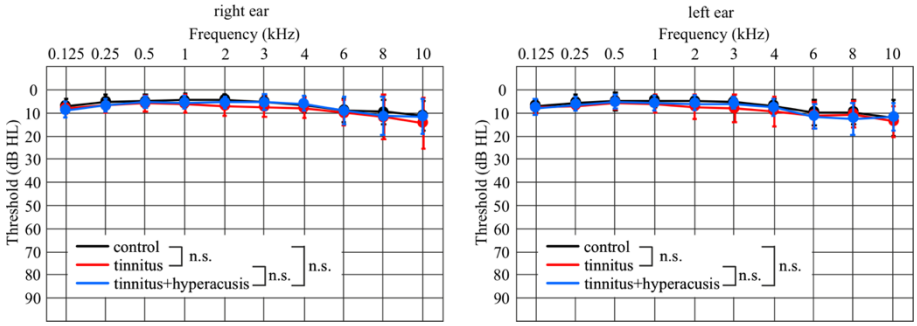

Figure 20 Pure tone audiometry measurement for the control, tinnitus and tinnitus+hyperacusis group

Averaged PTA (mean value ± SD) for the control group (black, *n = 43*), the tinnitus group (red, *n = 30*) and the tinnitus+hyperacusis group (blue, *n = 20*) separated for the right and left ear.

3.7.2 Identification of individual perceived tinnitus frequency and loudness in the tinnitus and tinnitus+hyperacusis group

Tinnitus localization was performed to quantify the individual perceived tinnitus loudness a frequency for the participants in both tinnitus groups.

Table 12 summarized the G-H-S (named as 'Tinnitus Score' in **Table 12**) and tinnitus localization (named as 'Tinnitus Frequency & Intensity' in **Table 12**) for the tinnitus group, together with the Tinnitus-CRF (named as 'Tinnitus laterality' for the tinnitus percept during the last days before the first examination day in **Table 12**).

The tinnitus group perceived the tinnitus tone at a mean frequency of 5.53 kHz for the right and 6.15 kHz for the left ear (SD 2.98 kHz and SD 2.83 kHz respectively), with an averaged loudness of 18.28 dB HL for the right and 14.83 dB HL (SD 11.29 dB HL and SD 9.81 dB HL respectively). In general, the participants in the tinnitus group perceived their tinnitus bilateral (60 %, 23.33 % single-sided for the right and 16.66 % single-sided for the left ear), with nearly the same frequency for both sides. Whereas the tinnitus intensity differed in some cases in a larger range within a participant with bilateral tinnitus percept.

Table 12 Tinnitus specific characteristics for the tinnitus group

Individual tinnitus characteristics with total tinnitus score, tinnitus percepts over the last days (before examination day 1) and tinnitus localization.

Group		Tinnitus laterality			Tinnitus frequency & Intensity			
Tinnitus	Tinnitus score	Right	Left	Inner Head	kHz (right)	dB (right)	kHz (left)	dB (left)
T001	0	low	moderate	moderate	8	15	8	15
T002	3	low	low	---	10	6	10	4
T006	21	moderate	moderate	---	6	27	6	27
T009	7	---	moderate	---	---	---	8	34
TN01	24	moderate	---	---	10	35	---	---
TN03	48	---	low	---	---	---	10	31
TN04	7	inaudible	inaudible	---	4	5	4	6
TN05	12	very low	inaudible	---	10	28	---	---
TN08	13	inaudible	inaudible	very low	10	13	10	10
TN10	7	inaudible	inaudible	inaudible	4	12	8	7
TN11	10	very low	very low	very low	6	14	8	7
TN16	19	high	low	moderate	8	15	6	3
TS004	22	---	low	---	---	---	1	13
TS005	7	moderate	low	inaudible	3	15	3	21
TS008	19	---	moderate	---	---	---	0.5	10
TS010	13	---	---	low	8	14	8	18
TS017	18	inaudible	low	low	6	18	6	14
TS019	7			very low	2	14	2	11
TS021	10	low	low	inaudible	8	13	8	15
TS031	38	high	high	---	6	34	6	34
TS032	4	moderate	---	inaudible	4	57	---	---
TS036	5	very low	---	inaudible	1.5	15	---	---
TS049	15	low	very low	low	4	5	4	1
TS053	16	---	very low	---	6	17	6	17
TS054	9	low	very low	low	6	11	8	9
TS059	13	moderate	low	---	3	23	3	26
TS062	9	---	low	very low	---	---	8	8
TS068	8	low	---	inaudible	4	24	---	---
TS070	26	inaudible		inaudible	0.5	16	---	---
TS073	6	very low	---	---	0.125	11	---	---

Table 13 summarized the G-H-S (named as 'Tinnitus Score' in **Table 13**) and tinnitus localization (named as 'Tinnitus Frequency & Intensity' in **Table 13**) for the tinnitus+hyperacusis group, together with the Tinnitus-CRF (named as 'Tinnitus

laterality' for the tinnitus percept during the last days before the first examination day in **Table 13**).

In the tinnitus+hyperacusis group, for the tinnitus localization, the tone was perceived at a mean frequency of 5.79 kHz for the right and 5.71 kHz for the left ear (SD 3.22 kHz and SD 2.85 kHz respectively), with an averaged loudness of 14.59 dB HL for the right and 13.58 dB HL (SD 9.08 dB HL and SD 6.69 dB HL respectively). In general, the participants in the tinnitus+hyperacusis group perceived their tinnitus bilateral (80 %, 15 % single-sided for the right and 5 % single-sided for the left ear), with nearly the same frequency for both sides. Whereas the tinnitus intensity differed in some cases in a larger range within a participant with bilateral tinnitus percept.

Table 13 Tinnitus specific characteristics for the tinnitus+hyperacusis group

Individual tinnitus characteristics with total tinnitus score, tinnitus percepts over the last days (before examination day 1) and tinnitus localization.

Group		Tinnitus laterality			Tinnitus frequency & Intensity			
Tinnitus+ Hyperacusis	Tinnitus score	Right	Left	Inner Head	kHz (right)	dB (right)	kHz (left)	dB (left)
TN12	18	low	very low	very low	1	11	8	22
TN13	9	---	very low	---	---	---	10	5
TN17	31	very low	very low	very low	8	8	8	8
TN19	16	low	---	---	10	20	---	---
TN20	30	low	high	inaudible	10	21	1	16
TN21	19	very low	very low	moderate	1	11	0.75	13
TN22	45	moderate	moderate	---	6	9	6	10
TN23	31	low	low	---	1	13	10	9
TN25	23	low	moderate	inaudible	4	15	4	15
TN28	28	high	moderate	inaudible	8	40	6	23
TS020	35	---	moderate	---	---	---	6	20
TS033	32	low	moderate	low	6	10	6	18
TS037	53	high	very low	low	4	15	4	20
TS040	27	low	moderate	---	8	9	6	11
TS044	23	---	inaudible	inaudible	---	---	0.75	5
TS048	32	---	---	moderate	8	30	6	9
TS050	22	low	low	---	6	2	6	1
TS057	18	moderate	moderate	inaudible	6	9	6	25
TS061	57	moderate	moderate	inaudible	10	15	10	16
TS067	23	very low	inaudible	inaudible	1.5	10	4	12

Summarized, both groups showed a bilateral tinnitus percept (60% for the tinnitus group and 80% in the tinnitus+hyperacusis group), while for single-sided tinnitus a right-sided percept predominated. The precepted frequency appeared in both groups for a similar mean (tinnitus group - 5.53 kHz for the right and 6.15 kHz for the left side, tinnitus+hyperacusis group 5.79 kHz for the right and 5.71 kHz for the left side), while the precepted loudness not significantly differed between them.

3.7.3 Identification of difference in early and late supra-threshold sound induced brainstem response

To analyze possible differences between the groups in the early auditory processing alongside the auditory brainstem we performed supra-threshold ABR measurements. The fine structure analysis of the ABR wave form allows a differential view for temporal processing according to the latency and amplitude.

In the tinnitus group compared to the control group, we observed for the ABR wave V amplitudes at the stimulus intensity of 75 dB nHL a significant reduction in the tinnitus group, compared to the control group in both ears (one-way ANOVA, for the right ear $p = 0.029$, for the left ear $p = 0.05$) (**Figure 21A**, first row). A reduced wave VI amplitude for the right ear was observed in the tinnitus group ($p = 0.004$), wave I and III showed no significant difference. At 65 dB nHL, we observed for the right ear a significant reduced ($p = 0.008$) amplitude in the tinnitus group (**Figure 21A**, second row).

For the latencies a significant delay was observed for wave V at 75 dB nHL for both ears (one-way ANOVA, for the right ear $p = 0.001$, for the left ear $p = 0.001$), at 65 dB nHL for both ears (for the right ear $p = 0.045$, for the left ear $p = 0.003$), at 55 dB nHL for the right ear ($p = 0.013$), at 45 dB nHL for both ears (for the right ear $p < 0.001$ and for the left ear $p = 0.027$), at 35 dB nHL for the right ear ($p < 0.001$), and at 25 dB nHL for the right ear ($p = 0.014$) (**Figure 21A**, third row).

The tinnitus group had a significant larger IPL at 75 dB nHL for wave III-V in both ears (one-way ANOVA, for the right ear $p = 0.048$, for the left ear $p < 0.001$), for IPL wave I-V in both ears (one-way ANOVA, for the right ear $p = 0.002$, for the left ear $p = 0.002$) was observed (**Figure 21A**, first row). At 65 dB nHL IPL wave III-V for the left ear was also significantly larger ($p = 0.046$) (**Figure 21A**, second row).

In the tinnitus+hyperacusis group compared to the control group, larger amplitudes for the ABR waves at 75 dB nHL in the group of tinnitus+hyperacusis were observed. For wave III, we observed a significant increased amplitude for the left ear at 75 dB nHL (one-way ANOVA, $p < 0.001$) (**Figure 21B**, first row).
Shorter latencies were observed in the tinnitus+hyperacusis group at 65 dB nHL for wave III in both ears (one-way ANOVA, for the right ear $p = 0.039$, for the left ear $p = 0.012$) and wave VI for the left ear (one-way ANOVA, $p = 0.04$). For wave V latencies, shorter response was observed in the tinnitus+hyperacusis group at 45 and 25 dB nHL for the right ear (one-way ANOVA, $p = 0.038$ and $p = 0.018$ respectively) and at 35 dB nHL for the left ear ($p = 0.024$) (**Figure 21B**, third row).

In the tinnitus+hyperacusis group compared to the tinnitus group, we observed for 75 dB nHL significant larger amplitudes in the tinnitus+hyperacusis group for wave III, V, and VI for both ears (one-way ANOVA, wave III for the right ear $p = 0.006$ and for the left ear $p = 0.008$, wave V for the right ear $p = 0.002$ and left ear $p = 0.001$, and wave VI for the right ear $p = 0.036$ and for the left ear $p = 0.04$) (**Figure 21C**, first row).
At 65 dB nHL, the tinnitus+hyperacusis group showed generally larger amplitudes. For wave III and V in the left ear (one-way ANOVA, $p = 0.045$, $p = 0.03$ respectively) significantly larger amplitudes were observed (**Figure 21C**, second row).
For wave I shorter latency in the tinnitus+hyperacusis group was found at 65 dB nHL for both ears (one-way ANOVA, for the right ear $p = 0.019$ and for the left ear $p = 0.05$), for wave III at 75 and 65 dB nHL (one-way ANOVA, 75 dB nHL for the right ear $p = 0.013$ and for the left ear $p = 0.018$, 65 dB nHL for the right ear $p = 0.008$ and for the left ear $p = 0.004$) and for wave VI at 65 dB nHL for the left ear (one-way ANOVA, $p = 0.013$).
In wave V we observed for all stimulus intensities significant shorter latencies in the tinnitus+hyperacusis group (one-way ANOVA, 75 dB nHL for the right ear $p = 0.05$ and for the left ear $p = 0.001$, 65 dB nHL for the right ear $p = 0.017$ and for the left ear $p = 0.002$, 55 dB nHL for the right ear $p = 0.007$ and for the left ear $p = 0.006$, 45 dB nHL for the right ear $p < 0.001$ and for the left ear $p = 0.011$, 35 dB nHL for the right ear $p < 0.001$ and for the left ear $p = 0.011$, 25 dB nHL for the right ear $p < 0.001$ and for the left ear $p = 0.02$) (**Figure 21C**, third row).

Smaller IPL between wave I-V was found for the left ear at 75 and 65 dB nHL in the tinnitus+hyperacusis group, compared to the group of tinnitus (one-way ANOVA, 75 dB nHL $p = 0.039$, 65 dB nHL $p = 0.023$) (**Figure 21C**, first row).

Summarized, we observed in the tinnitus group, compared to the control group significant reduced und prolonged late (central) brainstem regions (ABR wave V). In the group of tinnitus+hyperacusis, compared to the control group, we did not see prolonged ABR response, instead the ABR response is larger in early (wave III) and late (ABR wave V) brainstem regions. In the tinnitus+hyperacusis group compared to the tinnitus group significantly larger early (wave III) and late (ABR wave V) amplitudes with shorter latencies were observed.

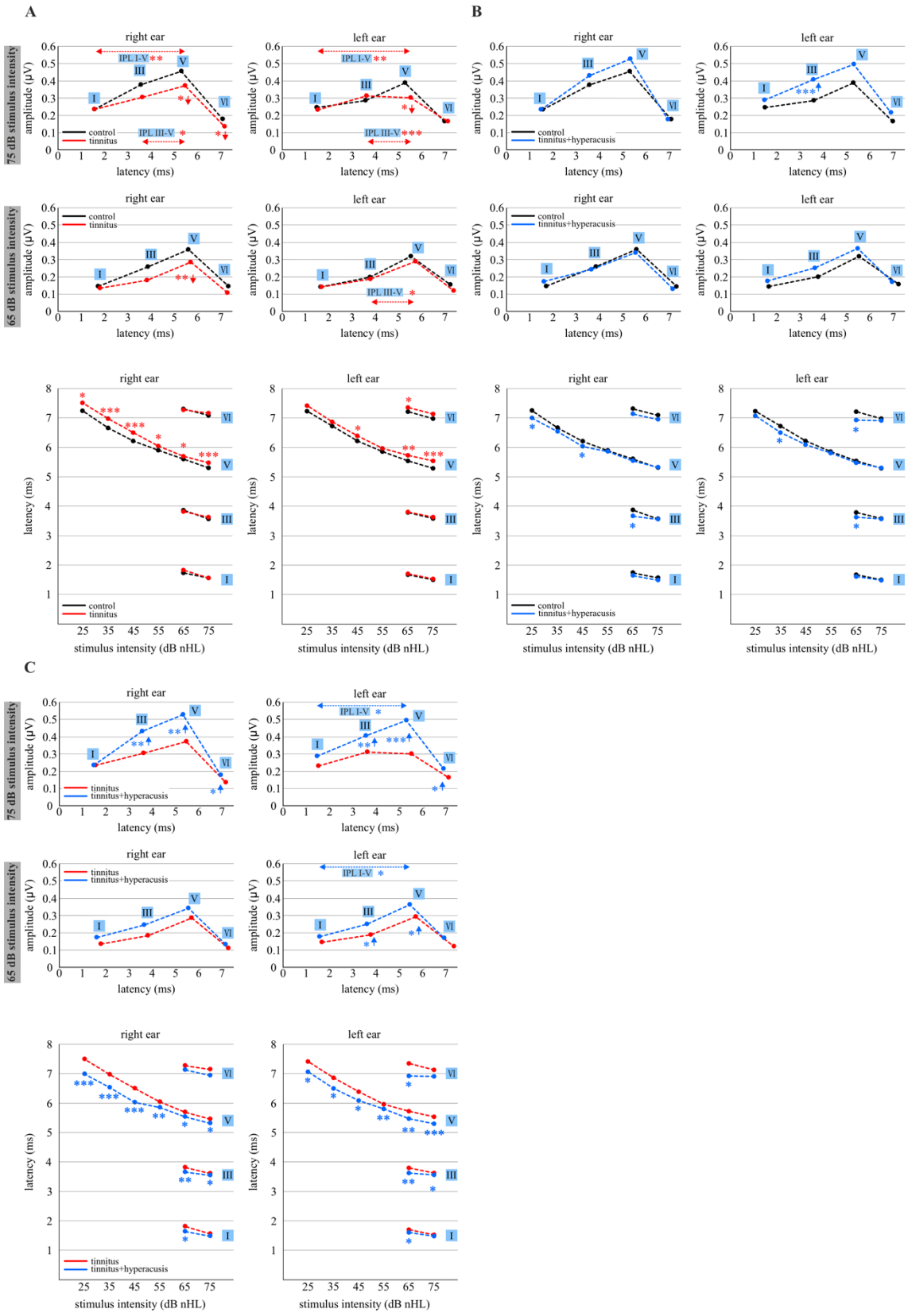

Figure 21 Supra-threshold auditory brainstem response analysis for the control, tinnitus and tinnitus+hyperacusis group

Averaged ABR wave I, III, V, and VI as amplitude-latency plot at 75 dB nHL (**first row** of **A**, **B**, and **C**) and 65 dB nHL (**second row** of **A**, **B**, and **C**) and averaged ABR wave I, III, V, and VI as intensity-latency plot at 75 and 65 dB nHL and Wave V also at 55, 45,

35 and 25 dB nHL (**third** row of **A**, **B**, and **C**). **A** Control (black, *n = 43*) and tinnitus group (red, *n = 30*). **B** Control (black, *n = 43*) and tinnitus+hyperacusis group (blue, *n = 20*). **C** Tinnitus+hyperacusis (blue, *n = 20*) and tinnitus group (red, *n = 30*).

3.8 Analysis of task-evoked BOLD response in the control, tinnitus and tinnitus+hyperacusis group

3.8.1 Identification of differences in sound induced BOLD response between the tinnitus+hyperacusis and control group for different auditory stimuli

Task-evoked fMRI measurements were used to proof, if the ABR measurement results were reflected in sound induced BOLD response in the tinnitus and tinnitus+hyperacusis group, in predefined ROIs (**Table 2**).

In the tinnitus group (compared to the control group), we observed for the music piece significant lower BOLD response in primary and secondary auditory cortex regions (BA41A-R, BA41P-R, BA42A-R, BA42-R and BA41-L), in regions responsible for sound detection (BA13P-R, BA22A-L, and BA13P-L), in subcortical auditory regions (SOC-R), and in somatosensory cortex regions (BA1-R, BA2-R, BA2-L, and BA1-L) (**Figure 22A**). Regions associated with pain (Job, Paucod et al. 2011) as the postcentral gyrus (PCG2-L, PCG1-L) and for sound caused pain (Segerdahl, Mezue et al. 2015) as the dorsal posterior insula (DpIns-R, DpIns-L) were also lower in the tinnitus group.
Significant higher BOLD response for the music piece was observed in the hippocampus (Hipp-L) (**Figure 22A**).
For the LF-chirp stimuli we observed similar to the music piece stimuli a reduction in the subcortical auditory region (SOC-R) (**Figure 22B**).
We found in the subcortical auditory regions a single significantly higher activity (CN-L) for the LF-chirp, with, in general higher but not statistically significant activity in most analyzed regions compared to the control group (**Figure 22B**).
For the HF-chirp as stimuli, we observe significant lower BOLD response in regions for sound detection (BA22A-R, BA21A-L, and BA22A-L) and in the subcortical auditory region (MGB-R) (**Figure 22C**). While in general, in primary auditory cortex regions, the BOLD response was lower in the tinnitus group, but not significant. No significant higher BOLD response to the HF-chirp was observed in the tinnitus group compared to the control group in primary auditory regions.

For the BB-chirp, we found in regions for sound detection (BA21P-L, and BA22P-L) and somatosensory regions (BA2-L, BA1-L, and Mam.-Body-L) significant lower activity in the tinnitus group, compared to the control group (**Figure 22D**). While also here the pattern for primary auditory cortex regions seems similar to the HF-chirp, with insignificant but generally lower BOLD response in the tinnitus group.

As similar to the results for the HF-chirp, no significant enhanced BOLD response to the BB-chirp was observed in the tinnitus group (**Figure 22D**).

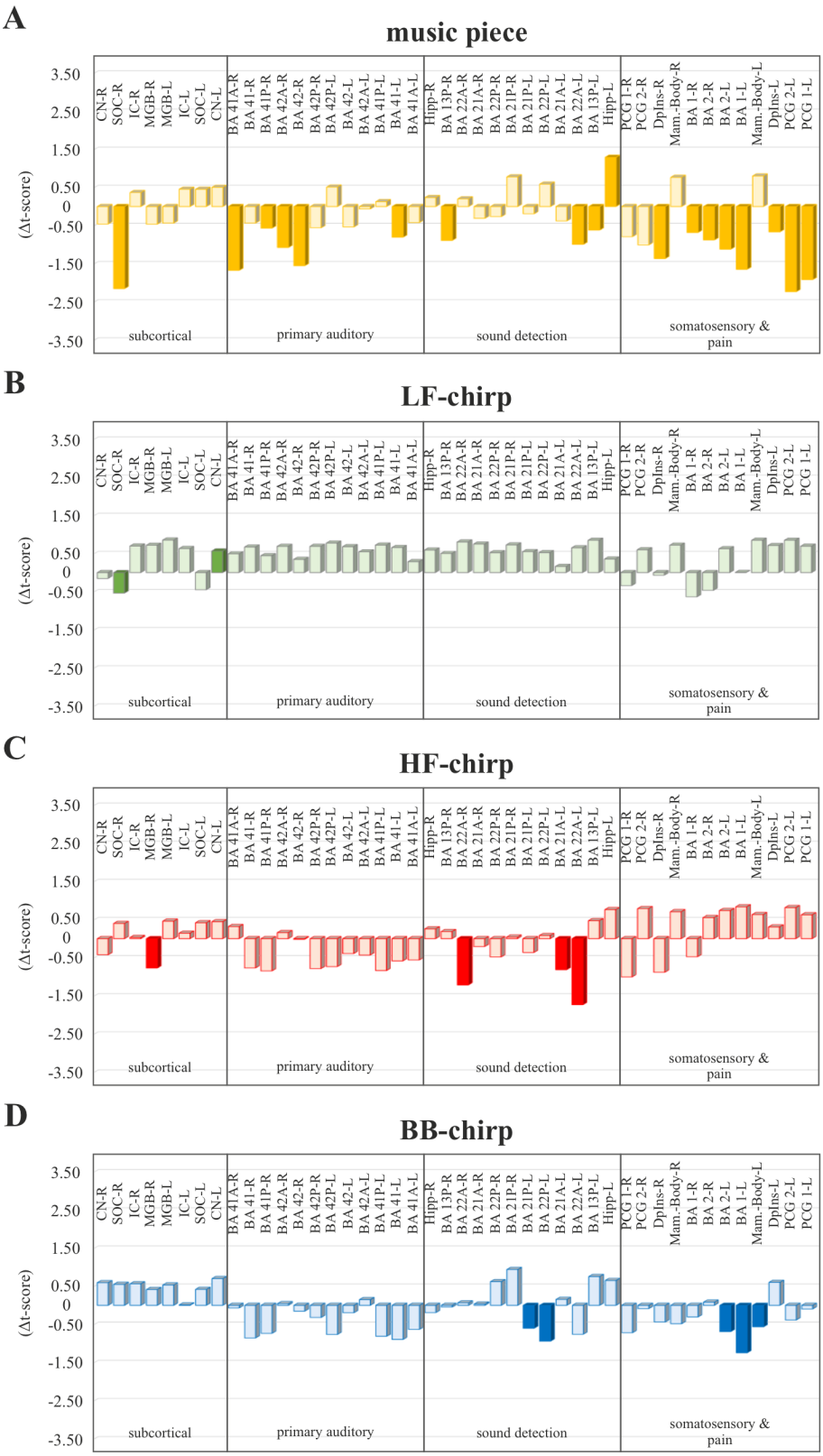

Figure 22 Task-evoked fMRI response differences for group comparison between tinnitus and the control group

Group comparison for task-evoked BOLD response in the tinnitus group (*n = 30*) compared to the control group (*n = 43*). The bars showed lower or higher BOLD response in the tinnitus group compared to the control group for different stimuli in predefined ROIs

(**Table 2**). Significant differences ($p < 0.05$, FDR corrected) are highlighted in the full color for every panel, shaded colors did not reach the significant level. (**A**) music piece stimuli (**B**) LF-chirp stimuli (**C**) HF-chirp stimuli (**D**) BB-chirp stimuli.

In the tinnitus+hyperacusis group (compared to the control group), we observed for the music piece significant lower BOLD response in subcortical auditory regions (CN-R, SOC-R, and MGB-L) (**Figure 23A**).

Significant higher BOLD response to the music piece was not observed in the group comparison, but in general for primary auditory regions, regions responsible for sound detection and in somatosensory regions, values were larger than in the controls (**Figure 23A**).

For the LF-chirp we observed significant lower BOLD response in subcortical auditory regions (CN-R, SOC-L, and CN-L) and in the hippocampus (Hipp-R) (**Figure 23B**).

We found for the LF-chirp in primary auditory cortex regions (BA41P-L, BA42-R, and BA42P-L) significantly higher activity in the group of tinnitus+hyperacusis, with a general pattern of higher but not significantly higher activity in the primary auditory cortex, sound detection and somatosensory regions (**Figure 23B**).

For the HF-chirp we observed significant lower BOLD response in regions for sound detection (BA22A-R, BA21A-R, BA22P-R, BA21A-L, and BA22A-L), in the subcortical auditory region (SOC-R, MGB-R, and SOC-L), in the primary auditory cortex region (BA41A-L), and the somatosensory regions (Mam.-Body-R, and Mam.-Body-L) (**Figure 23C**). Significant higher BOLD response to the HF-chirp was observed in regions for sound detection (Hipp-R).

For the BB-chirp, we found in regions for sound detection (Hipp-R) and somatosensory regions (Mam.-Body-R) significant lower BOLD response in the tinnitus+hyperacusis group, compared to the control group (**Figure 23D**).

While we did not observe significant higher BOLD response to the BB-chirp in the tinnitus+hyperacusis group compared to the control group, but the group of tinnitus+hyperacusis generally showed higher BOLD response in primary auditory cortex regions, similar to the LF-chirp and music piece stimuli (**Figure 23D**).

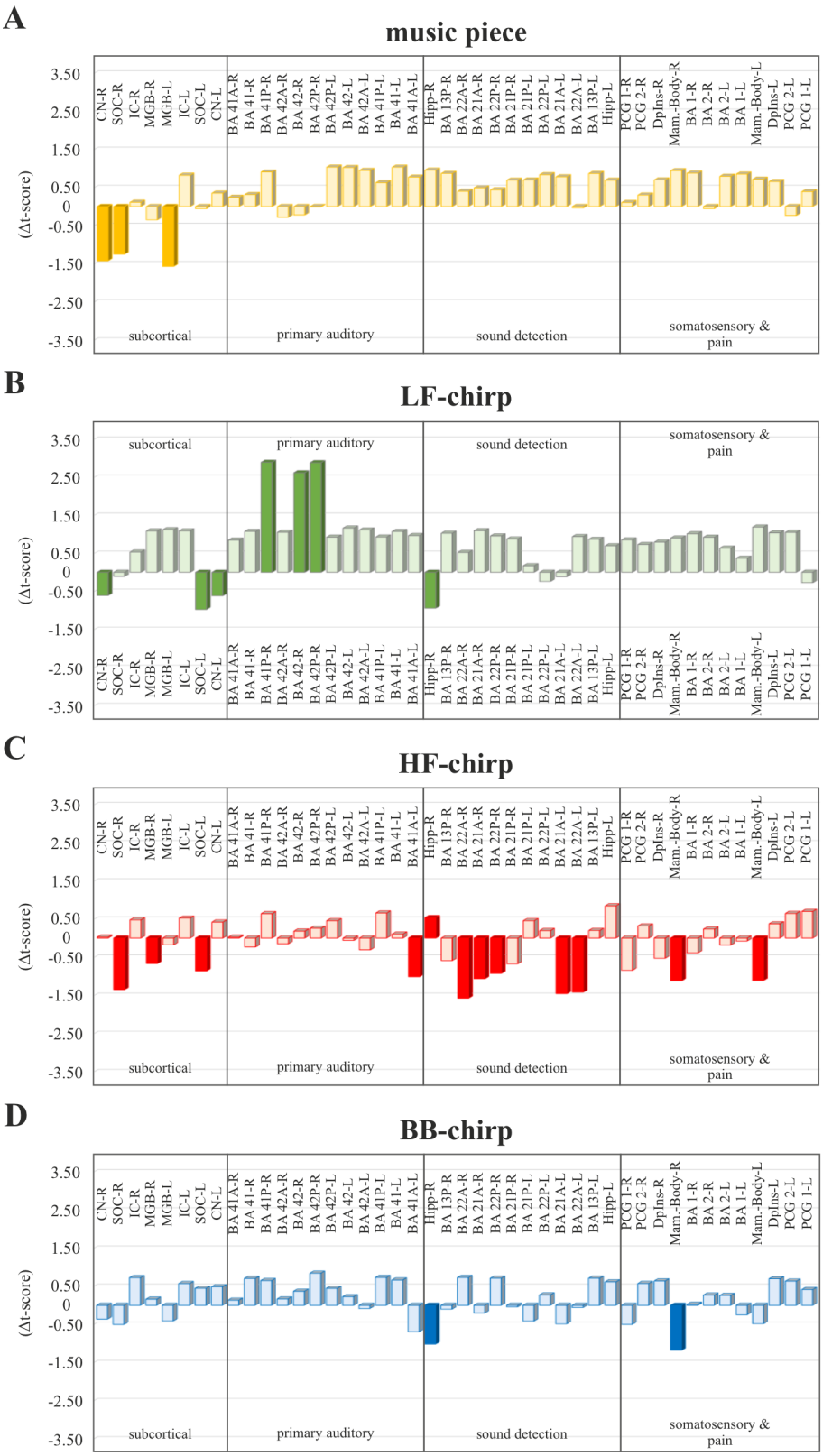

Figure 23 Task-evoked fMRI response differences for group comparison between tinnitus+hyperacusis and the control group

Group comparison for task-evoked BOLD response in the tinnitus+hyperacusis group ($n = 20$) compared to the control group ($n = 43$). The bars showed lower or higher BOLD response in the tinnitus+hyperacusis group compared to the control group for different stimuli in predefined ROIs (**Table 2**). Significant differences ($p < 0.05$, FDR corrected) are highlighted in the full color for every

panel, shaded colors did not reach the significant level. (**A**) music piece stimuli (**B**) LF-chirp stimuli (**C**) HF-chirp stimuli (**D**) BB-chirp stimuli.

In the tinnitus+hyperacusis group (compared to the tinnitus group), we observed for the music piece significant higher BOLD response in primary auditory cortex regions (BA42-L, BA42A-L, BA41P-L, and BA41-L) and in regions responsible for sound detection (BA13P-R, and BA13P-L) (**Figure 24A**). In regions associated with pain (Job, Paucod et al. 2011) as the postcentral gyrus (PCG2-L, PCG1-L) we also observed significant higher BOLD response in the tinnitus+hyperacusis group compared to the tinnitus group.
Significant lower BOLD response to the music piece was observed in subcortical regions (CN-R, and SOC-L) (**Figure 24A**).
For the LF-chirp, we did not observe significant differences between the tinnitus+hyperacusis group and the tinnitus group for BOLD response (F**igure 24B**). But the tinnitus+hyperacusis group showed higher (not significant) activity in all primary auditory cortex regions and in most regions for sound detection and subcortical auditory regions.
For the HF-chirp, we did not find significant higher BOLD response in all inspected regions (**Figure 24C**). Significant lower BOLD response in subcortical regions (SOC-R, and SOC-L) and also in somatosensory regions (Mam.-Body-R, BA1-L, and Mam.-Body-L) was observed (**Figure 24C**).
For the BB-chirp we observed no significant difference (**Figure 24D**), as we observed it for the LF-chirp. The tinnitus+hyperacusis group showed higher (not significant) BOLD response in most primary auditory cortex regions, compared to the tinnitus group (**Figure 24D**).
Summarized, the tinnitus group showed generally lower BOLD response in regions of the primary and secondary auditory cortex in response to acoustic stimuli in comparison to the control group, while the tinnitus+hyperacusis group responded to sound with higher BOLD activity in the primary and secondary auditory cortex compared to the control group.

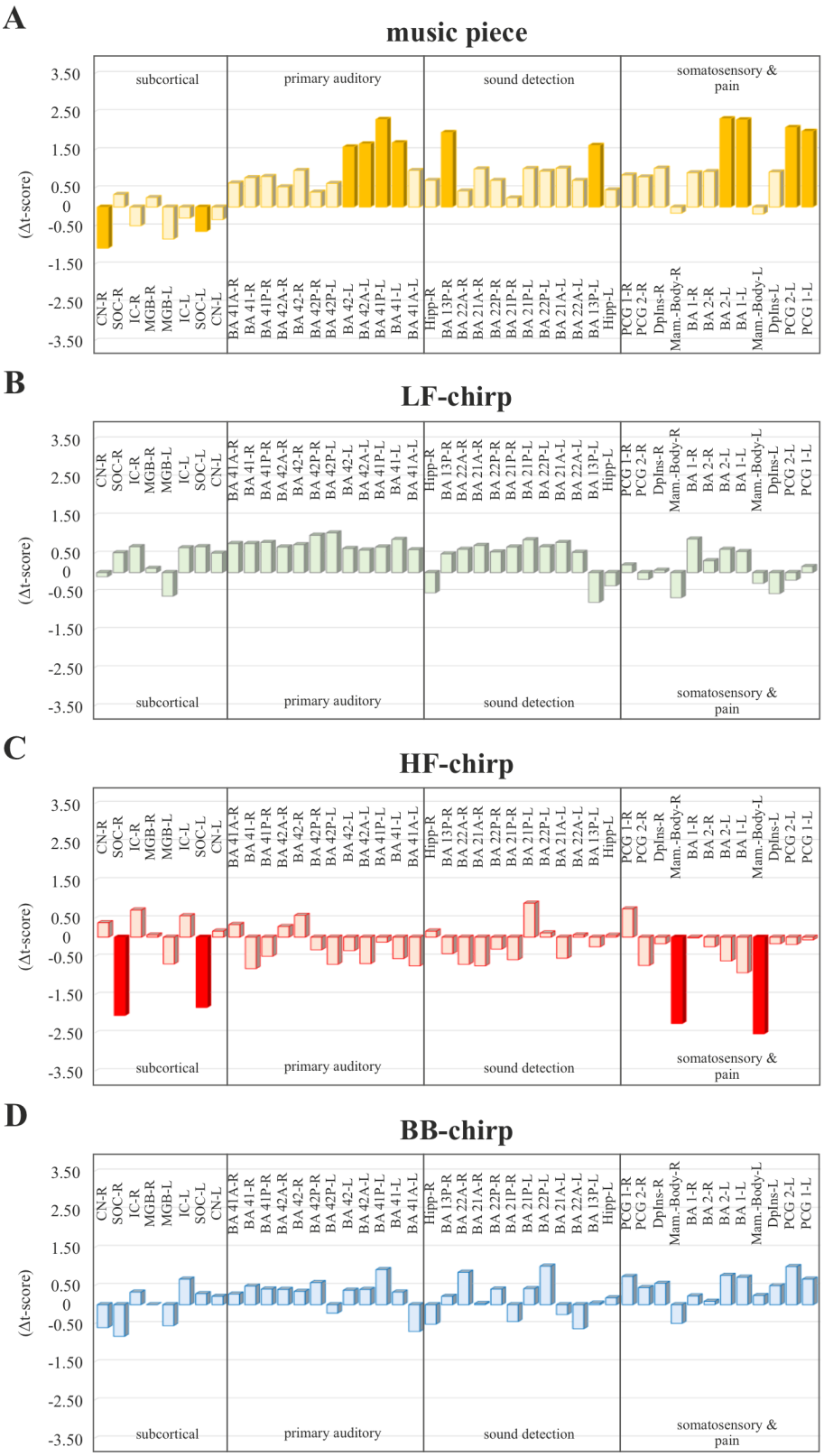

Figure 24 Task-evoked fMRI response differences for group comparison between tinnitus+hyperacusis and the tinnitus group

Group comparison for task-evoked BOLD response in the tinnitus+hyperacusis group ($n = 20$) compared to the tinnitus group ($n = 30$). The bars showed lower or higher BOLD response in the tinnitus+hyperacusis group compared to the tinnitus group for different stimuli in predefined ROIs (**Table 2**). Significant differences ($p < 0.05$, FDR corrected) are highlighted in the full color for every

panel, shaded colors did not reach the significant level. (**A**) music piece stimuli (**B**) LF-chirp stimuli (**C**) HF-chirp stimuli (**D**) BB-chirp stimuli.

3.9 Analysis of cortisol in saliva between the control, tinnitus and tinnitus+hyperacusis group

Since tinnitus is associated with stress (Hébert, Canlon et al. 2012), we analyzed cortisol in saliva to detect possible differences between both groups.

We took three samples of saliva for defined time points (8:00 am, 4:00 pm, and 11:00 pm) and analyzed the time points between the groups and also within the group over the time points.

We observed generally an extreme large distribution of single values within the groups, additionally there is often a measurement limitation for values smaller than 1.1 µg/l especially for time-points at 4:00 and 11:00 pm. These sample values, which could not be determined by the laboratory, were plotted below the groupwise bar and were not contributing to the group mean (**Figure 25**).

The tinnitus+hyperacusis group showed over all time points a slightly higher cortisol level, compared to the tinnitus group, but through the large distribution and the limited sample size no significant difference was found (two-way ANOVA with repeated measurement, for the group comparison $p > 0.05$). For the measured time points at 11:00 pm, only one sample for the tinnitus and tinnitus+hyperacusis group was determined.

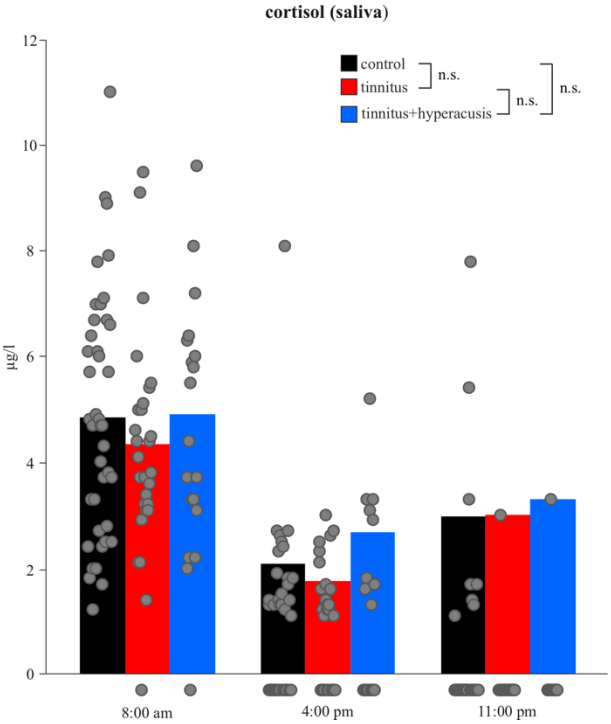

Figure 25 Cortisol in saliva for the control, tinnitus and tinnitus+hyperacusis group

Cortisol level in saliva as mean for the control group (black, *n = 43*), the tinnitus group (red, *n = 30*) and the tinnitus+hyperacusis group (blue, *n = 20*). Single cortisol values for each participant were plotted as grey dots. Lowered grey dots (below the zero-line) are values smaller 1.1 µg/l, which could not be quantified due to technical limitations and were not included in the mean bar chart value.

3.10 Presentation of identified tinnitus+hyperacusis-specific biomarkers

We identified in normal hearing matched participant with tinnitus+hyperacusis, increased early and late brainstem responses together with higher task-evoked BOLD response compared to the control and tinnitus group. The tinnitus group showed instead reduced and prolonged late brainstem responses, with reduced task-evoked BOLD response.

The group of tinnitus+hyperacusis showed significant higher tinnitus burden, compared to the tinnitus group, which was also reflected in slightly higher cortisol level compared to the tinnitus group.

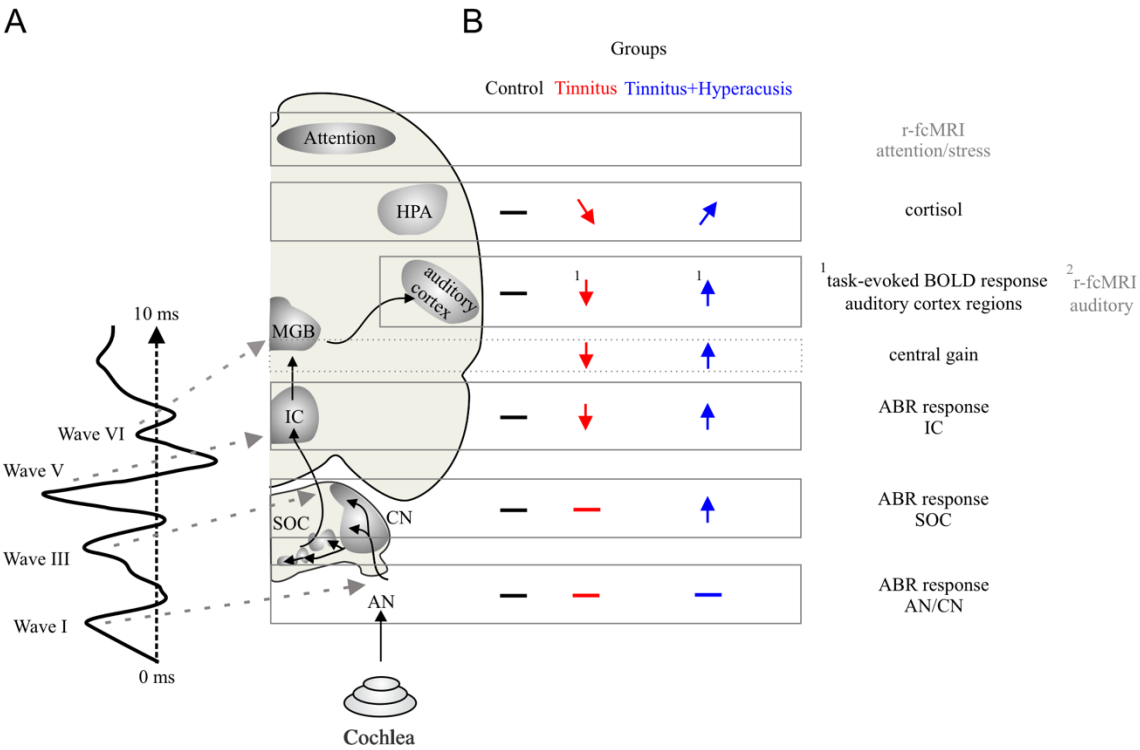

Figure 26 Summarized biomarker for tinnitus and tinnitus+hyperacusis

Identified biomarkers in tinnitus participants for supra-threshold sound induced ABR, task-evoked BOLD fMRI and cortisol analysis. **A** Example of an auditory signal in the brainstem and a schematic drawing of the human ascending auditory pathway. **B** Alterations in the tinnitus and tinnitus+hyperacusis group, compared to the control group for different level of analysis. Modified according to (Möhrle, Hofmeier et al. 2019)

4. Discussion

In the present study functional biomarkers are described that distinguish tinnitus without hyperacusis from tinnitus with hyperacusis in patients. Using audiometry, functional BOLD fMRI and resting state r-fcMRI overall signs of reduced auditory-specific responsiveness was observed as a characteristic feature of tinnitus but not tinnitus+hyperacusis. The details of the finding are discussed in the following for the individual sub-categories identified.

4.1 Tinnitus participants without hyperacusis exhibit reduced and prolonged supra-threshold sound response

In the first part of the study, we excluded patients with the co-occurrence of hyperacusis, to avoid effects from this symptom and included only participants with a tinnitus percept and controls with a normal hearing or a mild hearing loss (< 40 dB). We could not identify any difference in hearing loss between controls and the tinnitus group (**Figure 9**). This confirms previous hypotheses that postulate that hearing loss per se does not lead to tinnitus. This hypothesis was based on observations in animal and humans that demonstrated that tinnitus can occur without a shift in the hearing threshold (Geven, de Kleine et al. 2011, Knipper, Van Dijk et al. 2013, Boyen, de Kleine et al. 2014, Gilles, Schlee et al. 2016, Guest, Munro et al. 2017). Interestingly, our tinnitus participants show a significantly reduced amplitude and prolonged latency for the late auditory brainstem response in the midbrain (wave V – inferior colliculus/lemniscus lateralis) derived from supra-threshold fine structure (**Figure 10**). Reduced and delayed auditory brainstem responses in the tinnitus group were moreover not related to differences in age, which has a similar mean between both groups, nor with the sex, which is also equal distributed in both groups (**Table 8**).

While it is generally accepted, that tinnitus is associated to increased spontaneous firing rates (Jastreboff 1995, Brozoski, Bauer et al. 2002, Bauer, Brozoski et al. 2007, Roberts, Eggermont et al. 2010), the maladaptive mechanism that finally lead to the tinnitus percept is still controversially discussed. In the majority of literature the tinnitus percept is suggested to be the result of increased spontaneous firing rates that are translated to tinnitus through central auditory gain (Roberts, Eggermont et al. 2010, Noreña and Farley 2013, Shore, Roberts et al. 2016). Other studies link tinnitus percept to increased cortical

neural synchrony, cortical tonotopic reorganization or predictive coding errors (Roberts, Eggermont et al. 2010, Schaette and Kempter 2012, Noreña and Farley 2013, Sedley, Friston et al. 2016, Wu, Stefanescu et al. 2016, Gentil, Deverdun et al. 2019, Hullfish, Sedley et al. 2019, Sedley 2019).

In contrast the present study found reduced and delayed sound responsiveness (**Figure 11**, (Hofmeier, Wolpert et al. 2018) in tinnitus patients negative tested for co-occurrence of hyperacusis. Central neural gain would expectedly be correlated with an disproportionally enhanced or recovered late ABR wave as previously described (Schaette and Kempter 2009, Schaette and Kempter 2012). Our results (Hofmeier, Wolpert et al. 2018) thus, cannot confirm the central auditory gain theory. The findings in the present study rather suggest that tinnitus is associated with a loss of central auditory gain. This confirmed previous findings in equally exposed animals with and without tinnitus (Rüttiger, Singer et al. 2013). Strikingly in animals (Möhrle, Hofmeier et al. 2019) and humans (Hofmeier, Wolpert et al. 2018, Hofmeier, Wolpert et al. 2020, in preparation) tinnitus was linked with reduced and delayed late ABR wave responses.

This indicates a contribution of a distinct auditory fiber type that with high-spontaneous firing rates and low activation thresholds (high-SR auditory fibers) (Liberman and Kiang 1978) is suggested to define the shortest sound responses along the ascending auditory path as well as the perceptional hearing threshold (Meddis 2006, Heil, Neubauer et al. 2008). Thereby a loss of high-SR auditory fibers, which may contribute to improved signal-to-noise ratio (Knipper, Van Dijk et al. 2013), could explain reduced and delayed late ABR wave amplitudes observed in the present study (Hofmeier, Wolpert et al. 2018, Möhrle, Hofmeier et al. 2019). Also, latest multi-center clinical trials of tinnitus patients distinguished reduced and delayed ABR wave I amplitudes (Milloy, Fournier et al. 2017, Bramhall, Beach et al. 2019) or unchanged ABR wave I amplitudes (Paul, Bruce et al. 2017, Hofmeier, Wolpert et al. 2018) that also could be explained by loss of high-SR auditory fiber responses.

In conclusion, in contrast to the majority of literature that predict central neural gain as a neural substrate of tinnitus, the present study rather found evidence of reduced central neural gain through reduced and delayed sound induced brainstem responses, as a characteristic feature of tinnitus.

4.2 Tinnitus participants without hyperacusis exhibit lower stimuli-induced BOLD response compared to control participants

The fMRI signal reflects the change in hemodynamics within a macro-scale (1~27 mm^3) brain region, which is related to that region's neural activity through a cascade of events referred to as neurovascular coupling (Raichle and Mintun 2006). Henceforth, a e.g. activation in response to external stimuli as an acoustic sound signal of a population of neurons in a brain region might give rise to a change in the fMRI signal that may represent a neurophysiological process (Parker and Razlighi 2019).

When the control and tinnitus group were exposed to different low, high, broadband or music stimuli, a differential task-evoked response in ROIs in auditory, attentional, emotional networks became visible.

Focusing first on the auditory network, we observed reduced response to sound stimuli for the tinnitus participants compared to the controls in primary and secondary auditory cortex regions to almost all stimuli, but also in the MGB (**Table 10**). Reduced thalamocortical responsiveness between the MGB and auditory cortex was suggested from other imaging studies, in tinnitus (Smith, Mennemeier et al. 2007, Landgrebe, Langguth et al. 2009). Other groups showed reduced GM volume in auditory cortex for tinnitus (Schneider, Andermann et al. 2009, Schecklmann, Lehner et al. 2013) or in the inferior colliculus (Landgrebe, Langguth et al. 2009), which may point to reduced dendritic connections (Teipel, Stahl et al. 2007, Broad, Gabel et al. 2019). While thereby our results and these mentioned previous studies point to lower evoked BOLD responses in the auditory cortex of tinnitus patients, few previous studies also found higher BOLD response to stimuli in tinnitus participants (Lanting, de Kleine et al. 2014), a contrasting finding that may be due to a co-occurrence of hyperacusis with tinnitus (discussed in the next chapters).

Beside the lower BOLD response in the primary and secondary auditory cortex of the tinnitus group, we also observed lower BOLD response in the posterior insula (BA13P) and the hippocampus. The posterior insula, is among others, responsible for sound detection (Sadaghiani, Hesselmann et al. 2009), while the hippocampus contributes to hearing through learning dependent shaping and improving of auditory skills through attention (Kilgard and Merzenich 1998, Kraus and White-Schwoch 2015, Weinberger 2015). Lower BOLD responses in the posterior insula (BA13P), hippocampus and

secondary auditory cortices in response to auditory stimuli (**Table 10**), would thereby point to reduced connectivity of auditory-specific regions to fronto-striatal networks that control memory-linked attentional cues.

In the tinnitus group we observed higher BOLD response to sound in regions which are not associated to sound processing. The anterior insula (BA13A), a part of the limbic system, is known to be involved for distress (Wang, Rao et al. 2005) and also the mammillary body is involved in distress as another study showed (Bubb, Kinnavane et al. 2017). We here demonstrated that both, the anterior insula (BA13A) and mammillary body (**Table 10**) appeared to respond to sound with higher BOLD response. This was also shown in other tinnitus studies (Vanneste, Plazier et al. 2010, Carpenter-Thompson, Akrofi et al. 2014), and supports our findings.

Beside the higher BOLD response for these limbic regions in the tinnitus group, we also observed higher BOLD response in regions of the somatosensory cortex the BA1 and BA2 to sound. This BOLD response could be explained by a loss of specificity for auditory information processing, due to the fact that both regions are anatomically situated near the auditory cortex. The loss of auditory responsiveness in the ascending auditory path may point to less auditory-specific clustering of auditory information.

Overall the lower BOLD responses to auditory stimuli in regions responsible for accurate sound perception in tinnitus patients suggest a causal relationship to the observed reduced and delayed late ABR wave amplitudes in these patients.

This challenges to consider the value of previously suggested therapeutic intervention strategies for tinnitus – as e.g. transcutaneous vagus nerve stimulation (tVNS), in the context of these findings (Meyers, Kasliwal et al. 2019). Yakunina and colleagues (Yakunina, Kim et al. 2018) observed in tinnitus patients higher BOLD response in the CN while stimulating with tVNS, together with decreased BOLD response in limbic brain regions associated for distress, and discussed this method in the context of the common theory of deafferentation in tinnitus on the CN-level (Jastreboff 1995, Norena and Eggermont 2003, Weisz, Moratti et al. 2005, Weisz, Hartmann et al. 2006, Eggermont and Roberts 2012, Eggermont 2015, Shore, Roberts et al. 2016) and the role of the limbic system in the neurophysiological tinnitus model by Jastreboff (Jastreboff 1990). On the basis of our present findings we suggest that is might be challenging to consider for future studies variants of cochlear or brain stimulation devices to cure tinnitus.

4.3 Tinnitus participants without hyperacusis show less functional connectivity in auditory specific regions for resting state fMRI compared to control participants

Beside task-evoked fMRI measurements, where BOLD response after stimulation shows a robustly correlation to neural activity (Logothetis and Pfeuffer 2004, Drew 2019), resting state functional connectivity measurements by fMRI (r-fcMRI) is used to analyze spontaneous activity in anatomically separated brain areas, which have a dependency in temporal neural activity (Van Den Heuvel and Pol 2010, Shen 2015, Lv, Wang et al. 2018). A strongly positive correlated functional connectivity between spatially separated anatomically regions are discussed in the context of short time-lagged neuronal synchronicity. In contrast negative correlated functional connectivity is associated with a larger time-lag and a loss of neuronal synchrony (Goelman, Gordon et al. 2014). There is first evidence that a relation exist between more synchronic correlation in resting state measurements and higher synchronization in task-evoked fMRI, leading to a better sensory performance (Haag, Heba et al. 2015, Parker and Razlighi 2019).

We here observed reduced positive functional connectivity in the tinnitus group, especially for interhemispheric connectivities in auditory-specific regions as the auditory cortex, the MGB (**Figure 12**). Up to now interhemispheric connectivity changes in tinnitus patients were observed only in cortical regions (Kim, Kim et al. 2012, Lanting, de Kleine et al. 2014). Reduced interhemispheric connectivities in the sensory system are discussed in an animal model, to be an indicator for a pre-state of depression (Ben-Shimol, Gass et al. 2015).

We observed in the tinnitus group less positive functional connectivity between auditory cortex regions and regions associated for distress as the anterior insula (BA13A), but also in auditory cortex regions responsible for emotional distress in the temporofrontal cortex, which was as well described in the literature for attentional regions (BA45, BA46, BA47) (Vanneste and De Ridder 2012, Rauschecker, May et al. 2015, Husain 2016, Schmidt, Carpenter-Thompson et al. 2017) and distress regions (Leaver, Turesky et al. 2016, Chen, Xia et al. 2017) (**Figure 13**, **Figure 14**). Our participants with tinnitus did exhibit a low-grade form of tinnitus (Goebel and Hiller 1994), that is currently assumed not to be associated to psychiatric comorbidities (Langguth, Landgrebe et al. 2010). It thus may be interesting in future studies to test if the here observed reduced interhemispheric

connectivity in tinnitus patients with low-grade tinnitus scales, are characteristics of a pre-state of depression in the tinnitus patient group.

Particularly interesting is the differential connectivity of auditory cortex regions to regions in the prefrontal cortex (BA9) in tinnitus patients (**Figure 14**). The prefrontal cortex is suggested to be involved in the regulation of the HPA axis that controls stress responses (Sullivan and Gratton 2002). The observed enhanced positive connectivities between the auditory cortex and the medial part of the prefrontal cortex (BA9M) in tinnitus patients (**Figure 14**) points to the involvement of stress excitation (McKlveen, Myers et al. 2013, Utevsky, Smith et al. 2014, McKlveen, Morano et al. 2016). On the other side our findings demonstrate more negative functional connectivities in the tinnitus group to the dorsolateral part of the prefrontal cortex (BA9DL), that is suggested to reduce stress responses (Shallice, Stuss et al. 2008). Thus, the finding in the present study suggest an imbalance of the HPA-axis.

This finding may be linked to the tendency of higher cortisol levels found in the saliva of the tinnitus group over the day, which may point to a hint for blunted or modified stress controlling mechanism (**Figure 15**).

Numerous previous findings suggested a correlation of tinnitus and stress (Wang, Rao et al. 2005, Vanneste, Plazier et al. 2010, Hébert, Canlon et al. 2012, Mazurek, Haupt et al. 2012, Carpenter-Thompson, Akrofi et al. 2014). These higher stress related correlations, here observed in the tinnitus group, could be linked to the previously described neurophysiological model (Jastreboff 1990), where a negative emotional influence in the limbic system is described, to be essential for the tinnitus on-set.

Functional connectivity analysis for higher auditory cortex regions and regions in limbic and attentional areas are well described in the literature, but lower auditory brainstem regions were not. This could be due to a commonly used parameter for whole brain resting state measurements, where the FOV is aligned to the AC-PC line and often not covering lower auditory brainstem regions as the CN and SOC.

4.4 Tinnitus participants with and without hyperacusis do not differ in middle ear function and hearing threshold

Having now identified reduced auditory-specific functional responsiveness as a characteristic feature of tinnitus patients without co-occurrence of hyperacusis, we next

were curious if patients that exhibit tinnitus with co-occurrence of hyperacusis display similar or different functional characteristics.

Hyperacusis, as idiopathic symptom, can be caused by noise-induced hearing loss and is often associated with headache or forms depression, hormonal/infectious diseases or other factors (Lockwood, Salvi et al. 2002, Goebel and Büttner 2004). While noise-induced hearing loss seems to be one of the biggest risk factors for hyperacusis, it is still not clear how the pathophysiology mechanisms fully work and why hyperacusis is often related to painful experience of mild or moderate sounds. A wide range of the literature assumes similar maladaptive functions like for tinnitus by a central neural hyperactivity (Schaette and Kempter 2006, Gu, Halpin et al. 2010, Auerbach, Rodrigues et al. 2014). Other studies suggest hyperacusis to be linked to a disbalance in the serotonin metabolism (Marriage and Barnes 1995, Gopal, Daly et al. 2000, Pfadenhauer, Weber et al. 2001).

In up to 50 % of the people with tinnitus, hyperacusis occur as comorbidity (Hesse, Rienhoff et al. 1999, Pilgramm, Rychlik et al. 1999). In other cases is occurs as single symptom with prevalence ranging between 2 - 15 % according to different populations (Fabijanska, Rogowski et al. 1999, Pilgramm, Rychlik et al. 1999, Sammeth, Preves et al. 2000, Sheldrake, Diehl et al. 2015).

Important in the context of the present study, no study exists that distinguishes in tinnitus groups, those individual patients with and without a co-occurrence of hyperacusis.

Hypothesizing that various contrasting views about tinnitus may be linked to different sub-entities, we analyzed idiopathic tinnitus symptoms by splitting them into two different groups, using a tinnitus questionnaire (G-H-S) and a hyperacusis questionnaire (HKI) (**Figure 17**). In a first trial, as described previously, we recruited only participants with normal hearing or mild hearing loss, mainly through flyers in the clinic, while participants with a comorbidity of hyperacusis were excluded. For a second trial, we were able to address a larger and younger group of participants, who had to generally better hearing in higher frequencies. Here, the group with hyperacusis as comorbidity beside tinnitus is the third group, alongside the group of tinnitus and control (**Table 11**). We demonstrated that controls and tinnitus with and without the comorbidity of hyperacusis are not distinguishable from hearing thresholds. In all three groups, even up to 10 kHz, there is no hearing loss observed (**Figure 20**). Also, the middle ear function and stapedius reflex measurement is for all participants inconspicuous and seems not to affect the

tinnitus or hyperacusis symptom (**Figure 18**). This supports previous suggestions that tinnitus but also hyperacusis can occur without a shift in the hearing threshold (Geven, de Kleine et al. 2011, Knipper, Van Dijk et al. 2013, Boyen, de Kleine et al. 2014, Gilles, Schlee et al. 2016, Guest, Munro et al. 2017).

For non-audiological characteristics, we found differences for the sex distribution in our groups. We did not recruit participants according to their gender to fill the groups equally. So, it is interesting that we finally got different incidences of sex according to the symptoms of hyperacusis and tinnitus (**Table 11**), while we recruit a control group with nearly the same number of women and men. The tinnitus group contained significantly more men than women, compared to the control group. While vice versa, the group with tinnitus and co-occurrence of hyperacusis contained significantly more women, than the tinnitus group. This may point to a population characteristic for women being affected more by sound over-sensitivity as observed in different studies (Fabijanska, Rogowski et al. 1999, Khalfa, Dubal et al. 2002, de Magalhaes, Fukuda et al. 2003). Previous studies explained this phenomenon with anatomical circumstances, that the ear canal in women is smaller, leading to higher sound condensation for the same applied sound intensity, compared to men (Hellbrück 1983). In this context it is interesting to note that all 7 patients recruited for a study on hyperacusis without co-occurrence of tinnitus (a study not part of this thesis) are women. Even the group of 7 participants is very small, this gives a first hint, according to the literature (Hellbrück 1983, Fabijanska, Rogowski et al. 1999, Khalfa, Dubal et al. 2002, de Magalhaes, Fukuda et al. 2003), that hyperacusis could be gender biased. Conclusively, we currently cannot exclude that unbiased gender differences between groups may limit interpretation of possibly identified differences between groups. However, the previous findings (Hofmeier, Wolpert et al. 2018) with gender matched groups showed similar results to the latest ones with larger groups, leading to the assumption that sex would not affect the results per se.

Using the hyperacusis questionnaire (HKI) (Fischer 2013) the tinnitus and tinnitus+hyperacusis group could be clearly distinguished. We moreover surprisingly observed that the overall tinnitus score as well as the sub scores of the tinnitus questionnaire (G-H-S), giving information about the emotional and cognitive distress, annoyance, penetrants, sleeping disturbance and somatic complaints were significantly higher in the tinnitus+hyperacusis group (**Figure 17**). This finding appeared to be related

to differences in the tinnitus ratings between groups that evidenced in > 90 % of the Tinnitus group (29 of 30) a mild low-grade (I-II) tinnitus grade (Goebel and Hiller 1994, Goebel and Floezinger 2008), while – in contrast – in only 60 % of the tinnitus+hyperacusis group mild tinnitus grade I-II was identified (12 of 20) (**Figure 17**, **Table 12**, **Table 13**). Also, in the tinnitus group the tinnitus percept occurred with a prevalence of 60 % bilateral, 20 % right-side and 20 % left-side, while in the tinnitus+hyperacusis group, the tinnitus percept occurred with a prevalence 80 % bilateral and only 5 % on right, 15 % on left-side (**Table12**, **Table 13**). The tinnitus group perceived tinnitus generally bilateral, with nearly the same high frequency (mean around 6 kHz) and intensity as a sine-tone-like percept, on both sides (**Table 12**). This frequency range is also seen in the literature (Meikle and Griest 1992), where more than 50 % of tinnitus participants perceived their tinnitus at frequencies from 3.5 - 8.5 kHz. The group with tinnitus and hyperacusis as comorbidity had nearly the same frequency and intensity for the tinnitus localization as the tinnitus group.

An elevated tinnitus questionnaire score through co-occurrence of hyperacusis was observed in previous studies (Gabriels 1995, Jastreboff and Jastreboff 2000). Tinnitus patients with the comorbidity of hyperacusis are also affected more by clinical indicated forms of depression and anxiety, compared to patients with tinnitus (Goebel and Floezinger 2008), providing a possible rational for the elevated prevalence of lower tinnitus grades in the tinnitus+hyperacusis group. While the penetrance and influence of tinnitus on the patient's life is correlated to the tinnitus intensity (Aazh and Salvi 2019), the difference we saw in the tinnitus questionnaire is due to the impact of hyperacusis, according to the fact that we saw no difference for the measured tinnitus intensity in the tinnitus localization measurement. Conclusively, The co-occurence of hyperacusis to tinnitus appears to worsen signficantly the tinnitus score for emotional and cognitive distress, annoyance, penetrance, sleep disturbance and somatic complaints, linked to a lower prevalence of low-grade mild tinnitus score and higher prevalence of bilateral tinnitus percept.

4.5 Tinnitus participants with hyperacusis show enhanced early and late supra-threshold ABR compared to the control group and tinnitus group

Although no difference in hearing thresholds was observed between the tinnitus group and tinnitus+ hyperacusis groups, a significant reduced amplitude and prolonged latency for the late auditory brainstem response in the midbrain (wave V – inferior colliculus/lemniscus lateralis) was observed in the tinnitus groups upon supra-threshold fine structure analysis (**Figure 21**). The tinnitus+hyperacusis group exhibited reduced latencies in comparison to tinnitus and rather enhanced amplitudes for the late auditory brainstem response in the midbrain (wave V – inferior colliculus/lemniscus lateralis) and the early ABR wave III (superior olivary complex) compared to the control group. As the participants of all groups have no differences in hearing thresholds, even in the highest measured frequencies, a link to differences in late supra-threshold ABR waves between groups cannot be associated with differences in basic hearing function.

We here suggest that these identified differences in late ABR wave amplitude and latencies between tinnitus and tinnitus+hyperacusis may explain the numerous existing differences in sound responsiveness described for tinnitus groups. Central ABR in tinnitus patients were either not reduced (ABR wave V inferior colliculus/lemniscus lateralis) or even increased (Schaette and McAlpine 2011, Gu, Herrmann et al. 2012, Bramhall, Konrad-Martin et al. 2018).

Conclusively, among the participants with and without tinnitus, that did not differ in hearing thresholds, the tinnitus group showed a significantly reduced and delayed ABR wave V, a feature that would argue for reduced, rather than elevated, response gain in the ascending auditory pathway of the tinnitus group.

4.6 Tinnitus participants with hyperacusis show higher BOLD response, compared to the control and tinnitus group

Task-evoked fMRI measurements in the auditory cortex to music stimuli were significantly lower in the tinnitus group but not in the tinnitus+hyperacusis group. The lower acoustic stimuli-induced BOLD responses in tinnitus was also seen in our previous study (Hofmeier, Wolpert et al. 2018), (**Table 10**). The differences in BOLD responses to music stimuli in the auditory cortex may mirror functional sound response changes reflected contrasting late ABR wave amplitudes between both groups. The same may be

realized for LF-chirp stimuli that exhibit significantly higher BOLD responses in the auditory cortex of tinnitus+hyperacusis but not tinnitus patients (**Figure 23).** In other predefined ROIs, higher activity was observed in the tinnitus+hyperacusis group compared to the control group, but they did not reach the significance level in most cases. These findings strongly indicate that numerous studies that describe either higher responses in auditory cortex regions of tinnitus animals (Roberts, Eggermont et al. 2010), or e.g. reduced grey matter volume in auditory cortex for tinnitus (Schneider, Andermann et al. 2009, Schecklmann, Lehner et al. 2013) or the inferior colliculus (Landgrebe, Langguth et al. 2009), or higher BOLD fMRI activities in cortical or subcortical regions of tinnitus patients (Gu, Halpin et al. 2010, Husain 2016) may describe a mixture of patient groups that cover either tinnitus with and without hyperacusis.

As a next interesting finding we observed that regions responsible for sound sensitivity or attention showed likewise lower BOLD responses in the tinnitus group and tinnitus+hyperacusis groups, although in the latter to a significant higher extend. Interestingly, for the HF-chirp stimulus, both tinnitus groups show significantly lower activity in regions of sound detection (**Figure 22**, **Figure 23**).

In the comparison between the tinnitus+hyperacusis group and the tinnitus group, also for the music piece stimulus, the tinnitus+hyperacusis group showed significant higher activity in primary and secondary auditory cortex regions (BA42, BA42A, BA41P, BA41), regions for sound detection like the posterior insula (BA13P), and regions in the somatosensory cortex/pain associated areas (BA1, BA2, PCG1 and PCG2) (**Figure 23**).

Conclusively, we here could first distinguish tinnitus and tinnitus+hyperacusis through sound induced BOLD responses. The lower music-induced BOLD fMRI activities in the auditory cortex of tinnitus but not tinnitus+hyperacusis groups suggest that tinnitus is not linked to central neural gain, as still discussed in numerous tinnitus literature. Higher LF chirp stimulus induced BOLD responses in the auditory cortex of tinnitus+hyperacusis may motivate to consider that numerous animal and clinical studies describing characteristics of tinnitus, have identified instead characteristics of the underlying entity hyperacusis.

It is also interesting, that in a context of distress or burden associated with tinnitus, the somatosensory regions in the postcentral gyrus show also higher responses in BOLD to visual stimuli, depicting painful situations (Gu and Han 2007, Decety, Michalska et al.

2008) or also with tactile painful stimulation (Bingel, Lorenz et al. 2004). A relation to pain-associated regions can be an explanation for our observations of higher BOLD response in somatosensory regions for participants with tinnitus+hyperacusis, because the over-sensitivity to sound can also be painful.

The lower HF chirp-induced BOLD responses in tinnitus and even more pronounced in tinnitus+hyperacusis (**Figure 23**) suggest that both entities are linked to reduced auditory-specific resolution or capacities to properly focus attention to the tone through a memory-linked facilitation process (Irvine 2018).

4.7 No difference for cortisol in saliva was observed between the groups

While we observed in the smaller tinnitus group a tendency of higher cortisol level in saliva during the day, compared to the controls, in the larger groups we observe even a slight reduction, even though the means are not significantly different due to the large deviation of the single values (**Figure 25**).

Interestingly for the control group data, we could not observe the typical circadian rhythm, with a flatten of the cortisol level over the day/night (Oster, Challet et al. 2017). Instead outlier seem to affect the mean value for the 11.00 pm time-point in the control group, therefore larger groups are recommended.

The tinnitus group with hyperacusis appeared in the afternoon with a slightly higher average cortisol level than the control group and even the tinnitus group, additionally also slightly higher in the morning, compared to the tinnitus group. This may reflect the higher burden that we postulate from the results score of the tinnitus questionnaire (**Figure 17**), and from observations by other research groups, that tinnitus patients suffer more with an additional hyperacusis than with a tinnitus percept only (Gabriels 1995, Jastreboff and Jastreboff 2000).

Conclusion

The finding in the present study evidenced for the first-time objective differences between tinnitus and tinnitus+hyperacusis. Up to now no study exists that subdivide the tinnitus group in clinical trials to identify diagnostic or therapeutic intervention strategies for tinnitus independent of co-occurrence of hyperacusis. Differences between groups could be identified through audiometric differences as well as task-evoked BOLD responses

from fMRI measurements. In the present study spontaneous r-fcMRI activities are shown for the tinnitus group only. Although not shown in the present thesis, the study provided evidence that r-fcMRI activities in tinnitus+hyperacusis groups may significantly contribute to a differential diagnosis as well.

The study thus contributes to identification of distinguished characteristics for sub-entities behind tinnitus. In future animal and human studies, this sub-classification should be strictly followed, to potentially support the monitoring of therapeutically approaches and accelerate the development of a roadmap to cure tinnitus with and without hyperacusis.

5. Summary

Tinnitus is known as the sound people perceive, without an external stimulation. It affects around 15 – 20 % of the population (Baguley, McFerran et al. 2013, Bauer 2018). Until now, there is no single treatment to eliminate the tinnitus percept permanently (Zenner, Delb et al. 2017), which is also owed by the fact, that the pathophysiological mechanisms are poorly understood and controversial debated. The majority of tinnitus literature suggests the tinnitus percept to be the result of elevated spontaneous activity that is translated to a tinnitus percept by neural gain. Few other studies – including our own – suggest tinnitus rather to be linked to reduced central gain. We here hypothesized that contrasting views about the neural correlate of tinnitus may be due to possible comorbidities as hyperacusis that might be linked to central activity patterns different from tinnitus and thereby might hide characteristics of tinnitus-specific brain activities. To test this hypothesis, we analyzed participants with tinnitus, tinnitus+hyperacusis and control participants, using different methods from audiometry (pure tone audiometry, auditory brainstem response measurements), imaging (evoked and resting state fMRI) and analysis body fluids (cortisol in saliva) to possibly identify differences in tinnitus and tinnitus+hyperacusis specific characteristics. Participants with severe hearing loss or neurological disorder were excluded.

We showed, that neither the middle ear function nor the hearing threshold was different between control, tinnitus or tinnitus+hyperacusis group. However, emotional, cognitive distress, annoyance, penetrance, sleep disturbance and social complaints as sub scores identified through a tinnitus questionnaire were significantly elevated in the tinnitus+hyperacusis group in comparison to the tinnitus group. While tinnitus was characterized by reduced and delayed supra-threshold sound induced brainstem responses to click stimuli, the tinnitus+hyperacusis group exhibited elevated and rather shortened sound induced brainstem responses in comparison to the tinnitus and control group. The differences remained unaffected by non-audiometric parameters like age, sex or handedness. Reduced and delayed sound induced brainstem responses in the tinnitus group were reflected by auditory task evoked BOLD response in the auditory cortex to music stimuli and reduced spontaneous resting state r-fcMRI response in ascending auditory nuclei. In contrast the slightly higher sound evoked ABR brainstem responses in the tinnitus+hyperacusis group were reflected in higher low-frequency (LF)-chirp stimuli

induced BOLD responses in the auditory cortex regions. In both groups high-frequency (HF)-chirp stimuli resulted in significant reduced response pattern in regions responsible for sound sensitivity like the posterior insula (BA13P) and the hippocampus, although with a significant higher extend in tinnitus+hyperacusis groups. This indicates that tinnitus and tinnitus+hyperacusis exhibit distinguished and rather contrasting response patterns in the auditory path. In contrast both groups exhibit an obviously reduced capacity to properly discriminate and focus attention to tones, a feature that is more pronounced when hyperacusis co-occurs with tinnitus.

Summarized, these results suggest that tinnitus is linked to reduced central auditory gain. The study also unravels that the comorbidity of hyperacusis with tinnitus may be possibly linked to enhanced central response patterns. Thereby, we suggest that previous assumption of central neural gain as a neural correlate of tinnitus may have described a neural correlate that is rather characteristic for hyperacusis than for tinnitus. Finally, the study identifies first objective functional biomarkers that have the potential to be used in clinical trials to sub-classify tinnitus and hyperacusis and thereby improve therapeutic intervention strategies for both entities.

We here overall discuss and summarize studies shown in (Hofmeier, Wolpert et al. 2018, Möhrle, Hofmeier et al. 2019, Hofmeier, Wolpert et al. 2020, in preparation).

German Summary

Tinnitus ist eine auditorische Phantomwahrnehmung die bis zu 15 - 20 % der Bevölkerung betrifft (Baguley, McFerran et al. 2013, Bauer 2018). Bis heute gibt es keine Kausaltherapie (Zenner, Delb et al. 2017). Der nach wie vor fehlenden Therapieansatz ist u.a. der Tatsache geschuldet, dass die pathophysiologischen Mechanismen von Tinnitus bis heute nicht genau verstanden sind und zudem die Evidenzen kontrovers diskutiert werden. In der Mehrheit der Tinnitus-Literatur wird die Meinung vertreten, dass die Tinnituswahrnehmung das Ergebnis einer erhöhten spontanen Nervenaktivität ist, die durch neuronale Verstärkung zu einer kortikalen Tinnituswahrnehmung führt. Nur wenige andere Studien – einschließlich unserer eigenen – vermuten, dass Tinnitus eher durch ein vermindertes, auditorisch-spezifisches, zentrales Antwortverhalten und erhöhtes Rauschen verursacht wird, das unspezifisch verstärkt und zur Tinnitusperzeption führt. Wir haben hier die Hypothese aufgestellt, dass die konträren Ansichten über das neuronale Korrelat von Tinnitus auf mögliche Komorbiditäten wie Hyperakusis zurückzuführen sein könnten – welche die Tinnitus-spezifischen Merkmale der Gehirnaktivität überlagern. Um diese Hypothese zu überprüfen, untersuchten wir Kontrollprobanden und Probanden mit Tinnitus im Vergleich zu Probanden mit Tinnitus+Hyperakusis. Es wurde ein multimodaler Methodenansatz gewählt, der Audiometrie (Reintonaudiometrie, akustische Hirnstammaudiometrie), bildgebende Verfahren (evozierter fMRT-Messung wie auch Ruhe-fMRT-Messung) und die Analyse von Stresshormonen (Cortisol im Speichel) kombiniert. Teilnehmer mit einem größeren Hörverlust (> 40 dB) ebenso wie Patienten mit neurologischen Erkrankungen wurden zur Gruppenharmonisierung ausgeschlossen.

Wir zeigten, dass weder die Mittelohrfunktion noch die Hörschwelle zwischen der Kontroll-, Tinnitus- oder Tinnitus+Hyperakusis-Gruppe unterschiedlich ist, d.h. die Hörschwelle selbst kein charakteristisches Kriterium der Sub-Entitäten darstellt. Ein erster Unterschied zwischen den Gruppen zeigte sich jedoch schon für bei der Analyse des Tinnitusfragebogens. Sämtliche Belastungskriterien für Tinnitus wie emotionale und kognitive Beeinträchtigungen, emotionale Schwere und Penetranz durch den Tinnitus, Schlafstörungen und soziale Beeinträchtigungen waren in der Tinnitus+Hyperakusis-Gruppe im Vergleich zur Tinnitus-Gruppe signifikant erhöht. Tinnitusprobanden zeigten

ein reduziertes und verzögertes überschwelliges Antwortverhalten auf akustische Klicks, während die Tinnitus+Hyperakusis-Gruppe im Vergleich zur Tinnitus- und Kontrollgruppe eher erhöhte, verkürzte Antworten für Klicks zeigte. Die Unterschiede blieben unbeeinflusst von nicht-audiometrischen Parametern wie Alter, Geschlecht oder Händigkeit. Die reduzierte und verzögerte akustische Hirnstammantwort in der Tinnitus-Gruppe spiegelte sich in einer verminderten Stimulus-induzierten BOLD fMRT-Antwort im auditorischen Kortex auf Musikstimuli wider und ging mit einer verminderten spontanen Ruheaktivität (r-fcMRI-Reaktion) in der aufsteigenden Hörbahn einher. Im Gegensatz dazu wurden die geringfügig höheren akustisch evozierten BERA-Hirnstammantwort in der Tinnitus+Hyperakusis-Gruppe durch höhere niederfrequente Chirp-induzierte BOLD fMRT-Antwort in den Regionen des auditorischen Kortex reflektiert. In beiden Gruppen führten hochfrequente Chirp-Stimuli zu einer signifikant reduzierten Antwort in Regionen, die für gedächtnisabhängige Diskriminationsschärfe verantwortlich sind, wie die posteriore Insula (BA13P) und der Hippocampus, allerdings mit einem signifikant höheren Ausmaß in der Tinnitus+Hyperakusisgruppe. Dies deutet darauf hin, dass Tinnitus und Tinnitus+Hyperakusis bezüglich des zentralen Antwortverhaltens der aufsteigenden Hörbahn offenbar gegensätzlich sind. Im Gegensatz dazu zeigen beide Gruppen gemeinsam eine offensichtlich verminderte Fähigkeit, Töne richtig zu unterscheiden und die Aufmerksamkeit auf Töne zu lenken – ein Merkmal, das ausgeprägter ist, wenn Hyperakusis als Co-Morbidität mit Tinnitus auftritt.

Zusammengefasst deuten diese Ergebnisse darauf hin, dass die frühere Annahme der zentralen neuronalen Verstärkung als neuronales Korrelat von Tinnitus offenbar fälschlicherweise ein neuronales Korrelat von gleichzeitig auftretender Hyperakusis beschrieben hat, anstatt ein eindeutiges Tinnitusmerkmal. Die Studie identifiziert erstmals objektive funktionelle Biomarker für Tinnitus mit und ohne Hyperakusis, die das Potenzial haben, in klinischen Studien verwendet zu werden und kausale therapeutische Interventionsstrategien für Tinnitus mit und ohne Hyperakusis zu verbessern.

Wir diskutieren und fassen hier Studien zusammen, die in (Hofmeier, Wolpert et al. 2018, Möhrle, Hofmeier et al. 2019, Hofmeier, Wolpert et al. 2020, in Vorbereitung) gezeigt wurden.

Literature

Aazh, H. and R. Salvi (2019). "The relationship between severity of hearing loss and subjective tinnitus loudness among patients seen in a specialist tinnitus and hyperacusis therapy clinic in UK." Journal of the American Academy of Audiology **30**(8): 712-719.

Anari, M., A. Axelsson, A. Eliasson and L. Magnusson (1999). "Hypersensitivity to sound: questionnaire data, audiometry and classification." Scandinavian audiology **28**(4): 219-230.

Ashburner, J. and K. J. Friston (2005). "Unified segmentation." Neuroimage **26**(3): 839-851.

Auerbach, B. D., P. V. Rodrigues and R. J. Salvi (2014). "Central gain control in tinnitus and hyperacusis." Frontiers in neurology **5**: 206.

Axelsson, A. and A. Ringdahl (1989). "Tinnitus--a study of its prevalence and characteristics." Br J Audiol **23**(1): 53-62.

Baguley, D., D. McFerran and D. Hall (2013). "Tinnitus." Lancet **382**(9904): 1600-1607.

Bauer, C. A. (2018). "Tinnitus." N Engl J Med **378**(13): 1224-1231.

Bauer, C. A., T. J. Brozoski and K. Myers (2007). "Primary afferent dendrite degeneration as a cause of tinnitus." Journal of neuroscience research **85**(7): 1489-1498.

Ben-Shimol, E., N. Gass, B. Vollmayr, A. Sartorius and G. Goelman (2015). "Reduced connectivity and inter-hemispheric symmetry of the sensory system in a rat model of vulnerability to developing depression." Neuroscience **310**: 742-750.

Bernal, B., N. R. Altman and L. S. Medina (2004). "Dissecting nonverbal auditory cortex asymmetry: an fMRI study." International journal of neuroscience **114**(5): 661-680.

Bingel, U., J. Lorenz, V. Glauche, R. Knab, J. Glascher, C. Weiller and C. Buchel (2004). "Somatotopic organization of human somatosensory cortices for pain: a single trial fMRI study." Neuroimage **23**(1): 224-232.

Boyen, K., E. de Kleine, P. van Dijk and D. R. Langers (2014). "Tinnitus-related dissociation between cortical and subcortical neural activity in humans with mild to moderate sensorineural hearing loss." Hearing research **312**: 48-59.

Bramhall, N., E. F. Beach, B. Epp, C. G. Le Prell, E. A. Lopez-Poveda, C. J. Plack, R. Schaette, S. Verhulst and B. Canlon (2019). "The search for noise-induced cochlear synaptopathy in humans: Mission impossible?" Hearing research **377**: 88-103.

Bramhall, N. F., D. Konrad-Martin and G. P. McMillan (2018). "Tinnitus and Auditory Perception After a History of Noise Exposure: Relationship to Auditory Brainstem Response Measures." Ear and Hearing **39**(5): 881-894.

Broad, R. J., M. C. Gabel, N. G. Dowell, D. J. Schwartzman, A. K. Seth, H. Zhang, D. C. Alexander, M. Cercignani and P. N. Leigh (2019). "Neurite orientation and dispersion density imaging (NODDI) detects cortical and corticospinal tract degeneration in ALS." J Neurol Neurosurg Psychiatry **90**(4): 404-411.

Brozoski, T., C. Bauer and D. Caspary (2002). "Elevated fusiform cell activity in the dorsal cochlear nucleus of chinchillas with psychophysical evidence of tinnitus." Journal of Neuroscience **22**(6): 2383-2390.

Bubb, E. J., L. Kinnavane and J. P. Aggleton (2017). "Hippocampal–diencephalic–cingulate networks for memory and emotion: An anatomical guide." Brain and neuroscience advances **1**: 2398212817723443.

Campbell, K., T. Picton, R. Wolfe, J. Maru, J. Baribeau-Braun and C. Braun (1981). "Auditory potentials." Sensus **1**: 21-31.

Carpenter-Thompson, J. R., K. Akrofi, S. A. Schmidt, F. Dolcos and F. T. Husain (2014). "Alterations of the emotional processing system may underlie preserved rapid reaction time in tinnitus." Brain research **1567**: 28-41.
Chan, Y. (2009). "Tinnitus: etiology, classification, characteristics, and treatment." Discov Med **8**(42): 133-136.
Chen, Y. C., W. Xia, H. Chen, Y. Feng, J. J. Xu, J. P. Gu, R. Salvi and X. Yin (2017). "Tinnitus distress is linked to enhanced resting-state functional connectivity from the limbic system to the auditory cortex." Human brain mapping **38**(5): 2384-2397.
Cieslik, E. C., V. I. Mueller, C. R. Eickhoff, R. Langner and S. B. Eickhoff (2015). "Three key regions for supervisory attentional control: evidence from neuroimaging meta-analyses." Neuroscience & biobehavioral reviews **48**: 22-34.
Clare, S. (1997). Functional MRI: Methods and Applications. Doctor of Philosophy, University of Nottingham.
de Magalhaes, S. L. B., Y. Fukuda, R. I. G. Liriano, F. A. I. Chami, F. Barros and F. L. Diniz (2003). "Relation of hyperacusis in sensorineural tinnitus patients with normal audiological assessment." International Tinnitus Journal **9**(2): 79-83.
Decety, J., K. J. Michalska and Y. Akitsuki (2008). "Who caused the pain? An fMRI investigation of empathy and intentionality in children." Neuropsychologia **46**(11): 2607-2614.
Demeester, K., A. van Wieringen, J. J. Hendrickx, V. Topsakal, E. Fransen, L. Van Laer, D. De Ridder, G. Van Camp and P. Van de Heyning (2007). "Prevalence of tinnitus and audiometric shape." B-ent **3 Suppl 7**: 37-49.
Drew, P. J. (2019). "Vascular and neural basis of the BOLD signal." Current opinion in neurobiology **58**: 61-69.
Eggermont, J. (2015). "Neural substrates of tinnitus in animal and human cortex." hno **63**(4): 298-301.
Eggermont, J. J. and L. E. Roberts (2012). "The neuroscience of tinnitus: understanding abnormal and normal auditory perception." Frontiers in systems neuroscience **6**: 53.
Eggermont, J. J., F.-G. Zeng, A. N. Popper and R. R. Fay (2012). Tinnitus, Springer Science & Business Media.
Fabijanska, A., M. Rogowski, G. Bartnik and H. Skarzynski (1999). Epidemiology of tinnitus and hyperacusis in Poland. Proceedings of the sixth international tinnitus seminar, Citeseer.
Fischer, A. (2013). "Hyperakusis: Neues Screening-Instrument vorgestellt." HNO Nachrichten **43**(3): 38-38.
Fobel, O. and T. Dau (2004). "Searching for the optimal stimulus eliciting auditory brainstem responses in humans." J Acoust Soc Am **116**(4 Pt 1): 2213-2222.
Gabriels, P. (1995). Tinnitus/Hyperacusis and the family. Fifth International Tinnitus Seminar.
Gentil, A., J. Deverdun, N. Menjot de Champfleur, J.-L. Puel, E. Le Bars and F. Venail (2019). "Alterations in regional homogeneity in patients with unilateral chronic tinnitus." Trends in hearing **23**: 2331216519830237.
Geven, L. I., E. de Kleine, R. H. Free and P. Van Dijk (2011). "Contralateral suppression of otoacoustic emissions in tinnitus patients." Otology & Neurotology **32**(2): 315-321.
Gilles, A., W. Schlee, S. Rabau, K. Wouters, E. Fransen and P. Van de Heyning (2016). "Decreased speech-in-noise understanding in young adults with tinnitus." Frontiers in neuroscience **10**: 288.

Goebel, G., E. Biesinger, W. Hiller and K. Greimel (2005). Der Schweregrad des Tinnitus. Tinnitus, Springer**:** 19-42.
Goebel, G. and U. Büttner (2004). "Grundlagen zu Tinnitus: Diagnostik und Therapie." psychoneuro **30**(06): 322-329.
Goebel, G. and U. Floezinger (2008). "Pilot study to evaluate psychiatric co-morbidity in tinnitus patients with and without hyperacusis." Audiological Medicine **6**(1): 78-84.
Goebel, G. and W. Hiller (1994). "Tinnitus-Fragebogen (TF). Standardinstrument zur Graduierung des Tinnitusschweregrades. Erbebnisse einer Multicenterstudie mit dem Tinnitus-Fragebogen (TF)." HNO. Hals-, Nasen-, Ohrenärzte **42**(3): 166-172.
Goelman, G., N. Gordon and O. Bonne (2014). "Maximizing negative correlations in resting-state functional connectivity MRI by time-lag." PloS one **9**(11).
Gopal, K. V., D. M. Daly, R. G. Daniloff and L. Pennartz (2000). "Effects of selective serotonin reuptake inhibitors on auditory processing: case study." JOURNAL-AMERICAN ACADEMY OF AUDIOLOGY **11**(8): 454-463.
Gu, J. W., C. F. Halpin, E.-C. Nam, R. A. Levine and J. R. Melcher (2010). "Tinnitus, diminished sound-level tolerance, and elevated auditory activity in humans with clinically normal hearing sensitivity." Journal of neurophysiology **104**(6): 3361-3370.
Gu, J. W., B. S. Herrmann, R. A. Levine and J. R. Melcher (2012). "Brainstem Auditory Evoked Potentials Suggest a Role for the Ventral Cochlear Nucleus in Tinnitus." Journal of the Association for Research in Otolaryngology **13**(6): 819-833.
Gu, X. and S. Han (2007). "Neural substrates underlying evaluation of pain in actions depicted in words." Behav Brain Res **181**(2): 218-223.
Guest, H., K. J. Munro, G. Prendergast, S. Howe and C. J. Plack (2017). "Tinnitus with a normal audiogram: Relation to noise exposure but no evidence for cochlear synaptopathy." Hearing Research **344**: 265-274.
Guimaraes, A. R., J. R. Melcher, T. M. Talavage, J. R. Baker, P. Ledden, B. R. Rosen, N. Y. Kiang, B. C. Fullerton and R. M. Weisskoff (1998). "Imaging subcortical auditory activity in humans." Hum Brain Mapp **6**(1): 33-41.
Haag, L. M., S. Heba, M. Lenz, B. Glaubitz, O. Höffken, T. Kalisch, N. A. Puts, R. A. Edden, M. Tegenthoff and H. Dinse (2015). "Resting BOLD fluctuations in the primary somatosensory cortex correlate with tactile acuity." Cortex **64**: 20-28.
Hébert, S., B. Canlon and D. Hasson (2012). "Emotional exhaustion as a predictor of tinnitus." Psychotherapy and psychosomatics **81**(5): 324.
Heil, P., H. Neubauer, M. Brown and D. R. Irvine (2008). "Towards a unifying basis of auditory thresholds: distributions of the first-spike latencies of auditory-nerve fibers." Hearing research **238**(1-2): 25-38.
Hellbrück, J. (1983). "GESCHLECHTSSPEZIFISCHE UNTERSCHIEDE IN DER LAUTSTAERKENEMPFINDUNG: REALITAET ODER ARTEFAKT."
Hesse, G., N. Rienhoff, M. Nelting and D. Brehmer (1999). "Medikamentenkosten bei Patienten mit chronisch komplexen Tinnitus." HNO **47**(7): 658-660.
Hofmeier, B., S. Wolpert, E. S. Aldamer, M. Walter, J. Thiericke, C. Braun, D. Zelle, L. Rüttiger, U. Klose and M. Knipper (2018). "Reduced sound-evoked and resting-state BOLD fMRI connectivity in tinnitus." NeuroImage: Clinical **20**: 637-649.
Hofmeier, B., S. Wolpert, L. Rüttiger, U. Klose and M. Knipper (2020, in preparation). "in preparation."
Hoth, S. (2016). "Der Freiburger Sprachtest." HNO **64**(8): 540-548.
Hullfish, J., W. Sedley and S. Vanneste (2019). "Prediction and perception: insights for (and from) tinnitus." Neuroscience & Biobehavioral Reviews.

Husain, F. T. (2016). "Neural networks of tinnitus in humans: elucidating severity and habituation." Hearing research **334**: 37-48.
Irvine, D. R. (2018). "Plasticity in the auditory system." Hearing research **362**: 61-73.
Janssen, T. (2000). "Schwellennahe und überschwellige Schallverarbeitung des Innenohres, Teil I: Physiologie und Pathophysiologie." Z Audiol **39**(4): 100-117.
Jastreboff, P. J. (1990). "Phantom auditory perception (tinnitus): mechanisms of generation and perception." Neuroscience research **8**(4): 221-254.
Jastreboff, P. J. (1995). "Salicylate-induced abnormal activity in the inferior colliculus of rats." Hearing research **82**(2): 158-178.
Jastreboff, P. J. and M. M. Jastreboff (2000). "Tinnitus retraining therapy (TRT) as a method for treatment of tinnitus and hyperacusis patients." Journal of the American Academy of Audiology **11**(3): 162-177.
Job, A., J. C. Paucod, G. A. O'Beirne and C. Delon-Martin (2011). "Cortical representation of tympanic membrane movements due to pressure variation: an fMRI study." Human brain mapping **32**(5): 744-749.
Johnson, D. and N. Kiang (1976). "Analysis of discharges recorded simultaneously from pairs of auditory nerve fibers." Biophysical journal **16**(7): 719-734.
Josephs, O., R. Turner and K. Friston (1997). "Event-related f MRI." Hum Brain Mapp **5**(4): 243-248.
Kawamura, M. (2017). "[Korbinian Brodmann's Scientific Profile, and Academic Works]." Brain Nerve **69**(4): 301-312.
Khalfa, S., S. Dubal, E. Veuillet, F. Perez-Diaz, R. Jouvent and L. Collet (2002). "Psychometric normalization of a hyperacusis questionnaire." Orl **64**(6): 436-442.
Kilgard, M. P. and M. M. Merzenich (1998). "Cortical map reorganization enabled by nucleus basalis activity." Science **279**(5357): 1714-1718.
Kim, J.-y., Y.-h. Kim, S. Lee, J.-H. Seo, H.-J. Song, J. H. Cho and Y. Chang (2012). "Alteration of functional connectivity in tinnitus brain revealed by resting-state fMRI?: a pilot study." International journal of audiology **51**(5): 413-417.
Knipper, M., M. Müller and U. Zimmermann (2012). Molecular mechanism of tinnitus. Tinnitus, Springer**:** 59-82.
Knipper, M., P. Van Dijk, I. Nunes, L. Ruttiger and U. Zimmermann (2013). "Advances in the neurobiology of hearing disorders: recent developments regarding the basis of tinnitus and hyperacusis." Prog Neurobiol **111**: 17-33.
Kraus, N. and T. White-Schwoch (2015). "Unraveling the biology of auditory learning: a cognitive–sensorimotor–reward framework." Trends in cognitive sciences **19**(11): 642-654.
Kruschwitz, J. D., D. List, L. Waller, M. Rubinov and H. Walter (2015). "GraphVar: a user-friendly toolbox for comprehensive graph analyses of functional brain connectivity." J Neurosci Methods **245**: 107-115.
Landgrebe, M., B. Langguth, K. Rosengarth, S. Braun, A. Koch, T. Kleinjung, A. May, D. De Ridder and G. Hajak (2009). "Structural brain changes in tinnitus: grey matter decrease in auditory and non-auditory brain areas." Neuroimage **46**(1): 213-218.
Langers, D. R., E. de Kleine and P. van Dijk (2012). "Tinnitus does not require macroscopic tonotopic map reorganization." Frontiers in systems neuroscience **6**: 2.
Langguth, B., T. Kleinjung, B. Fischer, G. Hajak, P. Eichhammer and P. G. Sand (2007). "Tinnitus severity, depression, and the big five personality traits." Prog Brain Res **166**: 221-225.

Langguth, B., M. Landgrebe, T. Kleinjung, G. P. Sand and G. Hajak (2011). "Tinnitus and depression." World J Biol Psychiatry **12**(7): 489-500.
Langguth, B., M. Landgrebe, T. Kleinjung, J. Strutz and G. Hajak (2010). "Tinnitus and psychiatric comorbidities." HNO **58**(10): 1046.
Lanting, C. P., E. de Kleine, D. R. Langers and P. van Dijk (2014). "Unilateral tinnitus: changes in connectivity and response lateralization measured with fMRI." PloS one **9**(10).
Leaver, A. M., T. K. Turesky, A. Seydell-Greenwald, S. Morgan, H. J. Kim and J. P. Rauschecker (2016). "Intrinsic network activity in tinnitus investigated using functional MRI." Human brain mapping **37**(8): 2717-2735.
Lehnhardt, E. (2009). Praxis der Audiometrie, Georg Thieme Verlag.
Leto, J. (2010). Closer to the edge, Virgin EMI.
Liberman, M. C. and N. Y. Kiang (1978). "Acoustic trauma in cats: cochlear pathology and auditory-nerve activity." Acta oto-laryngologica.
Lockwood, A. H., R. J. Salvi and R. F. Burkard (2002). "Tinnitus." N Engl J Med **347**(12): 904-910.
Logothetis, N. K. and J. Pfeuffer (2004). "On the nature of the BOLD fMRI contrast mechanism." Magnetic resonance imaging **22**(10): 1517-1531.
Lv, H., Z. Wang, E. Tong, L. M. Williams, G. Zaharchuk, M. Zeineh, A. N. Goldstein-Piekarski, T. M. Ball, C. Liao and M. Wintermark (2018). "Resting-State Functional MRI: Everything That Nonexperts Have Always Wanted to Know." American Journal of Neuroradiology.
Marriage, J. and N. Barnes (1995). "Is central hyperacusis a symptom of 5-hydroxytryptamine (5-HT) dysfunction?" The Journal of Laryngology & Otology **109**(10): 915-921.
Mazurek, B., H. Haupt, H. Olze and A. J. Szczepek (2012). "Stress and tinnitus—from bedside to bench and back." Frontiers in systems neuroscience **6**: 47.
McKlveen, J. M., R. L. Morano, M. Fitzgerald, S. Zoubovsky, S. N. Cassella, J. R. Scheimann, S. Ghosal, P. Mahbod, B. A. Packard and B. Myers (2016). "Chronic stress increases prefrontal inhibition: a mechanism for stress-induced prefrontal dysfunction." Biological psychiatry **80**(10): 754-764.
McKlveen, J. M., B. Myers, J. N. Flak, J. Bundzikova, M. B. Solomon, K. B. Seroogy and J. P. Herman (2013). "Role of prefrontal cortex glucocorticoid receptors in stress and emotion." Biological psychiatry **74**(9): 672-679.
Meddis, R. (2006). "Auditory-nerve first-spike latency and auditory absolute threshold: a computer model." The Journal of the Acoustical Society of America **119**(1): 406-417.
Meikle, M. and S. Griest (1992). "Asymmetry in tinnitus perceptions. Factors that may account for the higher prevalence of left-sided tinnitus." Tinnitus **91**: 231-237.
Melcher, J. R. and N. Y. Kiang (1996). "Generators of the brainstem auditory evoked potential in cat III: identified cell populations." Hearing research **93**(1-2): 52-71.
Meyers, E. C., N. Kasliwal, B. R. Solorzano, E. Lai, G. Bendale, A. Berry, P. D. Ganzer, M. Romero-Ortega, R. L. Rennaker and M. P. Kilgard (2019). "Enhancing plasticity in central networks improves motor and sensory recovery after nerve damage." Nature Communications **10**(1): 1-14.
Milloy, V., P. Fournier, D. Benoit, A. Noreña and A. Koravand (2017). "Auditory brainstem responses in tinnitus: a review of who, how, and what?" Frontiers in aging neuroscience **9**: 237.

Mirz, F., T. Ovesen, K. Ishizu, P. Johannsen, S. Madsen, A. Gjedde and C. B. Pedersen (1999). "Stimulus-dependent central processing of auditory stimuli: a PET study." Scandinavian Audiology **28**(3): 161-169.
Möhrle, D., B. Hofmeier, M. Amend, S. Wolpert, K. Ni, D. Bing, U. Klose, B. Pichler, M. Knipper and L. Ruttiger (2019). "Enhanced Central Neural Gain Compensates Acoustic Trauma-induced Cochlear Impairment, but Unlikely Correlates with Tinnitus and Hyperacusis." Neuroscience **407**: 146-169.
Møller, A. R., P. J. Jannetta and H. D. Jho (1994). "Click-evoked responses from the cochlear nucleus: a study in human." Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section **92**(3): 215-224.
Nelting, M. and N. K. Finlayson (2004). Geräuschüberempfindlichkeits-Fragebogen: GÜF; Manual, Hogrefe.
Norena, A. and J. Eggermont (2003). "Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus." Hearing research **183**(1-2): 137-153.
Norena, A. J. (2015). "Revisiting the cochlear and central mechanisms of tinnitus and therapeutic approaches." Audiol Neurootol **20 Suppl 1**: 53-59.
Noreña, A. J. and B. J. Farley (2013). "Tinnitus-related neural activity: theories of generation, propagation, and centralization." Hearing Research **295**: 161-171.
Oster, H., E. Challet, V. Ott, E. Arvat, E. R. de Kloet, D. J. Dijk, S. Lightman, A. Vgontzas and E. Van Cauter (2017). "The Functional and Clinical Significance of the 24-Hour Rhythm of Circulating Glucocorticoids." Endocr Rev **38**(1): 3-45.
Parker, D. B. and Q. R. Razlighi (2019). "Task-evoked Negative BOLD Response and Functional Connectivity in the Default Mode Network are Representative of Two Overlapping but Separate Neurophysiological Processes." Scientific reports **9**.
Paul, B. T., I. C. Bruce and L. E. Roberts (2017). "Evidence that hidden hearing loss underlies amplitude modulation encoding deficits in individuals with and without tinnitus." Hearing Research **344**: 170-182.
Pfadenhauer, K., H. Weber, A. Rösler and M. Stöhr (2001). "Zentrale hyperakusis mit phonophobie bei multipler sklerose." Der Nervenarzt **72**(12): 928-931.
Picton, T. W., D. R. Stapells and K. B. Campbell (1981). "Auditory evoked potentials from the human cochlea and brainstem." The Journal of Otolaryngology. Supplement **9**: 1-41.
Pilgramm, M., R. Rychlik, H. Lebisch, H. Siedentop, G. Goebel and D. Kirchhoff (1999). "Tinnitus in der Bundesrepublik Deutschland – Eine repräsentative epidemiologische Studie." HNO aktuell **7**: 261-265.
Raichle, M. E. and M. A. Mintun (2006). "Brain work and brain imaging." Annu. Rev. Neurosci. **29**: 449-476.
Ralli, M., A. Greco, F. Cialente, A. Di Stadio, A. de Virgilio, L. Longo, A. Ciofalo, R. Turchetta, G. Cianfrone and M. Vincentiis (2017). "Somatic Tinnitus." Int Tinnitus J **21**(2): 112-121.
Rauschecker, J. P., E. S. May, A. Maudoux and M. Ploner (2015). "Frontostriatal gating of tinnitus and chronic pain." Trends in cognitive sciences **19**(10): 567-578.
Roberts, L. E., J. J. Eggermont, D. M. Caspary, S. E. Shore, J. R. Melcher and J. A. Kaltenbach (2010). "Ringing ears: the neuroscience of tinnitus." Journal of Neuroscience **30**(45): 14972-14979.
Rüttiger, L., W. Singer, R. Panford-Walsh, M. Matsumoto, S. C. Lee, A. Zuccotti, U. Zimmermann, M. Jaumann, K. Rohbock and H. Xiong (2013). "The reduced cochlear

output and the failure to adapt the central auditory response causes tinnitus in noise exposed rats." PloS one **8**(3).
Rüttiger, L., U. Zimmermann and M. Knipper (2017). "Biomarkers for hearing dysfunction: facts and outlook." ORL **79**(1-2): 93-111.
Sadaghiani, S., G. Hesselmann and A. Kleinschmidt (2009). "Distributed and antagonistic contributions of ongoing activity fluctuations to auditory stimulus detection." Journal of Neuroscience **29**(42): 13410-13417.
Sammeth, C. A., D. A. Preves and W. T. Brandy (2000). "Hyperacusis: case studies and evaluation of electronic loudness suppression devices as a treatment approach." Scandinavian audiology **29**(1): 28-36.
Saunders, J. C. (2007). "The role of central nervous system plasticity in tinnitus." Journal of communication disorders **40**(4): 313-334.
Schaette, R. and R. Kempter (2006). "Development of tinnitus-related neuronal hyperactivity through homeostatic plasticity after hearing loss: a computational model." European Journal of Neuroscience **23**(11): 3124-3138.
Schaette, R. and R. Kempter (2009). "Predicting tinnitus pitch from patients' audiograms with a computational model for the development of neuronal hyperactivity." Journal of neurophysiology **101**(6): 3042-3052.
Schaette, R. and R. Kempter (2012). "Computational models of neurophysiological correlates of tinnitus." Frontiers in systems neuroscience **6**: 34.
Schaette, R. and D. McAlpine (2011). "Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model." Journal of Neuroscience **31**(38): 13452-13457.
Schecklmann, M., A. Lehner, T. B. Poeppl, P. M. Kreuzer, R. Rupprecht, J. Rackl, J. Burger, E. Frank, G. Hajak and B. Langguth (2013). "Auditory cortex is implicated in tinnitus distress: a voxel-based morphometry study." Brain Structure and Function **218**(4): 1061-1070.
Schmidt, S. A., J. Carpenter-Thompson and F. T. Husain (2017). "Connectivity of precuneus to the default mode and dorsal attention networks: A possible invariant marker of long-term tinnitus." NeuroImage: Clinical **16**: 196-204.
Schmitt, C., M. Patak and B. Kroner-Herwig (2000). "Stress and the onset of sudden hearing loss and tinnitus." Int Tinnitus J **6**(1): 41-49.
Schneider, P., M. Andermann, M. Wengenroth, R. Goebel, H. Flor, A. Rupp and E. Diesch (2009). "Reduced volume of Heschl's gyrus in tinnitus." Neuroimage **45**(3): 927-939.
Sedley, W. (2019). "Tinnitus: does gain explain?" Neuroscience.
Sedley, W., K. J. Friston, P. E. Gander, S. Kumar and T. D. Griffiths (2016). "An Integrative Tinnitus Model Based on Sensory Precision." Trends Neurosci **39**(12): 799-812.
Sedley, W., K. J. Friston, P. E. Gander, S. Kumar and T. D. Griffiths (2016). "An integrative tinnitus model based on sensory precision." Trends in neurosciences **39**(12): 799-812.
Segerdahl, A. R., M. Mezue, T. W. Okell, J. T. Farrar and I. Tracey (2015). "The dorsal posterior insula subserves a fundamental role in human pain." Nature neuroscience **18**(4): 499-500.
Shallice, T., D. T. Stuss, T. W. Picton, M. P. Alexander and S. Gillingham (2008). "Mapping task switching in frontal cortex through neuropsychological group studies." Frontiers in neuroscience **2**: 13.

Sheldrake, J., P. U. Diehl and R. Schaette (2015). "Audiometric characteristics of hyperacusis patients." Frontiers in neurology **6**: 105.
Shen, H. H. (2015). "Core concept: Resting-state connectivity." Proceedings of the National Academy of Sciences **112**(46): 14115-14116.
Shiomi, Y., J. Tsuji, Y. Naito, N. Fujiki and N. Yamamoto (1997). "Characteristics of DPOAE audiogram in tinnitus patients." Hearing research **108**(1-2): 83-88.
Shore, S. E., L. E. Roberts and B. Langguth (2016). "Maladaptive plasticity in tinnitus—triggers, mechanisms and treatment." Nature Reviews Neurology **12**(3): 150.
Singer, W., A. Zuccotti, M. Jaumann, S. C. Lee, R. Panford-Walsh, H. Xiong, U. Zimmermann, C. Franz, H.-S. Geisler and I. Köpschall (2013). "Noise-induced inner hair cell ribbon loss disturbs central arc mobilization: a novel molecular paradigm for understanding tinnitus." Molecular neurobiology **47**(1): 261-279.
Sirimanna, T., D. Stephens and T. Board (1996). "Tinnitus and audioscan notches." Journal of Audiological Medicine **5**: 38-48.
Smith, J. A., M. Mennemeier, T. Bartel, K. C. Chelette, T. Kimbrell, W. Triggs and J. L. Dornhoffer (2007). "Repetitive transcranial magnetic stimulation for tinnitus: a pilot study." The Laryngoscope **117**(3): 529-534.
Stefanescu, R. A. and S. E. Shore (2017). "Muscarinic acetylcholine receptors control baseline activity and Hebbian stimulus timing-dependent plasticity in fusiform cells of the dorsal cochlear nucleus." J Neurophysiol **117**(3): 1229-1238.
Stouffer, J. L. and R. S. Tyler (1990). "Characterization of tinnitus by tinnitus patients." J Speech Hear Disord **55**(3): 439-453.
Sullivan, R. M. and A. Gratton (2002). "Prefrontal cortical regulation of hypothalamic–pituitary–adrenal function in the rat and implications for psychopathology: side matters." Psychoneuroendocrinology **27**(1-2): 99-114.
Tan, C. M., W. Lecluyse, D. McFerran and R. Meddis (2013). "Tinnitus and patterns of hearing loss." Journal of the Association for Research in Otolaryngology **14**(2): 275-282.
Teipel, S. J., R. Stahl, O. Dietrich, S. O. Schoenberg, R. Perneczky, A. L. Bokde, M. F. Reiser, H.-J. Möller and H. Hampel (2007). "Multivariate network analysis of fiber tract integrity in Alzheimer's disease." Neuroimage **34**(3): 985-995.
Tyler, R., C. Coelho, P. Tao, H. Ji, W. Noble, A. Gehringer and S. Gogel (2008). "Identifying tinnitus subgroups with cluster analysis." Am J Audiol **17**(2): S176-184.
Utevsky, A. V., D. V. Smith and S. A. Huettel (2014). "Precuneus is a functional core of the default-mode network." Journal of Neuroscience **34**(3): 932-940.
Van Den Heuvel, M. P. and H. E. H. Pol (2010). "Exploring the brain network: a review on resting-state fMRI functional connectivity." European neuropsychopharmacology **20**(8): 519-534.
Vanneste, S. and D. De Ridder (2012). "The involvement of the left ventrolateral prefrontal cortex in tinnitus: a TMS study." Experimental brain research **221**(3): 345-350.
Vanneste, S., M. Plazier, E. Van Der Loo, P. Van de Heyning, M. Congedo and D. De Ridder (2010). "The neural correlates of tinnitus-related distress." Neuroimage **52**(2): 470-480.
Wang, J., H. Rao, G. S. Wetmore, P. M. Furlan, M. Korczykowski, D. F. Dinges and J. A. Detre (2005). "Perfusion functional MRI reveals cerebral blood flow pattern under psychological stress." Proceedings of the National Academy of Sciences **102**(49): 17804-17809.
Weinberger, N. M. (2015). New perspectives on the auditory cortex: learning and memory. Handbook of clinical neurology, Elsevier. **129:** 117-147.

Weisz, N., T. Hartmann, K. Dohrmann, W. Schlee and A. Norena (2006). "High-frequency tinnitus without hearing loss does not mean absence of deafferentation." Hearing research **222**(1-2): 108-114.
Weisz, N., S. Moratti, M. Meinzer, K. Dohrmann and T. Elbert (2005). "Tinnitus perception and distress is related to abnormal spontaneous brain activity as measured by magnetoencephalography." PLoS Med **2**(6): e153.
Wu, C., R. A. Stefanescu, D. T. Martel and S. E. Shore (2016). "Tinnitus: Maladaptive auditory-somatosensory plasticity." Hear Res **334**: 20-29.
Xia, M., J. Wang and Y. He (2013). "BrainNet Viewer: a network visualization tool for human brain connectomics." PLoS One **8**(7): e68910.
Yakunina, N., S. S. Kim and E. C. Nam (2018). "BOLD fMRI effects of transcutaneous vagus nerve stimulation in patients with chronic tinnitus." PLoS One **13**(11): e0207281.
Yan, C. G., X. D. Wang, X. N. Zuo and Y. F. Zang (2016). "DPABI: Data Processing & Analysis for (Resting-State) Brain Imaging." Neuroinformatics **14**(3): 339-351.
Yang, S. and S. Bao (2013). "Homeostatic mechanisms and treatment of tinnitus." Restorative neurology and neuroscience **31**(2): 99-108.
Yang, S., B. D. Weiner, L. S. Zhang, S.-J. Cho and S. Bao (2011). "Homeostatic plasticity drives tinnitus perception in an animal model." Proceedings of the National Academy of Sciences **108**(36): 14974-14979.
Zenner, H.-P., W. Delb, B. Kröner-Herwig, B. Jäger, I. Peroz, G. Hesse, B. Mazurek, G. Goebel, C. Gerloff and R. Trollmann (2017). "A multidisciplinary systematic review of the treatment for chronic idiopathic tinnitus." European Archives of Oto-Rhino-Laryngology **274**(5): 2079-2091.

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Declaration of contribution

The present work was carried out under the supervision of Prof. Dr. rer. nat. Marlies Knipper in the group of Molecular Physiology of Hearing at the Department of Otolaryngology, Head and Neck Surgery Tübingen and Prof. Dr. rer. nat. Uwe Klose, from the Department of Diagnostic and Interventional Neuroradiology Tübingen. The clinical part of the work was supervised by Senior Physician Dr. med. Stephan Wolpert from the Department of Otolaryngology, Head and Neck Surgery Tübingen.

- The concept of the study was developed in a team led by Prof. Dr. rer. nat. Marlies Knipper, Prof. Dr. rer. nat. Uwe Klose, Prof. Dr. apl. rer. nat. Lukas Rüttiger, and Senior Physician Dr. med. Stephan Wolpert.

- The ethics application (German Clinical Trials Register DRKS0006332) was designed by Senior Physician Dr. med. Stephan Wolpert, Prof. Dr. rer. nat. Uwe Klose, Prof. Dr. apl. rer. nat. Lukas Rüttiger and Prof. Dr. rer. nat. Marlies Knipper. Senior Physician Dr. med. Stephan Wolpert, took over the coordination as study physician and was therefore responsible, together with Dr. med. John Thiericke, for the study clarification and privacy policy.

- My contribution for the tinnitus questionnaire, hyperacusis questionnaire, tinnitus-case report and the tinnitus localization in Table 8, Table 9 and Figure 7 (Hofmeier et. al. 2018, Figure 2, Figure Supplementary 2, Supplementary Table 1) is about 76 % (29 of 38 tinnitus participants). Training was done by Senior Physician Dr. med. Stephan Wolpert. Data analysis was done by me, Benedikt Hofmeier. From these 29 tinnitus participants, 11 were included in the study (Hofmeier et al. 2018).

- My contribution for pure tone audiometry, tympanic membrane measurements in Figure 8, Figure 9, Figure 10 (Hofmeier et. al. 2018, Figure 1, Figure Supplementary 1) is about 77 % (54 of 70 participants). Training was done by Senior Physician Dr. med. Stephan Wolpert. Data analysis was done by me,

Benedikt Hofmeier. From these 54 participants, 23 were included in the study (Hofmeier et al. 2018).

- My contribution for ABR measurements in Figure 11 (Hofmeier et. al. 2018, Figure 3) is about 77 % (54 of 70 participants). Training was done by Senior Physician Dr. med. Stephan Wolpert. Data analysis was done by me, Benedikt Hofmeier. From these 54 participants, 23 were included in the study (Hofmeier et al. 2018).

- My contribution for fMRI measurements/analysis in Table 10, Figure 12, Figure 13, Figure 14 (Hofmeier et. al. 2018, Table 2, Figure 4, Figure 5, Figure 6) is about 77 % (54 of 70 participants). Training was done by Prof. Dr. rer. nat. Uwe Klose and MTRA Anja Stierl. Data analysis was done by me, Benedikt Hofmeier and Prof. Dr. rer. nat. Uwe Klose. From these 54 participants, 23 were included in the study (Hofmeier et al. 2018).

- My contribution for cortisol analysis in Figure 15 (Hofmeier et. al. 2018, Figure 7) is about 77 % (54 of 70 participants). Training was done by Senior Physician Dr. med. Stephan Wolpert. Data analysis was done by me, Benedikt Hofmeier. From these 54 participants, 23 were included in the study (Hofmeier et al. 2018).

- My contribution for the tinnitus questionnaire, hyperacusis questionnaire, tinnitus-case report and the tinnitus localization in Table 11, Table 12, Table 13 and Figure 17 is about 50 % (15 of 30 tinnitus participants). Training was done by Senior Physician Dr. med. Stephan Wolpert. Data analysis was done by me, Benedikt Hofmeier and Fatma Mohammed Refat.

- My contribution for pure tone audiometry, tympanic membrane measurements in Figure 18, Figure 19, Figure 20 is about 60 % (36 of 59 participants). Training was done by Senior Physician Dr. med. Stephan Wolpert. Data analysis was done by me, Benedikt Hofmeier and Fatma Mohammed Refat.

- My contribution for ABR measurements in Figure 21 is about 60 % (36 of 59 participants). Training was done by Senior Physician Dr. med. Stephan Wolpert. Data analysis was done by me, Benedikt Hofmeier and Fatma Mohammed Refat.

- My contribution for fMRI measurements/analysis in Figure 22, Figure 23, Figure 24 is about 60 % (36 of 59 participants). Training was done by Prof. Dr. rer. nat. Uwe Klose and MTRA Silke Buschbach. Data analysis was done by me, Benedikt Hofmeier and Prof. Dr. rer. nat. Uwe Klose.

- My contribution for cortisol analysis in Figure 25 is about 60 % (36 of 59 participants). Training was done by Senior Physician Dr. med. Stephan Wolpert. Data analysis was done by me, Benedikt Hofmeier and Fatma Mohammed Refat.

I certify that I wrote the manuscript independently according to the instructions of Prof. Dr. rer. nat. Marlies Knipper and that I did not use any sources other than those I indicated.

Tübingen Date: Signature: Benedikt Hofmeier

Publications

Hofmeier, B., S. Wolpert, E. S. Aldamer, M. Walter, J. Thiericke, C. Braun, D. Zelle, L. Rüttiger, U. Klose and M. Knipper (2018). "Reduced sound-evoked and resting-state BOLD fMRI connectivity in tinnitus." NeuroImage: Clinical **20**: 637-649.

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Prof. Dr. apl. Lukas Rüttiger, who relentlessly provided good and critical suggestions for each of my concerns, for which I am very grateful.

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Not least in importance. To my beloved Ariane for the support and cohesion during this time, the time before and the time we will still have.

Appendix

Appendix A - Participant information/Declaration of consent/Privacy policy

Probandendaten

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Probandeninformation

über die Teilnahme und die Durchführung der klinischen Studie mit dem Titel:
„Funktionelle MR-Tomographie zur Darstellung der Hirnaktivität und der Hörbahn bei Tinnitus-Patienten mit und ohne Hyperakusis und Hyperakusis ohne Tinnitus und Vergleichspersonen“

German Clinical Trials Register DRKS0006332

Sehr geehrte Damen und Herren,

wir möchten Sie über eine Untersuchung des Tinnitus (Ohrgeräusch) und der Hyperakusis (Lärmempfindlichkeit) informieren, in der erforscht werden soll, wie unser Gehirn unter den krankhaften Veränderungen des Tinnitus und der Hyperakusis abweichend funktioniert und welche Funktionsmechanismen zu den krankhaften Veränderungen führen (pathophysiologische Veränderungen).

Tinnitus lässt sich in Tieren über operantes Verhaltenstraining im Tier nachweisen. Hierbei wird ein Ohrgeräusch ohne Ton durch Aspirin oder Schall-exposition induziert. Über ein Verhaltenstraining der Tiere - in Stille ruhig zu sitzen und bei Geräuschen Zuckerwasser trinken zu dürfen - lässt sich Tinnitus im Tier nachweisen. Wir wissen aus Untersuchungen an Tieren mit Tinnitus, dass bei Stress typischerweise Hormone (Corticoide) im Blut erhöht sind, die auch erhöht im Urin nachgewiesen werden konnten. Diese Hormone haben auch Einfluss auf die Gehirnaktivität und tragen zur Tinnitus-Entstehung bei. Die Beobachtungen bei Tieren konnten bei Patienten mit Tinnitus bestätigt werden. In vorangegangenen Studien konnten

Unterschiede in Aktivierungen in Bereichen des Gehirns zwischen Normalhörenden und Tinnitus-Betroffenen gezeigt werden. Bis heute sind die genauen Funktionsmechanismen unbekannt.

Hyperakusis gehört genau wie der Tinnitus zu einem Symptom mit aktueller und zukünftiger sozialmedizinischer Bedeutung. Zurzeit ist nicht bekannt, ob Tinnitus und Hyperakusis möglicherweise Pathologien ein und derselben Erkrankung im unterschiedlichen Schweregrad darstellen oder ob es sich um verschiedene Entitäten mit völlig unterschiedlichen neuronalen Korrelaten handelt.

Jede/r für diese Studie in Frage kommende Patient/Patientin erhält diese Patienteninformation während eines Besuches in der HNO-Klinik Tübingen, um sich über die Gründe zur Durchführung der Studie zu informieren. Alle auftretenden Fragen können Sie dem Prüfarzt stellen und Sie können sich Unklarheiten erläutern lassen. Ihr Prüfarzt (Ambulanzarzt) wird Sie nochmals mündlich über den Studienablauf informieren und Sie fragen, ob Sie mit der Durchführung der Studie und der Weitergabe Ihrer Daten einverstanden sind.

Warum wird diese Prüfung durchgeführt?

Durch diese Studie möchten wir ein näheres Verständnis für die Zusammenhänge des Tinnitus- und Hyperakusisleidens bekommen und eine bessere Behandlungsmöglichkeit, die an der Beseitigung der Krankheitsursache (kausale Therapie) ansetzt, entwickeln zu können.

Es sollen **60 Probanden (jeweils 10-20 pro Fallgruppe und 20 in der Kontrollgruppe)** an der Studie teilnehmen.

Welche Maßnahmen werden ausschließlich aus Studiengründen durchgeführt?

Die in dieser Studie durchgeführte Blutentnahme, Speichel- und Urinproben und Fragebögen (für Studienteilnehmer mit Tinnitus) werden ausschließlich aus Studiengründen durchgeführt.

Die Teilnahme an der Studie schließt die Untersuchung in einem 3.0 Tesla-Magnetresonanztomographen ein. Bei der Magnetresonanztomographie (MRT) handelt es sich um ein Verfahren zur Erstellung von Bildern des menschlichen Körpers, das seit 30 Jahren auch in der radiologischen Diagnostik eingesetzt wird. Es beruht auf der Aufnahme von Signalen, die von Atomkernen im Körper ausgesendet werden, wenn zuvor eine Anregung mit einem Radiowellenimpuls in einem äußeren Magnetfeld erfolgt ist. Es werden keine Röntgenstrahlen verwendet.

Wie ist der Ablauf der Studie?

Bei Aufnahme in diese Studie wird die Vorgeschichte Ihrer Krankheit erhoben und Sie werden einer HNO-ärztlichen Untersuchung unterzogen. Danach werden vier Hörtests zur Bestimmung des Hörvermögens und, falls bei Ihnen vorhanden, eine Bestimmung des Tinnitus (Lautstärke, Tonhöhe, Unterdrückbarkeit) durchgeführt sowie, falls Hyperakusis vorhanden, eine sogenannte Unbehaglichkeitsschwelle bestimmt. Die Möglichkeit Ihrer weiteren Teilnahme an dieser klinischen Prüfung wird von den Ergebnissen dieser Voruntersuchung abhängen (Zeitaufwand: 45-60 Minuten).

Bei Einschluss in diese Studie erfolgt einmalig eine Blutentnahme (ca. 20 ml), was ca. 4% der bei einer Blutspende entnommenen Menge entspricht. Studienteilnehmer mit Tinnitus werden zudem gebeten zwei Fragebögen auszufüllen. Für die Proben zur Hormonbestimmungen im Speichel und Urin werden Sie entsprechende Materialien für zu Hause mitbekommen. Der Prüfarzt wird Ihnen genau erklären, wie diese anzuwenden sind.

Innerhalb von 4 Wochen erfolgt eine weitere Visite (Visit 2). Hierzu müssen Sie in die Radiologische Klinik Tübingen (Abteilung für Diagnostische und Interventionelle Neuroradiologie) kommen und einen Aufenthalt von ca. 2 Stunden einplanen.

Circa ein bis zwei Wochen nach diesem Termin werden wir Sie nochmals telefonisch kontaktieren, um nachzufragen, wie Sie die Untersuchung vertragen haben.

Aufwandsentschädigung: 50 Euro.

Worauf ist vor/nach der MRT-Untersuchung zu achten?

Metallteile, die in das Magnetfeld gelangen, können zu Verletzungen und Bildstörungen führen. Deshalb müssen sämtliche metallischen, magnetischen und elektronischen Gegenstände vor Betreten des Untersuchungsraums abgelegt werden. Hierzu zählen z.B.:

- Uhren, Brillen;
- Ohrringe und anderer Schmuck (inkl. Piercing-Schmuck) sowie Haarnadeln oder Haarspangen;
- Brieftasche bzw. Portemonnaie inklusive Kreditkarten (die Magnetstreifen werden durch die MRT gelöscht!), einzelne Geldmünzen;
- Metallteile an der Kleidung (z.B. Gürtelschnallen); Kleidungsstücke mit einem Reißverschluss oder mit Metallfäden, Metallknöpfen oder Ähnlichem (z.B. Metallverschluss am BH) sollten nicht getragen werden;
- Kugelschreiber, Schlüssel, Taschenmesser und andere Metallteile;
- herausnehmbarer Zahnersatz, Zahnspangen,
- Hörhilfen.

Weitere Infos s.u.

Sollte während oder nach der Untersuchung oder in den darauffolgenden Tagen ein plötzliches Unwohlsein auftreten (z.B. Juckreiz, Niesreiz, Schwindel, Kopfschmerzen, Übelkeit, Atembeschwerden, Durchfall, Schmerzen o. ä.) sollten Sie bitte sofort Ihren Studienarzt informieren.

Was sind die Risiken der Untersuchungen?

Gesundheitliche Beschwerden wie Verschlechterung des Hörvermögens, des Tinnitus oder der Hyperakusis durch **Hörtests** (audiologische Untersuchungen und Messungen) sind bis heute nicht beobachtet worden.

Bei ***Blutentnahmen*** mit einer Einmalkanüle und durch das Legen einer Verweilkanüle für das fMRT kann es in seltenen Fällen zu Reizungen, Entzündungen oder Verlegungen von Venen, zu Infektionen bzw. zur Bildung von Hämatomen kommen. Sollte es zu den genannten Komplikationen kommen, führt eine lokale Behandlung mit Heparinsalbe in der Regel zu einer raschen Abheilung. In sehr seltenen Fällen kann es im Rahmen einer Blutentnahme zur generalisierten Entzündungsreaktion kommen, die auch zu einer eventuell lebensbedrohenden

Infektion führen könnte. Diese sollte im weiteren Verlauf medizinisch kontrolliert und ggf. intensiv behandelt werden. Irrtümlicherweise könnte es im Rahmen des Legens einer Verweilkanüle zur Punktion einer Arterie bzw. eines Nerven kommen, dies kann wiederum zur Hämatombildung führen, letzteres kann im ungünstigsten Fall auch zu einer Nervenschädigung an der Punktionsstelle führen, die mit einem anhaltenden Taubheitsgefühl in der entsprechenden Hautregion einhergeht. Es werden etwa 20 ml Blut entnommen, was ca. 4% der bei einer Blutspende entnommenen Menge entspricht.

Bei der ***MR-Untersuchung*** sind generell keine gesundheitlichen Schäden oder Beeinträchtigungen zu erwarten. Es gibt Richtlinien und Grenzwerte für MR-Untersuchungen zum Schutz von Patienten oder Probanden. Eine spezielle technische Überwachung an den verwendeten Magnetresonanztomographen sorgt dafür, dass diese Werte nicht überschritten werden. Die Untersuchung wird von medizinisch geschultem Fachpersonal vorgenommen, und es werden keine intravenösen Kontrastmittel verabreicht. Alle MR-Tomographen verfügen über eine Sprechverbindung zwischen Proband und Untersucher sowie über eine Alarmeinrichtung, die der Studienteilnehmer während der Untersuchung betätigen kann, um durch einen lauten Alarmton auf sich aufmerksam zu machen. Alle Studienteilnehmer werden visuell durch direkte Anwesenheit des Untersuchers während der Messung oder durch ein Fenster überwacht.

Folgende Risiken und Begleiterscheinungen sind möglich, auf die Sie achten sollten:

- Hautreizungen, die durch Tätowierungen oder Make-up, in denen metallhaltige Farbstoffe enthalten sind, hervorgerufen werden.
- Leichte bis mäßige Kopfschmerzen durch die lauten Geräusche, die in der Regel von selbst wieder abklingen und meist keiner Behandlung bedürfen.
- Extrem selten: Auftreten von Ohrgeräuschen (Tinnitus), die zumeist nach der Untersuchung wieder verschwinden, ausgesprochen selten aber auch bleiben können.
- Kurzzeitiges Schwindelgefühl oder sensorische Reizungen beim Einfahren in den Tomographen.
- Bei Hautberührungen der Beine oder der Arme oder Hände kann es in seltenen Fällen durch die eingestrahlte Hochfrequenz zu lokalen Erhitzungen oder Hautverbrennungen an den Kontaktstellen kommen. Bei der Lagerung von Personen im Magnetresonanztomographen ist deshalb darauf zu achten, dass solche Berührungen nicht auftreten.

<u>Wer darf an dieser klinischen Prüfung nicht teilnehmen?</u>

An dieser klinischen Prüfung dürfen Sie nicht teilnehmen, wenn Sie gleichzeitig an anderen klinischen Prüfungen oder anderen klinischen Forschungsprojekten teilnehmen oder vor kurzem (weniger als 30 Tage) teilgenommen haben.

Schwangere und stillende Frauen dürfen an dieser Studie nicht teilnehmen. Zu Beginn der Untersuchungen in der Radiologischen Klinik Tübingen müssen sich deshalb alle Frauen im gebärfähigen Alter einem Schwangerschaftstest unterziehen. Durch einen Schwangerschaftstest kann jedoch eine Schwangerschaft erst einige Tage nach der Empfängnis verlässlich nachgewiesen werden.

Aufgrund der MRT-Untersuchung können Sie <u>nicht</u> teilnehmen, falls eine der im Folgenden aufgeführten Bedingungen auf Sie zutrifft:

- Sie tragen nicht entfernbare Metallteile im oder am Körper wie z.B.:
  - Herzschrittmacher

- künstliche Herzklappen
- Metallprothesen
- implantierte magnetische Metallteile (Schrauben, Platten von Operationen)
- Spirale
- Metallsplitter/Granatsplitter
- feste Zahnspange
- Retainer an bis zu 4 Zähnen pro Zahnreihe sind in Ordnung
- Akupunktur-Nadel
- Insulinpumpe
- Intraport etc.
- Tätowierungen, Lidschatten

- Sie zählen zu den Personen mit eingeschränkter Temperaturempfindung und/oder erhöhter Empfindlichkeit gegenüber Erwärmung des Körpers.
- Eine Kreislauferkrankung kann nicht ausgeschlossen werden.
- Sie haben eine Gehörerkrankung.
- Sie haben Angst vor Enge (Klaustrophobie).

Welchen Nutzen haben Sie von der Untersuchung?

Da diese Untersuchung einen rein wissenschaftlichen Charakter hat, haben Sie keinen direkten Nutzen durch Ihre Teilnahme. Die Entwicklung neuer Messmethoden auf dem Gebiet der MRT hat jedoch für die zukünftige klinische Diagnostik einen äußerst hohen Stellenwert. Es handelt sich bei dieser Untersuchung nicht um eine diagnostische Kernspintomographie, so dass etwaiger krankhafte Veränderungen nicht zielgerichtet untersucht werden. Wenn sich aber aus den aufgenommenen Messdaten ein Hinweis auf das Vorliegen einer Erkrankung ergibt, werden Sie darüber informiert, insofern Sie damit einverstanden sind. In diesem Fall werden Sie Hinweise bekommen, welche diagnostischen Untersuchungen Sie durchführen lassen sollten.

Entstehen für mich Kosten durch die Teilnahme an der Studie?
Erhalte ich eine Aufwandsentschädigung?

Durch Ihre Teilnahme an dieser Studie entstehen für Sie keine Kosten. Für die Teilnahme erhalten Sie eine Aufwandsentschädigung entsprechend den folgenden Bedingungen: Durchführung von insgesamt 2 Visiten über insgesamt 4 Wochen in der Universitätsklinik Tübingen. Aufwandsentschädigung: 50 Euro

Bin ich während der klinischen Prüfung versichert?

Für die geplante Studie wird keine separate Versicherung abgeschlossen, da bei ordnungsgemäßer Handhabung für Sie kein erkennbares und erhöhtes Risiko besteht.

Was geschieht mit meinen Blut-, Speichel- und Urinproben?

Die Proben werden zunächst für diese klinische Prüfung verwendet. Etwaiges Restmaterial wird im Rahmen der Routineentsorgung durch das Zentrallabor vernichtet, bzw. im Labor der Abteilung Molekulare Psychiatrie pseudonymisiert für die Dauer von längstens 10 Jahren aufbewahrt.

Kann meine Teilnahme an der klinischen Prüfung vorzeitig beendet werden?

Ihre Studienteilnahme ist freiwillig, Sie können jederzeit, auch ohne Angabe von Gründen, Ihre Teilnahme beenden, ohne dass Ihnen dadurch irgendwelche Nachteile bei Ihrer medizinischen Behandlung entstehen.

Unter gewissen Umständen ist es aber auch möglich, dass der Prüfarzt entscheidet, Ihre Teilnahme an der Studie vorzeitig zu beenden, ohne dass Sie auf die Entscheidung Einfluss haben. Die Gründe hierfür können z. B. sein:

- Ihre weitere Teilnahme an der klinischen Prüfung ist ärztlich nicht mehr vertretbar;
- es wird die gesamte klinische Prüfung abgebrochen.

Was geschieht mit meinen Daten?

Während der Studie werden medizinische Befunde und persönliche Informationen erhoben und in der Prüfstelle in Ihrer persönlichen Akte niedergeschrieben oder elektronisch gespeichert. Die für die klinische Prüfung wichtigen Daten werden zusätzlich in pseudonymisierter Form gespeichert, ausgewertet und gegebenenfalls weitergegeben.

Pseudonymisiert bedeutet, dass keine Angaben von Namen oder Initialen verwendet werden, sondern nur ein Nummern- und/oder Buchstabencode, evtl. mit Angabe des Geburtsjahres.

Die Daten sind gegen unbefugten Zugriff gesichert. Eine Entschlüsselung erfolgt nur unter den vom Gesetz vorgeschriebenen Voraussetzungen.

Der Datenschutz ist in jeder Hinsicht gewahrt.

Die im Rahmen der wissenschaftlichen Untersuchung erhobenen Daten werden vertraulich behandelt und ausschließlich in verschlüsselter Form weitergegeben. Die Aufzeichnung der erhobenen Daten erfolgt zunächst in den Originalunterlagen, der Krankenakte, in die der Arzt auch bisher alle Befunde eingetragen hat. Die für die wissenschaftliche Untersuchung wichtigen Daten werden in verschlüsselter Form (pseudonymisiert, ohne Namensnennung) in einen gesonderten Dokumentationsbogen eingetragen.

Die Zuordnung Ihrer verschlüsselten Daten ist nur anhand einer Probandenliste möglich, die in einem verschlossenen Schrank, getrennt von den Studienunterlagen, aufbewahrt wird und nur dem Studienleiter und den Ärztlichen Direktoren der Kliniken zugänglich ist. Die Daten werden für die Dauer von 10 Jahren in der HNO-Klinik Tübingen (Hörforschungszentrum: Prof. Dr. Marlies Knipper) aufbewahrt. Ohne die Einwilligung können Sie nicht in diese Klinische Studie eingeschlossen werden.

Es ist geplant, nach Abschluss der Studie die Ergebnisse in einer wissenschaftlichen Zeitschrift zu veröffentlichen.

Wir bitten Sie mit der nachfolgenden Datenschutzerklärung um die Erlaubnis, die erhobenen Daten auswerten zu dürfen.

An wen wende ich mich bei weiteren Fragen?

Sie haben stets die Gelegenheit zu weiteren Gesprächen in Ihrem Prüfzentrum in der HNO- oder Radiologischen Klinik Tübingen mit den unten genannten Personen oder einem anderen Prüfarzt.

Oberarzt Dr.med. Stephan Wolpert
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Tel. 07071-2988001

Dr. med. Florian Hennersdorf
Universitätsklinikum Tübingen
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Radiologische Klinik
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Fax. 07071-29-5638
florian.hennersdorf@med.uni-tuebingen.de

Probandendaten

Einverständniserklärung für Probanden

über die Teilnahme und die Durchführung der klinischen Studie mit dem Titel:
„Funktionelle MR-Tomographie zur Darstellung der Hirnaktivität und der Hörbahn bei Tinnitus-Patienten mit und ohne Hyperakusis und Hyperakusis ohne Tinnitus und Vergleichspersonen“

Über den Zweck und den Ablauf der Studie, sowie die aus meiner Teilnahme an dieser Studie resultierenden Rechte und Pflichten und darüber, keinen Nutzen und kein Gewinn aus der Studienteilnahme zu ziehen, bin ich informiert worden.

Ich bin insbesondere auch über Wesen, Bedeutung und Tragweite sowie über mögliche Risiken und Nachteile aufgeklärt worden. Ich habe eine schriftliche Patienteninformation erhalten und konnte in einem Gespräch meine Fragen klären. Alle mich interessierenden Fragen wurden in für mich verständlicher Weise beantwortet.

Ich wurde darüber aufgeklärt, dass die Teilnahme freiwillig ist und ich meine Einwilligung zur Untersuchung jederzeit ohne Angabe von Gründen ohne Nachteile für mich zurückziehen kann. Ich wurde darüber informiert, dass sämtliche erhobenen personenbezogenen Daten vertraulich behandelt und pseudonymisiert ausgewertet werden.

Eine Kopie der schriftlichen Probandeninformation und dieser Einverständniserklärung habe ich erhalten.

Ich willige in die Studienteilnahme ein.

Tübingen, den_____ _____ _____

Name, Vorname des/der Probanden/in Unterschrift

Tübingen, den_____ _____ _____

Name, Vorname der Studienarztes Unterschrift

☐ Im unwahrscheinlichen Fall eines Zusatzbefundes möchte ich informiert werden

Anschrift:

_____

Telefonnummer: _____

Tübingen, den_____ _____ _____

Name, Vorname des/der Probanden/in Unterschrift

Hiermit erkläre ich mich zusätzlich einverstanden mit

- ☐ der wissenschaftlichen Verwendung der Biomaterialien
- ☐ der Blutentnahme von insgesamt 10 ml Blut

Tübingen, den_____ _____ _____

Name, Vorname des/der Probanden/in Unterschrift

Besondere Bestimmungen für den Umgang mit den pseudonymisierten Biomaterialien.

Ich bin damit einverstanden, dass das pseudonymisierte Biomaterial zusammen mit meinen Daten **(Zutreffendes bitte ankreuzen)**

☐ für das Projekt „**MR-Tinnitus/-Hyperakusis“** verwendet werden darf

☐ in der Biobank der Klinik für Psychiatrie und Psychotherapie Tübingen zeitlich unbeschränkt
- aufbewahrt werden darf

☐ **für zukünftige wissenschaftliche Projekte (**d.h. auch für die Erforschung anderer
- Erkrankungen) verwendet werden darf

☐ **im Rahmen wissenschaftlicher Kooperationsprojekte verwendet** und in diesem
- Zusammenhang an andere Kliniken oder Institute im In- oder Ausland **(anonymisiert)**
- versandt werden darf.

Tübingen, den_____ _____ _____

Name, Vorname des/der Probanden/in Unterschrift

Information zum Datenschutz

Probandendaten

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florian.hennersdorf@med.uni-tuebingen.de

Informationen zum Datenschutz

über die Teilnahme und die Durchführung der klinischen Studie mit dem Titel:
„Funktionelle MR-Tomographie zur Darstellung der Hirnaktivität und der Hörbahn bei Tinnitus-Patienten mit und ohne Hyperakusis und Hyperakusis ohne Tinnitus und Vergleichspersonen“

Ihre im Rahmen der wissenschaftlichen Untersuchung erhobenen Daten werden vertraulich behandelt und ausschließlich in verschlüsselter Form verarbeitet, d.h. erhoben, gespeichert, übermittelt, genutzt oder gelöscht. Für Probanden bedeutet das, dass die Aufzeichnung der im Rahmen dieser wissenschaftlichen Untersuchung erhobenen Daten zunächst in den Krankenunterlagen erfolgt, in die der Arzt auch bisher alle Befunde eingetragen hat. Die für die wissenschaftliche Untersuchung wichtigen Daten werden dann in verschlüsselter Form, d.h. pseudonymisiert, nur mit einer sinnfreien Kodierziffer versehen, in einen gesonderten Dokumentationsbogen eingetragen.

Die Zuordnung dieser pseudonymisierten Daten zu Ihrer Person ist nur anhand einer Liste möglich, die in einem verschlossenen Schrank, getrennt von den Studienunterlagen aufbewahrt wird und nur dem Studienleiter und dem Ärztlichen Direktor der Abteilung zugänglich ist. Die Daten werden für die Dauer von 10 Jahren in der HNO-Klinik Tübingen im Zentralarchiv des Universitätsklinikums Tübingen aufbewahrt.

Sollten Sie von der Studie zurücktreten, können Sie entscheiden, ob die bereits vorliegenden Daten vernichtet werden müssen oder weiterverwendet werden dürfen.

Einwilligungserklärung zur Verarbeitung der erhobenen Daten

Ich erkläre mich der Verarbeitung der im Rahmen der Studie „Funktionelle MR-Tomographie zur Darstellung der Hirnaktivität und der Hörbahn bei Tinnitus- und Hyperakusis-Patienten und Vergleichspersonen“ erhobenen Daten in der oben beschriebenen Weise einverstanden. Ich kann jederzeit meine Daten beim Studienleiter (o.a.) einsehen.

Tübingen, den	Unterschrift	Name des Probanden/der Probandin in Blockschrift
Tübingen, den	Unterschrift	Name des aufklärenden Arztes/Wissenschaftlers in Blockschrift

Appendix B - Recruitment flyer

Standort/Lageplan/Anfahrt

Parkmöglichkeiten

UKT Parkhaus Crona P4

Hoppe-Seyler-Straße 1

72076 Tübingen

Ansprechpartner/Info/Beteiligte

Kontakt

Moritz Walter
Tel. 07071/29-88202
Email: Moritz.Walter@med.uni-tuebingen.de

Universitätsklinikum Tübingen
Klinik für Hals-Nasen-Ohren Heilkunde
Dr. med. Stephan Wolpert (Studienleiter)
Dr. med. John Thiericke
Elfriede-Aulhorn-Str. 5
72076 Tübingen

Tübinger Hörforschungszentrum (THRC)
Prof. Marlies Knipper
Sektion Molekulare Hörphysiologie
Elfriede-Aulhorn-Str. 5
72076 Tübingen

Bereich MR Forschung
Prof. Klose
Abteilung für Diagnostische und Interventionelle Neuroradiologie
Hoppe-Seyler-Straße 3
72076 Tübingen

Universitätsklinik für Hals-Nasen-und Ohrenheilkunde

UNIVERSITÄTS KLINIKUM TÜBINGEN

fMRI-Hirnstamm Aktivitätsdarstellung von Tinnitus-Patienten und Vergleichspersonen

(klinische Studie)

Allgemeines

Was ist Tinnitus?

Tinnitus (Ohrensausen) ist eine Fehlfunktion unseres Hörsinns, bei der ohne eine äußere Geräuschquelle Phantomgeräusche wahrgenommen werden. Die neurophysiologischen Grundlagen bei der Entstehung von Tinnitus sind bisher noch nicht vollständig verstanden.
Tierexperimentelle und molekularbiologische Forschungsergebnisse aus unserem Hörforschungszentrum geben Hinweise darauf, dass bei der Tinnitusentstehung u.a. Stresshormone, fehlende spezifische Aktivierung von Hirnstammstrukturen und Schädigungen des Innenohrs eine wichtige Rolle spielen.
In der vorliegenden klinischen Studie soll in einem interdisziplinären Ansatz mittels hörphysiologischer Untersuchungen (Hörtests), funktioneller Magnetresonanztomographie (fMRT) und der Untersuchung von Stresshormonen dieser Zusammenhang überprüft werden.

Hierfür suche wir freiwillige Teilnehmer mit / ohne einem chronischen Ohrgeräusch (Tinnitus).
Eine Teilnahme ist sowohl bei normalem Gehör als auch bei moderatem Hörverlust möglich.

Studienziel

Ziel der Studie ist, mittels fMRT den Aktivitätszustand verschiedener Hirnareale mit und ohne Tinnitus zu bestimmen und den Zusammenhang von Innenohrschäden, Stresshormonen und weiteren Faktoren bei der Entstehung von Tinnitus zu ergründen. Mit den Erkenntnissen erhoffen wir uns neue Möglichkeiten zur Entwicklung diagnostischer und individualisierter Therapien für Tinnitusbetroffene.

Wie läuft die Studie ab?

Studienablauf

Wir planen für die Teilnehmer der Studie **zwei Besuche** ein:
In der HNO-Universitätsklinik werden wir beim **ersten Besuch** verschiedene Höruntersuchungen mit Tinnitus-Bestimmung und eine Speichel-,Urin-, und Blutentnahme durchführen.
Die Blutentnahme ist notwendig um die Verträglichkeit der fMRT zu untersuchen und um Ihre Stresshormon (Cortisol)- Konzentration zu bestimmen.
(Dauer ca. 4 ½h)

Wir werden Sie auch bitten uns zwei weitere Urin- und Speichelproben zum zweiten Besuch mitzubringen, in denen wir durch eine neues Verfahren ebenfalls die Stresshormon-Konzentrationen bestimmen wollen.

In der Abteilung Neuroradiologie findet der **zweite Besuch** statt, hier wird die fMRT-Untersuchung durchgeführt.
Sollte bei Ihnen ein Tinnitus vorliegen wird während der Messungen noch zusätzlich eine spezielle MRT-Aufnahme mit Kontrastmittel angefertigt um ein sog. „Akustikusneurinom" auszuschließen. Das „Akustikusneurinom" ist eine gutartige Geschwulst des Hörnerves, welche in seltenen Fällen Ursache für das Ohrgeräusch sein kann. Bei Vorliegen eines chronischen Tinnitus sollte einmalig eine solche MRT Untersuchung durchgeführt werden.
(Dauer ca. 2h)

Aufwandentschädigung:

Für die von Ihnen bereitgestellte Zeit können wir Ihnen, bei einem Studieneinschluss, eine Aufwandsentschädigung von **50€** erstatten.

Komme ich für die Studie in Frage?

Einschlusskriterien für die Studie:

(Sie können teilnehmen wenn die unten genannten Punkte zutreffen)

- **Kein Tinnitus** oder **Tinnitus einseitig/beidseitig**
- **Kein Hörverlust** oder **nur leichter Hörverlust**
- **Alter**: 18 – 70 Jahre.

Ausschlusskriterien für die Studie:

(Sie können nicht teilnehmen wenn die unten genannten Punkte zutreffen)

- Schwangerschaft
- Tinnitus-Therapie
- Klaustrophobie (Platzangst)
- Metallimplantate die nicht MRT fähig sind

Risiken

Welche Risiken bestehen für mich?

Es besteht ein sehr geringes Risiko bei Blutentnahme und fMRT.

(i) fMRT-Untersuchung findet in einem in der klinischen Routine (Neuroradiologie) verwendeten und zertifizierten MR-Tomographen statt.

Appendix C - Recruitment e-mail

Liebe Studien-Interessierte,

die HNO-Klinik sucht für eine Studie Probanden, um die Ursachen für Tinnitus (Ohrgeräusch) und Hyperakusis (Lärmempfindlichkeit) zu untersuchen.
Hierzu werden verschiedene Untersuchungen der Hörfunktion, der Körperflüssigkeiten und auch der funktionellen Magnetresonanztomographie (fMRI) durchgeführt. Dadurch soll der Aktivitätszustand der Patienten bestimmt werden, um den Zusammenhang von Innenohrschäden und bspw. Stressereignissen bei der Entstehung von Tinnitus und Hyperakusis zu ergründen und die Möglichkeit neuer diagnostischer und individualisierter therapeutischer Ansätze zu eröffnen.
Die Studie beinhaltet drei Untersuchungstermine. In einer Voruntersuchung (ca. 1,5h) wird mittels Hörmessungen (Hörtest, BERA, Sprachtest) das Hörvermögen überprüft; ebenfalls werden Körperflüssigkeiten abgenommen (Blut, Speichel und Urin). In einer zweiten Untersuchung (ca. 1.5h) wird an einem separaten Termin eine fMRI Untersuchung durchgeführt. In einem dritten Termin (ca. 1h) beinhaltet eine Nachmessung der zuvor durchgeführten BERA-Untersuchung.
Für die drei Termine können wir Ihnen eine Aufwandsentschädigung von 50€ gewährleisten.

Für die Teilnahme an dieser Studie gelten folgende Rahmenbedingungen und Einschlusskriterien:

- Alter zwischen 18 und 50 Jahren
- Ausreichende Deutschkenntnisse

- OHNE Tinnitus, OHNE Hyperakusis (Kontrollgruppe): kein Tinnitus- und Hyperakusisleiden zum Zeitpunkt der Untersuchung
- MIT Tinnitus, OHNE Hyperakusis: kontinuierliche Tinnitusanamnese von mehr als 4 Wochen
- MIT Hyperakusis UND Tinnitus: kontinuierliche Hyperakusisanamnese von mehr als 4 Wochen, Tinnitus wie o.a.
- MIT Hyperakusis OHNE Tinnitus: kontinuierliche Hyperakusisanamnese von mehr als 4 Wochen

- keine Teilnahme an der zuvor durchgeführten Tinnitusstudie in der HNO-Klinik Tübingen (2016-2018)

- keine Taubheit oder Schwerhörigkeit (Hörverlust größer als 40dB)
- keine chronischen Gehörgangs- und Mittelohrenzündungen
- keine neurologischen und psychiatrischen Grunderkrankungen
- keine Einnahme von Psychopharmaka
- keine Zuckerkrankheit, Bluthochdruck, Lungen- oder Herzerkrankung, Blutarmut oder neurologische Erkrankungen

- keinerlei magnetisches Material im Körper (z.B. Metallsplitter, Metallstaub durch Unfall oder Verletzungen, Implantate, Prothesen, Herzschrittmacher, künstliche Herzklappe, Shunt oder Port, Clips, Coils, Filter, Katheder, Hormonspirale, Platten, Nägel, Drähte, Klammer, Nähte, Zahnplomben mit Metall, Zahnspangen, Gelenkimplantate, Intrauterinpessar etc.)
- Retainer an bis zu 4 pro Zahnreihe sind in Ordnung
- keine großflächigen Tätowierungen
- keine Klaustrophobie
- keine Schwangerschaft
- keine Abhängigkeit (auch frühere) von Alkohol, Drogen oder Medikamenten

Erläuterungen zu den Rahmenbedingungen bzw. genauere Angaben können gerne auf Anfrage erteilt werden.

Wenn Sie Interesse haben bzw. weitere Informationen erhalten möchten, melden Sie sich bei Benedikt Hofmeier unter folgender E-Mail-Adresse: tinnitus.studie@hno.uni-tuebingen.de

oder Mo-Fr 9-18 Uhr telefonisch:
07071/29-88240 (Benedikt Hofmeier)

Die personenbezogenen Angaben werden anonymisiert bzw. pseudonymisiert, nur zu diesem Forschungszweck verwendet und nicht an Dritte weitergegeben. Auf die Freiwilligkeit der Teilnahme wird ausdrücklich hingewiesen.

Vielen Dank für die Unterstützung,
mit freundlichen Grüßen,

Benedikt Hofmeier

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Appendix D - List of inclusion/exclusion criteria

Checkliste für Probanden-Einschluss

Name: _____

Einschlusskriterien

○ **Fallgruppe (mit Tinnitus, ohne Hyperakusis)**

○ **Kontrollgruppe (ohne Tinnitus, ohne Hyperakusis)**

○ **Fallgruppe (mit Tinnitus UND Hyperakusis)**

○ **Fallgruppe (ohne Tinnitus, MIT Hyperakusis)**

Ja	Nein		Ja	Nein	
O	O	kontinuierl. Tinnitus (>4 Wochen)	O	O	Kein Tinnitusleiden
O	O	nicht pulsatil	O	O	Keine Hyperakusis
O	O	Tinnitus nicht als Begleiterkrankung			

Wenn Tinnitus, seit wann (Jahr):

Händigkeit (unzutreffendes bitte durchstreichen): rechts // links

Ausschlusskriterien

Ja	Nein	**Hörsystem**
O	O	Hörverlust über 40dB
O	O	Knalltrauma
O	O	Jahrelange Lärmexposition
O	O	Chronische Gehörgang- oder Mittelohrentzündung
O	O	Ertaubung (ein-/beidseitig)
O	O	Morbus Menière
O	O	Retrocochleäre Hörstörung (Nachweis durch BERA)
O	O	Schallleitungsschwerhörigkeit (über 10dB bei mehreren Frequenzen)

Ja Nein **Allgemeine Krankengeschichte**

O O Schädelhirntrauma(Grad II oder III)

O O Herz-Kreislauferkrankungen

O O Diabetes

O O Nierenerkrankungen

O O Behandlung von Krebsleiden (Leukämie)

O O Allergien (gegen Kontrastmittel)

O O Eingeschränkte Temperaturempfindung/erhöhte Empfindlichkeit gegenüber Erwärmung des Körpers

O O Ototox. Medikamente (Schleifendiuretika, Aminoglykoside, Chemo)

O O Behandlung von neurologischen oder psychiatrischen Erkrankungen (auch medikamentös: Neuroleptika, Haloperidol, L-Dopa)

O O Alkohol, Drogen

Ja Nein **Sonstiges**

O O Schwangerschaft

Ja Nein **Therapie Hörsystem**

O O Hörgeräteversorgung

O O Ohroperationen

O O Tinnitus Therapie (medikamentös, Masker/Noiser, HBO, Akupunktur)

Ja Nein **MRT spezifische Fragen**

O O Platzangst

O O Kontrastmittelallergie

O O Großflächige Tätowierungen

O O Permanente Metallteile

- Herzschrittmacher, Künstliche Herzklappe
- Granatsplitter
- Schrauben, Platten von früherer OP
- permanente Zahnspangen
- Prothesen
- Insulinpumpe
- (Retainer über 4 Zahnreihen sind in Ordnung)

Datum, Ort
Unterschrift

Einschlusskriterien für die Tinnituspatienten (Fallgruppe)

1. Kontinuierliche Tinnitusanamnese von mehr als 4 Wochen
2. Tinnitus ein- oder beidseitig
3. Klinisch unauffälliger mikroskopischer Ohrbefund, klinisch unauffällige Trommelfellbeweglichkeit
4. Alter ≥18 bis ≤50 Jahre
5. Unterschriebene Einverständniserklärung (die schriftliche und mündliche Information erfolgt in der Muttersprache des Studieninteressierten)
6. Deutschkenntnisse ausreichend

Einschlusskriterien für die Hyperakusispatienten (Fallgruppe)

1. Kontinuierliche Hyperakusisanamnese von mehr als 4 Wochen
2. Klinisch unauffälliger mikroskopischer Ohrbefund, klinisch unauffällige Trommelfellbeweglichkeit
3. Alter ≥18 bis ≤50 Jahre
4. Unterschriebene Einverständniserklärung (die schriftliche und mündliche Information erfolgt in der Muttersprache des Studieninteressierten)
5. Deutschkenntnisse ausreichend

Einschlusskriterien für die Vergleichsgruppe (Kontrollgruppe)

1. Kein Tinnitusleiden zum Zeitpunkt der Untersuchung und anamnestisch
2. Tonaudiometrische geringgradiger Hörverlust bei einer Hörschwelle von maximal 40 dB

Ausschlusskriterien für Tinnitus-/Hyperakusis und Kontrollgruppe

1. Immunsupressiva (bspw. tägliche Cortisoneinnahme)
2. Pulsatiler Tinnitus

3. Intermittierender nicht persistenter Tinnitus
4. Retrocochleäre Hörstörung (z.B. nachgewiesen durch BERA)
5. Hörgeräteversorgung
6. Vertigo
7. Z.n. Knalltrauma
8. Diabetes mellitus Typ I & Typ II
9. Schwangerschaft
10. Medikamentöse Tinnitustherapie in den letzten 4 Wochen
11. Z.n. Ohroperationen (bspw. Tympanoplastiken, einliegende Mittelohrimplantate, mit Ausnahme von Parazentese in der Vorgeschichte)
12. Tinnitus als Begleitsymptom im Rahmen einer anderen Grunderkrankung (bspw. Akustikusneurinom oder medikamentöser Tinnitus)
13. M. Menière, endolymphatischer Hydrops
14. Ein- oder beidseitige Ertaubung
15. Z.n. jahrelanger Lärmexposition (C5-Senke)
16. Z.n. Schädel-Hirntrauma (Grad II/III)
17. Schallleitungsschwerhörigkeit mit einem Hörverlust von mehr als 10 dB bei mehr als 2 Frequenzen
18. Chronische Gehörgangs-oder Mittelohrentzündungen
19. Anamnestisch Epilepsieleiden, Parkinson und/oder dementielle Erkrankung
20. Anamnestisch zeitgleiche Behandlung neurologischer bzw. psychiatrischer Störungen (bspw. Schizophrenie, Depression etc.)
21. Drogen-, Alkoholabhängigkeit
22. Eingeschränkte Nierenfunktion mit einem erhöhten Kreatininwert (>160 µmol/l = 1,8 mg/dl)
23. Aktuell in Behandlung aufgrund eines Krebsleidens (bspw. Leukämie)
24. Angst vor Enge (Klaustrophobie)
25. Anamnestische Hinweise auf Herz-Kreislauferkrankungen (hochgradige KHK)
26. Anamnestische eingeschränkte Temperaturempfindung und/oder erhöhte Empfindlichkeit gegenüber Erwärmung des Körpers
27. Nicht einwilligungsfähige Probanden

28. Probanden, die der deutschen Sprache nicht ausreichend mächtig sind, um die Anweisungen im Rahmen der Studie und die Fragen des Tinnitusfragebogen zu verstehen
29. Permanente Metallteile im oder am Körper (Herzschrittmacher, künstliche Herzklappen, Metallprothesen, implantierte magnetische Metallteile (Schrauben, Platten von Operationen), Spirale, Metallsplitter/Granatsplitter, feste Zahnspange, Akupunkturnadel, Insulinpumpe, Intraport, großflächige Tätowierungen)
30. Kontrastmittelallergie oder Überempfindlichkeit gegenüber Kontrastmittel
31. Behandlungen des Tinnitus mit Maskern, Noisern, hyperbarem Sauerstoff oder Akupunktur $\leq$4 Wochen

Appendix E – HKI questionnaire

Mini-Geräuschüberempfindlichkeits-Test				
	Stimmt immer	*Stimmt oft*	*Stimmt manchmal*	*Stimmt nicht*
1. Bestimmte Geräusche muss ich meiden.				
2. Ich habe sehr große Angst vor Lärm.				
3. Ich ärgere mich über Geräusche, die mir zu laut und unangenehm sind.				
4. Ich glaube, ich werde meinen Alltag nicht bewältigen können, wenn die Geräuschempfindlichkeit so schlimm bleibt.				
5. Bei lauten/unangenehmen Geräuschen ziehe ich mich sofort zurück.				
6. Haben Sie wegen der Geräuschempfindlichkeit Schwierigkeiten, an geräuschvollen Orten Gespräche zu führen?				
7. Empfinden Sie Lärm in manchen Umgebungen als unangenehm (zum Beispiel in Gaststätten, Lokalen, Konzerten, bei Feuerwerk)?				
8. Wenn Sie jemand bittet, mit Ihnen auszugehen (zum Beispiel ins Kino, ins Konzert, ins Restaurant), denken Sie dann als erstes an die Schwierigkeiten mit den Geräuschen?				
9. Können Sie sich in geräuschvollen Umgebungen schlechter konzentrieren, wenn Sie müde sind oder unter Stress stehen?				

Appendix F - Tinnitus-CRF

Universitäts-HNO-Klinik Tübingen

Tinnitus-CRF (case report form)

Minimaler Datensatz 1

Patient: _____

Datum_____

Hören Sie zur Zeit Ihr Ohrgeräusch? Wenn ja, wo hören Sie es? Wie laut?			
Tinnituslautheit	Rechtes Ohr	Linkes Ohr	Kopfmitte
6-stellige Digitalscala			
Nicht hörbar	○	○	○
Sehr leise	○	○	○
Leise	○	○	○
Mittel	○	○	○
Laut	○	○	○
Sehr laut	○	○	○

Wie war die Belästigung durch den Tinnitus in den letzten Tagen?								
Belästigung	8-stellige Digitalscala							
	1	2	3	4	5	6	7	8
	keine Belästigung	sehr leicht	leicht	mäßig	mittel	stark	sehr stark	extrem

[___] Göbel-Hiller-Score

Appendix G – Handling information for saliva samples

Handhabung der Speichelproben:

Um Ihre Stresshormon-Konzentration (Cortisol) zu verschieden Zeitpunkten bestimmen zu können, benötigen wir Speichelproben von Ihnen. Sie erhalten für die Entnahme zu Hause 3 kleinere Röhrchen. Die Probenentnahme soll am Tag und am Morgen vor dem zweiten Klinikbesuch begonnen werden.
(Sollte Ihnen dies nicht möglich sein und Sie führen die Probenentnahme früher durch, so bewahren Sie die Proben bitte bis zum zweiten Termin im Kühlschrank auf)

- Die erste Speichelprobe soll am Mittag um 16.00 Uhr angefertigt werden
  - Notieren Sie bitte auf dem Probenbehälter die Uhrzeit und das Datum der Probenentnahme
- Die zweite Speichelprobe soll am Abend um 23.00 Uhr angefertigt werden
  - Notieren Sie bitte auf dem Probenbehälter die Uhrzeit und das Datum der Probenentnahme
- Die dritte Speichelprobe soll am Morgen direkt nach dem Aufstehen angefertigt werden
  - Notieren Sie bitte auf dem Probenbehälter die Uhrzeit und das Datum der Probenentnahme
- Bringen Sie die Proben bitte zum zweiten Besuch in die Klinik mit
- Wichtiger Hinweis zur Speichelprobe:
  - Vor den Speichelproben bitte 30 Minuten keine Nahrung und Medikamente einnehmen, da sonst die Proben verunreinigt werden.

Probenentnahme:

- Watterolle aus dem Gefäß entnehmen
- In den Mund nehmen und etwa 1 Minute lang leicht kauen
- Das Wattestäbchen wieder in das Gefäß zurückgeben und verschließen
- Schreiben Sie bitte die Uhrzeit und das Datum auf das Etikett

Falls Sie Fragen haben sollten oder sich über die Probenentnahme unsicher sind, zögern Sie bitte nicht, uns zu kontaktieren:

Benedikt Hofmeier
Tel: 07071 / 29 88240 (in der Zeit von 08.30-18.00Uhr)
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