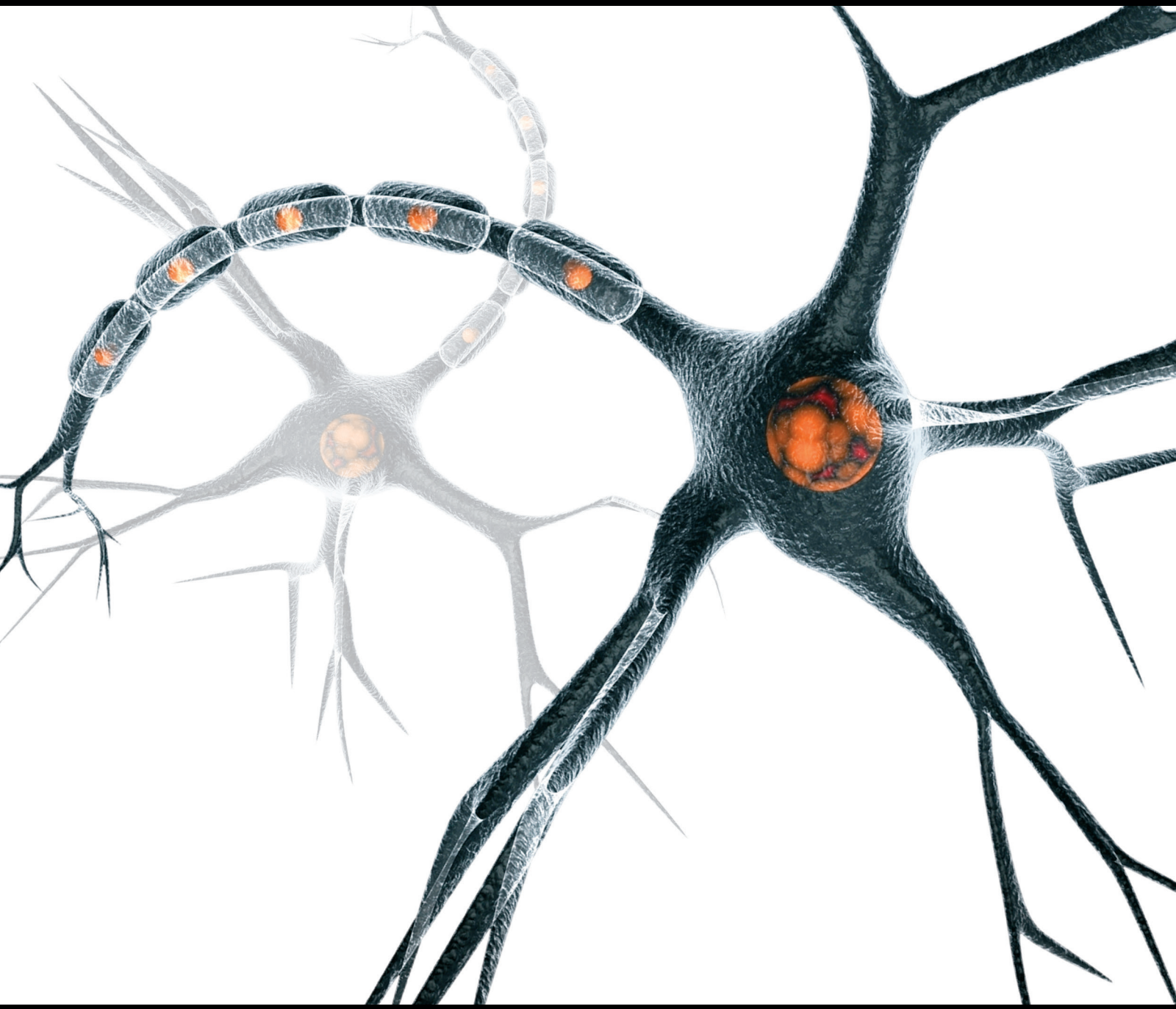
Neural Plasticity

Plasticity of Neural Systems in Tinnitus

Guest Editors: Martin Meyer, Berthold Langguth, Tobias Kleinjung, and Aage R. Møller



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Editorial Board

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Editorial

Plasticity of Neural Systems in Tinnitus

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This special issue of the journal is dedicated to tinnitus and the role of neuroplasticity in its symptomology. In western industrial countries with a steadily aging population, the number of individuals who suffer from tinnitus is immense. Approximately 50 million people in the USA and 70 million individuals in the European Union, that is, approximately 10% of the population, are affected. A fraction of those concerned individuals indicate a significant loss of quality of life. Tinnitus has two main forms: objective and subjective. Objective tinnitus, caused by sounds actually generated in the body, is rare. Subjective tinnitus, the more common form, is a phantom sensation. Tinnitus may be intermittent or constant (chronic) and its strength and its nature may vary. The causes of many forms of tinnitus are unknown and the treatments, therefore, focus on the management of symptoms.

Meanwhile, it is widely accepted that tinnitus must not be conceived as a sole dysfunction of the inner ear. It has rather been agreed that tinnitus emanates from a perplexing network that includes the ear and the auditory pathway but primarily resides in the human brain.

It is generally accepted that people with subjective tinnitus may experience two kinds of symptoms: one is the hearing of a sound that does not come from the environment and the other experience is a form of distress or suffering. These two kinds of symptoms are not directly related and an individual who experiences a weak tinnitus sound may nonetheless experience severe suffering. Others may experience a strong sound but suffer little or not at all. It seems likely that these two expressions of tinnitus have different pathologies and may engage different circuits in the brain.

The key to development of new treatments is a better understanding of the pathology of the disorder. Recent years have seen important progress in the understanding of pertinent aspects of the neuropsychology and neurobiology of subjective idiopathic tinnitus but many questions remain unanswered in that rapidly burgeoning field of neuroscience. The anatomical location of the pathology that causes the phantom sound is not completely known nor is it known what changes in the brain are directly or indirectly associated with distress or suffering. Recent advances in neuroscience and clinical medicine have introduced new models and frameworks that help elucidate the mechanisms underlying the pathology of subjective tinnitus.

Recent studies indicate that changes in connections in many parts of the brain play an important role in causing the symptoms of tinnitus mentioned above. The networks formed by these connections consist of cortical and subcortical areas that serve auditory as well as other functions. Understanding the abnormalities in these networks and their dynamic interactions (connectivity) is of utmost importance for understanding different people's experience of tinnitus. Such knowledge is naturally also important for developing effective treatments of tinnitus and of the associated symptoms of distress and suffering.

Management of idiopathic tinnitus is a challenge and effective treatment options are still limited. The main reason for these obstacles in management of the tinnitus patient is insufficient knowledge and understanding of the pathology of the many forms of tinnitus. The tinnitus patient is a challenge to the physician or neuropsychologist for several reasons.

Idiopathic tinnitus is not a single well-defined disease but a series of very different disorders. There are no objective tests that can distinguish between the different forms of tinnitus. This means that the patient's own description is so far the only basis for treatment. In those few forms of tinnitus known to stem from a specific treatable disease, the treatment of that underlying cause is often the best option though tinnitus without a known treatable underlying cause must be alleviated through the management of its symptoms.

This special issue has 14 articles covering the underpinning pathology, diagnosis, and treatments of chronic tinnitus. The articles report results of experimental studies in animals and studies in persons with tinnitus using different forms of imaging and electrophysiological techniques. Psychiatric, neurological, neuropsychological, and otological facets are comprehensively covered and discussed. The papers provide novel approaches for understanding the pathology and accordingly potential treatment of many forms of tinnitus. Some of the articles discuss abnormalities in EEG-based connectivity and others discuss the changes in the brain after specific treatments for tinnitus. Several of the articles in the special issue provide critical analysis of the efficacy of different forms of treatment of tinnitus while one of the articles discusses the psychiatric comorbidity of tinnitus.

We are convinced that this compilation of inspiring papers will be evidently well received as a crucial step towards better dealing with chronic tinnitus and we are delighted to introduce this special issue to the readers.

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Research Article

Disentangling Tinnitus Distress and Tinnitus Presence by Means of EEG Power Analysis

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The present study investigated 24 individuals suffering from chronic tinnitus (TI) and 24 nonaffected controls (CO). We recorded resting-state EEG and collected psychometric data to obtain information about how chronic tinnitus experience affects the cognitive and emotional state of TI. The study was meant to disentangle TI with high distress from those who suffer less from persistent tinnitus based on both neurophysiological and behavioral data. A principal component analysis of psychometric data uncovers two distinct independent dimensions characterizing the individual tinnitus experience. These independent states are distress and presence, the latter is described as the perceived intensity of sound experience that increases with tinnitus duration devoid of any considerable emotional burden. Neuroplastic changes correlate with the two independent components. TI with high distress display increased EEG activity in the oscillatory range around 25 Hz (upper β-band) that agglomerates over frontal recording sites. TI with high presence show enhanced EEG signal strength in the δ-, α-, and lower γ-bands (30–40 Hz) over bilateral temporal and left perisylvian electrodes. Based on these differential patterns we suggest that the two dimensions, namely, distress and presence, should be considered as independent dimensions of chronic subjective tinnitus.

1. Introduction

Tinnitus is an auditory phantom percept of chronic high-pitched sound, noise, or ringing, typically in the frequency range of 6–8 kHz, without any objective external sound source [1]. For this reason, reliable, objective measures of tinnitus are difficult to obtain and require sophisticated acoustic and psychometric techniques. Despite patients' occasional descriptions of immensely loud and tantalizing sounds, it has been shown that tinnitus occurs at intensities only 5–10 dB above hearing threshold [2]. In Western industrial countries with a steadily aging population, the number of individuals who suffer from tinnitus is immense. According to Cederroth and colleagues [3] approximately 50 million people in the US and 70 million individuals in the European Union, that is, approximately 10% of the population, are

affected. Meanwhile, it is widely accepted that tinnitus must not be considered as a sole dysfunction of the inner ear even though tinnitus is normally preceded and accompanied by transient or permanent hearing loss [2, 4]. It has rather been agreed that tinnitus emanates from a perplexing network that includes the ear and the auditory pathway but primarily resides in the human brain [5–9]. Tinnitus is highly subjective in nature and for this reason it is not considered as a physical disease but a heterogeneous diffuse phenomenon that lacks a clearly defined neurological pathogenesis [10]. Thus, it comes as no surprise that several, partly conflicting, neurobiological models exist that sketch the complex interplay between multiple cortical and subcortical human brain areas which may mediate the subjective experience of chronic tinnitus [6, 10–14]. These models agree in that they describe tinnitus as the unwanted result of an imbalance between inhibition and excitation of thalamocortical circuits [15]. According to this view, peripheral hearing loss caused by damage to hair cells in the inner ear results in deficient auditory information transfer to the auditory brain. The loss of sensory input instantiates low frequent self-oscillations of thalamic cells which activate the auditory cortex. This aberrant pattern of activity becomes fortified in thalamocortical feedback loops [12]. In analogy to the aching "phantom limb" sensation, tinnitus can also be considered as a phantom pain phenomenon that results from neuroplastic alterations during remapping of the auditory cortex [16]. To this end, tinnitus should be viewed as an unwanted perceptual state and function of incremental maladaptive learning. In the absence of externally generated inflowing information the phantom sound sensation is gradually but steadily reinforced internally. Top-down processes of attentional allocation become more and more dominant because concerned individuals are increasingly irritated and become aware of this disturbing noise in their heads. In absence of any reasonable and appropriate coping strategies these persons consider the permanent sound as extremely detrimental. Consequently, the neural thalamocortical circuit that maintains the phantom sound connects with attentional circuits. This neural loop is accelerated by aversive emotional attributions, is continuously updated, and eventually becomes established. Thus, chronic subjective tinnitus could be considered as a learned disorder that results from maladaption of several overlapping brain systems that bind together in a "vicious circle" [6].

To date there is no medical, neurological, or neuropsychological therapy that has been proved as universal treatment to cure tinnitus [4]. This lack of a standard treatment can be taken as an obvious evidence that subjective tinnitus is an exceptionally dynamic and complex phenomenon that emerges from a cascade of neuroplastic processes. In vivo morphometry studies indirectly also confirmed this notion in that they draw a complex picture of the structural neuroarchitecture of tinnitus. According to these studies a loose ensemble of cortical and subcortical limbic brain regions in TI have increased or decreased in volume, thickness, or surface [17–23]. One recent study applied an advanced approach in that the authors correlated neuroanatomical traits with tinnitus-related distress within a TI large sample [24]. According to these authors an inverse relationship between cortical volume in bilateral auditory areas and distress can be observed. However, due to the constant emission of detrimental scanner noise and other uncomfortable aspects of scanning environment many TI are reluctant towards participation in MRI studies so that magnetic resonance imaging cannot be considered as a suitable technique to explore the functional signature of tinnitus.

Alternatively, spectral power and connectivity analyses of resting-state EEG have been turned out as advantageous tools because recent research has demonstrated that EEG parameters obtained from TI generally deviate from EEG patterns of people without tinnitus symptoms [25]. Enhanced EEG activities in δ-, θ-, and β-bands have been described as indicative of a chronic dysrhythmia of thalamocortical circuits following auditory deafferentation. Along the same vein, chronic tinnitus has been associated with increased γ-band activity in the contralateral auditory cortex [26, 27]. However, tinnitus appears not only to affect neural circuits in the auditory cortex but also ensembles residing in the associative/paralimbic system, anterior cingulate, insula, prefrontal cortex, posterior cingulate, and (pre)cuneus [9]. To date several studies have been published that sought to identify deviant profiles in neurophysiological or neuromagnetic recordings that help better describe the interplay between different resting state networks that partly overlap and form larger oscillatory networks, thereby amalgamating brain circuits and form novel—partly maladaptive—associations [6, 11]. With respect to the α-band the situation is less clear. In TI both decrease and increase of α-activity have been observed [11]. While some authors surmise that a decay of α-oscillations mandatorily precedes an increase of γ- and θ-oscillations as a function of dysrhythmia, other scholars conjecture that an observed increase of α-activity is a proactive mechanism of the brain to suppress other tinnitus-related EEG frequencies [11].

Interestingly, a fraction of, but not all, individuals suffering from subjective tinnitus also show symptoms of psychiatric disorder and moderate symptoms of depression or anxiety or indicate considerable emotional distress. Zöger and colleagues [28] report a high prevalence of psychiatric, clinically pertinent diagnoses in a sample of TI. Depressive (62%) and anxiety (45%) disorders were noted in the investigated TI population underlining the paramount importance to carefully identify these affective disturbances in individuals suffering from subjective chronic tinnitus. More recent approaches using power spectrum analysis aim at correlating clinically pertinent psychometric measurements with specific increases or decreases of frequency-band-specific oscillatory modulations [10, 29–34]. Three issues are primarily discussed as reliable predictor variable, namely distress, duration, and loudness. Duration is understood as the amount of time that has passed from onset of the tinnitus sensation until the measurement. This variable can be assessed quite easily. Loudness (or intensity) is more difficult to measure. It can be either assessed by means of a visual analog device [34] or by means of an acoustic tinnitus simulation. In the latter case TI are able to adjust the subjective loudness of their individual tinnitus sensation. By means of standard questionnaires [35], distress has been identified as the most pertinent predictor

[11]. Recent studies describe distress as a serious and grave dimension [34]. Apparently, distress can be delineated as an emotional state that is frequently, but not necessarily, coupled with tinnitus because distress may be present in TI, but its strength may be completely independent from duration, loudness, or depression [11, 29–31].

The Present Study. The aim of the current study is to further explore the complex interplay between the multitude of variables that contribute to the heterogeneity of tinnitus. We investigated individuals suffering from tinnitus and nonaffected persons. However, our main interest comprises the heterogeneity of psychological and neural patterns of tinnitus. To account for this heterogeneity we did not take the standard approach (comparing tinnitus patients and control subjects). We rather thoroughly elucidate the differential psychopathological and neurophysiological individual profiles within a population of TI. Our psychometric toolbox includes standard questionnaires on tinnitus experience, depression, and other biographical details (see Section 2.3). Individual hearing thresholds will be determined to control the effect that hearing loss may have on distinct components in the tinnitus network. Furthermore we will test the application of a nonverbal self-evaluation for pictorial representation of illness and self measure (PRISM) [36, 37] as an alternative instrument to determine the relevance of tinnitus in the life of concerned individuals.

Akin to Vanneste and coworkers [34] a principal component analysis (PCA) is used to identify the independent dimensions underlying our comprehensive psychometric data. This procedure is advantageous in that it is devoid of any a priori constraints about latent relationships between behavioral variables.

Additionally, resting-state EEG was recorded to investigate whether the identified behavior and self-evaluation-based components may predict distinct neural signatures in the EEG power spectrum. Increase in γ-power, presumably generated in the auditory regions, has been discussed as manifestation of activity in the core tinnitus network [11, 27]. There is uncertainty regarding the role of the α-band originating from auditory fields which has been observed to increase and decrease in TI. According to Vanneste and collaborators [34], the auditory component may explain only 4-5% of total signal variance while other components, that is, contributions of extraauditory circuits, may account for the remaining variance. Tinnitus-related emotional distress has been associated with EEG modulation preponderance at different oscillations, namely, α-band [30, 32, 34], β-band [29, 32, 34], and γ-band [34]. With respect to this incongruous scheme and due to the data-driven PCA-based approach we only devise careful predictions. First, we hypothesize that the PCA will identify independent dimensions that underlie the psychometric data within the TI population. The identification of these traits will help better distinguish between differential tinnitus profiles. Secondly, we predict a significant relationship between the independent components and corresponding distinct neural signatures that can be evinced by EEG power spectrum and topographical maps of EEG signal scalp distribution. Finally, we expect a correlation between an established verbal and a not yet established nonverbal self-evaluation tool (PRISM). The investigation of the latter in the context of diagnosis of tinnitus is novel and may have interesting implications as the application of PRISM, the nonverbal device, takes only a fraction of time relative to the standard verbal tinnitus questionnaire.

2. Methods

2.1. Participants. We recruited a sample of 24 TI (age M = 39.75, SD = 12.11, and range 20–62) and 24 control subjects without tinnitus (CO; (age M = 37.04, SD = 9.97, and range 20–62)). All participants were comprehensively informed about the background and the aim of the study. They all gave written informed consent. Table 1 shows the demographics and clinical details of TI. As apparent from this table the included TI suffer from tinnitus of heterogeneous origins. Right- and left-handed individuals were accepted for the study, as there were no indications for a relation between tinnitus laterality and handedness. Tinnitus severity, as assessed by the Tinnitus Questionnaire (TQ), varied between 4 and 65 (TQ total score range 0–84, with higher scores indicating higher grades of distress, M = 26.75, and SD = 16.43). No participant was reported to suffer from any neurological disorder. The control group was matched to the tinnitus group with regard to age, years of education, sex, handedness, musical background, and time of day during the EEG recording. The years of education in the tinnitus group (M = 17.83, SD = 3.87) did not differ from the control group (M = 17.88, SD = 4.08 $t(46) = -.036$, and $P = .971$). All volunteering participants gave written informed consent. The study is in accordance with the ethical principles that have their origin in the Declaration of Helsinki, adopted and revised by the World Medical Association.

2.2. Design. The study design combined between-subjects and within-subject approaches. The independent variable for the between-subject comparisons consisted of the two levels TI and CO. As dependent variables questionnaire scores and EEG measurements in the conditions resting state ("eyes open" (EO) and "eyes closed" (EC)) were used. Since the comparison between TI and CO without consideration of specific differences in individual psychopathology did not open up compelling insights, the main focus of the analysis was on comparisons and differences in individual psychopathology within TI. Hence, correlational analyses between perceptual characteristics of tinnitus and EEG data were conducted.

2.3. Questionnaires. A range of questionnaires was applied to assess multiple psychological variables, namely, depression and emotional burden induced by tinnitus in all TI.

To assess tinnitus-related information, a German adaptation of the "Tinnitus Questionnaire" (TQ) [38] and a questionnaire of our own design were used. TQ is the most extensively used device to assess tinnitus-related distress [32, 39–42]. It comprises 52 statements, which are judged on a three-point Likert scale ("true," "partially true," and "not

Table 1: Demographics and clinical details of the patient group.

Number	Sex	Age	Tinnitus				
			Localization	Duration	Quality	TQ	Cause, as reported by the patient
1	Male	28	Right ear	12 y	Noise	4	Exposure to loud music
2	Male	26	Both ears	16 y	Tone	6	Playing drums otitis media (inflammation of the middle ear)
3	Male	28	Both ears	10 y	Tone	14	
4	Female	32	Right ear	8 y	Noise	14	Noise trauma, allergies
5	Female	55	Both ears	7 y	Tone	14	?[a]
6	Male	58	In the head	38 y	Noise	15	Noise trauma
7	Female	24	Both ears	4 y 6 m	Tone	16	?[a]
8	Male	32	Right ear	15 y	Tone	16	Pressure trauma from diving noise trauma, after "power meditation"
9	Male	62	In the head	33 y	Noise	16	
10	Female	31	Both ears	10 y	Tone	20	Stress
11	Male	52	In the head	5 y	Tone	20	?[a]
12	Female	31	Both ears	4 y 3 m	Tone	21	Stress
13	Male	47	In the head	15 y	Tone	21	Exposure to loud music
14	Female	29	Both ears	2 y 9 m	Tone	23	Hearing loss exposure to loud music, stress, otitis media thyroid dysfunction, and low blood pressure
15	Female	25	Right ear	1 y 1 m	Tone	28	
16	Female	41	Both ears	7 y	Noise	28	
17	Female	33	Right ear	0 y 2 m	Noise	29	Ménière's disease
18	Male	61	In the head	13 y	Tone	31	Stress, noise trauma
19	Male	31	Both ears	16 y 4 m	Tone	39	Exposure to loud music noise trauma, high blood pressure
20	Male	50	In the head	20 y	Tone	41	
21	Female	44	Both ears	1 y 6 m	Tone	47	Stress, SSRI[b]
22	Male	46	Left ear	0 y 10 m	Noise	53	SSRI[b]
23	Male	41	In the head	20 y	Noise	61	Occurred after road accident
24	Female	47	Both ears	1 y 6 m	Tone	65	Stress, otitis media, and SSRI[b]

Note. TQ: total score of the Tinnitus Questionnaire. Tinnitus duration is provided in years (y) and months (m). [a]Patient did not know what caused the tinnitus. [b]Selective serotonin reuptake inhibitor.

true"). Besides a total score for tinnitus distress and severity, six subscores ("Psychic Distress," "Intrusiveness," "Auditory Perceptual Difficulties," "Sleep Disturbances," and "Somatic Complaints") are derived. The subscore "Psychic Distress" is further subdivided into "Emotional Distress" and "Cognitive Distress." Our own questionnaire collected information about the tinnitus such as origin, duration, perceived side effects, or previous treatment options.

To determine hearing thresholds in all participants, we used the Home Audiometer software [43] in a sound-proofed room.

2.4. Distress Rating Tools

2.4.1. Beck Depression Inventory. Signs of depressions were measured by means of "Beck Depression Inventory" (BDI) [44]. The BDI contains 21 items which assess various symptoms of depression. The sum score over all items imply the degree of depressive mood or depression.

2.4.2. The PRISM Task. PRISM has been validated to measure burden of suffering in a variety of chronic diseases [37, 45–47] and was applied in this study as described elsewhere [36, 48].

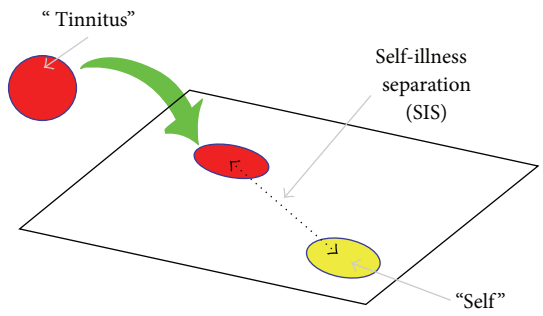


Figure 1: Self-evaluation of individual tinnitus-related distress by means of PRISM. TI imagined a metal board representing her life and a small yellow circle on the board representing her self. The task was to place a small magnetic red disk on the board to indicate the current salience and distress of tinnitus in the patient's life. Afterwards, the distance between the self and the tinnitus disk was measured as a quantitative measure of the burden of individual distress.

Briefly, the patient is shown a white A4-sized metal board with a fixed yellow disk (representing the patient's self) at the bottom right-hand corner and asked to imagine that the board represents her life as it is at present (see Figure 1). The

patient is then handed a red disk, asked to imagine that this represents her illness, and then asked one question: "Where on the board (representing your life) would you place the disk (representing tinnitus) at the moment?" The purpose of this task is to reflect the importance of the illness in her life. The main quantitative measure derived from PRISM is the self-illness separation (SIS), namely, the distance in millimeters between the centers of the illness disk and the self disk.

2.4.3. Visual Analog Scale. We applied "visual analog scales" (VAS) as a tool to measure the current tinnitus sensation. Participants rated the instantaneous strength of their tinnitus sensation on a paper sheet several times during the recording session. We opted for the term "strength of tinnitus sensation" in an attempt to capture the full qualitative spectrum of tinnitus perception, including loudness, disturbance, and intensity.

2.5. EEG Recordings. The recordings were made utilizing a dense array EEG system with 129 channels and were saved electronically with Net Station, both developed by Electrical Geodesics, Inc. [49]. The sampling rate was set to 500 Hz and impedances were kept below 40 kΩ. The CZ electrode was used as reference for online recording.

2.5.1. Procedure. Presentation software [50] was used to control the measurement procedure. Participants were informed about the course of events before the recording session was started. They were instructed to assume a comfortable position in the chair and to remain calm for the recording. EEG parameters were continuously monitored and checked for abnormalities during the recording sessions. A resting EEG was obtained during six minutes. It consisted of alternately 20 seconds of EO and 40 seconds of EC. Subjects were instructed via a prerecorded voice to open or close their eyes, respectively. During the EO periods, a cross mark was shown on the computer screen and participants were instructed to fixate it. TI were subsequently asked to rate the strength of the tinnitus sensation for EC and EO separately on a VAS (ranging from "not strong at all" to "very strong"). The EO periods are primarily meant to maintain a constant level of vigilance.

2.6. Data Analysis

2.6.1. Behavioral Data and Questionnaires. For TQ, we put special emphasis on the total score and the subscore of "Emotional Distress" because these two measures reliably predict tinnitus-related distress. In an exploratory manner, several variables of tinnitus distress were correlated with various scores of tinnitus strength, tinnitus duration, tinnitus pitch, tinnitus loudness estimations, and further behavioral variables addressed by the questionnaires. According to the study by Schlee and collaborators [39] who showed that individual tinnitus duration significantly contributed to tinnitus-related brain activity, we identified *tinnitus presence* as an important component for further analysis. To this end, tinnitus duration was transformed to the total amount of months to gain a parametric scale. Tinnitus pitch estimations (as obtained by means of sine wave generator of the MAX software suite [51]) were included to test whether it shares commonalities with the other subjective tinnitus variables.

2.6.2. Preprocessing of EEG Data. The raw data files from Net Station were transformed into EDF file format in order to preprocess them with BrainVision Analyzer [52]. Butterworth zero phase filters were applied: low cutoff was set at 0.5 Hz and high cutoff at 100 Hz. A notch filter was implemented at 50 Hz to reduce effects of the electric circuit on the EEG signal. For the PCA, the entire data set of each participant was used. Components containing eye movements or heart beats were identified and removed after a visual check of their impact on the EEG signal. Topographical interpolation was performed in order to recalculate rejected channels based on the signal mean of the four nearest electrodes. Next, the signal was re-referenced to the average amplitude of all electrodes at each sampling point (average reference). Data was segmented into 2 s epochs. After identification of remaining artifacts (e.g., muscle artifacts) based on visual inspection and supporting algorithms, respective segments were excluded from further processing. For EC this procedure resulted in a range of 72 s to 238 s of analyzable EEG data (M = 185.54, SD = 36.01).

2.6.3. Power Spectral Analysis. The number of electrodes was reduced to 109 channels by omitting the outermost ring of electrodes, as they usually show high amounts of noise. A power spectral analysis was applied using Fast Fourier transformation (FFT) with a Hanning window of 10% segment length. The spectrum from 0.5 Hz to 45 Hz was used with a resolution of 0.5 Hz. The power spectrum was averaged over all available 2 s segments for each subject. Next, segments were averaged over all available segments for each subject and electrodes separately. Data was then exported to MATLAB [53] for statistical analysis with custom scripts.

In analogy to the behavioral data, statistical comparisons between groups, conditions, and regression analyses were conducted. Student's t-tests were used to compare TI to CO during resting state. One-tailed tests were applied as we expected higher power values in the TI. Tests were done for each electrode and frequency bin separately. Behavioral data and EEG data were correlated by means of Pearson product-moment correlations within the TI population and cross-validated with nonparametric tests where indicated. Variables were controlled by means of partial correlations where appropriate. We generated topographical maps on the basis of the outcome of the statistical analysis. The topographical maps visualize the mean correlation between one of the two components and spatial distribution of EEG signal distribution across the scalp.

3. Results

3.1. Behavioral Data. For all questionnaire variables, psychometric data between TI and CO were compared. For the BDI, TI (M = 11.63, SD = 10.21) exhibited a higher extent of depression than the CO (M = 4.04, SD = 5.64, $t(36.16)$ =

Table 2: Correlations between various tinnitus-related measures.

		TQ total score	TQ emotional distress	PRISM	Tinnitus pitch	Tinnitus duration	VAS	BDI
TQ Total score	r	1	.971***	.763***	.589**	−0.276	.409*	.700***
	P		<.001	<.001	.004	.192	.047	<.001
TQ Emotional Distress	r		1	.728***	.696***	−.245	.427*	.760***
	P			<.001	<.001	.248	.037	<.001
PRISM	r			1	.576**	−.381	.498*	.423*
	P				.005	.067	.013	.039
Tinnitus Pitch	r				1	−.143	.380	.475*
	P					.526	.081	.026
Tinnitus Duration	r					1	.193	−.178
	P						.365	.406
VAS	r						1	.457*
	P							.025
BDI	r							1
	P							

Note. Pearson correlations. $N = 24$. From the TQ, only the total score and the subscore "Emotional Distress" are depicted because these scores yielded high correlations with most other measures. The BDI scale is included as the sole questionnaire measure in the table because the other measures did not correlate significantly with any tinnitus-related variables. */**/*** Significant correlation with $P < .05/.01/.001$.

3.167, and $P = .003$). Of additional interest, BDI scores varied more strongly in TI relative to CO ($F(45) = 5.045$, $P = .030$). A closer look revealed that 12 TI (out of 24) and 21 CO (out of 23, missing data from one control subject) showed normal scores.

To analyze the audiometric assessment, an average hearing threshold was built across all measured hearing thresholds (125, 250, 500, 1000, 2000, 4000, 6000, and 8000 Hz) for each ear separately. We found the average hearing threshold to be significantly higher in TI ($M = 18.98$, SD $= 7.47$) than in CO ($M = 13.07$, SD $= 3.63$, $t(33.29) = 3.489$, and $P = .001$).

Correlations were computed between all subjective measures of tinnitus characteristics and all questionnaire variables. A summary of the relevant data can be seen in Table 2. Interestingly, the PRISM tool has turned out to be highly indicative of tinnitus-related burden, as it correlates highly with TQ total score ($r = .763$, $P < .001$) and with TQ subscale "Emotional Distress" ($r = .728$, $P < .001$), with VAS ($r = .498$, $P < .013$) as well as with BDI ($r = .423$, $P < .039$). Tinnitus loudness (not depicted) did not correlate with other tinnitus-related variables. However, tinnitus pitch correlated positively with all measures related to tinnitus distress and tinnitus VAS scores on a moderate level. The highest correlation was observed between pitch and "Emotional Distress" ($r = .696$, $P < .001$).

3.1.1. Principal Component Analysis. As apparent from Table 2, the measures for tinnitus-related distress (TQ, PRISM, and tinnitus disturbance) showed intercorrelations on a moderate to high level. Thus it can be assumed that they are valid traits of tinnitus distress. Furthermore this pattern suggests that a single dimension may account for the majority of variation in the variables. Tinnitus duration correlates negatively with all measures of tinnitus distress but correlates positively with subjective strength of the tinnitus sensation as measured by the VAS. We thus concluded that other dimensions besides tinnitus distress contribute to the present strength of the tinnitus sensation. In order to extract converging information of the different psychometric measurements of tinnitus, we explored the available data with a PCA. PRISM, VAS, tinnitus duration, and TQ total score were included in the PCA. For the TQ, we decided to focus on the total score and not on "Emotional Distress" because items from other subscores also contained information relevant for evaluating the burden of suffering (e.g., item 10, which belongs to the subscale "Intrusiveness": "The ear sounds are really unpleasing."). The Kaiser-Meyer-Olkin measure (KMO) verified the sampling adequacy for the analysis; KMO = .55 [54]. Bartlett's test of sphericity, ($P < .001$), indicated that correlations between measures were sufficiently large for PCA. The extracted components were rotated with Varimax rotation with Kaiser normalization. An initial analysis revealed two components with eigenvalues >1, and the scree plot showed an inflexion which also justified to retain two components. In total the two components accounted for 85% of the total variance, which is satisfactory. Table 3 summarizes the results from the PCA.

The two applied measures of tinnitus-related distress (PRISM, TQ total score) and tinnitus strength as measured by VAS loaded strongly positively on the first component. We considered the first component to capture tinnitus-related distress, that is, the amount or burden of subjective suffering caused by the tinnitus. Tinnitus strength as measured by VAS loaded also strongly positively on the second component, together with tinnitus duration. We interpreted the second component as tinnitus presence, a perceptive aspect of tinnitus capturing the conscious awareness of the noise which increases with tinnitus duration but is unrelated to

Table 3: Rotated factor loadings.

	Component	
	1	2
PRISM	**.907**	−.247
TQ total score	**.865**	−.206
VAS (eyes closed)	**.744**	**.532**
Tinnitus duration	−.216	**.917**
Eigenvalues	2.171	1.229
% of variance	55.336	29.664

Note. The table lists rotated factor loadings (i.e., correlations), eigenvalues, and variance explained by each component. By convention, factor loadings above .40 appear in bold.

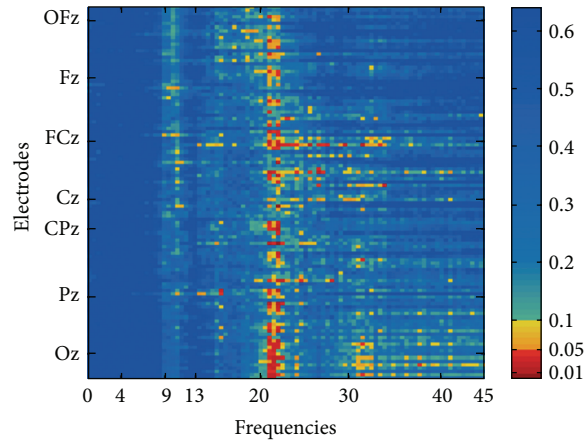


Figure 2: Comparison of TI versus CO normalized EEG spectral power adjacency matrix of the group comparison (TI versus CO): significance levels (P values) of the correlations are color-coded (one-sided). On the y-axis electrodes are aligned from rostral (top) to caudal (bottom), irrespective of laterality. Positions of electrodes on the central line are marked.

tinnitus distress. Of note, tinnitus strength as quantified by VAS is the only variable that loaded on both components, distress and presence. Based on the pattern of results we reasoned that there is a part of the tinnitus experience that is emotionally neutral, as expressed in the second component. Akin to our approach Schlee and colleagues [39] observed brain responses in TI that correlated with tinnitus duration, but not with tinnitus distress.

As apparent from Table 4 the two components do not correlate with the hearing threshold. This finding allows an interpretation of the data independent of the individual extent of hearing loss. While distress does not correlate with age we observed a weak correlation between age and presence for obvious reasons ($r = .380$, $P = .067$). BDI scores show a significant positive correlation with distress but not with presence. These findings support our interpretation that the first component delineates tinnitus-related distress whereas the second component depicts an emotionally neutral aspect of tinnitus. In conclusion, we identified two independent components that comprehensively explain the experience of subjective tinnitus in our sample of TI.

3.1.2. EEG Data. We analyzed EEG data for both EO and EC separately. The results between these two conditions did not differ considerably. Hence, we only report analyses for EC condition because it contains fewer artifacts from eye and head movements.

Figures 2–4 present the topographic maps and results of FFT-based power analyses. Please note that Figure 2 depicts normalized EEG power between the TI and CO groups, while Figures 3 and 4 show nonnormalized EEG pattern with the TI group. For the comparison between TI and CO segments were normalized by dividing each frequency bin by the total area under the curve of the according spectrum, thereby ascertaining that the total power of each spectrum was one unit. As apparent from Figure 2 TI showed weakly increased EEG power in the upper β-band between 20 and 22 Hz compared with CO. However, as outlined above, we did not expect substantial differences between TI and CO as the first group cannot be considered homogeneous due to the independent components—distress and presence—we identified. Thus, we did not further explore the difference between TI and CO but analyzed the two component scores obtained from the PCA. For this procedure, we used the components distress and presence to correlate behavioral data and the EEG signal.

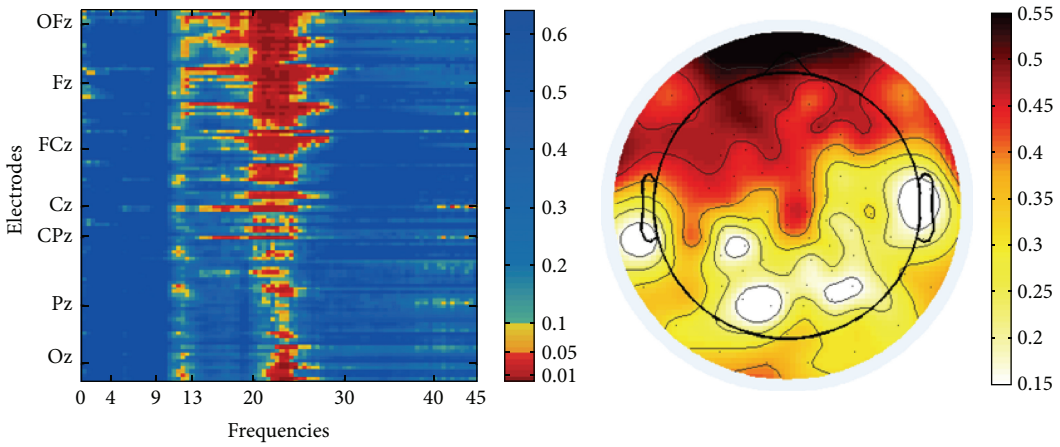
The EEG data partly confirmed this data-based differentiation. Negative correlations with tinnitus distress and presence, respectively, were not observed. Both Figures 3 and 4 visualize positive correlations between components and EEG signal. For the analyses of FFT-based power of TI we refrained from normalization of EEG data because we consider normalization only necessary when datasets from different groups are compared. Furthermore the results obtained from both normalized and nonnormalized data did not differ qualitatively. With respect to tinnitus-related distress we observed a strong correlation between distress and the EEG signal in the oscillatory range between 20 and 25 Hz (upper β-band) (cf. Figure 3). As apparent from the topographical map the maximum of activity agglomerates over frontal electrodes. A differential pattern of responses was observed for the second component. We performed partial correlations between presence and the EEG signal to control for age effects as this component weakly correlated with age ($r = .380$, $P = .067$). Presence corresponded to enhanced EEG signal predominantly in the δ-band (0.5–4 Hz), α-band (9–13 Hz), and lower γ-band (30–40 Hz) (cf. Figure 4). Unlike the first component, presence corresponded to a bilateral, but left dominant, maximum of activity over temporal auditory and adjacent left extraauditory recording sites for all frequency bands in general, but with a salient maximum for γ-activity.

Hence, the topographical maps and the EEG power analysis clearly confirm the results of the behavioral data. In the TI population differential subgroups can be identified who show differential psychometric and neurophysiological profiles.

Table 4: Correlations between components and other variables.

		Tinnitus distress	Tinnitus presence	Hearing threshold	Age	BDI
Tinnitus distress (first component)	r	1	.000	−.024	.056	.627**
	P		1.000	.911	.795	.001
Tinnitus presence (second component)	r		1	.151	.380	−.033
	P			.481	.067	.877
Hearing threshold	r			1	.594**	.123
	P				.002	.567
Age	r				1	.208
	P					.329
BDI	r					1
	P					

Note. Pearson correlations. $N = 24$. **Significant correlation with $P < .01$.



(a) (b)

Figure 3: (a) Nonnormalized EEG spectral power adjacency matrix of Pearson product-moment correlations with tinnitus distress. Significance levels (P values) of the correlations are color-coded (one-sided, uncorrected for multiple comparisons). On the y-axis electrodes are aligned from rostral (top) to caudal (bottom), irrespective of laterality. Positions of electrodes on the central line are marked. (b) Topographic plot of EEG power correlations in the upper β-band (20–25 Hz) with *tinnitus distress* and strength of correlations (Pearson's r) are color-coded.

4. Discussion

Even though 5–10% of the population in Western countries suffer from chronic tinnitus, it is bewildering to realize that only little is known about the neuroplastic changes and individual stamping of this phenomenon. This holds in particular because a significant fraction of TI (approximately 20%) [30] develop serious symptoms of distress that gravely affect their quality of life. The major reason for the immense lack of knowledge is the vast heterogeneity of tinnitus generators, the individual severity of primary and secondary, comorbid symptoms, the differences in individual coping strategies, and attribution of the life-illness relationship. Tinnitus is a phenomenon that is indicated by one sole major symptom, namely, a constant ringing in the ears or in the head but may manifest itself in multiple different forms and conditions. A recent integrative framework of auditory phantom perception proposes a compelling view that describes the interplay of several separable subnetworks in the human brain that are involved in tinnitus experiences [11]. According to this model tinnitus can be understood as a "unified coherent percept" that is modulated by a complex compound consisting of various psychological and neural traits. Moreover, correlating psychometric and clinical traits with EEG signal modulations offer a powerful option to systematically research the differential tinnitus profiles.

According to this framework we performed a study which collected psychometric, biographical, and neurophysiological resting state data in order to elucidate underlying mechanisms and opaque relationships between behavioral traits and EEG signal modulations.

The TI and CO samples are well matched in sex, age, handedness, and other biographical variables. Within the two

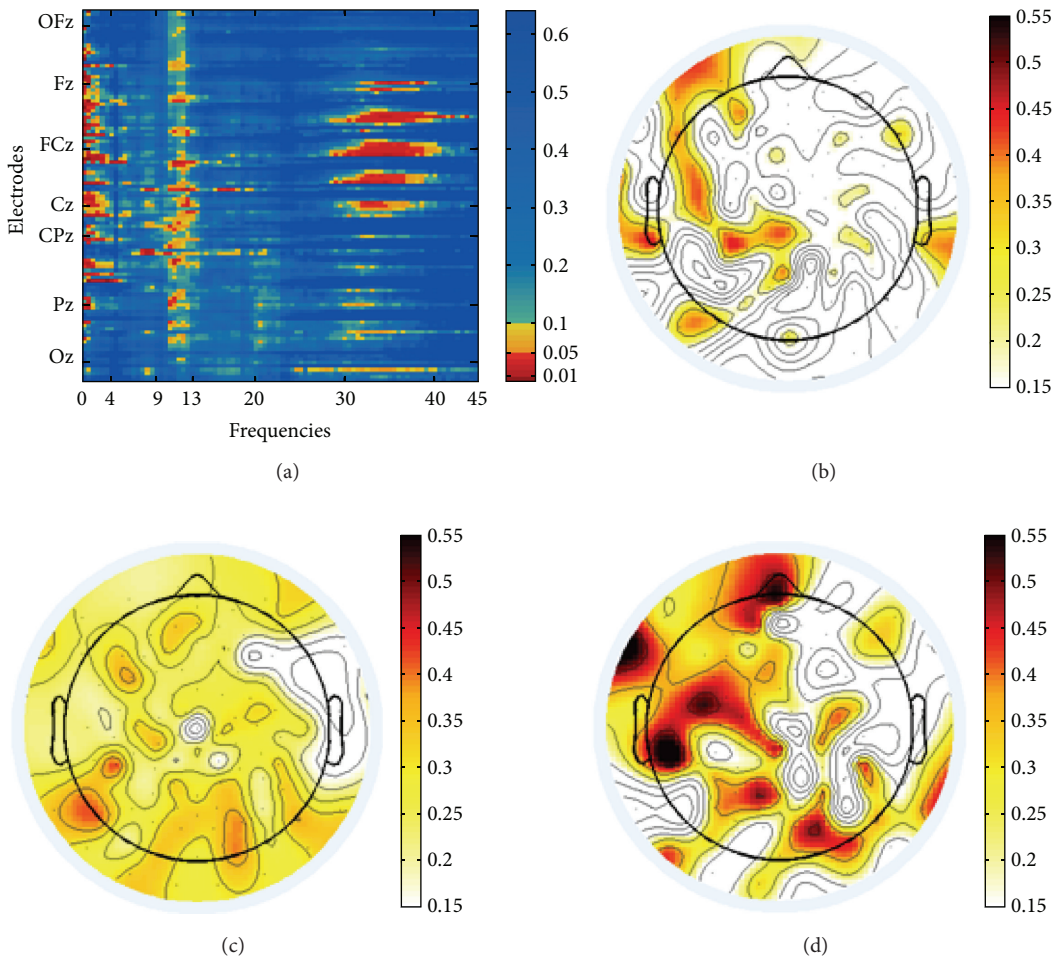


Figure 4: (a) Nonnormalized EEG spectral power adjacency matrix of Pearson product-moment correlations with tinnitus presence. Significance levels (P values) of the correlations are color-coded (one-sided, uncorrected for multiple comparisons). Electrodes on the y-axis are aligned from rostral (top) to caudal (bottom), irrespective of laterality. Positions of electrodes on the central line are marked. (b)–(d) Topographic plots of EEG power correlations in the δ-band (0.5–4 Hz) (b), in the α-band (9–12 Hz) (c), and in the γ-band (30–40 Hz) (d) with *tinnitus presence* and strength of correlations (Pearson's r) are color-coded.

samples we had an even distribution of age with approximately 25% of participants in each out of four lifetime decades. Based on this distribution age-biased effects (irrespective of hearing loss) in the TI population can be excluded. However, it should be mentioned that hearing loss was more prominent in the TI compared to the CO group. As apparent from Table 1 the TI indicate various effective or apparent elicitors that may have caused chronic "auditory pain." It thus comes as no surprise that we eventually identified two independent factors that characterize tinnitus.

The correlations between various tinnitus-related measures yield a plausible pattern (cf. Table 2). Generally, we noted a dense relationship between TQ total score, a comprehensive measurement of tinnitus-related annoyance, and BDI scores that indicate symptoms of depression. The same holds for other applied self-evaluation tools of subjective distress, namely, VAS and PRISM. A study by Joos and colleagues [30] provided evidence for the view that depression and distress could be disentangled in TI and are likely to recruit distinct neural circuits. However, they report a significant positive correlation between BDI and mini-TQ ($P < .05$) which is generally indicative of an existing relationship between these two emotional states. Maybe the fact that they used a reduced version of the TQ may account for the weaker correlation they observed. Based on our results we cannot make any statements about potential distinct neural circuits that mediate depressive symptoms in TI. Notably, Joos and colleagues observed correlations between activity in the β-band and both BDI and mini-TQ scores. This observation is similar to our finding of increased EEG activity in the β-band in TI who suffer from emotional distress. Thus, we suggest that tinnitus can be accompanied by both a transient state of distress and annoyance and a more constant depressive mood. Likely, the availability of appropriate coping mechanisms is supposed to modulate this relationship [55].

PRISM has been used successfully in several different clinical settings to gain a better understanding about the

self-assessed relationship between a patient and their illness [37, 45–47]. The high correlations between PRISM and other tinnitus-related measures, namely, TQ ($r = .763$, $P < .001$), VAS ($r = .498$, $P < .013$), and BDI ($r = .423$, $P < .039$), are in line with our predictions. PRISM scores showed the highest loading on the first PCA component distress, providing further evidence for the pertinent role of PRISM in measuring tinnitus distress (cf. Table 3, (.907)). Hence, we suggest that PRISM may be a quick, easy, and effective alternative to the application of the verbal TQ. Evidently, PRISM nonverbally measures tinnitus-related distress by means of solely one perspicuous question and achieves a validity that is similar to the TQ but takes considerably less time and is easy to perform.

Another interesting finding pertains to the relationship between tinnitus pitch, self-assessed by a standard sine wave generator to measure the approximate individual pitch height of the chronic noise (cf. Table 2). Tinnitus pitch correlates significantly with TQ total score ($r = .589$, $P < .004$), TQ "Emotional Distress" ($r = .696$, $P < .001$), PRISM ($r = .576$, $P < .005$), and BDI ($r = .475$, $P < .026$). In other words this finding suggests that the higher the subjective tinnitus the higher is the amount of distress. In our view this relationship has not yet been observed before and it is by all means worth reporting because it may imply that the determination of tinnitus pitch might reflect the interplay between subjective distress and objective features of the percept.

The First Component: Tinnitus-Related Distress. By means of comprehensive psychometric and neurophysiological data we identified two independent components that are supposed to characterize different TI profiles. The first component, "distress" can be straightforwardly interpreted because of the high loadings of PRISM, TQ-evaluated annoyance, and VAS. Across several psychometric measurements this component explains a high amount (55%) of the data. Interestingly, distress does not correlate with hearing loss and thus is probably not mechanically linked to the deafferentiated and dysfunctional auditory system. Previous studies that also applied hypothesis-blind approaches have identified a variable termed "distress" or "annoyance" [29, 34]. Thus, it is plausible to reason that tinnitus is frequently but not necessarily all the time related to transient emotional distress.

According to our results there is a relationship between the strength of distress and neural modulations in the upper β-band (20–25 Hz). While the tinnitus percept is frequently reported in association with γ-band increase [27, 31], studies that particularly elucidated the neural underpinnings of tinnitus-related distress noted changes across the entire β-band [29, 30]. In comparison to CO without tinnitus percept Vanneste and colleagues noted increased α- and β-oscillations originating from the dorsal anterior cingulate cortex in TI with distress [32]. Akin to our finding Joos and coauthors [30] observed in TI with unilateral tinnitus the occurrence of β-waves, predominantly in frontal areas that showed a strong positive correlation with distress. However, our finding of β-band activity is not perfectly comparable to the aforementioned studies as the present study does not provide results obtained from source estimation. This constraint notwithstanding the topographical map (cf. Figure 3(b)) shows the maximum of the distributed activity over frontal recordings sites which strongly speaks in favor of involvement of frontally situated top-down mechanisms that are recorded primarily when individuals pay attention to internal or external (auditory) stimuli [11, 30]. Presumably, dominant frontal signal power may be indicative of top-down driven evaluation processes that are closely related to the distress condition. However, based on our present data, it cannot be proposed whether this signal increase reflects successful coping with the tinnitus percept (competence) or whether it corresponds to a strenuous but unavailing attempt to get along with the disturbing sensation of chronic noise (incompetence). Actually we consider the latter interpretation more reasonable in the context of the existing literature. In their position paper De Ridder and coauthors [11] also suggest that preponderant β-oscillations can be attributed to dysfunctional noise canceling mechanisms. In our view this explanation can be brought in line with both the first and the latter interpretations. However, it should be mentioned that the distress circuit obviously at work in TI is not specifically related to the tinnitus percept but is probably identical with the general distress network that is part of a large-scale brain system. This network is supposed to mediate other aching percepts, namely, chronic pain [6, 56].

The Second Component: Tinnitus Presence. The second component we unveiled and named presence captures a perceptive aspect of tinnitus sensation. This dimension of tinnitus can be clearly distinguished from distress as it does not load on the distress-sensitive measurement tools but has high loadings from tinnitus duration (.917), that is, the period of onset from tinnitus experience until the screening session (see Tables 3 and 4, Figure 4). Apparently, presence as well as long-term duration of the tinnitus percept does not necessarily result in emotional distress and annoyance. Like distress, presence only correlates weakly with hearing loss and should thus be considered as independent from hearing integrity. Hence our data indicate that the presence, that is, the conscious awareness, of tinnitus increases as a function of tinnitus duration while this relationship does not hold for distress. Probably a fraction of TI have developed appropriate coping strategies to inhibit tinnitus-related annoyance. Interestingly, the two separate dimensions of tinnitus experience also indicate that some concerned individuals habituate to the chronic noise and consider it less annoying after some time, despite increasing presence of tinnitus sensation.

The results of the power analysis in TI show a more complex neurophysiological pattern correlating with presence as compared to the distress-related EEG modulations. As apparent from Figure 4(a) we observed minor but nonetheless significant signal increase in the δ- (Figure 4(b)) and α-band (Figure 4(c)). Furthermore we noted increased oscillations in the lower γ-band. For this frequency band the topographical map shows a maximal distribution of local power over (predominantly) left and (less prominently) right centrolateral recording sites (Figure 4(d)) which may be indicative of neural origins residing in auditory fields. This view concurs with the model proposed by De Ridder

and coworkers [11] who describe "persisting gamma activity localized in one brain area" as "pathological" (page 8). In this view γ-activity signals the breakdown of thalamocortical balance and reflects the binding of abnormally "distributed neural gamma activity into one coherent percept" (page 8). Thus, it should be regarded as the neural signature of abnormal synchronous oscillations, that is, the chronic tinnitus percept [12]. The occurrence of increased awareness of the tinnitus mirrored by enhanced δ- and α-activity in the EEG power spectrum can also be explained in the context of a complex network architecture. Usually α-waves are recorded from the auditory cortex during resting state and are indicative of a normally functioning system [11]. This statement notwithstanding increased α-band modulations have been observed in other former studies that investigated TI [16, 30, 57]. One possible explanation may reconcile these two apparently contradictory findings. It is conceivable that the default mode α-band activity serves as part of an active noise canceling system that (pro)actively eliminates detrimental noise in both TI (who are not this distressed) and nonaffected individuals. In other words, the significant α-band activity we revealed as a dimension of tinnitus presence can be considered a normal pattern of auditory activity that blocks any disturbing acoustic signal, amongst others' internally generated chronic noise. In TI who suffer more strongly and display more symptoms of tinnitus-related distress the noise-canceling system has been broken down due to maladaptive coping mechanisms. Increased δ-oscillations have also been associated with a deficient noise suppression mechanism [11] and should be considered a consequence of sensory deprivation that may result sooner in θ-γ instability and later in decoupling.

Limitations. Some limitations that may narrow down the significance of the present study should be mentioned. For an appropriate comparison between TI and CO it would have been indicated to match the auditory thresholds. Since there was greater hearing loss in the TI population we cannot clearly sort out the influence that hearing loss per se may have had on the comparison of EEG signal activity between TI and CO.

We concede that the age range (20–62) in our TI sample is relatively large. Little is known about the life-long neuroplastic changes of tinnitus experience on brain structure and function, but it seems that TI with an earlier onset of tinnitus apparently display less symptoms of suffering than individuals with a later onset in life [58]. Maybe the lack of cognitive coping strategies in older adults which may be a result of normal age-related frontal atrophy may account for this finding. Even though we did not notice any significant relationship between age and other variables, namely distress, depression, or disturbance, we think that a better exploration to what extent and how chronic tinnitus experience may differently affect elderly other than young(er) TI is needed [59].

Unlike previous studies [10, 29, 30, 33, 34, 60] that have also addressed the issue of neural signatures of tinnitus-related profiles we have not performed a source estimation. Of course it would have been interesting to complement our results with estimations of the EEG source generators to better understand the perplexing compound of the various facets of tinnitus. Unfortunately, probably due to the relatively small sample size, our source estimation did not yield results that weathered the conservative statistical testing we performed.

Finally it should be mentioned that the mean TQ-based distress score in our study was relatively low (TQ = 22) compared to other studies (TQ = 40.93) [30], (TQ = 40.2) [60], and (TQ = 42) [24]. Thus, it cannot be ruled out that the potential existence of behavioral and EEG effects has even been underestimated.

4.1. Conclusion. The present study is novel in that it—based on principal component and neurophysiological analysis—identifies two independent psychological dimensions, namely, distress and presence, that correlate with differential symptoms of chronic tinnitus and distinct neural signatures in the EEG power spectrum. While distress can be considered as a well-established factor that affects TI to a substantial degree, tinnitus perception seems to become more stable and present as a function of tinnitus duration. Interestingly, this dimension of tinnitus sensation is independent of emotional distress. The differential neural profiles observed for the two dimensions of chronic tinnitus suggest that differential adaptive and coping mechanisms in TI do exist. Hence the study makes a significant contribution to the underinvestigated field of neuroplasticity of tinnitus because it proceeds with the most recent initiatives to better understand the differences of individual psychological and neural profiles within the TI sample. We think that this approach is more promising than investing further research in the comparison between TI and nonaffected CO because our investigation has corroborated previous observations of other groups evincing that the population of TI is extremely heterogeneous. Hence, future research should concentrate on the exploration of specific behavioral and neural profiles within TI—as it has already been introduced [55, 61, 62]—to form a basis for the development of appropriate neuropsychological treatment approaches.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Martin Meyer and Matthias S. Luethi contributed equally to this paper.

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Research Article

Tinnitus-Related Distress and the Personality Characteristic Resilience

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It has been suggested that personality traits may be prognostic for the severity of suffering from tinnitus. Resilience as measured with the Wagnild and Young resilience scale represents a positive personality characteristic that promotes adaptation to adverse life conditions including chronic health conditions. Aim of the study was to explore the relation between resilience and tinnitus severity. In a cross-sectional study with a self-report questionnaire, information on tinnitus-related distress and subjective tinnitus loudness was recorded together with the personality characteristic resilience and emotional health, a measure generated from depression, anxiety, and somatic symptom severity scales. Data from 4705 individuals with tinnitus indicate that tinnitus-related distress and to a lesser extent the experienced loudness of the tinnitus show an inverse correlation with resilience. A mediation analysis revealed that the relationship between resilience and tinnitus-related distress is mediated by emotional health. This indirect effect indicates that high resilience is associated with better emotional health or less depression, anxiety, and somatic symptom severity, which in turn is associated with a less distressing tinnitus. Validity of resilience as a predictor for tinnitus-related distress is supported but needs to be explored further in longitudinal studies including acute tinnitus patients.

1. Introduction

Subjective tinnitus, an internal sound generated by aberrant activation within the auditory system, is a widespread phenomenon which constitutes a severe problem for 10%–20% of the tinnitus population [1]. The distress associated with tinnitus shows closer relation with factors related to emotional health as depression, anxiety, and somatic symptom severity than with the loudness of the tinnitus [2]. Moreover, depression and anxiety were found to be enhanced at tinnitus onset in patients who later develop high tinnitus-related distress [3, 4] suggesting that emotional health may be prognostic for future tinnitus-related distress. It cannot be excluded, however, that distressing tinnitus adversely influences emotional health and that its association with depression and anxiety is overestimated due to content overlap in the questionnaires [5, 6]. Therefore, alternative predictors, which are largely independent of the actual tinnitus-related distress, are needed for the prognosis of future tinnitus-related distress.

Personality characteristics display continuity throughout life, they have a predictive role for mental and physical health, and content overlap with the tinnitus questionnaires is not an issue. Personality characteristics associated with distressing tinnitus are emotional lability indicated by increased neuroticism and decreased extraversion [7–10] and the tendency to experience fear when perceiving body signs of arousal (rev. in [11]). Consequently, trait anxiety correlates with tinnitus-related distress, and distressed type D personality is overrepresented in tinnitus populations (rev. in [11, 12]). Also, significant numbers of somatic symptoms, which are linked

to the personality traits of neuroticism or negative affectivity [13], are found in a substantial portion of tinnitus patients [2], and depression and anxiety decreased with time only in those tinnitus patients that did not exhibit personality disorders [9]. Although there is no consensus about the role of personality for tinnitus severity [8], personality may influence the way tinnitus is dealt with and especially influence the persistence of tinnitus through a personality-driven tendency to be aware of it [9].

The concept of the positive personality characteristic resilience delineates capabilities of an individual to cope effectively with adverse life conditions such as chronic disease [16, 17]. The personality traits emotional stability and extraversion are associated with resilience [18], whereas depression and anxiety are inversely related to it [19]. Resilience was linked to psychobiological mechanisms that keep the hypothalamic-pituitary-adrenal (HPA) axis and the noradrenergic system, which are suspected to promote tinnitus-related distress [20] within an optimal range during stress exposure and terminate the stress response early [21, 22]. This is thought to be largely determined by genetic disposition in conjunction with early life experiences [23, 24].

The Wagnild and Young resilience scale was shown to be an appropriate instrument to study the personality characteristic resilience in adult populations (rev. in [16, 23–28]), and short versions of this scale are increasingly being used [25, 26, 29–31]. The German short version (RS-13) has been validated in representative clinical and nonclinical samples [25, 26].

Aims of the present study were to assess trait aspects of resilience in a tinnitus population and to relate these to measures of tinnitus-related distress and subjective tinnitus loudness. To gain an understanding for causal relationships between the personality characteristic resilience, the emotional health measures depression, anxiety, and somatic symptom severity, and the tinnitus-associated symptoms tinnitus-related distress and tinnitus loudness, we established a mediator model [32]. We hypothesized that the personality characteristic resilience is an important factor for determining the reaction on tinnitus as reflected in the amount of tinnitus-related distress, and that much of its influence is conveyed through a factor emotional health generated from current status of depression, anxiety, and somatic symptom severity. Furthermore, we hypothesized that the influence of resilience on tinnitus-related distress is higher than its influence on the subjectively perceived loudness of the tinnitus, which is thought to be affected primarily by hearing-related pathologies [2].

2. Methods

2.1. Data Collection and Sample. A questionnaire was sent to all 13,349 patient members of the German Tinnitus Association (DTL) together with a letter informing the participants that by filling out and sending in the questionnaire they agreed to the use of their data for research purposes. The DTL is a registered charity that provides information, support, and advice about tinnitus and funds research thereby aiming to raise awareness about the condition. 4752 questionnaires (35.6%) were received, and the data of 4705 questionnaires were entered into the data base. The rest was omitted mainly because of invalid membership numbers [2]. Questionnaires were pseudonymised in that they contained the membership code but not the participants' names. The study protocol was approved by the ethics committee and by the data safety commissioner of the Medical Faculty Mannheim of Heidelberg University according to the principles expressed in the Declaration of Helsinki. The following parts of the questionnaire were used.

2.2. Measures. Tinnitus-related distress was assessed with the 12-item Mini-Tinnitus Questionnaire (MTQ [14]). The MTQ represents an abridged version of the Tinnitus Questionnaire. It defines a general dimension of distress that has a high degree of correlation ($r = .90$) with the full Tinnitus Questionnaire. The test-retest reliability of the MTQ was .89 [14]. Sum scores range from 0 (no distress) to 24 (maximum distress) and were derived only from cases with complete MTQ-scales. Subjectively perceived tinnitus loudness was recorded on a numeric rating scale (T-NRS) from 0 (tinnitus audible only during silence) to 10 (tinnitus louder than all external sounds).

Resilience was addressed with the RS13 questionnaire [26]. The RS13 has a high correlation with the longer 25-item form of the resilience scale ($r = .95$), and its internal consistency is high with a Cronbach's alpha of .91 [26]. Response options ranged from 1 (strongly disagree) to 7 (strongly agree). A sum score was calculated from the 13 items with scores between 13 and 91, and higher scores indicating better resilience.

In addition three modules of the Patient Health Questionnaire (PHQ) addressing depression (PHQ9), generalized anxiety (GAD7), and somatic symptom severity (PHQ15) were included [15, 33]. These PHQ-scales have been used in clinical studies across a variety of medical conditions; their internal consistency is high with a Cronbach's alpha of .8 or above for PHQ15 and PHQ9 and a test-retest reliability around .83 for the three scales [33]. Response options for PHQ9 and GAD7 were 0 (not bothered at all) to 3 (bothered almost every day), and for PHQ15 they were 0 (not bothered at all) to 2 (bothered a lot). In all PHQ modules higher scores indicated greater symptom severity [15]. A case was eliminated for classification in a scale if a single item was missing, but if the two items addressing premenopausal women and sexually active persons in PHQ15 were left blank, they were scored as 0 [2].

2.3. Data Analysis. Analyses were performed with SPSS22. Bivariate and partial correlation coefficients were calculated to verify relations among the variables. Because correlation between the variables depression, anxiety, and somatic symptom severity were high, a variable "emotional health" (EH) was generated from the z-standardized PHQ-scales. Low values in the EH variable represent the more favourable condition of better emotional health. For the following analyses the variables RS13, MTQ, and T-NRS were z-standardized as well. Two stepwise regression analyses quantified the extent to which EH and RS-13 explained variance in tinnitus-related

Table 1: Descriptive statistics.

	Number of valid answers	Mean [SD] or %	Q1–median–Q3	Range	Female//male mean [SD] or %
Male	4606	59.1			
Age	4490	58.6 [11.8]	50–59–68	18–94	57.4 [12.2]//59.5 [11.4]
Tinnitus duration >5 years	4608	84.0			81.1//87.7
Tinnitus-related distress (MTQ)	4661	10.4 [6.5]	5–10–15	0–24	10.3 [6.2]//10.5 [6.5]
Subjective tinnitus loudness (T-NRS)	4372	6.0 [2.5]	4–6–8	0–10	5.9 [2.5]//6 [2.5]
Depression (PHQ9)	4369	7.1 [5.4]	3–6–10	0–27	7.5 [5.2]//6.9 [5.5]
Anxiety (GAD7)	4546	6.0 [4.8]	3–5–8	0–21	6.4 [4.8]//5.7 [4.8]
Somatic symptom severity (PHQ15)	4131	8.4 [5.2]	4–7–11	0–32	9.4 [5.3]//7.7 [5.1]**
Resilience (RS13)	4396	66.4 [15.1]	57–69–78	13–91	65 [15.1]//67 [15]

Demographic, psychological, and tinnitus characteristics of the study sample. Gender differences were minor, except for the somatic symptom scale PHQ15 (**), in which females could reach higher scores than males (see Section 2).

Table 2: Bivariate correlations.

	MTQ r (95% CI)	T-NRS r (95% CI)	RS13 r (95% CI)	PHQ9 r (95% CI)	GAD7 r (95% CI)
MTQ	1				
T-NRS	.526 [.498––.551]**	1			
Resilience (RS13)	−.399 [−.428––.369]**	−.132 [−.165––.098]**	1		
Depression (PHQ9)	.667 [.646–.687]**	.352 [.322–.382]**	−.559 [−.584––.533]**	1	
Anxiety (GAD7)	.616 [.593–.637]**	.303 [.271–.333]**	−.548 [−.574––.523]**	.805 [.790–.819]**	1
Somatic symptom Severity (PHQ15)	.540 [.514–.564]**	.303 [.271–.333]**	−.440 [−.468––.413]**	.758 [.742–.773]**	.655 [.634–.675]**

Bivariate Spearman-Rho correlation coefficients and their 95% confidence limits (95% CI) are reported. Confidence limits that do not include 0 are considered significant. MTQ—tinnitus-related distress assessed with the 12-item Mini Tinnitus Questionnaire [14], T-NRS—tinnitus loudness rated on a numeric rating scale. $^{**}P < .001$.

distress and subjective tinnitus loudness. Finally, direct and indirect effects of the personality characteristic resilience on tinnitus-related distress were assessed in a mediation analysis, using the SPSS macro provided by [31]. In this model, EH which was significantly correlated with both RS13 and MTQ was considered to be a potential mediator between the personality trait resilience and tinnitus-related distress. Causal order of the variables with the personality characteristic resilience as independent variable, emotional health as mediator, and tinnitus-related distress as outcome was based on theoretical grounds. As recommended by [32], significance of the indirect effect was also tested by means of a bootstrap analysis with 5000 bootstrap samples.

3. Results

3.1. Sample Characteristics and Bivariate Correlations. The sample has been described in detail in a preceding publication [2]. Resilience had been recorded along with the other variables but was not included in the previous analysis [2]. 4705 participants provided their data; 59.1% of them were male. Since results did not deviate substantially between genders (see Table 1), results are reported for the whole sample. Mean age was 58.6 [SD = 11.8] and 84% experienced tinnitus for more than 5 years. With a mean of 10.4 [6.5] the average sum score of the MTQ (Table 1) fell into the category of moderate tinnitus-related distress, with 37.6% reporting mild distress (MTQ ≤ 7), whereas 13.4% felt severely distressed by their tinnitus (MTQ ≥ 19). Cronbach's alpha for MTQ was high in this sample with 0.91, as well as for the three PHQ-scales with .87 for PHQ9; .90 for GAD7; and .81 for PHQ15. In the PHQ scales a score of 10 and above is the most commonly recommended cut point for clinically significant symptoms on all three scales [33]. Averages for each of the scales were below 10 (Table 1), but 20.6%, 27%, and 35.8% reached scores of 10 or above in the PHQ9, GAD7, or PHQ15, respectively. In contrast to the other scales, higher scores in the RS13 scale are desirable. Average of RS13 was 66.4 [15.1] (Table 1) which is slightly lower than that found in a normative sample ([26]: 70.0 [9.0]). Again, Cronbach's alpha for RS13 was high with .93.

3.2. Bivariate Correlations and Regression Analyses. All bivariate correlations were highly significant. The highest correlations were observed among the PHQ variables (Table 2). For tinnitus-related distress correlations were higher with depression and anxiety than with the subjectively perceived tinnitus loudness or somatic symptom severity. Inverse relations existed between all these variables and the RS13 resilience scale. Correlations of RS13 with the three PHQ-scales were higher than with tinnitus-related distress. Thus higher or more positive resilience scores were linked to lower

levels of depression, anxiety, and somatic symptom severity as well as to lower tinnitus-related distress. All correlations with subjective tinnitus loudness were conspicuously lower, and the lowest was the inverse correlation between T-NRS and RS13 (Table 2). Subsequently, two stepwise regression analyses were performed, one with MTQ and the other with T-NRS as dependent variable, to quantify the extent to which the PHQ-measures and RS13 explain variance in tinnitus-related distress and subjective tinnitus loudness, respectively. For these analyses, the three PHQ-scales were comprised into the variable emotional health (EH). Since the PHQ-scales have different ranges (Table 1), the variables were z-standardized prior to averaging. In addition, the other variables included in the regression analyses were z-standardized as well. Results of the regression analysis with MTQ as dependent variable evidenced that EH contributed 43.3% to the total of 43.4% of the explained variance in MTQ, while the influence of RS13 on MTQ was negligible (Table 3(a)). The second regression analysis with T-NRS as dependent variable showed that EH and RS13 only explained about 12% of the variance in T-NRS, and again the effect of RS13 was negligible (Table 3(b)).

3.3. Indirect Effect of Resilience on Tinnitus-Related Distress. Finally, a mediation analysis was conducted with the z-standardized values of the variables MTQ, RS13, and EH. For this analysis RS13 served as independent variable, EH served as mediator, and MTQ was the dependent variable. Results of this analysis were in line with the assumption that resilience has a significant, although indirect, effect on tinnitus-related distress. The total effect of RS13 on MTQ expressed as β was −.399. Most of this effect was indirect ($\beta = -.360$) and in the model was conveyed via the mediator variable EH. The direct effect of resilience on tinnitus-related distress was of much smaller magnitude with a β of −.038. Moreover, whereas the direct effect barely reached significance with $P = .048$, the total and the indirect effects of RS13 on MTQ through the mediator EH were significant (Table 3(c)).

Table 3: (a) Results of regression analysis 1 with MTQ as dependent variable. (b) Results of regression analysis 2 with T-NRS as dependent variable. (c) Results of mediation analysis with MTQ as dependent variable.

(a)

Independent variables	β Step 1	β Step 2
Step 1. adj. $R^2 = .433$, $F(1, 4327) = 3301.43^{***}$		
Emotional health	$.658^{***}$	
Step 2. adj. $R^2 = .434$, $\Delta F(2, 4326) = 7.97^{**}$		
Emotional health		$.636^{***}$
Resilience		$-.039^{**}$

(b)

Independent variables	β Step 1	β Step 2
Step 1. adj. $R^2 = .119$, $F(1, 4092) = 552.65^{***}$		
Emotional health	$.345^{***}$	
Step 2. adj. $R^2 = .123$, $\Delta F(2, 4091) = 21.05^{**}$		
Emotional health		$.391^{***}$
Resilience		$.081^{***}$

(c)

Effect	β	BCa 95%	
		Lower	Upper
IV (RS13)—mediator (EH)	$-.614^{***}$		
Mediator (EH)—DV (MTQ)	$.586^{***}$		
IV—DV direct effect	$-.038^{*}$		
IV—DV indirect effect	−.360	−.385	−.324
IV—DV total effect	$-.399^{***}$		
Adj. $R^2 = .434$, $F(2, 4326) = 1657.36^{***}$			

(a) A stepwise regression analysis with the z-standardized variables emotional health (EH) and resilience (RS13) as independent and tinnitus-related distress (MTQ) as dependent variable.
(b) A stepwise regression analysis with the z-standardized variables emotional health (EH) and resilience (RS13) as independent and subjective tinnitus loudness (T-NRS) as dependent variable.
(c) Mediation was subsequently tested with z-standardized RS13 as independent (IV) and z-standardized MTQ as dependent (DV) variable and the z-standardized variable EH as mediator. The mediation effects were estimated by bootstrap analyses [15].
BCa 95% CI = bias corrected 95% confidence interval based on 5000 bootstrap samples.
A confidence interval that does not contain 0 indicates a significant effect.
$^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$.

4. Discussion

To the best of our knowledge this is the first study to explore the relation of the positive personality characteristic resilience with tinnitus-related distress and subjective tinnitus loudness in a large tinnitus population. Results of a bivariate analysis indicate that the correlations of resilience and of emotional health (a factor generated from depression, anxiety, and somatic symptom severity scores) with tinnitus-related distress are higher than with perceived tinnitus loudness confirming the distinction between these tinnitus characteristics reported earlier [14]. Results of the bivariate analysis furthermore indicate a significant correlation between resilience and emotional health corroborating earlier findings in population samples that were selected for characteristics other than tinnitus [25, 34]. Results of a regression analysis that considers resilience and emotional health in conjunction indicate that current emotional health has a large effect on tinnitus-related distress but a small effect on subjective tinnitus loudness, whereas resilience has a negligible effect on both tinnitus characteristics. Finally, results of a mediator analysis which serves to reveal indirect effects of a factor on an outcome variable are in line with the interpretation that resilience has an indirect effect on tinnitus-related distress conveyed by the present status of emotional health. As the personality trait resilience is fairly stable throughout life [16, 23, 24] while tinnitus usually arises at middle or older age [2], low resilience is unlikely to develop as a result of current low emotional health or through experiencing distressing tinnitus. Rather, low resilience may promote an unfavourable emotional health status which in turn may promote high tinnitus-related distress. Along this line of reasoning, the study extends prior research on

an association between personality and tinnitus characteristics by suggesting that personality has an indirect influence on tinnitus severity conveyed via general emotional health.

Resilience is a personality characteristic associated with adaptation to adverse chronic health conditions. Individuals with high resilience scores exhibit emotional stability and possess a behavioural repertoire that allows them to face stress and adversity in such a way that they retain their emotional balance. High resilience has been associated with an internal locus of control [35], that is, the extent to which an individual perceives an event to be under his own control, and an internal locus of control was found to be associated with lower tinnitus-related distress [36]. Usually highly distressed tinnitus patients believe that they cannot influence their tinnitus (external locus of control) and as a consequence they do not apply effective coping strategies [36, 37]. Interestingly, a mediating effect of coping on the relation between illness representations and adjustment to the tinnitus has been reported recently [38].

Although results have to be interpreted within the limits of a cross-sectional design, they are consistent with the interpretation that resilience has an indirect effect on tinnitus severity which is mediated by current emotional health. This interpretation is corroborated by longitudinal studies, which suggest that depression and anxiety levels at tinnitus onset are related to the progression of tinnitus-related distress [3, 4]. Furthermore, it was observed that depression and anxiety in tinnitus sufferers decreased with time only in those tinnitus patients that did not exhibit personality disorders [9]. Even though ultimate proof for the validity of these interactions requires further prospective studies, testable interactions between the variables are suggested.

Some other limitations of the present study should be noted. As the members of the DTL are a self-selected sample, they may not be representative of the general tinnitus population. The distribution of resilience in the study sample is comparable to that of other studies with the same instrument, however [26, 27]. Furthermore, it cannot be excluded that some questions of the self-report questionnaire were misunderstood or were reported incorrectly. The resilience scale does not contain items to control for response biases. Though high consistency within the scale as indicated by a high Cronbach's alpha as well as the distribution of resilience in the study sample which is comparable to that of other studies with the same instrument, together with data obtained with other personality inventories [39, 40], argue against intentional bias in tinnitus populations.

5. Conclusions

Analysing data from a large tinnitus population we found that low resilience is associated with low emotional health and with distressing tinnitus. When considering the personality trait resilience and the current status of emotional health in conjunction, resilience has only a minor effect on tinnitus characteristics. Because of its association with emotional health, resilience may nevertheless serve as an indicator for future development of tinnitus-related distress, since it is less likely to be influenced by adverse transient life conditions and by distressing tinnitus than emotional health. This needs to be verified in longitudinal studies involving patients with acute tinnitus.

Conflict of Interests

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Research Article

The Enigma of the Tinnitus-Free Dream State in a Bayesian World

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There are pathophysiological, clinical, and treatment analogies between phantom limb pain and phantom sound (i.e., tinnitus). Phantom limb pain commonly is absent in dreams, and the question arises whether this is also the case for tinnitus. A questionnaire was given to 78 consecutive tinnitus patients seen at a specialized tinnitus clinic. Seventy-six patients remembered their dreams and of these 74 claim not to perceive tinnitus during their dreams (97%). This can be most easily explained by a predictive Bayesian brain model. That is, during the awake state the brain constantly makes predictions about the environment. Tinnitus is hypothesized to be the result of a prediction error due to deafferentation, and missing input is filled in by the brain. The heuristic explanation then is that in the dream state there is no interaction with the environment and therefore no updating of the prediction error, resulting in the absence of tinnitus.

1. Introduction

Fundamental concepts in psychology and philosophy of the mind are the notion of sensation and perception [1]. When a stimulus produces an effect on different sensory receptors it induces sensation. Subsequent interpretation and organization of this sensory stimulus produce a meaningful experience of the world and of one's perception [1]. Although in most cases perception is conscious, perception without awareness does exist, that is, the interpretation of semantic stimuli [2]. Normally wakefulness and awareness are related; one has to be awake; that is, there has to be a certain level of consciousness to be aware of something; that is, there is content in consciousness [3]. In states of deep sleep, anesthesia, and coma there is little or no wakefulness and hence no awareness. In drowsiness and light sleep there is more awareness. However, in certain states, dissociations exist between wakefulness and awareness, such as in the vegetative state, when there is wakefulness presumably without awareness (eyes open, brain shut) [4]. In the dream state there is awareness (content in consciousness) with decreased wakefulness (level of consciousness) [3]. Dreams are succession of images, ideas, emotions, and perceptions without sensations that occur involuntarily in the mind predominantly during rapid eye movement (REM) sleep.

Nonpulsatile subjective tinnitus is considered a phantom perception [5], the conscious awareness of a percept in the absence of an external stimulus. It is characterized by the perception that the phantom sound comes from an external sound source, even though the sound might be pulled from memory [1, 6, 7]. This is reminiscent of a dream state, when there is awareness, with stimuli attributed to the external world but generated internally [8]. Whereas tinnitus can be considered a simple phantom percept, dreams could be considered complex phantom percepts, like hallucinations and hallucinosis [9, 10]. However, in contrast to hallucinations and hallucinosis that occur during wakefulness, dreams occur during certain stages of sleep.

Stimulus-evoked auditory cortical activation does not necessarily produce conscious auditory perception [11], and

auditory perception is possible in the absence of auditory input: more than 80% of people with normal hearing perceive phantom sounds when placed in a soundproof room [12]. Likewise, after limb amputation almost all people experience a phantom limb [13], whereas 70% suffer from severe phantom pain [13].

A clear clinical analogy exists between phantom pain and disabling tinnitus [1, 14, 15]. There are also parallels between the pathophysiology of tinnitus and pain [1], as well as in the treatment [16, 17]. However, there are also differences between tinnitus and pain. While physiological pain is mediated via nociceptive pathways, no analogous physiological tinnitus pathways exist. This could explain why commonly available analgesics that suppress acute physiological body pain are inefficient in ameliorating tinnitus [18]. Also, medications such as antiepileptics and antidepressants, which are effective in the treatment of neuropathic pain [18], tend to be ineffective for tinnitus [19].

Many to most (33–100%) patients who suffer from phantom limb percepts do not experience phantom limb percepts in a dream state [20–23]. This has been explained as follows [8, 21, 23]: neural representation of the body derives from sensory and proprioceptive feedback from the body. During sleep, when the brain/mind is actively kept offline, this sensory feedback is lacking. Moreover, during REM sleep and in the absence of external inputs, dreaming could activate a set of innate or early life spatial-temporal categories [8]. So if REM sleep is a state of protoconsciousness, that is, a contextually emergent property of self-sustaining systems, the self as it appears in REM sleep dreams is no longer affected by waking experiences because it feeds from an embodied and functionally intact body scheme [8, 21].

In view of the pathophysiological analogy between tinnitus and pain, it can be hypothesized that tinnitus is absent in the dream state as well. We therefore explored this in a group of 78 consecutive tinnitus patients attending the Multidisciplinary Tinnitus Research Initiative Clinic at the University of Antwerp. A recently proposed pathophysiological model of phantom sound based on a predictive brain concept with Bayesian updating [24] might explain why tinnitus is not perceived during dreaming.

2. Methods

2.1. Participants. Seventy-eight patients (57 males and 21 females) with chronic, nonpulsatile tinnitus were included in this study with an average age of 48.78 years (Sd = 12.87) and an average tinnitus duration of 5.74 years (Sd = 6.96). Thirty-five patients perceive noise-like tinnitus, while 43 patients experience pure tone tinnitus. Forty-three patients had bilateral tinnitus; 12 patients perceive tinnitus holocranially, 12 on the left side and 11 on the right side. Antwerp University Ethics Committee reviewed and approved the study. All patients signed an approved informed consent in order to enroll into the study.

2.2. Questionnaire. A questionnaire was created based on previous research in phantom limb pain and dreaming [20]. The first question asked whether the tinnitus patient recalled if they dreamed during the night (1), followed by the question whether in their dreams they perceive tinnitus (2).

3. Results

Of the 78 participating patients only 2 (2.56%) declared that they do not recall their dreams, while 76 (97.44%) do. Of those 76 patients that do recall their dreams 74 (97.73%) state that they do not perceive tinnitus while dreaming or are not aware of having tinnitus during sleep.

4. Discussion

People with tinnitus do not perceive tinnitus in their dreams analogous to what is reported for many phantom limb perceptions [21, 25]. Dreams and wakefulness are both associated with awareness, but in one state of awareness there is no tinnitus (dreams), whereas in the other (wakefulness) there is tinnitus.

The reason why patients with tinnitus do not perceive tinnitus in their dream state can be theoretically explained by the Bayesian brain model which has been used as an explanation for the development of tinnitus in relation to auditory deafferentation [24]. This Bayesian brain model is founded on an extension of a predictive brain model (see Figure 1(a)).

Whereas other models (see [26] for an overview) can explain the tinnitus in the presence of deafferentation, they cannot explain why it would be absent in the dream state. The Bayesian model is compatible with both the deafferentation and noise-cancelling models [24] and provides a rationale why tinnitus develops in a wake state and not in a dream state. Previously proposed models rather describe how tinnitus would develop.

Physiologically the brain can be conceptualized as a Helmholtz machine [27] that constantly makes one or possibly multiple [28] predictions about the world. A Helmholtz machine tries to find a hidden structure in unlabeled data. Since the examples given to the learner are unlabeled, there is no error or reward signal to evaluate a potential solution; in other words, there is no updating of the predictions. A Bayesian brain however updates predictions based on what it actively explores in the environment by means of the senses [24, 29, 30]. Bayesian inference can therefore be conceptualized in a way that would be familiar to John Hughlings-Jackson as using sensory information from the environment to update memory-based expectations (held before acquiring sensory inputs) to produce posterior beliefs represented as percepts. This mechanism permits decision making based on predictions updated by actively sampling the environment for confirmation or rejection of expectations (see Figure 1(b)) [24].

Auditory deafferentation limits the amount of information the brain can acquire to make sense of the world. The topographically specific deafferentation induces a topographically specific prediction error hypothetically based on temporal incongruity [1]. In other words, it is inconsistent

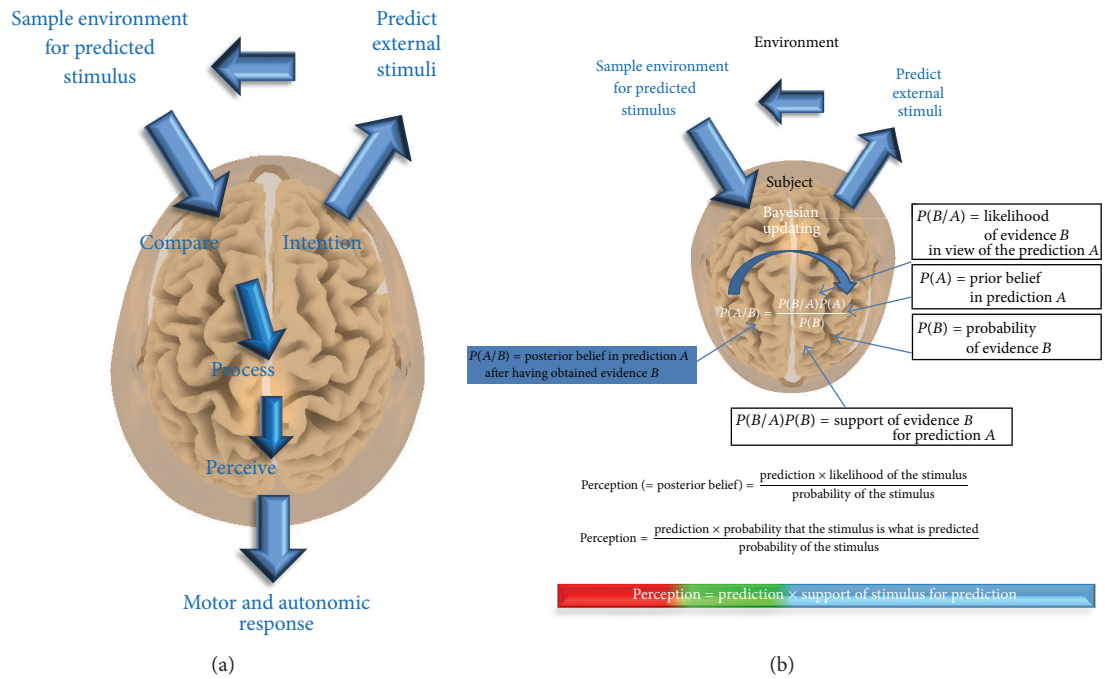

Figure 1: (a) The concept of the predictive brain; (b) the concept of Bayesian updating.

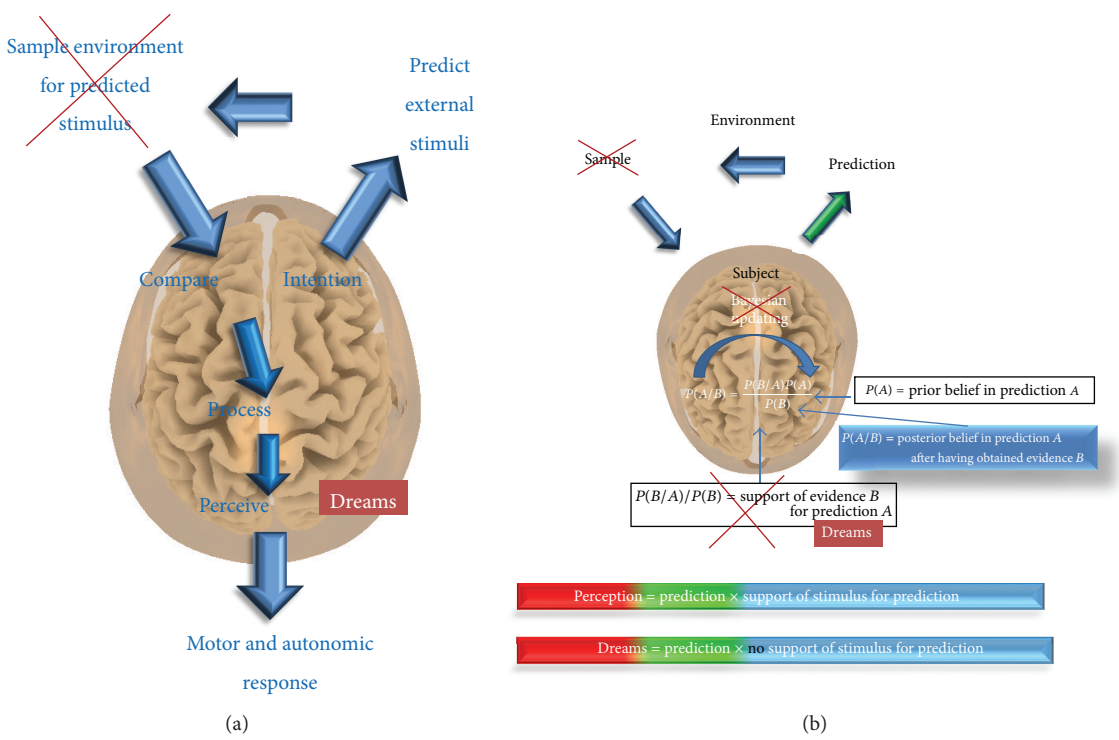

Figure 2: (a) Hypothetical explanation of the absence of tinnitus in dreams as seen from the predictive brain; (b) hypothetical explanation of the absence of tinnitus in dreams as seen from the Bayesian brain.

with what is stored in memory and should be updated. The model hypothesizes that deprived auditory information depends on the amount (bandwidth) of deafferented auditory channels [24]. Limited damage to auditory receptors causes loss of functional surround inhibition in the cortex, unmasking of latent inputs, and significantly altered neural coding. However, these changes do not lead to plasticity of the cortical map [31]. This suggests that the missing information can be obtained via access of overlapping tuning curves of the neighboring cortical cells. If the deafferentation is somewhat larger, a widening of auditory receptive fields [32] will permit pulling the missing information from the auditory cortical neighborhood. If this is insufficient, due to a still larger deafferentation, dendritic and axonal rewiring can occur [33]. If this is still insufficient, the missing auditory information can be pulled from (para)hippocampal memory [24].

When we dream, we create an image of the world that is entirely detached from sensory feedback [34]; that is, it cannot be updated. This is under influence of decrease in monoamines in REM sleep. Aminergic activity is highest during waking, declines during NREM sleep, and is lowest during REM sleep. Cholinergic activity on the other hand shows the reverse pattern [34]. Sensory prediction errors are suppressed by aminergic influence during sleep [34]. This means that the discrepancy between top-down predictions and (the absence of) sensory signals received will not be registered, and the auditory deafferentation will not be filled in, resulting in the absence of tinnitus in the dream state (see Figures 2(a) and 2(b)) [26].

Indirect arguments for this hypothesis come from recent research on cerebellar influences in tinnitus. It has been argued that the cerebellum is involved in motor, sensory, and cognitive predictions [35]. It is therefore possible that auditory predictions are made in the paraflocculus, as removing this cerebellar structure can prevent tinnitus from arising and arrest the presence of tinnitus in animals [36]. This conceptually suggests that removing the prediction can prevent or abolish tinnitus, which is in accordance with the concept that tinnitus could be a malprediction [1].

However, apart from its theoretical implications, the data might also help to find the neural correlates of tinnitus. The putative on/off switch for tinnitus is to be found in these areas that differ between waking and REM state [26], that is, the ventrolateral prefrontal cortex/frontopolar-inferior parietal-cerebellar-parahippocampal network [10]. These areas overlap with a recent meta-analysis of PET studies in tinnitus [37] and provide a framework for zooming in on the pathophysiology of this enigmatic symptom.

In addition to its evident benefit for tinnitus research, it could also provide clues for consciousness research, by delineating the core areas involved in the neural correlates of consciousness; that is, minimal assembly of brain areas required for consciousness per se [38, 39].

Other potential explanations for the absence of tinnitus in the dream state have to be considered. It is possible that during the dream state there is an attention shift from the tinnitus to the dream, analogous to what is noted in patients who do not perceive their tinnitus when intensely engaged in a task.

In conclusion, this report demonstrates that tinnitus perception is switched off during dream sleep even though there is awareness, like in wakefulness. This suggests that it is theoretically possible to find the neural correlates of phantom sound and thereby find a potential avenue for suppressing this enigmatic symptom.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Diffusion Imaging of Auditory and Auditory-Limbic Connectivity in Tinnitus: Preliminary Evidence and Methodological Challenges

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Subjective tinnitus, or "ringing in the ears," is perceived by 10 to 15 percent of the adult population and causes significant suffering in a subset of patients. While it was originally thought of as a purely auditory phenomenon, there is increasing evidence that the limbic system influences whether and how tinnitus is perceived, far beyond merely determining the patient's emotional reaction to the phantom sound. Based on functional imaging and electrophysiological data, recent articles frame tinnitus as a "network problem" arising from abnormalities in auditory-limbic interactions. Diffusion-weighted magnetic resonance imaging is a noninvasive method for investigating anatomical connections in vivo. It thus has the potential to provide anatomical evidence for the proposed changes in auditory-limbic connectivity. However, the few diffusion imaging studies of tinnitus performed to date have inconsistent results. In the present paper, we briefly summarize the results of previous studies, aiming to reconcile their results. After detailing analysis methods, we then report findings from a new dataset. We conclude that while there is some evidence for tinnitus-related increases in auditory and auditory-limbic connectivity that counteract hearing-loss related decreases in auditory connectivity, these results should be considered preliminary until several technical challenges have been overcome.

1. Introduction

Subjective tinnitus, an auditory phantom percept often described as "ringing in the ears," affects about 10 to 15% of the adult population [1] and significantly impairs quality of life in a subset of those affected by it. While being often perceived "in the ears" and linked to hearing loss in the vast majority of cases, chronic subjective tinnitus appears to be a problem of the central nervous system rather than the ear, since it can persist or even start when the auditory nerve is cut [2]. Numerous studies in human tinnitus patients as well as animal models of tinnitus have provided evidence for structural and functional changes at multiple locations of the central auditory system, and it is widely assumed that central auditory system plasticity is at the root of the aberrant neural activity that gives rise to the perception of tinnitus [3].

However, central auditory system plasticity alone cannot explain the phenomenon of tinnitus. First, compensatory plasticity should occur in all cases of significant deafferentation, yet tinnitus is only reported by a subset of patients with measurable hearing loss [4]. Second, tinnitus patients often report that tinnitus is exacerbated or even triggered by stress [5, 6], which suggests influences of the limbic system. Indeed, there is mounting evidence that limbic system involvement in tinnitus goes beyond merely determining the emotional response to a chronic and sometimes debilitating condition but may instead modulate whether and to what extent aberrant auditory system activity results in a conscious tinnitus percept [7–12]. Of particular interest in this context are previous findings indicating reduced gray matter (GM) in subcallosal prefrontal cortex [10, 11, 13] and the amygdala-hippocampal area [14]; tinnitus-related hyperactivity in the ventral striatum near the nucleus accumbens (NAc) whose strength was correlated with prefrontal GM reductions [10]; correlations between ventromedial prefrontal cortex (vmPFC) function and tinnitus-related variables in ventromedial prefrontal cortex [12, 15]; and modulation of the tinnitus percept by electrical stimulation of the striatum [7].

In line with these findings, many theoretical models frame tinnitus as a network problem, arising from altered interactions between multiple auditory and limbic-related brain structures [10, 16–21]. Consequently, tinnitus research has increasingly employed methods that interrogate large-scale brain networks and interactions between them, in studies of functional connectivity using whole-head magnetoencephalography [22–24], EEG [25–27], and fMRI [28–33]. In addition to investigating tinnitus-related abnormalities in functional connectivity, there is also an increasing interest in assessing potential alterations in anatomical connectivity that might arise from or underlie the observed alterations in functional connectivity and other imaging measures. An increasingly popular tool for assessing structural connectivity in the human brain in vivo is diffusion tensor imaging.

Diffusion-weighted magnetic resonance imaging (DWI) is noninvasive means of measuring water diffusion in tissue. Because water diffusion is hindered by myelin sheaths, axonal cell membranes, and neurofilaments, it is much stronger in the direction parallel to major fiber tracts than in the direction perpendicular to the tracts [34]. By measuring water diffusion along multiple noncollinear directions and fitting a "diffusion tensor" describing diffusion in each direction as well as the correlations between the directions, diffusion tensor imaging (DTI) allows the derivation of measures such as mean diffusivity (MD), fractional anisotropy (FA), and the principal diffusion direction. One can visualize a diffusion tensor as an ellipsoid; the principal axis corresponds to the principal diffusion direction, the average volume corresponds to MD, and the elongation corresponds to FA.

All three measures are used to make inferences about white matter [35]. The principal diffusion direction is interpreted as an estimate of the dominant direction of the fiber tracts, which is then used to track fibers between remote locations in vivo. Fractional anisotropy is commonly used as an indicator of white matter microstructural integrity. The reasoning behind this interpretation is that FA should be the largest in regions where strongly myelinated fiber tracts run in parallel, permitting free diffusion along, but preventing diffusion perpendicular to, the fibers. Thus, reductions in myelination or in the number of parallel fibers result in lower FA. Low FA should be observed when diffusion is equally strong in all directions, for example, where oriented microstructures are essentially absent (such as the ventricles) and in regions with a high density of fibers oriented in many different directions. Because it is a measure of overall diffusion regardless of direction, mean diffusivity can distinguish between these cases: in the former, MD would be high because diffusion would be unconstrained in all directions and in the latter, MD would be low due to the presence of myelinated fibers through which water molecules cannot easily cross.

Note, however, that making these inferences requires several strong assumptions and that the tensor (ellipsoid) model of diffusion is only appropriate if there is only one fiber bundle running in a straight line through the voxel. As pointed out by Jones and colleagues [36], due to the relatively large voxel sizes necessary for maintaining reasonable signal-to-noise ratio (SNR), it can be assumed that at least 90% of voxels contain more than one fiber population, thus violating the core assumption behind the tensor model. Moreover, large voxels are also likely to contain different tissue types (i.e., not only white, but also gray matter) and/or cerebrospinal fluid (CSF), resulting in partial-volume effects. Thus, while DTI measures such as FA and MD are certainly sensitive to changes in tissue microstructure (e.g., axon diameter and density, myelination, and membrane permeability), one should bear in mind that interpreting a difference in these measures with regard to a particular anatomical change (e.g., loss of fibers) or even to a rather vague property such as "white matter integrity" or using them to quantify the strength of anatomical connections between brain areas is a huge leap from the data based on assumptions that in all likelihood are severely violated [36].

Few studies to date have investigated tinnitus using DTI, and their results have been somewhat divergent. The first DTI studies of tinnitus in humans constrained their analyses to predefined regions of interests (ROIs). Lee et al. [37] compared average FA in small, circular ROIs in the corpus callosum, frontal arcuate fasciculus, and parietal arcuate fasciculus between a group of 28 tinnitus patients and 12 normal hearing controls. Average FA was found to be significantly lower in the left frontal arcuate fasciculus and the right parietal arcuate fasciculus. However, both age and hearing loss were higher in the patient group than in the control group. While the authors could mitigate age differences as an alternative explanation for the FA reductions in patients by showing that there were no significant correlations between FA and age in the relevant ROIs, hearing loss differences could not be similarly ruled out. Crippa et al. [38] used DTI-based probabilistic fiber tracking to assess white matter tracts connecting the inferior colliculi (IC), auditory cortices (ACx), and amygdalae (AM) in 15 tinnitus patients and 10 control participants, matched for age. A higher percentage of fibers tracked from ACx reached the ipsilateral AM in tinnitus patients than in controls, and the same held for tracking

success from left ACx to left IC and from right IC to right AM. The authors interpret these findings as indicating stronger auditory-limbic connectivity in tinnitus patients.

Three additional DTI studies investigating tinnitus in humans did not constrain their analysis to certain ROIs but instead searched for tinnitus-related connectivity changes along all major white matter tracts. Aldhafeeri et al. [39] found decreased FA in the left longitudinal fasciculus, as well as in the left superior longitudinal fasciculus, the left anterior thalamic radiation, the body and splenium of the corpus callosum, and the right prefrontal cortex of tinnitus patients compared to controls. These FA reductions are reminiscent of those reported by Lee et al. [37]. In that study, it was unclear whether the FA reductions were due to tinnitus or hearing loss. Aldhafeeri et al. [39] report that average hearing thresholds did not differ significantly between tinnitus patients and controls, suggesting that, in their data, FA reductions are indeed due to tinnitus, not hearing loss. However, the only two test frequencies specifically mentioned in the article are 2 kHz and 4 kHz, and it is unclear whether the comparison of hearing thresholds included the higher frequency range that is most commonly affected in tinnitus patients. Thus, differences in high-frequency hearing loss could still have contributed to the observed FA reductions. The remaining two studies [40, 41] took hearing loss into account but used different approaches and had quite different results. Husain et al. [40] used a three-group design comparing participants with tinnitus and hearing loss to participants with hearing loss but no tinnitus and to participants with neither hearing loss nor tinnitus. This study observed only differences due to hearing loss; compared to controls, patients with hearing loss had reduced FA in a right hemisphere cluster including the anterior thalamic radiation, inferior longitudinal fasciculus, and inferior frontooccipital fasciculus. Benson et al. [41] compared two groups of patients with noise-induced hearing loss, differing only in tinnitus status, and found that the tinnitus group had increased FA in several clusters along the left anterior thalamic radiation, as well as in some clusters along the left and right superior longitudinal fasciculi, the left inferior longitudinal fasciculus, and the right interior frontooccipital fasciculus.

Taken together, the results of these studies seem to hint at FA reductions associated with hearing loss (directly demonstrated by Husain et al. [40] and indirectly by Lee et al. [37] and Aldhafeeri et al. [39]) and FA increases and increased fiber tracking success between auditory and frontal/limbic areas in tinnitus patients [38, 41]. However, considering the technical difficulties associated with diffusion imaging analysis in general and probabilistic fiber tracking in particular, the small number of studies, and the even smaller number of studies controlling for age and especially hearing loss, more research is clearly needed. Most studies so far have focused on FA as a measure of tract integrity; however, as argued above, this interpretation is problematic due to the high likelihood of crossing fibers and partial-volume effects in the assessed voxels. We believe that including MD as an additional measure can alleviate at least part of the interpretation problem by assessing general (nondirectional) changes in diffusivity, thus providing a clue as to whether the part of the observed FA changes that reflects changes in tissue microstructure should be attributed to changes along the principal diffusion direction. Moreover, despite the increasing interest in auditory-limbic interactions and the role of the limbic system in tinnitus, none of the diffusion imaging studies to date has considered factors such as depression and anxiety, which are often elevated in tinnitus patients and may contribute to the observed connectivity changes. The present study thus investigates FA and MD while looking at effects of tinnitus unrelated to hearing loss and depression/anxiety.

Based on the results of earlier studies (summarized above), we expected to find hearing-loss related FA decreases and MD increases along auditory pathways (specifically, in the white matter near the inferior colliculi, medial geniculate nuclei, and auditory cortices) and tinnitus-related increases in FA (and decreases in MD) along auditory and auditory-limbic pathways. We assumed that these effects would be most clearly reflected in group differences and also expected correlations between subjective tinnitus ratings and diffusion measures in vmPFC and NAc, which according to our model of tinnitus are the key areas modulating the tinnitus percept [19]. Regarding behavioral measures, we expected (based on our own experience with the population as well as on the literature) that tinnitus patients would be more noise sensitive [42], have higher depression and anxiety scores [43], and have stronger hearing loss [44] than age-matched controls.

2. Materials and Methods

2.1. Participants. DTI data were acquired from 24 tinnitus patients (TPs) and 19 controls (CTs). Both groups comprised a wide range of ages (TPs: 23–66, mean = 50.13, and sd = 14.64; CTs: 27–67, mean = 48.32, and sd = 12.04), included both men and women (12 women in each group), and included left- and right-handers (2 left-handers among the CTs and 3 among the TPs). For various reasons (see "Quality Control and Preprocessing" below), data from several subjects were excluded from the analyses. The results reported here are based on data from 18 TPs (9 female, 3 left-handed) and 14 CTs (10 female, 1 left-handed). The groups did not differ significantly regarding age (TPs: mean = 44.71 and sd = 11.42; CTs: mean = 46.50 and sd = 13.08; $t = 0.40$, $P = 0.6888$) or regarding the proportion of female and left-handed participants ($\chi^2 = 1.50$, $P = 0.2208$ for sex; $\chi^2 = 0.65$, $P = 0.4190$ for handedness).

2.2. Behavioral Data Acquisition. All participants underwent audiometry at Georgetown University Hospital's Department of Otolaryngology, assessing pure-tone thresholds for both ears from 200 to 20,000 Hz. However, only thresholds up to 8 kHz could be established in all participants. We thus computed average hearing loss (HL) for all frequencies up to 8 kHz for use as a covariate. In addition, all participants completed the Patient Health Questionnaire (PHQ9 [45]), the Generalized Anxiety Disorder questionnaire (GAD7 [46]), and the Hospital Anxiety and Depression Scale (HADS [47]) to assess symptoms of depression and anxiety. All TPs also completed the Tinnitus Handicap Inventory (THI [48]), and

both TPs and CTs completed the Tinnitus Sample Case History Questionnaire (TSCHQ [49]; CTs only completed those items that did not specifically address the participant's tinnitus). Of this latter instrument, the items of particular interest in TPs were "*Describe the loudness of your tinnitus using a scale from 1 to 100 (1 = very faint, 100 = very loud)*" and "*What percent of your total awake time, over the last month, have you been aware of your tinnitus? For example, 100% would indicate that you were aware of your tinnitus all the time, and 25% would indicate that you were aware of your tinnitus* 1/4 *of the time.*" Of particular interest in both CTs and TPs were the items "*Do you have a problem tolerating sounds because they often seem much too loud? That is, do you often find too loud or hurtful sounds which other people around you find quite comfortable?*" and "*Do sounds cause you pain or physical discomfort?*" the answers to which were provided on a rating scale (a slight deviation from the original TSCHQ) and combined into a single noise sensitivity measure for the purpose of the present analysis.

2.3. MRI Data Acquisition. Two diffusion-weighted datasets were acquired in immediate succession for each participant on a 3-Tesla Siemens TIM Trio scanner using a 12-channel birdcage head coil. Each dataset contained five non-diffusion-weighted images (gradient value $b = 0\ \mathrm{s/mm^2}$—later referred to as "$b0$") and 30 diffusion-weighted images (gradient value $b = 1000\ \mathrm{s/mm^2}$) in which the gradients were applied in 30 noncollinear directions. The parameters used for the DTI sequence were as follows: repetition time (TR) = 7,700 ms, echo time (TE) = 100 ms, 55 horizontal slices, acquired in interleaved order, and $2.5 \times 2.5 \times 2.5\ \mathrm{mm^3}$ resolution. A high-resolution T1-weighted structural scan (MPRAGE, TR = 2,530 ms, TE = 3.5 ms, inversion time = 1,100 ms, flip angle = 7°, 176 sagittal slices, and $1 \times 1 \times 1\ \mathrm{mm^3}$ resolution) was also acquired in the same session.

2.4. MRI Data Analysis. Data analysis was performed using FSL (version 5.0.0) as provided by the University of Oxford's Center for Functional Magnetic Resonance Imaging of the Brain (FMRIB). Descriptions of the FSL software have been provided in multiple publications [50–52]. At the core of the present analysis were several functions of FMRIB's diffusion toolbox (FDT [53, 54]) which will be described in more detail below.

2.5. Quality Control and Preprocessing. The high-resolution T1-weighted scan was inspected to confirm that none of the participants had any large-scale structural abnormalities (e.g., lesions or atrophy unusual for their age). Data from one CT were excluded because of strongly enlarged ventricles.

The two diffusion-weighted datasets acquired from each participant were concatenated in time and visually inspected for excessive motion and artifacts. Based on this inspection, single bad images (displaying an obvious offset between odd and even slices due to in-volume motion) were removed from the datasets of five participants (2 TPs and 3 CTs). Datasets with more than three bad images or excessive motion between subsequent volumes were excluded from the analysis, which removed an additional 8 participants (5 TPs and 3 CTs). Two more datasets were discarded, one (1 CT) because of significant signal loss ("hole artifact") in superior regions of the brain and one (1 TP) because it was missing the superior-most slices of the brain due to volume placement issues.

Following quality control and exclusion of bad datasets, FSL's "eddycorrect" function was used to automatically align all images acquired for a subject to the first non-diffusion-weighted ($b0$) image of that subject using 12-parameter affine transformation. This function corrects for motion between successive images as well as for image distortions caused by eddy currents, which differ for images acquired with diffusion weighting in different directions. In order to obtain a mask for limiting further analysis steps to voxels inside the brain, FSL's brain extraction tool (BET [55]) was used on the first $b0$ image of each subject. Parameters were adjusted and manual corrections were made as necessary to ensure that the resulting mask included all brain tissue while excluding most or all of the surrounding skull and meninges.

2.6. Tensor Fitting. Following the above preprocessing, a diffusion tensor was estimated for each voxel in each subject's concatenated dataset using FSL's "dtifit" function. Aside from the 4D dataset and the mask file constraining the analysis to voxels in the brain, this function also takes as input two text files, one describing the gradient directions with which each of the images in the 4D dataset was acquired (the bvecs file) and one describing the diffusion weighting applied when acquiring each image (b values, which in the present dataset were $0\ \mathrm{s/mm^2}$ for non-diffusion-weighted images and $1000\ \mathrm{s/mm^2}$ for diffusion-weighted images). Like the two 4D datasets acquired for each subject, these files, too, were concatenated, and where single images had been removed from the 4D files during the quality control process, the corresponding entries were removed from the bvecs and bvals files.

After tensor fitting, the resulting functional anisotropy (FA) and mean diffusivity (MD) maps were inspected, separately, for each subject. All maps looked as expected (i.e., higher FA values in locations of major white matter tracts, such as the corpus callosum, and higher MD in locations of cerebrospinal fluid, such as the ventricles), and no artifacts were found.

2.7. Preparation for Tract-Based Spatial Statistics (TBSS). Whole-brain analyses aiming to compare groups of subjects require that all subjects' 3D datasets be aligned in a shared standard space (such as Talairach or MNI space). Because of individual differences in brain anatomy, perfect alignment cannot be achieved by linear affine transformations, and alignment procedures allowing for local distortions (i.e., nonlinear alignment) run the risk of resulting in perfectly aligned datasets that no longer reflect the original data well. This is particularly problematic in regions of high individual variability. Residual misalignments between subjects can, to a certain degree, be overcome by large-scale smoothing of the data (which "blurs away" individual differences), but large amounts of smoothing also introduce partial-volume effects and can conceal small, spatially circumscribed effects.

We thus decided to use a method that avoids excessive smoothing.

The tract-based spatial statistics (TBSS) approach [56] to analyzing multisubject diffusion data overcomes these problems in two ways. First, it constrains the analysis to the main white matter tracts that can be assumed to be present and laid out similarly in all subjects. Second, rather than aiming to transform all subjects' diffusion data such that their main white matter tracts are perfectly aligned, it only roughly aligns all subjects' data, thus avoiding excessive distortion of individual datasets. This results in a multisubject dataset in which the mean white matter tracts are not perfectly aligned but sufficiently well aligned to allow derivation of an average white matter (WM) skeleton containing only the main tracts shared across subjects. After eroding the average skeleton such that only the centers of the main tracts are left, individual datasets are searched in the direction perpendicular to the average tract until the center (i.e., the maximum FA value) of the corresponding individual tract is found. The data from the individual tract center are then projected onto the average WM skeleton. This ensures that subsequent statistical analyses compare corresponding points of all individuals' white matter tract centers.

In the present analysis, we prepared for TBSS by using nonlinear transformations to roughly align all subjects' FA data with the FMRIB58 FA template, an average FA image based on the datasets of 58 healthy subjects aged between 20 and 50 years transformed into MNI space. An average FA image was then created from the aligned data and thresholded at FA >0.35 to ensure that only voxels with reasonably high likelihoods of containing white matter tracts in most subjects were retained. (We chose an FA threshold stricter than the recommended range of 0.2 to 0.3 [56] because visual inspection of the average FA image indicated that, at more lenient thresholds, the skeleton included small peripheral WM tracts for which intersubject correspondence cannot safely be assumed.) The resulting image was then eroded such that only the centers of the white matter tracts (i.e., the voxels with highest FA values) remained. Data from each individual subject's tract center were then projected onto this average WM skeleton as described above.

2.8. Group Comparisons across the Entire WM Skeleton. Data were analyzed using an analysis of covariance (ANCOVA) approach, investigating group differences in FA and MD while controlling for the effects of age and hearing loss (two variables known to affect connectivity from many previous studies), using a design matrix including four predictors: two binary predictors coding group membership (CT and TP) and two continuous predictors coding age and average hearing loss, respectively. The hearing loss predictor was orthogonalized with respect to age to account for the known positive correlation between age and hearing loss.

Across the entire WM skeleton, we tested both FA and MD values for group differences, as well as correlations with age and hearing loss, using FSL's "randomise" tool with 10,000 iterations and threshold-free cluster enhancement (TFCE [57]). The advantage of the TFCE approach is that, unlike other cluster-size- (or cluster-mass-) based approaches, it does not require the user to arbitrarily define a cluster-forming threshold (i.e., a threshold that voxels have to exceed in order to be counted as part of a cluster whose size or mass is then evaluated for significance by testing it against a null distribution obtained via permutation testing). Instead, for each voxel, it essentially uses all possible cluster-forming thresholds from 0 up to the statistical value of the voxel, establishing the cluster extent at each of these thresholds, and then summarizes the results as a weighted sum of all extents at all thresholds (for more details, see [57]). The resulting statistical image retains important spatial features of the original statistical image, such as local maxima, while at the same time enhancing each voxel's signal depending on how much "support" it receives from neighboring voxels with high statistical values. Like the original statistical image, the TFCE image can be converted into a map of P values corrected for multiple comparisons using nonparametric permutation testing, as implemented in FLS's randomise function. Because the WM skeleton is only two-dimensional at any given point in space, we used the "−T2" option of the FSL randomise tool, which is optimized for 2D data.

2.9. Group Comparisons across Auditory and Limbic Regions of Interest. To increase power for detecting smaller effects in locations where such effects were expected, we repeated our search for group differences while constraining single-voxel analyses to one region of interest (ROI) at a time. Twelve auditory and limbic ROIs were defined based on our theoretical framework and previous findings: left and right auditory cortices (ACx), medial geniculate nuclei (MGN), inferior colliculi (IC), amygdalae (AM), accumbens nuclei (NAc), and ventromedial prefrontal cortex (vmPFC) white matter.

Left and right ACx ROIs were defined as all voxels on the average WM skeleton of Heschl's gyrus (HG) and thev planum temporale (PT) that were anterior to $y = -36$. Medial geniculate nucleus ROIs were defined as all WM skeleton voxels falling within a sphere of an 8 mm radius around MNI coordinates +/−17, −24, −2 (following Mühlau et al. [13]), and ROIs for the inferior colliculi (IC) were defined as all WM skeleton voxels falling between the MNI coordinates of $x =$ +/−3 to +/−8, $y = -30$ to −35, and $z = -9$ to −17, that is, as the WM tracts inferior to the IC (since the IC themselves were not part of the WM skeleton). Amygdala ROIs were defined as all voxels on the WM skeleton falling within the area defined by the Harvard-Oxford Subcortical Structures Atlas as having a nonzero probability of belonging to the amygdala (the extremely lenient lower bound was chosen so that nearby white matter would be included) while at the same time falling within the WM tracts identified by Crippa et al. [38] as connecting the amygdala with the auditory system. The white matter tracts of the anterior limb of the internal capsule nearest to the left and right NAc were defined as our NAc ROIs, and inferior frontal WM tracts extending from just anterior to the head of the caudate into the area inferior to the genu of the corpus callosum were defined as our vmPFC ROIs. Details about the ROIs can be found in Table 1 and illustrations can be found in Figure 1.

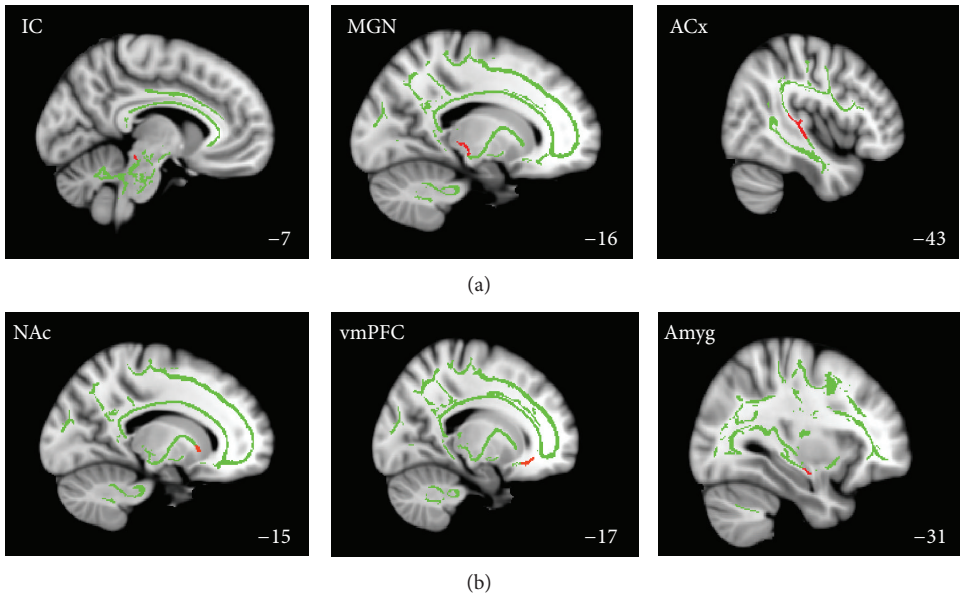

Figure 1: *Regions of interest.* Auditory (a) and limbic (b) ROIs (red) overlayed on the WM skeleton (green) superimposed on the MNI152 brain template. Numbers in the bottom right corner indicate the MNI x-coordinate of the illustrated sagittal slice.

Table 1: ROIs.

ROI name (ROI actually refers to the WM tracts adjacent to the named structure)	Center of gravity (MNI coordinates)	Size (mm^3)
Left inferior colliculus (lIC)	−6, −32, −15	27
Right inferior colliculus (rIC)	6, −32, −15	30
Left medial geniculate nucleus (lMGN)	−18, −24, −2	348
Right medial geniculate nucleus (rMGN)	18, −23, −2	349
Left auditory cortex (lACx)	−44, −29, 2	594
Right auditory cortex (rACx)	46, −26, 5	570
Left amygdala (lAM)	−28, −15, −10	70
Right amygdala (rAM)	28, −14, −10	42
Left nucleus accumbens (lNAc)	−15, 14, −1	48
Right nucleus accumbens (rNAc)	14, 14, −1	63
Left ventromedial prefrontal cortex (lvmPFC)	−19, 28, −9	183
Right ventromedial prefrontal cortex (rvmPFC)	20, 29, −9	199
Average WM skeleton (WM skeleton)		76259

2.10. Analyses for Correlations between Tinnitus Loudness and DTI Measures. In addition to testing for group differences, we also tested average FA and MD for correlations with tinnitus loudness ratings. Tinnitus loudness ratings were chosen as the tinnitus-related variable of interest because they, in contrast to THI scores and tinnitus awareness ratings, did not show strong correlations with depression and anxiety scores (see results for "Behavioral Data" below) and thus appeared to be the "purest" measure of the tinnitus percept itself (as opposed to tinnitus-related distress). We limited our search for correlations to only one tinnitus-related variable of interest to avoid the strict error-level adjustment that would be required if we tested multiple correlations.

We also performed post hoc correlation analyses across voxels displaying a significant group difference or tinnitus-loudness correlation to investigate whether the observed effects might be due to any of the other variables (e.g., depression, anxiety, and noise sensitivity). (We chose this post hoc approach rather than including these variables as covariates of no interest because inspection of the scores made it obvious that the homogeneity of regression slopes assumption was not met for these variables, making them unfit for inclusion as covariates.) While this involves multiple tests, we nevertheless used an uncorrected error level as our significance criterion, reasoning that since we were trying to demonstrate the absence of correlations, using a more lenient threshold would make the test stricter. At an uncorrected significance threshold of $P < 0.05$, correlations across the 18 TPs would have to exceed an absolute value of 0.469, correlations across the 14 CTs an absolute value of 0.532, and correlations across all 32 participants an absolute value of 0.347 to be considered significant.

3. Results

3.1. Behavioral Data. While matched for age, the two participant groups nevertheless differed significantly regarding auditory behavioral measures. Averaged across all frequencies of the standard audiogram (i.e., up to 8 kHz), TPs had significantly more hearing loss than CTs (27.14 dB HL versus 13.60 dB HL; $P = 0.0013$). In addition, TPs indicated significantly higher sensitivity to noise on the TSCHQ ($P = 0.0005$). The groups did not differ regarding depression and anxiety measures, although there were strong tendencies for TPs to score higher on the associated questionnaires

($P = 0.1572$ for the PHQ9 and $P = 0.0506$ for the GAD7). In addition, both tinnitus awareness ratings and THI scores were strongly correlated with depression and anxiety scores (correlations ranging from 0.53 to 0.78). In contrast, tinnitus loudness ratings were comparatively unrelated to depression and anxiety measures (all correlations below 0.25).

3.2. Whole-Skeleton Analysis regarding FA and MD. Our first DTI analysis tested all voxels of the average WM skeleton for significant group differences and correlations with age and hearing loss, using the ANCOVA TFCE approach described above. While no significant group differences emerged, there were significant correlations between DTI measures and both age and hearing loss across groups.

3.3. FA Decreases and MD Increases with Age. Across the entire skeleton, but especially in the frontal WM tracts and the corpus callosum (see Figure 2(a)) and mostly sparing subcortical WM tracts and brainstem, we observed significant positive correlations between MD and age and, more constrained to frontal WM tracts, corresponding negative correlations between FA and age. These age-related decreases in FA and increases in MD (regardless of tinnitus status) are well in line with the results of other DTI studies that specifically investigated the effect of age on white matter tracts (for a recent review, see [58]). No significant effects in the opposite direction (i.e., positive correlations between age and FA and negative correlations between age and MD) were observed. These findings serve as a "sanity check" of sorts, indicating that well-known and robust effects are replicated in our dataset.

3.4. FA Decreases with Hearing Loss. More interestingly, we also observed significant negative correlations between FA and average hearing loss in the WM tracts near left auditory cortex and in the WM tracts between left auditory cortex and the corpus callosum (Figure 2(b)). Corresponding effects in right auditory cortex could be seen at slightly reduced thresholds ($P_{corr} < 0.1$). Positive correlations between MD and average hearing loss were evident in the same locations at more lenient thresholds but did not reach significance. In addition, significant negative correlations between FA and average hearing loss were also observed in several voxels of the corpus callosum. Because the variable "average hearing loss" was orthogonalized with respect to age in our analysis, these effects are unlikely to be related to the known age-related decline in FA.

3.5. Negative Correlations between MD and Tinnitus Loudness Ratings in Left Anterior Thalamic Radiation and Anterior/Superior Corona Radiata. Significant negative correlations between MD and tinnitus loudness ratings were observed in the anterior thalamic radiation and the anterior and superior corona radiata of the left hemisphere (Figure 2(c)). Corresponding trends (at $P_{corr} < 0.1$) were also evident in the right hemisphere. Interestingly, these effects were found in locations corresponding well to those in which Benson et al. [41] observed higher FA in tinnitus patients than in controls; however, the present dataset did not show any FA effects in these areas.

To investigate whether the observed correlation might be driven by variables other than tinnitus loudness, we extracted average MD across all voxels identified as significant in this correlation analysis (i.e., defining a post hoc ROI) and computed correlations with the remaining tinnitus-related variables. (Note that this post hoc analysis is not statistically independent since the ROI was chosen for correlation between MD and tinnitus loudness and is now being tested for correlations between MD and other tinnitus variables, some of which are correlated with tinnitus loudness.) The negative correlation between MD and tinnitus loudness ratings was corroborated by similarly strong negative correlations between MD and tinnitus awareness ($r = -0.64$, $P = 0.004$) and between MD and noise sensitivity ($r = -0.60$, $P = 0.008$) in TPs; in contrast, CTs did not show a substantial correlation between MD and noise sensitivity ($r = -0.27$, $P = 0.31$). None of the other tinnitus-related variables (hearing loss and depression/anxiety scores) were correlated with MD, neither across nor within groups. In line with the widespread age-related MD increases described above, there was a strong positive correlation ($r = 0.59$, $P = 0.0004$ across groups) between MD and age in this ROI.

3.6. ROI Analyses regarding FA and MD. Next, we constrained our search for group differences to certain auditory and limbic ROIs in which we expected significant effects based on our theoretical framework and the results of previous studies. The following paragraphs highlight all significant results (and, where applicable, nonsignificant trends in the contralateral hemisphere); ROIs not mentioned here (MGN, NAc, and AM) did not show significant effects.

3.7. FA Increases and MD Reductions in ACx WM. As shown in Figure 3(a), several voxels of our rACx ROI at the confluence of Heschl's gyrus (HG) and the superior temporal gyrus (STG) had significantly higher FA values in TPs than in CTs (cluster center of gravity (CoG) = 41, −28, 4), and a similar trend was evident (at $P_{corr} < 0.1$) in lACx (CoG = −39, −32, 2). For MD, opposite results were observed: significantly lower MD values in TPs than in CTs in lACx (CoG = −42, −28, −1) and a nonsignificant trend in rACx (CoG = 40, −28, 3). To ensure that the observed effects were not simply due to correcting for hearing loss (which was higher in TPs and, as we mentioned above, associated with lower FA and higher MD), we also repeated the analysis without covariates and found somewhat reduced effects in the same direction, with only the MD effect in lACx remaining significant, the FA effect in lACx trending at $P_{corr} < 0.1$, and the effects in rACx trending at $P_{corr} < 0.2$.

To test whether the observed group differences might be due to noise sensitivity, which was significantly higher in TPs but was not included as a covariate of no interest in the group analysis, we also computed correlations between noise sensitivity and the DTI measure showing the group difference, averaged across all voxels for which the group difference was significant. Across groups and within CTs,

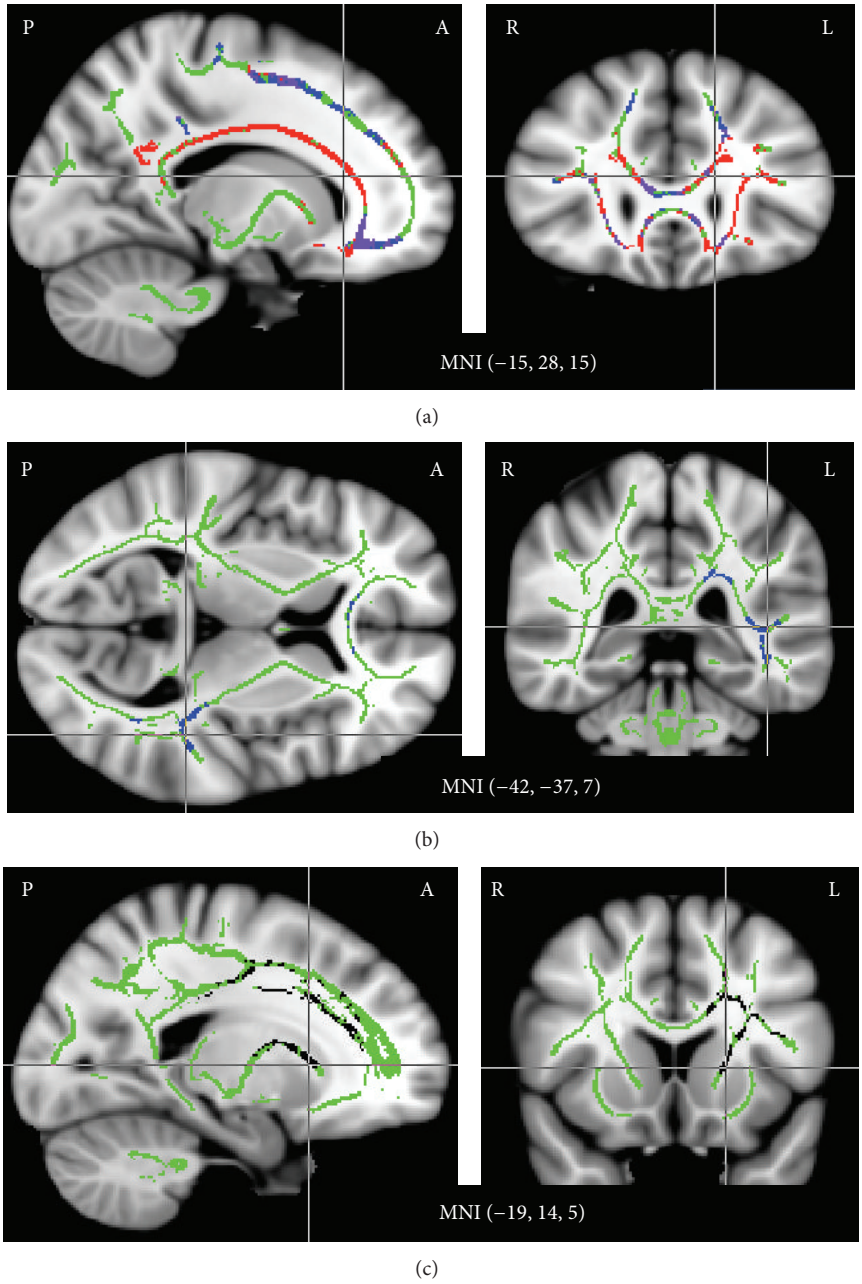

Figure 2: *Correlations between FA/MD and age (a), hearing loss (b), and tinnitus loudness (c).* (a) Many voxels of the average WM skeleton (green), especially in frontal cortex and the corpus callosum, showed a significant ($P < 0.05$, corrected) positive correlation between age and MD (red), a significant negative correlation between age and FA (blue), or both (purple). (b) In addition, several voxels in the WM tracts near left auditory cortex and in the WM tracts connecting left ACx to the corpus callosum showed a significant negative correlation between average hearing loss and FA (blue). This negative correlation was also observed in anterior portions of the corpus callosum. (c) Significant negative correlations (black) were also observed between tinnitus loudness ratings and MD in the anterior thalamic radiation and the anterior and superior left corona radiata. Results are superimposed on the MNI152 brain template.

these correlations were close to zero. In rACx only, TPs showed a strong negative correlation ($r = -0.67$) between FA and noise sensitivity. Interestingly, this correlation is in opposition to the group difference. As a group, TPs had higher FA and higher noise sensitivity, but within TPs, FA decreased with increasing noise sensitivity so that the TPs with the highest noise sensitivity ratings had FA values more like CTs. Considering these findings, it is highly unlikely that group differences in noise sensitivity were responsible for the observed group difference in the DTI measures. The strong negative correlation between TPs' noise sensitivity and FA in rACx is somewhat puzzling; however, since no corresponding correlation was evident in lACx ($r = -0.08$) or in CTs ($r = 0.07$), it is likely a spurious result.

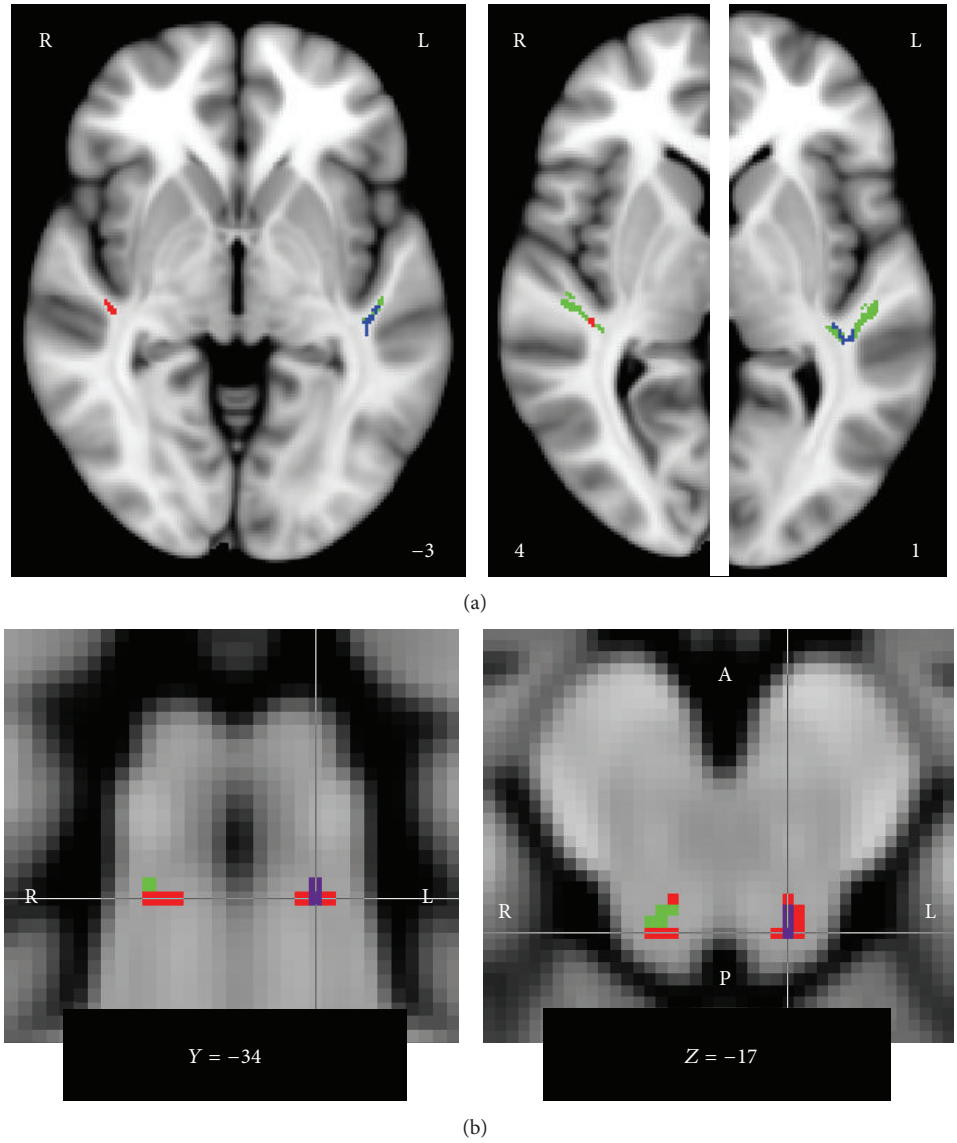

Figure 3: *Group differences regarding FA and MD in auditory ROIs.* (a) Compared to controls, tinnitus patients showed significantly increased FA (red) in right ACx and significantly decreased MD (blue) in left ACx, with corresponding but nonsignificant trends in the opposite hemisphere. The ROIs are indicated in green, and the numbers at the lower edges of the images indicate the MNI z-coordinate of the illustrated horizontal slice. (b) Tinnitus patients also showed significantly increased FA (red) in the WM underneath left and right IC and significantly decreased MD (blue) in the WM underneath left IC; a corresponding trend was also present in right IC. Voxels for which both FA increases and MD increases were significant are shown in purple. The numbers at the lower edges of the images indicate the MNI coordinate of the illustrated coronal/horizontal slice.

3.8. FA Increases and MD Reductions in the WM Inferior to the IC. We also observed significantly increased FA values and decreased MD values for TPs compared to CTs in the WM inferior to our lIC ROI and significantly increased FA values in rIC (Figure 3(b)). A trend for decreased MD in the rIC ROI was also present (at $P_{\text{corr}} < 0.1$). As above, we confirmed that this effect also held when age and hearing loss were not included as covariates. We also checked for correlations between FA/MD (averaged across all voxels of the ROI for which the group difference was significant) and noise sensitivity and found the correlations to be near zero across groups and tending to oppose the group difference when looking at the groups separately, making it extremely unlikely that the observed DTI results reflect differences in noise sensitivity.

3.9. The Louder the Tinnitus, the Higher FA and the Lower MD in vmPFC. In addition to ruling out correlations between DTI measures and noise sensitivity in regions showing significant DTI group differences, we also tested all ROIs for correlations between FA/MD (averaged across all voxels of the ROI) and our tinnitus variable of interest: tinnitus

loudness ratings. The only ROIs in which these correlations exceeded the significance criterion (absolute correlation value of 0.47 or larger) were left and right vmPFC. Both showed significant positive correlations between FA and tinnitus loudness ratings ($r = 0.49$, $P = 0.039$ and $r = 0.53$, $P = 0.024$ for left and right vmPFC, resp.); in addition, left vmPFC also showed a corresponding negative correlation between MD and tinnitus loudness ratings ($r = -0.51$, $P = 0.031$). A trend in the same direction ($r = -0.26$, $P = 0.297$) was also evident in right vmPFC. The vmPFC ROIs and scatter plots illustrating the correlations are shown in Figure 4.

These correlations between DTI measures and tinnitus loudness ratings opposed the ones observed in the same ROIs for age and HL: FA decreased and MD increased with age and hearing loss both across and within groups. Correlations with noise sensitivity went in the same direction as those with tinnitus loudness ratings but were weaker and did not reach significance even if an uncorrected criterion was applied or did any of the correlations with depression and anxiety scores. Thus, we are confident that the observed correlations were indeed related to tinnitus rather than to age, hearing loss, noise sensitivity, or depression and anxiety. The correlations were further supported by nonsignificant trends regarding group differences: FA tended to be higher and MD tended to be lower in TPs compared to CTs when correcting for the influence of age and hearing loss.

4. Discussion

4.1. Summary of Results. In the present dataset, we observed (1) that age was positively correlated with mean diffusivity (MD) and negatively correlated with fractional anisotropy (FA), especially in frontal white matter tracts; (2) that FA was negatively correlated and MD tended to positively correlate with hearing loss in the white matter (WM) between left auditory cortex and the corpus callosum and including both structures; (3) that tinnitus loudness ratings were negatively correlated with MD in the anterior thalamic radiation and anterior and superior corona radiata (although significantly so only in the left hemisphere); (4) that compared to age-matched controls, tinnitus patients had higher FA and lower MD in anatomically defined regions of interest in the white matter tracts underneath both auditory cortices (ACx) and inferior colliculi (IC); and (5) that in anatomically defined ROIs in ventromedial prefrontal cortex (vmPFC), FA correlated positively and MD correlated negatively with tinnitus loudness ratings. Depression and anxiety, while tending to be higher in tinnitus patients than in controls, could be ruled out as alternative explanations for these findings.

Bearing in mind that making inferences from FA/MD findings about microstructural changes is problematic for the reasons outlined in the Introduction section and summarized by Jones and colleagues [36], the age-related findings may be cautiously interpreted as a widespread decline in white matter tract integrity with age. This conclusion has been drawn from a number of studies investigating aging with diffusion-weighted imaging [58]. The present study found hearing loss to be associated with an additional decline in the white matter tracts of the auditory cortices. Interestingly, the presence of a tinnitus percept seemed to more than compensate for this hearing-loss related decrease, since tinnitus patients, despite having significantly more hearing loss than controls, showed an FA/MD pattern commonly interpreted as indicating increased tract density in auditory ROIs. The fact that the same pattern (increased FA and reduced MD) was found to correlate with tinnitus loudness ratings within frontal white matter, especially in the vmPFC ROIs, provides additional evidence for a role of these limbic-related areas in tinnitus.

4.2. Comparison with Other DTI Studies of Tinnitus. The present results both confirm and complement those of earlier studies. Hearing-loss related reductions in WM tract integrity have been inferred from several studies for a number of major WM tracts [40, 59] as well as for subcortical auditory ROIs [60–62]; however, the only study [59] reporting such effects in the white matter tracts of Heschl's gyrus and the superior temporal gyrus (i.e., in auditory cortical white matter) was based on a group comparison of young adults without hearing loss and older adults with hearing loss, so that the results might have been due to age rather than hearing loss. Thus, the present finding of a correlation between hearing loss and reduced FA/increased MD in auditory cortical white matter after controlling for age (Figure 2(b)) adds to these previous findings. What none of the DTI studies to date can determine is whether the apparent WM tract reductions are the cause or the consequence of hearing loss. They might reflect the effects of peripheral hearing loss on the central auditory system, that is, a degeneration of connections used less due to reduced input. Alternatively, they may be at the heart of central hearing loss, where an auditory signal is taken up in the periphery but insufficiently propagated through the central auditory system. Lastly, both relationships may hold to some extent, even within the same patient, and contribute to hearing problems.

Our observation of increased FA (Figure 3(b)) near the inferior colliculi of human tinnitus patients compared to controls adds to previous findings of FA increases in subcortical auditory structures (IC and MGN) in a rat model of blast-induced tinnitus [63]. Interestingly, Lutz et al. [59] also reported FA increases in the inferior colliculi for a comparison of older participants with hearing loss with a young normal-hearing control group. In contrast, Lin et al. [61] reported hearing-loss related FA decreases, and our study did not find effects of either age or hearing loss on FA in the inferior colliculi but did find increased FA in tinnitus patients. Considering the high incidence of tinnitus among older participants with hearing loss (which makes it likely that at least some of the older participant in Lutz et al.'s study had tinnitus), this may suggest that, rather than reflecting normal aging of the acoustic pathway (as Lutz et al. [59] conclude), the observed FA increases may instead be a sign of excessive compensatory plasticity following hearing loss that results in tinnitus.

The present results also indicate tinnitus-related FA increases/MD decreases in auditory cortical WM

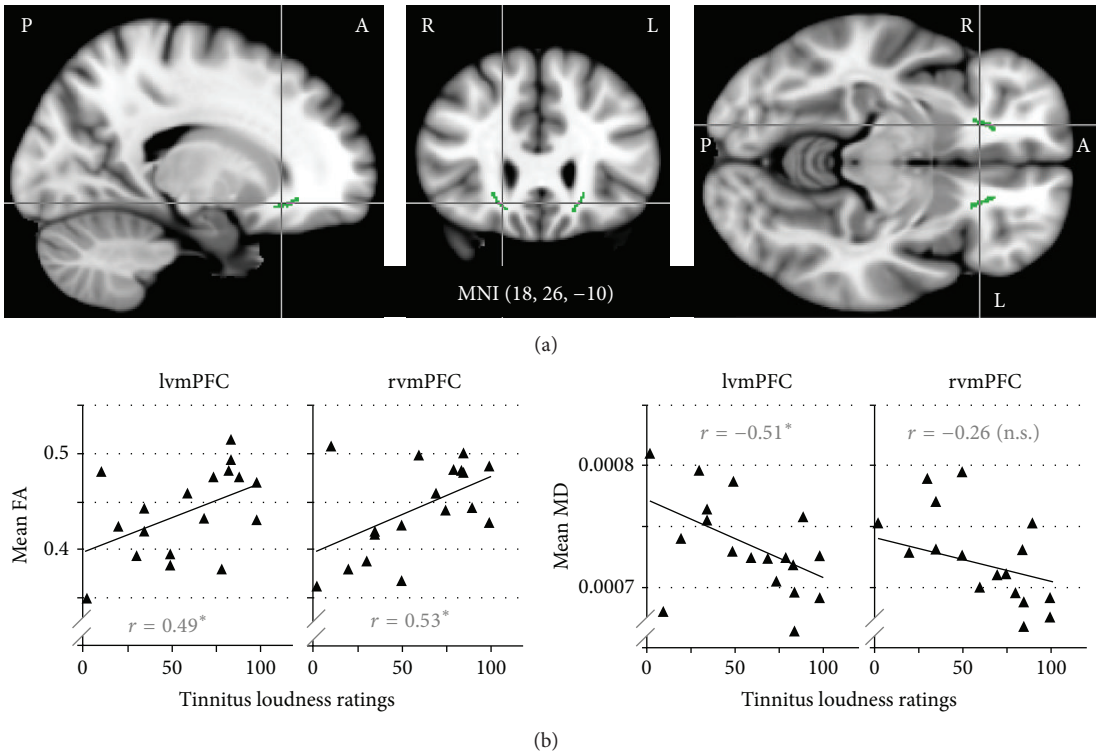

Figure 4: *Correlations between DTI measures (FA and MD) and tinnitus loudness ratings in vmPFC.* (a) Sagittal, coronal, and horizontal views of the anatomically defined vmPFC ROIs (green) superimposed on the MNI 152 brain template. MNI coordinates indicate the crosshairs intersection. (b) Scatter plots illustrating correlations between DTI measures and tinnitus loudness ratings in both ROIs.

(Figure 3(a)). This is well in line with the increased tracking success in tinnitus patients compared to controls for fibers leaving auditory cortex in the direction of the amygdala and in the direction of the inferior colliculi as reported by Crippa et al. [38]. While it may appear puzzling that no more diffusion studies have observed effects in auditory cortical WM, this is actually not surprising for the following reasons. First, of the five studies investigating tinnitus with diffusion imaging, only four included this region (Lee et al. [37] instead focused on small ROIs in major WM tracts). Of those four, one [39] used an intersubject alignment approach that is bound to fail in auditory cortex, where anatomy can differ vastly between subjects. Two others [40, 41] overcame these alignment issues by using the tract-based spatial statistics (TBSS) approach described above, but neither used threshold-free cluster enhancement (TFCE), which may have led them to miss spatially small effects that did not meet their cluster-size threshold. Also, in the TBSS approach, how much auditory cortical WM is actually included in the average WM skeleton critically depends on the number of participants (which was relatively small in Husain et al.'s study) and on the FA threshold chosen to limit the analysis to major WM tracts; it is thus unclear how much of auditory cortex was included in these previous analyses. In the only two studies that did detect auditory cortex effects (Crippa et al. [38] and the present study), auditory cortex was specifically chosen as an ROI. This suggests that while tinnitus-related changes in this area are present, they may not be easily detected with a spatially rather coarse technique like diffusion imaging. A possible reason for this is the potentially large heterogeneity across study participants regarding auditory experience aside from tinnitus and hearing loss (e.g., musical training) that can also influence auditory cortex connectivity (e.g., [64]).

Lastly, our finding of positive correlations between tinnitus loudness ratings and DTI measures in the vmPFC ROIs (Figure 4), the anterior thalamic radiation, and the anterior and superior corona radiata (Figure 2(c)) and the fact that these effects were stronger in the left than in the right hemisphere fits well with the left-dominant FA increases in frontal and thalamic white matter recently reported by Benson et al. [41] for a comparison of tinnitus patients to controls matched for age and hearing loss. We did not observe such group differences in our own data, but that may be due to the fact that our groups were much more heterogeneous than those studied by Benson et al. [41], whose inclusion criteria required noise-induced hearing loss and, for tinnitus patients, a THI score of at least 35. However, allowing for a wide range of hearing loss and tinnitus severity in our sample enabled us to find correlations that would otherwise have been missed and that nicely complement the group differences observed in previous studies.

4.3. Interpretation

4.3.1. Increased Connectivity within the Auditory System. The number of studies investigating anatomical connectivity in

tinnitus by means of diffusion imaging is still relatively small, and no single study so far has given a conclusive picture. This is partly owed to technical difficulties of diffusion imaging (e.g., intersubject alignment issues and limited success of fiber tracking attempts) and partly to the fact that it is nearly impossible to control for and/or investigate the influence of the many potential confounding variables (such as hearing loss, noise sensitivity, and depression/anxiety) at once. Nevertheless, taken together, the evidence is solidifying to suggest that, while hearing loss is related to changes in diffusion measures commonly taken to indicate decreases in white matter integrity within the auditory system at both the subcortical and the cortical level, there is a converse relationship in tinnitus, which is associated with increases in white matter density.

A possible interpretation is that the reduced auditory input associated with hearing loss of peripheral origin results in a weakening of the connections previously carrying this signal. In contrast, propagation of a constant tinnitus signal (arising, e.g., from an increase in spontaneous firing rates of neurons deafferented by hearing loss, as has been observed in several studies and proposed as a potential mechanism for tinnitus generation—e.g., [65]) might lead to preservation or even strengthening of connections. However, since results so far are purely correlational and thus cannot speak to causality, it is also possible that tinnitus is the consequence, rather than the cause, of the observed increase in auditory system connectivity. For example, several studies have found tinnitus to be associated with reorganization of tonotopic maps in auditory cortex. Frequency regions that have lost their normal inputs due to peripheral hearing loss start responding to the same stimuli as adjacent regions, resulting in an overrepresentation of certain frequencies that may be at the heart of the tinnitus signal (e.g., [66, 67]). Such local map reorganization is bound to involve strengthening of local connectivity, although it is unclear whether this effect could be detected given the current resolution of diffusion tensor imaging, where voxels are often several cubic millimeters large. Lastly, it is also possible that both interpretations are true at different locations within the auditory system. Increases in local connectivity may drive the generation of the tinnitus signal, which, being passed on to more remote regions, might in turn drive increased long-range connectivity.

4.3.2. Involvement of the Limbic System. All of the four studies using diffusion imaging in humans and reporting effects that the authors associate with tinnitus [37–39, 41] interpret at least part of their results in terms of altered limbic and/or auditory-limbic connectivity. However, none of these studies attempted to differentiate whether the observed effects were associated with the tinnitus percept per se or rather with its concomitant emotional phenomena. The present study revealed that diffusion measures in vmPFC, as well as along WM tracts containing fibers connecting temporal and thalamic with prefrontal regions, were strongly correlated with tinnitus loudness ratings but not with measures of depression, anxiety, or tinnitus distress. Thus, it provides the first diffusion-imaging evidence for a role of prefrontal, limbic-related areas in determining the tinnitus percept, that is, its intensity, itself.

This evidence adds to previous findings indicating (1) gray-matter reductions in subcallosal prefrontal cortex of tinnitus patients [10, 11, 13] whose magnitude correlates with tinnitus-related hyperactivity in the ventral striatum [10]; (2) correlations between vmPFC activation and tinnitus-related variables [12, 15]; (3) modulatory effects of deep-brain stimulation in the striatum on tinnitus percept [7]. Based on these findings, we have previously proposed a "noise cancellation" model of tinnitus according to which limbic and prefrontal areas work together to evaluate the tinnitus signal and, depending on the relevance assigned to it, enhance or suppress it via feedback to the auditory system. Gray-matter reductions in vmPFC result in a reduced ability to cancel "noise" and are associated with tinnitus-related hyperactivity in ventral striatum and auditory cortex. By demonstrating what might be interpreted as a tinnitus-related increase in auditory-limbic connectivity, diffusion imaging studies like the present one quite literally provide the "missing link" in this model.

In this context, it is interesting to note that the present study observed tinnitus loudness correlations, but no group differences in vmPFC and along some of the same white matter tracts for which Benson et al. [41] reported larger functional anisotropy in tinnitus patients compared to controls. A potential explanation for this discrepancy is that Benson et al. only included tinnitus patients with THI scores of 35 or higher, whereas the majority (14 out of 18) of tinnitus patients included in the present study had THI scores below 35. As mentioned at the beginning of the results section, THI scores in the present study were strongly correlated with depression and anxiety scores (correlations between 0.60 and 0.78). If the same relationship held in Benson et al.'s [41] sample, their patient group was not only more bothered by their tinnitus than ours, but also considerably more depressed and anxious. It is conceivable that increased depression/anxiety results in larger relevance being assigned to the tinnitus signal, leading to its enhancement in a sort of self-perpetuating loop, which goes along with increased limbic-auditory connectivity. The same mechanism could be responsible for the enhanced tracking success between auditory cortex and amygdala reported by Crippa et al. [38]. In other words, the widespread group differences described by Benson et al. [41] and the increased tracking success between auditory cortex and amygdala described by Crippa et al. [38] may be less associated with the tinnitus percept itself and more with the patients' emotional sequelae. These differences would not have shown up in our study because our patients overall had comparatively low depression and anxiety scores. In contrast, connectivity within the circumscribed areas identified in the present study (particularly vmPFC) may modulate the tinnitus percept even in the absence of depression and anxiety and prior to any additional widespread increases in auditory-limbic connectivity that may result from a distressed, self-perpetuating response to it.

4.4. Limitations. While diffusion-imaging studies of tinnitus aim to investigate changes in long-range connectivity, most findings to date (including those of the present study) are in fact quite localized, being based either on comparisons of diffusion measures in fairly small predefined regions of interest or a more global analysis yielding small, localized clusters of voxels showing significant effects. Drawing inferences about the integrity of long-range connections between fairly remote brain regions (as suggested by terms like "auditory-limbic connectivity") from such localized results is difficult. Ideally, one would instead trace connections between auditory and limbic regions of interest and then assess the patency of these connections along their entire length (i.e., the approach taken by Crippa et al. [38]).

Unfortunately, the suitability of current fiber tracking methods for identifying the auditory and auditory-limbic connections of interest is questionable. Deterministic fiber tracking, which relies on the dominant diffusion direction as an indicator of fiber orientation in a voxel, cannot identify even major auditory pathways because they are crossed by more dominant orthogonal pathways into which the tracking algorithm gets diverted. Probabilistic fiber tracking is more flexible and able to trace nondominant fibers, but regarding current methods, its flexibility is also its biggest weakness, because it allows fiber samples to take quite implausible routes. Our own experience from attempting probabilistic fiber tracking in the present dataset (results not reported for reasons that will be laid out in the following) is well in line with the complications reported by Crippa et al. [38]. When tracking from one seed ROI to a target ROI, a significant number of fiber samples tend to take "shortcuts" through gray matter or even cerebrospinal fluid or they reach the target only after highly unlikely detours through remote brain areas. A common remedy for these problems is the use of "exclusion masks" defining areas where fibers are not allowed to go (such as the ventricles); if a fiber sample nevertheless reaches this area, it is aborted and does not figure into the results. In addition, Crippa et al. [38] also report manual removal of fiber samples leading to the cerebellum or motor cortex. Despite all this, fiber tracking in their study was successful for only 50 percent (or fewer, depending on the tract) of the participants. Crippa et al.'s [38] data, along with our own experience, indicate that probabilistic fiber tracking can accurately identify known auditory pathways (e.g., from the inferior colliculi via the medial geniculate nuclei to primary auditory cortex) if sufficiently constrained by the researcher based on prior knowledge. However, the fact that unconstrained fiber tracking can lead to quite implausible results casts significant doubt on the validity of plausible tracts that remain after effectively preventing implausible behavior. Furthermore, it also means that one cannot rely on the results of fiber tracking between ROIs whose actual anatomical connection is not known (e.g., on the basis of tracer studies in animals).

Moreover, even if tracking is successful and the identified tracts are plausible, making quantitative inferences about connectivity (whether interpreted as fiber density, myelination, or something else) from diffusion data is highly problematic for several reasons discussed elsewhere [68], which will be briefly summarized here. One approach for quantifying connectivity is to compute average FA along the identified tract (based on the reasoning that a loss of axons or a reduction in myelination of the dominant fiber bundle will reduce diffusion obstacles in the direction perpendicular to the bundle, thus reducing FA). Considering that, as outlined in the Introduction section, the vast majority of voxels contain more than one bundle of parallel fibers, it is obvious why this does not yield an interpretable measure of connectivity: FA is influenced by the crossing fibers as well, so that, for example, a reduction of FA can result not only from reductions of connectivity (e.g., fiber density or myelination) in the tract of interest, but also from an increase in connectivity along perpendicular, crossing tracts. Another approach related to probabilistic fiber tracking is to evaluate tracking success (e.g., assessing which proportion of streamlines sent out from a user-defined seed region reaches a user-defined target region); a related measure is to average tracking success from the seed region to each voxel along the tract of interest to obtain a measure of "connection probability." However, these measures depend crucially on the tracking parameters (e.g., the maximum angle a streamline is allowed to bend from one voxel to the next without being aborted as biologically implausible), the length and curvature of the path to be tracked, the presence of crossing fibers or tract "branching" that might divert the streamline, and the overall signal-to-noise ratio in the images.

For these reasons, we think that diffusion imaging of tinnitus cannot yet confidently draw conclusions about "auditory system connectivity" or "auditory-limbic connectivity." The results of studies performed to date are called into question by considerable technical difficulties (e.g., with intersubject alignment, fiber tracking, or controlling for potential confounding variables such as hearing loss, noise sensitivity, and depression), and inferences made from the results often go far beyond the data. Thus, while what we have so far may be valuable puzzle pieces, the emergence of the whole picture illustrating tinnitus-related changes in anatomical connectivity will likely have to wait until diffusion imaging and fiber tracking techniques are more advanced.

4.5. Future Directions. While it was the goal of this paper to alert the tinnitus research community to proceed with caution when using and interpreting the results of diffusion MRI, we by no means intend to discourage the use of the technique for tinnitus research. On the contrary, since tinnitus is increasingly thought of as a network problem and, due to its subjective nature, is most easily investigated in humans, thus requiring noninvasive methods, diffusion imaging may be one of the better tools available. Discontinuing its use altogether would be akin to throwing out the baby with the bathwater. We thus want to end this discussion with a few recommendations for future use of diffusion imaging in tinnitus research.

In general, as with any research method, it is important that those using diffusion imaging as a research tool understand the limitations of the technique. To this end, we refer the reader to Jones et al.'s "Do's and Do not's of diffusion MRI" [36], a recent review of diffusion imaging and related analysis

methods that also makes recommendations for good practice. In addition to those recommendations, we would like to stress the importance of using intersubject alignment methods ensuring coregistration of corresponding WM tracts (as opposed to approaches that align based on the entire brain and apply large amounts of smoothing to "smooth over" intersubject differences in tract location). The TBSS approach described above has been successful in this respect, and a recent publication promises further improvements in this direction [69]. Also, to facilitate interpretation of the results, we recommend combining multiple diffusion measures, such as FA and MD.

Specifically for tinnitus research, it is crucial to assess tinnitus-related variables such as age, hearing-loss, noise sensitivity, depression, and anxiety and to take them into account when performing analyses. (While this has become common practice in fMRI studies of tinnitus, it seems to have been mostly neglected in diffusion imaging studies so far.) Since tinnitus-related changes in tissue microstructure are likely subtle, the small number of participants commonly enrolled in fMRI studies is probably insufficient for achieving the statistical power necessary to detect such small effects. (This criticism clearly applies to the present study, as evidenced by the fact that, for many of the reported findings, the opposite hemisphere showed clear trends in the same direction, which however failed to reach statistical significance.) It would thus be highly desirable if tinnitus researchers could agree upon collecting diffusion data in the same way and combining them into one big dataset. This could be achieved by polling tinnitus researchers interested in pursuing diffusion imaging at one of the upcoming tinnitus conferences and drafting a "consensus" paper, as has already been done for "tinnitus patient assessment and treatment outcome measurement" [49]. In addition to higher-powered cross-sectional studies, longitudinal studies, especially prospective ones that follow participants at high risk for developing tinnitus (e.g., active-duty military prior to deployment), could help answer the question of whether specific tinnitus-related changes identified in cross-sectional studies precede the tinnitus (i.e., there is a preexisting vulnerability) or develop after tinnitus onset (and thus likely reflect a consequence of the phantom percept rather than a cause).

5. Conclusions

Diffusion imaging is a noninvasive tool for assessing anatomical connectivity in the brain in vivo. As such, it has the potential to provide anatomical evidence for those tinnitus-related changes in auditory and auditory-limbic connectivity that have been proposed based on previous functional imaging and electrophysiological data. The few diffusion-imaging studies of tinnitus performed to date (including the present one) have somewhat divergent results whose interpretations are complicated by various technical difficulties. Despite this, there seems to be some converging evidence that hearing loss is associated with changes in diffusion measures thought to reflect decreases in anatomical connectivity of central auditory pathways that go beyond those that occur in the course of aging. In contrast, tinnitus is associated with changes that might reflect increases in auditory and auditory-limbic connectivity. Future research will have to confirm these findings and establish whether these connectivity increases are the cause of the tinnitus percept or rather a consequence of the tinnitus signal being continually propagated through the system. This enterprise will be greatly facilitated if researchers are willing to contribute to a shared dataset and agree upon and adhere to certain best practice approaches to analyzing and interpreting diffusion data.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Protective Effects of *Ginkgo biloba* Extract EGb 761 against Noise Trauma-Induced Hearing Loss and Tinnitus Development

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Noise-induced hearing loss (NIHL) and resulting comorbidities like subjective tinnitus are common diseases in modern societies. A substance shown to be effective against NIHL in an animal model is the *Ginkgo biloba* extract EGb 761. Further effects of the extract on the cellular and systemic levels of the nervous system make it a promising candidate not only for protection against NIHL but also for its secondary comorbidities like tinnitus. Following an earlier study we here tested the potential effectiveness of prophylactic EGb 761 treatment against NIHL and tinnitus development in the *Mongolian gerbil*. We monitored the effects of EGb 761 and noise trauma-induced changes on signal processing within the auditory system by means of behavioral and electrophysiological approaches. We found significantly reduced NIHL and tinnitus development upon EGb 761 application, compared to vehicle treated animals. These protective effects of EGb 761 were correlated with changes in auditory processing, both at peripheral and central levels. We propose a model with two main effects of EGb 761 on auditory processing, first, an increase of auditory brainstem activity leading to an increased thalamic input to the primary auditory cortex (AI) and second, an asymmetric effect on lateral inhibition in AI.

1. Introduction

A universal characteristic of modern societies, both in developing and developed countries, is the steadily increasing level of noise exposure within our working environments and during leisure time activities (for review see [1]). Consequently, an increasing number of people suffer from hearing disorders that result from an overexposure to noise, that is, noise-induced hearing loss (NIHL). Statistical data show that in 2000 about 9% of the general population in the USA display hearing impairments [2]. Similar data were published for Germany in 2006, where 8% of the 18- to 79-year-old adults appear to be hearing impaired [3]. Even more alarming are current data on the hearing loss in schoolchildren and teenagers. Several studies from North America and Europe report up to 15% of this age group to readily display significant hearing deficits [4], which reflects an increase of hearing loss among children and adolescents of around 10% [2, 3]. As the prevalence of hearing impairments increases with age, it appears to be sensible to assume that when these children grow up the number of patients with hearing impairments will increase dramatically in the future. Furthermore, as hearing loss may be etiologically responsible for the development of a number of secondary diseases, like hyperacusis (for review see [5]), tinnitus [6, 7], or depression due to social isolation [8], the problem of NIHL should be of immensely growing importance. For tinnitus alone, between 5% and 15% of the general population report to be affected and around 1% state that their quality of life is considerably impaired by their persistent perception of a phantom sound [9].

Effective strategies for protective measures against the development of hearing loss after noise exposure are therefore gaining increasing relevance in health care policies. In this context, two main types of strategies are conceivable: reducing noise exposure by technical measures or preventing the development of NIHL via pharmacological interventions. Whereas many technical measures, for example, for noise reduction in working environments or public, have the advantage that they may be effective for a large number of

people, they are often very expensive and have no effect if the source of noise is self-inflicted [10].

During the last few years a large number of substances have been tested both in animal and human studies in search of a powerful drug that is able to prevent NIHL. Based on their physiological mechanisms of effectiveness against NIHL, a number of substance classes may be distinguished. Among these, antioxidants that reduce oxidative stress by elimination of reactive oxygen species (ROS) [11], glucocorticoids, and substances that improve cochlear blood flow (for review see [12]), activate inhibitory transmitter systems [13], or block apoptosis pathways in hair cells were most successfully employed (for review see [14]).

A substance that has been shown to protect against NIHL in guinea pigs is the *Ginkgo biloba* extract EGb 761 [15, 16]. Stange and coworkers demonstrated that animals which were treated with EGb 761 before exposing them to different types of noise trauma exhibited a smaller reduction in auditory nerve compound action potentials (CAP) than untreated controls. EGb 761 is a plant extract that is composed of about 80 different compounds which deploy not only one but also a number of different mechanisms presumed to counteract the development of NIHL. Well-documented are the protection of neuronal mitochondrial ATP synthesis in the presence of oxidative stress [17, 18], the protection of erythrocyte membranes against oxidative damage, which results in reduced blood viscosity and improved blood flow [19, 20], and neuroprotection through antiapoptotic properties [21–25]. In addition, the extract displays a number of effects that may counteract the development of secondary consequences of NIHL like tinnitus. These include increased extracellular dopamine levels in the prefrontal cortex [26], which may reduce depressive behavior that may foster the development of tinnitus [27], by partial inhibition of the norepinephrine transporter [28] or adult neurogenesis of hippocampal neurons [29], which additionally could both lead to cognition increasing effects. In clinical trials, the safety profile of the compound was similar to placebo [30]. Therefore, EGb 761 is a promising candidate substance for protective measures against NIHL and its consequences.

As detailed above, one of the consequences of NIHL may be the development of a tinnitus percept. In a previous study we have described the development of noise trauma-induced tinnitus in the *Mongolian gerbil* both on a behavioral and neurophysiological level [31]. We were able to detect a number of neuroplastic changes in auditory brainstem and cortex that were correlated with the development of a tinnitus percept as tested with a well-characterized behavioral paradigm [32]. In particular we could demonstrate a neuronal predisposition for the development of tinnitus. We were able to show that animals developing such a mispercept after an acoustic trauma show significantly less cortical activity already in the healthy state compared to tinnitus-resistant animals. The latter animals are able to counteract tinnitus development after noise trauma while animals without this ability develop a chronic tinnitus percept.

While studies in humans with different *Ginkgo biloba* extracts so far failed to show any reliable effect on tinnitus perception when given after its development [34, 35], we here tested the effectiveness of a prophylactic treatment with EGb 761 in the context of NIHL and tinnitus development in this animal model. We describe the effects of the EGb 761 extract on the behavioral level (acoustic startle response (ASR) audiometry), the auditory brainstem level (electrophysiological recordings of auditory brainstem responses (ABR)), and the central level (electrophysiological recording of local field potentials (LFP) and single and multiunit responses in auditory cortex (AC)). Our results point to massive neuroplastic effects of EGb 761 on auditory processing both at the peripheral and central level. These changes in processing may underlie the observed protective effects against NIHL and in the consequence tinnitus development.

2. Material and Methods

2.1. Ethics Statement and Animals. Mongolian gerbils (*Meriones unguiculatus*) were housed in standard animal racks (Bio A.S. Vent Light, Ehret Labor-und Pharmatechnik, Emmendingen, Germany) in groups of 2 to 3 animals per cage with free access to water and food at 20 to 24°C room temperature under 12/12 h dark/light cycle. The use and care of animals were approved by the state of Bavaria (Regierungspräsidium Mittelfranken, Ansbach, Germany).

A total of 36 ten- to twelve-week-old male gerbils purchased from Charles River Laboratories Inc. (Sulzfeld, Germany) were used in this study. All methods used in this paper have been described previously (Ginkgo treatment [36]; tinnitus model, behavioral audiometry, and electrophysiology [31]) but still will be recapitulated here for easier intelligibility.

2.2. Treatment with EGb 761 and Time Regime. EGb 761 is a dry extract from *Ginkgo biloba* leaves (35–67 : 1), extraction solvent: acetone 60% (w/w). The extract is adjusted to 22.0%–27.0% ginkgo flavonoids calculated as ginkgo flavone glycosides and 5.0%–7.0% terpene lactones consisting of 2.85%–3.4% ginkgolides A, B, and C and 2.6%–3.2% bilobalide and contains less than 5 ppm ginkgolic acids.

EGb 761 provided by Dr. Willmar Schwabe Pharmaceutics (Karlsruhe, Germany) was diluted in 2% agar in water. As illustrated in Figure 1 the animals were either fed daily with the extract in agar (100 mg extract/kg body weight) via a feeding cannula over two weeks before start of the experiments (EGb 761 ginkgo group E, 17 animals), or they were fed over the same time with the same volume of agar only (vehicle control group V, 19 animals).

During the oral administration the measurements were started (cf. below and Figure 1). These included the behavioral testing in the first week of application, the pretrauma auditory brainstem response (ABR) measurements, and the pretrauma recording from the AC within the second week of substance administration. Subsequently, an acoustic trauma at 2 kHz was inflicted and all postmeasurements were done within 7 to 8 days after trauma.

2.3. Behavioral Measurements. For behavioral testing, animals were placed into a transparent acrylic tube (length: 10 cm; inner diameter 4.3 cm). This tube was placed 10 cm

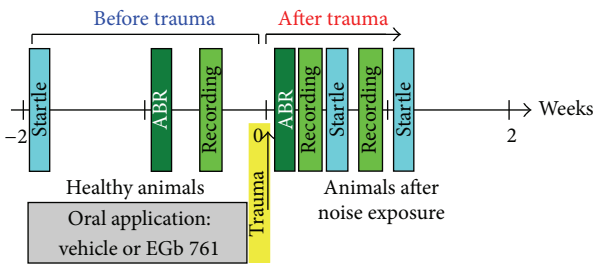


Figure 1: Timeline of the experiments. Two weeks prior to trauma (yellow bar) oral application of vehicle or EGb 761 was performed on a daily basis. Pretrauma measurements included behavioral startle responses (turquoise; hearing threshold and gap-noise tinnitus paradigms), ABR measurements (dark green), and electrophysiological recordings in auditory cortex (light green) both under anesthesia. After the acoustic trauma the measurements were repeated within the first seven days after the trauma.

from a speaker (Canton Plus X Series 2) onto a Honeywell FSG15N1A piezo force sensor (sensitivity 0.24 mV/gram; null shift at ±25°C is ±1 mV; force range 0 to 1500 gram), assembled within an IAC acoustic chamber on a TMC low-vibration table. The front end of the tube was closed with a stainless steel grate (wire mesh width 0.5 mm) allowing acoustic stimulation with no detectable distortion (signal to noise ratio at least 70 dB). Sound pressure level was controlled via a B&K Type 2610 measuring amplifier fed with a B&K Type 2669 preamplifier/B&K Type 4190 condenser microphone combination. Stimulus generation and data acquisition were controlled using custom-made Matlab 2008 programs (MathWorks, Natick, MA, USA; stimulation/recording sampling rate 20 kHz). For sound generation the frequency response function of the speaker was calibrated to produce an output spectrum that was flat within ±1 dB.

Three different types of prepulse inhibition (PPI) modulated auditory startle response (ASR) paradigms [31] were performed to assess, first, hearing capacities (behavioral audiogram, [37]) and, second, the potential existence of a tinnitus percept [38] after the noise trauma (cf. below). For obtaining the behavioral hearing thresholds we used a PPI of ASR paradigm in all animals. We startled the animals with 90 dB SPL pure tones (6 ms length including 2 ms rise and fall ramps) ranging from 0.5 kHz to 16 kHz in octave steps and used the same pure tones as prestimulus probes ranging from 0 to 50 dB SPL in 10 dB steps 100 ms before the startle stimulus. Each pure tone frequency and prestimulus intensity were repeated 15 times. This procedure was performed before the acoustic trauma and during the week after that event. The data obtained were checked by eye via a custom-made Matlab program; trials in which the animals moved within 100 ms before the startle stimulus were discarded; in the valid trials only peak-to-peak amplitudes of responses within the first 50 ms after startle stimulus onset were used for further analysis. The evaluation was performed independently by the principal investigator and a technical assistant, who was blind to the state of the animal. Evaluations of both experimenters led to identical results. This reduction of data led to a final valid trial number of 12260 of 20520 (59.7%) in the V group and 10719 of 18360 (58.4%) in the E animals. We made sure that the animals were always (pre- and posttrauma = trauma status) responding to the 90 dB SPL startle stimulus (cf. Figure 2). For validation of the PPI effect of the prestimuli we performed 1-factiorial ANOVAs for the valid response amplitudes dependent on the prestimulus intensity for each frequency and trauma status separately for each individual animal. The mean responses of all 36 animals (19 V, 17 E) before and after trauma are given in Figure 2. Responses in this threshold paradigm were fitted with a sigmoidal Boltzmann functions for each frequency, trauma status, and animal separately. Hearing thresholds were defined as the sound level at the inflection point of the Boltzmann function at each frequency before and after trauma [33] and are depicted in Figure 3.

For tinnitus testing we used two modified ASR paradigms in all animals before and after trauma. These consisted of either a 90 dB SPL pure tone startle stimulus of 1 kHz, 2 kHz, or 4 kHz within a 50 dB SPL continuous white noise, or a 90 dB SPL click startle pulse within a 50 dB SPL band pass filtered noise with a center frequency of 1 kHz, 2 kHz, or 4 kHz and a band width of one octave (cf. [31]). In both cases, a silent 20 ms gap within the noise 100 ms before the startle stimulus served as prepulse. The rational of these paradigms is that an animal that perceives tinnitus would be impaired in gap detection because it would hear its own tinnitus within the silent gap (cf. [32]). Consequently, when using a gap as prepulse, animals with tinnitus should produce smaller PPI of ASR than animals without tinnitus (cf. Figure 4). If tinnitus is detected, the different tone frequencies and noise spectra used should give a rough estimate of the spectral content of the tinnitus percept. Each frequency and gap-condition were repeated 15 times and each test was performed before and after the acoustic trauma. Again the data obtained were checked by eye by the same two experimenters as above via a custom-made Matlab program. Trials in which the animals moved within 100 ms before the startle stimulus were discarded; in the valid trials only peak-to-peak amplitudes of responses within the first 50 ms after startle stimulus onset were used for further analysis [31]. This reduction of data led to a final valid trial number of 4582 of 6840 (67.0%) in the V group and 3630 of 6120 (59.3%) in the E animals. This approach allowed the determination of a possible frequency-specific tinnitus-related behavior at one octave precision. We tested the gap-effect on the response amplitude separately in each individual animal before and after trauma for each tested frequency by t-tests ($\alpha = 0.05$) and found in all pretrauma data a significant PPI effect (i.e., a reduction of startle amplitude in the condition with the gap in the background noise) in each individual animal ($P < 0.05$). After the trauma only a part of the animals showed an undisturbed gap-effect at all frequencies tested while other animals showed no gap-effect at some but not all frequencies (cf. Figure 4) which gave a first hint of a possible tinnitus percept but was not yet used as the final classification of the animals in the tinnitus or nontinnitus group (cf. below). To avoid possible effects of the acoustic trauma on different stimulation frequencies all startle response data were normalized to minimize variance

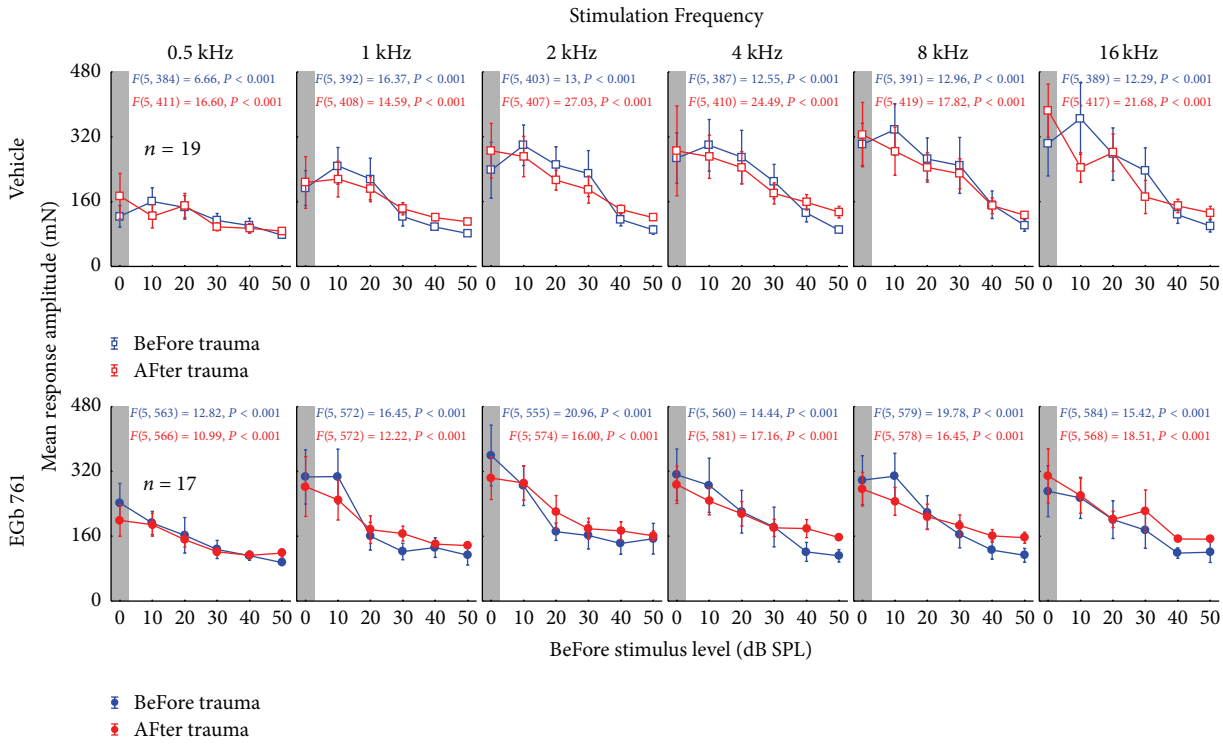


Figure 2: Validation of the hearing threshold ASR paradigm. Given are the mean response amplitudes in mN (±95% confidence interval) for all vehicle treated animals (upper panels) and all EGb 761 treated animals (lower panels) over all prestimulus intensities in dB SPL for the different stimulation frequencies of pre- and startle stimuli. The "pretrauma" (blue) and "posttrauma" (red) data are presented with the corresponding F statistics of the 1-factorial ANOVAs. Note that all statistics were significant, demonstrating that the animals were always (pre- and posttrauma = trauma status) responding to the 90 dB SPL startle stimulus and the different prestimuli.

of the response amplitudes. Normalization was performed as described earlier [31, 39]; briefly, we divided each amplitude by the corresponding median amplitude of the 90 dB SPL only condition (which reflects the full startle response of the animal for the loudest condition at each specific frequency, grey area in Figure 2). Thus we were able to control for differences in the startle amplitudes resulting from the hearing loss at the trauma frequency. This normalization also guarantees that the reduced ASR response after acoustic trauma is not due to hearing loss rather than a tinnitus percept [39]. Finally, the PPI of ASR in the healthy animal (before trauma) and after the trauma was calculated and the change in PPI relative to pretrauma (in %) was tested against 0 (no change) for each frequency separately with a t-test ($\alpha = 0.025$). Significant positive values for PPI change reflect impaired PPI and therefore indicate the development of a tinnitus percept. Only such animals were therefore classified as probably perceiving tinnitus (T groups) that showed at least one impaired frequency after the trauma independent of the affected frequency itself. As it turned out, in all cases where tinnitus was detected according to one of the two gap-ASR paradigms used, the second gap-ASR paradigm was also positive for tinnitus. Only the affected frequencies could differ between gap-ASR paradigms. Animals without such a significant increase in PPI change were classified as nontinnitus perceiving animals (NT groups) (cf. Figure 5). As it turned out, animals classified as T or NT based on these behavioral measures also differed in neurophysiological response measures (ABR and AC; for example, Figures 7 and 12), thereby strengthening the classification (cf. also [40]).

2.4. Acoustic Trauma and Auditory Brainstem Recordings (ABR). A bilateral acoustic trauma at 2 kHz (Canton Plus X Series 2 speaker frontal at 10 cm distance from animals head, 115 dB SPL at animals head, 75 min duration) in deep ketamine xylazine anesthesia (mixture of ketamine, xylazine, NaCl, atropine at a mixing ratio of 9 : 1 : 8 : 2, initial dose: 0.3 mL s.c.; continuing application at a rate of 0.2 to 0.3 mL/h) was used to induce a frequency-specific NIHL in all 36 animals and possibly the subsequent development of a tinnitus percept. The animals' body temperature was kept constant at 37°C by a warming pad.

ABRs were measured via subcutaneously placed thin silver wire electrodes (0.25 mm diameter) using a Plexon Multichannel Acquisition Processor (HLK2, Plexon Inc., Dallas, TX, USA) after amplification by a JHM NeuroAmp 401 (bandpass filter 400 Hz to 2000 Hz, 50 Hz notch filter) and stored with a custom-made Matlab program (10 kHz sampling rate). Auditory stimuli were presented free field to one ear at a time via a frequency response function corrected speaker (SinusLive neo 25S, pro hifi, Kaltenkirchen, Germany) at circa 0.5 cm distance from the animal's pinna

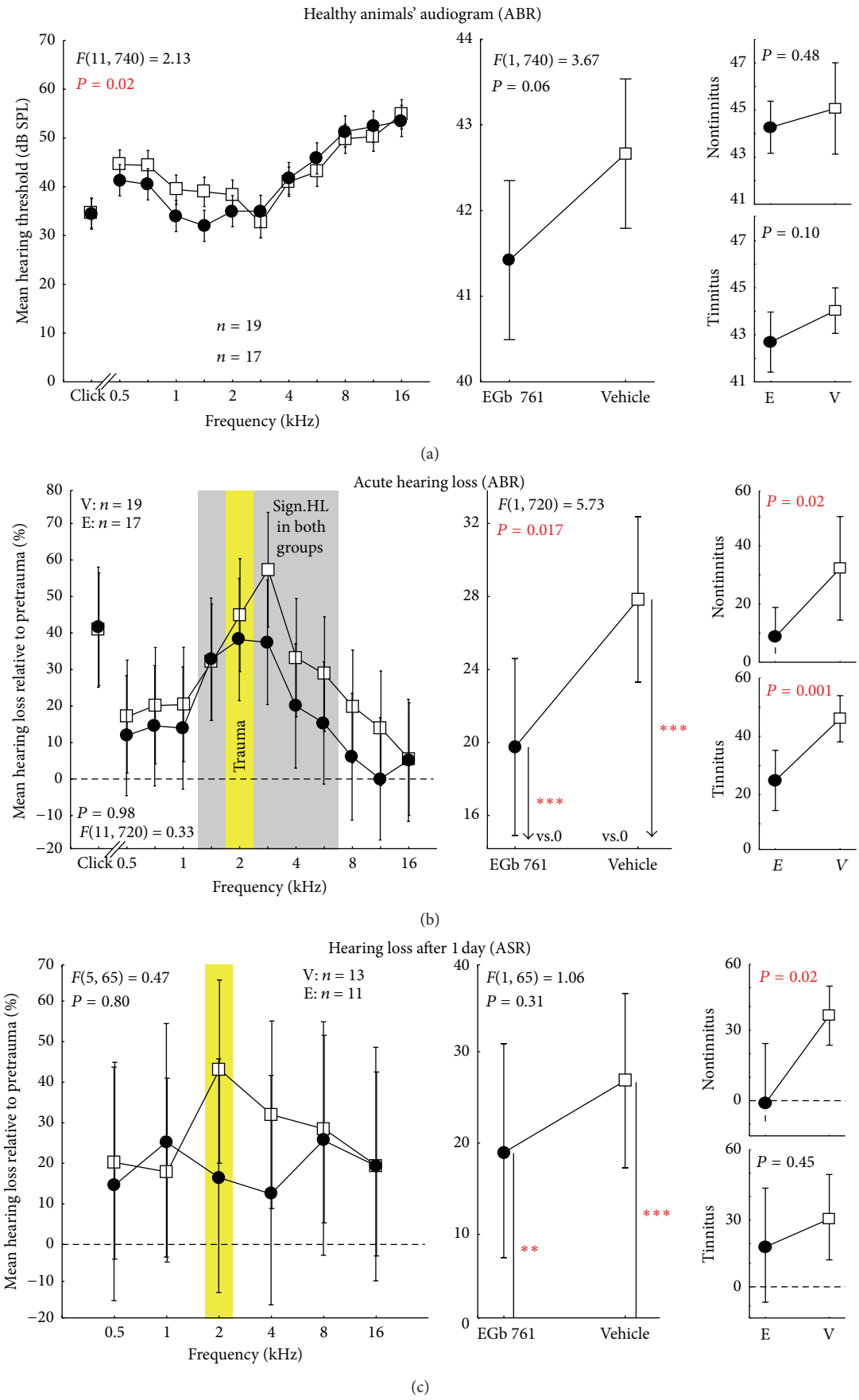
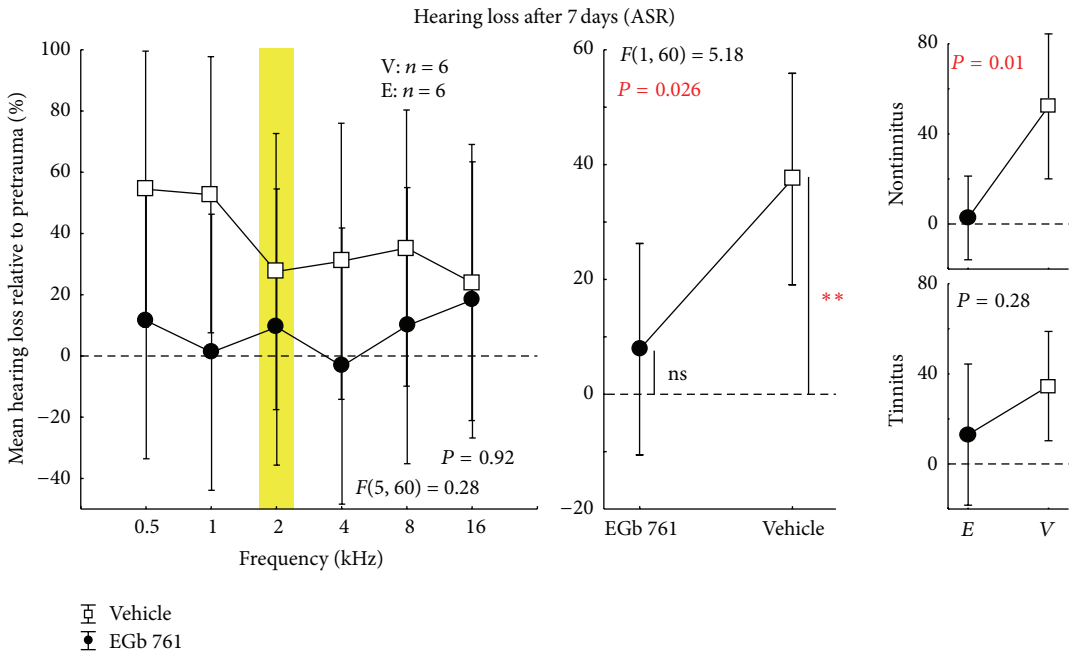


Figure 3: Continued.



(d)

Figure 3: Hearing threshold and NIHL of all tested animals. (a) Auditory brainstem response (ABR) based audiogram of the healthy animals (before acoustic trauma) of vehicle group (black open squares) and EGb 761 treated group (black solid circles). The left panel documents the mean hearing thresholds with their 95% confidence interval for clicks and all tested tone frequencies with the F-statistics of the interaction of the 2-factorial ANOVA. The center panel depicts the 1-factorial part of the same ANOVA with the factor group (mean values over all tested frequencies and click). Right panels show the same data separated into animals that do not develop a tinnitus percept (upper panel) and those that did show a tinnitus percept after the trauma (lower panel). (b) Acute NIHL, relative to pretrauma in percent (change of ABR threshold relative to pretrauma) of both groups obtained by ABR, measured immediately after trauma at 2 kHz (yellow bar) with their 95% confidence interval. The grey area in the left panel indicates significant hearing loss (single sample t-test versus 0) in both groups (V = vehicle, E = EGb 761), which is also significant if averaged over all tested frequencies and the click stimulus (center panel, asterisks). (c) Hearing loss one day after trauma and (d) 7 days after trauma obtained by auditory startle response audiometry (see Section 2 for details). Note that relative changes of thresholds measured with either ABR or ASR have been demonstrated to be identical [33]. Symbols and abbreviations as above, single sample t-test: ns = not significant, $^{**}P < 0.01$, $^{***}P < 0.001$.

while the contralateral ear was tamped with an ear plug as previously described [41]. Stimuli presented were clicks (0.1 ms duration) and pure tones (4 ms duration including 1 ms cosine-squared rise and fall times) ranging from 0.5 to 16.0 kHz in half-octave steps. 120 stimuli were presented in pairs of two-phase inverted stimuli (intrastimulus interval 100 ms) and an interstimulus interval of 500 ms between stimulus pairs. Stimulation was pseudo-randomized using a fixed list of all combinations of stimulus frequencies and sound pressure levels (0 to 90 dB SPL in 5 dB steps). To obtain ABR-based audiograms the mean ABR waves were compared to the mean amplitude 200 to 100 ms before the stimulus (baseline). Thresholds were defined automatically by a custom-made Matlab program at the highest attenuation at which the evoked amplitude raised over 2 standard deviations of the baseline; data were discarded at frequencies where this procedure was not possible, for example, at low signal to noise ratios. For additional analysis the root mean square (RMS) value of the ABR signal was calculated from 1 to 5 ms after stimulus onset. For further analysis data from both ears of each animal were used.

As behavioral audiograms using PPI of ASR (cf. above) could be obtained much faster—although with lower frequency resolution—than the ABR recordings (1.5 h compared to 6 h) we decided to measure fine-grain audiograms before and immediately after noise trauma only. For later audiogram measurements we rely on behavioral audiograms only (cf. Figure 1). We have demonstrated earlier [33] that these different methods to assess audiograms in our animal model yield different absolute thresholds but identical relative shifts in hearing thresholds after noise trauma. As we compare only relative shifts in this study (cf. Figure 3) this is not expected to introduce any bias to the interpretation of our findings presented here.

2.5. Electrophysiological Unit Recordings in Primary Auditory Cortex (AI). In a subset of the 36 animals (3 vehicle, 4 EGb 761) used in this study we performed electrophysiological recordings in auditory cortex in addition to the behavioral and ABR measurements described above. Two to three days after obtaining baseline ASR and ABR data, that is, before the acoustic trauma, the skull of the anesthetized animals

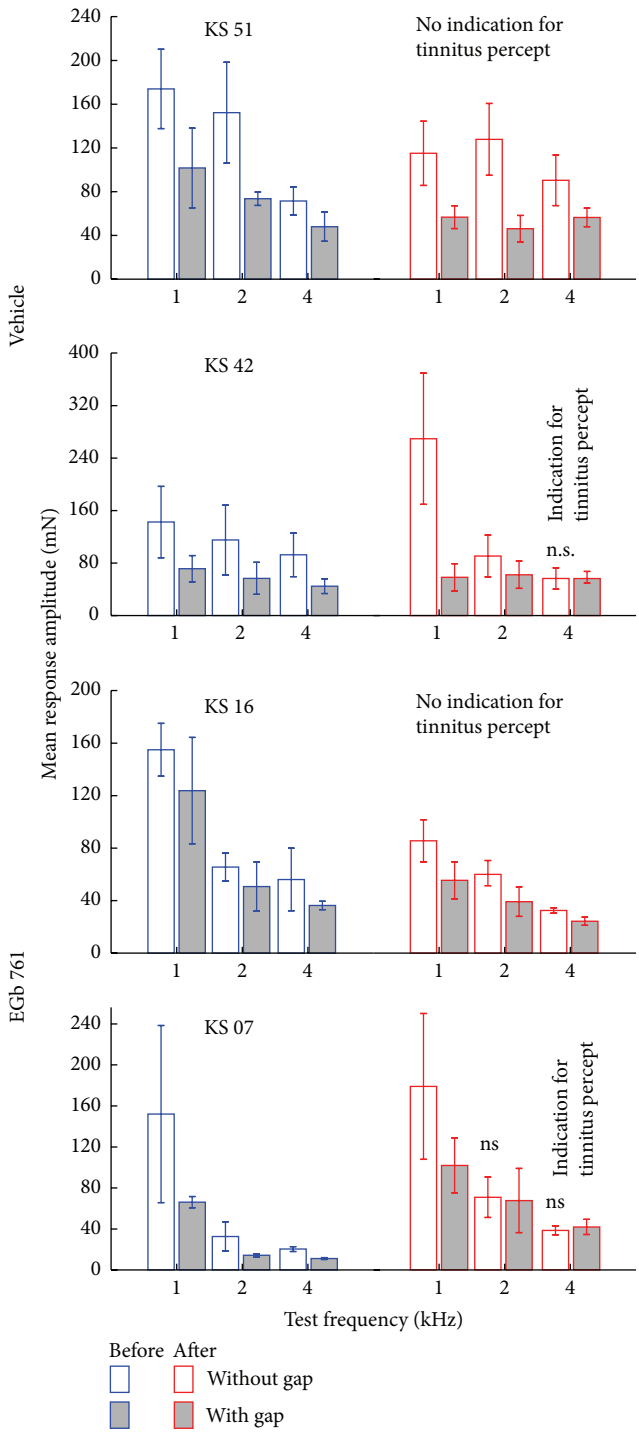


Figure 4: Results of the gap-noise PPI of the ASR in four exemplary animals. Given are the mean response amplitudes in mN (±standard deviation) for the two noise conditions: without gap (open bars) and with gap (filled bars) before (blue) and after (red) the trauma for all 3 frequencies tested (averaged for both gap noise paradigms). The upper two animals received the vehicle and the lower two animals received the EGb 761 extract before the trauma. All gap conditions produced significantly (t-tests) lower ASR amplitudes before the trauma. In some animals (KS 51 and KS 16) this was also true for the "posttrauma" condition and was a first indication for NT categorization. In other cases (KS 42 and KS 07) gap detection was impaired and did not show any significant change after the trauma at least at some frequencies; this was a first indication of T categorization (cf. Section 2).

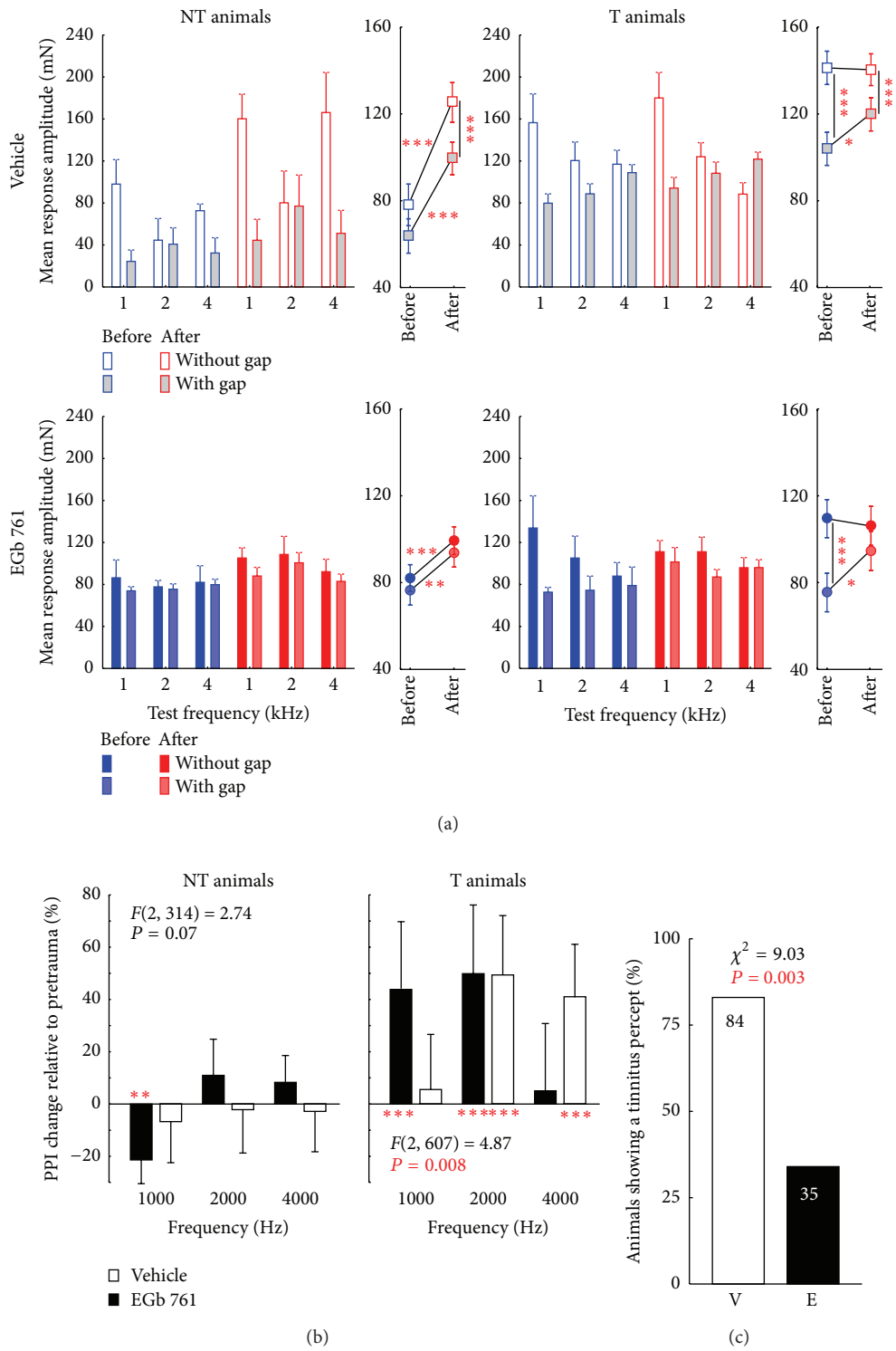


Figure 5: Development of tinnitus percept after acoustic trauma at 2 kHz. (a) Mean startle amplitudes in mN (+95% confidence interval) for no-gap (open and solid symbols) and gap condition (gray and shaded filled symbols) of all animals separated by tinnitus development, treatment, and test frequency. 2-factorial ANOVA (only interaction shown) depict the changes in no-gap and gap conditions before and after trauma. Note that even when gap-effects are small on the group level they were always significant in the single animals before trauma. Asterisks indicate significance levels of post hoc Tukey tests: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (b) Change of PPI relative to pretrauma data. Significant positive values of PPI change reflect an impaired PPI, indicating the development of a tinnitus percept. 2-factorial ANOVA indicates that EGb 761 treated animals develop tinnitus percepts at lower frequencies than vehicle treated controls. Asterisks below or above the abscissa indicate significant change of PPI (t-test versus 0): $^{**}P < 0.01$, $^{***}P < 0.001$. (c) Percentage of animals that develop a tinnitus percept after an acoustic trauma at 2 kHz. EGb 761 treated animals show significantly less signs of a tinnitus percept (chi^2 test).

was trepanned to expose the left auditory cortex. A 2.5 cm aluminum head-post for fixation and a recording chamber were implanted. Recording under deep ketamine-xylazine anesthesia began two days after surgery. Single and multiunit responses to tones were recorded in primary auditory cortex (AI) using acutely inserted single tungsten microelectrodes (1 MΩ impedance, 1-2 μm tip diameter, Plexon microelectrodes PLX-ME-W-3-PC-3-1.0-A-254). Verification of recording sites was done using neuronal response characteristics (latency, tuning sharpness (Q_{30}), temporal response patterns (phasic/tonic), tonotopic organization [42]). We concentrated our investigation on units with phasic response patterns.

Stimulation consisted of pure tones (200 ms including 1 ms cosine-squared rise and fall times) ranging from 0.25 to 16.0 kHz in quarter-octave or half-octave steps presented pseudo-randomly at 70 dB SPL with 500 ms interstimulus intervals. In addition to these iso-intensity measurements, tuning curves were recorded using pure tones in the mentioned frequency range but at different intensities ranging from 0 to 90 dB SPL. The recorded unit activity was analyzed with custom-made Matlab and IDL programs (IDL 7.06, Exelis Visual Information Solutions, McLean, VA, USA). Best frequency (BF; frequency with highest discharge rate at 70 dB SPL) as well as spontaneous rate (mean activity within a time window from 50 ms before to stimulus onset), evoked rate at BF, and evoked rates at all tested stimulation intensities and frequencies were calculated for each unit individually (evoked rate was calculated as the mean firing rate in a time window comprising the onset response, usually ranging from stimulus onset to 60 ms after stimulus onset). Statistics were performed with Statistica 8 (StatSoft, Hamburg, Germany). Where appropriate, either parametric statistics (Student's t-test, one- and two-factorial ANOVA with Tukey post-hoc-test) or nonparametric statistics (Kolmogorov-Smirnov-test, Kruskal-Wallis ANOVA with post hoc Median-test, and Mann-Whitney U-test) were applied.

3. Results and Discussion

3.1. Effects of Prophylactic EGb 761 Treatment on NIHL and Tinnitus Emergence. We used the PPI of the ASR to obtain behavioral audiograms from all 36 animals before and after the acoustic trauma. The responses to the different prestimulus intensities were checked by 1-factorial ANOVAs for each frequency and trauma status (before or after trauma) and for each individual animal separately. For an overview of the different response characteristics before and after trauma the mean response amplitudes of the 19 vehicle treated and 17 EGb 761 treated animals are given in Figure 2. Note that we find different response characteristics dependent on the stimulation frequency and trauma status but always find the significant prestimulus intensity effect of the PPI, that is, a decrease in ASR amplitude with increasing prestimulus intensity, which is not only true for the mean response but also for the individual responses. To these individual responses we fitted sigmoidal Boltzmann functions obtaining the individual behavioral hearing thresholds for each frequency.

Additional to these behavioral thresholds we obtained individual ABR based hearing thresholds under anesthesia before and after the trauma. Interestingly, when comparing the ABR thresholds of EGb 761 treated and vehicle treated animals, treatment led to slightly improved hearing thresholds in the low frequency range between 0.5 and 1.4 kHz already before induction of NIHL (Figure 3(a) left, filled circles and open squares, resp.), while across all frequencies, only a tendency for better hearing (Figure 3(a) center) could be found. Between the treated animals that later (cf. below) developed a tinnitus percept (T animals) and those that did not (NT animals) there was no significant difference in overall hearing level (Figure 3(a) right).

The traumatizing pure tone at 2 kHz led to significant and frequency specific NIHL immediately after the traumatizing event in both the EGb 761 treated group (E) and the vehicle treated control group (V) (Figure 3(b)). In both groups, significant elevations of hearing thresholds could be detected for frequencies between 1.4 and 5.6 kHz, indicating a stronger effect on the high frequency range compared to the low frequency range relative to the traumatizing pure tone (gray area in Figure 3(b) left). Nevertheless, the impact of the trauma on the hearing thresholds was significantly stronger in group V compared to group E (Figure 3(b) center). Whereas the mean threshold increase across all frequencies in group V was 27.8% relative to "pretrauma" (=9.0 dB), it was only 19.8% (=4.7 dB) in group E (Figure 3(b) center). There was no difference between NT and T animals in the relative NIHL (Figure 3(b) right). Furthermore, the degree of NIHL that developed differed between the V and E groups during the first week after trauma (Figures 3(c) and 3(d)). Whereas the frequency specificity of the NIHL vanished in both groups resulting in flat NIHL functions, the overall threshold shift completely recovered in group E, resulting in audiograms not significantly different from pretrauma conditions 7 days after trauma. In contrast, the NIHL in group V increased within the same time period (Figure 3(d)). The temporal development was entirely different between NT and T animals (Figures 3(c) right, 3(d) right). While NT animals showed no remaining hearing loss only 1 day after trauma, all vehicle treated animals and the T animals of the E group showed increased NIHL until day 7 after trauma. Note that we show the relative hearing loss compared to pretrauma level as we want to keep the two different methods of hearing threshold level measurements comparable. As we have demonstrated in an earlier study [33] ABR and ASR thresholds differ in absolute values so that audiograms show a parallel upward or downward shift. Relative changes due to hearing loss on the other hand were identical for both methods.

As described above, both EGb 761 and vehicle treated groups contained T and NT animals. Exemplarily the mean response amplitudes to both gap-ASR paradigms of four animals are depicted in Figure 4. The two upper animals were treated with the vehicle and the two lower ones with the substance. In animals KS 51 and KS 16 significant gap detection (t-tests, $P < 0.05$) was found before and after the trauma at all frequencies tested. In animals KS 42 and KS 07 that was only the case before the trauma, after the acoustic

trauma gap detection was impaired at 4 kHz or 2 kHz and 4 kHz, respectively. This impairment was a first hint for the classification of these two animals into the tinnitus group (cf. Section 2). The mean startle response amplitudes for all these animals before and after the acoustic trauma are depicted in Figure 5(a). Four 2-factorial ANOVA revealed especially in NT animals of both groups (Figure 5(a), left panels) significantly increased startle amplitudes after the trauma in the no-gap and in the gap condition (2-factorial ANOVAs; V group: trauma status: $F(1, 324) = 90.73$, $P < 0.001$; gap presence: $F(1, 324) = 21.18$, $P < 0.001$; interaction trauma status and gap presence: $F(1, 324) = 1.75$, $P = 0.19$; E group: trauma status: $F(1, 788) = 27.34$, $P < 0.001$; gap presence: $F(1, 788) = 3.97$, $P = 0.04$; interaction trauma status and gap presence: $F(1, 788) = 0.001$, $P = 0.97$) while in the T animals (Figure 5(a), right panels) only the gap conditions showed significantly elevated amplitudes (2-factorial ANOVAs; V group: trauma status: $F(1, 1964) = 3.80$, $P = 0.051$; gap presence: $F(1, 1964) = 56.27$, $P < 0.001$; interaction trauma status and gap presence: $F(1, 1964) = 4.70$, $P = 0.03$; E group: trauma status: $F(1, 968) = 3.04$, $P = 0.08$; gap presence: $F(1, 968) = 25.26$; interaction trauma status and gap presence: $F(1, 968) = 6.14$, $P = 0.01$). Please note that in this plot we show the means of the unnormalized response amplitudes of the animals. By that the gap-effect—especially in the "pretrauma" condition—is not always visible. As we used the individual data of each animal separately and calculated the PPI change relative to pretrauma the mean amplitudes give only a raw picture of the classification method (e.g., of individual data refer to Figure 4). So obviously, whereas the relative change in PPI after trauma relative to pretrauma conditions leads to stronger or nonsignificant PPI change in the NT animals (Figure 5(b), left panel), relative PPI amplitudes were significantly reduced in T animals (Figure 5(b), right panel; note that a positive relative PPI change in Figure 5(b) refers to a reduction in absolute posttrauma PPI amplitude). Consequently, in NT animals a two-factorial ANOVA showed no significant interaction of group (V versus E) and frequency in PPI change (Figure 5(b), left panel) and also no difference in the one-factorial part of the analysis (V versus E, $F(1, 314) = 0.34$, $P = 0.56$). As a result of the categorization of the individual data, no significant impairment of the PPI could be found. Interestingly, a significant decrease of PPI change emerged at 1 kHz in E group but not in V group animals (t-tests versus 0), indicating an improved PPI in this group. On the other hand, the PPI data of T animals from both groups showed significant interaction in the two-factorial ANOVA (Figure 5(b), right panel), indicating a spectrally different percept of animals in the E group compared to V group, namely, a tinnitus precept with lower frequencies. By contrast, across all frequencies we did not find any significant difference between both groups ($F(1, 607) = 0.01$, $P = 0.93$). It should be noted that, since EGb 761 treatment obviously provides considerable protection against NIHL, the impact of the tinnitus-inducing event affected the auditory system less severe in group E compared to group V. Consequently we found fewer animals in group E that developed a tinnitus percept compared to group V (cf. Figure 5(c)). Whereas 84% (16/19) of animals in group V showed clear signs of tinnitus in our behavioral paradigms, significantly fewer (chi^2 test, $P = 0.003$), namely, 35% (6/17) of the animals in group E seemed to have developed tinnitus.

3.2. Neurophysiological Effects of Prophylactic EGb 761 Treatment in AI

3.2.1. Overall Neuronal Activity. In total, 663 units could be recorded in 7 of 36 treated animals (418 units in 4 E animals; 245 units in 3 V animals. Note that all statistics in this paragraph are based on unit numbers, not animal numbers). We first investigated the general effect of the application of the EGb 761 extract on cortical responses and compared it to the vehicle treated group and to an untreated group (U) of 6 animals from an earlier study (627 units) [31]. Neurophysiological responses to tones of single and multiunits in AI showed a number of significant differences between EGb 761 and vehicle treated animals (group E versus V), both before the induction of NIHL and in response to the noise trauma while group V showed nearly identical responses to the U animals (2-factorial ANOVA: group: $F(2, 18480) = 27.83$, $P < 0.001$; trauma status: $F(1, 18480) = 1.05$, $P = 0.31$; interaction: $F(2, 18480) = 1.24$, $P = 0.29$). Post hoc Tukey tests revealed a significant difference in mean responses before the trauma between U and E ($P < 0.001$) and V and E ($P < 0.001$) but not between U and V ($P = 0.79$) which was also true for the responses after the trauma (U versus E: $P = 0.03$; V versus E: $P < 0.001$; U versus V: $P = 0.11$). Figure 6 gives an overview of the mean neuronal discharge activity in AI as a function of stimulation frequency and trauma status. We here compared pre- and posttrauma evoked responses across all stimulation frequencies by group with 2-factorial ANOVAs. Responses of the untreated group (Figure 6(a)) show no change in mean (± standard deviation) pre- and posttrauma response rates averaged across all frequencies (before: 7.57 ± 11.76 spk/sec; after: 7.00 ± 13.24 spk/sec; $F(1, 7846) = 0.003$, $P = 0.95$) but a frequency dependency ($F(13, 7846) = 24.22$, $P < 0.001$) while the interaction of both factors ($F(13, 7846) = 2.86$, $P < 0.001$) indicates a change of responses dependent on frequency and trauma status. Basically we see the same results in the vehicle treated group (Figure 6(b)) with no effect of the trauma status on mean response rate (before: 7.99 ± 10.91; after: 8.01 ± 16.00; $F(1, 3938) = 1.19$, $P = 0.28$) but a frequency dependency ($F(13, 3938) = 20.27$, $P < 0.001$) and the significant interaction of both factors ($F(13, 3938) = 2.50$, $P = 0.002$), demonstrating that handling and vehicle treatment per se had no effect on our measurements. The EGb 761 treated animals (Figure 6(c)) showed a somewhat dampened response when comparing it with the two other groups (cf. analysis above); the responses did not show an overall effect of the trauma (before: 6.28 ± 11.20 spk/sec; after: 6.26 ± 9.19 spk/sec; $F(1, 6618) = 0.23$, $P = 0.63$), although they did show a frequency dependency ($F(13, 6618) = 18.63$, $P < 0.001$) but no interaction ($F(13, 6618) = 1.37$, $P = 0.17$).

Of the 7 animals where single unit AC responses were recorded in this study, only one in the E group developed a tinnitus percept but two in the V group and therefore the following detailed analysis has a preliminary character, but as group V and group U show basically the identical response

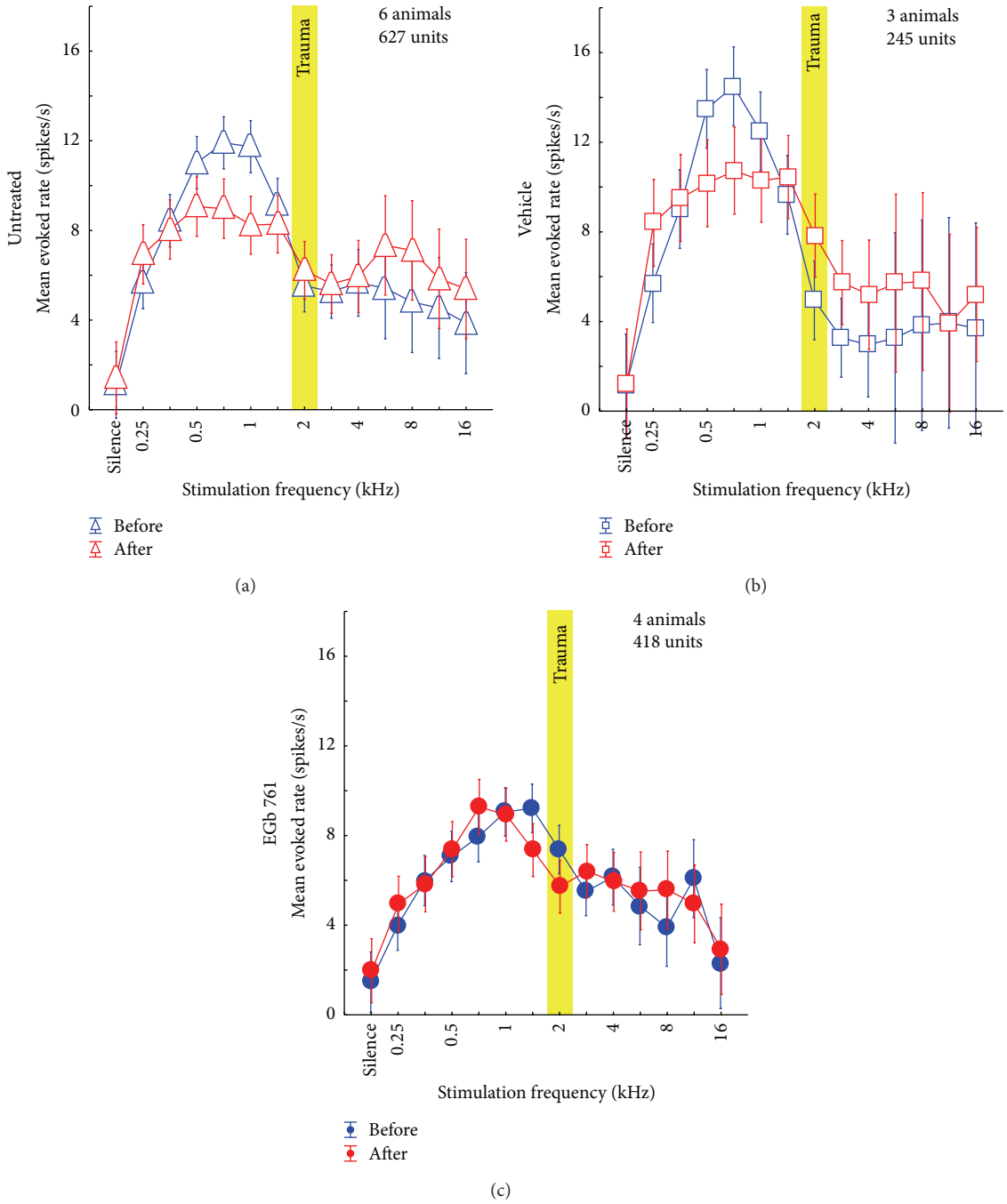


Figure 6: Mean evoked neuronal response (±95% confidence interval) to iso-intensity pure tone stimulation across all recorded units in (a) untreated animals from an earlier study [31], (b) vehicle treated animals, and (c) EGb 761-treated animals. Depicted are the mean evoked rates (spikes/s) before (blue) and after (red) acoustic trauma at 2 kHz (yellow bar). For statistical values please refer to Section 3.2.1.

patterns in NT and T animals it still seems likely that we found a valid effect in our animal model. Figure 7 gives an overview of the mean activity in AI as a function of stimulation frequency and trauma status. Mean response rates across all recorded units in completely untreated animals (Figure 7(a), data replotted from [31]), vehicle treated controls (Figure 7(b)), and EGb 761 treated animals (Figure 7(c)) are compared. Panels in the left column show data from animals that did not develop a tinnitus percept (NT); right panels depict data from those animals that did develop tinnitus (T) after NIHL as determined by the behavioral gap-noise paradigms.

The data of group V were very similar to our recently published results [31] with untreated animals (Figure 7(b) versus Figure 7(a)). In the vehicle treated animals we found a comparable overall activity in AI, both before and after trauma,

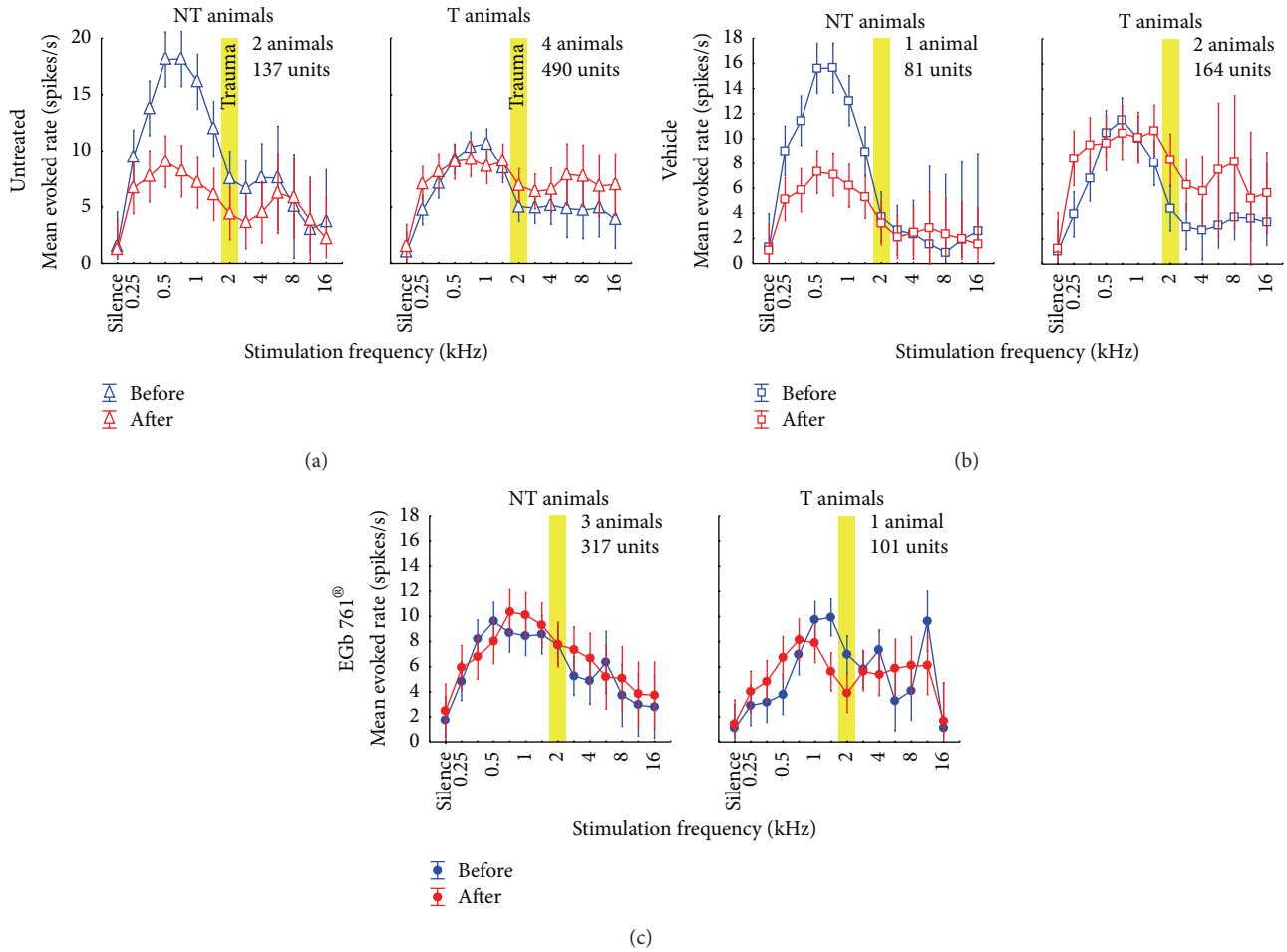


Figure 7: Mean evoked neuronal response (±95% confidence interval) to iso-intensity pure tone stimulation across all recorded units in non-tinnitus and tinnitus perceiving animals. Animals that did not develop a tinnitus percept are grouped in the left column while animals that perceived tinnitus are shown in the right column. Depicted are the mean evoked rates (spikes/s) before (blue) and after (red) acoustic trauma at 2 kHz (yellow bar). (a) Data from untreated animals replotted from an earlier study [31]. 2-factorial ANOVA interaction F statistics: NT: $F(13, 1866) = 3.12$, $P < 0.001$; T: $F(13, 5952) = 1.54$, $P = 0.10$. (b) Data from vehicle treated animals. 2-factorial ANOVA interaction F-statistics: NT: $F(13, 838) = 8.46$, $P < 0.001$; T: $F(13, 2772) = 1.42$, $P = 0.14$. Note the similarity between these and the untreated animals in the NT as well as in the T group. (c) Data from EGb 761 treated animals showing clear differences to the other two animal groups. The 2-factorial ANOVA shows strong interaction of time of measurement (before versus after trauma) and stimulation frequency in the T ($F(13, 1970) = 5.58$, $P < 0.001$), but not in the NT group ($F(13, 4420) = 0.86$, $P = 0.59$).

for animals with and without tinnitus (mean responses averaged across all frequencies grouped by tinnitus status, t-tests always $P > 0.05$). Again, a predisposition for the development of tinnitus, obvious from an overall lower cortical activity before trauma, compared to the animal group that does not develop tinnitus after NIHL, could be demonstrated. Furthermore, the reduction of the initially high response rates in the low frequency range in the NT groups after trauma was similar in the vehicle treated and the untreated group and not seen in the T groups of animals that did develop a tinnitus percept (t-tests before versus after, low frequency range (mean (± standard deviation)): untreated NT: 14.6 (±15.9) spk/sec versus 7.5 (±8.7) spk/sec, $P < 0.001$; vehicle NT: 12.3 (±11.9) spk/sec versus 6.2 (±5.5) spk/sec, $P < 0.001$; untreated T: 8.4 (±11.7) spk/sec versus 8.5 (±16.5) spk/sec, $P = 0.82$; vehicle T: 10.4 (±12.3) spk/sec versus 12.0 (±21.8) spk/sec, $P = 0.04$). We hypothesize this high response rate to be a correlate of the mechanisms that prevents the development of tinnitus in these animals [31]. On the contrary, T group animals showed increased posttrauma response rates in the high frequency range above the trauma frequencies, corresponding to the behaviorally determined frequency range of their tinnitus percept [31] (t-tests before versus after, high frequency range: untreated NT: 3.9 (±6.9) spk/sec versus 3.9 (±7.9) spk/sec, $P = 0.99$; vehicle NT: 1.8 (±2.7) spk/sec versus 1.9 (±2.4) spk/sec, $P = 0.82$; untreated T: 4.5 (±7.8) spk/sec versus 7.2 (±16.2) spk/sec, $P = 0.002$; vehicle T: 4.2 (±4.1) spk/sec versus 7.4 (±8.7) spk/sec, $P = 0.004$). We conclude from this comparison of untreated and vehicle treated animals that the mere handling of animals that was associated with vehicle (or EGb 761) administration had no effect on overall activity in AI, neither "pre-" nor posttrauma.

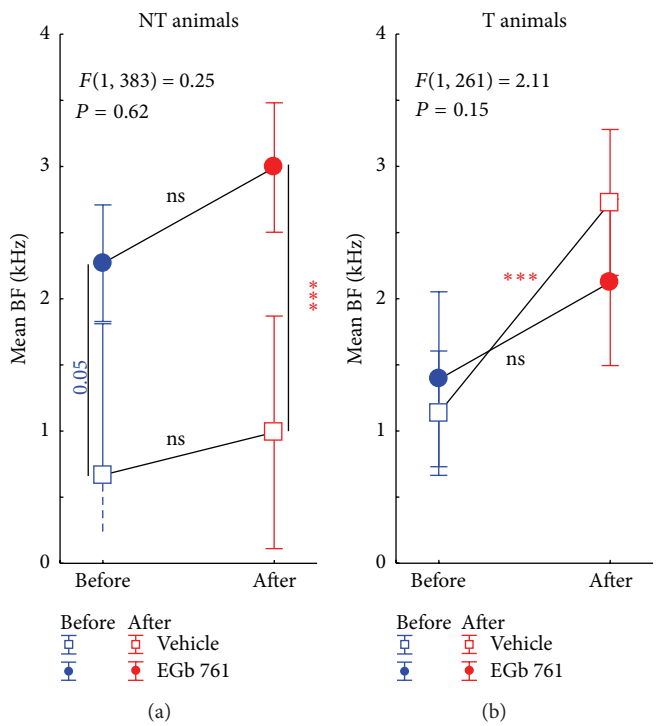


Figure 8: Effect of noise trauma on mean best frequency (BF) ±95% confidence interval in NT and T animals. Depicted are the statistical interactions of time of measurement (before versus after trauma) and animal group (V versus E) with the F-statistics of the 2-factorial ANOVAs. Asterisks indicate significant Tukey post-hoc-tests levels (ns = not significant, $^{***}P < 0.001$). Note the offset between vehicle and EGb 761 treated animals in the nontinnitus animals' data (a).

In contrast to this high degree of similarity between data from untreated and vehicle treated animals, large differences were found in the overall activity of units in AI of the EGb 761 treated animals (group E, Figure 7(c)). In NT animals (Figure 7(c), left panel) we observed low overall activity similar to the activity seen in the group V-NT after trauma (Figure 7(b), left panel, red curve) even before inflicted trauma (Figure 7(c), left panel, blue curve). At the same time, the overall activity in AI as a function of stimulation frequency in group E-NT before trauma was also similar to that of group V-T before trauma (Figure 7(c), left panel, blue curve versus Figure 7(b), right panel, blue curve). However, in contrast to the latter, the EGb 761 treated group did not display increased response rates in the high frequency region after trauma (Figure 7(c), left panel, red curve versus Figure 7(b), right panel, red curve) and did not develop behavioral signs of tinnitus. Rather, the mean activity in AI in group E-NT showed no significant changes post trauma (2-factorial ANOVA: before versus after: $F(1, 3420) = 2.67$, $P = 0.10$; interaction: $F(13, 3420) = 0.86$, $P = 0.59$) and therefore seemed to be resistant against such NIHL induced plasticity. This stabilizing effect of EGb 761 on overall activity in AI seemed to be less effective in group E-T (2-factorial ANOVA: before versus after: $F(1, 3170) = 0.24$, $P = 0.62$; interaction: $F(13, 3170) = 3.58$, $P < 0.001$; cf. also Figures 8 and 10(b)), resulting in more noisy frequency response functions compared to group E-NT (Figure 7(c), right panel), which may be the reason why animals in this group V could not withstand the development of NIHL induced tinnitus.

3.2.2. Spectral Tuning. In addition to these overall changes in AI activity, we also found plastic changes of the tonotopic organization in AI, as evident from changes in mean BF (Figure 8) and BF frequency distributions (Figure 9). These were different between E and V as well as T and NT animals. In NT animals we saw an effect of EGb 761 treatment already before the induction of NIHL. Treated animals showed a frequency distribution of BFs that was significantly shifted to higher frequency ranges compared to vehicle treated controls (Figure 8(a): Tukey post-hoc-test, $P = 0.05$; and Figure 9, compare blue bars in first versus third column: Kolmogorov-Smirnov-test, $P = 0.04$). In both NT groups (V-NT and E-NT), NIHL introduced no further effect on mean BF (Figure 8(a); Tukey post-hoc-tests, $P > 0.05$), but a significant flattening of the BF frequency distribution after 4 to 5 days after trauma (Figure 9, third column; Kolmogorov-Smirnov-test, $P = 0.018$). By contrast, in T animals no significant difference was found in BF distribution between the V and E groups before NIHL (Figure 8(b); Tukey post-hoc-test, $P > 0.05$; and Figure 9; compare blue bars in second versus fourth column; Kolmogorov-Smirnov-test, $P > 0.05$), pointing to a neurophysiological correlate of a possible division of EGb 761 treated animals in responders and nonresponders (blue circles in Figure 8; compare also blue

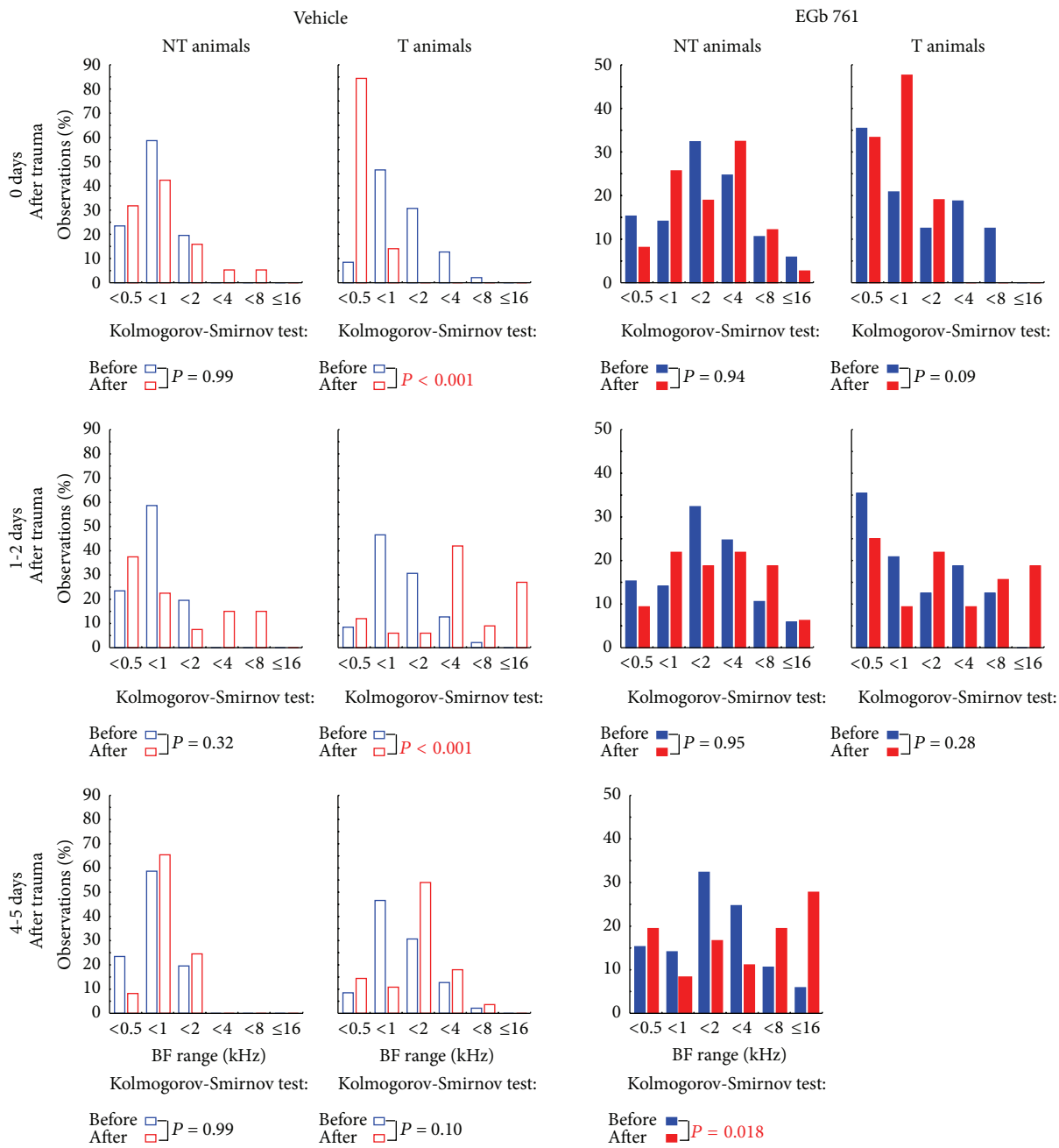


Figure 9: Changes in BF frequency distributions over time. Shown are the comparisons of the frequency distributions of BF (observations in %) binned in one octave step of vehicle treated animals (left two columns) and EGb 761-treated animals (right two columns). Treated and untreated animal groups are further subgrouped into NT (first and third column) and T animals (second and fourth column) before the trauma (blue) with the data obtained during 3 different time points windows after trauma (red), from top to bottom: day of trauma, 1 to 2 days after trauma, and 4 to 5 days after trauma. The distributions are tested by Kolmogorov-Smirnov tests corrected for multiple comparisons. Note that we were not able to record from the single animal in the EGb 761 tinnitus group at days 4 to 5.

curves in Figure 7(c)). In response to NIHL, V-T animals showed disturbances in tonotopic organization, as evident by significant shifts in mean BF (Figure 8(b); Tukey post-hoc-test, $P < 0.001$); and shifts in BF frequency distribution (Figure 9, second column day 0 and day 1-2; Kolmogorov-Smirnov-test, $P < 0.001$) that normalized after 4 to 5 days. In the E-T group, no such shifts were seen (Figure 8, right panel; Tukey post-hoc-test, $P > 0.05$; see also Figure 9 fourth column; Kolmogorov-Smirnov-test, $P > 0.05$). Note that in group E-T no data could be measured 4 to 5 days after trauma due to problems with the recording chamber after day 3 after trauma in the only T animal of the E group.

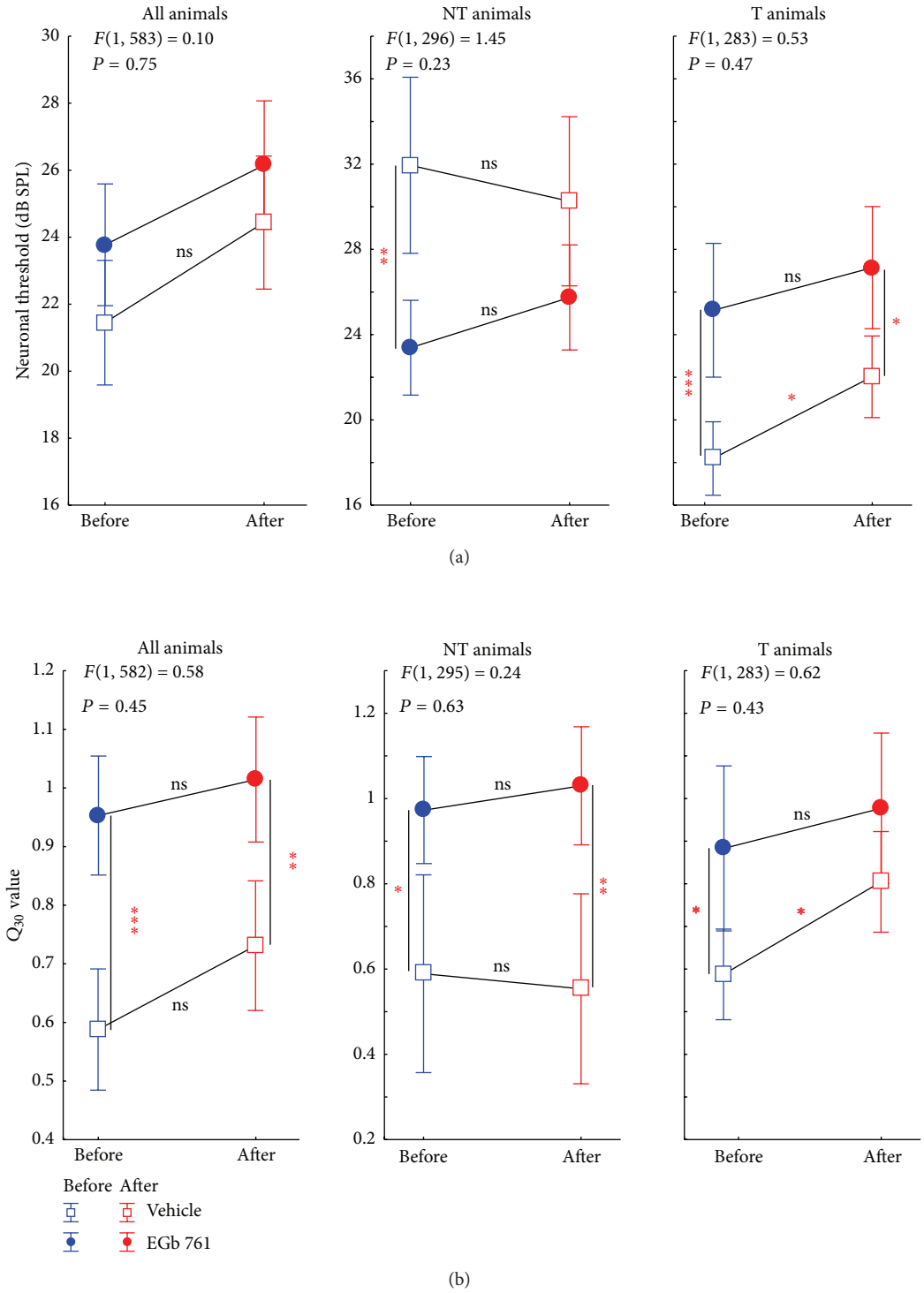


FIGURE 10: Trauma and treatment induced changes of neuronal response characteristics in NT and T animals. (a) Statistical interaction (with F-statistics) of time of measurement (before versus after trauma) and animal group (V versus E) with the mean neuronal threshold (±95% confidence interval) averaged across all animals (left panel) or separated into nontinnitus (center panel) and tinnitus animals (right panel). (b) Statistical interaction of time of measurement and animal group on spectral tuning sharpness (Q_{30} value) with the same grouping as above. Note that none of the statistical interactions become significant while most data show significant differences between V and E animals in the Tukey post-hoc-tests indicated by the asterisks (ns = not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

3.2.3. Neuronal Threshold and Spectral Tuning Sharpness. NIHL and EGb 761 treatment also affected neuronal thresholds and sharpness of spectral tuning measured in AI (Figure 10). When mean neuronal thresholds in AI were compared across all experimental groups, no significant differences were observed between V and E animals, neither before nor after NIHL (Figure 10(a), left panel; Tukey post-hoc-tests, all $P > 0.05$). Interestingly, when T and NT animals were analyzed separately (Figure 10(a), middle and right panel), another possible predisposition for the development of tinnitus after NIHL was seen in the V group: NT animals showed much higher neuronal thresholds in AI before NIHL compared to T animals (Student t-test, $P < 0.001$). These low thresholds in V-T increased after NIHL (Tukey post-hoc-test, $P = 0.02$), whereas no significant changes were observed in V-NT animals (Tukey post-hoc-test, $P > 0.05$). In the EGb 761 treated group, these pre-NIHL differences vanished, resulting in an intermediate level of neuronal thresholds that did not differ between T and NT animals (Student's t-test, $P > 0.05$) and remained stable even after NIHL (Tukey post-hoc-tests, $P > 0.05$).

Analysis of spectral tuning sharpness as specified by Q_{30} values revealed another pre-NIHL effect of EGb 761 treatment (Figure 10(b)): E animals showed significantly increased tuning sharpness across most experimental groups and conditions tested (Tukey post-hoc-tests, $P < 0.05$), with the exception of the V-T versus E-T comparison, where the Tukey post-hoc-tests showed $P > 0.05$. At least for the NT groups, where the E animals had lower neuronal thresholds compared to the controls, this result points to a neuroplastic process triggered by the EGb 761 treatment, which is effective in off-BF frequency ranges.

3.2.4. Response Latency and Response Duration. The details of our analyses of temporal neuronal response properties to tones in AI are shown in Figure 11. Here Figure 11(a) gives an overview of the frequency distribution of response latencies measured in V animals (open bars) and E animals (filled bars) both before NIHL (blue) and after NIHL induction (red) summarized across all recording sessions from 0 to 5 days after trauma. As revealed by both the Kolmogorov-Smirnov test for the comparison of distributions and the Mann-Whitney U-tests for the comparisons of the median values (see insets) there were no differences in pre- versus post-NIHL latency distributions in either V or E animals. Nevertheless, E animals had significantly shorter latencies than V animals under both conditions. When analyzing NT and T animals separately (Figure 11(b), Kruskal-Wallis ANOVA with post-hoc Median-tests), the difference in mean latency in NT animals was 5 ms before and 3 ms after NIHL. Such differences were not seen in T animals, pointing to another possible distinction of EGb 761 responders and non-responders. In contrast, when comparing response duration (Figure 11(c)), there were no significant differences between pre- and post-NIHL conditions, neither in the V-NT nor in the E-NT group, although in the latter there may be a trend to shorter response durations after the trauma (Figure 11(c), left panel). In the T groups on the other hand, NIHL induced a significant shortening in response duration in controls while it induced significant increase in response duration after EGb 761 treatment.

3.3. Neurophysiological Effects of Prophylactic EGb 761 Treatment: Comparison between Rate-Intensity Functions in Brainstem and Auditory Cortex. Finally, we compared the mean rate-intensity functions based on tone evoked ABR as a measure of brainstem activity (Figure 12); local field potentials (LFP) in AI, as an estimate of AI input (Figure 13; although LFP reflect thalamic as well as intracortical input [43]); and neuronal spike counts in AI, as a measure of AI output (Figure 14). In each of these figures, data are given for V (left) and E groups (right), and each of these separately for NT (first and third columns) and T animals (second and fourth columns). Furthermore, data are grouped for responses to three ranges of stimulation frequencies, namely, low (upper row, 0.5 to 1.4 kHz for ABR and 0.25 to 1.4 kHz for LFP and spiking activity), medium (middle row, 2.0 to 4.0 kHz), and high (lower row, 5.6 to 16.0 kHz) frequency tones. In each single panel, the effect of NIHL on the neuronal activity can be estimated by comparing pretrauma conditions (blue) with the posttrauma status (red). A 2-factorial ANOVA is used for this comparison. In addition to this comparison of pre- versus post-NIHL neuronal activity shown in Figures 12 to 14, the same data are replotted in Figures 15 to 18, respectively, to allow for an easier comparison of neuronal activity in groups V (open symbols) versus E (filled symbols). That is, Figures 12 to 14 show the effects of NIHL on neuronal activity throughout the auditory system; Figures 15 to 18 show the effects of prophylactic EGb 761 treatment on this activity. Insets in each panel in these figures give mean values across the respective rate-intensity function (as 1-factorial part of the 2-factorial ANOVA).

In general, both NIHL and prophylactic EGb 761 treatment led to significant changes in rate-intensity function at all levels of the auditory pathway (brainstem, AI presynaptic, AI postsynaptic), and within all frequency ranges analyzed here. These changes may be evident in absolute shifts of the function (significant change in mean values in the 2-factorial ANOVA), different shapes of the function (significant interaction in the 2-factorial ANOVA), or both. For better readability the F and P values of all tests are only shown in the appropriate figures and not mentioned again in the text.

Evaluating the changes at the level of the auditory brainstem (Figures 12 and 15), a general decrease in neuronal activity after NIHL was evident in all groups except for the group V-NT (Figure 12, first column), where a slight increase in neuronal activity was observed that was manifest especially at high stimulus intensities. EGb 761 treatment led to a slight increase in auditory brainstem activity before NIHL in all groups and frequency ranges (Figure 15, compare blue functions on the right to the blue functions on the left). NIHL led to a decrease in brainstem activity in all E animals (Figure 12, right), but the decrease was stronger in animals that did not develop tinnitus (group E-NT, Figure 12, third column), which is clearly different from the V-NT ABR described above. In other words, whereas the ABR in T animals

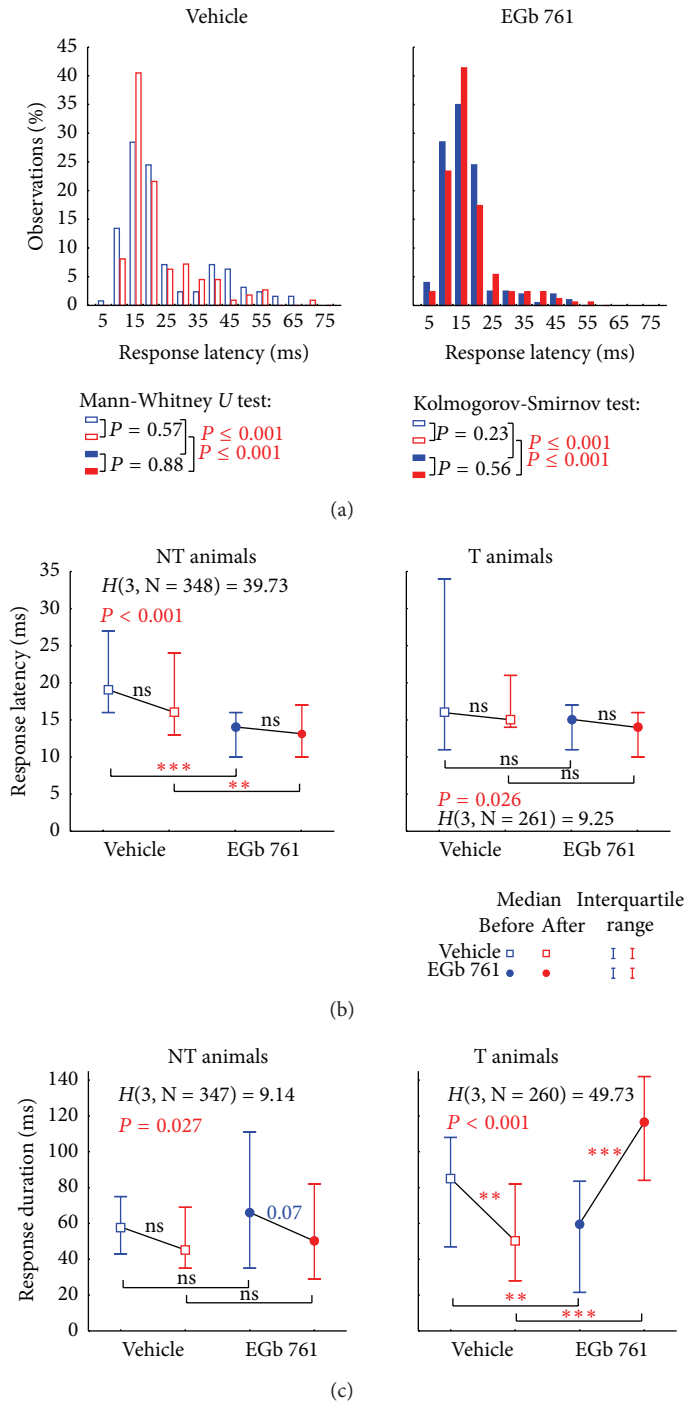


Figure 11: Distributions and Kruskal-Wallis-ANOVAs of neuronal response latency and duration in NT and T animals. (a) Distribution of response latencies (given in % observations binned into 5 ms bins) before (blue) and after (red) trauma in vehicle treated (open symbols) and EGb 761-treated animals (solid symbols) with the median values and interquartile range given above. Additionally, the statistics of the Mann-Whitney U-tests (corrected for multiple comparisons)—testing of median and interquartile range—and the Kolmogorov-Smirnov tests for the testing of the whole distributions against each other are plotted. Note that in both tests only the two pre- and two postdatasets between the groups are significantly different from each other while pre- versus posttrauma data are equal in both animal groups. (b) Median neuronal response latency (in ms ± interquartile range) tested by Kruskal-Wallis-ANOVAs (H-statistics) and multiple comparisons between the subgroups (ns = not significant, $^{**}P < 0.01$, $^{***}P < 0.001$) separated in nontinnitus and tinnitus perceiving animals treated with vehicle or EGb 761 before and after trauma. (c) Median neuronal response duration (in ms ± interquartile range) was analyzed as above.

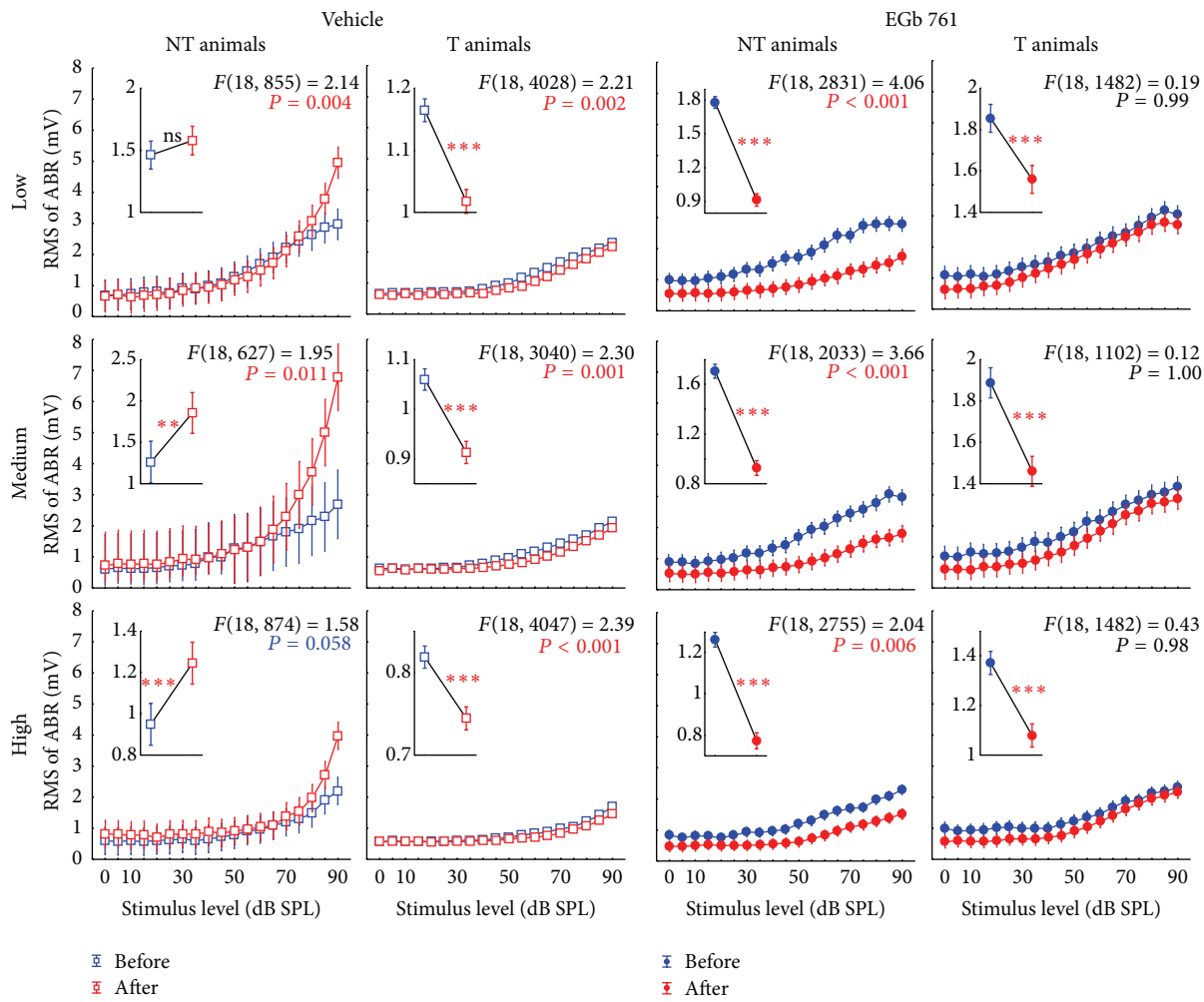


Figure 12: Level functions of the auditory brainstem responses (ABR) in NT and T animals grouped for vehicle and EGb 761 treated groups. Given are the mean root mean square (RMS) values of the ABR amplitudes (±95% confidence intervals) as a function of stimulus level before (blue) and after trauma (red) for the four subgroups for low (0.5 to 1.4 kHz), medium (2.0 to 4.0 kHz), and high stimulation frequencies (5.6 to 16.0 kHz). The F-statistics of the 2-factorial ANOVAs are shown for each panel and the corresponding 1-factorial part grouped for time of measurement (pre versus post trauma) is given in each inset (also with the RMS of ABR in mV) with the asterisks indicating the significance level (ns = not significant, $^{**}P < 0.01$, $^{***}P < 0.001$).

reacted similarly both in EGb 761 and vehicle treated animals, NT animals in groups V and E obviously deployed different neuroplastic mechanisms in the auditory brainstem that prevent the development of tinnitus after NIHL.

When looking to the synaptic input into AI further upstream in the auditory pathway (Figures 13 and 16), the picture becomes less clear compared to the auditory brainstem. For the control group V, effects of NIHL in general seem to point into the opposite direction as we just described for the ABR measurements: where we saw increases in activity after NIHL in the ABRs (Figure 12, NT animals, first column) we now find decreased LFP activity (Figure 13, first column) and vice versa (Figures 12 and 13, T animals, second columns). As in the brainstem activity, LFP changes were again mainly restricted to high stimulus intensities. In the EGb 761 treated animals, in contrast to the ABR results, NIHL-induced changes were much more specific and restricted to small ranges of frequency and intensity. In E-NT animals, for example, (Figure 13, third column), significant decreases in activity were exclusively seen for medium frequencies at 50 to 60 dB SPL, pointing to a very specific mechanism focused on the traumatized frequency range to prevent tinnitus development. Interestingly, a change in activity was already obvious before the induction of NIHL (Figure 16, first column). Prophylactic treatment with EGb 761 led to strong increases in LFP activity before noise trauma, possibly enabling the system to react to NIHL with LFP decreases focused to the traumatized frequency range to prevent tinnitus development. Changes in the E-T group (i.e., animals that were not able to prevent the development of tinnitus) after NIHL were much less focused and showed increases in activity rather than decreases (Figure 13, fourth column). Furthermore, in this group, we did not observe increases in LFP activity before noise trauma, which differ from the E-NT

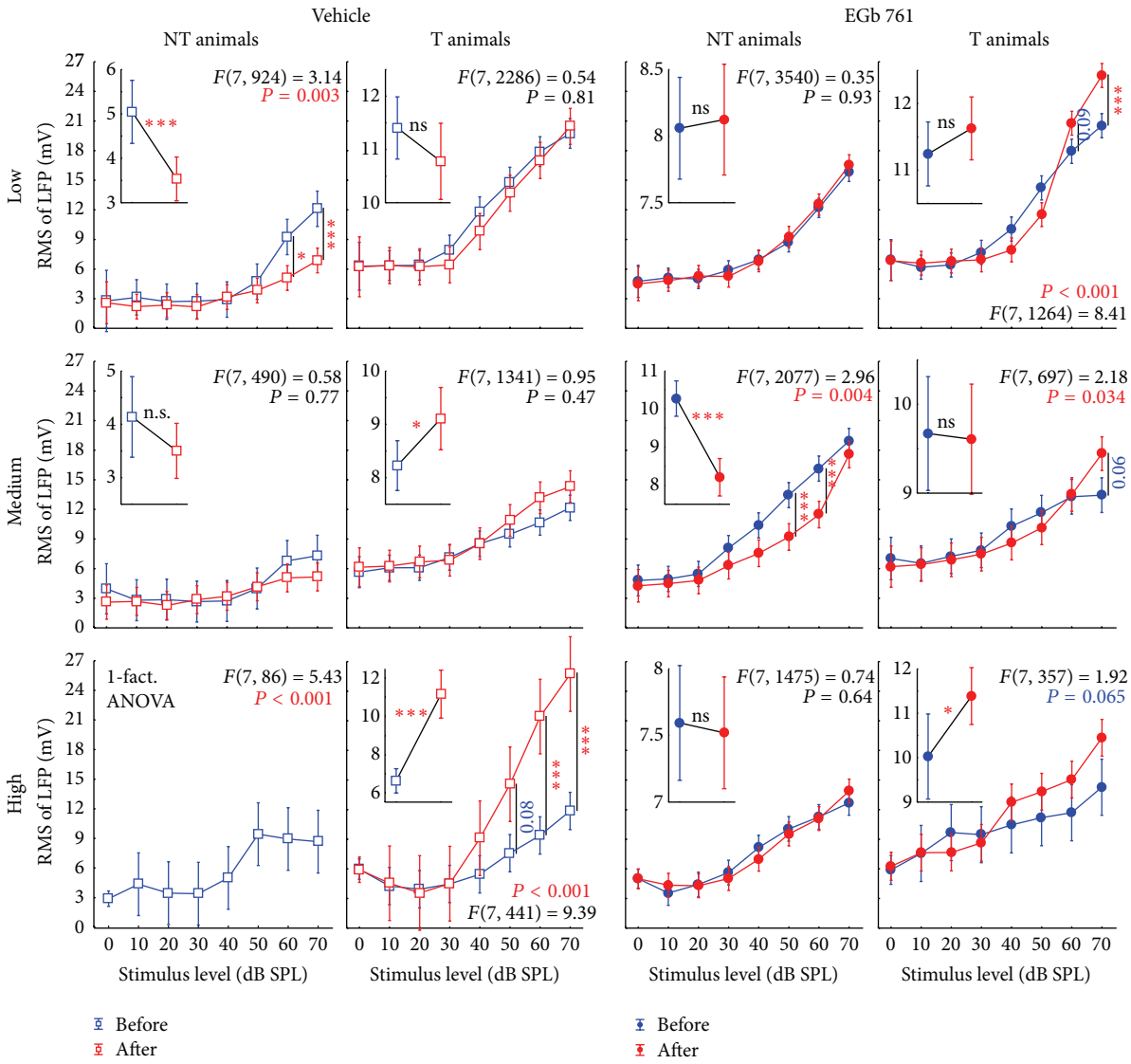


Figure 13: Level functions of the local field potential (LFP) amplitudes in the auditory cortex of NT and T animals grouped into vehicle and EGb 761 treated groups. Presented are the mean RMS values of the LFP amplitudes (±95% confidence intervals) as a function of stimulus level grouped as in Figure 8.

group (Figure 16, second column), at least in low and medium frequency ranges, pointing to another possible distinction between EGb 761 responders and nonresponders.

Finally, analyzing AI output activity (Figures 14 and 17), we generally found similar effects of NIHL in the V group, except for high stimulation frequencies in T animals. There, the strong increase that was evident in the LFP data (Figure 13, second column, bottom panel) turned into a general decrease in spiking output functions (Figure 14, second column, bottom panel). In the E group, the picture was again similar in AI output compared to AI input functions in NT animals, except for an increase in AI responses after NIHL to low stimulus frequencies (cf. Figure 14, third column versus Figure 13, third column). E-T animals on the other hand showed strong differences in the NIHL-induced changes in AI input versus output functions, with a general increase in AI spiking responses in all stimulation frequency ranges that was not evident in LFP functions and may be a correlate of tinnitus. Note that the increase in spiking activity increased from low to high stimulation frequencies; that is, it was particularly strong at frequencies corresponding to the behaviorally determined perceived tinnitus frequencies [31]. Evaluating the effect of EGb 761 treatment on spiking activity in AI (Figure 17), spike rates tended to be increased for most frequency and intensity ranges in the E-NT compared to V-NT animals, both before and after NIHL. By contrast, in E-T animals, we generally saw decreases in evoked spike rates in AI before NIHL compared to V-T animals. After NIHL, differences between E-T and V-T animals differed as a function of stimulation frequency, with least differences seen at low stimulation frequencies and strongest differences at high stimulation frequencies.

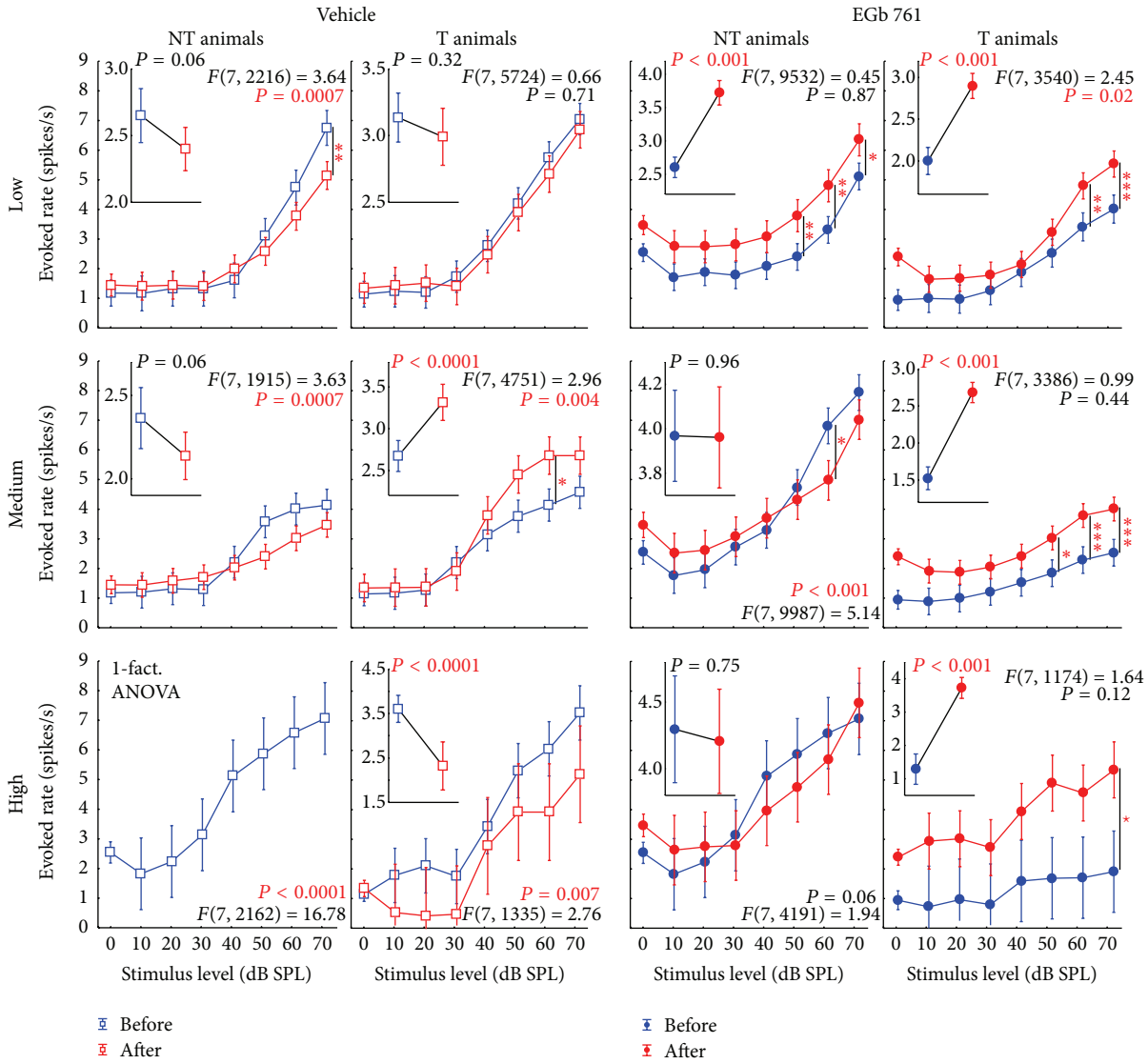


Figure 14: Level functions of the evoked spike rates in the auditory cortex of NT and T animals grouped into vehicle and EGb 761 treated groups. The mean evoked response rates of the auditory neurons in AI (±95% confidence interval) as a function of stimulus level grouped as in Figure 8 are shown.

3.4. Known Physiological Effects of EGb 761. EGb 761 is a standardized extract of dried green leaves of *Ginkgo biloba*. It contains numerous different compounds (see Section 2 and [17, 19, 44]). A number of different physiological effects are described for the EGb 761 [19, 20], but given the nature of such plant extracts as being composed of a variety of different components, it is not always easy (if at all possible) to attribute a particular effect to a single compound (although in some cases at least the effective compound class could be determined, [26, 28, 45]). In the context of the present study, four physiological effects described for EGb 761 seem to be most important for the prophylactic effects on NIHL and tinnitus development reported here. These are, first, stabilization of mitochondrial respiratory chain metabolism and ATP production due to antioxidant effects that reduce oxidative stress by elimination of ROS [17, 18]; second, increase of extracellular dopamine levels in prefrontal cortex that may improve mood and thereby reduce stress [26], based on blocking dopamine reuptake via the norepinephrine transporter [28]; third, reduction of hormonal stress responses by reduction of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), or corticosterone [46]; and fourth, improved blood flow [47, 48].

In the final section we will discuss how these known physiological effects of EGb 761 may be beneficial in the context of a reduction of NIHL and central tinnitus development.

3.5. Possible Mechanisms of EGb 761 Protective Effects against NIHL and Noise Trauma-Induced Tinnitus. A number of studies have demonstrated that the prophylactic use of several antioxidant substances may reduce NIHL [14, 49]. Models for NIHL assume that the cochlea is damaged mechanically by

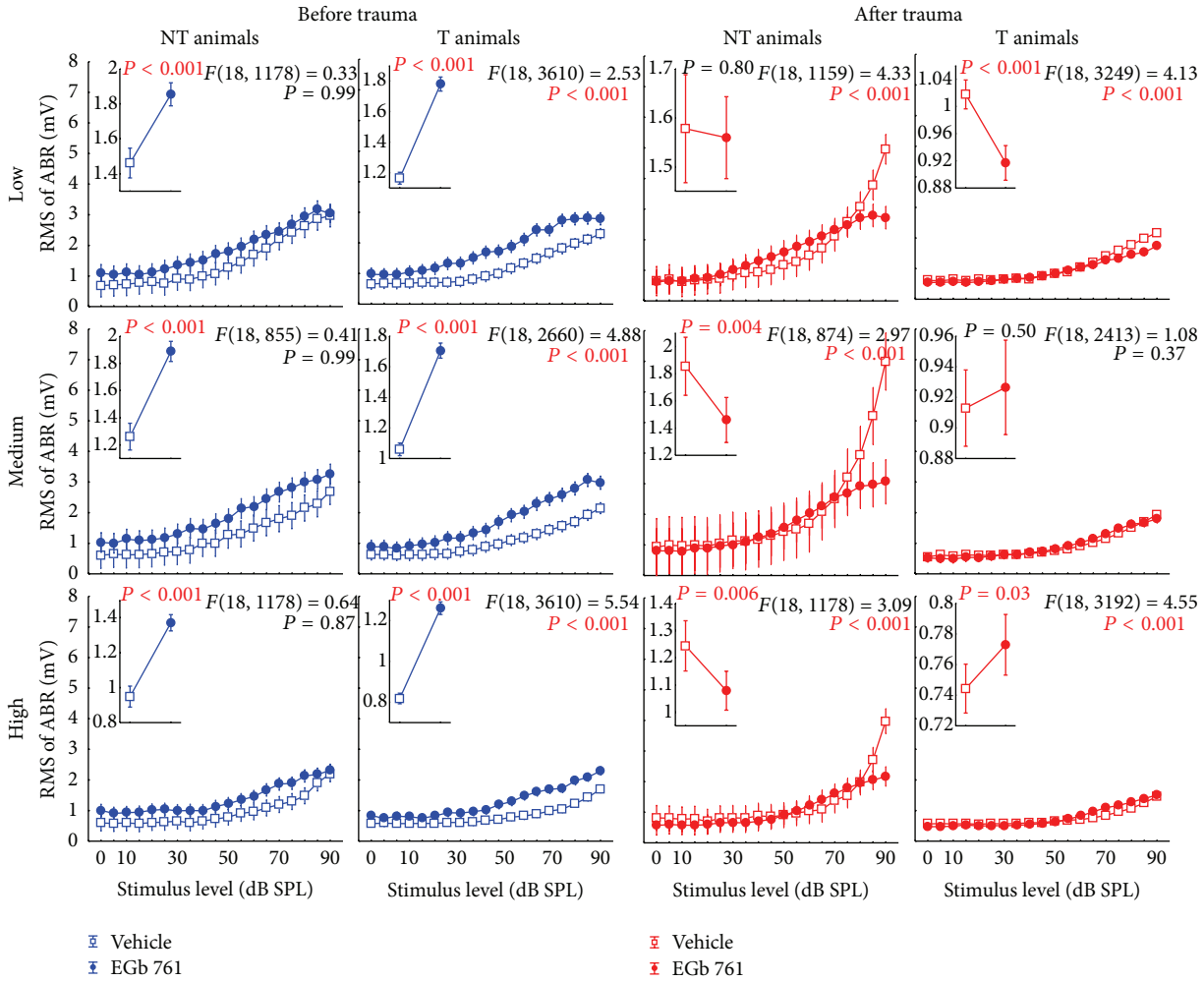


Figure 15: Replotted data of Figure 8, grouped according to the time of measurement (before versus after trauma) to allow for an easier comparison of vehicle versus EGB 761 treated animals. Note the consistent differences in the ABR amplitudes, especially for pretrauma in vehicle versus EGB 761 treated animals.

intensive noise, and in addition that metabolic stress induces hair cell death via ROS, which activate apoptotic pathways. According to these pathological mechanisms antioxidant substances are thought to protect the cochlea from hair cell loss after intensive noise exposure by reducing ROS. Over the last few years it has been demonstrated numerous times that this strategy can successfully be applied using different antioxidants [50–58]. It is therefore plausible to assume that the antioxidant effect described for EGb 761 is responsible for the protective effect of the extract against NIHL as reported here (Figure 3) and earlier [15, 16]. In addition, as noise trauma also decreases cochlear blood flow [59], the improvement of blood flow after EGb 761 administration [19] may also add to the cochlea protective effect of the extract.

As a consequence, it seems self-evident that a reduced amount of NIHL will also lead to a reduced percentage of animals that develop noise trauma-induced tinnitus (Figure 5). In this context it remains unclear if the tinnitus we observed in our animal model in this study does reflect acute tinnitus or already a chronic manifestation of tinnitus. But independent of the type of tinnitus, we observed only in T animals an isolated increase in gap response amplitude (cf. Figure 5(a)) after the acoustic trauma, while NT animals showed a generally similar pre- and posttrauma gap response. We know from our earlier study [31] that central neuroplastic changes in AC which correlate with the development of tinnitus in our animal model are restricted to the first week after trauma, although the tinnitus percept itself is stable for at least 16 weeks. It may therefore be the case that in this model system chronic tinnitus already manifests after one week after trauma. However, this is still an open question that needs to be addressed in future studies. Independent of this question, we were able to describe a number of additional effects of the extract on central auditory processing, which make it unlikely that all beneficial effects we observed on NIHL and tinnitus development may be based exclusively on the abovementioned protective effects on the peripheral auditory system, that is, on hair cells.

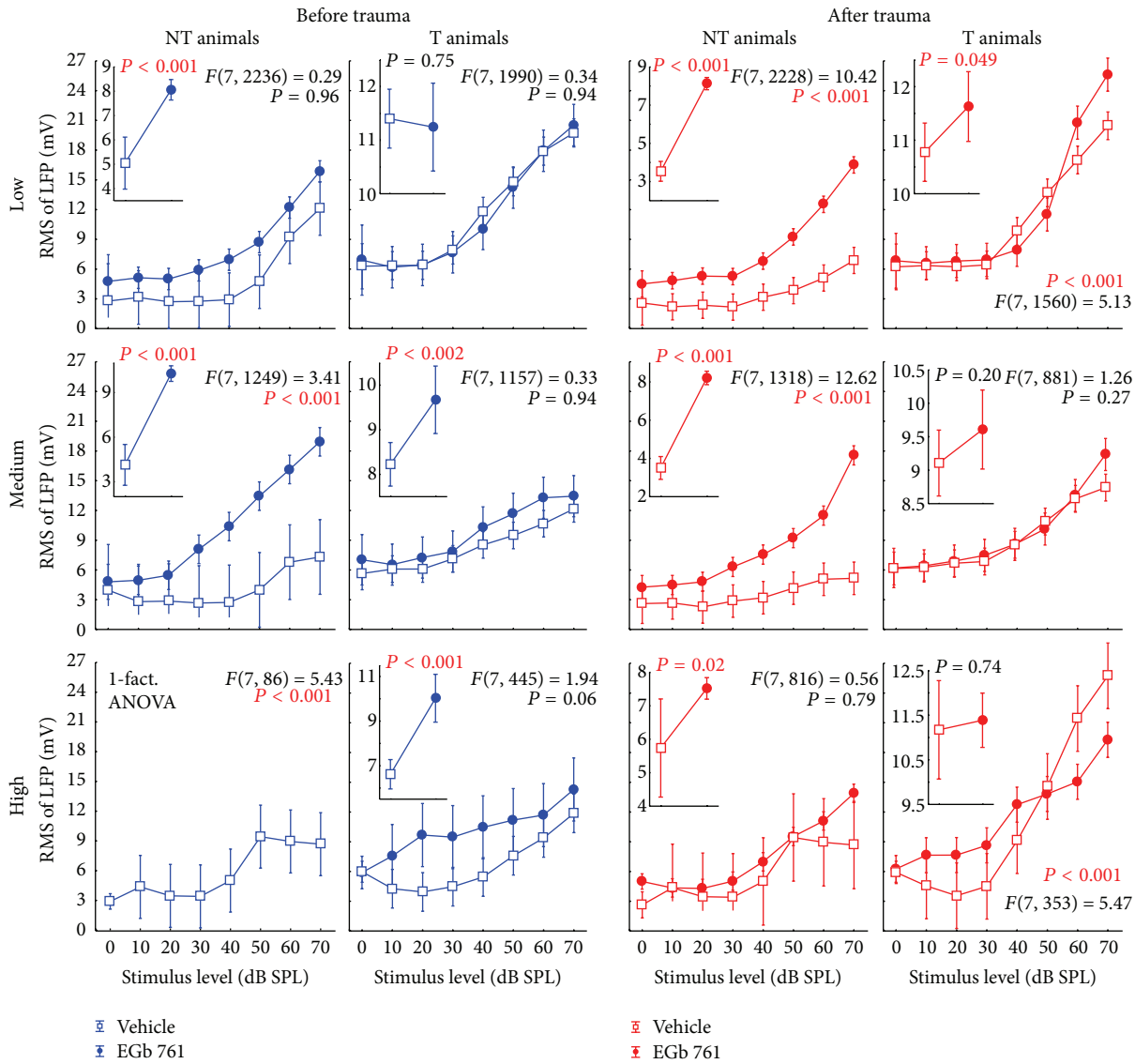


Figure 16: Replotted data of Figure 9, grouped according to the time of measurement (before versus after trauma) to allow for an easier comparison of vehicle versus EGB 761 treated animals. Note the consistent differences in the LFP amplitudes, in particular in the NT animals in vehicle versus EGB 761 treated groups.

In a previous publication on tinnitus development in untreated animals [31] we proposed a global inhibitory mechanism in AI that should be able to successfully counteract the development of tinnitus in a subset of animals by decreasing overall activity in AI. In this report, we found that administration of Ginkgo extract before the trauma already leads to a reduction in AI activity, which is comparable to that observed in untreated animals after trauma (cf. Figure 7). Nevertheless, closer inspection of the data showed that the pretrauma effects of EGb 761 on activity in the central auditory system do not resemble the posttrauma mechanism that prevents some of the untreated animals from the development of tinnitus. Importantly, we saw an increase in activity in auditory brainstem before the trauma in EGb 761 treated animals, whereas there was a slight decrease of ABR amplitudes in untreated animals after trauma [31] (cf. Figure 14). Furthermore, EGb 761 treatment led to increased mean BFs, which indicate plastic changes in tonotopic organization in AI that were not seen in untreated animals without tinnitus.

Based on these differences we propose here the hypothesis that EGb 761 treatment activates a lateral inhibition mechanism rather than a global inhibitory mechanism as proposed for untreated animals. Figure 18 illustrates the details of this model. The model assumes two main effects of the Ginkgo extract on central auditory processing, namely, an increase of gain in auditory brainstem, as evident from increased ABR amplitudes (Figure 15), and an activation of intracortical lateral inhibition, as indicated by increased tuning sharpness

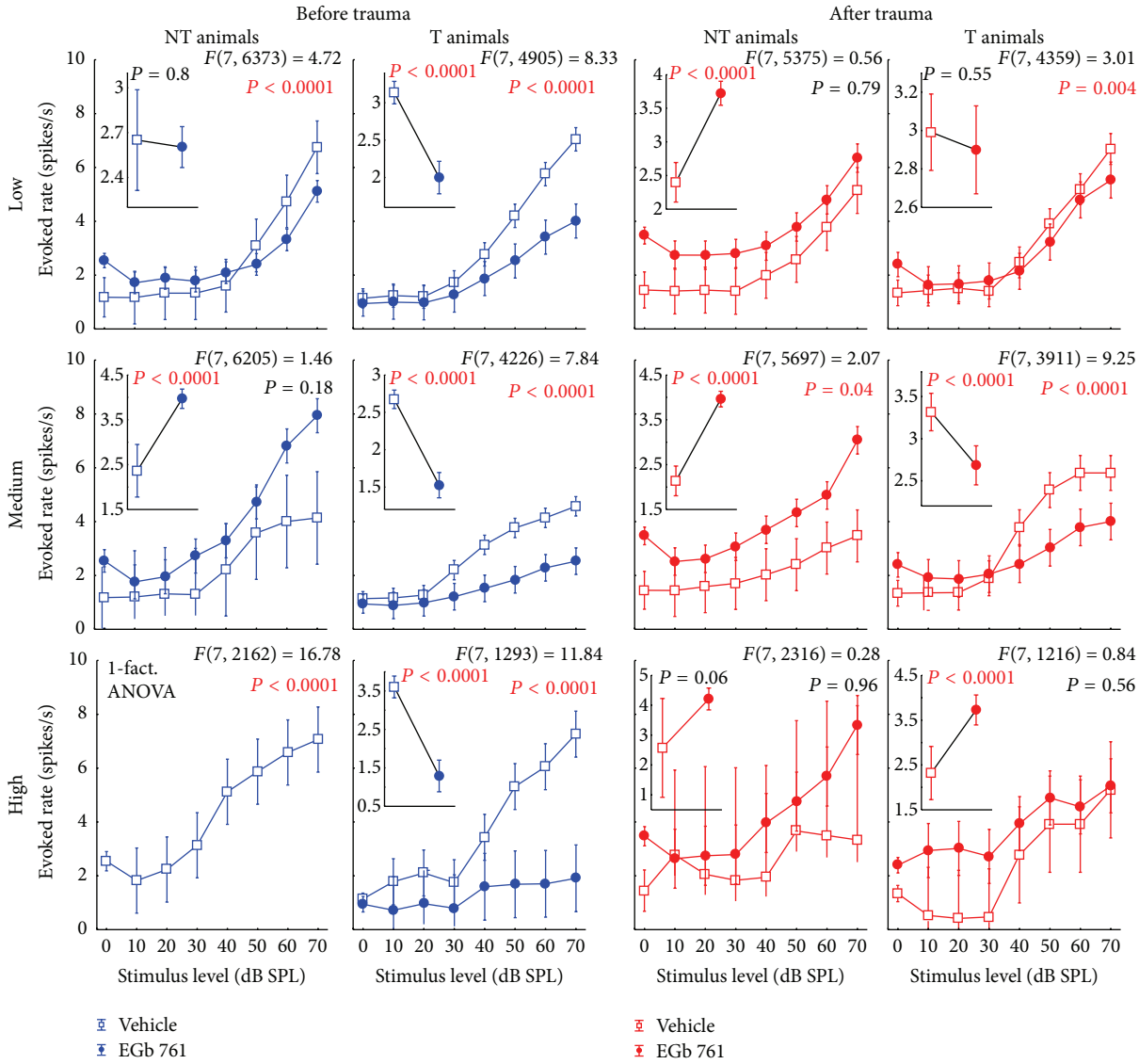


FIGURE 17: Replotted data of Figure 10, grouped according to the time of measurement (before versus after trauma) to allow for an easier comparison of vehicle versus EGB 761 treated animals.

(Table 1, Q_{30}). We believe that the increased ABR activity leads to an increased thalamic input to AI, which is responsible for the decreased neuronal thresholds we observed after Ginkgo treatment (Table 1, top row; Figure 18). The reduced response latency after EGb 761 treatment is probably a consequence of these reduced thresholds. Nevertheless, as the evoked response rate is decreased in AI, some intracortical inhibition must be activated simultaneously. The increased Q_{30} values point to local, lateral inhibitory influences, while the shifted mean BF points to an asymmetric distribution of this lateral inhibition, with stronger inhibition at the low frequency side compared to the high frequency side of a given tuning curve (Figure 14, bottom panel). The fact that the main reduction in overall activity in AI is in the low frequency rather than in the high frequency range (Figure 7, left column; Figure 18, top panel) is in line with this interpretation. As a result, EGb 761 induced changes in activity throughout the auditory system seem to lead to processing characteristics in AI that are more stable (Figures 7(c), 8 and 10) and therefore less prone to the development of central tinnitus after noise trauma (Figure 5). The fact that most of these effects of EGb 761 treatment on AI activity were not seen in animals that did develop tinnitus and that most pretrauma changes remained stable in the NT but not in T animals (Table 1, third row) leads us to speculate that these central effects of EGb 761 in responders compared to nonresponders substantially add to the protective effect of the antioxidant characteristics in the cochlea that counteract NIHL, but revealing the exact mechanism needs further investigation.

Finally, one could speculate about these mechanisms by which EGb 761 leads to the changes in central auditory processing as described above. One factor in this context may be

Table 1: Overview of parameter change EGb 761 versus vehicle control.

	Hearing threshold	Evoked rate	Mean BF	BF distrib.	Neuronal threshold	Q_{30}	Response latency	Response duration
Nontinnitus animals								
Before	—	↓	↑	↑	↓	↑	↓	—
After	↓ (acute & 1–7 d)	—	↑	↑	—	↑	↓	—
Tinnitus animals								
Before	—	—	—	—	↑	↑	—	↓
After	— (1 & 7 d)	—	—	—	↑	—	—	↑

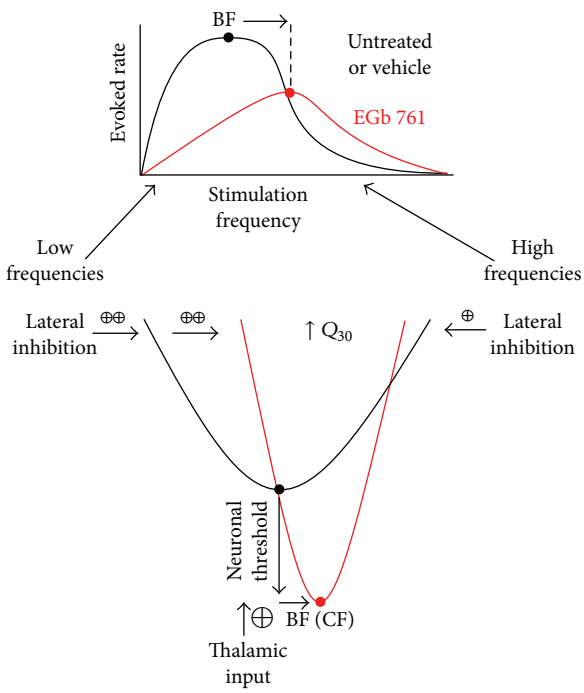


Figure 18: Proposed model of the effects of EGb 761 treatment on auditory processing. Upper panel: evoked neuronal response rate in AI for iso-intensity pure tone stimuli in vehicle (black) and EGb 761 treated animals (red). Lower panel: neuronal threshold and tuning of cortical neurons in both animal groups. Based on our data, we propose two main effects of EGb 761 on auditory processing: first, an increase of auditory brainstem activity leading to an increased thalamic input to AI, which results in lower response thresholds and shorter response latencies, and second, an asymmetric effect on lateral inhibition in AI that reduces overall response rates, shifts the best frequency (BF) to higher values, and sharpens spectral tuning (Q_{30}-values).

the increased dopamine level in prefrontal cortex that was found under EGb 761 treatment [26]. Dopamine is known to foster several neuroplastic processes [60–62] so that it may be possible that dopamine effects are also involved in the neuroplasticity described here under treatment, although the mechanisms that trigger this plasticity still remain unclear. In addition, dopamine is known to improve mood, and when combined with the EGb 761 effects on hormonal stress responses, these factors may lead to decreased stress in the animals that could also be beneficial in the context of tinnitus development [63].

As described above, the exact mechanism that leads to the increased lateral inhibition in AI after the treatment with EGb 761 remains unclear. But the concept that such lateral inhibition may counteract the development of central tinnitus—especially in the acute phase after a noise trauma—seems to be straightforward based on current models of central tinnitus [64] and was already used in new, promising treatment strategies in both animal models for tinnitus [65] and human patients [66]. Possibly, additional administration of EGb 761 might further improve the outcome of such treatment regimens.

4. Conclusion

In this report we were able to demonstrate that the prophylactic treatment of animals with the *Ginkgo biloba* extract EGb 761 elicits a number of protective effects on the development of NIHL as well as on subjective tinnitus, both on peripheral as well as central levels of the auditory pathway. Although the fact that only a subset of animals that have been characterized behaviorally could also undergo detailed electrophysiological recordings might pose a limitation to this study (EGb 761 animals: 3 NT, 1 T; vehicle animals: 1 NT, 2 T), the observed effects of EGb 761 on central auditory processing still revealed a number of significant changes of response parameters that allow us to speculate about the neurophysiological mechanisms underlying these changes. A qualitative overview of these effects is given in Table 1.

In general, when comparing EGb 761 effects between NT and T animals (upper two rows versus lower two rows in Table 1) it is obvious that in NT animals much more significant effects of the extract can be seen than in T animals. Furthermore, where effects were found in T animals, they sometimes pointed into the opposite direction as in NT animals (Table 1, threshold), or there were no effects in NT animals (e.g., Table 1, response duration). Based on these differences,

in particular those that were already seen before trauma, animals can be separated in EGb 761 responders and nonresponders. This distinction seems to correlate with the distinction between NT and T animals, respectively. That is, the extract, if prophylactically applied, obviously is able to reduce NIHL (Figure 3) and the probability to develop subjective tinnitus after noise trauma (Figure 5), and this outcome is based on a whole number of neurophysiological effects (Figures 7 to 17).

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Konstantin Tziridis and Sabine Korn contributed equally to the study.

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Research Article

Modulation of Electrocortical Brain Activity by Attention in Individuals with and without Tinnitus

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Age and hearing-level matched tinnitus and control groups were presented with a 40 Hz AM sound using a carrier frequency of either 5 kHz (in the tinnitus frequency region of the tinnitus subjects) or 500 Hz (below this region). On attended blocks subjects pressed a button after each sound indicating whether a single 40 Hz AM pulse of variable increased amplitude (target, probability 0.67) had or had not occurred. On passive blocks subjects rested and ignored the sounds. The amplitude of the 40 Hz auditory steady-state response (ASSR) localizing to primary auditory cortex (A1) increased with attention in control groups probed at 500 Hz and 5 kHz and in the tinnitus group probed at 500 Hz, but not in the tinnitus group probed at 5 kHz (128 channel EEG). N1 amplitude (this response localizing to nonprimary cortex, A2) increased with attention at both sound frequencies in controls but at neither frequency in tinnitus. We suggest that tinnitus-related neural activity occurring in the 5 kHz but not the 500 Hz region of tonotopic A1 disrupted attentional modulation of the 5 kHz ASSR in tinnitus subjects, while tinnitus-related activity in A1 distributing nontonotopically in A2 impaired modulation of N1 at both sound frequencies.

1. Introduction

Forms of neural plasticity are expressed by many neurons in central auditory structures and are believed to sculpt the neural changes that underlie the development of tinnitus and hyperacusis associated with hearing loss [1, 2]. Examples of neural changes attributed to neural plasticity in animal models include upregulation of somatosensory inputs to principal neurons in the dorsal cochlear nucleus (DCN) following section of the cochlear nerve [3] and broadening of the temporal integration window of spike-timing dependent plasticity for neurons in the DCN [4] and auditory cortex [5] in animals exhibiting behavioral evidence of tinnitus. Neural changes taking place after deafferentation may in turn affect how neural activity is modified when auditory training is applied to individuals with tinnitus, as is done by sound therapies intended to treat this condition. Roberts et al. [6] trained individuals with tinnitus and age and hearing-level matched controls to detect an auditory target embedded in a 5 kHz 40 Hz amplitude modulated (AM) sound. The 5 kHz 40 Hz AM sound was in the tinnitus frequency region (TFR) of the tinnitus subjects and evoked the stimulus-driven 40 Hz auditory steady response (ASSR) known to localize to sources in primary auditory cortex (A1) [7–10]. In agreement with earlier results obtained from normal hearing subjects [11, 12], the phase of the ASSR phase (the time delay between the 40 Hz stimulus and response waveforms) decreased progressively over training sessions in the control group, but ASSR phase did not change in the tinnitus group. In contrast, the amplitude of the ASSR (which was known from earlier research to be resistant to change) did not increase with training in controls, but ASSR amplitude increased with training in the tinnitus group, as did online ratings of the loudness of their tinnitus percept. It was suggested that abnormal synchronous neural activity underlying the tinnitus percept may have obstructed changes in ASSR phase in the tinnitus group, whereas reduced inhibition in A1 associated with tinnitus may have permitted an expansion of

the cortical representation for 5 kHz that was prevented by competitive interactions within the tonotopic map of control subjects without tinnitus [6].

These results suggest that the effects of plasticity are modified in tinnitus sufferers by tinnitus-related neural activity occurring in auditory pathways. Further findings of Roberts et al. [6] suggested that effects of attention on neural responses are also modified in the tinnitus brain. In hearing-intact animals, neural plasticity is modulated by subcortical cholinergic and other neuromodulatory systems that receive top-down input from prefrontal cortex and project widely to the neocortex where they perform an attention-like function, making neurons more sensitive to their afferent inputs [13–17]. These mechanisms may account for the observation in normal hearing humans that auditory tasks that require top-down attention increase not only the amplitude of the ASSR localizing to A1, but also the amplitude of the N1 transient response whose cortical sources localize to secondary auditory cortex (A2) in the region of the planum temporale [7, 18]. In agreement with results obtained in normal hearing subjects, control subjects in the study of Roberts et al. [6] showed increased ASSR and N1 amplitude on active trials where detection of targets was required, compared to a passive condition where subjects were told to ignore the sounds and rest until the next active block was presented. However, modulation of ASSR and N1 amplitude by attention was abolished in the tinnitus group for N1 in all sessions of training and for ASSR amplitude on the first session with a weak modulation appearing subsequently as ASSR amplitude increased over trials. The results suggested that, although the top-down auditory attention system may work normally in tinnitus, its expression was obstructed by tinnitus-related neural activity occurring in the TFR of the tinnitus group where the sound to be detected (a 40 Hz AM 5 kHz carrier frequency) was located.

The present experiment evaluated this hypothesis by determining whether deficient modulation of ASSR and N1 amplitude by attention is observed when subjects with tinnitus are required to detect auditory targets embedded in a 40 Hz AM carrier of 500 Hz, which is well below the region where tinnitus-related neural activity is expected to occur. The results were compared in a unified analysis to the 5 kHz groups reported by Roberts et al. [6] which performed the same auditory detection task except for the carrier frequency chosen. In addition, two additional long-latency responses, namely, the N2 transient response (latency ~325 ms) and the auditory sustained response (SR, commencing after N2 and persisting to the end of stimulation), were studied in both groups, to determine whether modulation of these late responses was similarly affected by tinnitus.

2. Methods

2.1. Participants and Design. 60 subjects (30 tinnitus and 30 controls) were recruited via McMaster University faculty and staff by email list servers and from our laboratory archive. One control subject was excluded from analysis due to noise in the electroencephalogram (EEG) that could not meet the requirement for artifact rejection in offline processing. Two further controls withdrew for unrelated medical reasons. Two tinnitus participants withdrew after expressing concern that the procedures might worsen their tinnitus. Of the remaining 55 subjects, 22 completed the 5 kHz study of Roberts et al. [6] and 33 subjects were new recruits assigned to 500 Hz and tested here. No subjects in the total sample reported use of medication during the time of the study; controls reported no history of tinnitus or ear diseases. Participants received an honorarium of $10 CAD per hour as well as reimbursement for parking fees. Subjects provided informed consent using procedures approved by the Research Ethics Board of McMaster University and consistent with the Declaration of Helsinki.

Tinnitus subjects completed a preliminary interview (intake session, about 90 minutes) that collected detailed information on personal history of their tinnitus. The Tinnitus Handicap Questionnaire (THQ) was administered to assess tinnitus attributes and impact on quality of life [19]. Pure-tone audiometric thresholds were measured using a GSI 61 audiometer with Telephonics 296 D200 (0.125–8.0 kHz) and Sennheiser HDA 200 (8.0–16 kHz) headphones using the pulsed-tone method. Properties of tinnitus were measured by computerized tools described by Roberts et al. [12]. Using the tools, subjects first identified the ear of tinnitus (left, right, or both) and tinnitus bandwidth (tonal, ringing, or hissing) following which they rated tinnitus loudness on a Borg CR100 visual analog scale. Next, subjects adjusted the loudness of each of 11 pure tones between 0.5 and 12.0 kHz to equal that of their tinnitus. The tinnitus frequency spectrum (likeness rating) was then taken for the same pure tones at the determined loudness level, followed by a brief test for residual inhibition. Control subjects completed the same intake procedure as tinnitus subjects except for procedures pertaining to tinnitus.

Four groups of subjects were studied: controls tested at 500 Hz (Cont500 Hz), tinnitus subjects at 500 Hz (Tinn500 Hz), control subjects at 5 kHz (Cont5 kHz), and tinnitus subjects at 5 kHz (Tinn5 kHz). The tinnitus and control groups were matched for age within the two stimulus frequencies and as much as possible between the two frequencies. The number, age, and gender of subjects in each group and the sound levels experienced by the subjects are given in Table 1 where properties of tinnitus are also reported for the two tinnitus groups. Figure 1 shows audiometric thresholds for each group and, for tinnitus subjects only, the tinnitus spectrum and loudness matches for sound frequencies between 500 Hz and 12 kHz. Approximately one week lapsed between the intake session and the experimental session described next.

2.2. Stimuli. The stimuli were 500 Hz and 5 kHz pure tones AM by a 40.96 Hz sinusoid (called 40 Hz, 100% modulation depth following the modulation wave). Tone duration was 975.56 ms, such that each stimulus contained 40 AM pulses. Stimuli were generated by a digital signal processor (Tucker-Davis RP.2) and presented binaurally through ear inserts (Etymotic ER2). Sound levels were determined by a loudness

Table 1: Participant demographics.

	Tin500 Hz group	Cont500 Hz group	Tinn5 kHz group	Cont5 kHz group
Characteristics of participants				
Number (male)	17 (10)	16 (5)	11 (7)	11 (8)
Age in years, mean (SE)	62.0 (3.31)	62.0 (2.29)	48.6 (4.75)	53.9 (5.86)
Age range in years	22–77	42–74	22–68	22–76
Audiometric Data				
Mean (SE) threshold @ 500 Hz (dB HL)	10.6 (3.49)	11.9 (2.90)	8.0 (2.56)	10.5 (2.88)
Mean (SE) threshold @ 1 kHz (dB HL)	18.4 (3.76)	13.1 (3.25)	7.9 (2.70)	10.7 (3.67)
Mean (SE) threshold @ 2 kHz (dB HL)	24.5 (4.24)	12.5 (3.97)	9.8 (3.65)	13.6 (3.61)
Mean (SE) threshold @ 5 kHz (dB HL)	43.8 (5.04)	33.3 (5.19)	28.5 (6.24)	25.7 (7.01)
Sound levels				
Standard for matching	1 kHz pure tone 65 dB SL	1 kHz pure tone 65 dB SL	2 kHz 40 Hz AM tone at 65 dB SPL	2 kHz 40 Hz AM tone at 65 dB SPL
Mean (SE) stimulus intensity (dB SPL)	81.2 (1.68)	78.7 (2.90)	60.0 (2.05)	58.5 (2.01)
Stimulus intensity range (dB SPL)	69–93	47–93	50–74	40–66
Tinnitus characteristics				
Mean (SE) duration in years	12.5 (2.68)		11.7 (3.03)	
Mean (SE) loudness rating Borg CR100 scale	44.8 (5.62)		57.1 (6.21)	
Mean (SE) loudness match (1 kHz tone, dB SPL)	36.7 (9.05)		53.9 (6.32)	
THQ Mean Total Score (SE)	32.5 (5.64)		48.9 (6.66)	
Tinnitus bandwidth (number of participants)				
Tonal	12		6	
Ringing	2		2	
Hissing	3		3	
Tinnitus ear				
Bilateral	15		11	
Left	1		0	
Right	1		0	

matching paradigm in which subjects in the 500 Hz groups matched the loudness of the stimulus to a reference pure tone of 1 kHz presented at 65 dB SL and subjects in the 5 kHz groups to a reference tone of 2 kHz presented at 65 dB SPL. These matching procedures aligned the groups with those of earlier research [2, 6] and equated subjective stimulus loudness between the tinnitus and control groups at each probe frequency. However, it was inevitable that probe intensity measured in SPL would vary between the 500 Hz and 5 kHz groups as a consequence of threshold shifts at 5 kHz and hyperacusis in the tinnitus groups. Possible effects of probe intensity were evaluated by regressing effects of attention expressed in each brain response on probe intensity in SPL, which was known for each subject.

2.3. Auditory Task. The auditory task is described in Figure 2. Subjects sat in a sound-attenuated (ambient noise level 16 dBA SPL) and electrically shielded booth, comfortably in a chair distanced 1.4 m from a computer monitor. There were two types of stimuli: standard stimuli and stimuli containing a target. The two stimuli were identical except that target stimuli contained a single 40 Hz pulse of variable increased amplitude (target) that occurred randomly at 415 ms, 610 ms, or 805 ms after stimulus onset. Approximately 2/3rd of the stimuli contained a target; however, because approximately 1/3rd of the targets were expected to be below or close to the threshold of detection, target stimuli likely were heard on about 50% of trials. Trials of both types (standard and target) unfolded in either active blocks or passive blocks with each block containing 54 stimuli and lasting roughly 2.5 minutes. On active blocks, the word "Listen" appeared in a text box on the computer screen, instructing participants to attend to the trial for a target event. After stimulus completion text on the screen prompted, "Did you hear a target?" As per instructions on the screen, participants pressed the left mouse button "yes" if they had detected a target and a right mouse button "no" if they had not. Correct responses (hits and correct rejections) generated a green text box for 400 ms providing appropriate feedback. Incorrect responses (misses and false alarms) produced a red text box for the same duration. An intertrial interval (ITI) varying between 1400 and 1600 ms commenced with each behavioral response, giving a variable interval of about 1900 ms including the feedback cue and depending on behavioral response latency. During passive blocks, the text "Stop responding and ignore

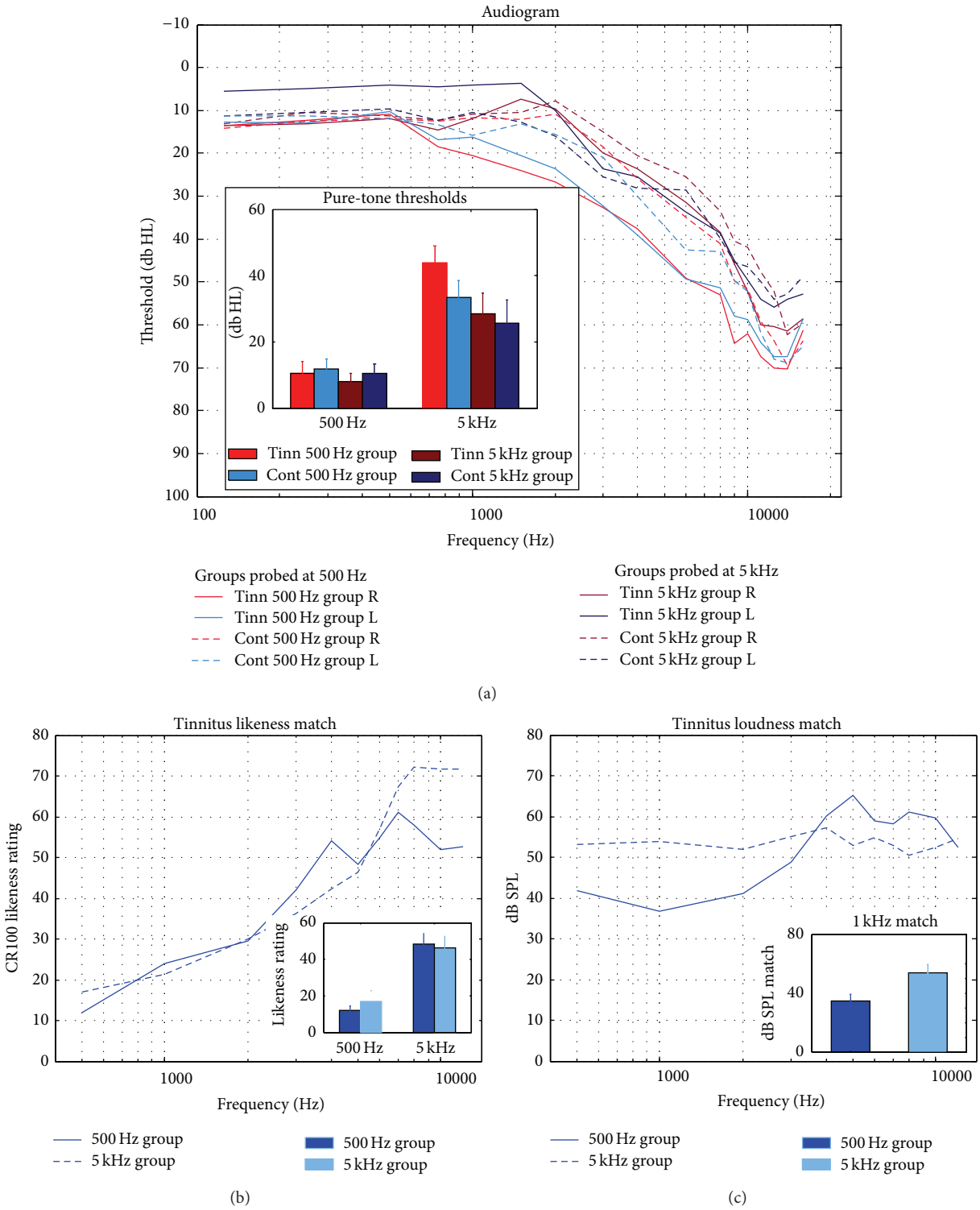

Figure 1: Audiogram, tinnitus spectrum, and tinnitus loudness matches. (a) Pure-tone audiograms (pulsed-tone method) from 0.125 to 16 kHz showing each ear and group separately. Comparisons of thresholds averaged across ears at 500 Hz and 5 kHz are shown in the inset bar graph (5 kHz interpolated between 4 kHz and 6 kHz) separately for groups probed with 500 Hz and 5 kHz sounds. (b) Tinnitus likeness ratings from 0.5 to 12 kHz for both tinnitus groups and an inset bar graph comparing 500 Hz ratings to 5 kHz ratings in each group. 500 Hz ratings are below the tinnitus spectrum which commences above a likeness rating of 40 (a sound beginning to resemble tinnitus; Roberts et al. 2008). (c) Tinnitus loudness matches from 0.5 to 12 kHz for both tinnitus groups. Inset bar graphs compare loudness matches at a common 1 kHz frequency.

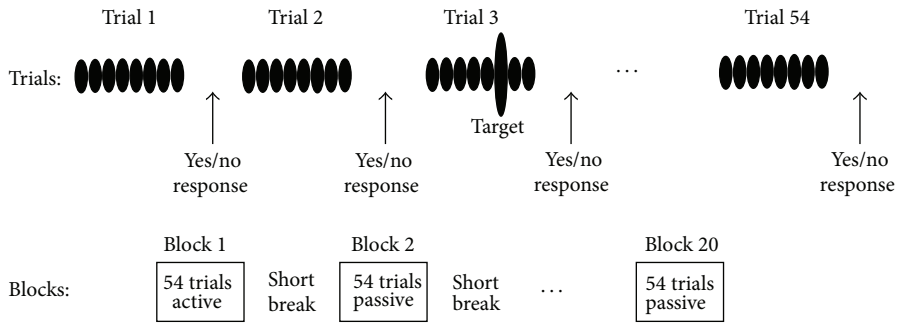

Figure 2: Auditory task. Upper panel: three standard 40 Hz AM stimuli and one target stimulus containing a single amplitude-enhanced AM pulse (target) are illustrated by cartoons containing 8 AM pulses (40 pulses were delivered on each trial). Approximately 2/3rd of the stimuli contained a target of variable enhanced amplitude such that not all targets were detectable. Lower panel: on active blocks participants identified whether a target was present or not; on passive blocks participants ignored the sounds and waited for the next active block. Blocks contained 54 trials and alternated between active and passive blocks for a total of 20 blocks per session.

stimulus" appeared continuously on the computer screen, indicating that participants should ignore the sounds and wait until the next active block. The ITI was randomly varied between 1600 and 1900 ms (stimulus offset to onset) for passive blocks, to be comparable to active blocks. Each session began with an active block and alternated with passive blocks for a total of 20 blocks (10 active and 10 passive) with 54 trials in each (Figure 2).

It should be noted that active trials on this task not only required attention to the stimuli but also involved other cognitive functions such as processing of target events, behavioral response selection, and perhaps also anticipation of correctness feedback. Short latency responses such as the ASSR and N1 are likely to be dominated by attention since this process was necessarily deployed commencing at trial onset with other functions following after target detection. Consistent with this expectation, Gander et al. [7] found that attention modulated ASSR amplitude in a dual auditory-visual task when all other task requirements (processing of feedback events, response selection, and correctness feedback) were held constant. We refer herein to the active/passive manipulation as one affecting attention but acknowledge that long-latency brain responses in particular may reflect overlapping cognitive functions.

Immediately prior to the session, each subject completed a staircase procedure in order to determine a set of target amplitudes suitable for the detection task. 80 stimuli were presented each containing a target, commencing with a 200% amplitude increase known to be detectable by inexperienced subjects. Target amplitude decreased after each "yes" response and increased after each "no"; target amplitude at the end of 80 trials was taken as the amplitude corresponding to the subject's threshold of detection (TH). A set of six target stimuli was then generated for each subject consisting of TH, TH ± 5%, TH ± 10%, and TH + 20% for use on the detection task. TH varied between subjects and averaged 47% over all subjects.

2.4. Electrophysiological Recording. The EEG was recorded from a 128-channel Biosemi ActiveTwo amplifier (Cortech Solutions, Wilmington, NC) and sampled at 2048 Hz. Before recording, the electrode array positions were digitized for each participant (Polhemus Fastrak). EEG data were stored as continuous data files referenced to the vertex electrode.

2.5. Signal Processing. Eyeblink and other movement artifacts were removed from raw continuous data by the spatial filtering option of BESA (version 5.1.8; MEGIS Software GmbH, Grafelfing, Germany). Responses were epoched around 100 ms pre- and poststimulus baselines.

40 Hz Steady-State Response. EEG responses for ~85% of trials (rejecting trials with artifacts exceeding 100 μV between 30 and 50 Hz) were used for analysis of the ASSR. Data were converted to the average reference and filtered 40 to 42 Hz (zero phase). For each of the 128 channels, data between 244 and 952 ms poststimulus were collapsed to a two-AM cycle average waveform for each subject (see Figure 3). Because the ASSR is reflected in most electrodes, ASSR amplitude was calculated as the total field power (TFP) determined by Fourier transform summed over 128 electrodes, following Gander et al. [20] and Roberts et al. [6].

Transient Responses. EEG responses for ~80% of trials (rejecting trials with artifacts exceeding 150 μV between 1 and 20 Hz) were used for analysis of transient responses. Epoched data were averaged and interpolated to the 81-channel "reference free" average reference montage of BESA using each participant's digitized electrode array positions, which reduced individual differences in electrode cap placement between subjects. Data were then filtered from 0.2 to 20 Hz (zero phase). The latencies of P1 (from time window 30–85 ms), N1 (85–140 ms), P2 (140–230 ms), and N2 (250–350 ms) transient responses were identified from electrode Fz where the responses typically reached their amplitude maximum [7]. TFP for each response was calculated as the sum of each channel's squared voltage at the peak latency of electrode Fz (Figure 3). The auditory sustained response (SR) was calculated as the TFP over the time interval 400–900 ms (Figure 3). Two subjects (both in the 500 Hz tinnitus

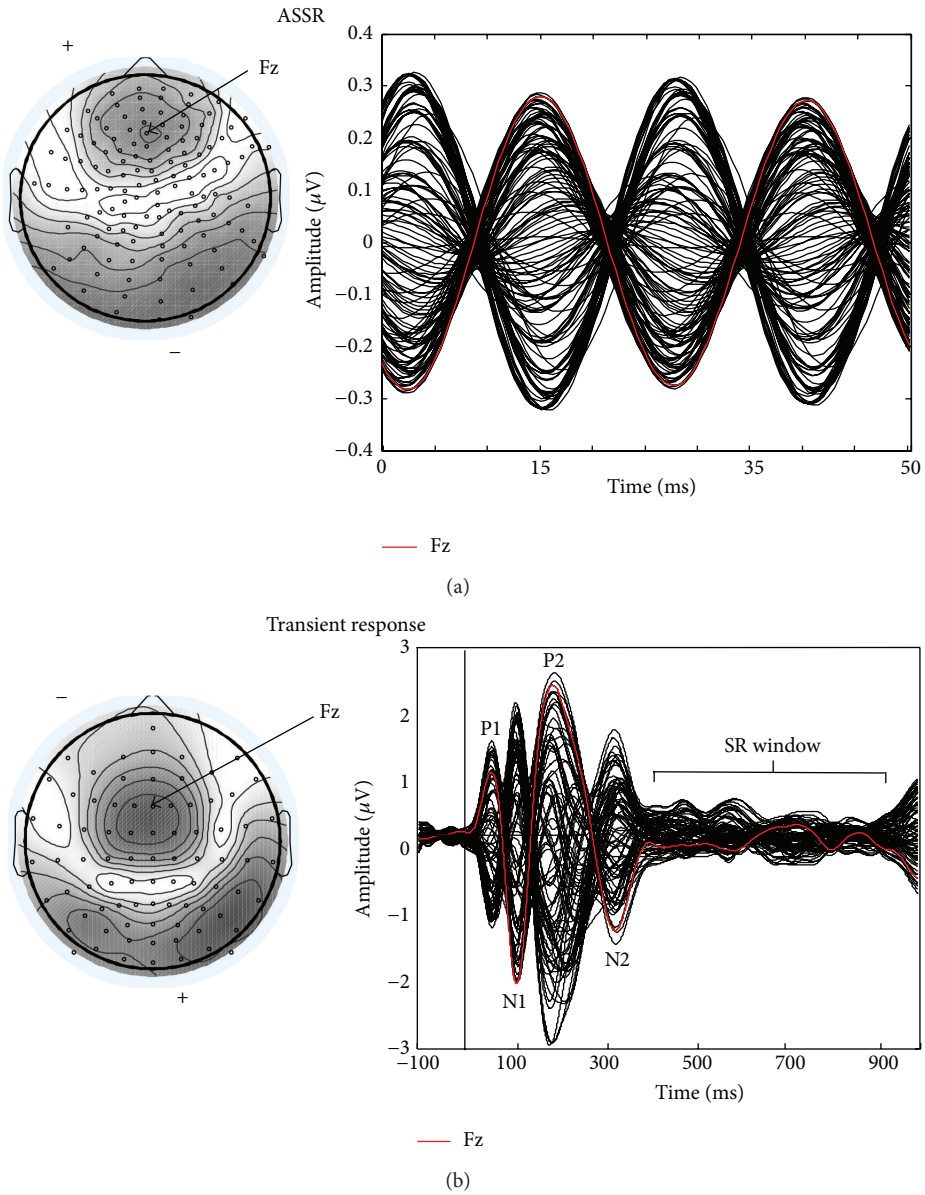

Figure 3: Representative topographies and time domain waveforms for the 40 Hz auditory steady-state response (ASSR) and N1 response derived from the grand average of active trials from control subjects probed with a 500 Hz stimulus. (a) shows the ASSR during the interval 244–952 ms poststimulus, collapsed down to two 40 Hz AM cycles. An alternating dipolar waveform is observed (one for each AM cycle). ASSR amplitude was calculated as the total field power of all electrodes in the two-cycle AM waveform. (b) A dipolar N1 is seen peaking at 100 ms poststimulus. N1 amplitude was calculated as total field power at the peak of the dipolar waveform. The transient responses P1, P2, and N2 and the time range for the auditory sustained response (SR) are also labeled in the waveform. For the purpose of visualization, the trace in the right panel is high pass filtered at 2 Hz to distinguish N2 from the SR which is attenuated as shown here. In each panel the Fz electrode is shown in red.

group) were omitted from the analysis of the SR because of the electrode drift exceeding −50 μV past 400 ms.

2.6. Statistical Evaluation. Repeated measures ANOVAs were performed using the General Linear Model of Statistica (version 6.0). Least significant difference (LSD) tests were used to describe significant main effects and interactions. Group comparisons not addressed by ANOVA were evaluated by t-tests. Significance level was set at $\alpha = 0.05$. Further details regarding statistical approach are reported where appropriate in Section 3.

3. Results

3.1. Behavioral Responses. Performance on the behavioral task is presented in Figure 4. The probability of a hit (P(H)) exceeded the probability of a false alarm (P(FA)) for all subjects with no differences between the tinnitus and control

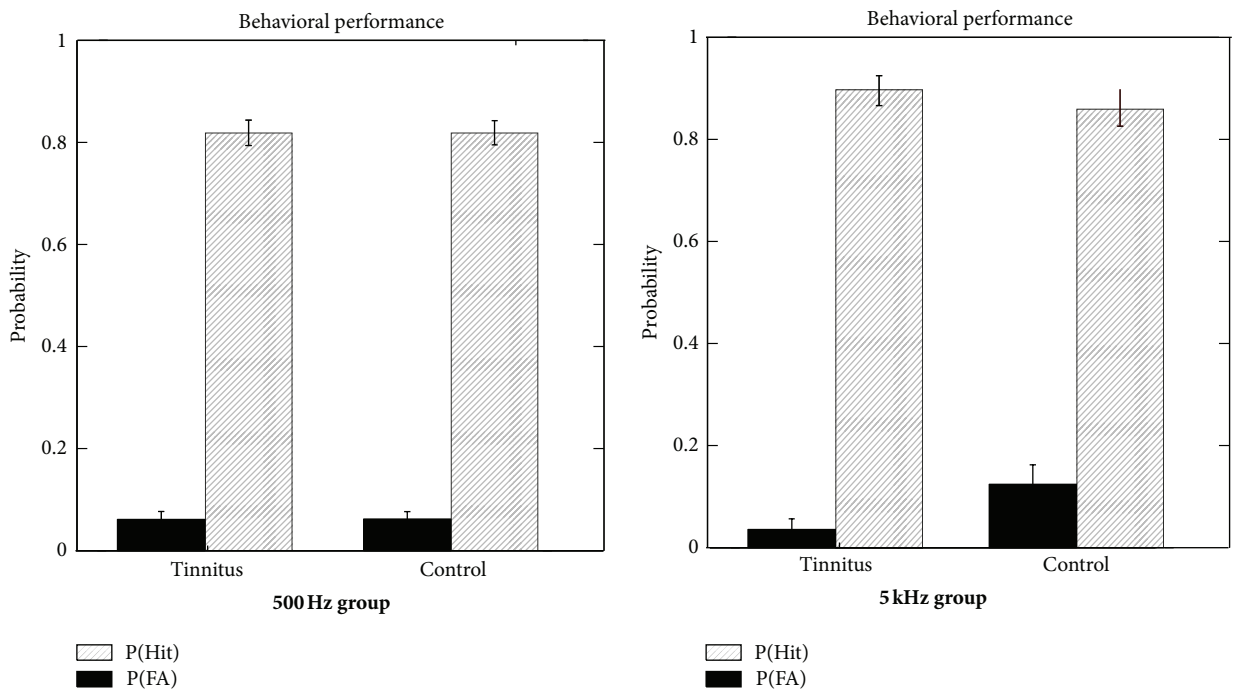

Figure 4: Performance on the behavioral task for both tinnitus and control groups probed at 500 and 5 kHz. The probability of a hit (P(Hit)) is averaged across the six target amplitude enhancements. The probability of a false alarm (P(FA)) was determined from trials with no target.

groups or the two carrier frequencies on this measure. P(H) averaged 0.85 overall indicating that for most subjects at least one of the six target stimuli was not detectable.

3.2. Electrophysiological Responses

3.2.1. Effects of Carrier Frequency and Group on Passive Blocks. In the first analysis, ANOVAs including the variables group (tinnitus/control) and frequency (500 Hz and 5 kHz) were applied to passive blocks for each brain response to identify effects of these variables on brain activity in the absence of attended performance. ANOVA returned main effects for carrier frequency (500 Hz versus 5 kHz groups) on these blocks for the ASSR ($F(1, 51) = 10.38$, $P = 0.002$), P1 ($F(1, 51) = 11.87$, $P = 0.001$), N1 ($F(1, 51) = 10.17$, $P = 0.002$), P2 ($F(1, 51) = 12.93$, $P = 0.001$), and the SR ($F(1, 49) = 5.31$, $P = 0.025$), with similar results for N2 ($F(1.51) = 2.40$, $P = 0.127$). For each response TFP was larger in the 500 Hz groups than in the 5 kHz groups in accordance with the known dependence of the amplitude of the ASSR and transient responses on carrier frequency [21]. No main effects involving group reached significance for any response on passive blocks, although P2 tended to be larger in control subjects than in the tinnitus groups ($P = 0.078$) on these blocks. Interactions between carrier frequency and group did not reach significance for any response on passive blocks.

3.2.2. Effects of Attention (Active versus Passive Blocks). Effects of attention were evaluated first by comparing response TFP on active blocks where attention to the probe stimuli was required with that on passive blocks where subjects were instructed to ignore the stimuli and rest. No main effects or interactions involving active/passive were found for P1 and P2 responses, and these responses are not discussed further. However, the main effects of attention were found for the ASSR ($F(1, 51) = 10.38$, $P = 0.002$), N1 ($F(1, 51) = 7.51$, $P = 0.008$), N2 ($F(1, 51) = 29.12$, $P < 0.001$), and the SR ($F(1, 49) = 28.71$, $P < 0.001$).

Effects of attention on these responses were examined in more detail, as follows. For each subject and response, the effect of attention was calculated (1) as the difference in TFP between active and passive blocks (passive subtracted from active) and by (2) representing the attention effect as TFP on active trials divided by TFP on passive trials (this ratio minus 1, to represent no effect of attention as zero). Distributions of these measures ($n = 55$ subjects) were then examined for kurtosis, which can be pronounced for the ASSR where large but repeatable individual differences are known to occur (test-retest reliability $r > 0.90$, [2]), likely reflecting summation of ASSR fields across two tonotopic maps sharing a common low frequency border in Heschl's gyrus. For ASSR amplitude kurtosis was lower for the ratio measure (2.94) than for the difference measure (19.4), whereas the reverse was true for N1 (5.95/2.37), N2 (13.4/10.37), and the SR (9.50/2.64). Thus for the additional analyses reported below, effects of attention were analyzed as the ratio measure for the ASSR and as the difference in TFP between active and passive blocks for N1, N2, and the SR. Effects were evaluated statistically by t-tests and by ANOVA applied to

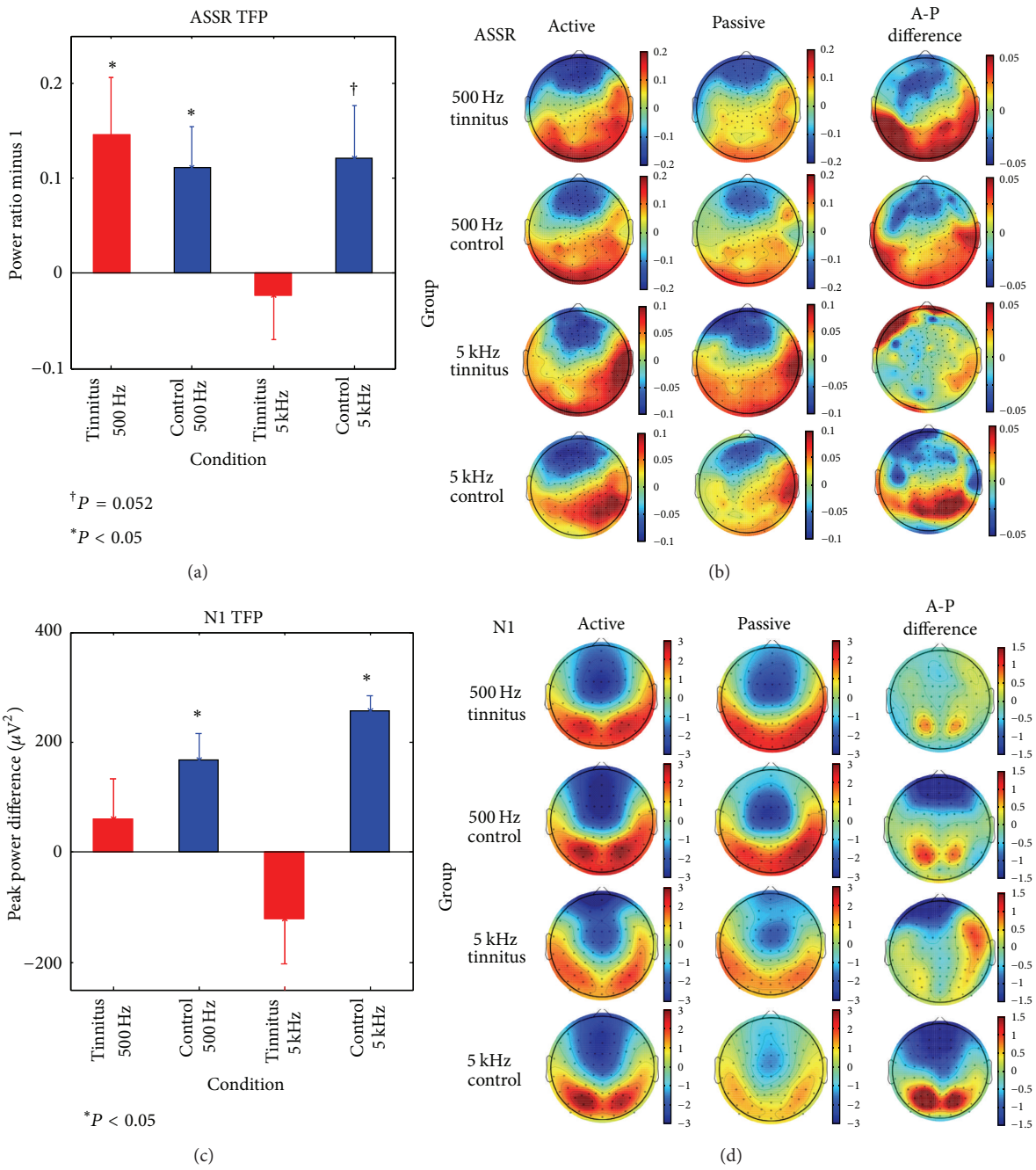

FIGURE 5: ASSR and N1 attention effects. (a) Effect of attention on ASSR TFP in each group (A/P TFP-1). (b) Voltage map of the ASSR taken at the time point of maximum total field power on active and passive blocks and the voltage difference map (active-passive blocks). (c) Effect of attention on N1 TFP in each group (active-passive blocks). (d) Active, passive, and difference voltage maps for N1 at the peak latency of electrode Fz. The error bars in (a) and (c) are one standard error ($^{*}P < 0.05$; $^{\dagger}P = 0.052$).

these measures. In addition, the topography of TFP on active and passive blocks and the difference in TFP (active-minus passive) are shown for all responses.

ASSR. Effects of attention on the ASSR are shown in each group as TFP ratios in Figure 5(a) and as voltage difference maps in Figure 5(b). TFP ratios increased on active compared to passive blocks in the Cont500 Hz group ($t(15) = 2.53$, $P = 0.023$), Cont5 kHz group ($t(10) = 2.199$, $P = 0.052$), and in the Tinn500 Hz group ($t(16) = 2.42$, $P = 0.028$), but the TFP ratio did not increase on active blocks in the Tinn5k group ($t(10) = -0.49$, $P = 0.628$). This pattern can also be seen in the voltage difference maps presented for the four groups in Figure 5(b) (right column) where the voltage difference was minimal in the Tinn5 kHz condition. When the four groups were collapsed into one, the TFP ratio

differed significantly from zero ($t(54) = 3.54$, $P = 0.001$) confirming the sensitivity of ASSR amplitude to attention. An ANOVA applied subsequently to TFP ratios with group and frequency as between-subjects variables found no significant effects, although the interaction of group and frequency approached significance ($F(1, 51) = 2.69$, $P = 0.106$) reflecting the pattern seen in Figure 5(a). LSD tests within this interaction found the 5 kHz and 500 Hz tinnitus groups to be different from one another ($P = 0.032$) whereas contrasts of the Cont500 Hz group and the Cont5khz group to the Tinn5 kHz group reached $P = 0.09$ in each case. Effects of attention on ASSR amplitude were unrelated to ASSR amplitude on passive blocks when correlations were calculated between the two responses for the total sample ($r = 0.09$, $P > 0.53$) or for the tinnitus and control groups separately collapsing over probe frequency (rs $= 0.24$ and 0.09, resp., Ps ≥ 0.21).

N1. Effects of attention on the N1 are shown for each group in Figure 5(c) (TFP difference between active and passive blocks) and in Figure 5(d) (voltage difference maps, right column). TFP increased on active compared to passive blocks in Cont500 Hz group ($t(15) = 3.35$, $P = 0.043$) and in the Cont5 kHz group ($t(10) = 9.48$, $P < 0.001$), but this difference did not reach significance in tinnitus groups probed at either frequency (Ps > 0.17) notwithstanding a weak posterior modulation which can be seen in the voltage difference map for the Tinn500 Hz group. ANOVA applied to the difference in TFP between active and passive blocks returned the main effects of group ($F(1, 51) = 13.37$, $P = 0.001$) and a significant interaction between group and frequency ($F(1, 51) = 4.12$, $P = 0.048$). LSD tests within the interaction found that the N1 TFP difference was larger in the Cont5 kHz group than in either tinnitus condition ($P < 0.04$ or better) and also larger in the Cont500 Hz control group than in the Tinn5 kHz group ($P < 0.004$). Correlations between N1 TFP on passive blocks and the effect of attention on N1 TFP did not reach significance when the four groups were collapsed into a single sample ($r = -0.23$, $P = 0.09$) or when correlations were calculated for the tinnitus and control subjects separately collapsing over probe frequency (rs $= -0.26$ and -0.13, resp., Ps ≥ 0.19).

N2. Effects of attention on N2 are shown for each group in Figure 6(a) (TFP difference measure) and as voltage difference maps in Figure 6(b) (right column). TFP increased on active compared to passive blocks in Cont500 Hz ($t(15) = 4.42$, $P < 0.001$), Cont5 kHz ($t(10) = 4.47$, $P = 0.001$), and Tinn500 Hz ($t(16) = 2.21$, $P = 0.042$) groups, while the difference in Tinn5 kHz approached significance ($t(10) = 1.94$, $P = 0.081$). Comparison of the groups by ANOVA found no significant main effects or interactions involving group or frequency, although the TFP difference between active and passive blocks tended to be larger in the control groups than in the tinnitus groups at both probe frequencies (main effect of group $P = 0.105$). The voltage maps of Figure 6(b) show further that N2 reached its maximum negativity at central electrodes, as did the TFP difference between active and passive blocks. This contrasts with the ASSR and N1 where amplitude maxima were focused frontocentrally on active trials (see Figures 5(b) and 5(d), resp.), particularly for the ASSR whose sources are localized tonotopically in the region of Heschl's gyrus.

Sustained Response. SR TFP increased on active compared to passive trials in all groups (Figure 6(c)). The results for each group were Tinn500 Hz ($t(14) = 2.27$, $P = 0.039$), Cont500 Hz ($t(15) = 2.78$, $P = 0.0139$), Tinn5 kHz ($t(10) = 3.07$, $P = 0.012$), and Cont5 kHz ($t(10) = 5.46$, $P < 0.001$). While active-passive differences in SR TFP tended to be larger in the control groups than in tinnitus, SR TFP differences for each group subjected to ANOVA revealed no main effects or interactions of group or frequency. On active blocks the SR showed a predominant negativity at central electrodes (Figure 6(d)) where the effect of attention was also predominantly expressed.

3.3. Demographics. The mean age of the subjects, their hearing thresholds at four sound frequencies, the intensity of the probe stimuli they received, and, where applicable, properties of their tinnitus are summarized for each group in Table 1. Correlations between several of these variables and (1) ASSR and N1 responses measured on passive blocks in the absence of attended performance and (2) effects of attention on ASSR and N1 TFP are reported in Table 2.

3.3.1. Age. Subjects in the 500 Hz groups of Table 1 were on average 60.0 years old and those in the 5 kHz groups were 51.3 years old, a difference that was significant ($F(1, 51) = 8.33$, $P = 0.005$). However, age range was similar among the four groups, and the tinnitus and control groups within each frequency were matched with no significant differences found in age between them. Age did not correlate significantly with ASSR and N1 responses measured on passive blocks or with effects of attention expressed in these responses when the tinnitus and control groups were collapsed at each frequency (Table 2).

3.3.2. Hearing Thresholds. The audiograms for each group and ear measured to 16 kHz are reported in Figure 1(a). All groups exhibited thresholds exceeding 25 dB HL above 3 kHz while for the Tinn500 Hz group this criterion was met at 2 kHz. Threshold shifts were similar in both ears, with the only difference being thresholds about 7 dB greater in the right ear than in the left ear in the Tinn5 kHz group at the audiometric frequencies of 500 Hz and 1 kHz. To compare audiometric thresholds across all groups, 5 kHz thresholds were interpolated from 4 and 6 kHz thresholds, collapsed over left and right ears, and submitted to repeated-measures ANOVA with 500 Hz thresholds (see inset, Figure 1(a)). ANOVA returned the main effect of audiometric threshold frequency confirming higher thresholds at 5 kHz than 500 Hz in each subject group ($F(1, 51) = 66.23$, $P < 0.001$). The main effect of group (tinnitus versus control) on 500 Hz and 5 kHz audiometric thresholds was not significant. Audiometric thresholds at 500 Hz and 5 kHz did not correlate with ASSR or N1 amplitude measured on passive blocks or with effects

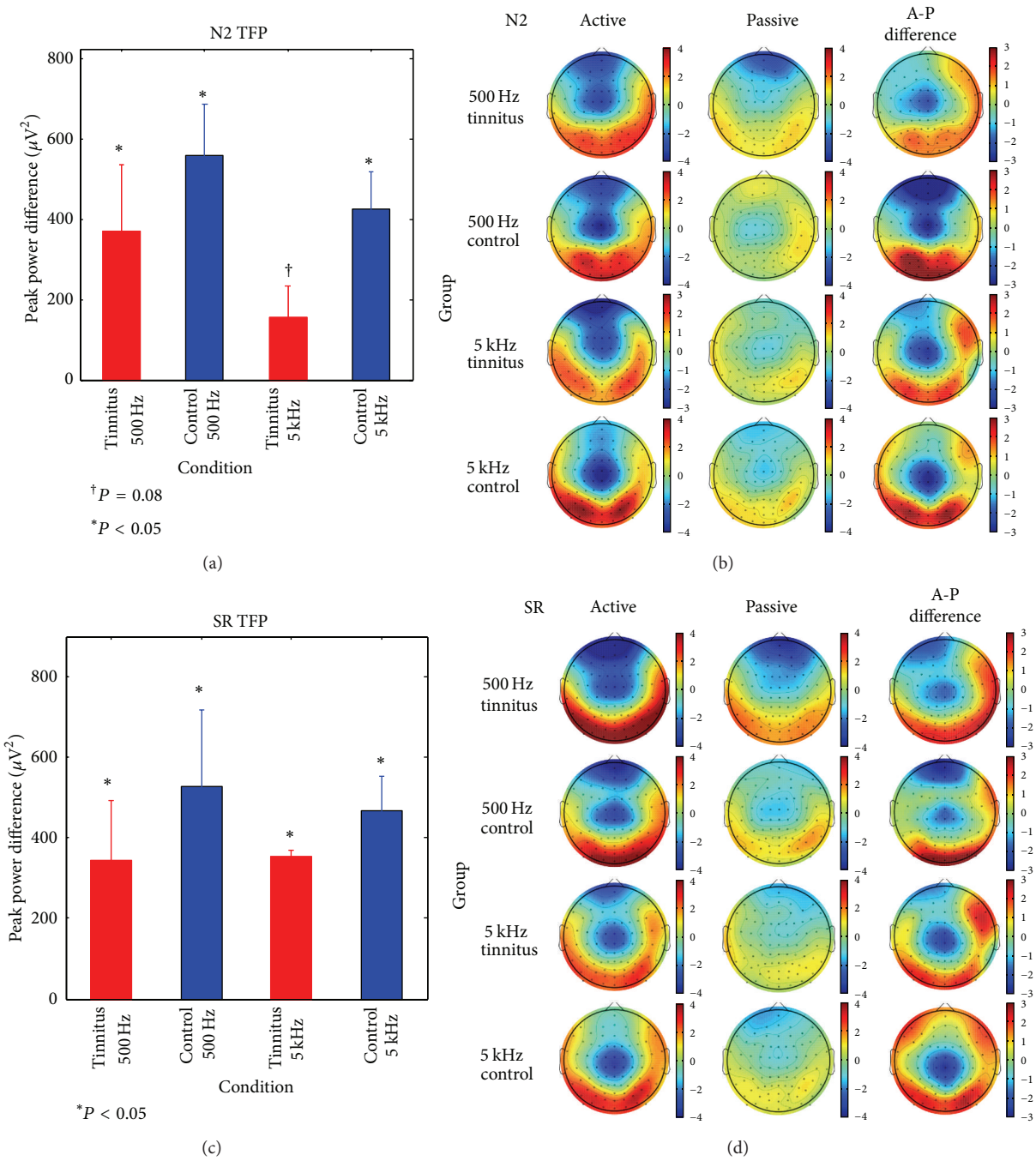

Figure 6: N2 and auditory SR scalp topography and attention effects. (a) Effect of attention on N2 TFP in each subject group (active-passive blocks). (b) Active, passive, and difference voltage maps for N2 at the peak latency of electrode Fz. (c) Effect of attention on SR TFP in each subject group (active-passive blocks). (d) Active, passive, and difference voltage maps for SR averaged from 400 to 900 ms. The error bars in (a) and (c) are one standard error ($^{*}P < 0.05$; $^{\dagger}P = 0.08$).

of attention in these responses when the tinnitus and control groups were collapsed at each frequency (Table 2).

3.3.3. Probe Intensity. Probe intensity ranged from 47 to 93 dB SPL ($M = 79.9$) in the 500 Hz probe groups and from 40 to 74 dB SPL ($M = 59.3$) in the 5 kHz groups. Differences in probe SPL between the tinnitus and control groups tested at each carrier frequency averaged 2.5 dB or less (Ps > 0.51), indicating that sound level matching between the groups was achieved within the 500 Hz and 5 kHz conditions. However, probe intensity collapsed over the tinnitus and control groups differed between the 500 Hz (80.0 dB SPL) and 5 kHz (59.2 dB SPL) conditions ($F(1, 51) = 73.05$, $P < 0.001$). This difference was a function of several factors including a 15.7 dB HL threshold shift at 1 kHz in the 500 Hz groups (who

Table 2: Relationship of ASSR and N1 responses on passive blocks and ASSR and N1 attention effects to subject and tinnitus variables. The table entries are product-moment correlations reported for the 500 Hz and 5 kHz conditions separately.

	Subject variables*				Tinnitus variables			
	Age	500 Hz threshold†	5 kHz threshold†	Probe SPL	Loudness match (1 kHz)	Borg CR100	THQ	Years with tinnitus
				500 Hz condition				
ASSR TFP passive	0.16	0.30	0.06	0.55‡	0.44	−0.03	0.44	−0.24
N1 TFP passive	0.10	0.24	−0.14	0.07	−0.25	−0.52‡	0.04	0.14
ASSR TFP ratio	−0.30	0.27	0.12	0.32	0.14	0.14	0.14	0.13
N1 TFP diff.	−0.28	0.06	0.05	0.02	−0.08	−0.26	0.10	0.13
				5 kHz condition				
ASSR TFP passive	0.25	0.11	−0.19	−0.01	0.24	0.19	−0.40	0.05
N1 TFP passive	0.18	0.32	0.14	−0.04	0.52	0.19	−0.62‡	0.57
ASSR TFP ratio	−0.13	0.02	−0.20	−0.04	0.43	0.23	−0.41	0.27
N1 TFP diff.	−0.07	−0.10	−0.18	−0.06	−0.46	−0.11	0.46	−0.43

*Tinnitus and control subjects combined.
†Left and right ears combined.
‡$P < 0.05$.

would have experienced their 500 Hz probes at about 65 dB SL when matching to a 1 kHz 65 dB SL standard), a tendency for subjects to find 5 kHz 40 Hz AM sounds perceptually more salient than 500 Hz 40 Hz AM sounds, the presence of threshold shifts at 5 kHz in groups tested at this frequency, and some degree of unreported hyperacusis for a 5 kHz sound in the 5 kHz groups (which would have reduced probe SPL when matching a 65 dB SPL 2 kHz standard).

To assess whether probe intensity affected the brain responses, probe SPL was correlated with ASSR and N1 amplitude on passive blocks in the absence of attended performance and with effects of attention observed for these two responses. A correlation between probe level and ASSR amplitude was found on passive blocks in the 500 Hz group ($r(31) = 0.55$, $P = 0.001$; Table 2), indicating that louder 500 Hz probe stimuli evoked large ASSR responses in this group on passive trials. Probe intensity did not correlate significantly with ASSR responses evoked by 5 kHz probes or with N1 evoked by probes of either frequency on passive blocks. We also correlated probe intensity with effects of attention on ASSR and N1 amplitude collapsing the tinnitus and control groups within the 500 Hz and 5 kHz conditions. There was a weak tendency for stronger probe stimuli to be associated with larger effects of attention on ASSR amplitude in the 500 Hz groups ($r = 0.32$, $P < 0.07$), but no correlations between probe intensity and ASSR and N1 attention effects reached significance in the 500 Hz and 5 kHz conditions (see Table 2).

3.3.4. Tinnitus Characteristics. The tinnitus likeness matches obtained in the Tinn500 Hz and Tinn5 kHz groups are shown in Figure 1(b) where a likeness rating of 40 indicates a sound that is beginning to resemble tinnitus [12]. In each group the likeness matches given for 500 Hz sounds were well below the tinnitus spectrum and those for 5 kHz sounds well within it (effect of sound frequency $F(1, 26) = 58.74$, $P < 0.001$) with no difference observed between the likeness matches of the groups at either frequency. Tinnitus loudness was assessed by a Borg CR100 scale (range zero to 100) and by loudness matches obtained using a 1 kHz tone (after Roberts et al. 2008) and tinnitus handicap by the THQ (total score range zero to 100). Loudness matches given by Tinn5 kHz group were higher at 1 kHz (mean = 53.9 dB SPL, see Table 1) than those of Tinn500 Hz group (M = 36.7 dB SPL, $t(26) = 2.61$, $P = 0.014$), although when all matching frequencies were considered the groups did not differ from one another ($F(1, 26) = 1.13$, $P > 0.71$, Figure 1(c)). Loudness ratings on the BorgCR100 scale were nonsignificantly higher in the Tinn5 kHz group ($P = 0.16$) while THQ scores were significantly worse in this group compared to the Tinn500 Hz group ($t(26) = 2.14$, $P = 0.042$). To assess whether these results suggesting a stronger tinnitus in the Tinn5 kHz group may have influenced the attention effects, pairwise correlations were calculated between tinnitus loudness matches at 1 kHz, BorgCR100 ratings, and the THQ, on one hand, and ASSR and N1 attention effects, on the other hand. The resulting correlations were directionally inconsistent and did not reach significance either in the Tinn500HZ and Tinn5 kHz groups considered separately (see Table 2) or when the two groups were combined into one sample. When passive trials only were considered, N1 TFP correlated negatively with the BorgCR100 loudness in the Tinn500 Hz group and with the THQ score in the Tinn5 kHz group reflecting lower TFP for a more disturbing tinnitus (Table 2). When the tinnitus groups were collapsed together, correlations involving tinnitus loudness measures and brain responses on passive trials were near zero and not significant. The duration of tinnitus was similar in the Tinn500 Hz and Tinn5 kHz groups (M = 12.5 and 11.7 years, resp., Table 1) and did not correlate significantly with the two brain responses in either group (Table 2) or when the two groups were combined.

4. Discussion

We previously reported that the amplitude of the ASSR (localizing to cortical sources in A1) and the N1 transient

response (localizing to cortical sources in A2) was not modulated by top-down attention in tinnitus sufferers when the probe frequency was 5 kHz, a frequency known to be in the region in which tinnitus sufferers experience their tinnitus [6]. Conversely, age and hearing-threshold matched controls successfully modulated the amplitude of both responses [6] in accordance with prior evidence showing the responses to be sensitive to attention in normal hearing subjects [7, 8, 20]. It was suggested that tinnitus-related neural activity in central auditory pathways may have prevented modulation of the two responses by attention in the tinnitus sufferers. In the current experiment we tested this possibility by determining whether attention modulates these brain responses normally when evoked by a 500 Hz sound in tinnitus sufferers, which is a sound well below the TFR where tinnitus-related neural activity is believed to occur. The procedure used to assess modulation by attention was the same for the two groups, and the 500 Hz and 5 kHz datasets were combined into a single analysis which also included the long-latency auditory evoked potentials N2 and SR. We found that top-down attention modulated ASSR amplitude normally in tinnitus and control subjects probed with 500 Hz sounds and for control subjects probed with a 5 kHz sound, but not for tinnitus subjects probed with a 5 kHz sound. N1 amplitude was modulated by attention for control groups tested at each probe frequency, but modulation of N1 amplitude by attention failed for tinnitus groups tested at both frequencies. The amplitude of N2 and SR responses was modulated by attention in all groups. We discuss how attention may work in tinnitus sufferers compared to normal hearing individuals and consider how differences between these groups may be expressed in ASSR and N1 amplitude in the absence of attended performance.

4.1. Auditory Attention in Normal Hearing and in Tinnitus. Several lines of evidence have suggested that mechanisms that support auditory attention are persistently aroused in tinnitus [2]. One approach has been to compare the performance of subjects with chronic tinnitus with that of control subjects matched for age and verbal intelligence on cognitive tasks that require divided attention and access to memory. The rationale has been that obligatory attention to the tinnitus percept may deplete the cognitive resources needed to perform such tasks. Following this approach it has been shown that, while subjects with tinnitus perform as well as controls on tasks such as simple word naming, they do not perform as well on more complex tasks requiring retention of words in working memory over a series of sentences [22] or on Stroop tasks that divide attention between word naming and color naming [23]. The performance deficits observed in the tinnitus groups in these studies remained intact when measures of anxiety, depression, and hearing level were regressed out by covariate analyses. A more direct approach was followed by Cuny et al. [24]. In an initial demonstration based on research by Schröger [25], Cuny et al. presented normal hearing subjects with S1 stimuli in one ear that were to be ignored while they categorized S2 stimuli presented to the other (attended) ear. Performance on the S2 task was disrupted by infrequent deviant S1 stimuli, which appeared to draw attention away from the S2 task presented to the other ear. Cuny et al. subsequently found that when this task was presented to subjects with unilateral tinnitus, the interfering effect of deviant S1 stimuli was diminished when the S2 task was presented to the tinnitus ear compared to the reverse arrangement. It was suggested that persistent top-down auditory attention was directed to the tinnitus ear, such that deviant S1 stimuli presented to the nontinnitus ear could not draw attention away from it [24]. These results are in agreement with functional imaging studies of tinnitus [26, 27] which have reported increased activity in A1 and in auditory association areas that are modulated by attention when normal hearing subjects perform auditory detection tasks [2].

The presence of tinnitus did not impair behavioral performance during auditory discrimination under the conditions of our test, likely because there was no competing task requirement and most of the targets presented on the discrimination task were easy to detect. However, while ASSR and N1 responses known to be attention sensitive were modulated normally by attention in our control groups, modulation of these responses by attention was modified in tinnitus subjects. The pattern of impairment we observed could reflect differences in the functional organization of A1 and A2 and aberrant neural activity occurring in these regions in tinnitus sufferers. Unlike ASSR sources in A1 that show a frequency (tonotopic) organization in the region of Heschl's gyrus, N1 sources localize to lateral aspects of the superior temporal gyrus [18], are weakly or not tonotopic [28], and appear to reflect contributions arising from several cortical areas that comprise A2. A2 regions exhibit a heterogeneous cytoarchitectonic structure [29, 30] in which layer II/III pyramidal neurons receive inputs from diverse regions of the brain and in turn form intrinsic contacts that are more distal than in A1 where links are made in more localized modules [31]. Frequency representations which are prominent in A1 are virtually absent in A2, which appears to be specialized for processing of multidimensional auditory objects and for conveying perceptual information to higher cortical structures [30, 32, 33]. Hence it is possible that neural changes related to tinnitus (such as reduced intracortical inhibition [34], increased spontaneous activity [34, 35], and increased synchronous firing [34]) occurring in tonotopic regions of A1 may have diffusely activated A2, impairing modulation of N1 responses at both probe frequencies in tinnitus subjects. However, because A1 regions coding 500 Hz sounds are below the frequency region of A1 where tinnitus-related activity is presumed to occur, attentional modulation of the ASSR was expressed normally when tinnitus subjects were probed with this sound frequency. This interpretation is consistent with evidence from animal [1, 36] and human [37] studies which suggests that aberrant neural activity occurring in frequency regions of A1 affected by hearing impairment contributes to tinnitus percepts. It can also be aligned with previous results [38] showing that the mismatch negativity (a brain response initiated in A1 by bottom-up auditory attention, [39]) was increased in individuals with tinnitus when evoked by unexpected frequency deviants adjacent to

the audiometric edge but not one octave below it. Overall it appears that persistent tinnitus-related activity occurring in the frequency region of A1 affected by hearing loss may impair modulation of the ASSR by top-down attention in this frequency region in tinnitus, but bottom-up disparities may still evoke larger responses near the lesion edge where cortical reorganization may be present [36].

Notwithstanding prior evidence for persistent auditory attention in tinnitus [24], this interpretation suggests that mechanisms of top-down auditory attention functioned normally in tinnitus sufferers under the conditions of our test, but their expression was modified by the presence of tinnitus-related neural activity occurring in central auditory pathways. Other findings of the study can be aligned with this interpretation. Subjects in the Tinn5 kHz and Cont5 kHz groups received an additional six sessions of training on the auditory detection task in the earlier study of Roberts et al. [6]. ASSR amplitude increased over training sessions in the tinnitus subjects but not in their matched controls [6] nor in previous studies using subjects with normal hearing [11, 20], possibly reflecting reduced lateral inhibition in the tinnitus subjects [1]. As training progressed, ASSR amplitude began to modulate on active blocks compared to the passive baseline in tinnitus subjects revealing an effect of attention on this response, although this modulation subsequently declined and was weak compared to that seen in controls (N1 did not modulate with attention during any session of training in the tinnitus subjects). New analyses reported in the present paper have gone further to show that the long-latency responses N2 and SR (which reach their negative maxima at central electrodes) were modulated between active and passive trials in our tinnitus groups as well as by control subjects. It is possible that these responses reflect communication between auditory regions and global networks in frontoparietal cortex that are involved in memory processing and response preparation [40]; moreover, the performance deficits cited above in tinnitus [22, 23] may derive in part from competition for resources in these pathways. In this respect we note that, while N2 and SR responses were modulated by attention in our tinnitus subjects, there was a tendency toward stronger effects in the control groups at both probe frequencies.

4.2. Group Differences in the Absence of Attention. Neuromodulatory systems in the basal forebrain and midbrain tegmentum are widely believed to be activated by tasks requiring attention and serve to make neurons more sensitive to their afferent input [2]. On this basis, evidence for persistent auditory attention in tinnitus could be expected to modulate the amplitude of brain responses evoked by auditory stimuli under passive conditions where tinnitus sufferers would experience tinnitus but control subjects would not. In a previous study using 40 Hz AM stimuli similar to those used here but different groups of subjects [2], we found that ASSR amplitude was larger in a tinnitus group than in controls when the carrier frequency of the probe was 500 Hz ($P = 0.004$), but this difference was reversed in groups for whom the carrier frequency was 5 kHz ($P = 0.045$). Reduced ASSR amplitude at 5 kHz was attributed to tinnitus-related synchronous activity occurring in the TFR of the tinnitus subjects (a busy line effect). Additionally, N1 amplitude was larger in the tinnitus groups compared to controls at both probe frequencies ($P = 0.023$). These results were obtained during a continuous 20-minute baseline condition in which individuals in the tinnitus groups would have heard their tinnitus. To compare these findings with the current dataset, we performed paired t-tests contrasting the tinnitus and control groups on passive blocks for the ASSR measured as TFP and N1 amplitude measured at electrode Fz (as in the previous work). ASSR TFP tended to be smaller in tinnitus than control subjects at 5 kHz ($P = 0.26$) and N1 larger ($P = 0.18$) at this frequency in qualitative agreement with previous results, but no group differences in ASSR or N1 amplitude reached significance in the present dataset. Overall, current evidence suggests that ASSR amplitude is larger in tinnitus subjects than in controls, at least for sounds below the TFR [2, 37]. Results regarding N1 are less consistent [2] and may reflect differences among studies with regard to the conditions of testing, stimulus procedure, and other variables that have yet to be identified.

4.3. Limitations and Future Directions. Within each probe frequency, our tinnitus and control groups were well matched for tinnitus characteristics, age, hearing status, and stimulus levels. Group differences in the effects of attention on brain responses at each probe frequency could not be attributed to these variables which did not differ between tinnitus and control subjects. However, while our 5 kHz and 500 Hz groups were well matched for hearing function, age range, and years of tinnitus, subjects in the 500 Hz groups tended on average to be 10 years older and their THQ scores lower than subjects in the 5 kHz groups. The intensity of the probe stimuli also differed between the 500 Hz and 5 kz conditions, in part because of the presence of threshold shifts at 5 kHz in the Tinn5 kHz and Cont5 kHz groups. To assess whether differences in these variables may have influenced our results, we correlated each variable with the effects of attention on ASSR and N1 responses at each probe frequency, collapsing tinnitus and control subjects within each frequency to increase the likelihood of uncovering alternative explanations for the findings. None of the variables correlated significantly with the effects of attention on ASSR and N1 responses, at either probe frequency. Within the limits of this analysis we conclude that differences between tinnitus and control groups in the effect of attention on ASSR and N1 amplitude reflected the presence of tinnitus in the tinnitus subjects and not the other attributes or the conditions of testing. Although interactions among different stimuli could be a limiting factor, looking forward it could be informative to modify our stimulus procedure to allow examining effects of tinnitus on attention-sensitive responses when both probe frequencies are tested within the same subjects.

A further possible limitation to consider is the extent to which a given brain response reflects the operation of an attention mechanism rather than brain processes concerned with other cognitive or behavioral functions. Active trials in our procedure required not only the deployment of attention

but also the processing of target events using memory, the preparation of behavioral responses depending on target occurrence or nonoccurrence, and likely the anticipation of correctness feedback depending on outcome. As we have noted, auditory attention is known to increase ASSR amplitude when these additional factors are held constant [7], confirming the sensitivity of this response specifically to attention. Although the transient N1 response is widely believed to be sensitive to attention, as far as we are aware similar detailed analyses precluding contributions from other task features are surprisingly lacking this response. In the absence of such studies it is reasonable to assume that brain responses with short latencies are likely to reflect attention, assuming that on any attention task this process is deployed at trial onset.

Many individuals with tinnitus also experience some degree of hyperacusis expressed either by verbal reports of sensitivity to environmental sounds [41] or by loudness growth functions that are steeper than those observed in individuals with similar audiometric profiles [42]. Because we did not have a basis in the present study to distinguish between these two conditions, failure of attentional modulation could relate in principle either to the presence of tinnitus or hyperacusis or to both. It is not easy to disentangle these correlated factors in tinnitus research. However, the current findings are not easily explained in terms of altered perceptual responses to the probe stimuli in the tinnitus groups. ASSR and N1 responses might have been expected to reflect such differences under passive conditions, but the differences we observed between tinnitus and control groups were small and did not reach significance. Our practice of requiring subjects to adjust probe sound intensity to comfortable-level standard sounds presented in the frequency range of normal hearing may have attenuated effects attributable to hyperacusis in our tinnitus samples. It is also relevant that effects of attention on ASSR and N1 responses did not correlate with physical sound intensity within tinnitus and control subjects tested at 500 Hz or 5 kHz. Had perceptual responses to the probe stimuli affected attentional modulations, such correlations might have been expected but did not occur.

5. Conclusion

Previous studies have provided behavioral evidence of impaired performance on tasks involving control of attention in individuals with tinnitus compared to individuals without tinnitus. Our study extended the analysis to compare, between age and hearing-level matched tinnitus and control groups, the effect of attention on brain responses known to be sensitive to attention in normal hearing subjects. We focused in particular on the 40 Hz ASSR which localizes to sources in tonotopically organized primary auditory cortex (A1) and the N1 transient response which localizes to sources in nontonotopic secondary auditory cortex (A2). We found that, unlike in controls where all responses were modulated by attention, the presence of tinnitus impaired attentional modulation of the ASSR evoked by a 5 kHz but not a 500 Hz sound and the N1 evoked at both sound frequencies. We suggest that impairments of auditory attention are expressed preferentially in the 5 kHz region of tonotopically organized A1 where tinnitus-related neural activity is typically expected to occur and more diffusely in nontonotopic A2 where neuron response properties are more broadly tuned for spectrotemporal and multisensory integration.

Abbreviations

A1: Primary auditory cortex
A2: Secondary (nonprimary) auditory cortex
AM: Amplitude modulated
ANOVA: Analysis of variance
ASSR: Auditory steady-state response
DCN: Dorsal cochlear nucleus
EEG: Electroencephalogram
ITI: Intertrial interval
SPL: Sound pressure level
SL: Sensation level
SR: Auditory sustained response
TH: Threshold
THQ: Tinnitus Handicap Questionnaire
TFP: Total field power
TFR: Tinnitus frequency region.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Review Article

A Brain Centred View of Psychiatric Comorbidity in Tinnitus: From Otology to Hodology

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Introduction. Comorbid psychiatric disorders are frequent among patients affected by tinnitus. There are mutual clinical influences between tinnitus and psychiatric disorders, as well as neurobiological relations based on partially overlapping hodological and neuroplastic phenomena. The aim of the present paper is to review the evidence of alterations in brain networks underlying tinnitus physiopathology and to discuss them in light of the current knowledge of the neurobiology of psychiatric disorders. *Methods*. Relevant literature was identified through a search on Medline and PubMed; search terms included tinnitus, brain, plasticity, cortex, network, and pathways. *Results*. Tinnitus phenomenon results from systemic-neurootological triggers followed by neuronal remapping within several auditory and nonauditory pathways. Plastic reorganization and white matter alterations within limbic system, arcuate fasciculus, insula, salience network, dorsolateral prefrontal cortex, auditory pathways, ffrontocortical, and thalamocortical networks are discussed. *Discussion*. Several overlapping brain network alterations do exist between tinnitus and psychiatric disorders. Tinnitus, initially related to a clinicoanatomical approach based on a cortical localizationism, could be better explained by an holistic or associationist approach considering psychic functions and tinnitus as emergent properties of partially overlapping large-scale neural networks.

1. Introduction

Comorbid psychiatric disorders are frequent among patients affected by tinnitus [1]. In ancient times, Hippocrates and then Galen remarked the frequent concomitant presentation of tinnitus and depressive symptoms (melancholia), hypothesizing that the effect of black bile (*atra bilis*) on the same organ, the brain, could represent the common etiopathogenetic factor of the two disorders [2]. In the course of history both tinnitus [3] and psychiatric disorders [4] have been considered the expression of pathological alterations of various different organs potentially having mystic or unknown aetiology.

Current medical literature indicates that the association between tinnitus and psychiatric disorders is complex [5]. Those elements underlying the frequent, multiform, and non-deterministic relation between the two classes of disorders will be evidenced in this introduction from epidemiological, clinical, and biological points of view.

Both the classes of disorders are common in the general population, with a prevalence of 15–20% of tinnitus and 27% of psychiatric disorders [6]. From an epidemiological point of view, the prevalence of comorbid psychiatric disorders among tinnitus patients ranges between 14% and 80% [7, 8], with such a large range probably due to the different methodologies of sampling and diagnosis used in the different clinical studies [9]. Two recent studies of our research team found comorbid psychiatric disorders in 48% [10] and 43% [11] of the enrolled tinnitus patients. It is also true, however, that patients suffering from tinnitus-related distress may more

frequently seek clinical help and thereby may have a better chance to get enrolled in clinical studies than patients with well compensated tinnitus; for this reason, the prevalence of high psychiatric comorbidity in tinnitus may be only representative of the subpopulation of clinical help seekers.

Although the majority of studies on the topic are focused on comorbid depression, other psychiatric disorders have also been found to be substantially present in tinnitus patients, such as anxiety, obsessive compulsive, mood, conversion, somatoform [12], sleep [13], psychotic [14], cognitive [15], substance use related [16], language [17], sexual [18], personality [18], and eating disorders [19]. In addition, some authors reported that the rate of suicide among tinnitus patients is 10 times higher than among general population [20].

The temporal relation between tinnitus and psychiatric disorders is not linear: psychiatric comorbidities are not simply reactive to tinnitus distress but they can even precede tinnitus onset [6]. It is still not possible to postulate the presence of a psychopathologically determined vulnerability to tinnitus onset but, on the other hand, preliminary studies of our research team on temperament and character provide evidence of a personological predisposition (scarce coping abilities and neurotic prone attitude) for the development of a disabling and distressful perception of tinnitus (i.e., severe tinnitus) [10]. A recent study of Sand et al. [21] on gene variants of glial cell-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) in tinnitus patients provided interesting links between coping skills and the degree of tinnitus-related distress; BDNF Val66Met gene has been further the object of extensive investigations in sensitivity to stress and adaptation to stress [22] and empirical data support its additional roles in the processing of auditory information [23] and in the tinnitus severity in women [24].

Stressful life events and daily hassles may precede tinnitus onset [25], can contribute to tinnitus physiopathology [26], or may be elicited by decompensated tinnitus [27]. Furthermore, there are mutual clinical influences between psychiatric disorders and tinnitus: tinnitus severity and its impact on quality of life lead to more severe presentations of the concomitant psychopathological disorders, while concomitant psychopathological disorders can strongly worsen the tinnitus-related distress potentially representing the milestones to shift from a compensated to a decompensated tinnitus [28].

The complex circular relationship between psychopathology and tinnitus has strongly stimulated the scientific debate; the major issue underlying the theoretical speculations about this comorbidity is the unobjectifiable nature of the clinical manifestations of the two classes of disorders: both of them are not identifiable through objective diagnostic markers but rather through subjective symptoms resulting from a functional impairment of the same organ, the brain [29].

Disturbances of connectivity and thus of neural dynamics are thought to underlie a number of disease states of the brain, and some evidence suggests that degraded functional performance of brain networks may be the outcome of a process of randomization affecting their nodes and edges [30].

In tinnitus, as well as in psychiatric disorders, neural plasticity, defined as the adaptation of central nervous system (CNS) to altered peripheral input and the compensation of the effects induced by injury or diseases, occurs in all parts of the central nervous system; it represents an allostasis attempt occurring after a deprivation of peripheral input, after an abnormal peripheral input or injury, learning, and adaptation, and even after behavioral training. A large amount of current researches focuses on the concept of maladaptive neural plasticity to explain the physiopathology of tinnitus and psychiatric disturbances [31], identifying the phenomenon leading to multiple pathological conditions that we globally define as "dysfunctional de-contextualizations from sensorial experience fields" (i.e., bodily perceptions, environmental embodiment, and the otherness) [32]. This plastic reorganization causes neuronal or even glial and vascular changes at molecular, cellular, and histological levels [33, 34].

The fundamental processes underlying neural plasticity at molecular levels may be traced to two mechanisms: protein phosphorylation (i.e., a rapid, easily reversible response) and regulation of genes expression (i.e., a more structured process) [33]. Brain reorganization may emerge quickly or slowly and may be permanent or labile, reflecting a shift in the influence of excitatory or inhibitory events in the brain [35]. The changes may involve the synaptic communication between neurons but also the cellular membrane properties [35].

According to the deafferentation-based pathogenetic model of tinnitus, it is possible to individuate two different stages or levels of neuronal plastic reorganizations and network reconfigurations in tinnitus. During the initial response to peripheral input deprivation, neural plasticity induces an allostatic response in the auditory cortex, consisting in a reduced GABAergic inhibition of dormant, glutamate excitatory synapses, and creates new excitatory connections through axonal sprouting and lateral spread of neural activity, resulting in enlarged regions of neural activity [34]. These reorganization processes and new axonal connections contribute to an excess of tonotopical cells representing a very restricted tonotopical area of the cochlea, perceived as tinnitus [34]. It is assumed that this "lateral spread" of these excitatory response areas creates conditions of hyperexcitability in the brain [34]. In the second stage of plastic reorganization the new auditory cortex neuronal restyling is punctuated and limited in function and extension by brain network gating systems; in case of a lack of gating system or in case of the presence of facilitating factors, the neuronal restyling affects several auditory (lemniscal and extralemniscal) and nonauditory pathways, leading to modifications in the location and crossmodal interplay of specific information processes. In fact, there is nowadays evidence that tinnitus phenomenon results from systemic neurootological triggers (Table 1) followed by neuronal remapping within several auditory (Figures 1 and 2) and nonauditory pathways [6].

According to this "remapping" hypothesis of tinnitus, the reorganization process usually begins with a loss of hair cells in the inner ear, a "sensorineural" hearing loss (SNHL) [37–39]. Notably, tinnitus has been reported to occur more

Table 1: Common systemic neurootological risk factors for developing tinnitus [36].

Otological, infectious	Otitis media, labyrinthitis, mastoiditis
Otological, neoplastic	Vestibular schwannoma, meningioma
Otological, labyrinthine	Sensorineural hearing loss, Ménière's disease, vestibular vertigo
Otological, other	Impacted cerumen, otosclerosis, presbycusis, noise exposure
Neurological	Meningitis, migraine, multiple sclerosis, epilepsy
Traumatic	Head or neck injury, loss of consciousness
Otofacial	Temporomandibular joint disorder
Cardiovascular	Hypertension
Rheumatological	Rheumatoid arthritis
Immune-mediated	Systemic lupus erythematous, systemic sclerosis
Endocrine and metabolic	Diabetes mellitus, hyperinsulinaemia, hypothyroidism, hormonal changes during pregnancy
Ototoxic medications	Analgesics, antibiotics. Antineoplastic drugs, corticosteroids, diuretics, immunosuppressive drugs, nonsteroidal anti-inflammatory drugs, steroidal anti-inflammatory drugs

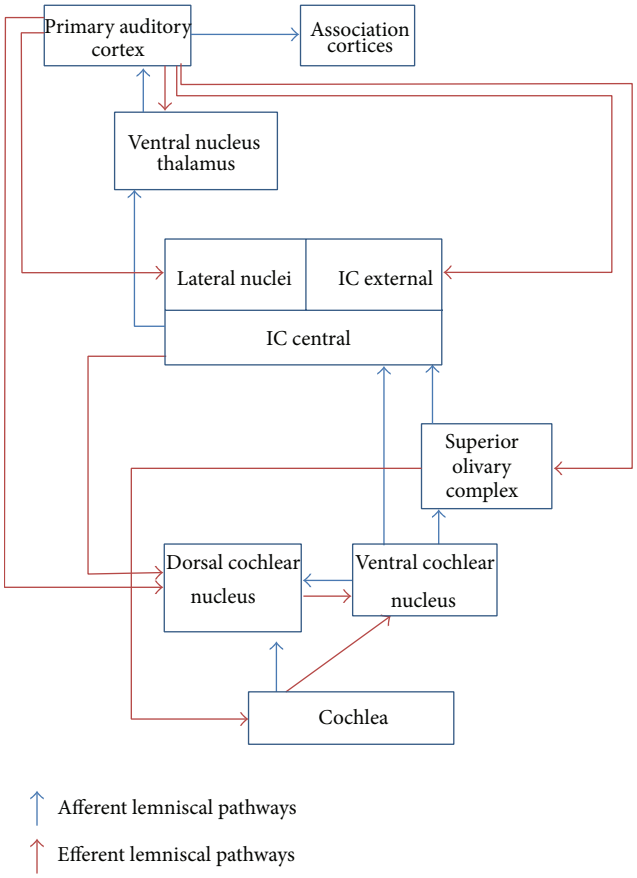

Figure 1: Lemniscal pathways, modified from [34]. Abbreviations: IC = Inferior Colliculus.

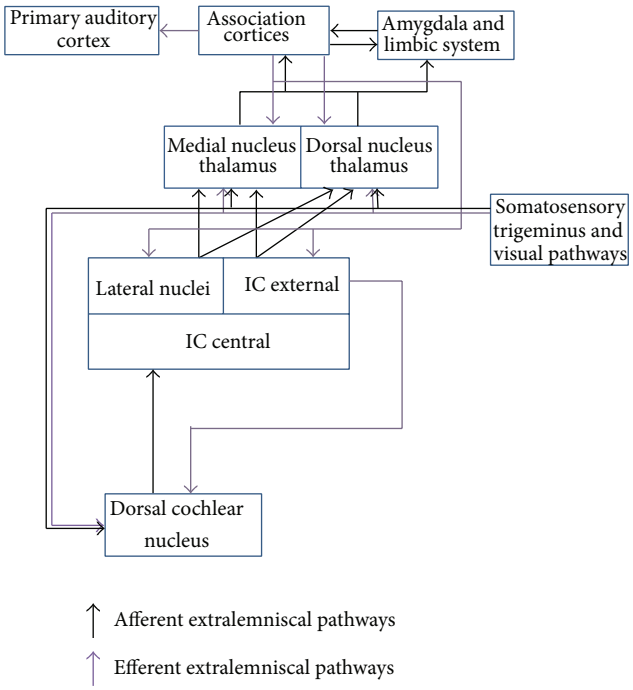

Figure 2: Extralemniscal pathways, modified from [34]. Abbreviations: IC = Inferior Colliculus.

frequently in patients with hearing loss, but it occurs even in individuals with normal hearing [40]. When audiological testing is performed at finer intervals and at frequencies above 8 kHz, cases of tinnitus with absolutely no hearing loss become more rare in our hands and in those of other investigators [41]. It is safe to say, therefore, that the great majority of tinnitus cases do involve SNHL, that is, damage to the sensory periphery. Importantly, the reverse is not true; that is, not everyone with SNHL develops tinnitus.

Data on complexity of global interrelation between different brain areas in tinnitus patients derive from resting-state functional magnetic resonance imaging (rfMRI). rfMRI allows to study functional connectivity in the brain by acquiring fMRI data while subjects lie inactive in the MRI scanner and taking advantage of the fact that functionally related brain regions spontaneously coactivate.

In healthy subjects, the identified auditory resting-state network encompasses bilateral primary and associative auditory cortices, insula, prefrontal, sensorimotor, anterior cingulate, and left occipital cortices. On the other hand, in

tinnitus patients the identified auditory resting-state network has been found to encompass all previously mentioned areas (excluding the anterior cingulate cortex) and also included the brainstem, thalamus, nucleus accumbens (NAc), isthmus of cingulate gyrus, and right occipital, parietal, and prefrontal cortex (PFC). In addition, chronic tinnitus patients as compared to controls showed increased connectivity in the brainstem, cerebellum, right basal ganglia/NAc, parahippocampal areas, right frontal and parietal areas, left sensorimotor areas and left superior temporal region and decreased connectivity in right primary auditory cortex, left fusiform gyrus, and left frontal and bilateral occipital regions [42].

Utilizing fMRI to study psychopathological dimensions, some authors found that diverse forms of psychopathology are characterized by breakdowns (disconnectivity) in interregional relationships between networked brain regions leading to cognitive, affective, motivational, and social dysfunctions [63].

What results clear from the studies of the last decades is that tinnitus and psychiatric disorders cannot be considered only diseases of specific anatomically defined parts of the brain, but rather disorders resulting from complex wide subtle dysfunctions of multiple CNS regions and networks, leading to the idea of diffuse rather than localized disorders potentially sharing common neurobiological substrates [64].

Given the increasing amount of data evidencing epidemiological, clinical, and neurobiological relations and mutual influences between tinnitus and psychiatric disorders, the aim of the present paper is to review the evidence of alterations in brain networks underlying tinnitus physiopathology and to discuss them in light of the current knowledge of the neurobiology of psychiatric disorders.

2. Methods

Relevant literature was identified through a search on Medline and PubMed. Search terms included tinnitus[ti] AND brain AND plasticity OR tinnitus[ti] AND brain AND cortex OR tinnitus[ti] AND brain AND network OR tinnitus[ti] AND brain AND pathways. Through these search terms, 139 papers have been found. Among these, we considered only those studies written in English and conducted on humans (109 papers); reviews, meta-analysis, editorials, and letters were excluded, resulting in a total of 66 papers. Among these, we manually selected only those studies fitting the purpose of the review study and investigating alterations in brain networks through neuroimaging and neurophysiological techniques (Tables 2 and 3). Results have been discussed in the light of the current data available about psychiatric disorders neurobiology.

3. Results

An association of tinnitus with changes in the function and structure of auditory pathways has been demonstrated in many studies; however, tinnitus-related activity changes within CNS are not restricted to the auditory pathways [74] but rather they can be conceived as alterations of a network involving both auditory (lemniscal and extralemniscal) and nonauditory structures [75, 76].

Auditory networks can be divided into three streams that convey information "*into*," "*within*," and "*beyond*" auditory cortex [77].

The primary auditory cortex, in fact, receives projections of the acoustic radiations from both the medial geniculate nuclei and it represents the final step of lemniscal and extralemniscal ways ("into" pathway).

The information then flows within auditory cortex ("within" pathway) and connects to adjacent areas through U-shaped fibres. The local connections of each auditory area are unique, complex, and characterized by the following properties: (1) a single area typically has reciprocal connections with several others; (2) adjacent areas tend to be more densely interconnected than nonadjacent areas; (3) the densest connections link neurons within a single area; and (4) laminar and sublaminar patterns of connections vary systematically.

Ultimately, information flows "beyond" auditory cortex ("beyond" pathway) toward the auditory-related areas. In particular, from the auditory cortex information moves in four principal directions (1) rostral, (2) caudal, (3) medial, and (4) lateral. The rostrally directed stream has auditory-related targets in temporal pole, ventral, rostral, and medial prefrontal areas, rostral cingulate, parahippocampal areas, and the amygdala, while the caudally directed stream flows from the caudal belt and parabelt regions into temporoparietal junction, posterior parietal and occipital regions (such as secondary visual cortex), caudal and dorsal prefrontal areas, dorsal cingulate, and parahippocampal areas; the rostral and caudal areas of auditory cortex project, therefore, to auditory-related targets that are largely segregated, many of which are located in regions of the brain associated with the ventral and dorsal networks of the extrastriate visual system. The other two "streams" (medial and lateral) flow laterally from the belt and parabelt regions to the superior temporal sulcus and medially into the insula and retroinsular areas within the lateral sulcus [77].

The results of the present review are given in Tables 2 and 3; they will be presented into separate sections focusing on afferent ("into"), intracortical ("within"), and efferent ("beyond") structures discussing the specific brain networks underlying tinnitus physiopathology.

3.1. Tinnitus-Related Brain Structures "into" and "within" Auditory Pathways (Table 2). Both anatomical and functional alterations of auditory pathways are nuclear findings related to tinnitus perception; the auditory cortex has been found to be reduced in volume [43, 50] and altered in functionality [48, 51–55, 59, 61, 62, 67, 68] in numerous studies and its hyperactivity plays a critical role in tinnitus.

fMRI data showed symmetrical activation in the primary auditory cortex in patients with bilateral tinnitus and homolateral activation towards the side of perceived tinnitus in patients with lateralized tinnitus [45, 49], supporting the idea that tinnitus may be considered as an auditory phantom phenomenon.

Table 2: Tinnitus-related alterations "into" and "within" auditory pathways.

Methods	Alterations observed	References
MRI	Reduced grey matter volume in bilateral auditory areas including the Heschl's gyrus.	[43]
	Significant grey matter decrease in the right IC.	[44]
fMRI	Abnormal asymmetric IC activation in patients with lateralized tinnitus.	[45]
	The ratio of activation between right and left IC did not differ significantly between tinnitus and non-tinnitus patients or in a manner dependent on tinnitus laterality.	[46]
	Tinnitus-induced hyperactivity in the dorsal cochlear nucleus.	[47]
	Tinnitus-related hyperexcitability of auditory cortex.	[48]
	Significant signal change lateralized towards the side of perceived tinnitus in primary auditory cortex and IC in patients with right sided tinnitus and towards the medial geniculate body in patients with left sided tinnitus.	[49]
	Smaller medial partition of Heschl's gyrus gray matter volume.	[50]
PET	Tinnitus-related elevated blood flow in auditory cortex.	[51]
	Focal metabolic activation in the predominant left auditory cortex.	[52]
	Significantly increased metabolic activity in the left primary auditory cortex; increased metabolic activity in temporal and parietal brain regions (in female tinnitus patients) and in frontal and occipital regions (in male tinnitus patients).	[53]
	Asymmetric activation of the auditory cortex, predominantly on the left side and independently from tinnitus laterality.	[54]
	Activation of left and right posterior inferior temporal gyrus as well as left and right posterior parahippocampal-hippocampal interface; overactivation of left in contrast to right Heschl's gyrus independently from tinnitus laterality.	[55]
MEG	Reduced alpha activity (8–12 Hz) and increased slow wave activity (delta and theta 1–6 Hz) and gamma activity (>30 Hz) in the temporal cortex.	[56]
EEG	Abnormal gamma band activity (>30 Hz) generated as a consequence of hyperpolarization of specific thalamic nuclei.	[57]
	Correlation between electroencephalographic gamma band activity in the contralateral auditory cortex and the presence of tinnitus.	[58]
	Discrete localised unilateral foci of high frequency activity in the gamma range (>40–80 Hz) over the auditory cortex.	[59]
	Reduced wave I (indicating reduced auditory-nerve activity) and elevated waves III and V amplitude (indicating hyperactivity of pathways originating from ventral cochlear nucleus) assessed via auditory brainstem responses.	[60]
	Increased neuronal activity in auditory pathways (long latency auditory evoked potentials).	[61]
	Cortical information processing dysfunction in chronic tinnitus patients associated with auditory stimuli.	[62]

IC: Inferior Colliculus.

Simple phantom sounds like tinnitus are related to an increased neuronal activity within the auditory cortex secondary to the imbalance between excitatory and inhibitory mechanisms or an adjustment of auditory gain mechanisms [78]. One major psychoacoustic finding is that the dominant tinnitus pitch generally falls within the area of hearing loss; this is consistent with the theory of deafferentation as the main trigger of hyperactivation of tonotopic cortex in tinnitus pathogenesis [36]. The side of perception of tinnitus can be linked to the side of the altered structures of the auditory pathways.

Altered auditory inputs may support in tinnitus patients widespread functional reorganization of synaptic connections leading to dysfunctional activity in several subcortical lemniscal structures [36, 49, 53, 60–62, 67, 68, 78, 79] (cochlear nuclei, inferior colliculi (IC), and medial geniculate bodies) and associative auditory cortex [67]; Cochlear nuclei (ventral and dorsal) have been found hyperactive [51, 52, 54, 55, 59, 61, 62], IC has been found reduced in volume [44] and both hyper and hypoactive [45, 46, 49] and Medial geniculate bodies have been found hypoactive in left sided tinnitus patients [49]. The contrasting findings could be explained by the different methodologies of the studies and could be interpreted as the effect of a neuroplastic attempt to gate aberrant signals by saturation [80].

In tinnitus the long-term reorganization of central auditory pathways appears to lead to changes at cortical as well as thalamic level, resulting in structural changes (increase of grey matter density in posterior thalamus associated with significant volume loss in subcallosal area [66]) and altered

Table 3: Tinnitus-related alterations "beyond" auditory pathways.

Methods	Alterations observed	References
fMRI	Increased connectivity in extra-auditory regions (brainstem, basal ganglia/NAc, cerebellum, parahippocampal, and right prefrontal, parietal, and sensorimotor areas); reduced connectivity in right primary auditory cortex, left prefrontal, left fusiform gyrus, and bilateral occipital regions.	[42]
	Reduced grey matter volume in bilateral insula.	[43]
	Significant grey matter decrease in right IC and left hippocampus.	[44]
	Hyperactivity in the anterior cingulate cortex, midcingulate cortex, posterior cingulate cortex, left middle frontal gyrus, retrosplenial cortex and insula.	[65]
	Highly significant volume loss in the subcallosal area; significant increase of grey-matter density in the posterior thalamus.	[66]
	Activation of primary auditory cortices, associative auditory cortices, and left hippocampus.	[67]
PET	Hyperactivity of NAc and primary auditory cortex; increased gray matter and decreased white matter concentrations in the ventromedial PFC.	[68]
	Increased metabolic activity in temporal and parietal brain regions (in female tinnitus patients) and in frontal and occipital regions (in male tinnitus patients) associated with significantly increased metabolic activity in the left primary auditory cortex.	[53]
DTI	Decreased FA in the left frontal arcuate fasciculus and the right parietal arcuate fasciculus.	[69]
	Increased FA in the inferior frontooccipital fasciculus and superior longitudinal fasciculus; decreased FA in the superior longitudinal fasciculus of the left parietal lobe.	[70]
	Disrupted white matter integrity in tracts involving the connectivity of PFC, temporal lobe, thalamus, and limbic system.	[71]
EEG	Increased alpha activity in both left and right anterior insula in patients with severe tinnitus-related distress who can or cannot cope with these phantom sounds.	[72]
	In the right anterior insula increased delta and gamma activity related to increased tinnitus distress; in the left anterior insula decreased theta and gamma activities.	[58]
	Gamma-band activity in the parahippocampal area contralateral to the tinnitus lateralization.	[72]
MEG	Marked reduction in alpha (8–12 Hz) power associated with enhancement in delta (1.5–4 Hz) neuronal activity particularly in right temporal and left frontal areas	[56]
	In patients with significant tinnitus-related distress, more synchronized alpha activity in subcallosal anterior cingulate cortex, insula, parahippocampal area, and amygdala; less synchronized alpha activity in posterior cingulate cortex, precuneus, and DLPFC.	[58]
	Tinnitus-related distress correlated with a right sided connectivity increase between the anterior cingulate and the frontal and parietal cortices.	[46]
	Altered role of frontal cortex in the modulation of sensory inputs.	[73]

IC: Inferior Colliculus
NAc: Nucleus Accumbens
PFC: Prefrontal Cortex
DLPFC: Dorsolateral Prefrontal Cortex
FA: Fractional Anisotropy.

thalamocortical lemniscal and extralemniscal oscillations [81]. According to this model tinnitus perception is related to an abnormal, spontaneous, and constant gamma band activity (>30 Hz) generated as a consequence of hyperpolarization of specific thalamic nuclei [57]; moreover, it was found that tinnitus perceived loudness is correlated with increased contralateral gamma band activity in the auditory cortex indicating that gamma band activity is a frequent founding in tinnitus patients [53, 58]. Based on magnetoencephalography (MEG) data, the emergence of gamma band activity could be also enabled by the absence of thalamus inhibitory function in the auditory cortex, which in turn is shown by reduced alpha band activity (8–12 Hz) [56]. Direct connections from the thalamic nuclei of the nonlemniscal pathway to the limbic system may explain these components often accompanying tinnitus [34].

The limbic system is a group of interconnected cortical and subcortical structures dedicated to linking visceral states and emotion to cognition and behavior; it has always been considered to be a complex arrangement of transitional structures situated between a visceral "primitive" subcortical brain and a more evolved cortical one. It is affected by

a wide range of disorders including neurodevelopmental conditions and neurodegeneration [65]. Limbic structures are also considered a part of extralemniscal auditory pathway [34].

Among the limbic structures, the subgenual anterior cingulate cortex extending into nucleus accumbens-ventral tegmental area is involved in the processing of aversive sounds and unpleasant music as well as tinnitus [82]; it is functionally connected to the amygdala, insula, parahippocampus, orbitofrontal cortex, and ventrolateral PFC and anticorrelated with the dorsal anterior cingulate cortex and precuneus and, as such, the subgenual anterior cingulate cortex could be thought to be important as an emotional component for tinnitus [83]. By comparison of patients with tinnitus with high and low distress, differences in neuronal activity were identified in a network of the anterior cingulate cortex, the anterior insula, and the amygdale; this nonspecific distress network is similarly activated in chronic pain or somatoform disorders [65, 72].

Evidence from neuroimaging studies in patients with tinnitus reports increased connectivity in basal ganglia parahippocampal, right prefrontal, parietal, and sensorimotor areas [42] and hyperactivity in the associative auditory cortices and in the left hippocampus [67]. Hippocampal involvement in tinnitus pathophysiology is also documented by MRI evidence of decreased grey matter volume in this area: this result confirms histopathological findings of hippocampus lesions in patients who experience tinnitus as a symptom of methyltin intoxications [84, 85]. Other relevant findings (fMRI and encephalographic studies) focus on parahippocampal area whose involvement in tinnitus might be related to the establishment of auditory memory for tinnitus [86].

Even if the limbic activation has traditionally been interpreted as a reflection of the emotional reaction of tinnitus patients to the tinnitus sound, limbic and paralimbic structures may play a more extended role than previously proposed. According to a recent paper [30], efferents from structures in the subcallosal area, which includes the nucleus accumbens of the ventral striatum and the ventral medial PFC, are involved in the cancellation of the tinnitus signal at the thalamic level. Although the tinnitus signal may initially be generated in parts of the auditory system, it is the failure of the limbic regions to block this signal that leads to the tinnitus percept becoming chronic [30]. Limbic areas seem to be involved both in chronicization and in decompensation of tinnitus.

Tinnitus distress is related to neural activity in left and right anterior insula according to some authors [58, 72, 76]. The insula is part of auditory pathways and, together with the dorsal anterior cingulate cortex, has also been referred to as the salience network [87]. This network has been implicated in bottom-up detection of salient events and coordinating appropriate responses and its activity is correlated with improved sound detection thresholds, showing a role in the direction of attentional resources toward audition. Main encephalographic findings linked to the salience network in patients with tinnitus report: (1) increased delta and gamma activity in the right anterior insula [72], (2) decreased theta and gamma activities in the left anterior insula [72], and (3) increased alpha activity in both the left and the right anterior insula [58]. The activation of the salience network in tinnitus patients suggests that the brain allocates an importance to the auditory stimulus and might as such also signify importance to the internally generated tinnitus sound. In addition, the insula cortex has distinct auditory and multisensorial connections (with the prefrontal and auditory cortices, amygdala, thalamus, parabrachial nucleus, orbitofrontal cortex, striate, cuneus, and cerebellum) that have been identified through functional imaging techniques to be dysfunctional in cases of severe tinnitus [88].

3.2. Tinnitus-Related Brain Structures "beyond" Auditory Pathways (Table 3). Auditory cortex is connected to several other brain areas through extralemniscal auditory pathway elements such as limbic structures and through temporal lobe efferences [77]. The involvement of these areas seems to be concomitant to auditory structures dysfunctions and not exclusive of tinnitus pathogenesis.

fMRI studies show a complex involvement of multiple areas in tinnitus patients in comparison to healthy controls: auditory resting-state network has been found to encompass bilateral primary and associative auditory cortices, insula, prefrontal, sensorimotor areas, the brainstem, thalamus, NAc, isthmus of cingulate gyrus, right and left occipital, parietal, and PFC; in chronic tinnitus patients, as compared to controls, increased connectivity was found in the brainstem, cerebellum, right basal ganglia/NAc, parahippocampal areas, right frontal and parietal areas, left sensorimotor areas, and left superior temporal region. In addition, chronic tinnitus patients as compared to controls showed decreased connectivity in right primary auditory cortex, left fusiform gyrus, and left frontal and bilateral occipital regions [42]. Concomitant nucleus accumbens and primary auditory cortex hyperactivity associated with increased gray matter and decreased white matter concentrations in the ventromedial PFC were also found in a recent study of Leaver et al. [68].

Several MRI studies evidenced structural alterations in tinnitus patients involving grey matter decrease in auditory and nonauditory brain areas [67].

Diffusion tensor imaging (DTI) is an in vivo imaging tool for studying CNS microstructure [44]. That is, whereas conventional structural MRI is relatively insensitive to the white matter microstructure, DTI reveals the orientation of the white matter tracts in vivo and yields an index of microstructural integrity through quantification of the directionality of water diffusion [69]. Lee et al. used DTI to compare tinnitus subjects with control populations [89]: a statistically significant reduction in the fractional anisotropy (FA) value was found in frontal and parietal arcuate fasciculus in the tinnitus groups compared with the healthy control group. Another recent study by Benson et al. [70] showed increased FA in the inferior frontooccipital fasciculus and superior longitudinal fasciculus and decreased FA in the superior longitudinal fasciculus of the left parietal lobe. The arcuate fasciculus is a white-matter fibre tract, part of the superior longitudinal fasciculus, that links lateral

temporal cortex with frontal cortex via a dorsal projection that arches around the Sylvain fissure; it connects Broca's area and Wernicke's area, playing a critical role in language functions. Other authors also confirmed the findings about "disconnectivity" in extra-auditory pathways involving PFC, temporal lobe, thalamus, and limbic system [71] in DTI studies. On the other hand, some authors described a right sided connectivity increase between the anterior cingulate and the frontal cortex and parietal cortex [76].

Among tinnitus patients there is a large heterogeneity of findings about functionality of brain structures and a positron emission tomography (PET) study evidenced gender-related differences in female tinnitus patients increased metabolic activity of left primary cortex was associated with a similar finding in temporal and parietal brain areas while in male patients an increased metabolic activity was found in frontal and occipital regions [53]. A concomitant involvement of right temporal and left frontal areas (marked reduction in alpha (8–12 Hz) together with an enhancement in delta (1.5–4 Hz) neuronal activity) was also reported in a study utilizing MEG [71].

Recently also dorsolateral prefrontal cortex (DLPFC) dysfunctions have been associated with tinnitus and tinnitus-related distress [53]. DLPFC exerts early inhibitory modulation of input to primary auditory cortex in humans [90] and has been found to be associated with auditory attention [91] resulting in top-down modulation of auditory processing [92]. As electrophysiological data indicated that tinnitus might occur as the result of a dysfunction in the top-down inhibitory processes [73], it has been hypothesized that the hypofunctioning of DLPFC may contribute to the hyperfunctioning of auditory cortex observed in tinnitus patients, representing a neurophysiological substrate of tinnitus perception and related distress [69]. An electroencephalographic (EEG) study recently confirmed the involvement of DLPFC (associated with a less synchronized alpha activity in the posterior cingulate cortex and precuneus and with a concomitant more synchronized alpha activity in subcallosal anterior cingulate cortex, the insula, parahippocampal area, and amygdala) in tinnitus distressed patients [72].

A tinnitus distress MEG study, in addition, associated tinnitus with an increased right sided connectivity between the anterior cingulate and the frontal cortex and parietal cortex [76].

4. Discussion

Far from being considered only an otological disorder, tinnitus is a frequent and heterogeneous symptom of various underlying pathologies, resulting in most cases from neuronal changes occurring in the CNS as a reaction to auditory deprivation. As tinnitus-related plastic rearrangements of auditory pathways involve brain structures such as insula, IC, thalamus, and PFC that are important nodes of various other brain circuits, it can be hypothesized that these rearrangements lead not exclusively to auditory symptoms but also to other symptomatology involving psychic functions.

Results from neuroimaging (MRI, fMRI, and PET) and encephalographic (MEG and EEG) studies widely documented tinnitus-related processes of neural plasticity that affect neuronal activity of the auditory system at several levels along the auditory pathway as well as cortical regions involved in perceptual, emotional, memory, attentional, and salience functions [93]. Among the alterations observed in tinnitus, some altered networks are also involved and play a critical role in the physiopathology of emotional and psychiatric disturbances, supporting the idea of overlapping neurobiological substrates between decompensated tinnitus and psychopathology.

Consistently with the presented results, tinnitus, initially related to a clinicoanatomical approach based on a narrow cortical localizationism within an otological perspective, could be better explained by an holistic approach [94] considering all regions to be mutually interconnected through a network of homogeneously distributed association fibres or by associationist models considering the brain organized in parallel distributed networks around cortical epicentres [95].

Considering that psychological functions and symptoms are the result of the simultaneous activity of all brain regions acting as a whole through association pathways, psychic functions and tinnitus may be considered emergent properties of partially overlapping large-scale neural networks [96, 97].

The discussion section will be presented in two separate sections each section discussing those brain networks that could underlie the still not adequately understood connection between tinnitus and psychopathology.

4.1. Tinnitus-Related Brain Structures "into" and "within" Auditory Pathways. The hyperactivity of auditory cortex plays a critical role both in tinnitus and in auditory verbal hallucinations (AVHs); this evidence is supported by the fact that inhibitory temporal transcranial magnetic stimulation (TMS) protocols have successfully been used to treat both of the disorders [98]. For what concerns AVHs, defined as "the subjective experience of hearing voices speaking in the absence of corresponding physical stimulation," it has been proposed that the brain regions dedicated to auditory processing, especially the primary auditory cortex, are relevant to experiencing hallucinations. This idea is supported by the so-called "symptom capture" studies, which attempt to measure brain activity while subjects are experiencing AVHs [99–101].

Even if in the majority of cases relevant clinical differences between tinnitus and AVHs are present, both the clinical conditions may be considered forms of auditory perception alterations which present with a "continuum of complexity" and with subjective differences in the levels of insight and perceived distress, having potential similar neurobiological substrates [102].

Tinnitus differs from AVHs because it is perceived as a sound not having any complex, digitalized linguistic meaning, thus being typically recognized by patients as a pathological phenomenon. There is evidence suggesting that, while tinnitus and AVHs share common dysfunctions in auditory processing underlying phantom sound perceptions,

they present a different pattern of alterations of thalamocortical networks that are supposed to be related to conscious perception of auditory inputs [52, 102].

Behrendt [103] has provided a thought-provoking hypothesis based on the idea that perceptual experience arises from synchronization of gamma oscillations. This oscillatory activity is normally constrained by sensory input and also by prefrontal and limbic attentional mechanisms. There is evidence that in patients with schizophrenia (SCZ) there is impaired modulation of thalamocortical gamma activity by external sensory input, allowing attentional mechanisms to play a preponderant role in the absence of sensory input and thus potentially leading to hallucinations. While dysfunctions of auditory cortex are related to AVHs perception, functional alterations of extralemniscal auditory pathways structures represent a common field between tinnitus and other psychopathological dimensions. In fact, direct connections from the thalamic nuclei of the nonlemniscal pathway to the amygdala, the hippocampus, and other structures of the limbic system may explain, according to several authors, the affective components of tinnitus [34].

Limbic dysfunction underlies many symptoms (related to emotion regulation and social interaction and behaviour) of psychiatric conditions, including SCZ, affective disorders, psychopathy, and autism spectrum disorders (ASD) [83]. This system has often been considered a "switch" in the brain that can turn the tinnitus sensation on or off [94]. The first behavioral animal model of tinnitus developed by Jastreboff et al. in 1988 [104] has provided important insight into the neuronal mechanisms involved in the pathophysiology of tinnitus; it does not exist, however, an animal model of tinnitus-related distress potentially representing the psychopathological consequences of tinnitus. Increased activity in the auditory cortex as a consequence of auditory deprivation, in fact, is necessary but not sufficient for tinnitus perception: the patient becomes distressed by the phantom sound if auditory activity is connected to larger coactivated networks involving, also, the limbic system [105, 106]; related psychiatric symptoms could derive from dysfunction of circuits of the limbic system, not directly from topological structures [83].

The limbic structures that are known to be related to tinnitus pathophysiology (amygdala, hippocampus, parahippocampal gyrus, insula, cingulum, and, for extension, nucleus accumbens) are components of three distinct but partially overlapping networks and corresponding clinical syndromes [83]. The first network, composed of the hippocampal-diencephalic limbic circuit (connected through the fornix and mammillothalamic tract) and the parahippocampal-retrosplenial circuit (ventral cingulum), is dedicated to memory and spatial orientation, respectively; the second, the temporoamygdala-orbitofrontal network (connected through the uncinate fasciculus) is dedicated to the integration of visceral and emotional states with cognition and behavior; the third, the dorsomedial default-mode network consists of a group of medial regions (anterior cingulate-medial PFC and the posterior cingulate-precuneus interconnected through the dorsal cingulum). Psychiatric disorders associated with these networks are described in Table 4.

Table 4: Limbic networks and neuropsychiatric disorders [65].

Network	Disorder
Hippocampal-diencephalic and parahippocampal-retrosplenial	(i) Amnesias (ii) Korsakoff's syndrome (iii) Mild cognitive impairment (iv) Alzheimer's disease (early) (v) Balint syndrome
Temporoamygdala-orbitofrontal	(i) Alzheimer's disease (advanced) (ii) Semantic dementia (iii) Kluver-Bucy syndrome (iv) Temporal lobe epilepsy (v) Geschwind's syndrome (vi) Psychopathy (vii) Bipolar affective disorders
Dorsomedial default network	(i) Depression (ii) Autism (iii) Schizophrenia (iv) Obsessive compulsive disorder (v) Mild cognitive impairment (vi) Alzheimer's disease (early) (vii) Attention deficit hyperactivity disorder (viii) Anxiety

Tinnitus distress seems to be also related to neural activity in the left and right anterior insula. Insular cortex through interconnection with cingulate gyrus, orbitofrontal cortex, and parahippocampal gyrus (paralimbic areas) is believed to be involved in consciousness and plays a role in diverse functions including perception, motor control, self-awareness, social cognition, cognitive functioning, and interpersonal experience [107]. As written above, the insula together with the dorsal anterior cingulate cortex has also been referred to as the salience network [87]; the activation of the salience network in tinnitus patients suggests that the brain allocates high importance to the internally generated tinnitus sound. Anomalies of salience network have been implicated in different psychiatric disorders, especially SCZ [108], ASD, and attention-deficit hyperactivity disorder (ADHD) [109], as well as obsessive compulsive disorder (OCD) [110], anxiety, and mood disorders [111]. These clinical conditions (SCZ and ASD in particular) are characterized by difficulties in integrating external sensory stimuli with internal states, and several authors postulated the key role of aberrant salience in their physiopathology [112]. The paralimbic involvement in tinnitus patients may thus indicate tinnitus distress as a state of aberrant salience potentially comparable to the aberrant salience of other serious brain disorders.

Tinnitus usually becomes troublesome if patients focus their attention on it and the perception of tinnitus severity usually correlates more closely with psychological and general health (such as pain or insomnia) factors than with audiometric parameters [105]. The perception of tinnitus often extinguishes in a short time through habituation mechanisms: superior brain centres activate thalamic filters to "switch off" the signal, often independently of the resolution of the dysfunction that originally generated the tinnitus. On

the other hand, in case of emotional reinforcements caused by fear, anxiety, or tension, the continued perception of tinnitus is supported by the limbic system, primarily by the amygdala; this establishes a vicious circuit which leads to the amplification (increased excitability) and the chronicity (through neuronal plasticity mechanisms) of the signal [10].

From a clinical point of view, emotional "limbic" reinforcements can strongly worsen the tinnitus-related distress potentially representing the milestones to shift from a compensated to a decompensated tinnitus [113]. Consistently, pharmacological (selective serotonin reuptake inhibitors [114, 115], benzodiazepines [116], mood stabilizers [117, 118]), psychotherapeutic (cognitive behavioural therapy [119]) and neuromodulating (TMS [120, 121], tDCS [122], Neurofeedback [123]) treatments aimed at modulating the subjective emotional component of tinnitus showed to be among the best interventions to treat tinnitus distress and should always be integrated with regular otological interventions [10].

4.2. Tinnitus-Related Brain Structures "beyond" Auditory Pathways. Among the brain areas beyond auditory cortex, the frontal lobe seems to be the principal structure involved in the pathogenesis of tinnitus. The role of frontal lobe in tinnitus has been confirmed by studies using different brain mapping techniques and it involves frontocortical and frontosubcortical circuits.

Data from DTI studies in tinnitus patients show decreased fractional anisotropy in frontal and parietal arcuate fasciculus [69], increased FA in the inferior frontooccipital fasciculus and superior longitudinal fasciculus, decreased FA in the superior longitudinal fasciculus of the left parietal lobe [89], "disconnectivity" in extra-auditory pathways involving pathways involving PFC, temporal lobe, thalamus, and limbic system [71] and increased right sided connectivity between anterior cingulate, frontal and parietal cortices [76].

Of particular interest are data on the arcuate fasciculus, this pathway is critically involved with human language. Evidence of arcuate fasciculus damages in patients with tinnitus indicates a deterioration of white-matter fibres and underlines the importance of cortical interconnectivity in the pathogenesis of this disorder. Arcuate fasciculus has also been found damaged in several psychiatric disorders such as ASD, SCZ, dyslexia, and dyscalculia, supporting the idea that white matter deterioration may represent a common functional substrate of tinnitus and psychiatric disorders. Moreover, as Tim Crow assessed in the paper "Schizophrenia as the price that homo sapiens pays for language: a resolution of the central paradox in the origin of the species" [124] there is a well-established involvement of language development in psychiatric disorders, supporting the idea of a potential role of arcuate fasciculus damages in both the conditions.

The concomitant involvement of right temporal and left frontal areas (marked reduction in alpha (8–12 Hz) together with an enhancement in delta (1.5–4 Hz) neuronal activity) reported in a MEG study [71] could derive from the interconnection of the two lobes through the arcuate fasciculus. There is also evidence of the involvement of other long white tract fibres pathways in tinnitus and psychopathology: an altered network among frontooccipital connections [36, 53, 78] has been associated with behavioural syndromes like personality changes, emotional liability, and disinhibition [107]; lesion at the longitudinal superior fasciculus leading to an altered connectivity between frontal cortex, cingulus, and parietal cortex [76] has been hypothesized to determine derealization symptomatology and memory deficits [125]; OCD symptomatology has been suggested to be related to a dysfunction of frontoparietal connectivity [126].

Alterations in frontal-subcortical circuits [71] from PFC to thalamus and limbic system seem to be relevant for the onset of several psychiatric disorders such as depression, OCD, and SCZ [127]. Among frontal-subcortical circuits, DLPFC exerts early inhibitory modulation of input to primary auditory cortex in humans and several studies evidenced its involvement in tinnitus; as electrophysiological data indicated that tinnitus might occur as the result of a dysfunction in the top-down inhibitory processes [73], it has been hypothesized that the hypofunctioning of DLPFC may contribute to the hyperfunctioning of auditory cortex observed in tinnitus patients, representing a neurophysiological substrate of tinnitus [69]. Results from a large body of functional and structural brain imaging studies provide convergent evidence that DLPFC plays critical roles in mood regulation and DLPCF hypoactivity is nowadays considered a critical neural substrate for depression [128]. Impaired DLPFC functioning may thus represent a common neurobiological substrate of tinnitus symptomatology and depression, potentially explaining the high rate of comorbidity between the two disorders and the efficacy of prefrontal TMS in the treatment of both of the disorders [29, 103, 129, 130]. Consistently with this view, Gray described PFC as a "candidate for the integration of sensory and emotional aspects of tinnitus" [131].

Furthermore, concomitant hyperactivation of NAc and primary auditory cortex and decreased white matter concentrations in the ventromedial PFC [68] have been proposed as indirect findings related to frontosubcortical circuits involvement in patients with tinnitus. NAc is involved in both normal and abnormal reward processes, in the pathogenesis of anhedonia and loss of motivation. Due to its strategic location between emotional system, cognitive system, and motor control system, NAc has been proposed as a central node in mood and feeling regulation [132].

Finally, implication of extra encephalic structures as cerebellum in a circuit involving brainstem, basal ganglia/NAc, parahippocampal, right prefrontal, parietal, and sensorimotor areas [42] should be related to psychiatric manifestation (SCZ, bipolar disorder, major depressive disorder, anxiety disorders, dementia, and ADHD) [133, 134].

5. Conclusion

From an accurate analysis of scientific literature it emerges that tinnitus and psychiatric disorders share common neuronal network dysfunctions related to specific pathways; thalamus and limbic areas seem to represent the most relevant "nodes" of such altered networks linked to auditory extralemniscal areas, while multiple hodological alterations

of frontal circuits with others structures seem to emerge from extra-auditory involvements in tinnitus. The rearrangement of auditory cortex functionality is probably linked to tinnitus perception.

From a tractographic point of view, it is possible to hypothesize that neuroplastic rearrangements of auditory pathways in patients with tinnitus could affect the functionality of all those nonauditory brain areas connected with the auditory cortex through the plastic rearrangement of white matter pathways potentially leading to the onset of psychopathological symptoms. On the other hand it is possible that psychological stress, current or previous psychiatric disorders, and personality traits associated with a genetically or epigenetically determined vulnerability may represent a vulnerability factor giving rise to maladaptive tinnitus-related neuroplastic rearrangements [135] leading to tinnitus symptomatology. Given the above, our hypothesis is that patients' symptomatology may be considered the peculiar expression of an alteration of global brain hodological equilibrium.

Clinical trials concerning the use of psychotropic medications for the treatment of tinnitus evidence interesting issues supporting this hypothesis: standard tinnitus treatments often show poor outcomes on tinnitus-related distress [136–138] while the treatments focused on psychiatric comorbidities appear to be more effective than standard tinnitus treatments, achieving a response rate of up to 81.39% [139].

Among psychiatric treatments, the best outcomes have been obtained approaching psychopathological disturbances with a dimensional rather than a DSM-defined categorical point of view [139]; the categorical model of the DSM, in fact, provides a poor fit to the latent structure of psychopathology [140]. Dimensional approaches to psychiatric therapies have increasingly been supported; to this purpose, Buckholtz and Meyer-Lindenberg have recently proposed a dimensional transdiagnostic "common symptom, common circuit" model of psychopathology suggesting that specific clusters of psychic disturbances correspond to specific clusters of brain network alterations associated with tinnitus perception [63, 141]. We find reliability and promise in this kind of approach in order to diagnose and treat psychiatric comorbidities of tinnitus.

The hodological view of psychiatric comorbidities in tinnitus patients also gives rise to other considerations: (1) "*οδός*" means "way," but also "connection": the management of tinnitus complexity requires a multidisciplinary approach where otolaryngologists should involve and "connect" several different medical specialists; (2) clinicians should have more accurate instruments to assess the psychiatric comorbidities and the global neurofunctional activity [11]; (3) other nonpsychiatric comorbid conditions potentially able to induce plastic rearrangements, such as muscle tension [4] and hyperinsulinemia [142, 143], should be taken into consideration; (4) from a "*methodological*" point of view, the studies on tinnitus pathogenesis and on treatment response should be personalized rather than standardized.

Given the absence of objective diagnostic markers, tailored psychiatric treatments can currently be implemented exclusively on the basis of patients' reported complaints [144–146]. We hereby suggest a comprehensive approach to tinnitus treatment focused on 4 areas of intervention based on its clinical presentations: (A) predominantly audiological (deafferentation or deprivation tinnitus); (B) predominantly somatosensory (i.e., cross-modal tinnitus); (C) predominantly psychopathological (D) mixed-combined [10]. Further studies are needed to evaluate specific therapeutic approaches targeted on each of these 4 clinical domains. It is also probable that if functional and structural imaging studies will follow an adequate classification of tinnitus patients they will be able to provide more detailed and less confusing results.

Conflict of Interests

The authors of this paper have no relevant affiliations or financial involvement with any organization or entity with a financial interest in, or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. All authors acknowledge that the conflict of interest disclosures are complete for both themselves and their co-authors, to the best of their knowledge.

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Research Article

Structural Brain Changes Following Left Temporal Low-Frequency rTMS in Patients with Subjective Tinnitus

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Repetitive transcranial magnetic stimulation (rTMS) of the temporal cortex has been used to treat patients with subjective tinnitus. While rTMS is known to induce morphological changes in healthy subjects, no study has investigated yet whether rTMS treatment induces grey matter (GM) changes in tinnitus patients as well, whether these changes are correlated with treatment success, and whether GM at baseline is a useful predictor for treatment outcome. Therefore, we examined magnetic resonance images of 77 tinnitus patients who were treated with rTMS of the left temporal cortex (10 days, 2000 stimuli/day, 1 Hz). At baseline and after the last treatment session high-resolution structural images of the brain were acquired and tinnitus severity was assessed. For a subgroup of 41 patients, additional brain scans were done after a follow-up period of 90 days. GM changes were analysed by means of voxel based morphometry. Transient GM decreases were detectable in several brain regions, especially in the insula and the inferior frontal cortex. These changes were not related to treatment outcome though. Baseline images correlated with change in tinnitus severity in the frontal cortex and the lingual gyrus, suggesting that GM at baseline might hold potential as a possible predictor for treatment outcome.

1. Introduction

Subjective tinnitus is the phantom perception of a sound in the absence of a corresponding objective sound source. With about 25% of adults in the US having experienced a ringing in the ears at least once [1], transient tinnitus is a common phenomenon. About 10–15% of the world population experience tinnitus in its chronic form [2]. While the majority of those 10–15% gets used to their tinnitus and is able to lead a normal life, in 1–3% of the general population tinnitus is experienced as extremely bothersome and debilitating. It can severely affect patients' everyday lives and is often accompanied by psychiatric comorbidities such as depressive syndromes or sleep disturbances [2, 3]. In order to improve existing treatment options and also to generate new treatment strategies for subjective tinnitus, it is mandatory to broaden knowledge on the neural mechanisms underlying the tinnitus percept.

More than 15 years ago it has been suggested [4, 5] and demonstrated [6] that tinnitus is related to alterations in the central nervous system. Furthermore, recent functional neuroimaging studies suggest [7–10] that, apart from the auditory cortex, widespread neural networks involving many different brain areas seem to be involved in the generation and maintenance of the phantom sounds as well as in the distress accompanied by the tinnitus percept [11, 12]. In addition to functional alterations within the brain, tinnitus has also been shown to be related to structural brain changes [13].

Studies using high-resolution magnetic resonance imaging (MRI) to compare the grey matter (GM) volume and cortical thickness of tinnitus patients with healthy control subjects have revealed alterations in the auditory cortex [14–16] and in subcortical parts of the central auditory pathway like the thalamus [17] and the right inferior colliculus [18]. Furthermore, alterations in grey matter volume and cortical thickness were also found in nonauditory brain locations [15, 17–21].

The knowledge that subjective tinnitus is associated with neural alterations suggests the therapeutic use of brain stimulation techniques such as repetitive transcranial magnetic stimulation (rTMS). The early finding that the auditory cortex is overly active in tinnitus patients [6] led to the idea of using low-frequency repetitive transcranial magnetic stimulation to modify the cortical hyperactivity in patients with phantom sounds [22]. Ever since then low-frequency rTMS has been investigated in an increasing number of studies (for a review, see [23]) showing that rTMS is effective with high interindividual variability. However, it is still difficult to identify predictors for treatment success [24]. The idea to use and improve rTMS as a treatment for tinnitus is further pursued though. To gain deeper insight into the mechanisms of rTMS treatment —and consequently to facilitate improvement of the therapeutic approach— the complementary use of both longitudinal neuroimaging and clinical assessment to measure rTMS effects in tinnitus patients is an important next step in tinnitus research [25]. The number of studies addressing this issue is limited so far. Some studies investigated the effect of low-frequency rTMS treatment on auditory evoked potentials and auditory steady state responses using electro- and magnetoencephalography (EEG/MEG) [26–28]. Two studies using single-photon emission computed tomography and functional magnetic resonance imaging (fMRI) found changes of neural activity in the temporal lobe, the right cingulate gyrus, and the uncus [26, 29]. While those studies have provided first insight in the functional alterations that are associated with low-frequency rTMS of the auditory cortex, there is no study which adds knowledge about structural alterations induced by rTMS treatment in tinnitus patients. Until now, only one study examined the effect of low-frequency rTMS over the left auditory cortex in healthy subjects using voxel based morphometry (VBM) [30]. The results suggest that five days of rTMS treatment leads to GM changes in the auditory cortex and the thalamus.

Based on all those results the current study was conducted with the following three research questions in mind: (1) is there a change in grey matter detectable in tinnitus patients after 10 sessions of rTMS treatment and after a follow-up period of 90 days? (2) Is there a relationship between the clinical outcome and the grey matter changes? (3) Can structural imaging be used as a predictor for outcome? To answer these questions we evaluated MRI scans of patients suffering from subjective tinnitus which were done routinely before and after low-frequency rTMS of the temporal cortex.

2. Materials and Methods

2.1. Subjects. Data from 77 patients (59 male, 18 female) with chronic tinnitus were included in the analyses. Patients with cardiac pacemakers, history of seizures, or any severe somatic, neurologic, or psychiatric disorder were excluded. The decision whether a patient was suffering from any severe somatic, neurologic, or psychiatric disorder was made by the physician, who decided about study inclusion based on the global clinical impression. One criterion for a severe somatic, neurologic, or psychiatric disorder was the need for an immediate therapeutic action for the treatment of this disorder. Another criterion was current hospitalization because of such disorder.

All patients were treated with rTMS and underwent MRI scanning before (baseline) and after (day 12) ten sessions of rTMS treatment. In a subgroup of 41 patients, an additional measurement was done after a follow-up period of three months (day 90). The total sample of 77 patients was therefore divided into two independent subgroups of one sample with two scans ($n = 36$) and one sample with three scans ($n = 41$). Demographical and clinical characteristics for both subgroups are shown in Table 1. Audiological data and a measure of hyperacusis were not available for all patients and could therefore not be included in the further analyses. Standardized pure tone audiometry data was available for 57 patients and revealed a mean hearing loss of 20.38 ± 12.14 [dB HL] (average of all thresholds measured bilaterally ranging from 125 Hz to 8 kHz). As a screening measure of hyperacusis, patients were asked whether "sounds cause pain or physical discomfort" [31]. Of the 61 patients who answered this question, 35 said "yes" and are therefore supposed to suffer from hyperacusis. Independent samples t-tests and Chi^2-tests revealed no significant difference between the two independent subgroups concerning all variables reported in Table 1.

2.2. Repetitive Transcranial Magnetic Stimulation. rTMS treatment consisted of 10 treatment sessions on 10 consecutive working days. Patients were treated in the context of several clinical trials [32–34] or rTMS was done as compassionate use treatment between 2006 and 2009. Patients were stimulated over the left temporal cortex (1 Hz, 2000 stimuli/day, 110% resting motor threshold) which was localized either by using a standard procedure targeting the primary auditory cortex based on the 10–20 system [35, 36] or by using neuronavigation based on individual MRI/PET (positron emission tomography) images. In the latter cases, the area of increased activation within the primary auditory cortex was used as target area. Even if these two methods may have resulted in slightly different targets, the spatial difference is smaller than the spatial accuracy of rTMS treatment with the used figure-of-eight coil. For rTMS treatment, a Medtronic system with a figure-of-eight coil was used (90 mm outer diameter; Medtronic, Minneapolis, MN). The coil was held with a mechanical arm and placed over the left temporal cortex with the handle of the coil pointing upwards. During treatment, the patients were seated in a comfortable treatment chair. The resting motor threshold was measured once before the first

Table 1: Demographical data and clinical characteristics for both independent subgroups.

	VBM data at baseline, day 12, and day 90 (n = 41)	VBM data at baseline and day 12 (n = 36)	Group comparison	
				P value
Gender	32 m (78%) 9 f (22%)	27 m (75%) 9 f (25%)	$\chi^2(1.77) = 0.10$	0.752
Age (years)	50.72 ± 13.37	50.79 ± 13.28	$T(75) = -0.02$	0.983
Tinnitus laterality	10% right 15% left 75% bilateral	14% right 14% left 72% bilateral	$\chi^2(2.77) = 0.32$	0.853
Tinnitus duration (years)	8.97 ± 8.36	7.57 ± 6.74	$T(75) = 0.80$	0.427
TQ (baseline)	36.61 ± 17.78	39.56 ± 18.21	$T(75) = -0.72$	0.476
Loudness (baseline)	6.32 ± 2.04	6.00 ± 2.11	$T(75) = 0.67$	0.441
Mean hearing threshold [dB HL]	21.67 ± 11.49 (N = 29)	19.06 ± 12.85 (N = 28)	$T(55) = 0.81$	0.421
Hyperacusis	51% (n = 39)	68% (n = 22)	$\chi^2(2.61) = 2.31$	0.316

TQ: Tinnitus Questionnaire.
Loudness: how STRONG or LOUD is tinnitus at present (0 not at all, 10 extremely strong or loud).
Mean hearing threshold: average of all thresholds measured bilaterally ranging from 125 Hz to 8 kHz).

treatment session and was defined as the minimal intensity at which at least four out of eight magnetically evoked potentials were ≥50 μV in amplitude in the right abductor digiti minimi muscle [37]. All patients were treated at the Tinnitus Centre at the University of Regensburg, Germany, and provided written informed consent. The treatment protocol has been approved by the local ethics committee.

2.3. Clinical Assessment. For the assessment of demographical and clinical characteristics, the Tinnitus Sample Case History Questionnaire was used [38]. Tinnitus severity was assessed using the German version of the Tinnitus Questionnaire (TQ [39, 40]) and a numeric rating scale, which measured how loud the tinnitus was perceived (How strong or loud is your tinnitus at present?). This scale was rated from 0 (not loud at all) to 10 (extremely strong or loud). These measures were assessed before the first treatment session (baseline), after the last treatment session (day 12), and—for the subgroup of 41 patients with three images—after the follow-up period of three months (day 90).

2.4. Magnetic Resonance Imaging. A Siemens Sonata 1.5 Tesla whole body scanner (Siemens AG, Erlangen) with a standard 8-channel birdcage head coil was used to collect the anatomical images. For each subject and each time point, a high-resolution T1-weighted image was acquired using a magnetization-prepared-rapid-acquisition-gradient-echo-(MP-RAGE-) sequence (repetition time 1880 ms; echo time 3.42 ms; flip angle 15°; matrix size 256 × 256; number of slices 176; voxel size $1 \times 1 \times 1\,\text{mm}^3$).

2.5. Data Processing and Statistical Analysis. For statistical analyses of the clinical data, PASW statistics 18 (SPSS Inc., Chicago, IL) was used. To test for changes in tinnitus severity, an analysis of variance (ANOVA) with the within-subjects factor time (baseline, day 12, day 90) was calculated for both the TQ and the loudness scale. In case of significant results, post hoc paired t-tests were done. For the group of 36 patients with only two assessments, paired t-tests were used to compare the TQ and loudness on baseline and day 12. All statistical tests were two-tailed. The level of significance was set at .05.

Processing and statistical analysis of the anatomical data were performed with the SPM8 software package (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging; http://www.fil.ion.ucl.ac.uk/spm/). All anatomical images were visually examined for the presence of morphological abnormalities or artifacts. Preprocessing of the anatomical data was done using the standard procedure of the voxel based morphometry toolbox (VBM8 version 435, Structural Brain Mapping Group; http://dbm.neuro.uni-jena.de/vbm/) for longitudinal data and involved intrasubject realignment, bias correction, segmentation, and normalization to the Montreal Neurological Institute (MNI) space. The default options of the standard procedure were not changed. As modulation is not necessary for longitudinal data, unmodulated images were used. Afterwards, a quality check was done using VBM8 before smoothing data with a Gaussian kernel of 8 mm full width at half maximum. Only grey matter images were used for further analyses. For the statistical analyses all voxels with a grey matter value below 0.1 were excluded to avoid edge effects around the border between grey and white matter. All analyses were done for the overall group of 77 patients (baseline and day 12 scans) as this group provided the highest statistical power. Additionally, all analyses were also done for the independent subgroups with two (n = 36) and three (n = 41) MRI scans. The following whole-brain analyses were performed.

(1) Grey matter images acquired at every time point were compared by estimating a flexible factorial model in SPM8 with the factors subject and time (baseline, day 12, and day 90).

(2) To test for correlations between the grey matter changes over time and changes in the clinical outcome parameters,

difference images were calculated using the image calculator implemented in SPM8 (day 12-baseline; day 90-baseline) and correlated with the corresponding difference in the TQ and loudness scores.

(3) To find out whether grey matter images might be useful as a predictor for clinical outcome, baseline images were correlated with the difference in the TQ score (day 12-baseline). Please see Table 2 for an overview of all analyses done.

(4) For all analyses, the significance threshold was set to $P < .001$ (uncorrected) at voxel level and $P < .05$ (familywise error (FWE) corrected) at cluster level. Due to the nonisotropic smoothness of VBM data, correction for nonstationarity was applied. Anatomical Automatic Labeling (AAL; [41]) and the SPM Anatomy Toolbox [42] were used for anatomic labeling of significant clusters.

3. Results

3.1. Clinical Outcome. The paired t-tests comparing the TQ and the loudness differences between baseline and day 12 in the overall group of 77 patients revealed a significant decrease in the TQ score ($t(76) = 2.474$, $P = .016$) and a marginally significant decrease in the loudness rating ($t(76) = 1.745$, $P = .085$). The paired t-tests comparing the TQ and the loudness differences between baseline and day 12 in the subgroup with only two scans ($n = 36$) revealed a significant decrease in the TQ score ($t(35) = 2.292$, $P = .028$) and no significant change in the loudness rating ($t(35) = -0.099$, $P = .922$). The ANOVA comparing the TQ scores of the subgroup with three scans ($n = 41$) revealed no significant effect of time ($F(1.70, 67.82) = 1.743$, $P = .187$). The ANOVA comparing the loudness scores of all three time points suggested a significant difference between at least two time points ($F(2, 80) = 3.522$, $P = .034$). Post hoc paired t-tests revealed a significant decrease from baseline to day 12 ($t(40) = 2.529$, $P = .015$) and a marginally significant decrease from baseline to day 90 ($t(40) = 2.007$, $P = .052$). There was no significant change from day 12 to day 90 ($t(40) = -0.371$, $P = .713$). See Figure 1 for a line chart showing the development of the TQ and loudness scores over time.

3.2. VBM. (1) The flexible factorial models revealed significant grey matter concentration decreases from baseline to day 12 in the left and right insula as well as in the left and right inferior frontal gyrus (please see Figure 2 and Table 3 for MNI coordinates and statistical details). These GM changes were visible in both the $n = 41$ and the overall patient sample with 77 patients. It was not detected in the $n = 36$ sample though. If data of this group was analyzed with a more relaxed statistical threshold ($P < .05$ (uncorrected) at voxel level and $P < .05$ FWE corrected at cluster level), GM decreases were found in the right inferior frontal gyrus ($x = 40$, $y = 39$, and $z = 19$; $Z = 3.07$, $P = .059$). Please see Figure 3 for the mean GM concentration of the relevant clusters for all groups and all time points.

In addition, grey matter decreases were found in the left temporal pole and the left ventromedial prefrontal cortex. These GM changes were only visible in the $n = 41$ sample though. The contrast between baseline and day 12 in the overall patient sample ($n = 77$) additionally revealed decreased GM in the left inferior/medial temporal gyrus (Table 3). This was also visible in the $n = 41$ group ($x = -62$, $y = -36$, and $z = -20$; $Z = 4.08$, $P = .016$) if analyzed with a more relaxed statistical threshold ($P < .001$ (uncorrected) at voxel level and uncorrected at cluster level). In the $n = 36$ group, no significant GM decreases were visible. Overall, no grey matter increases from baseline to day 12 were visible in neither group. Neither grey matter increases nor decreases were found from baseline to day 90.

(2) The correlation analyses between the difference images and the difference in the TQ/loudness ratings revealed no significant results.

(3) The correlation analyses between the TQ difference and the baseline images revealed a positive correlation of the TQ with GM concentration in the left medial temporal pole and the right posterior cingulate cortex in the $n = 36$ group (Table 3). The correlations in the $n = 41$ group did not reach statistical significance. Furthermore, in the overall patient group, a positive correlation between the TQ difference and the baseline images was found in the left and right lingual gyrus. Additionally, a marginally significant positive correlation was detected in the right inferior/middle frontal gyrus. Using a more relaxed statistical threshold ($P < .05$ (uncorrected) at voxel level and $P < .05$ FWE corrected at cluster level), a marginally positive correlation in the lingual gyrus ($x = -4$, $y = -91$, and $z = 13$; $Z = 3.78$, $P = .064$) and in the inferior/middle frontal gyrus ($x = 40$, $y = 44$, and $z = 21$; $Z = 3.34$, $P = .093$) was also found in the $n = 41$ group.

4. Discussion

In order to improve rTMS treatment for patients suffering from subjective tinnitus, it is of particular importance to understand the neural alterations rTMS induces in tinnitus patients' brains in general and in treatment responders' brains in particular. The current study aimed at investigating the structural brain changes after rTMS treatment and the connection between these changes and clinical outcome. We examined grey matter alterations after ten sessions of low-frequency rTMS of the left temporal cortex. Besides the result that tinnitus severity and loudness were significantly reduced after rTMS treatment, the main findings of the present study were the following. (1) Transient GM decreases from baseline to day 12 were observed in several cortical areas. Neither GM increases nor GM changes from baseline to day 90 were detectable. (2) There was no correlation between GM changes and clinical outcome. (3) GM images at baseline correlated with treatment outcome suggesting that GM at baseline might be related to treatment response.

4.1. Grey Matter Changes from Baseline to Day 12. Bilateral GM decreases from baseline to day 12 were detectable in the insula and the inferior frontal gyrus (IFG). Those results were identical in the $n = 41$ group and the overall patient sample.

Table 2: Overview over all VBM analyses.

Research question	Statistics		
	$n = 41$ (3 scans)	$n = 36$ (2 scans)	$n = 77$ (whole group 2 scans)
(1) Grey matter changes after rTMS?	Flexible factorial models with factors subject + time		
	Time points: baseline, day 12, day 90	Time points: baseline, day 12	Time points: baseline, day 12
(2) Correlation between grey matter changes and clinical outcome parameters?	Correlation of difference in the TQ/loudness rating with difference images		
	Time difference: day 12–baseline day 90–baseline	Time difference: day 12–baseline	Time difference: day 12–baseline
(3) Grey matter as predictor for treatment response?	Correlation of difference in the TQ with baseline images		

On a more relaxed statistical threshold, the GM decreases in the right inferior frontal cortex were also visible in the $n = 36$ group. As it can be seen in Figure 3, this group also shows the tendency for GM decreases in both the right and left insula/frontal cortex. However, the difference is too small to reach statistical significance. Together with the anterior parts of the insula, the IFG is supposed to be a part of the ventral attention network (VAT), a mostly right-lateralized network responsible for a stimulus-driven "bottom-up" reorientation of attention to salient stimuli [43]. An altered connectivity between the VAT and the auditory and visual cortices in patients with bothersome tinnitus has recently been shown [44]. Furthermore, the insula has been reported to be part of a salience network [45], and both the IFG and the anterior insula are supposed to be involved in conflict processing [46]. If tinnitus is perceived as a permanent salient stimulus, it continuously attracts attention and conflicts with other salient stimuli. It is therefore not surprising that, as part of the VAT, alterations in the structure [15, 47] and function [10] of the IFG have been repeatedly reported in tinnitus research. While the insula is also a part of the VAT, it additionally plays an important role as part of a nonspecific distress network [11]. A relation between the insula and tinnitus distress has been consistently found in EEG studies [48, 49] and in studies examining structural brain alterations; decreased GM volume in the insula was reported in highly distressed patients [13] as well as a positive correlation between tinnitus distress and the cortical thickness in the anterior insula [19].

Notably, the GM decreases in the IFG and the insula seen in the current study were observed for the whole group independently of treatment outcome, indicating that these changes are rather related to the intervention than related to its clinical effect. The same is true for the remaining GM decreases observed. While GM alterations in the left temporal pole and the ventromedial prefrontal cortex were only visible in the small sample and are therefore not further discussed, the GM decrease in the inferior and middle temporal gyrus was only seen in the overall sample and—on a more relaxed statistical threshold—in the $n = 41$ sample. Again, the $n = 36$ sample showed the same tendency (see Figure 3) but not in a significant degree. Similar to the IFG and the insula, the medial temporal cortex has been previously reported to show functional alterations in tinnitus patients [10, 50]. However, GM changes in the medial temporal cortex might be rather linked to hearing loss than linked to tinnitus [14] and the same might be true for the inferior temporal cortex. Again, the morphological changes observed in the current study are not correlated with changes in the TQ or loudness scores. These results clearly suggest that rTMS leads to GM changes indeed but that these changes are an expression of "treatment" rather than an expression of "treatment outcome." All in all, those results are to be seen as preliminary and replications are clearly needed as the GM decreases were only statistically significant in the overall sample and one subsample but not in the second, smaller subgroup of 36 patients.

Besides the GM decreases reported above, no grey matter increases were found from baseline to day 12—a finding which is not in line with the results of May et al. [30] who found GM increases in the left superior temporal area after 5 days of rTMS stimulation of the temporal cortex. The absence of such a GM increase in the current study is presumably not a problem of too little statistical power as it was found neither in the subsamples nor in the larger sample with 77 patients. One of the main differences between the current study and the study of May et al. is that the latter applied rTMS to healthy subjects while we used rTMS as a treatment for patients with subjective tinnitus. Maybe, tinnitus brains react differently to low-frequency magnetic stimulation in comparison to control subjects. Knowing that there are both structural and functional alterations in the tinnitus brain in comparison to healthy controls [8, 9] and knowing that the effect of 1 Hz-rTMS is state-dependent [26, 51] the different study outcomes might be reconcilable.

4.2. Grey Matter Changes from Baseline to Day 90. Interestingly enough, no GM decreases (nor increases) were seen from baseline to day 90 which suggests that the decreases seen on day 12 are temporary in nature. This observation is in line with the results of May et al. who also found that the changes induced by rTMS are transient [30]. It remains to be seen at which point in time the regression of the GM changes happens exactly. Whether the observed transient nature of the rTMS effect on GM may also reflect a transiency of clinical effects of rTMS treatment should be explored in further

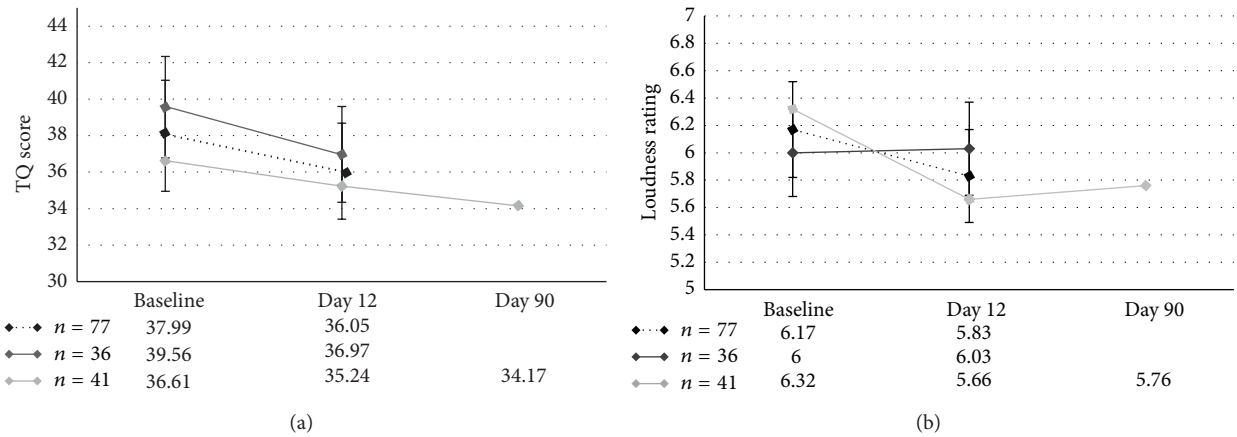

Figure 1: Line chart showing the time course of the TQ scores and the loudness ratings for both independent subgroups and the overall group.

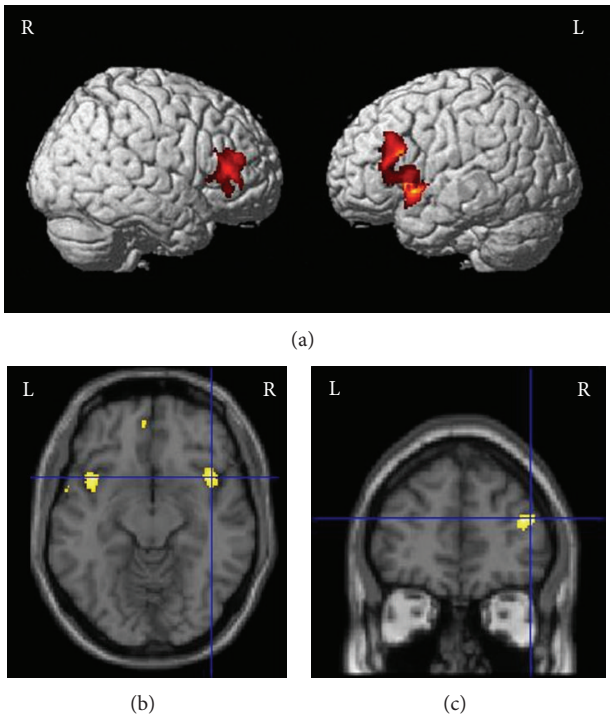

Figure 2: Grey matter decreases from baseline to day 12 in (a) the right and left inferior frontal gyrus and (b) the insula bilaterally. (c) Positive correlation of the TQ difference with the GM concentration at baseline in the right frontal gyrus.

studies. Notably, previous long-term follow-up investigations in tinnitus patients have suggested long-lasting effects over periods of up to four years in the majority of rTMS responders [52, 53].

4.3. Grey Matter Changes and Clinical Outcome. Obviously, rTMS treatment of the temporal cortex leads to alterations in cortical regions known to be important for subjective tinnitus. These alterations do not seem to directly cause change in tinnitus distress though. As we investigated 77 patients, the lacking correlations do probably not arise from too little statistical power. Rather, it has to be considered that VBM might not be a method sensitive enough to capture neural changes that are related to the slight change of tinnitus distress or loudness which can be obtained using rTMS. This might be different for TMS treatment protocols with larger treatment effects and this might also be different for neuroimaging methods more sensitive to function rather than structure—such as fMRI or EEG. The only study investigating functional changes induced by rTMS using fMRI measurements could in fact not detect a relationship between changes in brain activity and clinical outcome [26]. However, with only six patients the study might have lacked the required power to detect such an effect.

Taken together, the key message is that rTMS treatment of tinnitus patients affects brain structures different to the stimulation site which points to the importance of interconnections between distant cortical areas. It is well-known that TMS effects are not limited to the stimulated area and that functional changes can also be seen in remote cortical brain areas [54, 55]. What is true for functional changes might also be right for structural changes. While May et al. [30] found GM increases in the stimulated area, they also reported the trend of GM increases in the temporal cortex contralateral to the stimulation site as well as in the thalamus bilaterally. Together with the results of the current study this emphasizes the importance of having in mind that magnetic stimulation of one cortical hotspot results in functional and presumably also structural alterations in a whole network of interconnected areas.

In summary, the bilateral alterations in the IFG and insulae after rTMS, although not seen on a significant level in the $n = 36$ group subgroup, further support the notion of functional connectivity between the left temporal cortex and the ventral attention network in tinnitus patients. Whereas rTMS induces transient alterations in these areas and also in the inferior and medial temporal cortex, these changes do not determine the clinical effects.

Table 3: Results of all VBM analyses.

Laterality	Anatomical region	Cluster size in voxels	MNI coordinates x	y	z	Peak voxel Z-score	Cluster level P value
	GM decrease from baseline to day 12 (n = 41)						
L	Temporal pole, insula, and inferior frontal gyrus	1121	−56	8	−18	4.93	<0.001
R	Insula (extending into temporal pole)	565	33	10	−18	4.79	0.001
R	Inferior frontal gyrus	475	51	33	12	4.49	0.009
L	Ventromedial prefrontal cortex	355	−4	52	−8	3.72	0.026
	GM decrease from baseline to day 12 (n = 77)						
L	Inferior frontal gyrus, insula	1439	−46	12	−5	4.41	<0.001
R	Insula (extending into temporal pole)	684	42	16	−11	4.44	0.001
R	Inferior frontal gyrus	616	51	34	12	4.74	0.001
L	Inferior/medial temporal gyrus	558	−57	−42	−17	4.21	0.045
	Positive correlation of TQ difference with baseline images (n = 36)						
L	Medial temporal pole	460	−32	6	−33	4.67	0.014
R	Posterior cingulate cortex	430	6	−45	31	4.19	0.036
	Positive correlation of TQ difference with baseline images (n = 77)						
R + L	Lingual gyrus	534	4	−72	0	4.49	0.037
R	Inferior/middle frontal gyrus	413	52	30	19	3.86	0.089

FWE-corrected at cluster level $P < 0.05$.
L, left; R, right; MNI, Montreal Neurological Institute.

4.4. Baseline Grey Matter Images as Predictor for Treatment Outcome. Concerning the question whether grey matter images can serve as predictors for treatment response, the current results suggest that there are some cortical areas in which patients who will benefit from rTMS treatment have less GM at baseline than patients who will not benefit. In the right IFG and the lingual gyrus bilaterally, a positive correlation between GM at baseline and the TQ change was detected which means that an improvement in the TQ (implicated by negative values) is related to less GM at baseline. These results were seen in the overall patient group and in tendency also in the n = 41 group. Though a positive correlation was also found in the left medial temporal pole and the right posterior cingulate cortex, these results were only visible in the n = 36 sample and are therefore not further discussed. As mentioned above, the right IFG is part of the VAT and important for attention shifts to salient stimuli. The question arises however, what "reduced GM volume in the right IFG" actually means in terms of the function of the VAT. One could speculate that the VAT had been less sensitive to salient stimuli (e.g., the tinnitus) prior to rTMS treatment. As a consequence, a reduction of tinnitus severity might have been easier to accomplish in those patients. This is speculation though and—after replication—a challenging question for future research. The lingual gyrus has never been reported to play an important role for subjective tinnitus. However, functional and structural alterations in nearby occipital regions have been observed in tinnitus patients [14, 56], even if one of those studies suggests that GM decreases in occipital regions might be rather due to hearing loss than due to tinnitus [14]. Overall, these findings have to be considered as preliminary as the mentioned correlations reached statistical significance only in the overall patient group but not in the two independent subsamples. Therefore, replications are needed to confirm those results. Furthermore, there is some evidence that patients who benefitted from treatment once also benefit from a second treatment phase [57–59]. For that reason, future studies should also try to shed light on the question whether there are characteristics in the brain which predispose an individual to benefit from rTMS treatment in general while others do not.

4.5. Limitations. The current study has a number of limitations which should be considered in future studies. First, as just mentioned, hearing level was not available for all patients and could therefore not be integrated in the analyses. Although hearing loss is not supposed to be a predictor for response to rTMS treatment [24], previous studies have shown that hearing loss is an important confounder concerning GM changes in tinnitus patients [14, 60, 61]. To be able to thoroughly interpret research results, future work should try to include pure tone audiogram including high frequency audiogram [14, 60, 61] for all patients. Second, the lacking correlation between treatment outcome and GM changes might have been due to the small treatment effects. As already known from previous studies, the effect of rTMS treatment is small. Therefore, an even higher number of patients might have been necessary to ensure sufficient power for all analyses. The third and main limitation of the current study is the lack of a placebo condition. Without a patient group treated with sham stimulation we cannot definitely determine whether the observed GM changes were specific to rTMS treatment or unspecific effects. In the study of

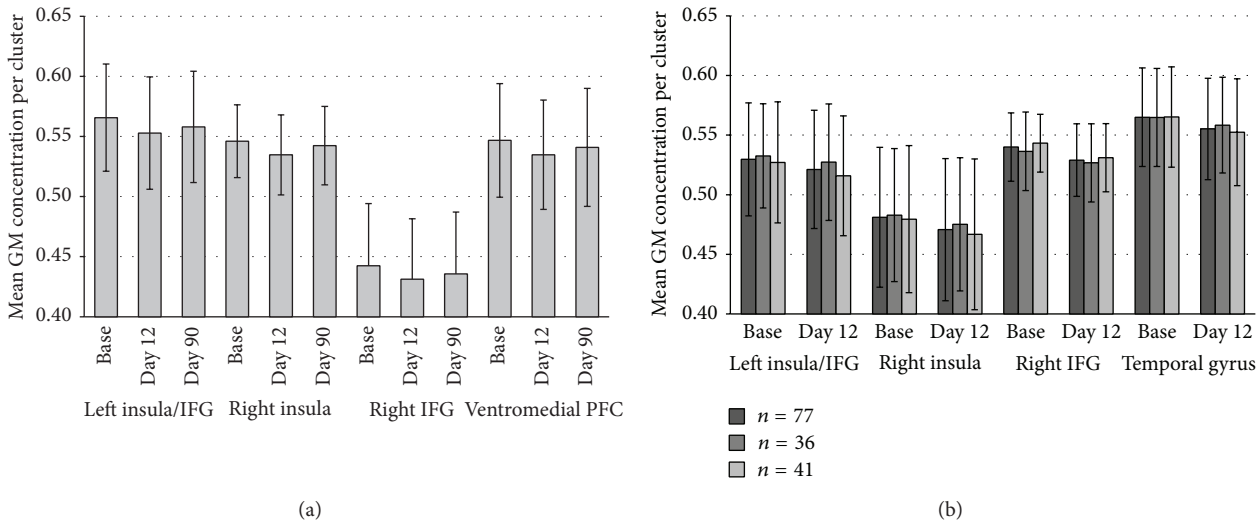

Figure 3: Mean grey matter concentration for each time point for the clusters with significant GM changes in (a) the subgroup of 41 patients and (b) the total group of 77 patients. For the clusters of (b) the mean GM concentration is also shown for the two independent subgroups.

May et al. [30], healthy control subjects showed no GM changes after sham rTMS as opposed to subjects treated with active rTMS. This finding has not been replicated for tinnitus patients yet.

5. Conclusions

To the best of our knowledge, this is the first study to combine clinical assessment and longitudinal structural MRI scans to measure rTMS effects in tinnitus patients. The major result of the study is that ten days of low-frequency rTMS treatment of the temporal cortex leads to transient GM decreases in cortical regions different from the stimulated area. This highlights the importance of considering that the brain is organized in networks and that this organization highly influences the outcome of an intervention. Transient GM decreases were seen bilaterally in the insula, the IFG, and the left inferior/middle temporal gyrus, indicating functional connectivity between the stimulation site in the left temporal cortex and the ventral attention network in tinnitus patients. Although these cortical areas are known to be important in the generation and maintenance of tinnitus, the GM decreases were independent of treatment success. Thus, they were rather related to the TMS intervention per se and not to its clinical effect. However, treatment outcome correlated with GM at baseline indicating reduced GM in the right IFG and the lingual gyrus in patients benefiting from treatment. Thus, baseline GM images might hold potential to be further investigated as predictor for rTMS response in the future.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Reduced Variability of Auditory Alpha Activity in Chronic Tinnitus

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Subjective tinnitus is characterized by the conscious perception of a phantom sound which is usually more prominent under silence. Resting state recordings without any auditory stimulation demonstrated a decrease of cortical alpha activity in temporal areas of subjects with an ongoing tinnitus perception. This is often interpreted as an indicator for enhanced excitability of the auditory cortex in tinnitus. In this study we want to further investigate this effect by analysing the moment-to-moment variability of the alpha activity in temporal areas. Magnetoencephalographic resting state recordings of 21 tinnitus subjects and 21 healthy controls were analysed with respect to the mean and the variability of spectral power in the alpha frequency band over temporal areas. A significant decrease of auditory alpha activity was detected for the low alpha frequency band (8–10 Hz) but not for the upper alpha band (10–12 Hz). Furthermore, we found a significant decrease of alpha variability for the tinnitus group. This result was significant for the lower alpha frequency range and not significant for the upper alpha frequencies. Tinnitus subjects with a longer history of tinnitus showed less variability of their auditory alpha activity which might be an indicator for reduced adaptability of the auditory cortex in chronic tinnitus.

1. Introduction

Subjective tinnitus is characterized by the conscious perception of a sound in the absence of a corresponding physical source. This auditory phantom sound is usually described as a pure tone, a hissing, or a roaring noise. Most of the people suffering from tinnitus report that the tinnitus sound is an ongoing and continuous perception which is typically more prominent in silent environments. Resting state measures in a silent environment should therefore be a useful tool to investigate the aberrant brain activity associated with the tinnitus. Comparison of resting brain activity of tinnitus patients and healthy controls under silent conditions should reveal the abnormal brain activity that is related to both tinnitus perception and tinnitus-associated distress.

Tinnitus-related alterations of resting state activity have indeed been demonstrated by several studies using electroencephalographic (EEG) and magnetoencephalographic (MEG) recordings. Changes in auditory areas of tinnitus sufferers comprise enhanced gamma activity [1–4], enhanced slow wave activity [1, 4–6], and reduced alpha activity [5]. The relation between these changes of spontaneous brain activity and the tinnitus has been further strengthened by longitudinal studies providing evidence that a temporary or long-lasting reduction of tinnitus symptoms is associated with a normalization of this abnormal brain activity. For instance, Adamchic and colleagues showed that reduced tinnitus severity after coordinated reset treatment relates to reduced delta and gamma power in temporal areas [7]. Kahlbrock and Weisz observed reduced auditory delta power during episodes of residual inhibition [8]. Müller and colleagues demonstrated increased auditory alpha power following successful tinnitus reduction with repetitive transcranial magnetic stimulation (rTMS) [9] and normalization of the delta and alpha power by means of neurofeedback has been shown to reduce tinnitus symptoms [10, 11].

In the current study, we sought to further investigate alpha activity in auditory areas of subjects with chronic

tinnitus perception. It has been hypothesized that the oscillatory activity of the alpha frequency range (8–12 Hz) in sensory regions of the human brain might represent a gating mechanism for incoming information that is not relevant to the subject and is therefore actively suppressed [12, 13]. It has been proposed that this reduced alpha power reflects a state of desynchronized neuronal networks, which is associated with auditory attention [14, 15]. In a normal hearing participant, this desynchronized state would be temporary and would enable neuronal couplings driven by correlated stimulus attributes. In tinnitus, however, the persisting desynchronization may be a consequence of ongoing auditory attention.

A study on visual attention demonstrated an increase of alpha power in parietooccipital regions ipsilateral to the attended side together with a decrease of alpha power at the contralateral side [16]. Furthermore, Thut and colleagues showed that the amount of this prestimulus hemispheric alpha lateralization is correlated with the reaction time of the participants [17]. In a somatosensory discrimination task, Haegens et al. were able to demonstrate that prestimulus alpha lateralization is associated with the cued target location and the reliability of the cue [18]. In a cross-modal paradigm in which subjects had to switch between visual and auditory targets, Mazaheri and colleagues recently showed that the prestimulus alpha activity switches between the respective sensory brain areas depending on the stimulus modality [19]. Taken together, all these results indicate an involvement of alpha oscillations in the active suppression of irrelevant and potentially distracting sensory information.

This view on alpha activity is complemented by another line of research investigating spontaneous fluctuation of cortical alpha activity. Romei and colleagues measured the spontaneous alpha fluctuation over visual areas and applied transcranial magnetic stimulation to evoke phosphenes. Trials with a conscious perception of the phosphene were associated with reduced visual alpha activity in the visual cortex [20] which suggests an enhanced excitability of the visual cortex during moments with low alpha power. Similarly, another study showed that the detection of visual stimuli near the perceptual threshold was more reliable during episodes of alpha desynchronisation than during episodes of alpha synchronisation [21].

Our knowledge about the underlying neuronal mechanisms for these spontaneous fluctuations in the resting state is currently still relatively limited. Nevertheless, evidence is accumulating that the variability of brain activity from one moment to the other is of functional importance for the central nervous system [22]. Measuring the variability of the blood oxygen level-dependent (BOLD) signal, Garrett and colleagues demonstrated that the moment-to-moment variability increases when participants are engaged in a task rather than being in the resting state. Furthermore, participants that are performing the task faster than average usually show larger variability of their BOLD signals [23]. In another study investigating the variability of EEG phase synchronization in children with acute traumatic brain injury (TBI), Nenadovic et al. reported that children with greater variability have higher chances for recovery from TBI [24]. Measurements of brain signal variability in other pathologies like Alzheimer's disease [25] and autism [26] also show large reductions, when patients are compared with healthy control groups.

A conceptual framework for these findings is provided by the notion that variability in cortical processing is an essential feature of learning [27]. According to this theory cortical map expansion occurs during learning processes for increasing processing capacities in order to enable replication with variation. Analogous to a Darwinian mechanism the behaviourally most useful circuit is then selected and consolidated [27].

Taken together, it can be hypothesized that disease-related alterations of neuronal plasticity should be reflected by alterations in the variability of neuronal activity. Reduced variability may indicate a reduction of the dynamic range of brain response and impaired neuroplastic capacity. Here we aimed to investigate whether the moment-to-moment variability of auditory alpha activity in tinnitus is altered as compared to controls. For this purpose, we used magnetoencephalographic recordings in the resting state and compared signal variability between subjects with chronic perception of tinnitus and healthy controls. Our hypothesis was that the variability of auditory alpha activity is reduced in individuals with tinnitus.

2. Materials and Methods

2.1. Participants. Data of 42 subjects who participated in different studies [28–31] were analysed retrospectively. All participants were right-handed according to the Edinburgh Handedness Inventory [32]. Twenty-one of the participants reported an ongoing perception of tinnitus for more than 6 months (mean duration: 4 years; standard deviation: 3.3 years; range: 5–12 years). Tinnitus-related distress was measured with the German version of Tinnitus Questionnaire [33] average distress of 21.5 points (standard deviation: 16.8; range: 3–59). The mean age of the tinnitus group was 44.4 years (standard deviation: 14.8 years; range: 22–69 years; 6 female). The data of the tinnitus group was compared to an age- and gender-matched healthy control group (mean age: 43.7 years; standard deviation: 15.3 years; range: 22–69 years; 6 female). All participants gave informed written consent and the study was approved by the ethical review board of the University of Konstanz.

2.2. Data Acquisition. Spontaneous brain activity was recorded using a whole-head MEG system with 148 magnetometer (MAGNES TM 2500 WH, 4D Neuroimaging, San Diego, USA) at a sampling rate of 678.17 Hz or 2034.51 Hz and a hard-wired high pass filter of 0.1 Hz. For data analysis, all data were downsampled to 600 Hz. Participants were instructed to relax in supine position with eyes open and fixating a point at the ceiling of the measuring chamber and not to engage in any deliberate mental activity. Five minutes of resting state data were analysed.

2.3. Data Analysis. The Matlab-based FieldTrip toolbox was used for analysing the MEG data [34]. Prior to data analysis, the MEG channel positions were realigned towards standard magnetometer positions for each individual subject [35]. A discrete Fourier transform (DFT) filter was used to reduce the 50 Hz line noise. The continuous data set was cut into epochs of two seconds and those epochs containing artefacts were manually excluded from data analysis. Following the artefact correction, we selected randomly a number of 90 epochs (i.e., 180 seconds of resting state data) from the remaining trials in order to make sure that the same amount of MEG data was used for all subjects.

We calculated the time-frequency representation of the spontaneous recordings from 1 to 100 Hz with an increment of 0.5 Hz. For each of the 90 trials, the MEG data was multiplied with a Hanning window before applying the fast Fourier transform.

2.4. Coefficient of Variation Computation. In order to measure the moment-to-moment variability of alpha power, we calculated the coefficient of variation (CV) across the 90 trials for each individual data set and each sensor [22]. The coefficient of variation for the frequency f and the trial t is defined as the ratio of the standard deviation $\sigma_{f,t}$ to mean $\mu_{f,t}$:

$$\mathrm{CV}_{f,t} = \frac{\sigma_{f,t}}{\mu_{f,t}}. \quad (1)$$

Thereby, the coefficient of variation expresses an unbound measure of the variability which is independent from the magnitude of the mean.

2.5. Statistical Analysis. Statistical analyses including the mixed models analysis of variance (ANOVA) were carried out using the open source R statistical software package available at http://www.r-project.org/ including the *nlme* library. Comparison between the tinnitus and the control group was performed with a mixed models ANOVA allowing a random intercept for each participant.

3. Results

3.1. Alpha Power Reduction in Tinnitus. In the first step of the analysis, we intended to reproduce the finding of reduced alpha activity in chronic tinnitus as proposed by previous studies [5]. While the study by Weisz et al. only investigated the reduction in the source space, we here show that the effects are also significant on the sensor level. Figure 1(a) shows the normalized power spectrum for the tinnitus and the control group averaged over all sensors. In Figures 1(b)–1(d) we illustrate the topographical map for each group and the group difference. For the analysis of the spectral power over auditory regions, regions of interest (ROI) were defined in order to cover the brain regions showing the strongest difference in alpha desynchronization between the tinnitus and the control group. Therefore, the ROI selection was based on the group difference in the 8–10 Hz frequency range (Figure 1(b)). Spectral power of the left and the right temporal areas were averaged for the analysis. The same ROIs were used for all of the following analyses. The analysis was done for the lower alpha band (8–10 Hz) and the upper alpha band (10–12 Hz) separately. A linear mixed models analysis of variance (ANOVA) for the lower alpha band revealed a significant group difference with $F(1, 40) = 9.58$ and a P value of $P = 0.0036$. The ANOVA for the upper alpha band revealed with $F(1, 40) = 3.86$ and $P = 0.056$ a trend towards significance. The group differences for the lower and upper alpha power are depicted in Figures 2(a) and 2(b).

3.2. Variability of Auditory Alpha Activity. In order to analyse the variability of alpha power, we calculated a spectral analysis for each single trial. To illustrate the alpha variability we selected an example control subject and plotted the temporal alpha power for all 90 trials in Figure 3. Topoplots for selected trials are shown above. Please note that this graph does not necessarily show a continuous timeline of temporal alpha activity since some trials in between might have been rejected during artifact correction and only 90 trials have been randomly selected. It rather demonstrates the relatively large variability of alpha activity from one trial to the other.

In order to measure this variability, coefficients of variation (CV) have been calculated for each subject. Statistical comparison of the CV in both groups indicated a strong reduction of temporal alpha variability in the tinnitus group. The analysis was done for the lower alpha band (8–10 Hz) and the upper alpha band (10–12 Hz) separately. A linear mixed models analysis of variance (ANOVA) for the lower alpha band revealed a significant group effect with $F(1, 40) = 9.04$ and a P value of $P = 0.004$. For the upper alpha band, the group difference was not significant with $F(1, 40) = 1.90$ and $P = 0.18$. Group differences for both alpha bands are illustrated in Figure 4.

3.3. Longer Tinnitus Duration Is Associated with Reduced Auditory Alpha Variability. Further analysis of the alpha variability in the tinnitus group suggested a nonlinear relationship between the variability and the duration of the tinnitus. In subjects with a shorter duration of tinnitus we measured larger alpha variability than in subjects with longer tinnitus duration (see Figure 5). A nonlinear function was fitted to the data explaining the auditory alpha variability by 1/tinnitus duration + a, with an estimate for a of 2.38 ($t = 6.28$, $P < 0.001$). Furthermore, a median split was used to divide the group in tinnitus subjects with a shorter (less than 3 years) and longer history of tinnitus (more than 3 years). A Welch two sample t-test revealed a significant lower variability measures for the group with the longer tinnitus duration: $t = 2.3$, P value = 0.038. No association was found between the alpha variability and the tinnitus-related distress ($P > 0.7$) or the age of the subjects ($P > 0.2$).

4. Discussion

In the current study, we were able to repeat previous results by demonstrating reduced alpha activity in temporal regions in people with tinnitus as compared to healthy controls. In

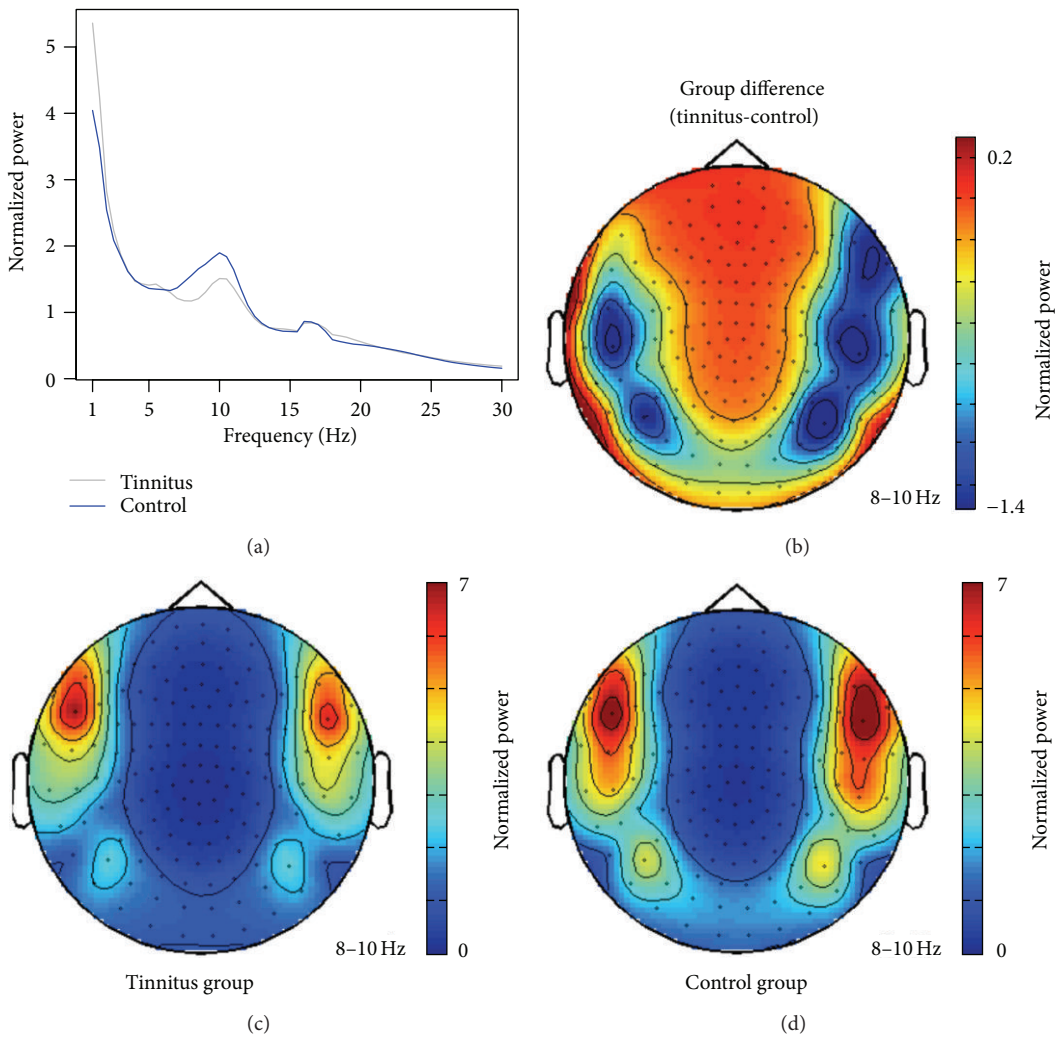

Figure 1: Normalized power. (a) illustrates the normalized global power averaged over all sensors. (b)–(d) show the topographical distribution of the low-frequency alpha power (8–10 Hz) for the group difference (tinnitus minus control, (b)), the tinnitus group (c), and the control group (d).

addition to former studies we differentiated in the current analysis between lower and higher alpha power and found evidence that this power reduction is more pronounced in the lower alpha frequency range (8–10 Hz) than in the upper alpha frequency range (10–12 Hz). Furthermore, we were able to show that the moment-to-moment variability of auditory alpha activity is significantly decreased in chronic tinnitus subjects. Again, this effect was more prominent in the lower than in the upper alpha frequency range. Moreover, alpha variability was more reduced in patients with a longer history of tinnitus.

Oscillatory activity in the alpha frequency range can be detected in all sensory areas and is by far the strongest oscillation that can be observed in the human brain [5, 15, 17–19]. It has been shown that episodes with enhanced alpha activity in sensory areas are characterized by reduced excitability in the respective sensory modality, while episodes with low alpha activity (i.e., alpha desynchronization) are associated with enhanced neuronal excitability of this area [20, 21, 36, 37]. In this context, electro- und magnetoencephalographic recordings of auditory activity over sensory areas can be interpreted as a measurement of a neuronal mechanism gating sensory information processing. Increases in alpha power recordings can therefore indicate suppression of sensory input that is currently not needed or even distracting, while reductions of alpha power suggest increased excitability of the sensory area for a more precise perception of potentially important sensory input. The link between enhanced neuronal excitability and reduced alpha power is currently not well established. Recently, it has been shown that the locally enhanced neuronal excitability can be also characterized by increased functional coupling with remote brain areas ([38]; see also Weisz and Obleser for a theoretical framework [39]), meaning that the respective sensory area is "ready" to receive information from distant brain regions via already established functional connections. Therefore, it

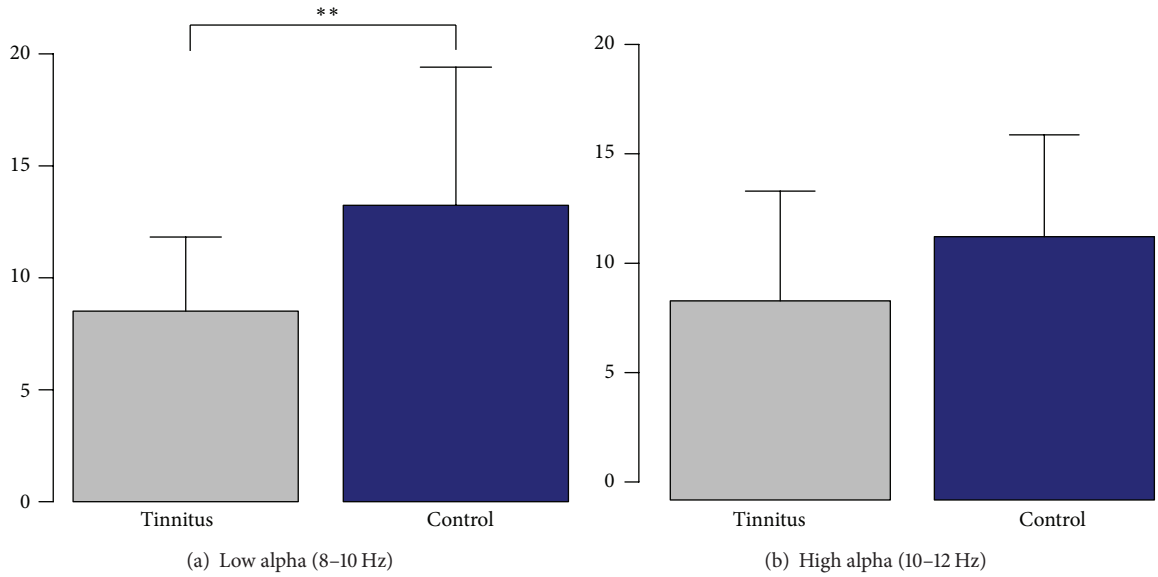

Figure 2: Alpha power group comparison. The bar plots demonstrate the group difference for the temporal alpha power for the lower alpha band from 8 to 10 Hz (a) and the upper alpha band from 10 to 12 Hz (b). The group difference is only significant for the lower alpha frequency range with $P = 0.0036$.

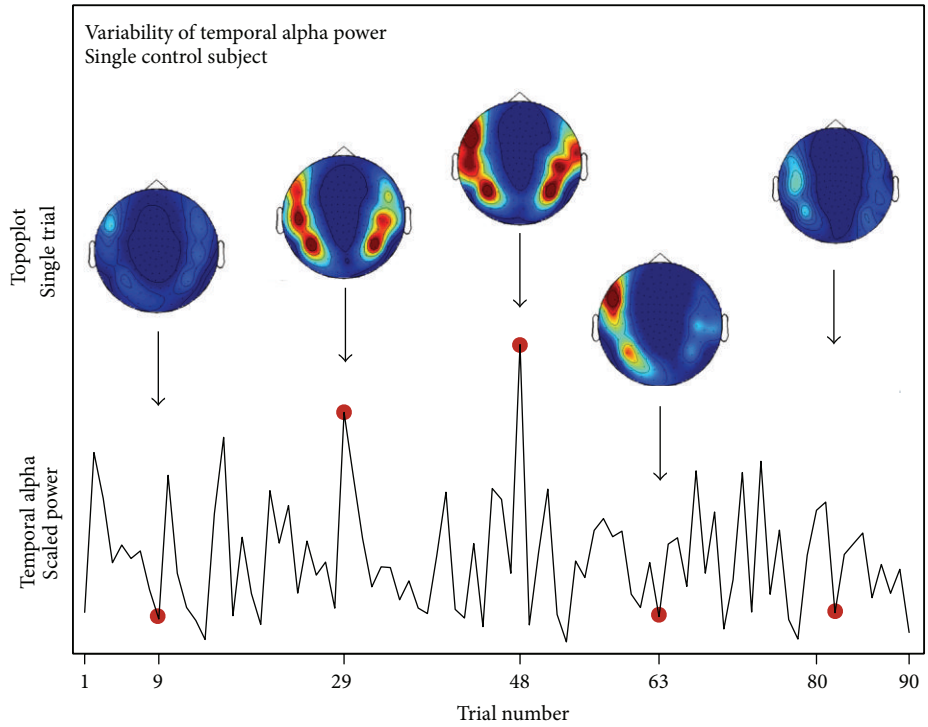

Figure 3: Illustration of the alpha moment-to-moment variability. Data are shown for an example control subject. Bottom: the variability of the temporal alpha activity is shown for the 90 trials. Top: topographical maps are plotted for five selected trials.

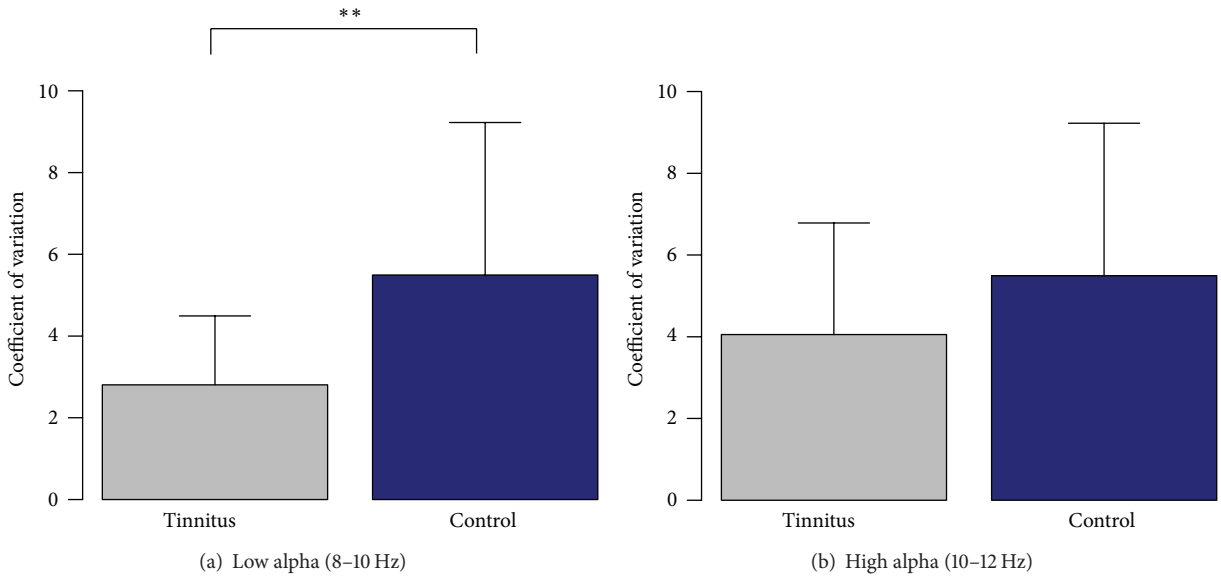

Figure 4: Alpha variability group comparison. The bar plots demonstrate the group difference for the variability of the temporal alpha power. Coefficients of variation are shown for both groups, for the lower and upper alpha band separately. Group differences are only significant for the lower alpha band from 8 to 10 Hz with $P = 0.004$.

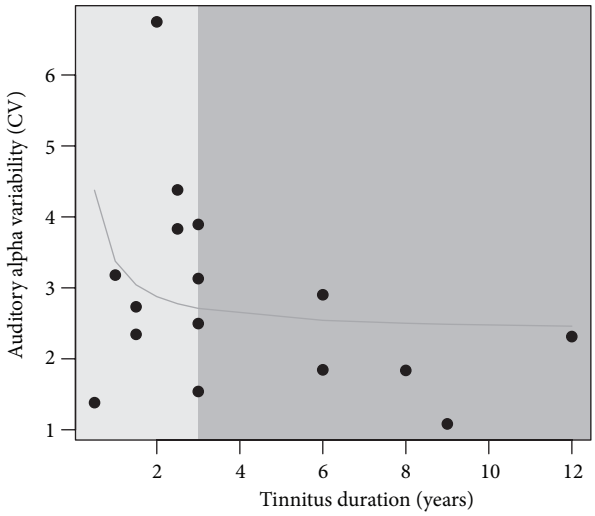

Figure 5: Association between tinnitus duration and alpha variability. Patients with a longer duration of tinnitus show lower levels of auditory alpha variability (8–10 Hz). A median split was calculated and revealed significant difference in the variability between patients with a long and a short duration of tinnitus with $P = 0.038$.

might be that the alpha desynchronization is just one indicator of the enhanced neuronal excitability; the integration in a distributed brain network might be another. How this state of enhanced excitability in the auditory areas is triggered in tinnitus remains a debate. Several explanations are possible and might also depend on individual patient characteristics: (1) top-down attention to the auditory stream might trigger this state (e.g., in patients that routinely "check" if their tinnitus is still there), (2) a mismatch between the auditory phantom perception and the environment without a physical source for it might enhance the excitability in order to dissolve this mismatch, or (3) bottom-up mechanisms might also trigger regularly and/or constantly the excitability state. Here, we used the reduced temporal alpha activity as a marker for the enhanced neuronal excitability. The recordings were done during resting state in a quite environment with no relevant auditory stimulation. During this resting state, we recorded strong alpha activity over temporal areas in healthy control subjects indicating reduced excitability of the auditory cortex. In tinnitus patients, however, we recorded reduced alpha activity over auditory areas indicating enhanced neuronal excitability.

With this study we showed that auditory alpha activity is variable and fluctuates from one moment to the other. The variability was significantly reduced in the tinnitus group. This result is in line with other research showing dynamic changes of brain activity under rest [22, 40, 41]. The dynamic change of alpha activity in sensory areas reflects thereby a variability of states with enhanced and reduced excitability. It has been hypothesized that this variability is beneficial to the system insofar as that it increases the dynamic range permitting more different responses to a broader range of incoming stimuli which finally leads to greater adaptability [22]. The current results show that the variability of auditory alpha activity is reduced for the tinnitus group. Furthermore, in recordings of tinnitus subjects with longer tinnitus duration even less variability was detected. Due to the cross-sectional design of the study we cannot distinguish whether the reduced variability reflects the predisposition to develop tinnitus or the consequence of tinnitus. Thus, if the reduced

moment-to-moment variability of brain activity represents a trait-marker reflecting reduced adaptability of the nervous system, one could conclude that only subjects with reduced alpha variability can enter the state of chronic tinnitus perceiving the phantom sound for many years. Tinnitus subjects with greater variability might be able to adapt to the tinnitus, which results in a spontaneous remission of the symptoms. Therefore, we do not see tinnitus subjects with great signal variability and a long history of tinnitus. If this hypothesis is true, the alpha variability should represent an indicator for spontaneous tinnitus remission.

The second explanation favours neuroplastic changes of the auditory cortex as a consequence of the chronic tinnitus perception over the years. The continuous auditory phantom perception might attract the attention to the auditory stream leading to an ongoing enhancement of excitability in the auditory cortex. This long-term potentiation might lead to plastic changes in auditory areas that finally reduce the variability of auditory alpha activity in the long run. Whether reduced alpha variability in tinnitus represents the predisposition or the consequence of tinnitus or in other words a trait or state marker should be addressed by longitudinal studies.

This pilot study which investigates for the first time the moment-to-moment variability of oscillatory brain activity in tinnitus patients has several limitations. First, the analysis focussed on alpha activity in temporal brain areas, because the reduced alpha activity in the auditory cortex is the most robust neuroimaging finding in tinnitus. However, alterations in other frequency bands [1–4] and in other brain areas [42–44] have been documented as well. Thus further studies should investigate variability in other frequency bands and other brain areas.

The variability measure that we used in this study was normalized to the mean power. Since we observed both reduced mean alpha power and reduced variability in the tinnitus group we can exclude that our finding of reduced variability is an artefact resulting from an increase in the mean alpha. Nevertheless, the used procedure is just one possibility to quantify the variability of neuronal oscillations.

In this study the control group was age- and gender-matched, but the groups were not matched for comorbidities of tinnitus like hearing loss, depression, or hyperacusis, which were not assessed in the whole study population. Therefore, we cannot rule out hearing loss, depression, or hyperacusis as alternative explanations for the reduced alpha variability. Therefore, further studies are needed which control for those potential confounding factors.

Our study has also revealed that both the reduction of alpha power and alpha variability was mainly driven by low alpha activity (8–10 Hz). This finding is new and somewhat unexpected and requires confirmation by investigations in independent samples.

5. Conclusions

The current study supports the idea of reduced auditory alpha activity in chronic tinnitus patients. Based on the concept that alpha activity reflects the level of inhibitory influence on sensory regions this finding can be interpreted as enhanced excitability of the auditory cortex in tinnitus. Furthermore, we showed that the auditory alpha activity in healthy controls is dynamic and varies within the range of seconds. The moment-to-moment variability of auditory alpha in tinnitus subjects is significantly reduced with a tendency that subjects with a longer tinnitus duration show less variability. This might be an indicator for reduced adaptive potential of the auditory cortex in tinnitus patients and—if confirmed by further studies—has important implications for understanding the pathophysiological underpinnings of tinnitus. Moreover, the reduced variability might represent a potential therapeutic target for neuromodulatory treatment approaches, for example, by auditory [45] or brain stimulation [46].

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Playing and Listening to Tailor-Made Notched Music: Cortical Plasticity Induced by Unimodal and Multimodal Training in Tinnitus Patients

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Background. The generation and maintenance of tinnitus are assumed to be based on maladaptive functional cortical reorganization. Listening to modified music, which contains no energy in the range of the individual tinnitus frequency, can inhibit the corresponding neuronal activity in the auditory cortex. Music making has been shown to be a powerful stimulator for brain plasticity, inducing changes in multiple sensory systems. Using magnetoencephalographic (MEG) and behavioral measurements we evaluated the cortical plasticity effects of two months of (a) *active* listening to (unisensory) versus (b) learning to play (multisensory) tailor-made notched music in nonmusician tinnitus patients. Taking into account the fact that uni- and multisensory trainings induce different patterns of cortical plasticity we hypothesized that these two protocols will have different affects. *Results*. Only the *active* listening (unisensory) group showed significant reduction of tinnitus related activity of the middle temporal cortex and an increase in the activity of a tinnitus-coping related posterior parietal area. *Conclusions*. These findings indicate that *active* listening to tailor-made notched music induces greater neuroplastic changes in the maladaptively reorganized cortical network of tinnitus patients while additional integration of other sensory modalities during training reduces these neuroplastic effects.

1. Introduction

Subjective tinnitus is an auditory perception in the absence of physical sources [1, 2]. While transient tinnitus lasts only a couple of seconds to a few hours, chronic tinnitus is an ongoing conscious perception of a sound for more than three months with low incidence of spontaneous remissions. Around 5–15% of the people in western countries suffer from chronic tinnitus affecting their quality of life, that is, sleep disturbance, work impairment, and psychiatric distress [3]. An investigation of the individual characteristics of tinnitus in 528 tinnitus patients by [4] showed that about 65% of the tinnitus patients suffer from tonal tinnitus.

Tinnitus perception is often associated with aging and hearing loss; it arises in auditory cortex, and the generation and maintenance have been associated with maladaptive reorganization of the auditory cortex [5]. Following certain tinnitus trigger events such as noise or stress, the central auditory pathway reorganizes itself, exhibiting excitatory-inhibitory network dysbalances and permitting increased spontaneous firing rates, burst firing, and neuronal hypersynchrony [6, 7]. Physiological studies in mice suggest that the most probable underlying mechanism of this reorganization consists of the loss of inhibitory drive to neurons, elicited by changes in glycinergic [8] and GABAergic [9] systems. Upregulations of glutamatergic and cholinergic systems may be involved as well [10, 11]. These changes affect several levels of the auditory pathway along with nonauditory centers comprising a network that includes posterior parietal, frontal, somatosensory, and limbic regions [12–14]. Importantly, the auditory cortex activity corresponding to the tinnitus frequency has been consistently shown to be enhanced and related to perceived tinnitus loudness [5, 15, 16].

Causal tinnitus therapies are not yet widely available, but recent neurophysiological studies indicate that modality appropriated training can reverse maladaptive cortical reorganization [17–19]. Recent MEG studies [20–23] indicate that short-term and long-term listening to spectrally "notched" music (tailor-made notch-music, TMNM) containing no energy in the frequency range at and around the individual tinnitus frequency can considerably reduce the tinnitus-related neuronal activity of primary and nonprimary auditory cortical structures and alleviate tinnitus perception through lateral inhibition. In the abovementioned studies that introduced this approach, TMNM did not include active engagement with the music. Instead, the task of the tinnitus patients during the training was merely to listen to their favorite music. Nevertheless, attention plays an important but still unclear role in tinnitus perception [24–27] and the corresponding change in cortical plasticity [28].

Music playing is a highly complex task. It involves almost all sensory systems as well as the motor system and requires high amount of precision and accuracy with regard to the coordination and integration of the different sensory systems [29]. Therefore, extensive music training induces plastic changes in the human brain on both functional [30] and structural [31] levels. Recent studies indicate that even short-term (1-2 weeks), laboratory controlled music training can induce cortical plasticity [32] while its multisensory component plays a crucial role increasing the resulting plasticity effects [33, 34].

Therefore the goal of this MEG and behavioral study was to compare the neuroplastic effects of uni- and multimodal music trainings by manipulating the focus of attention. Two groups of nonmusicians suffering from chronic, tonal tinnitus were investigated. In the multimodal group, subjects were trained to play simple melodies on a tablet computer accompanying preset music songs. Thus, their attention was almost equally divided to all sensory systems involved in the task: visual, sensory-motor, and auditory. In contrast, in the unimodal group, high degree of attentional demands was introduced by asking the subjects to detect small auditory variations in repeated runs of the songs. Hence, the *focus* of attention was either solely in the listening (auditory modality, unimodal group) or divided to the somatosensory, visual, and auditory modality (multimodal group). The music of both groups was filtered in real time over headphones with a notch filter surrounding the individual tinnitus frequency. Results were evaluated using neurophysiological and behavioral pre-, during, and posttraining measurements.

2. Materials and Methods

2.1. Subjects. Twenty-six tinnitus patients were recruited by advertisement in local newspapers. Informed consent was obtained by procedures consistent with the principles of the Declaration of Helsinki and approved by the Ethics Commission of the Medical Faculty, University of Münster. In order to participate in the study subjects had to fulfill the following criteria with respect to their tinnitus: (i) chronic (≥3 months), single stable (no pitch fluctuation) tonal tinnitus perception (beep- or whistle-like), (ii) tinnitus frequency ≤8.5 kHz (to ensure unrestricted sound stimulation), (iii) age ≤ 65 years, (iv) no severe hearing impairment (hearing loss ≤ 55 dB HL in the frequency range from 0.125 to 8.5 kHz), (v) no psychological or neurological diseases, (vi) no current alcohol or drug abuse, (vii) no parallel tinnitus treatment, and (viii) no training in playing an instrument. Subjects were pseudorandomly assigned to the unimodal (listening) or multimodal (playing) group. For homogeneity matching the following criteria were also considered: (i) tinnitus pitch, (ii) time since tinnitus onset, (iii) age, (iv) hearing loss, (v) subjective tinnitus loudness, (vi) Tinnitus Questionnaire (TF) [35], and (viii) Symptom Check List SCL 90R total score [36].

Over the course of the study, three subjects dropped out due to lack of time for training; one subject had the impression of possible tinnitus worsening; thus the dropout rate per group was playing group (2/13) and listening group (2/13). Three subjects were not included in the MEG analyses due to extensive hearing loss that did not allow sufficiently loud auditory stimulation: playing group (1/11) and listening group (2/11). Finally, 19 subjects completed the 3-month study (2 months of music training and 1 month followup) and were included in the MEG-data evaluation: playing group $n = 10$ and listening group $n = 9$.

On average (mean ± SD), the two groups did not differ significantly in age (46.3 ± 11.66 years, range 23–64 years, $P = 0.78$) and average hearing loss (19.93 dB SL ± 12.41; range 5–55 dB SL, $P = 0.66$) or the tinnitus characteristics (i) duration (35.42 ± 14.20 years; range 14.49–56.15 years, $P = 0.81$), (ii) frequency (5.954 kHz ± 2.136; range 1–8.5 kHz, $P = 0.86$), (iii) loudness estimate of tinnitus (55.64 ± 26.23; range 16–99; scale 0–100, $P = 0.13$), or tinnitus-related distress in the Iowa tinnitus handicap questionnaire total score (27.78 ± 15.77; range 5.93–57.78, $P = 0.69$) and in the SCL-90-R (0.32 ± 0.25; range 0.03–0.84, $P = 0.8$). The abovementioned characteristics of the sample are summarized in Table 1. Most of the subjects reported bilateral tinnitus (bilateral: $n = 17$; left lateralization: $n = 2$; right lateralization: $n = 3$).

2.2. Design. The design of the study comprised three parts. The first part (baseline) lasted 2 weeks and included 2 weekly measurements of the subjective tinnitus characteristics. The second part lasted 2 months and included, for both groups, one hour of daily training (described in detail below), 8 weekly measurements of the subjective tinnitus characteristics, and 3 MEG recording sessions: one prior to the training, one after one month of training, and one after completion of the training. The third part lasted one month and included 4 weekly measurements of the subjective tinnitus characteristics as a followup. An illustration of the design is shown in Figure 1.

During the performance of the experiment a tablet computer with a touch screen (iPad-II, Apple Inc) was provided to each patient including a music application (ThumbJam https://itunes.apple.com/us/app/thumbjam/id338977566?mt=8) that served as the basis for the musical

Table 1: Characteristics of the sample of the study.

	Group	Mean	SD	SEM
Age (y)	Playing	45.55	12.00	3.79
	Listening	46.97	11.86	3.43
Hearing loss (dB)	Playing	18.60	11.25	3.56
	Listening	21.04	13.69	3.95
Duration (y)	Playing	34.61	11.89	3.76
	Listening	36.09	16.38	4.73
Pitch (hz)	Playing	5865.00	2094.18	662.24
	Listening	6029.17	2261.18	652.75
Subjective loudness	Playing	46.41	24.08	7.62
	Listening	63.33	26.40	7.62
THI	Playing	26.20	20.01	6.33
	Listening	29.67	19.78	5.71
SCL90_R	Playing	0.34	0.26	0.08
	Listening	0.31	0.26	0.07

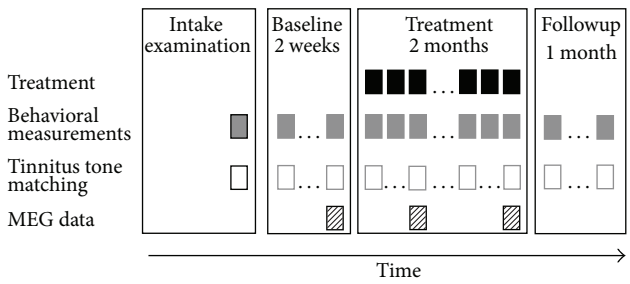

Figure 1: Illustration of the design. The red squares indicate the training sessions, the blue ones the behavioral measurements, the yellow ones the tinnitus tone matching measurements, and the green ones the MEG measurements.

training of the two groups. The main user interface of this music application resembled a simplified piano keyboard. An in-house application regarding tinnitus frequency likeness rating (TPLR) was also installed. Moreover, a set of headphones was provided (Sennheiser, PX360) which was modified by the company enabling us to program the filter coefficients of the (active) noise cancellation, based on the individual tinnitus frequency, in a way that an online notch filtering could be performed while listening to music.

2.3. Training

2.3.1. Multimodal (Playing) Group. The subjects of the playing group were instructed in detail how to use the provided tablet computer and the music application. Thirty different songs, each in three various tempi, had to be melodically accompanied by pressing the correct position on the touch screen of the tablet, on the basis of a self-created music book suitable for nonmusicians. The song difficulty increased over time and new learned songs had to be repeated the next day. Subjects could choose their favorite finger technique using either all fingers (as written in the music book) or only the thumbs of both hands. Training duration was one hour per day and the training sessions were recorded weekly. While playing, the subjects were listening to the melody they played along with the preset backing track. All music spectra were notched in real time via the provided headphones.

2.3.2. Unimodal (Listening) Group. The subjects of the listening group used the provided tablet for listening to the same 30 songs as the playing group. In order to increase the amount of attention needed, the subjects had to fulfill an auditory task while listening to the music. All songs were played in two runs. The first run was played in the correct way as it was written in the music book. A second run directly followed the first one providing the same song. The second run was either identical or contained up to six variations that had to be detected by the patient. After each pair of songs the identified number of variations had to be filled in a form. Each session lasted one hour comprising all 30 songs in a randomized order. In the course of the study the difficulty of the variations increased (from dissonant to consonant variations) and new variations were repeated the next day. As in the playing group, all music spectra were notched in real time via the provided headphones.

2.4. Intake Examination. All subjects were recruited by the tinnitus team and completed a structured interview that collected information on the nature and the personal history of their tinnitus. Audiological measurements included an otoscopic examination, securing that the subjects do not suffer from objective tinnitus. Then, measurements of the hearing threshold with a high-frequency audiometer (0.125 to 16 kHz) and determination of the tinnitus frequency following a structured audiological protocol, using a frequency resolution of 1/24 octave, were performed. Further, the subjects had to assess their tinnitus loudness, distress, awareness, and handicap over the last three days by visual analogue scales. An assessment of tinnitus distress followed with a battery of tests that are described in the subsequent section.

2.5. Measurement of Subjective Tinnitus Characteristics

2.5.1. Frequency. Two procedures were applied in order to determine the tinnitus frequency. (i) Seven "tinnitus frequency candidates" were collected by professional audiologists at the ENT department following the same procedure as described by H. Okamoto at al., 2010. Specifically, tinnitus pitch and loudness were matched ipsilaterally to the frequency and loudness of a pure tone starting from seven different anchor frequencies (1, 12.5, 2, 10, 4, 8, and 6 kHz). Next, two of the previously determined tinnitus frequency candidates were directly compared in a two-alternative forced-choice (2AFC) procedure and the winner of each comparison was tested against the lowest remaining candidate frequency until the winner tinnitus frequency was found. In an octave confusion test the loudness-matched harmonics of the winner tinnitus frequency were again directly compared in a 2AFC procedure. (ii) The subjects

were asked to assess their tinnitus frequency at home on the seven following days using a tinnitus pitch likeness rating (TPLR) application on the provided tablet computer. In this process 37 loudness-matched test tones (sinusoidal tones, two minutes duration, two seconds fadein, and one second fadeout) in frequency steps of 1/12 octave from 2 kHz to 16 kHz (three octaves) had to be rated according to the tinnitus likeness on a scale from 0 to 10 points. The test tones were presented in a randomized order each day. After seven days five tones with the highest ratings including the winner tinnitus frequency of (i) were directly compared in a 2AFC procedure. An octave confusion test was applied on the winner tinnitus-frequency of the TPLR. Afterwards, the audiometric pitch matching described in (i) was repeated: the winner tinnitus frequency of the audiometric approach was compared with the winner tinnitus frequency of the TPLR. This last 2AFC determined the tinnitus frequency for the following TMNM treatment. Over the course of the study, additional TPLR measurements were obtained regularly one time per week.

2.5.2. Tinnitus Related Distress. Tinnitus related distress was assessed with the German translations of (i) Tinnitus Handicap Questionnaire (THQ), (ii) the tinnitus handicap inventory (THI), and (iii) the Tinnitus Questionnaire (TQ). Hyperacusis was assessed with the Geräuschüberempfindlichkeits-Fragebogen (GÜF) [37] and subjective impairment was valued by the SCL-90R. Psychic constitution is estimated by the ADS-L and the state-trait-anxiety-inventory (STAI) [38]. All subjects were asked to estimate their duration of music listening per day, their fun, and relaxation while listening. All questionnaires were fulfilled at the beginning of the training and after each of the following three months.

2.5.3. Tinnitus Characteristics and Evaluation of TMNM Treatment. Tinnitus loudness, awareness, distress, and handicap were measured twice a week on a continuous visual analog scale performed on the provided tablet computer (scale poles: 0 (= tinnitus gone) versus 100 (= personal tinnitus loudness maximum experienced so far)). A baseline period of two weeks before the music training was surveyed. Subjects were also asked to estimate their progress in the music training, the difficulty of the training, their fun, and motivation to continue.

2.6. MEG Measurement Stimuli. Two different sound stimuli were prepared and delivered randomly to either the left or the right ear during the MEG measurement via 60 cm long silicon tubes. The frequency of one stimulus corresponded to the tinnitus frequency; the control stimulus had a frequency of 500 Hz. The loudness of the control stimulus was 20 dB above sensation level that was determined with an accuracy of at least 5 dB at the beginning of each MEG session for each ear. The tinnitus frequency was matched in loudness to the control stimulus before the baseline measurement and kept identical across all course measurements. The stimuli had duration of one second and a random onset asynchrony between two and three seconds. Four runs were presented lasting approximately 14.5 min each, with short breaks in between. The total amount of stimuli for each category was 500.

2.7. MEG Recordings. Evoked magnetic fields were measured with a 275-channel whole-head system (OMEGA, CTF Systems Inc, Port Coquitlam, Canada) in a magnetically shielded and acoustically silent room. MEG data were acquired continuously during each run with a sampling rate of 600 Hz. Subjects were seated upright, and their head position was comfortably stabilized with cotton pads inside the MEG dewar. During the four measuring runs the subjects watched a soundless video of their own choice projected onto the back of a semitransparent screen positioned 90 cm in front of the subjects' nasion. Between runs two and three and after the last run the subjects had to rate their tinnitus loudness on a visual analogue scale.

2.8. Data Analysis. Brain Electrical Source Analysis software (BESA research, version 5.3.7, Megis Software, Heidelberg, Germany) was used for the processing of the MEG data. The recorded data were separated into epochs of 700 ms including a prestimulus interval of 200 ms. The epochs were baseline corrected using the interval from −100 to 0 ms. Epochs with amplitudes larger than 2.5 pT were considered as artifacts and were excluded from the averaging procedure. Data were filtered off-line with a low-pass filter of 30 Hz and a high-pass filter of 1 Hz. Current density reconstructions (CDR) were calculated on the brain responses of each subject for each stimulus category (tinnitus tone and control tone) and each one of the four runs using the LORETA method [39]. LORETA directly computes a current distribution throughout the full brain volume instead of a limited number of dipolar point sources or a distribution restricted on the surface of the cortex. This method has been used successfully for the mapping of auditory evoked brain responses [34, 40] and has the advantage of not needing an a priori definition of the number of activated brain sources. A time window of 50 ms was used for the CDR (70–120 ms after stimulus onset). The chosen time window contains the typical latency of the N1 component ranging from 70–120 ms and includes the rising slope and the peak of the grand average global field power (GFP) of the responses within this time range. Each individual's mean CDR image over the selected time-window for each one of the 4 runs was calculated and projected onto a standard MRI template based on the Montreal Neurological Institute (MNI) template. The images were smoothed and their intensities normalized by convolving an isotropic Gaussian kernel with 7 mm full width half-maximum (FWHM) through BESA's smoothing utility. The smoothed images of each run were then averaged in order to achieve a sufficient signal to noise ratio, producing thus one image for each condition (control and tinnitus) and each time-point (before training, after one month of training, and after training) of each subject.

The software packages Statistical Parametric Mapping 8 (SPM8, http://www.fil.ion.ucl.ac.uk/spm) and GLM-Flex

(http://nmr.mgh.harvard.edu/harvardagingbrain/People/AaronSchultz/GLM_Flex.html) were used for the statistical analysis of the CDRs. Specifically, using GLM-Flex a $2 \times 2 \times 3$ mixed model ANOVA was designed with subjects factor group (playing and listening) and within subjects factors frequency (tinnitus and control) and time point (before training, after one month of training, and after training). Results were constrained in gray matter using a mask, thereby keeping the search volume small and in physiologically reasonable areas. A permutation method for peak cluster level error correction (AlphaSim) at $P = 0.05$ was applied for this whole head analysis, as implemented in REST software (Song et al., 2011), by taking into account the significance of the peak voxel (threshold $P < 0.001$ uncorrected) along with the cluster size (threshold size > 513 voxels), thereby controlling for multiple comparisons. The smoothness factor used for AlphaSim estimation was calculated from the residual image of the three-way interaction effect.

3. Results

3.1. Behavioral Results. The four items measuring the tinnitus severity via visual analogue scales (tinnitus loudness, awareness, distress, and handicap) were highly intercorrelated (Cronbach's $\alpha = 0.968$; averaged over the 14 weekly measurements). Consequently, we chose to average the four items to obtain a single measure of perceived tinnitus *severity*.

The first two time points defined the baseline. Severity values at baseline appeared to differ between the listening and the playing group ($M_{\text{listen}} = 43.14$, $\text{SEM}_{\text{listen}} = 7.41$; $M_{\text{play}} = 56.49$, $\text{SEM}_{\text{play}} = 6.98$). Although a t-test showed that this difference was not significant, $t(20) = 1.233$, $P = 0.232$, we chose to analyze the development of tinnitus severity by means of an analysis of covariance (ANCOVA) to control for possible baseline effects due to accidentally imbalanced sampling. For each of the 12 postbaseline measurement points we calculated the change-from-baseline severity and performed a 2 (hroup) × 12 (time point) ANCOVA, with *baseline severity* as a covariate.

For the factor time point we observed a violation of the sphericity assumption (Mauchly's $W = 0.00001$, $P < 0.001$) and will thus report Greenhouse-Geisser corrected P value where necessary. Only the interaction time point × group was marginally significant, $F(11, 209) = 1.784$, $P = 0.058$, indicating that the development of severity over time could be predicted from baseline severity. No other effects were significant (all $P > 0.149$). Figure 2 illustrates the development of severity change from baseline over time. No significant effects were seen in the other behavioral measurements used (THQ, THI, TQ, SCL-90R, ADS-L, and STAI).

3.2. MEG Results. Our main hypothesis states that the two training types should develop different effects between the groups over the course of the training, but exclusively for the tinnitus frequency, not for the control frequency. The relevant statistic test is therefore a three-way interaction of group, frequency, and time point. This analysis is run first and is henceforth used as a localizer; that is, all further analyses that are performed to resolve the three-way interaction will be restricted to cortical regions where the three-way interaction was found to be significant.

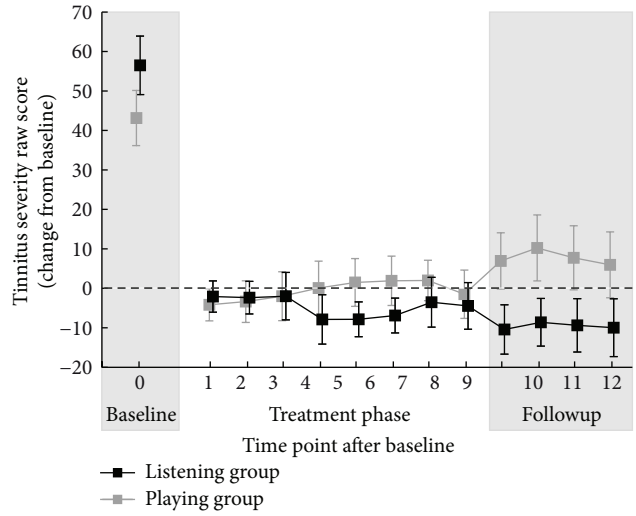

Figure 2: Behavioral data. Subjective tinnitus severity score (scale ranged from 0 to 100) for the playing group (gray) and listening group (black). Time point 0 corresponds to the baseline. For time points 1 to 12 the figure depicts the change from baseline in tinnitus severity. Error bars show ± 1 SEM.

The statistical comparison of the MEG results indicated that TMNM treatment affected differently the cortical responses of the two groups and two frequencies. Specifically, the three-way interaction of the mixed model ANOVA (group × frequency × time point) yielded two significant clusters: one at the right middle temporal cortex (peak coordinates: $x = 56$, $y = -28$, $z = -8$; $F(2, 34) = 11.492$; cluster size = 766 voxels; $P < 0.05$ AlphaSim corrected) and one in Brodmann area 7 at the posterior parietal cortex (peak coordinates: $x = 12$, $y = -66$, $z = 48$; $F(2, 34) = 11.816$; cluster size = 1208 voxels; $P < 0.05$ AlphaSim corrected). The statistical parametric map of this analysis is presented in Figure 3.

In order to investigate the origin of this result a mask was constructed that included only the two clusters that were found to have significant effects in the abovementioned three-way interaction (i.e., right temporal cortex and posterior parietal cortex). This mask was then used as region of interest (ROI) for the post hoc analyses of the two-way interactions of frequency × time point for each group. The two-way interaction in the analysis of the playing group revealed no significant activation differences, even when the threshold was lowered at an uncorrected $P < 0.01$ level. On the contrary the two-way interaction of the listening group showed significant activation differences in both cortical areas (right temporal cortex and posterior parietal cortex) in the AlphaSim corrected $P < 0.05$ threshold level, thereby indicating that the three-way interaction originated from a two-way interaction that was more pronounced in

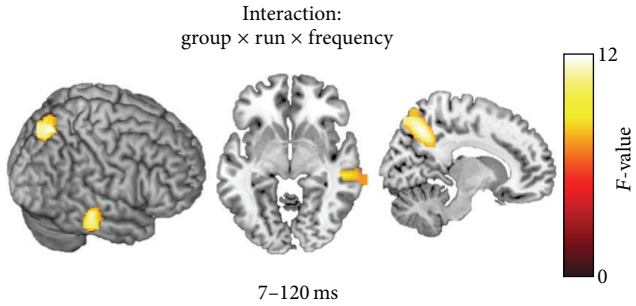

Figure 3: Statistical parametric maps of the group × frequency × time point interaction. The tailor made notched music training affected in a significantly different way the two groups and the two frequencies in two areas: right middle temporal cortex, (peak coordinates: $x = 56$, $y = -28$, and $z = -8$; $F(2, 34) = 11.492$; cluster size = 766 voxels; $P < 0.05$ AlphaSim corrected) and right posterior parietal cortex (peak coordinates: $x = 12$, $y = -66$, and $z = 48$; $F(2, 34) = 11.816$; cluster size = 1208 voxels; $P < 0.05$ AlphaSim corrected).

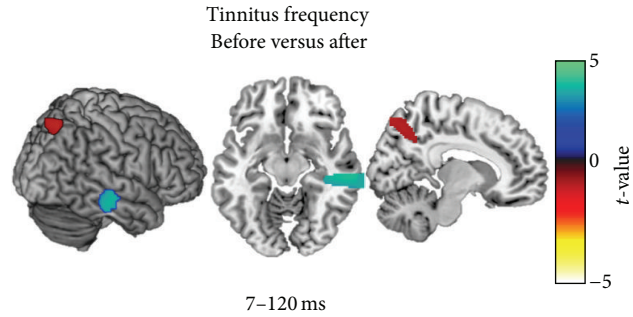

Figure 4: Statistical parametric maps of the post hoc paired samples t-tests ROI comparing the pre- and posttraining MEG results of the listening (unimodal) group with regard to the tinnitus frequency. Tailor made notched music training induced a decrease in the activity of the right temporal cortex (peak coordinates: $x = 68$, $y = -22$, and $z = -8$; $t(16) = 3.62$; cluster size = 472 voxels; $P < 0.05$ AlphaSim corrected) and an increase in the activity of the posterior parietal cortex (peak coordinates: $x = 12$, $y = -64$, and $z = 52$; $t(16) = -3.64$; cluster size = 155 voxels; $P < 0.05$ AlphaSim corrected).

the listening than in the playing group (peak coordinates for the right temporal activation: $x = 43$, $y = -29$, $z = -13$; $F(2, 16) = 12.4059$; cluster size = 569 voxels; $P < 0.05$ AlphaSim corrected; peak coordinates for the posterior parietal activation: $x = 7$, $y = -59$, $z = -42$; $F(2, 16) = 10.3954$; cluster size = 2097 voxels; $P < 0.05$ AlphaSim corrected). A two-way ANOVA of group × time point only for the tinnitus frequency was then calculated that revealed a significant activation difference between the two groups and the 3 time points (peak coordinates for the right temporal activation: $x = 43$, $y = -29$, $z = -13$; $F(2, 34) = 7.5943$; cluster size = 364 voxels; $P < 0.05$ AlphaSim corrected; peak coordinates for the posterior parietal activation: $x = 7$, $y = -59$, $z = -42$; $F(2, 34) = 6.82$; cluster size = 146 voxels; $P < 0.05$ AlphaSim corrected).

Subsequently, using the same mask and threshold two post hoc one-way ANOVAs (one for each frequency) with factor time point within the listening group were calculated. The analyses showed activation differences in the regions of interest defined by the three-way ANOVA only in the tinnitus frequency (peak coordinates for the right temporal activation: $x = 68$, $y = -20$, $z = -8$; $F(1, 16) = 13.50$; cluster size = 144 voxels; $P < 0.05$ AlphaSim corrected; peak coordinates for the posterior parietal activation: $x = 14$, $y = -66$, $z = 56$; $F(1, 16) = 13.27$; cluster size = 187 voxels; $P < 0.05$ AlphaSim corrected), while no activation difference was found for the control frequency, thereby indicating that the TMNM treatment affected the responses to the tinnitus frequency but not to the control frequency. To identify the direction of this result a paired sample t-test of before to after the tinnitus frequency was calculated for the listening group (again using the same threshold). Thereby it was revealed that the response of the right temporal cortex for the tinnitus pitch for the listening group decreased during the course of the treatment (peak coordinates: $x = 68$, $y = -22$, $z = -8$; $t(16) = 3.62$; cluster size = 472 voxels; $P < 0.05$ AlphaSim corrected), while the response of the posterior parietal cortex increased (peak coordinates: $x = 12$, $y = -64$, $z = 52$; $t(16) = -3.64$; cluster size = 155 voxels; $P < 0.05$ AlphaSim corrected). The statistical parametric maps of this analysis are presented in Figure 4 and the mean contrast estimates of the 2 regions for the 3 time points (i.e., middle temporal cortex and posterior parietal cortex) are presented in Figure 5.

4. Discussion

In this study we compared the cortical plasticity effects of multimodal and unimodal notched music treatment in tinnitus patients by means of MEG and behavioral measurements over a time period of three months (two months of TMNM treatment for one hour per day and one month followup). Results indicate a decrease in the cortical activity corresponding to the tinnitus frequency for the unimodal training group, while no significant effect was present in the multimodal group. Importantly, the MEG results reveal, for the first time according to our knowledge, that unimodal TMNM treatment induces favorable plastic cortical changes not only in the temporal cortex, but also in a posterior parietal region, which constitutes another node of the cortical network that underlies the generation and/or maintenance of the tinnitus perception [41–43].

The present study employed an active engagement with music in both the uni- and multisensory groups: one group detected variations in the preset music pieces and the other one melodically accompanied preset backing tracks. At the same time, the acoustic input was filtered in real time with a tailor made notch filter targeting the individual tinnitus frequency via the supplied special type of headphones. In the unisensory group this process caused a decrease in the temporal cortex responsiveness to the tinnitus frequency, while it did not affect the response to the control frequency. As shown in previous studies [18, 20, 22], listening to pleasurable tailor-made notched music can reduce tinnitus perception

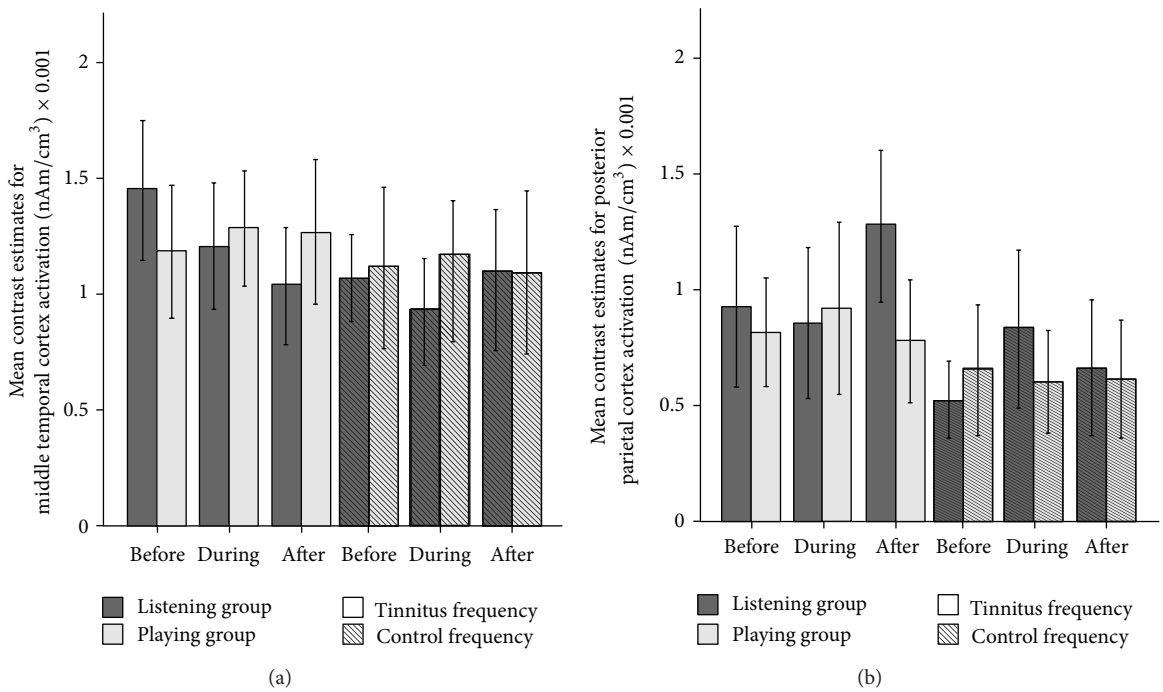

Figure 5: Mean contrast estimates for (a) the middle temporal cortex and (b) posterior parietal cortex before, during, and after the treatment. The solid dark grey bars indicate the contrast estimates of the listening group while the solid light grey indicates the activations of the playing group for the tinnitus frequency. The dark grey bars marked with lines indicate the contrast estimates of the listening group for the control frequency. The light grey bars marked with lines indicate the contrast estimates of the playing group for the control frequency. The treatment caused a gradual decrease of the activation of the middle temporal cortex and an increase in the activation of the posterior parietal cortex in the tinnitus frequency of the listening group. Error bars: 2 × standard error of mean.

and reduce the evoked activity in temporal cortex areas corresponding to the tinnitus frequency. This kind of individually modified music introduces a functional deafferentation of auditory neurons corresponding to the eliminated frequency while the surrounding neurons, which are still excited by the nonfiltered acoustic input presumably suppressing the tinnitus generating neurons via lateral inhibition [21, 44, 45]. Thus, the deprivation from auditory input in the frequency range of the tinnitus seems to induce long-term depression in auditory neurons corresponding to the tinnitus frequency via synaptic and/or cellular mechanisms [46, 47]. This process seems to affect mainly the right temporal cortex due to an increased predisposition of right auditory cortical neurons to synchronize their activity following deafferentation leading to tinnitus [48].

The specificity of the right auditory cortex in processing spectral information [49, 50] in contrast to the left one, that is, specified in the processing of temporal auditory information [51] along with the fact that tinnitus distress, is highly related to the activity of right temporal areas which [52] may be the reason for the right lateralized effect of the applied treatment. The neuroplastic effect of the treatment is located in the MTG. This area is correlated with auditory awareness of pitch [53] contributing, thus, to the perception that the tinnitus sound is externally located [54]. A recent voxel based morphometry study by Boyen et al. [55] revealed that tinnitus is associated with higher grey matter volume in MTG, while a meta-analysis of tinnitus related PET studies [56] indicated increased activation in MTG in tinnitus patients. Hence, the treatment effect of decreased activity in MTG may indicate a functional reorganization of the temporal network that subserves tinnitus [54].

Additionally, the training caused an increase of the activity of the posterior parietal cortex as a response to the tinnitus frequency in the unisensory group. For this region positive correlation between glucose metabolism and tinnitus was reported in a recent PET study [42], while its activation in tinnitus patients has been also shown in previous PET study [57]. Importantly, an increase of the activity in the posterior parietal cortex (precuneus) has been found to positively correlate with less tinnitus distress in recent EEG studies [14, 58, 59], indicating that it may constitute part of a tinnitus coping network. This interpretation seems plausible as this region has also been correlated with selective attention in the auditory modality [60]. Within this framework, the fact that TMNM treatment causes an increase in responsiveness of the posterior parietal cortex becomes increasingly important.

In a series of studies music making has been shown to be one of the most powerful stimulators for brain plasticity, inducing changes in multiple sensory systems [32, 61]. Three recent training studies using MEG indicated that music training based on a multisensory protocol that utilizes the auditory, visual, and motor modalities enhances the plastic changes induced by musical training in healthy adult

nonmusicians [33, 34, 62]. The reward and enjoyment of playing music compared to merely listening to it seems to cause an increase in dopamine release that can enhance cortical reorganization [63]. Nevertheless, in the present study only the unisensory (listening) but not the multisensory (playing) group revealed a significant effect. Thereby we assume that mechanisms reversing the changes in the auditory system, which have been already reorganized in a maladaptive manner generating tinnitus perception, are not the same as the mechanisms that drive the cortical plasticity induced by music training in healthy adults [32]. Instead, the amount of attention dedicated to the auditory input seems to be even more crucial. Attention strengthens not only the excitatory neural connections but also the inhibitory networks, thereby driving also the effectiveness of tailor-made notched music in the auditory system [60]. In the training protocol of the present study the listening group concentrated on the auditory input solely, while the playing group divided its attention to the different modalities, that is, the somatosensory (pressing a button with the right finger in time on the tablet), visual (reading the music book), and auditory system (listening to music).

The behavioral responses on the visual analog scales with regard to tinnitus severity do not reflect the changes observed in MEG. This null-finding is in contrast to former studies using TMNMT [20, 22, 23]. This result can be attributed to a combination of small sample size ($n = 9$ for the listening group and $n = 10$ for the playing group) and the great interindividual variance included in the data (cf. Figure 2). Moreover, the training lasted two months which is a considerably smaller time period compared to other studies [20] and therefore slight differences in tinnitus perception may have not been detected with the questionnaires [64].

5. Conclusion

Listening attentively to individually filtered music over a time period of two months, for one hour per day, led to plastic cortical changes in a network of sources that subserve the generation and/or maintenance of tinnitus, as revealed by MEG measurements (a decrease of auditory evoked activity in the right temporal cortex and an increase of activity in the posterior parietal cortex). The present study also indicates that unimodal tailor-made notched music training induces greater neuroplastic changes than multimodal training in nonmusician tinnitus patients. Thereby we assume that the mechanisms reversing the maladaptively reorganized auditory system that generates tinnitus perception are different from the mechanisms driving the cortical plasticity induced by music training in healthy brains. Thus, a training protocol based on attentive listening to tailor-made notched music can reverse the maladaptive reorganization of the cortical network that generates and supports tinnitus perception.

List of Abbreviations

MEG: Magnetoencephalography
TMNMT: Tailor-made notch-music training
ANOVA: Analysis of variance
AlphaSim: It is used in multiple regression analysis to find the minimum cluster size for certain alpha
TPLR: Tinnitus frequency likeness rating
2AFC: Two-alternative forced-choice
THQ: Tinnitus Handicap Questionnaire
THI: Tinnitus handicap inventory
TQ: Tinnitus Questionnaire
GÜF: Geräuschüberempfindlichkeits-Fragebogen
STAI: State-trait-anxiety-inventory
ADS-L: Allgemeine depressionskala lange version
BESA: Brain Electrical Source Analysis Software
CDR: Current density reconstructions
LORETA: Low resolution brain electromagnetic tomography
GFP: Global field power
MRI: Magnetic resonance imaging
MNI: Montreal Neurological Institute
FWHM: Full width half-,maximum
SPM8: Statistical parametric mapping 8
GLM-Flex: Set of second level neuroimaging analysis scripts.

Conflict of Interests

The authors declare that they have no financial or any other competing interests.

Authors' Contribution

Christo Pantev developed the concept of the study; Janna Pape, Evangelos Paraskevopoulos, Maximilian Bruchmann, Andreas Wollbrink, and Christo Pantev designed the experimental setup; Janna Pape developed the stimulus and training procedures and performed the experiments. Janna Pape, Evangelos Paraskevopoulos, Maximilian Bruchmann, and Andreas Wollbrink performed the data analysis and the statistical evaluation. All authors participated in the evaluation and interpretation of the results and in writing the paper. All authors have approved the final version of the manuscript.

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Research Article

Salicylate-Induced Auditory Perceptual Disorders and Plastic Changes in Nonclassical Auditory Centers in Rats

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Previous studies have shown that sodium salicylate (SS) activates not only central auditory structures, but also nonauditory regions associated with emotion and memory. To identify electrophysiological changes in the nonauditory regions, we recorded sound-evoked local field potentials and multiunit discharges from the striatum, amygdala, hippocampus, and cingulate cortex after SS-treatment. The SS-treatment produced behavioral evidence of tinnitus and hyperacusis. Physiologically, the treatment significantly enhanced sound-evoked neural activity in the striatum, amygdala, and hippocampus, but not in the cingulate. The enhanced sound evoked response could be linked to the hyperacusis-like behavior. Further analysis showed that the enhancement of sound-evoked activity occurred predominantly at the midfrequencies, likely reflecting shifts of neurons towards the midfrequency range after SS-treatment as observed in our previous studies in the auditory cortex and amygdala. The increased number of midfrequency neurons would lead to a relative higher number of total spontaneous discharges in the midfrequency region, even though the mean discharge rate of each neuron may not increase. The tonotopical overactivity in the midfrequency region in quiet may potentially lead to tonal sensation of midfrequency (the tinnitus). The neural changes in the amygdala and hippocampus may also contribute to the negative effect that patients associate with their tinnitus.

1. Introduction

One of the most reliable methods of inducing transient tinnitus involves administering a large dose of sodium salicylate (SS) [1, 2], the active ingredient in aspirin. Consequently, SS is often used to investigate the biological underpinning of tinnitus as well as the ensuing peripheral hearing loss that accompanies it [2–7]. The biological mechanisms underlying SS-induced hearing loss and tinnitus have been extensively studied in the classical auditory system. In the cochlea, salicylate competitively binds to prestin in outer hair cells (OHC); this attenuates OHC electromotility, distortion product otoacoustic emissions (DPOAE), and the cochlear compound action potential (CAP) and contributes to SS-induced hearing loss [2, 8, 9]. *In vitro*, SS suppresses GABAergic inhibition [10–12]; these changes are believed to contribute to neural hyperactivity, changes in gain control and synaptic rescaling, and plastic reorganization in the classical auditory pathway, effects that presumably contribute to the SS-induced auditory perceptual disorders [8, 13–21].

Neural signals in the classical auditory pathway make their way to many other brain regions involved in auditory learning/memory, sound-related emotional response, vocal production, multisensory integration, and motor control [22–55]. Brain regions outside the classical auditory system are postulated to gate or modulate the severity of tinnitus and hyperacusis [56–58]. Indeed, clinical evidence suggests that the amygdala, striatum (Str), hippocampus (HC), and frontal cortex participate in tinnitus and hyperacusis [59–62]. Consistent with clinical data, we found that SS enhanced sound-evoked responses and altered the tonotopy of neurons in the lateral amygdala (LA) [56]. Previous c-fos immunolabeling studies suggested that SS could induce electrophysiological changes in several other nonauditory structures [63, 64]. To investigate the functional changes induced by SS in other nonauditory structures linked to tinnitus, we recorded from

the Str, LA, HC, and cingulate (Cg) to determine how the electrophysiological properties of neurons in these structures were altered by a high dose of SS known to induced tinnitus and mild cochlear hearing loss.

2. Experimental Methods and Materials

2.1. Subjects. Forty-three Sprague-Dawley rats (3–5 months of age, Charles River Laboratories, Wilmington, MA) were housed in the Laboratory Animal Facility (LAF) at the University at Buffalo and given free access to food and water. The colony room was maintained at 22°C with a 12-hour light-dark cycle. All procedures used in this project were approved by the Institutional Animal Care and Use Committee (HER05080Y) at the University at Buffalo and carried out in accordance with NIH guidelines.

2.2. Salicylate Administration. SS (Sigma-Aldrich, no. S3007) was dissolved in saline (50 mg/mL). Rats were injected with saline (5 mL/kg, i.p.) or SS (200 or 250 mg/kg, i.p.); these doses of SS have previously been shown to consistently enhance the amplitude of acoustical startle responses and induce tinnitus in rats [4].

2.3. Behavioral Measurement of Auditory Threshold. Five rats were trained in a go/no-go operant conditioning paradigm to detect broadband noise bursts in a sound attenuating chamber. Rats were food restricted and kept at approximately 85% of their free-feeding weight during the course of experiment. The broadband noise burst (300 ms duration, 5 ms rise/fall time, cosine gated) used in this experiment contained frequencies up to 42 kHz.

A rat began a trial by placing its nose in a nose-poke hole, which initiated a variable waiting interval ranging from 1 to 4 s. The rat had to maintain its position in the nose-poke hole until it heard a noise burst or the trial was aborted. In the *go* condition, the target stimulus was the noise burst. If the rat detected this signal, it removed its nose from the nose-poke hole resulting in a food reward (45 mg dustless rodent grain pellets, Bio-Serv); a *hit* was recorded if the rat correctly responded to the broadband noise within 2 s. A *miss* was recorded if the rat failed to remove its nose from the nose-poke within the 2 s response interval. Approximately 30% of all trials were *catch* trials. These constituted the *no-go* part of the procedure; noise bursts were not presented during these trials. If the rat removed its nose during a *catch* trial, a *false alarm* was recorded and the rat received a 4 s timeout, during which the house light was turned off and the rat could not start another trial. However, if the rat continued to nose-poke, a *correct rejection* was recorded. No reinforcement was given for a *correct rejection*.

The noise bursts were presented according to the psychophysical method of constant stimuli (MOCS). Within each 10-trial block, seven predetermined target intensities were presented randomly along with 3 *catch* trials. The target intensities were chosen so that only the lowest one or two intensities were estimated to be below threshold, whereas the remaining intensities were well above threshold. Mean *hit* and *false alarm* rates were used to calculate thresholds using signal detection theory with a threshold criterion of $d' = 1.5$.

After baseline noise-burst thresholds were collected, the rats were tested once per week with either a single i.p. injection of sodium salicylate (200 mg/kg) dissolved in saline (50 mg/mL) or an equivalent volume of saline (control). The injections were administered 2 h before testing.

2.4. Behavioral Measurement of Tinnitus. Three rats were trained on a two-alternative forced choice identification task designed to detect tinnitus. The material and methods for this behavioral measure are similar to those described previously [65]. Rats were food restricted to 85–90% of free feeding weight during the course of the experiment. The rats were trained to activate the left feeder trough in the presence of a steady-state narrowband noise (NBN: 1/8 octave band, center frequencies randomized across trials: 4, 5, 6, 8, or 11 kHz at 70 dB SPL) and to activate the right feeder trough in the presence of an amplitude-modulated noise (AM: broadband noise at 70 dB SPL, 100% modulation depth at 5 kHz) or no sound (Quiet). One of the three acoustic conditions was continuously present in the chamber at the start of each trial. The rat would initiate a trial by holding its nose in the center nose-poke for a random interval ranging from 4 to 8 s. After this waiting interval, a white light above the nose-poke would illuminate, serving as a "go cue" that initiated the start of a trial. Directly after the go cue, the rats responded to the feeder associated with the acoustic condition. Correct responses were immediately rewarded with a food pellet (45 mg dustless grain pellets, Bio-Serv) delivered to the respective feeder associated with each of the three stimuli while incorrect responses were punished with a 60-second "time out" in which the rat was unable to initiate a new trial. After the rat responded to a feeder trough and received either a pellet or a time out, the acoustic condition changed and another trial began. Trial sequences were randomized using criteria outlined previously [66, 67] in order to minimize guessing and strategized behavior. Percentage of trial types was split up evenly between the two feeders (NBN at 50%; AM at 30%; Quiet at 20%). Throughout training the rate of reinforcement was progressively reduced from 100% to 70%, that is, partial reinforcement to minimize extinction of the learned behaviors. Rats were trained to a criterion of >80% correct response for each acoustic condition.

Once a rat met the criteria for at least 4 consecutive baseline days they were injected with either a 200 mg/kg (i.p.) dose of sodium salicylate dissolved in 50 mg/mL saline or an equivalent volume of saline 2 h before testing. On the tinnitus testing days with injections of either saline (control) or salicylate, Quiet trials were unreinforced, but a response to either feeder was required to complete the trial. Evidence of tinnitus was described as a shift in response on Quiet trials from the feeder previously associated with AM and Quiet trials to the feeder associated with the steady NBN trials; a shift in response preference from the Quiet feeder to the steady NBN feeder was interpreted as evidence that the rat perceived a steady state sound in the absence of any acoustic stimuli. On tinnitus testing days with either

saline (control) or salicylate, there was no reinforcement for Quiet trials; however, the rate of reinforcement for AM and NBN trials was increased from 70% to 90% in order to compensate for the lack of food reinforcement on Quiet trials. If animals shifted their responses on Quiet trials when they were injected with saline, then we would assume that the animals are only sensitive to the reinforcement probabilities of the testing schedule and may not be experiencing tinnitus. However, if the animals only show a shift during Quiet trials when injected with salicylate, while maintaining accurate performance on AM and NBN trials, then we can interpret this as evidence of tinnitus.

2.5. Estimates of Loudness Perception Using Reaction Time Measures. Using reaction time as a surrogate of loudness perception, 7 rats were tested on a go/no-go operant conditioning paradigm to detect broadband noise bursts in quiet. The procedure for this experiment was identical to the one used to obtain broadband noise thresholds. However, the intensity of the broadband noise bursts (300 ms duration, 5 ms rise/fall time, cosine gated) in this condition ranged from 30 to 90 dB SPL instead of near-threshold levels. Reaction times measures were taken from the onset of the noise burst to the time the rat removed its nose from the nose-poke hole. Only reaction times for "hits" (when the animal correctly detected the stimulus) were included in our analysis.

As in the broadband noise threshold condition, the rats were tested once per week with either a single i.p. injection of sodium salicylate (200 mg/kg) dissolved in saline (50 mg/mL) or an equivalent volume of saline (control). The injections were administered 2 h before testing and all 7 animals received saline and salicylate injections. Three of the rats received saline injections first while the other 4 received salicylate injections first.

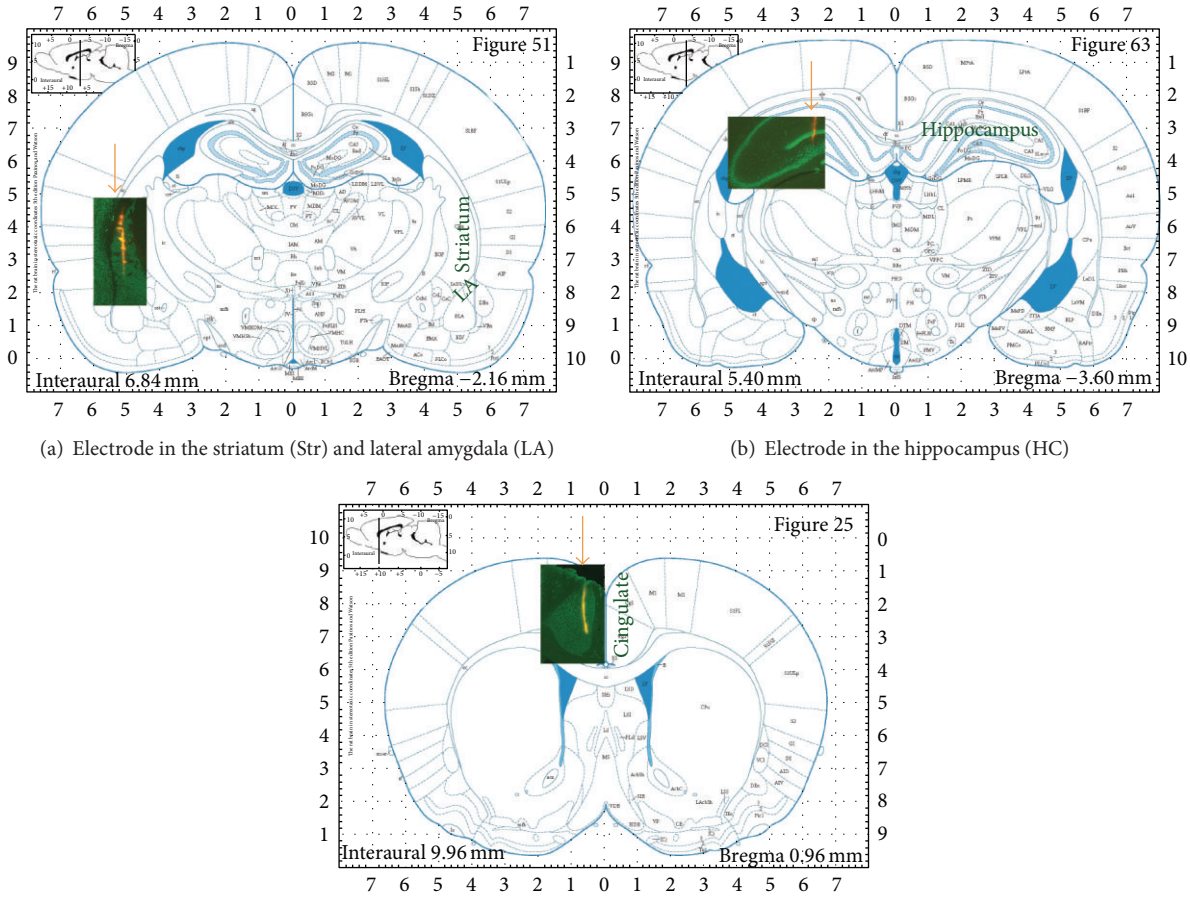
2.6. Acoustic Startle Reflex Amplitude. Six rats were tested on an acoustic startle reflex paradigm in order to assess the magnitude of the animal's reflexive motoric response to a sudden, unexpected loud sound [68]. As described in our previous publications, each rat was placed in an acoustically-transparent wire-mesh (0.5 cm × 0.5 cm) cage (20 cm × 7 cm × 6 cm) mounted on a Plexiglas base (20 cm × 10 cm) which rested on a pressure sensitive 35 mm piezoelectric transducer (MCM 28-745) that generated a voltage response proportional to the magnitude of the startle response [20, 69, 70]. Sound stimuli and startle responses were produced and measured with Tucker Davis hardware and custom software as described previously [71]. Stimuli were generated by a real-time processor (TDT RX6) with a ~100 kHz sampling rate, amplified, and delivered through a speaker (Fostex FT17H) placed approximately 25 cm above the startle platform. The startle stimulus consisted of a single broadband noise burst (20 ms duration, 0.1 nominal rise/fall time) presented at ten intensities from 70 to 115 dB SPL. Ten trials were presented in a pseudorandom order (15–25 s intertrial intervals) per intensity. Startle amplitudes for each rat were obtained following i.p. injections of either sodium salicylate (250 mg/kg) dissolved in saline (50 mg/mL) or an equivalent volume of saline (control condition). All six rats were tested with saline and SS; three rats received the saline control injection first while the other three rats received the salicylate injection first. The injections were always administered 2 h before testing.

2.7. Electrodes. A customized electrode assembly consisting of 2–4 polyimide-insulated tungsten electrodes (FHC Inc., impedance ~1 MΩ) or a 16-channel, linear silicon microelectrode (A-1x16–10 mm 100–177, NeuroNexus Technologies) was used to record neural activity in the LA, Str, HC, and Cg.

2.8. Surgery, Stimuli, and Physiological Recordings. Details of our electrophysiological techniques are described in detail in previous publications [8, 13, 56]. Briefly, rats were anesthetized with ketamine and xylazine (50 and 6 mg/kg i.m.) and placed in a stereotaxic apparatus with blunted ear bars. The dorsal surface of the skull was exposed and a head bar was firmly attached to the skull using a screw and dental cement. The head bar was attached to a rod mounted on a magnetic base. The assembly was used to hold the animal's head in the stereotaxic frame after removing the right ear bar. This allowed the right ear to be acoustically stimulated using a free-field loudspeaker. A craniotomy was performed on the skull (contralateral to the ear receiving acoustic stimulation) at the appropriate location to gain access to the left LA, Str, HC, and Cg. The dura of the brain was removed and an electrode was inserted into the brain and advanced into the desired brain region using stereotaxic coordinates [72].

Broadband noise and tone bursts (50 ms duration, 1 ms rise/fall time, cosine^2-gated) were generated (TDT RX6-2, ~100 kHz sampling rate) and presented at a rate of 2/s through a loudspeaker (FT28D, Fostex) located 10 cm in front of the right ear. Stimuli were calibrated using the electrical output from a sound level meter (Larson Davis model, 1/4 inch microphone, model 2520) which was delivered to a custom sound calibration program in the computer. Responses to the noise bursts were obtained at 11 intensities (0–100 dB SPL, 10-dB steps, 100 repetitions per intensity, pseudorandom presentation). Responses to tone bursts were collected at 10 frequencies (1.0, 1.5, 2.3, 3.5, 5.3, 8.0, 12.1, 18.3, 27.7, and 42.0 kHz) at 6 intensity levels (0–100 dB SPL, 20-dB steps, 50 repetitions per frequency-intensity combination, pseudorandom presentation order).

Local field potentials (LFPs) and spike discharges were sampled simultaneously from the same electrode with a resolution of 40.96 μs using a RA16PA preamplifier and RX5 base station (Tucker-Davis Technologies System-3, Alachua, FL) using custom-written data acquisition and analysis software (MATLAB R2007b, MathWorks) as previously described [13, 56]. Following digital bandpass filtering (2–300 Hz), LFP signals were down-sampled online to 610 Hz. Averaged evoked LFPs were computed from the down-sampled data over a 500 ms time window following stimulus onset. Spike detection was performed online using a manually set voltage threshold (spike signal filtered 300–3500 Hz). Peristimulus time histograms (PSTH) were constructed offline using custom software with a time window up to 500 ms and bin



(a) Electrode in the striatum (Str) and lateral amygdala (LA)

(b) Electrode in the hippocampus (HC)

(c) Electrode in the cingulate cortex (Cg)

Figure 1: The recording electrodes in the brain. The drawings of the brain coronal section are from the rat brain atlas [72] and the inserts are photomicrographs of the brain showing DiI labeling of the recording electrodes (pointed by the arrows) in the Str and LA (a), the HC (b), and the Cg (c).

widths of 1–10 ms. The root mean square (RMS) of LFP was measured and mean discharge rate of neuronal activity was obtained in a time window of 0–100 ms.

2.9. Anatomical Confirmation of Electrode Position. In addition to stereotaxic coordinates, the electrode position in the brain was verified in at least 2 animals per recording site by painting a fluorescent dye (DiI, Cat no. 42364, Sigma-Aldrich) on the surface of the electrode prior to penetration. After completing the recordings, the brain was removed, placed in 10% buffered formalin for 5–7 days, and immersed in 30% sucrose solution for two days. The brain was cryosectioned (50 μm) in the coronal plane. After blocking in normal horse serum, slices were incubated in a primary mouse antineuronal nuclei (NeuN) monoclonal antibody (1 : 1000, Chemicon, MAB377), washed three times with phosphate buffered saline (PBS), and incubated with a donkey anti-mouse secondary antibody conjugated to Alexa Fluor 488 (1 : 1000; Invitrogen, A21202). Sections were washed with PBS and mounted on Fisher Superfrost polarized slides and coverslipped with Prolong Antifade mounting medium (Invitrogen). Sections were visualized and photographed with a Zeiss Axio Imager Z1 Microscope equipped with a digital camera, and images were processed with Zeiss AxioVision software. Figure 1 presents the electrode penetration locations (pointed by arrows) in the Str-amygdala (a), HC (b), and Cg (c).

2.10. Statistical Analysis. One-and two-wayANOVAs(GraphPad ver. 5, Prism) and t-tests were used to evaluate the significance of the results.

3. Results

3.1. Behavioral Changes after SS Injection

3.1.1. Hearing Loss. To determine the magnitude of hearing loss resulting from SS treatment, five rats were trained to detect broadband noise bursts for a food reward. Normal untreated rats and saline treated rats could detect broadband noise bursts at ~2 dB SPL. However, after SS-injection, mean threshold was shifted to 19.4 dB SPL (Figure 2(a)). A one-way repeated measures ANOVA showed a significant difference between the treatments ($P < 0.0001$) and a Newman-Keuls

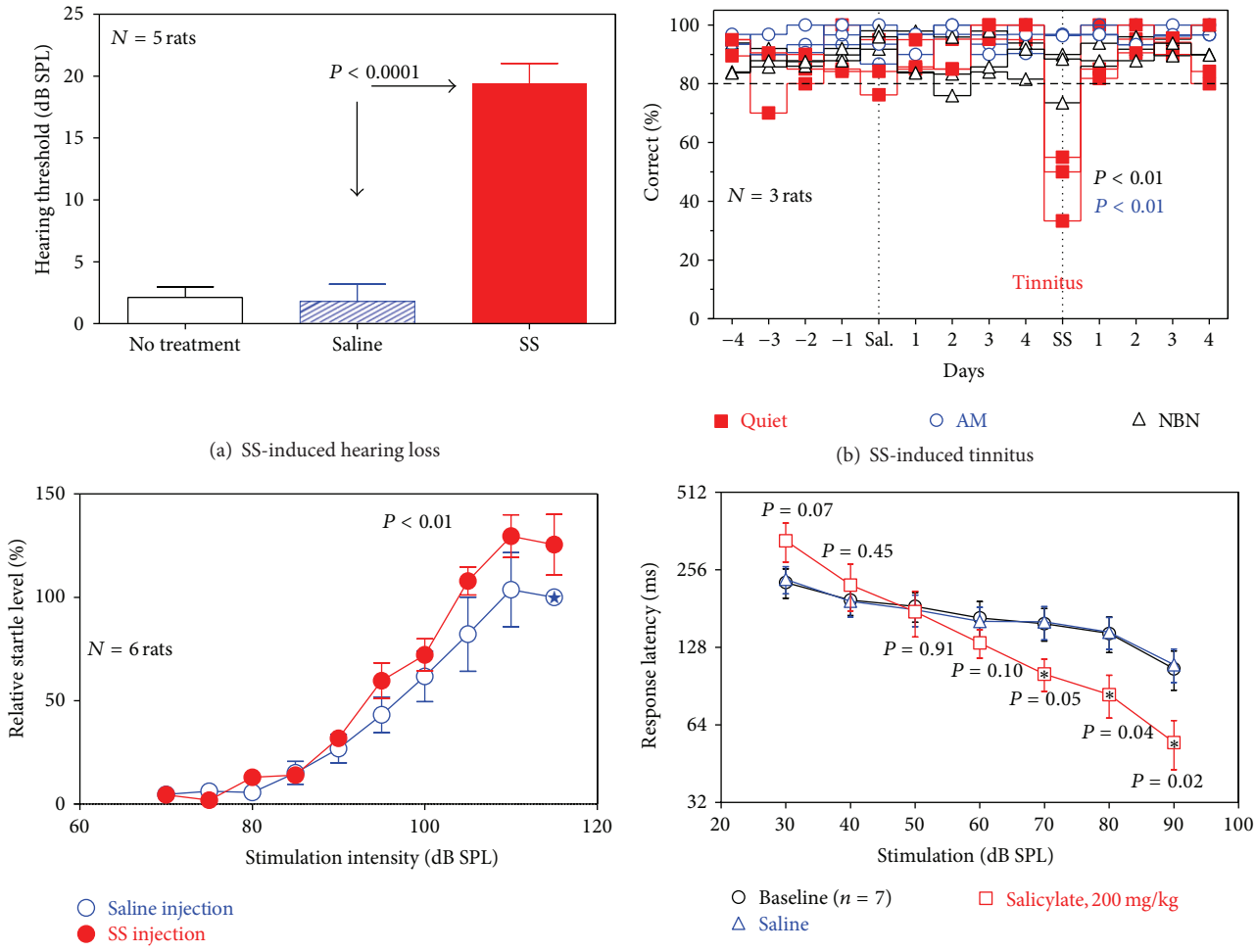


Figure 2: The effects of sodium salicylate (SS) injection on auditory perception. (a) Mean hearing thresholds for broadband noise bursts ($n = 5$). Baseline (black open bar), saline (blue shadowed bar), and salicylate (red filled bar) conditions are shown with standard error (SE) bars. Thresholds significantly increased by about 17 dB following salicylate administration; (b) salicylate-induced tinnitus. Rats ($n = 3$) were trained to respond to 3 stimuli. Quiet (red filled squares) and amplitude modulated (AM) (blue open circles) stimuli were paired with the right feeder while a narrowband noise (NBN) (black open triangles) was paired with the left feeder. Injection of saline (sal) showed no significant difference in responding during Quiet trials compared with baseline (no injection). However, an injection of 200 mg/kg SS showed a significant difference in response only during Quiet trials. This switch in response suggests that the rats perceived a steady state sound in the absence of an acoustic source. (c) Mean percentage (±SE) of startle amplitude relative to saline control startle amplitudes at 115 dB (marked with the star); note significantly increased startle amplitudes after salicylate injection, (d) Mean reaction time measures for broadband noise bursts ($n = 7$). Baseline (black circles), saline (blue triangles), and salicylate (red squares) are shown with standard error (SE) bars. Reaction times for 70, 80, and 90 dB SPL noise bursts decreased significantly with salicylate, suggesting an increased sensitivity to loud sounds.

Multiple Comparison Test showed that thresholds during SS treatment were significantly higher (~17.4 dB) than saline treatment ($P < 0.0001$).

3.1.2. Tinnitus. To confirm that tinnitus-like behavior was present after SS treatment, three rats were trained on our two-alternative forced choice paradigm to activate a left feeder in the presence of a steady NBN and to activate the right feeder in the presence of an AM noise or no sound (Quiet). Figure 2(b) presents the percentage of correct responses for each animal on AM, Quiet, and NBN trials 4 days prior to saline treatment (−4 to −1 days), ~2-3 h after saline treatment, days 1–4 after saline treatment, ~2-3 h after SS treatment, and days 1–4 after SS treatment. Prior to treatment (baseline control), the mean percentages of correct responses for NBN, AM, and Quiet trials were typically greater than 80% and never less than 70% correct for all three rats over the three conditions and four days; these results indicate that behavior was under stimulus control. Mean percentages of correct responses on NBN and AM trials were typically greater than 75% on the day of saline and greater than 80% on days 1–4

following saline treatment; these results indicate that animal behavior remained under stimulus control on the day of and the 4 days after saline treatment. Importantly, animals did not show a shift in responding during Quiet trials when treated with saline. However, on the day of SS treatment all three rats showed a dramatic change in their behavior on Quiet trials by shifting their response from the feeder associated with Quiet and AM to the feeder associated with a steady NBN, behavior consistent with hearing a steady phantom sound on Quiet trials rather than no sound. On days 1–4 following SS treatment, the percentages of correct responses reverted to 80% or more on Quiet trials behavior consistent with the absence of tinnitus. A repeated measures ANOVA showed significant differences on the Quiet trials between the saline treatment and SS treatment ($P = 0.0066$); a Newman-Keuls Multiple Comparison Test showed significant differences between Quiet and AM trials ($P < 0.01$) (i.e., fewer responses to the previously reinforced feeder on Quiet trials than during SS compared to saline) and between Quiet and NBN tests ($P < 0.01$) (i.e., a greater number of responses on Quiet trials to the NBN feeder during SS treatment compared to saline). Taken together, these results indicate that the rats can correctly discriminate AM and steady NBN during SS treatment; however, on roughly 45–65% of the Quiet trials the three rats mistakenly selected the feeder associated with a steady NBN suggesting that the rats are experiencing a phantom sound similar to the NBN stimulus.

3.1.3. Startle Response. To determine if SS treatment would alter the rat's suprathreshold response to sound, we measured startle reflex response amplitudes in six rats to broadband noise bursts (20 ms) presented at intensities from 70 to 115 dB SPL. For ease of comparison, startle reflex amplitudes in each animal were normalized to the startle reflex response measured at 115 dB SPL (star) during the saline-control condition. Figure 2(c) presents mean startle responses of the animals after saline control treatment (blue open circles) and after SS-injection (250 mg/kg, red filled circles). A two-way ANOVA (matching by rows) showed that the startle amplitudes in the SS-treated group were significantly larger than in the saline-treated group ($P = 0.009$).

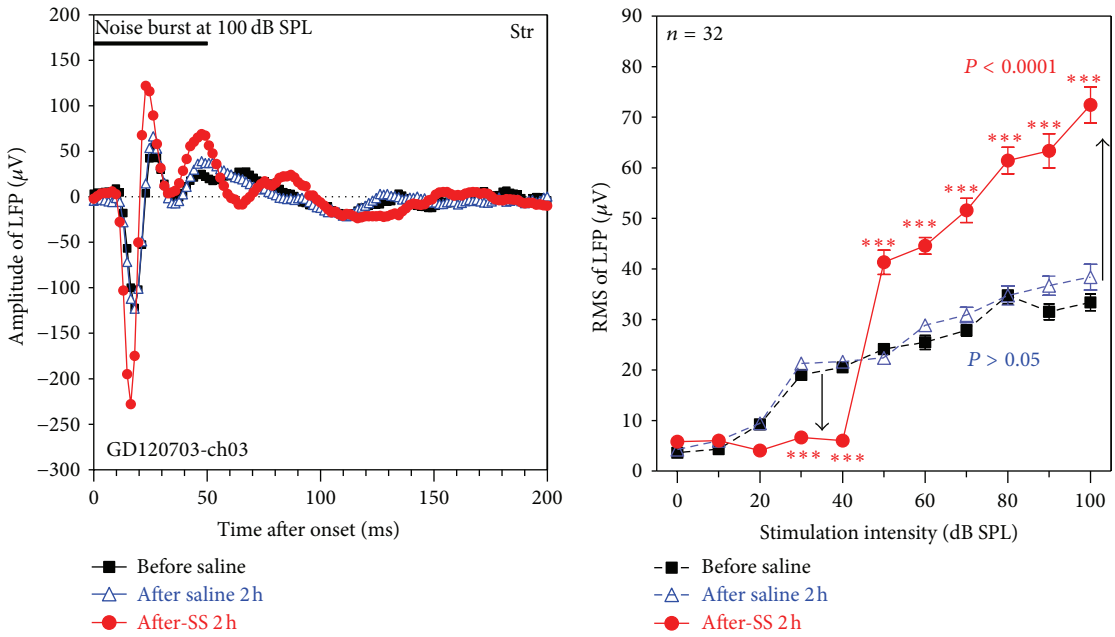
3.1.4. Loudness Perception. Previous researchers have used reaction time to estimate loudness perception in humans [7], monkeys [73], canaries [74], and cats [75]. Therefore, to confirm that hyperacusis-like behavior was present in our rats following an injection of SS, we measured reaction times to suprathreshold broadband noise bursts. Figure 2(d) shows mean reaction times for 7 rats for baseline (no injection), saline (control), and SS (200 mg/kg, i.p.) treatments. There were no significant differences between baseline and saline reaction times; but after SS treatment, the rats exhibited significantly faster reaction times to 70 ($P = 0.05$), 80 ($P = 0.04$), and 90 dB SPL ($P = 0.02$) noise bursts than after saline treatment. As in humans with hyperacusis, animals with hyperacusis-like behavior showed shorter than normal reaction times for suprathreshold stimuli, presumably because sound stimuli are perceived as being louder than in normal-hearing animals [74]. In other words, rats given an injection of SS became more sensitive to loud sounds.

3.2. Neurophysiological Changes in the Brain after SS Injection

3.2.1. Striatum. Previous studies indicate that some cells in the Str respond to sounds [76] that electrical stimulation of the Str can trigger phantom auditory percepts [61] and that SS induces c-fos expression in some cells in the Str [63]. To determine if and how SS altered its electrophysiological properties, we recorded from the Str before and after administering SS (250 mg/kg i.p.). Noise bursts (50 ms, 100 dB SPL) evoked a robust LFP in the Str (Figure 3(a)) with a large negative peak around 18 ms followed by positive peak near 23 ms. There was little or no change in the amplitude or waveform of the LFP 2 h after saline treatment. However, the negative and positive peaks of the Str LFP increased substantially 2 h after SS. To compare the LFP responses before and after the treatments, the RMS of the LFP was measured from 0 to 100 ms. Figure 3(b) presents the mean RMS of LFP of 32 recordings as a function of intensity; mean data are shown before saline, 2 h after saline, and 2 h after SS. Saline treatment had no effect on LFP amplitudes. In contrast, the LFP input/output function 2 h after SS was shifted roughly (20 dB) to the right, indicative of a threshold shift (hearing loss) and consistent with the behavioral threshold shift (Figure 2(a)). In addition, LFP amplitudes at 50 dB SPL or higher were roughly twice as large as presaline values. A two-way ANOVA showed that SS induced significant changes in LFP amplitude ($P < 0.0001$). Bonferroni posttests revealed a significant decrease of LFP at 30–40 dB 2 h after SS treatment and a significant increase from 50 to 100 dB SPL ($P < 0.001$).

Figure 4 demonstrates the effects of SS injection (250 mg/kg) on tone burst-evoked LFP in the Str of five rats (64 recordings from different Str locations). LFP response to midfrequency tones (Figure 4(a), 12.1 kHz as an example) increased at high stimulus levels (>50 dB SPL) and decreased at stimulus levels <50 dB SPL (Figure 4(a)) consistent with the noise-burst LFP. To determine if the changes in LFP amplitude were frequency dependent, LFP amplitudes were measured at 100 dB SPL before and 2 h after SS is plotted as a function of frequency (Figure 4(b)). Pretreatment LFPs were largest at low frequencies (1.5–8 kHz) and decreased at high frequencies (see blue open circles). However, 2 h after the SS injection, midfrequency region (3.5–18.3 kHz) LFPs were much larger than normal, whereas low-frequency and high-frequency LFPs showed much smaller increases (two-way ANOVA, Bonferroni posttests, $P < 0.001$). Similar results were observed at other stimulation levels (data not shown). These results are consistent with our previous observation of SS-induced hyperactivity at the midfrequencies in the inferior colliculus (IC) [14].

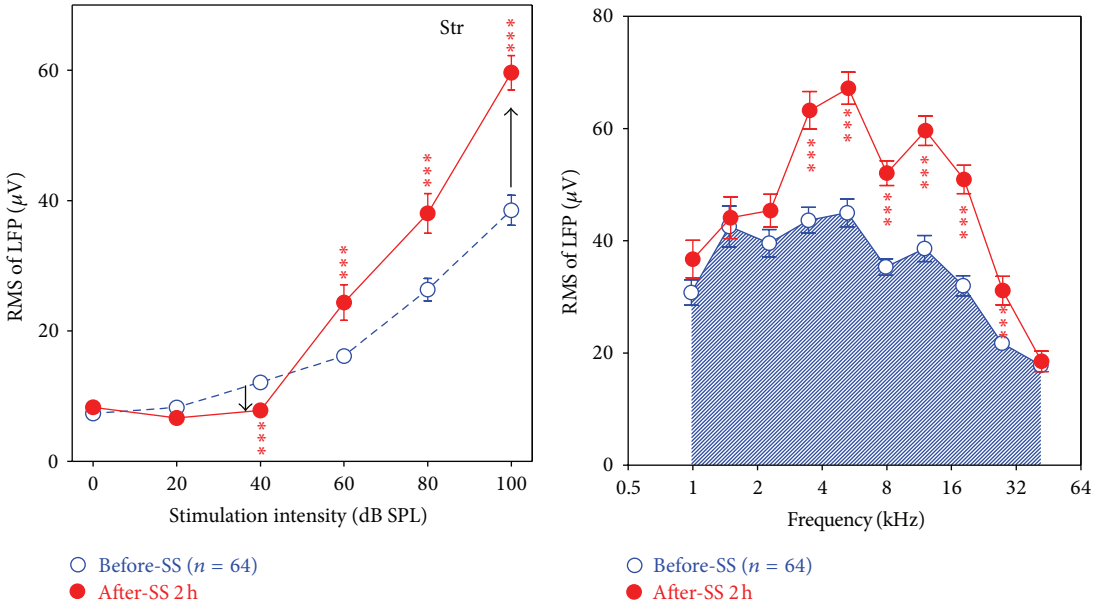
Figure 5(a) presents the mean spontaneous discharge rates of 32 multiunit clusters in the Str measured before (−2 h to 0 h) and after SS treatment (1 h, 2 h). Spontaneous rates were stable before SS treatment (−2 h to 0 h; one-way ANOVA, Newman-Keuls Multiple Comparison Tests,



(a) An example of LFP

(b) Mean LFP response levels

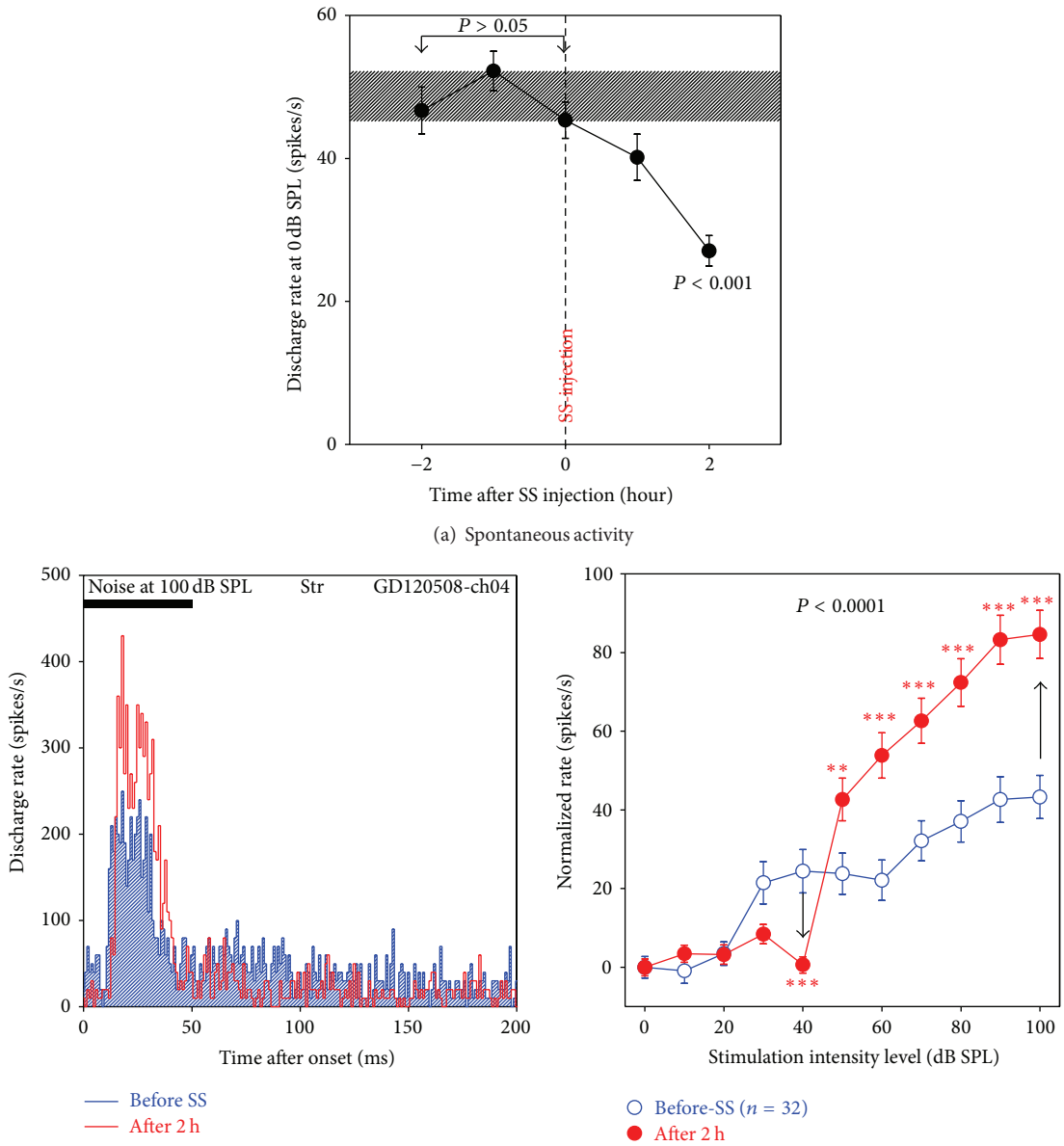
Figure 3: The effects of SS-injection on sound-evoked local field potential (LFP) elicited from electrodes in the Str. (a) An example of LFP at 100 dB SPL recoded before treatment (black filled squares), after saline injection (blue open triangles), and after SS injection (red filled triangles), showing an enhanced response following SS-injection. (b) Mean RMSs of LFP ($n = 32$) in a time window of 100 ms as a function of stimulation level, showing progressive increase of LFP amplitude at high stimulation levels but a reduction at low stimulation levels. Acoustic stimulation: 50 ms noise burst; treatments: saline (5 mL/kg, i.p.) and SS (250 mg/kg, i.p.); the vertical bars are standard errors (SEs) and the $***$ means $P < 0.001$; the arrows indicate increase and decrease of LFP amplitude.



(a) Mean response levels of LFP to 12.1 kHz

(b) Mean response levels of LFP at 100 dB SPL

Figure 4: The effects of SS-injection on the tone-evoked LFP in the Str. (a) Mean RMSs of LFP ($n = 64$) to 12.1 kHz in a time window of 100 ms as a function of stimulation level, showing increase of LFP amplitude at high stimulation levels but a reduction at low stimulation levels. (b) Mean RMSs of LFP ($n = 64$) at 100 dB SPL as a function of stimulation frequency, showing significant enhancement at midfrequencies (3.5–18.3 kHz). Acoustic stimulation: 50 ms tone bursts at different frequencies; treatment: SS (250 mg/kg, i.p.); the vertical bars are SEs and the $***$ means $P < 0.001$; the arrows indicate increase and decrease of LFP amplitude.



(a) Spontaneous activity

(b) An example of response

(c) Mean discharge rates

Figure 5: The SS effects on unit activity of neurons in the Str. (a) Mean spontaneous discharge rates ($n = 32$) as a function of time showing significant decrease after SS injection ($P < 0.001$). (b) An example of peristimulus time histograms (PSTH) obtained before SS (blue) and after SS (red), showing SS-induced increase of discharge rate. (c) Averaged discharge rates of neurons in the Str ($n = 32$) in a time window of 100 ms, showing a similar effect of SS injection as the LFP recorded in the nucleus. The discharge rates of each neuron were normalized to that at 0 dB SPL. Acoustic stimulation: 50 ms noise burst; treatment: SS (250 mg/kg, i.p.); the vertical bars are SEs; the arrows indicate increase and decrease of unit activity; $^{***}P < 0.001$; $^{**}P < 0.01$.

$P > 0.05$); the mean rate (±SEM) during the pretreatment period is shown by the dashed horizontal rectangle. Spontaneous rates began to decline 1 h after SS and were significantly below pretreatment firing rates 2 h after SS ($P < 0.001$).

Figure 5(b) shows a representative PSTH of a multiunit cluster in the Str in response to a noise burst (50 ms duration, 100 dB SPL). The firing rate was enhanced after SS injection (red PSTH above blue PSTH) resulting in a sharper onset peak. Because SS reduced spontaneous activity, the transient nature of the onset response was further accentuated by the reduced spontaneous rate prior to the onset response (red line below blue line 0–10 ms). Figure 5(c) presents the mean discharge rates of 32 multiunit clusters as a function of intensity. Since spontaneous activity decreased after the SS treatment, the sound-evoked discharge rate was normalized by subtracting the mean firing rate at 0 dB SPL from the mean firing rate at higher intensities. The normalized discharge rates represent the sound-driven responses. Similar to the LFP, the sound-evoked discharge rates were enhanced at intensities ≥50 dB SPL but reduced at levels <50 dB SPL

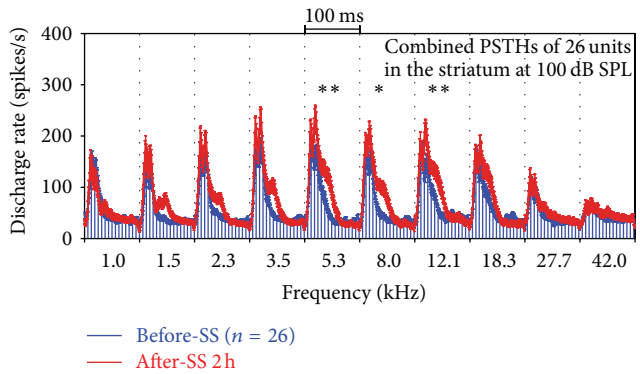


Figure 6: Averaged PSTHs of 26 units recorded in the Str showing significant effect (enhancement) in the midfrequency region (5.3, 8.0, and 12.1 kHz). The PSTHs were obtained before (blue) and after SS injection (red). Stimulation: 50 ms tone bursts at 100 dB SPL and at different frequencies; treatment: SS (250 mg/kg, i.p.). The vertical bars are SEs. $^{**}P < 0.01$; $^{*}P < 0.05$.

leading to a threshold shift of ~20 dB. A two-way ANOVA showed a significant change pre- and post-SS injection ($P < 0.0001$). The sound-evoked firing rate 2 h after SS was significantly below pretreatment values at 40 dB SPL, whereas firing rates 2 h after SS were significantly greater than normal from 50 to 100 dB SPL (Figure 5(c); Bonferroni posttests, $^{***}P < 0.001$, $^{**}P < 0.01$).

Similar to the tone-evoked LFP (Figure 4(b)), tone-evoked firing rates of multiunit clusters were affected in a frequency dependent manner after the SS-injection. To identify the SS-induced changes in the population of units from which we recorded, we computed the mean PSTH at each frequency-intensity combination from all 26 multiunit clusters as described previously [56]. Figure 6 presents the mean PSTHs (100 ms duration, 5 ms bin width) of 26 multiunit clusters obtained in the Str in response to 50 ms tone bursts presented at 100 dB SPL. Control responses are shown in blue; responses obtained 2 h after SS (250 mg/kg) are shown in red. The mean discharge rates of the PSTH were enhanced 2 h after SS. The firing rate increases, which was most pronounced between 1.5 and 18.3 kHz, resulting in a larger onset peak and a prolongation of the PSTH. To quantify the changes, mean discharge rates were calculated from 0 to 100 ms of each mean PSTH; SS treatment produced a significant increase in firing rate at 5.3, 8.0, and 12.1 kHz (one-way ANOVA, Newman-Keuls multiple comparison test). Altogether, SS increased sound-evoked activity at high sound levels predominantly at the midfrequencies, increased threshold, but decreased spontaneous activity and sound-evoked activity at low sound levels.

3.2.2. Lateral Amygdala. The amygdala, which assigns emotional significance to past experiences, has been implicated in tinnitus and hyperacusis [59, 63, 77], but its precise role in SS-induced tinnitus is poorly understood. Therefore we recorded from the LA to determine how SS would influence its electrophysiological properties. Noise-burst-evoked LFP from the LA had a longer latency, longer duration, and broader peaks than those from the Str (Figure 7(a)). The LFP from the LA evoked by a 100 dB SPL noise burst (black) consisted of a negative peak at ~25 ms and a positive peak at ~55 ms. The sound-evoked LFP increased substantially after SS injection (250 mg/kg, red line) and the peaks became narrower. The mean LFP amplitude-intensity function from the LA evoked by noise bursts is shown in Figure 7(b) before and 2 h after SS. SS treatment resulted in a slight-to moderate reduction in response amplitude at low intensities (<60 dB SPL), a threshold shift of approximately 20 dB, and a significant increase in response amplitude at high intensities (≥60 dB) (two-way ANOVA, Bonferroni post hoc tests significant at ≥60 dB SPL).

Figure 7(c) shows mean PSTHs of 4 multiunit clusters recorded in the LA in response to 1.0 kHz and 8.0 kHz tone bursts presented at 60 dB SPL. The 1.0 kHz tone induced a robust response (blue) with a large, narrow, short-latency peak followed by a smaller, long latency peak. SS induced striking changes in the temporal profile of the 1 kHz PSTH; the early part of the response was slightly enhanced while the late part of the response was completely suppressed. The mean PSTH to the 8.0 kHz, 60 dB tone burst was broad and lacked a sharp onset response (Figure 7(c), black); however, 2 h after SS injection, the same tone burst evoked a more robust response with a completely different temporal PSTH profile, one that consisted of a large, sharp onset peak followed by the loss of the delayed response (Figure 7(c), purple). To quantify the frequency effect of SS, mean discharge rates during SS treatment were normalized to the pretreatment firing rate and expressed as percentage of pretreatment rate (Figure 7(d)). At low frequencies (1.0–5.3 kHz) and a high frequency (42.0 kHz), the mean discharge rates either remained near pretreatment control levels (~100%, 1.5–3.5 kHz) or were significantly lower than the controls (<100%, 1.0, 5.3, and 42.0 kHz). In contrast, mean discharge rates at the midfrequencies (8.0–27.7 kHz) increased significantly (>100%).

The mean spontaneous rates of eight multiunit clusters increased slightly from 34.4 ± 21.1 spikes/s (mean ± SD) before treatment to 42.5 ± 20.4 spikes/s 1 h after SS; the increase did not reach statistical significance ($P = 0.12$, t-test).

3.2.3. Hippocampus. The HC, important in memory formation, has been implicated in tinnitus [59, 78, 79], but its functional contributions to SS-induced tinnitus are unclear. To evaluate its contributions, we recorded from the HC in six rats before and after SS treatment. Broadband noise bursts induced a clear LFP in the HC but seldom evoked strong neuronal discharges. Responses to tone bursts were also weak; therefore, we focused our analysis on noise-burst-evoked LFP. Figure 8(a) presents averaged LFP from 4 recordings obtained from electrodes in the dorsal HC of one animal in response to noise bursts. The LFP evoked by 100 dB noise bursts consisted of a broad negative peak around 30 ms followed by a much broader positive peak beginning around 50 ms (Figure 8(a),

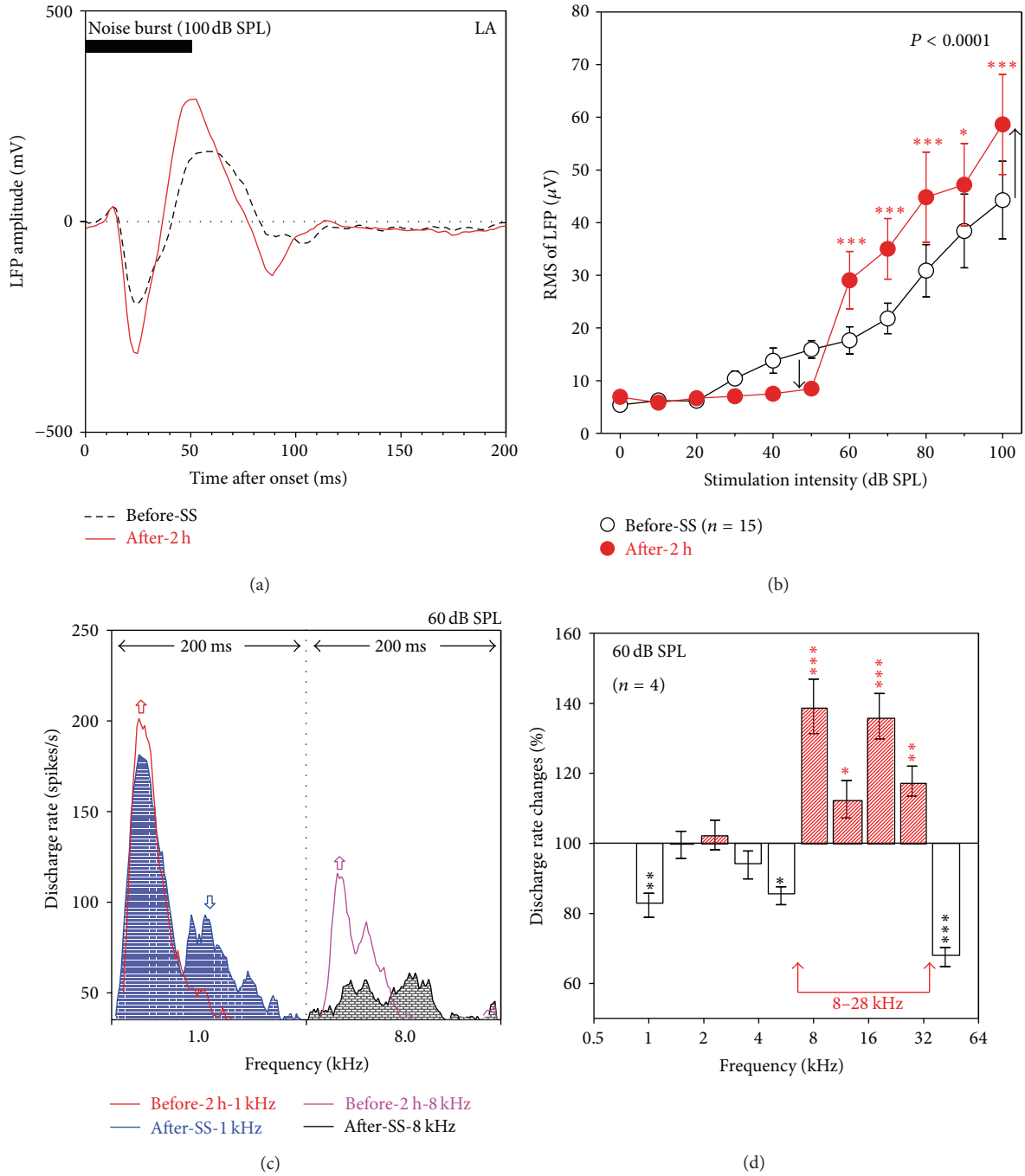


Figure 7: The effects of SS injection on auditory responses of the LA. (a) An example of LFP to noise burst at 100 dB SPL (black) showing an increase after SS injection (red). (b) Mean RMSs of LFP ($n = 15$) in a time window of 100 ms as a function of stimulation level, showing enhancement at high stimulation levels ≥60 dB SPL but reduction at low-stimulation levels <60 dB SPL. (c) Example of PSTHs in response to tones at 1.0 kHz (left) and 8.0 kHz (right) before and after SS injection showing greater increase after SS injection at the high-frequency; (d) SS-induced changes (%) of mean discharge rate in a time window of 100 ms showing SS-induced increase in the frequency range of 8–27.7 kHz. Stimulation: 50 ms noise or tone bursts; treatment: SS (250 mg/kg, i.p.). The vertical bars are SEs. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

black). Saline treatment had little or no effect on the amplitude or profile of the LFP (Figure 8(a), blue). However, the amplitude of the LFP increased and the positive peak became narrower 2 h after SS treatment. Figure 8(b) shows the RMS amplitude (100 ms window) of the LFP (n = 29) from the HC as a function of noise-burst intensity. Pretreatment LFP amplitudes increased slowly up to 70 dB SPL and then increased more rapidly at higher levels reaching a maximum of around 19 μV at 100 dB SPL, much smaller than the LFP in the Str and LA. LFP amplitude increased

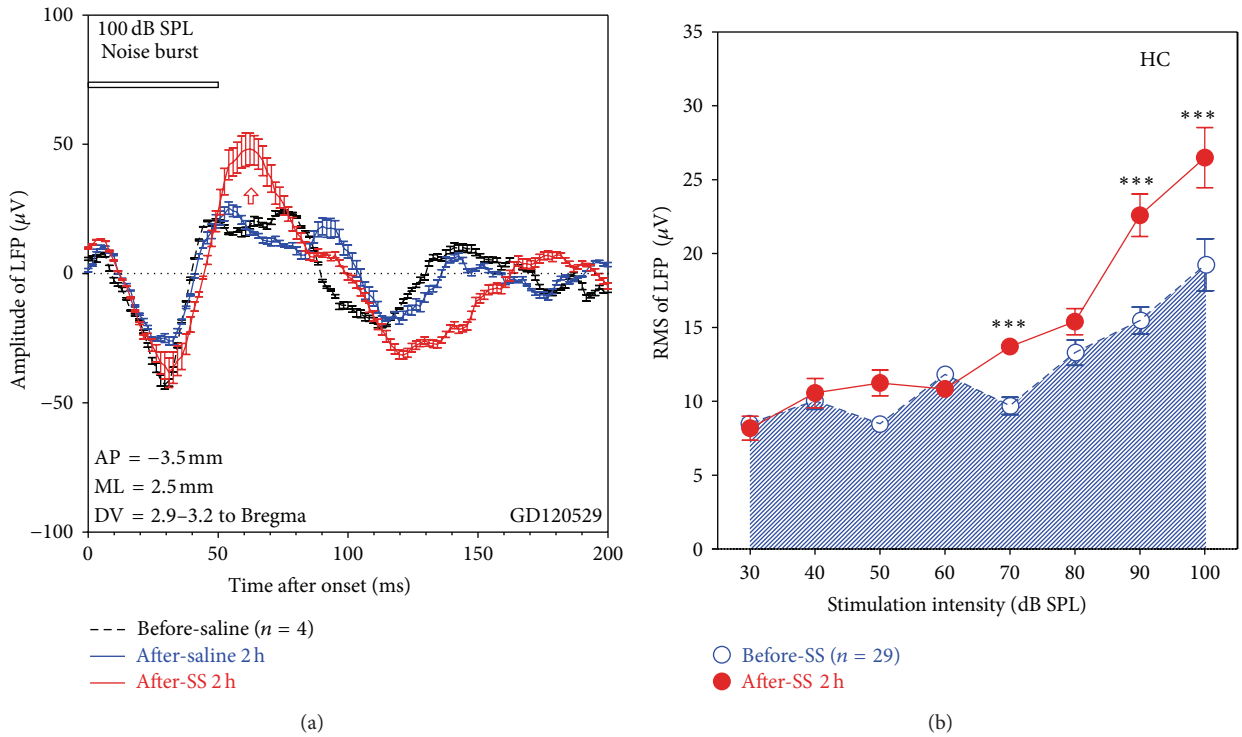


Figure 8: The effect of SS injection on noise-burst-evoked LFP elicited from electrodes in the hippocampus. (a) Averaged LFP ($n = 4$ recordings in one rat) at 100 dB SPL recoded before treatment (black), after saline injection (blue), and after SS injection (red), showing a slight increase after SS injection. (b) Mean RMSs of LFP ($n = 29$) in a time window of 100 ms as a function of stimulation level, showing enhancement at high stimulation levels. Stimulation: 50 ms noise burst; treatment: SS (250 mg/kg, i.p.). The vertical bars are SEs and the $***$ means $P < 0.001$.

significantly at 70, 90, and 100 dB SPL 2 h after SS treatment (two-way ANOVA, intensity a repeated measure, $P < 0.0001$; Bonferroni post hoc tests); the amplitude increase in the HC, about 35%, was less than in the Str and LA.

3.2.4. Cingulate Cortex. The Cg has been implicated in tinnitus distress and showed strong c-fos immunolabeling following salicylate treatment [63, 80, 81]. We recorded the LFP from the Cg to identify possible electrophysiological changes induced by SS. Noise-burst LFPs from the Cg were substantially smaller and broader than those from the HC, LA, and Str and few Cg multiunit clusters responded to tones or noise. Noise-burst-evoked LFPs were measured from the Cg of four rats. LFP waveforms varied with electrode depth. Upon penetrating area-1 of the cingulate Cg1 [72], an LFP was encountered with an initial positive peak (~35 ms latency; data not shown). With increasing electrode depth and entry into cingulate area-2 (Cg2), the LFP reversed polarity (Figure 9(a)) and increased amplitude. The LFP from Cg2 began with a negative peak (~35 ms latency) followed by an extremely broad positive peak (~70 ms latency). LFPs were measured from Cg2 region prior to treatment, 2h post-saline treatment, and 2h post-SS treatment (250 mg/kg). LFP amplitudes and waveforms remained largely unchanged after the saline (blue) and SS treatments (red, Figure 9(a)). Figure 9(b) presents mean (RMS, 100 ms window, $n = 16$) noise burst versus intensity functions measured in the Cg before, 2 h after saline, and 2 h after SS (250 mg/kg) treatments. In contrast to the large amplitude increases observed in other areas (St, LA, and HC), LFP amplitude-intensity functions in the Cg were largely unaffected by SS treatments.

4. Discussion

4.1. Behavioral Features. SS has long been known to induce sensorineural hearing loss by affecting the electromotile response of cochlear outer hair cells and neural activity in the cochlea [2]. The magnitude of the hearing loss is related to SS dose and serum salicylate levels [82]. The 200 mg/kg SS dose increased noise-burst behavioral thresholds ~17.4 dB (Figure 2(a)). Our noise-burst threshold shifts are slightly greater (~7.5 dB) than those reported previously with the same dose of SS, but with a different behavioral method and low-to-mid frequency tone bursts instead of noise bursts [83]. Our noise-burst threshold shifts, however, were similar to the noise-burst LFP threshold shifts observed in the Str and LA.

Despite the threshold elevation and reduced neural output at low stimulus levels (Figures 3, 4, 5, and 7), SS enhanced the amplitude of acoustic startle reflex at high stimulus levels (Figure 2(c)), consistent with previous results [20] and reduced animals' reaction times to loud sounds (Figure 2(d)). The enhanced motor response to suprathreshold sounds could conceivably be related to hyperacusis, a perceptual phenomenon whereby high intensity sounds

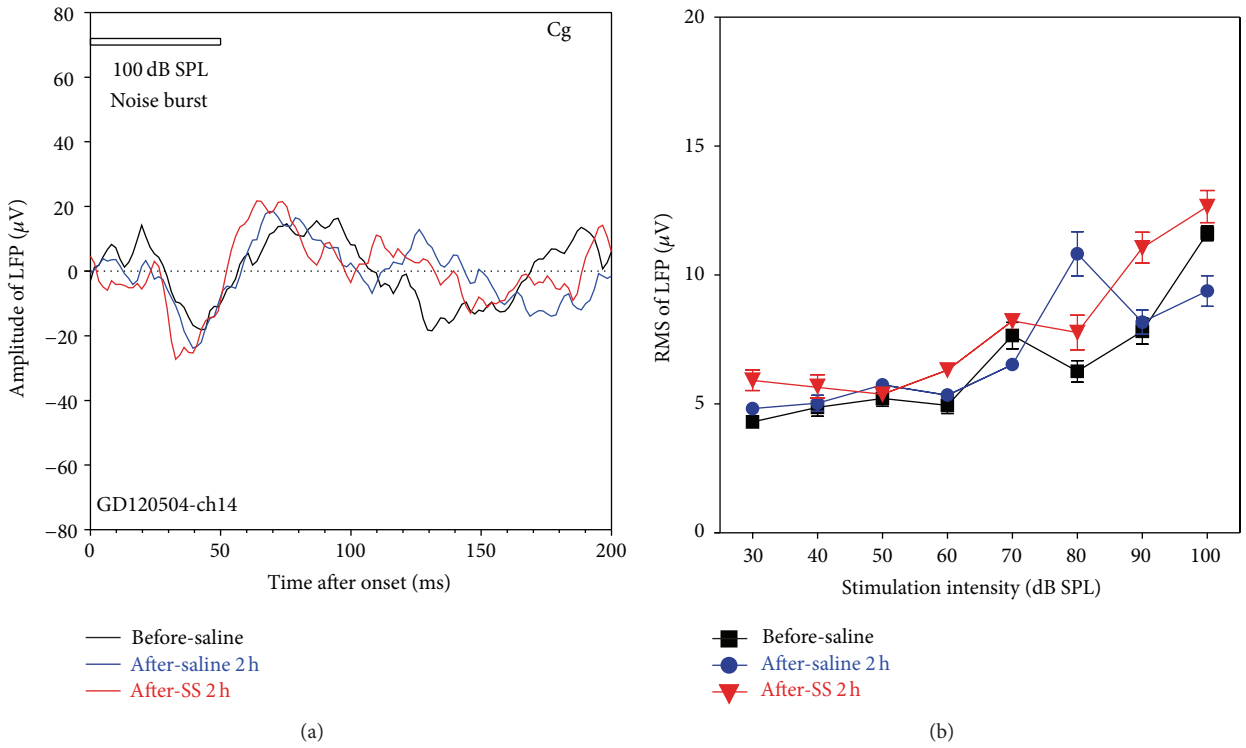


Figure 9: The effect of SS on noise-burst-evoked LFP elicited from electrodes in the cingulate cortex. (a) An example of LFP at 100 dB SPL recoded before treatment (black), after saline (blue), and after SS (red), showing no change during treatment. (b) Mean RMSs of LFP ($n = 16$) in a time window of 100 ms as a function of stimulation level, showing no significant change of the mean LFP. Stimulation: 50 ms noise burst; treatment: SS (250 mg/kg, i.p.). The vertical bars are SEs.

become intolerably loud, a condition that frequently accompanies tinnitus [84, 85]. However, this hyperactive motoric response may not be the perceptual equivalent of hyperacusis until further confirmatory data are obtained from human listeners with hyperacusis. Alternatively, the enhancement of suprathreshold startle reflex amplitudes could be related to the increased suprathreshold excitability seen within the central auditory pathway, as we have previously reported [8, 20, 86]. Greater neural activity within the LA, known to modulate the startle reflex [87, 88], and neural activity in the Str, which influences motor movements and vocalizations could enhance the startle reflex [51, 89–91].

Previous studies indicate that the minimum dose of SS needed to induce tinnitus in rats is 150–200 mg/kg [3, 4, 92]. In agreement with these earlier studies employing different techniques, we observed robust behavioral evidence of tinnitus on Quiet trials 2 h following the administration of 200 mg/kg SS; in contrast saline had no effect on Quiet performance. One day later, after SS washout, behavior on Quiet trials reverted to normal. Importantly, the performance of the rats to the steady NBN and the AM signal were unaffected by SS treatment indicating that the behavior remained under stimulus control. Taken together, the behavioral results confirm that our salicylate treatment induced mild, reversible hearing loss, tinnitus, increased sensitivity to suprathreshold sounds, and enhanced acoustic startle reflex motor activity to high intensity sounds.

4.2. SS and Nonauditory Structures. The ototoxic effects of SS on the cochlea have been well documented [9, 13, 20]; however, its effects on the central nervous system are only beginning to be explored, despite the fact that SS readily crosses the blood-brain barrier [93, 94]. The past two decades have seen a rapid increase in our understanding of how SS affects the function of neurons in the central auditory pathway, but comparatively little is known about the effects of SS on structures outside the classical auditory pathway. Insights likely affected brain structures can be gleaned from earlier c-fos immunolabeling studies [63, 95, 96]. Since SS increased c-fos labeling in the Str, LA, and Cg we investigated the electrophysiological changes in these areas along with the hippocampus where rather modest c-fos labeling occurred.

4.2.1. Brain Gain. Acoustic stimulation induced robust neural response in the LA and Str consistent with earlier reports [23, 76, 97]. SS produced a number of well-defined changes in the LA and Str. LFP thresholds increased approximately 20 dB following SS treatment similar to behavior thresholds (Figures 2(a), 3(b), and 7(b)). The threshold elevation in

the LA and Str is most likely due to a cochlear hearing loss which reduces the neural output of the cochlea [2, 13, 20]. Despite a reduced neural output from the cochlea after SS treatment [8], suprathreshold responses from the LA and Str were greatly enhanced (Figures 3 and 7) [56]. These results suggest that the neural output from the cochlea is amplified as it transits up the central nervous system. Previous reports indicate that LFPs in the inferior colliculus are nearly normal after SS treatment; this implies that some amplification is already occurring between the auditory nerve midbrain. Broadly speaking, neural amplification could result from increased excitation or decreased inhibition. *In vitro*, SS reduces γ-aminobutyric acid (GABA) mediated inhibitory currents in auditory cortex, hippocampus, inferior colliculus, and spinal neurons [10–12, 98] while acute SS treatment *in vivo* decreased GABA expression and increased glutamate expression in the inferior colliculus [99]. Since LFPs from the LA, Str, and HC, as well as auditory cortex and medial geniculate, become larger than normal after SS [8, 20], additional amplification, likely due to a reduction of GABA-mediated inhibition, must occur above the midbrain [20, 70]. Taken together, these results suggest that increased neural gain occurs at multiple sites within the central nervous system. In response to a reduced cochlear output, the central auditory system becomes more responsive to a reduced input indicative of increased central gain or sensory rescaling due to peripheral hearing loss.

The hyperexcitability in the Str and LA was frequency dependent, similar to that previously reported in auditory cortex [13]. Interestingly, tone evoked hyperactivity was maximal at the midfrequencies (Figures 4(b), 6, and 7(d)) where the pitch of SS-induced tinnitus occurs [100]. Salicylate is known to induce tinnitus with a pitch between 10 and 20 kHz [69, 101]. Physiologically, the CF of many neurons in the auditory cortex (AC) and LA shifted into the tinnitus frequency region after SS treatment [13, 56]. In the current report, enhancement of suprathreshold responses of the LA also occurred in the frequency range of 8–28 kHz (Figure 7(d)) and that of the Str occurred in the frequency range of 3.5–28 kHz (Figure 4(b)). The midfrequency hyperactivity could result from two factors. One is a cochlear frequency-dependent loss in sensitivity that was smallest at the midfrequencies and relatively greater at low and high frequencies [13]. The second is a SS-induced CF-shift in AC and LA such that many high-CF and low-CF neurons undergo a CF-shift towards the midfrequencies [13, 56]. One consequence of this CF shift is that many more neurons respond to the midfrequency tones than normal would do so.

4.2.2. Temporal Profiles. SS altered the temporal profile of LFP and PSTH from the Str and LA. In general, the onset component of the LFP was more robust, the latency shorter, and the width narrower after SS treatment (Figures 3(a) and 7(a)). PSTH onset responses were more pronounced in the Str (Figures 5 and 6) and LA (Figure 7). SS had the opposite effects on the duration of the PSTH response in the Str and LA. In the Str, SS prolonged the duration of the response and in some cases generated a secondary peak with a latency around 50 ms (Figure 6, 1.2–3.5 kHz). The latency of this secondary peak corresponds closely with the pronounced increase in the second positive peak of the LFP from the Str (Figure 3(a)). An LFP can be evoked in the Str by electrical stimulation of overlying cortex [102]. The electrically evoked LFP consisted primarily of a single onset peak; however, when the GABAa receptor antagonist, bicuculline, was infused into the striatum, the initial peak of the LFP became larger and a secondary peak appeared in the LFP. In addition, bicuculline increased the number of action potentials and the duration of the response merges effects similar to those induced by SS [10–12, 98]. This suggests that the amplitude enhancement and prolongation of the response in the Str are due to a loss of local GABA-mediated inhibition. Disruption of this circuit could impair auditory temporal processing. Indeed, high doses of aspirin, whose active ingredient is salicylate, lead to a slight impairment of temporal resolution [103].

SS increased the amplitude of the onset response in the LA, decreased the latency of the second peak of the LFP, and reduced the duration of the PSTH so that the response was more phasic than sustained. SS enhanced the onset response and shortened the duration of responses in the supragranular layer of the auditory cortex [86], changes attributed to reduced intracortical GABA-medicated inhibition [70, 104]. Electrical stimulation of the medial geniculate body, part of the auditory pathway, evoked a negative-positive LFP in the LA. Administration of baclofen, a GABAb agonist, significantly reduced the amplitude and increased the latency of the positive peak [105]. Since SS suppresses GABA-mediated inhibition [12], its effects on the LA, either directly or indirectly, would be expected to increase the amplitude and decrease the latency of the positive peak similar to what we observed (Figure 7).

4.2.3. Spontaneous Activity and Tinnitus. Models of tinnitus often assume that the phantom sound arises from an increase in spontaneous activity localized to the region of hearing loss and tinnitus pitch [106]. While there is a good deal of data in the auditory brainstem and midbrain to support this hypothesis for cases of chronic tinnitus [107–110], the effects of SS on spontaneous rates have varied across studies, region of the brain, and drug dose employed [14, 18, 19, 21, 56, 69, 111–113]. In this study we found a slight increase of spontaneous activity in the LA but a decrease in the striatum, which was inconsistent with the sound-evoked response. Our SS data suggest that different mechanisms modulate spontaneous activity and sound-driven responses in the Str. Although c-fos functional relationship to neuron firing is not well understood, c-fos immunolabeling has nevertheless often been used as a marker of neural activity [114]. Immunolabeling studies have identified many regions of strong c-fos expression after SS treatment. SS induced strong c-fos labeling in the LA; therefore, we assumed that spontaneous activity might increase in LA after SS treatment [63]. However, we found that SS induced an insignificant increase of spontaneous activity among LA neurons that responded to sound stimulation. Strong c-fos labeling was also reported in the Str after SS treatment but surprisingly SS

caused a significant decrease in spontaneous activity among Str neurons that responded to sound (Figure 5(a)). Thus, our results do not provide any support for the hypothesis that tinnitus is due to an increase in spontaneous activity in LA or Str. Moreover, the data suggest that the SS-induced change in c-fos expression is not a good predictor of spontaneous firing rate. However, this interpretation should be tempered by the fact that our assessment of spontaneous rate was obtained only from acoustically responsive neurons; it is conceivable that spontaneous rates may have increased among acoustically unresponsive neurons. Moreover, the effects of the anesthetics used in our study may have masked the effects of SS on spontaneous activity.

4.2.4. Hyperactivity and Hyperacusis. Among the most robust and consistent electrophysiological change induced by SS treatment is the enhancement of suprathreshold sound-evoked responses at multiple sites in the central auditory pathway [8, 20]. SS also enhanced sound-evoked responses in the Str, LA, and HC, regions outside the classical auditory pathway. One common factor that may be responsible for these enhanced neural responses is the SS-induced reduction of GABA-mediated inhibition [11, 12, 20, 99]. The robust increase in suprathreshold neural activity in several higher auditory centers could conceivably cause sounds to be perceived as much louder than normal (hyperacusis); this assumes that the amplitude of sound-evoked LFP is closely correlated with the loudness.

The Str is known to modulate the startle reflex [34, 115] and the SS-induced enhancement of neural activity in this motor area could conceivably contribute to the enhanced startle amplitudes (Figure 2(b)). Electrical stimulation of the amygdala can enhance the startle reflex [116]. Thus, the SS-induced enhancement of LA responses could be another factor that potentiates the startle reflex amplitude after SS. The HC also modulates the startle reflex [117]. Thus, the SS-induced enhancement of HC activity provides another means of increasing the startle responses. However, SS failed to enhance responses in the Cg indicating that this structure is unlikely to be involved with the startle response. The lack of functional change in Cg is rather surprising given that SS significantly increased c-fos labeling in this region [63].

4.3. Anesthesia. In this study, we administered SS to ketamine anesthetized rats. In an earlier study, we demonstrated that ketamine, noncompetitive NMDA antagonist, accentuated the SS-induced enhancement of sound-evoked activity in the auditory cortex [20]. Ketamine also enhanced the cortically generated 40 Hz auditory steady-state response but not the more peripherally generated auditory brainstem response [1, 118]. In contrast, isoflurane anesthesia, which enhances GABAergic activity, suppressed the SS-induced enhancement of the LFP [20]. Taken together, these results suggest that the SS-induced enhancement of sound-evoked activity may be due to its combined effects glutamatergic and GABAergic synapses.

5. Conclusion

Rats treated with SS doses of 200 and 250 mg/kg showed behavioral evidence of hearing loss and tinnitus and responded in a hyperactive manner to loud sounds. These SS-induced behavioral changes were accompanied by suprathreshold hyperexcitability in the Str, a motor area, the LA, an emotional center, and HC, involved in memory and spatial navigation. SS shortened temporal response in the LA, whereas in the Str it prolonged the response and reduced spontaneous activity. The SS-induced hyperactivity observed in the LA, Str, and HC implicates plastic change in the nuclei and may contribute to the enhancement of the startle reflex and hyperacusis.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Abnormal Baseline Brain Activity in Patients with Pulsatile Tinnitus: A Resting-State fMRI Study

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Numerous investigations studying the brain functional activity of the tinnitus patients have indicated that neurological changes are important findings of this kind of disease. However, the pulsatile tinnitus (PT) patients were excluded in previous studies because of the totally different mechanisms of the two subtype tinnitus. The aim of this study is to investigate whether altered baseline brain activity presents in patients with PT using resting-state functional magnetic resonance imaging (rs-fMRI) technique. The present study used unilateral PT patients ($n = 42$) and age-, sex-, and education-matched normal control subjects ($n = 42$) to investigate the changes in structural and amplitude of low-frequency (ALFF) of the brain. Also, we analyzed the relationships between these changes with clinical data of the PT patients. Compared with normal controls, PT patients did not show any structural changes. PT patients showed significant increased ALFF in the bilateral precuneus, and bilateral inferior frontal gyrus (IFG) and decreased ALFF in multiple occipital areas. Moreover, the increased THI score and PT duration was correlated with increased ALFF in precuneus and bilateral IFG. The abnormalities of spontaneous brain activity reflected by ALFF measurements in the absence of structural changes may provide insights into the neural reorganization in PT patients.

1. Introduction

Tinnitus is a serious public health problem. 10%–30% of people all over the world are influenced, while 5–26% of them are affected severely [1–5]. Tinnitus can be divided into nonpulsatile or pulsatile subtypes [6]. Most of the patients are nonpulsatile tinnitus (NPT) type. In contrast, pulsatile tinnitus (PT) is relatively rare and has been described in case reports or summaries with a relatively small sample [6–15].

The direct etiology of NPT remains unclear yet. The neurobiological basis may be ongoing abnormal spontaneous neural activity, brain plasticity, or disturbed brain network [16–27], while in contrast, PT is an auditory percept by stimulation of the hair cells in the inner ear. Complaints include a sound like water flow, wind blowing, or beat of the drum in the ear, and so forth. It may be low- to high-pitched tinnitus. Typically, symptomatic improvement could be achieved by external compression of the internal jugular vein in the neck on the symptomatic side. Possible etiologies include sigmoid sinus diverticulum, atherosclerosis, abnormal vascular loops, aneurysm of internal carotid artery, mastoid emissary vein, dural arteriovenous shunts, paraganglioma, involuntary contraction of muscles in the middle ear, and so forth [6–11, 28–36]. The abnormal blood flow induced by a focal defect of mastoid bone shell in the region of the transverse-sigmoid junction is a common etiology according to our daily work. PT is more likely a vascular or muscular originating disorder rather than a true neural activity disorder induced disease.

Increasing attention is being given on the brain functional activity of the tinnitus patients [16–27]. However, these studies were focused only on NPT patients; the PT patients were defined as exclusion criteria because of the totally different mechanisms of the two subtype tinnitus. Thus, it is still not known whether long time PT stimulation can cause the brain abnormalities at present. The totally different

etiology between NPT and PT may generate different brain activities. Besides, 20–60% of tinnitus patients have been reported to be depressed [22, 37–41]. The PT patients could also suffer from distress or depression. However, whether there is any corresponding abnormal neural activity remains unclear. An investigation using resting-state functional magnetic resonance imaging (rs-fMRI) technique focusing on brain activities of PT patients is an interesting and essential work.

In this study, we hypothesize that there is altered brain activity in PT patients. These alterations may be considered as neural reorganization or brain plasticity in patients with PT. To the best of our knowledge, there is still no research on the issue.

2. Subjects and Methods

2.1. Subjects. Forty two patients with persistent unilateral pulsatile tinnitus and 42 healthy controls, matched in gender, age, and right or left handedness, were enrolled in the study. Eighteen patients suffered from left-side pulsatile tinnitus and 24 from right-side pulsatile tinnitus. All of the patients complained of a sound like low- to median-pitched beats of the drum in the ear. Sounds were synchronous with the cardiac activity. Symptomatic improvement could be achieved by external compression of the internal jugular vein in the neck on the symptomatic side. CT angiography (CTA) examinations [42, 43] suspected the etiology of sigmoid sinus diverticulum caused by focal defect of mastoid bone shell in the region of the transverse-sigmoid junction. Digital subtraction angiography (DSA) examinations were also conducted. Other etiologies could be excluded [8]. After surgery, symptoms of all the patients were released. Thus, the etiology of all of the patients was confirmed as a focal defect of mastoid bone shell in the region of the transverse-sigmoid junction. All of the subjects had normal hearing threshold and magnetic resonance imaging findings of brain and had no history of neurological and psychiatric illness, alcohol or drug abuse, and severe visual impairment (the hearing threshold was determined by puretone audiometry (PTA) examination. Participants had hearing thresholds <25 dB HL at the frequencies of 0.250, 0.500, 1, 2, 3, 4, 6, and 8 kHz. This is the normal level of hearing thresholds). The severity of tinnitus and related distress were measured using the validated questionnaires such as Tinnitus Handicap Inventory (THI) originally developed by Kam et al. [44] and Newman et al. [45]. The characteristics of the participants are presented in Table 1.

This study was approved by medical research ethics committee and institutional review board of Beijing Tongren Hospital, Capital Medical University, Beijing, China, and written informed consent was obtained.

2.2. MRI Scanning. Imagines were acquired on a 3.0 T magnetic resonance scanner (General Electric Medical Systems, Milwaukee, WI, USA). The matched eight-channel phased array coil was used with foam padding to reduce head motion and scanner noise. Resting-state fMRI was obtained using an EPI (echo planar imaging) pulse sequence: TR (repetition time)/TE (echo time) = 2000/35 mm, flip angle = 90, field of view = 24 cm × 24 cm, matrix = 64 × 64. Twenty-eight axial slices were obtained with 4 mm thickness and a 1 mm gap. Each fMRI session lasted 400 seconds. A 3-dimensional brain volume (3D-BRAVO) technique was used to acquire high-resolution structural images (TR = 8.8 ms, TE = 3.5 ms, TI = 450 ms, FOV = 24 cm × 24 cm, matrix = 256 × 256, slice thickness = 1.0 mm without gap, 196 slices, 1 averages). During the scan, subjects were asked to remain motionless, not to think of anything particular during the functional scans.

2.3. Data Preprocessing. The preprocessing was carried out by using Data Processing Assistant for Resting-State fMRI (DPARSF) [46] (http://www.restfmri.net/) which is based on Statistical Parametric Mapping (SPM8) (http://www.fil.ion.ucl.ac.uk/spm/) and Resting-State fMRI Data Analysis Toolkit (REST) [47] (http://www.rest-fmri.net/), including removing the first 20 volumes for the signal equilibrium and participants' adaptation to the scanning noise, slice timing, head motion correction, spatial normalization to the Montreal Neurological Institute (MNI) template (resampling voxel size = 3 mm × 3 mm × 3 mm), linear trend removal, temporally bandpass filtering (0.01–0.08 Hz), and spatially smoothed with a Gaussian kernel of 4 mm full-width at half maximum. Exclusion criteria include a head motion larger than 1.5 mm maximum displacement in any direction or an angular rotation greater than 1.5° throughout the scan.

2.4. GM Volume Measurements. Many studies have suggested that regional ALFF results could be influenced by gray matter (GM) volume [48, 49]. We performed a voxel-based morphometry (VBM) analysis to investigate whether a changed functional result is related to changes of GM based on the comparison of volume and concentration of GM between PT patients and normal controls using the 3D-BRAVO sequence. DPARSF [46] was used to analyze the data. The preprocessing protocols including normalization, optional modulation, segmentation, and smoothing are similar to previous studies described [50]. GM intensity maps in the MNI space were obtained by the unified segmentation algorithm. Data were spatially smoothed with 8 mm full width at half maximum Gaussian kernel. Two-sample t-tests were performed between the patient and normal control groups. The threshold was set to $P < 0.01$ corrected for multiple comparisons using Monte Carlo simulation. If there is any positive result, the voxel-wise gray matter volume will be taken as covariates in REST calculations. The threshold was also set to $P < 0.01$ corrected for multiple comparisons using Monte Carlo simulation. Results were visualized by the REST Slice Viewer in the REST software.

2.5. ALFF Analysis. We applied REST [47] to calculate the ALFF, which is similar to previous studies [51–53]. Briefly, the time courses were first converted to the frequency domain using an FFT (Fast Fourier Transform). The square root of

Table 1: Characteristics of the participants.

	PT (n = 42)	HC (n = 42)	P-value
Gender (male/female)	3/39	3/39	1.000[a]
Age (year)	24–65 (37.2 ± 10.2)	22–64 (37.0 ± 10.0)	0.948[b]
Education (years)	4–19 (12.0 ± 4.3)	5–20 (13.3 ± 4.9)	0.260[b]
Handedness	42 right-handed	42 right-handed	1.000[b]
PT duration (months)	6–60 (31.6 ± 14.4)		
THI score	16–95 (51.5 ± 21.0)		

Data are presented as the range of min–max (mean ± SD); PT: pulsatile tinnitus; HC: healthy controls.
[a]Fisher's exact test.
[b]Two-sample t-tests.

the power spectrum was computed and then averaged across 0.01–0.08 Hz at each voxel. This averaged square root was taken as the ALFF. The ALFF of each voxel was divided by the global mean ALFF value for each subject, resulting in a relative ALFF. The global mean ALFF value was calculated for each participant within a group global mean mask.

2.6. Statistical Analysis. Two-sample t-tests and Fisher's exact test were used to compare demographic data between two groups. Two-sample t-tests were performed to calculate the GM volume and ALFF difference between groups. The ALFF values of all the pulsatile tinnitus patients and healthy controls will be compared by two-sample t-tests and the age was included as a covariate. Pearson's correlative analysis was performed using SPSS 12 software (SPSS, Inc., Chicago, IL) to explore the relationships between the THI result, PT duration, and ALFF values of the peak voxels in the patient group. Voxels with a P value <0.01 (corrected for multiple comparisons using Monte Carlo simulation) and cluster size >21 voxels were considered to show significant difference between the two groups. Results were visualized by the REST Slice Viewer in the REST software.

3. Results

3.1. ALFF Analysis with GM Volume as Covariates. We performed a VBM analysis to reveal the GM changes and its relationship with the ALFF results. The result showed there's no GM volume difference between the two groups after Monte Carlo simulation correction.

3.2. ALFF Changes in Pulsatile Tinnitus. Two-sample t-tests were performed to assess differences between groups. As shown in Figure 1 and Table 2, after statistically controlling for the age, the bilateral precuneus and inferior frontal gyrus (IFG) showed significantly increased ALFF in the pulsatile tinnitus patients than that in the controls. However, compared to the controls, the left cuneus, right precentral gyrus, and the bilateral middle-inferior occipital gyrus, lingual gyrus, and right superior parietal lobule significantly decreased ALFF in the pulsatile tinnitus patients.

Regions showed that significant increased ALFF values were bilateral precuneus and inferior frontal gyrus. Decreased ALFF values were found in left cuneus, bilateral middle-inferior occipital gyrus, lingual gyrus, right mPFC, right superior parietal lobule, and right precentral gyrus. The left side corresponds to the right hemisphere.

3.3. Correlations between THI Score, PT Duration, and ALFF Values. A significant positive correlation was found between the THI score and ALFF value in the precuneus ($r = 0.549$, $P < 0.001$) (Figure 2(a)). None of the other significant correlations was found.

A correlation trend was also found between the PT duration and ALFF in the precuneus, but it was not statistically significant ($r = 0.290$, $P = 0.062$) (Figure 2(b)). Significant positive correlation was found between the PT duration and left and right IFG ($r = 0.314$, $P = 0.043$; $r = 0.342$, $P = 0.027$, resp.) (Figure 2(c)). None of the other significant correlations was found.

4. Discussion

The ALFF could be influenced by regional GM atrophy. The GM atrophy may lead to artificial reduction in regional ALFF results. If there is any abnormality, all the functional results should be adjusted. But the VBM analysis performed in our study showed no significant change in the GM volume of PT patients. Thus, on the basis of our result, the altered ALFF in PT patients in our group are believable. It implies that our results could reflect the changes in intrinsic brain functional activities in the PT patients. Previously, Husain et al. [54] and Mühlau et al. [55] conducted research on patients with nonpulsatile tinnitus (NPT). They concluded that the brain volume could be changed in NPT patients. Apart from the totally different etiology between nonpulsatile and pulsatile tinnitus, there should be some other explanations. The most likely one is the different disease duration. The tinnitus course of those NPT patients was up to 240 months. What is more, a large sample ($n = 257$) makes it more possible to demonstrate statistically significant changed areas of GM in previous NPT patients [22]. However, our study contains relatively fewer patients (42 cases) because of the relatively rare PT. The disease duration of the PT patients (6–60 months) may not be long enough to exert changes in the volume of gray matter. Another possible hypothesis for the diminished gray matter of those NPT patients might be the hearing loss. Husain et al. investigated structural changes related to NPT and hearing

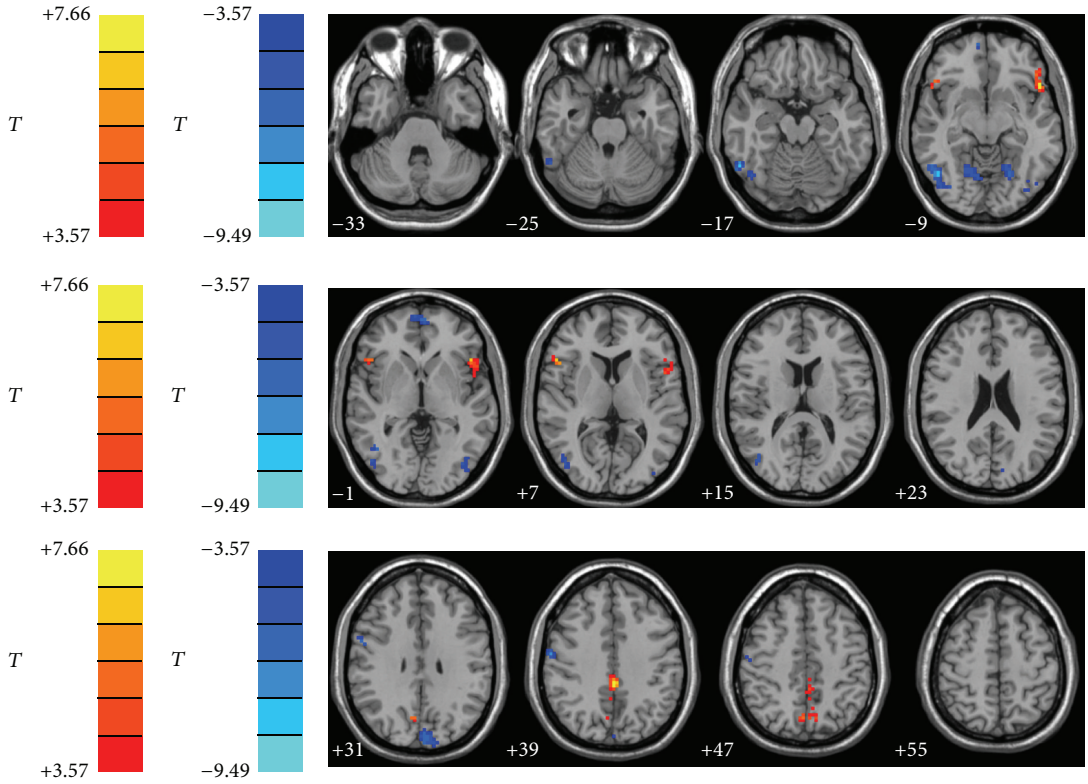

Figure 1: Altered ALFF in PT patients in comparison with controls (two-sample t-tests).

Table 2: Regions showing significant ALFF differences between PT patients and controls ($P < 0.01$ corrected for multiple comparisons using Monte Carlo simulation).

Brain region	Peak MNI, mm			Peak T value	Cluster size, mm^3
	x	y	z		
Precuneus	0	−36	39	7.4512	94
L inferior frontal gyrus	−48	21	−3	7.6632	63
R inferior frontal gyrus	54	21	6	6.8969	55
R mPFC	9	60	−6	−7.4832	27
L cuneus	−3	−87	30	−6.7625	60
L middle-inferior occipital gyrus	−45	−84	−3	−6.7819	49
R middle-inferior occipital gyrus	57	−57	−8	−9.4918	94
L lingual gyrus	−21	−63	−9	−6.7887	33
R lingual gyrus	21	−63	−9	−6.1621	44
R precentral gyrus	54	−27	21	−7.3635	38
R superior parietal lobule	24	−51	63	−7.3521	24

R: right; L: left; MNI: Montreal Neurological Institute. The threshold was set $P < 0.01$ corrected for multiple comparisons using Monte Carlo simulation. mPFC: ventromedial prefrontal cortex.

loss [54]. They found that they were both gray and white matter changes around the auditory cortex for subjects with hearing loss alone relative to those with tinnitus and those with normal hearing. However, there was no significant gray matter volume changes in NPT patients compared with normal controls. Although they studied relatively fewer NPT patients (only 8 cases) and hearing loss patients (only 7 cases), they concluded that hearing loss, rather than tinnitus itself, had the greatest influence on gray and white matter alterations based on their VBM study [54]. However, the PT patients enrolled in our study all confirmed normal hearing by hearing threshold examination. It is reasonable that no significant changes of brain volume were detected in PT patients. But we still need additional studies to make it clear whether there will be changes in the gray matter in PT patients with longer disease duration.

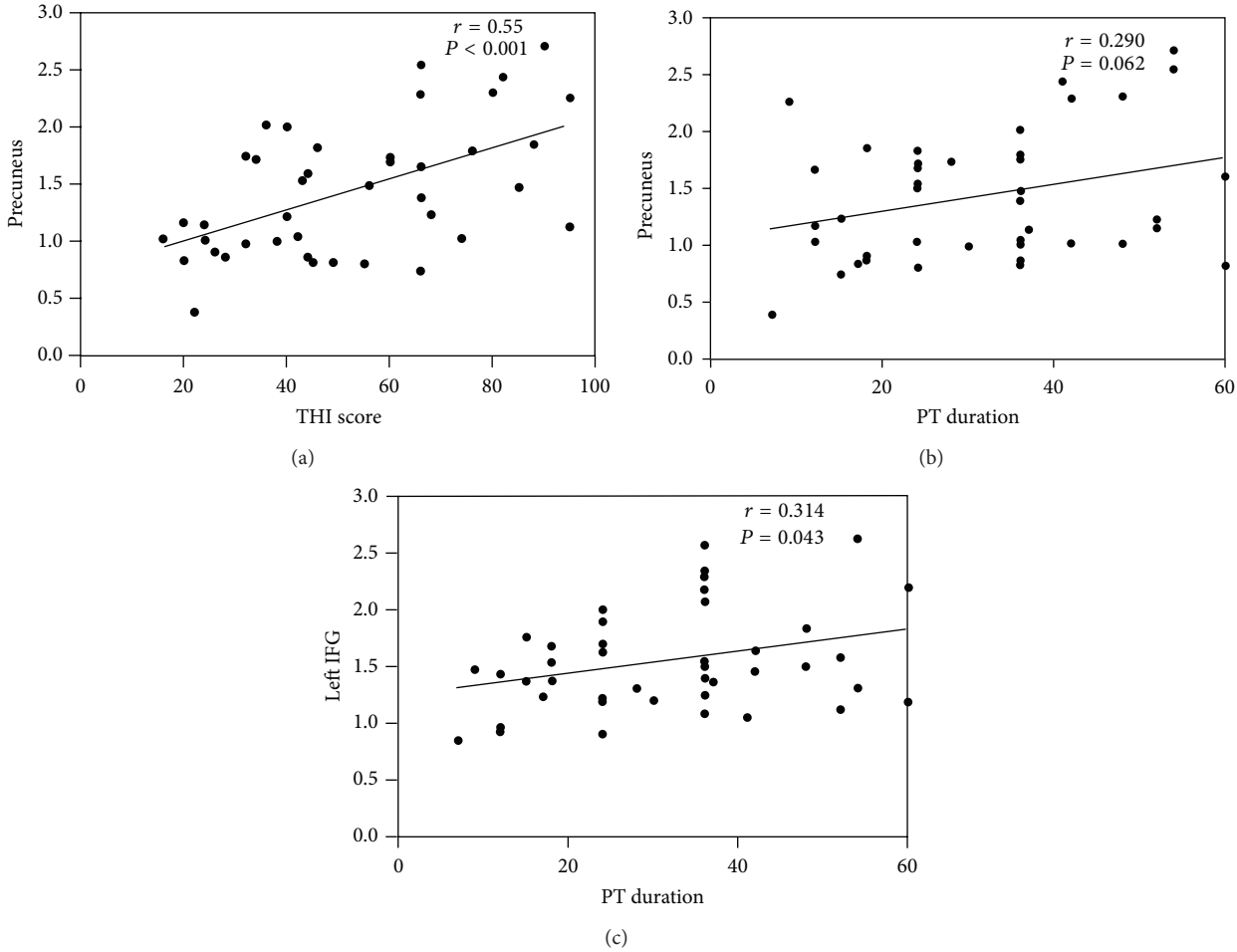

Figure 2: Correlations between THI score, PT duration, and ALFF values in precuneus and left IFG. (a) Correlation between the THI score and ALFF value in the precuneus ($r = 0.549$, $P < 0.001$). (b) Correlation between the PT duration and ALFF value in the precuneus ($r = 0.290$, $P = 0.062$). (c) Correlation between the PT duration and ALFF value in the left IFG ($r = 0.314$, $P = 0.043$). A similar result was also present in the right IFG ($r = 0.342$, $P = 0.027$) (not shown here). r = Pearson correlation coefficient. IFG = inferior frontal gyrus.

Abnormal baseline brain activity in several brain areas was found in the PT patients. Increased ALFF brain areas include the bilateral precuneus and bilateral inferior frontal gyrus (IFG). Decreased ALFF brain areas include left cuneus, bilateral middle-inferior occipital gyrus, lingual gyrus, right mPFC, right superior parietal lobule, and right precentral gyrus. Meanwhile, we also focused on the correlation between the ALFF values and clinical data such as THI score and PT duration.

The precuneus is part of the core structure in the limbic system. Parts of the limbic system play a central role in the development of tinnitus [56, 57]. The limbic network were believed to be closely associated with tinnitus distress [38–41, 56, 58, 59]. The precuneus is a highly integrated structure, supposed to be involved in self-consciousness, shifting of attention [60], auditory imagery [61], auditory memory retrieval [62], and memory-related aspects of the tinnitus percept [39, 63]. For those who can cope with the NPT and have only low distress, increased activities were presented in the PCC/precuneus area in previous studies [20, 39]. But there is something we need to pay attention to; these previous studies were based on continuous scalp EEG recordings and sLORETA (low resolution electromagnetic tomography), a tomographic inverse solution imaging technique. The direct relationship between the sLORETA analysis results and the BOLD signal remains unclear. Direct relationship between fMRI signal and EEG activity was reported in a study [64]. There is also some resting-state studies reporting that EEG is not directly linked to the changes in neural activity as measured by BOLD fMRI [65, 66]. On the other hand, Logothetis et al. [67] found that task-induced BOLD signal changes correlated better to local field potential (LFP) than to single unit spiking, indicating that the BOLD response reflects the integration of input and intraneuronal processing. Such a combination is a good way of understanding the nature of LFF [51]. Anyway, one could presume that the fMRI signal change should have a possible correlation with the EEG results but it may not always be accurate. Resting-state PET/SPECT, on the other hand, directly measures the metabolism of different brain areas, reflecting the neural

activity in a period of time (while the ALFF measures the deviation of the BOLD signal [51]). It may be a better comparable technique to the LFF according to some authors [68]. Increased activity in precuneus/PCC regions was observed in subjects with major depression (examined by PET (positron emission tomography) scan) [69] and unpleasant music perception (examined by CBF (cerebral blood flow) changes) [70]. In our study, our patients showed increased ALFF value in precuneus. Also we found that ALFF values in precuneus region have significant correlations ($r = 0.549$, $P < 0.001$) with THI score. Thus, even though there was no similar study using ALFF analytic technique to study distressed patients or tinnitus (NPT or PT) patients, the increased ALFF in precuneus of the PT patients is considered to be possibly related to PT awareness as well as the tinnitus related distress. A similar result was also reported in a connectivity analysis of NPT patients using ICA approach based on fMRI (significant correlation between the beta values of PCC/precuneus and THI score, $r = 0.68$, $P = 0.01$) [25]. In fact, nearly 20–60% of tinnitus patients have reported clinical depression [37]. Note also the correlation trend between the activity of precuneus with the disease duration ($r = 0.290$, $P = 0.062$). One can hypothesize that the PT sounds may integrate in the limbic system with time. The altered baseline brain activity in precuneus region should be considered as a kind of modulation secondary to pulsatile tinnitus.

The Precuneus/PCC is also an important part of the default system. This brain system is comprised of Precuneus/PCC, medial prefrontal cortex (MPFC), inferior parietal lobule (IPL), lateral temporal cortex (LTC), parahippocampal gyrus (PHG), and hippocampus. DMN is active with high metabolic rates at rest [71, 72], during self-referential behavior [73], episodic memory processing [74], and so forth but shows divergent fluctuations in spontaneous activities during the task [72, 75]. In our study, the patients maintained high activity in Precuneus/PCC, indicating preservation of normal autobiographical reveries, despite the presence of persistent pulsatile tinnitus. Also, there have been some researches indicating that a positive correlation between the strength of inflow to the temporal cortices and tinnitus-related distress was found in DMN, especially in Precuneus/PCC areas [76]. This corresponds with our correlation analysis between THI score and precuneus.

The role of IFG involved in tinnitus patients is still unclear. In a previous task-fMRI study [77], significant increased signal intensity was found in bilateral IFG after trials with stimulation at the tinnitus frequency. These clusters also showed significant correlation between the tinnitus loudness ratings and the BOLD signal change in that experiment. Even the result may be overestimated due to the task design, the correlation trend is still present. Our results provide further support for abnormal ALFF in bilateral IFG in tinnitus patients; even the loudness of pulsatile tinnitus cannot be measured because of the insufficient measurement and standard. Thus, the increased ALFF in bilateral IFG is considered to be vital areas to identify tinnitus awareness. Moreover, the IFG was critical for response inhibition [78]. The increased ALFF may reflect an inhibitory effort in patients with PT to suppress the sound which is in accordance with the heartbeat. Meanwhile, there are also researches indicating that the brain connectivity is widely disturbed in the tinnitus patients over time [16, 79]. Positive correlations were also found between the ALFF values and disease duration in the left and right IFG ($r = 0.314$, $P = 0.043$; $r = 0.342$, $P = 0.027$, resp.). Thus, we hypothesize that these results are possibly reflecting ongoing changes in neural networks of PT patients. The connectivity research in PT patients is required to confirm the theory above. Early treatment might be essential in ensuring better prognosis.

Decreased ALFF in multiple occipital areas are interesting findings in our research. In a previous PET study, decreased occipital blood flow was reported during auditory tasks in the normal control group without temporal activation [80]. Our results were just similar to this study. In our opinion, the connections between auditory and visual cortex make it possible to alter the brain activity in the occipital areas [27, 81–86]. In blind subjects, the visual cortex was recruited in the context of auditory localization [80, 81]. It was named "auditory occipital activations" (AOAs), which may reflect the visual region processing soon-to-appear objects after sound source stimulation [86]. Contrary to the AOAs, the decreased ALFF in occipital cortex may be caused by neural reorganization in this area. PT patients may need a downregulation adjustment of the AOAs to avoid misinterpreting the sounds around. This could be understood as a kind of "self-protect mechanism" in PT patients. A previous study proved the negative correlations reciprocally characterizing the functional connectivity between auditory and occipital/visual cortex in NPT patients. Could we find similar results in PT patients? What is the difference between these two groups of patients (NPT V.S. PT)? These questions should be answered by more investigations, especially functional connectivity analyses.

The auditory system was involved in patients with nonpulsatile tinnitus [16–27]. However, these areas did not show any difference compared with the normal control group. The possible reason is the totally different sensation and etiology of the pulsatile tinnitus from the nonpulsatile type. The pulsatile tinnitus usually sounds like wind blow, rushing water, or just rumbling in accordance with the heartbeat. But the nonpulsatile tinnitus is a kind of ringing sound with different frequencies. Pulsatile tinnitus is usually caused by vibrations from turbulent blood flow that reaches the cochlea, which often arises from sigmoid sinus diverticulum, atherosclerosis, aneurysm of internal carotid artery, abnormal mastoid emissary vein, dural arteriovenous shunts, paraganglioma, and so forth, [7, 28–30], while the exact etiology of nonpulsatile tinnitus remains unclear. This is the most possible reason that makes the pulsatile tinnitus unique. Thus, it is this particular kind of disease that presents us with results different from the previous ones. We just discuss our findings and try to explore the clinical meanings of the pulsatile tinnitus patients.

5. Limitation

There are also some limitations in our research. Firstly, the patients enrolled in this research lack of variance.

The etiology of PT is quite a lot. But the common etiology of PT, according to our daily work, was the abnormal blood flow induced by focal defect of mastoid bone shell in the region of the transverse-sigmoid junction. Also, hundreds of patients with this etiology were cured in our hospital, while cured patients with other etiology were not quite common. Thus, the underlying pathology of pulsatile tinnitus and the variability and the possibility that it might influence our results could not be discussed this time. Data of the patients with the most common etiology are easier to acquire and more important to analyze. Patients with different etiology or different kinds of sounds will be enrolled. We firstly investigate the relatively common type and then, we could continue our investigation for more details. Secondly, the neuropsychological tests, such as Mini Mental State Exam (MMSE), Auditory Verbal Learning Test (AVLT), and so forth, were not administrated. But THI is suitable in reflecting the psychological status of the tinnitus patients in our study. We will enroll more tests to evaluate individual's neuropsychological status. Thirdly, subjects in patient group with higher THI score and longer disease duration were not included. We only enrolled patients with disease duration between 6 and 60 months because previous results indicated that the activity and neural network of chronic tinnitus patients are quite different from that of patients with onset disease [16]. Further studies will enroll patients with extensively different disease duration to reveal the changes of brain activity or brain network over time. But the current study was the first to investigate the changes of baseline brain activity in patients with pulsatile tinnitus using resting-state fMRI. Our study is effective in offering information about understanding the changes in brain activity in PT patients. Last but not least, functional connectivity analysis of the tinnitus network is an important part of tinnitus fMRI research. We will apply functional connectivity analyses based on the ALFF results to study the altered brain network of PT patients.

6. Conclusion

In summary, multiple altered baseline brain activity areas in patients with pulsatile tinnitus were focused on part of the limbic system, the IFG, and multiple occipital areas. The activity of auditory system was not found to be significantly changed. Results confirmed the disturbances in PT-related neural networks, which may be potentially helpful in understanding the pathophysiology of PT.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Lv Han and Liu Zhaohui contributed equally to this paper.

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Review Article

Animal Models of Subjective Tinnitus

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Tinnitus is one of the major audiological diseases, affecting a significant portion of the ageing society. Despite its huge personal and presumed economic impact there are only limited therapeutic options available. The reason for this deficiency lies in the very nature of the disease as it is deeply connected to elementary plasticity of auditory processing in the central nervous system. Understanding these mechanisms is essential for developing a therapy that reverses the plastic changes underlying the pathogenesis of tinnitus. This requires experiments that address individual neurons and small networks, something usually not feasible in human patients. However, in animals such invasive experiments on the level of single neurons with high spatial and temporal resolution are possible. Therefore, animal models are a very critical element in the combined efforts for engineering new therapies. This review provides an overview over the most important features of animal models of tinnitus: which laboratory species are suitable, how to induce tinnitus, and how to characterize the perceived tinnitus by behavioral means. In particular, these aspects of tinnitus animal models are discussed in the light of transferability to the human patients.

1. Introduction

Subjective tinnitus is the phantom perception of sound in the absence of an external stimulus. In 1–3% of the general population it constitutes a significant impairment of the quality of life [1]. Despite significant research efforts, our understanding of the underlying neuronal mechanisms is far from complete. As a result the only approved therapies are symptomatic. One major obstacle arises from the fact that by its very nature tinnitus is a subjective phenomenon, and the only possible diagnosis relies on self-reports of the subjects [2]. This fact poses a problem not only for diagnosing tinnitus and identifying subtypes in human patients but also in animal models of tinnitus. At present, however, only research on animal models can provide us with the necessary understanding of the peripheral and central mechanisms that lead to the aberrant neuronal activity ultimately perceived as tinnitus. One proposed mechanism is that the pathological activity originates from plastic changes of the central auditory system following damages to the periphery. In a healthy system, this plasticity is essential for adjusting neuronal activity to changing acoustic environments. An acoustic trauma damaging the cochlea leads to a loss of input to the central stages of the auditory processing hierarchy. The lack of input is then overcompensated by increasing the spontaneous activity and neuronal synchrony. This proposed mechanism makes tinnitus a "plasticity disorder" [3] and it is this plasticity that should be targeted for treating tinnitus.

Results from animal models of tinnitus are an essential element in the combined efforts of different audiological specializations for developing new tinnitus therapies. The irreplaceable advantage of animal models lies in the possibility to study small neuronal networks and individual nerve cells through invasive methods such as extra- and intracellular recordings in potentially genetically engineered or sound exposed subjects. These means provide high spatial and temporal resolution (i.e., micrometer and millisecond range, resp. [4]) which is impossible in human studies applying electroencephalography or functional magnetic resonance imaging (exceptions are recordings during brain surgery). In fact, current hypotheses about the pathogenesis of tinnitus are mostly based on results from animal models, in particular from studies on tinnitus following noise-induced hearing loss [1]. However, since tinnitus is a conscious percept [5], many

aspects have to be studied and characterized in laboratory animals through behavioral means. Furthermore, physiological measurements of tinnitus-related neuronal activity should ideally be sampled in awake animals in order to exclude artefacts from anesthesia and to facilitate a comparison with human subjects who only perceive tinnitus when awake. In summary, any behavioral assessment of tinnitus in the animal model should try to mimic as closely as possible conditions under which tinnitus develops in humans.

The first section of this review provides an overview of the different species used as animal models in tinnitus research. Then, the different methods used for tinnitus induction in animal models are reviewed. Finally, the competing behavioral paradigms used for assessing subjective tinnitus in the animal model are discussed. This sequence reflects a natural order of the main decisions to be made when designing animal experiments. Which species mimics the human condition and pathology best? What is the most appropriate way to induce tinnitus? Which behavioral paradigm is best suited for addressing the research questions?

2. Species Used for Behavioral Testing of Tinnitus

The first behavioral test for tinnitus in an animal model was established by Jastreboff et al. [6, 7] in 1988 using rats. Since then a number of different laboratory animal species and various strains have been used for the behavioral assessment of tinnitus. Besides the laboratory rat (*Rattus norvegicus*) [8–51], these include the domestic house mouse (*Mus musculus*) [52–58], the chinchilla (*Chinchilla laniger*) [59, 60], the Syrian golden hamster (*Mesocricetus auratus*) [61–64], the guinea pig (*Cavia porcellus*) [65–67], and the Mongolian gerbil (*Meriones unguiculatus*) [68, 69]. Since the early studies by Jastreboff et al., the laboratory rat remains the most prominent species used for investigating tinnitus at the behavioral level. However, an increasing number of studies are being performed on mice since the wide range of genetically modified strains is not available for rats at present. Comparing the hearing abilities and the suitability of different species for tinnitus assessment reveals advantages and drawbacks of the different approaches.

2.1. Hearing Ranges of Different Species. Compared to research on other sensory systems (e.g., the somatosensory modality which is usually investigated in the rat or mouse barrel cortex), investigation on hearing in mammals is characterized by a larger variety of established animal models. Usually, the criteria for selecting one species over another are not documented in the literature, even though this choice has serious consequences for the interpretation of results and their transferability to human subjects. Despite the fact that all species mentioned above belong to the same systematic order (*Rodentia*), their acoustical and behavioral ecology and physiology varies significantly from one another, and more importantly from the final subject of interest, the *Homo sapiens*. These differences are revealed most clearly by comparing the different audiograms. In rodents, the hearing range mostly covers the high frequency range beyond the upper human limit (highest audible frequency at 60 dB SPL for human is 17.6 kHz [70], rat: 58 to 70 kHz [71, 72], mouse: 85.5 kHz [70], chinchilla: 33 kHz [73], hamster: 46.5 kHz [74], guinea pig: 50 kHz [75], and gerbil: 58 kHz [76]). The same applies to the low frequency hearing limit (at 60 dB SPL in humans: 0.03 kHz [74], rat: 0.52 kHz [71], mouse: 2.3 kHz [70], chinchilla: 0.05 kHz [73], hamster: 0.096 kHz [74], guinea pig: 0.05 kHz [75], and gerbil: 0.032 kHz [74]). It has been proposed that mammals that do not hear below 0.5 kHz do not use temporal encoding for pitch perception [74], with the exact frequency of this boundary being discussed. This suggests that the two most widely used behavioral models of tinnitus, rat, and mouse employ neuronal mechanisms for pitch perception that fundamentally differ from those of humans. It has been argued that this difference applies only to the lower frequency range (<5 kHz). Nevertheless, interpretation of animal studies in relation to a human disease would be more directed in species with audiograms similar to humans (e.g., gerbil or chinchilla). However, gerbils are prone to a certain degenerative disorder of the auditory system, at least when supplied by a commercial manufacturer [77]. This caveat has to be taken into account when considering the gerbil as a potential model for subjective tinnitus.

Furthermore, choosing an animal model with human-like audiograms would facilitate the comparability of tinnitus pitch. Many studies cited above induced tinnitus through a noise trauma centered at 16 kHz. This treatment is presumed to give rise to a phantom percept that has a higher frequency than the region of highest sensitivity in the rat (around 8 kHz [71, 72]). In the mouse studies mentioned above the tinnitus inducing noise is centered at 16 kHz as well. In contrast to the rat, the mouse has its highest sensitivity at 16 kHz [78]. In humans, the average tinnitus pitch is in the range of 5–8 kHz [79] and the highest sensitivity lies around 3 to 4 kHz [80]. Independent of the species this means that the tinnitus-inducing stimuli have to be carefully matched to the hearing range of subjects in order to achieve comparability with the human pathology.

2.2. Differences between Rats and Mice in Suitability for Behavioral Paradigms. In recent years, the mouse has become a widely used behavioral model for tinnitus research. One of the reasons why mice entered the scene so late could be their presumed limited cooperation in behavioral training paradigms assumed from the larger variability in the effects found in acoustic startle experiments. Characteristically, all mouse behavioral studies of tinnitus mentioned above use a paradigm (gap-startle paradigm, introduced in 2006 by Turner et al. [8]) that does not necessarily require a functional auditory cortex [81] and does not require any behavioral training beyond adaptation to the setup [8]. However, so far no evidence has been published that substantiates the cognitive difference between rats and mice. On the contrary, in the somatosensory modality, rats and mice exhibit similar performance levels and learning curves when facing a complex 2-alternative forced choice task, which requires the discrimination of simultaneously presented whisker deflections

at different frequencies [82]. The big advantage of the mouse model is the almost infinite range of genetically modified strains available. This allows the recording and manipulation of specific types of neurons (e.g., excitatory pyramidal neurons or inhibitory neurons of a certain cortical layer). However, so far no one has taken advantage of this feature of the mouse model. The downside, however, is that some mouse strains exhibit elevated auditory thresholds (measured as auditory brainstem responses) within 2 months after birth [83], a problem that may be aggravated in genetically modified lines. Rats do not exhibit this early onset of age-dependent hearing loss [84, 85].

3. Established Ways of Tinnitus Induction in Animal Research

Comparable to the diversity of species used in behavioral testing of tinnitus, there is a number of different ways of inducing tinnitus in animal models. In principle, there are two ways of inducing tinnitus. One way is through pharmacological means. Alternatively, tinnitus is induced by presenting high level stimuli for one hald to two hours. Both approaches try to mimic the etiology of tinnitus in humans even though the pathogenesis of subjective tinnitus remains poorly understood. However, it is commonly accepted that in many cases it commences with noise-induced damage to the hair cells of the inner ear, followed by deafferentation and hearing loss [86]. Such a trauma leads then to the initiation of compensatory processes in the central nervous system. In the healthy system, these processes warrant an activity level that is optimal for encoding the present acoustic environment. However, after a trauma and consequential deafferentation, this beneficial plasticity of the auditory system goes astray and overcompensates the missing input from the damaged region of the cochlea, leading to a permanently present phantom percept [87]. Hence, maladapted plasticity may underlie tinnitus and not the peripheral damage itself [88].

3.1. Pharmacologically Induced Tinnitus. The first study assessing subjective tinnitus in an animal model used a pharmacological method for induction [6]. The main advantages of a pharmacological tinnitus induction are its potential reversibility and its previous use in human subjects for inducing tinnitus as well (i.e., 3.9 g salicylate/day for 5 days [89, 90]). The two most commonly used substances where tinnitus is assessed by behavioral means in animal models are salicylate [9, 11–13, 16–20, 22, 32, 33, 35, 36, 38, 39, 43–45, 47, 50, 57, 58, 67] and quinine [10, 12, 18], an antimalarial drug. Other ototoxic drugs that have been investigated in animal studies are cisplatin (cis-diammine-dichloroplatinum (II)) and carboplatin [60]. Both are chemotherapeutics, with cisplatin predominantly targeting the outer hair cells [91] and carboplatin most likely affecting the inner hair cells [92]. Salicylate, the active component of Aspirin, is the most commonly used drug in animal models [93]. Therapeutically it is administered usually as a mild analgesic or in anti-inflammatory therapy (e.g., against rheumatic arthritis). Salicylate has the advantage of fast induction within minutes and its effects reverse within 72 hours of the last administration [94, 95]. In most studies cited above, salicylate was administered systemically, either orally or by injection. In some cases, salicylate was applied locally to the inner ear [39, 50] or central structures (e.g., auditory cortex) as well [34].

Salicylate most likely exerts its effects on hearing at high doses, both in the sensory periphery and in the central nervous system. In the auditory periphery it mainly targets outer hair cells, inhibiting their electromotility most likely by partitioning into the membrane [96] and blocking the prestin protein [97]. The consequence is a reduced cochlear sensitivity which manifests itself in a reduction of otoacoustic emissions (spontaneous and evoked), a decreased neural output, and ultimately a temporary hearing loss [94]. Long-term application of salicylate, however, leads to an increased expression of prestin, most likely as a compensatory reaction [98].

Parallel to these effects on the sensory epithelium, there is strong evidence that salicylate affects the central nervous system as well. Different levels of the auditory pathway have been identified as being modulated by salicylate. Amongst others these are the cochlear nucleus (CN), the inferior colliculus (IC), the medial geniculate body (MGB), and the auditory cortex (AC) [94]. The observed effects can either originate from changes of the input (i.e., altered cochlear output) or from direct action on the neuronal activity. In particular, it has been shown that different parts of the inhibitory GABAergic neurotransmission can be modulated by salicylate [94] and that a modulation of the GABAergic inhibition reduces salicylate-induced ototoxicity [50]. After chronic systemic administration, salicylate causes an increase in the expression of the GABA-synthesizing enzyme GAD [99]. In slice preparations, salicylate decreases GABAergic inhibition of auditory cortical pyramidal neurons, potentially facilitating hyperactivity [100]. These pieces of evidence indicate that acute salicylate administration reduces the GABAergic inhibition in the network, which is then compensated by an increased GABA synthesis. Other effects of salicylate are a reduced spontaneous firing rate in the inferior colliculus [101], adjustments in the tonotopy of the auditory cortex [102], and changes in the cochlear nucleus [103]. However, GABAergic transmission is most likely not the only target of salicylate. Another very likely target is the NMDA receptor (N-methyl-D-aspartate) [38, 104]. Finally, it has been proposed that salicylate acts on the extralemniscal pathway while noise trauma induces tinnitus in the lemniscal pathway [105].

The applied dosage of salicylate varies significantly between studies and species. However, it seems that with the right dosage (100 mg sodium salicylate/kg/day for two consecutive days) there is a reliable tinnitus induction, as shown with a behavioral test in rats [12]. How such a dosage in rats translates to a comparably critical serum level in humans is a source of uncertainty. In humans average salicylate serum concentrations of approximately 300 mg/L induce tinnitus [90]. 90 minutes after an i.p. injection of 350 mg/kg sodium salicylate (corresponding to 300 mg/kg salicylic acid), the salicylate serum level in the rat was 625 mg/L [106]. For

the dosage of 100 mg/kg inducing reliable tinnitus in the rat, the expected salicylate serum concentration is approiamtely 56 mg/L. These differences (56 mg salicylate/L in rat vs 300 mg salicylate/L in human serum concentration) might indicate a higher sensitivity of the rat, differences in underlying clearance mechanisms, or different threshold criteria and administration schedules.

3.2. Tinnitus Induced by Acoustic Trauma. The second established method for inducing tinnitus in behavioral models is through acoustic trauma [8, 15, 20, 21, 23, 25–27, 30, 31, 37, 39–42, 46–49, 51–56, 59–66, 68, 69, 107]. It is assumed that a cochlear damage is in most cases the trigger for a sequence of events leading to the development of tinnitus in humans. However, not every hearing loss resulting from a trauma gives rise to tinnitus and a subset of patients exhibit normal audiogram indicating that "hidden hearing losses" play a role as well [108]. Acoustic trauma and subsequent hearing loss induces a number of acute and chronic changes in the periphery and the central nervous system. At the periphery, an acoustic trauma results in outer hair cell damage, cochlear dead regions (no functional inner hair cells) [109], damaged stereocilia in both types of hair cells [110], and deafferentation of auditory nerve fibers [111]. Typically, the hearing loss accompanying tinnitus is located in the high-frequency range. The tinnitus pitch itself is either near the edge of the hearing loss or in the frequency range of the damaged region itself [112].

The parameters for inducing tinnitus through acoustic trauma in the animal model are quite variable. Typically, a high level noise stimulus is applied for 1 to 2 hours under anesthesia, either to one or both ears. For the rats, a widely used stimulation paradigm consists of an octave-band noise with a peak intensity of 116 dB sound-pressure level centered at 16 kHz for 1 hour [8]. However, sound level (80 dB SPL [62] to 130 dB SPL [39], [63]), duration (2 min [25] to 7 hours [28]), frequency (2 kHz [69] to 22 kHz [52]), frequency range (pure tones [27] to broadband noise [15]), and concerned ear (uni- or bilateral) vary a lot between studies. In rats, binaural exposure to a 10 kHz tone for 1-2 h leads to significant tinnitus when the sound level was 120 dB but not at 80, 100, or 110 dB SPL [51].

The primary criteria for selecting the stimulus parameters are usually the hearing range of the species, the targeted tinnitus pitch, and time course (temporary versus chronic). Mice exposed to noise centered at 16 kHz at 116 dB SPL for 1 hour exhibited signs of tinnitus for 25 months afterwards [54], while in rats exposed to 17 kHz pure tones at 115 dB SPL for 2 minutes tinnitus lasted only 13 min [25] (induction under isoflurane anesthesia). In gerbils, a reliable and chronically induced tinnitus can be achieved by noise stimuli with an exposure time of at least 1 hour and narrow bandwidth leading to a temporary threshold shift and ultimately to self-sustaining activity perceived as phantom sound. Such a protocol leads to a hearing loss that disappears after 3 to 6 weeks and a tinnitus percept centered at the center-trauma frequency appearing 5 to 7 weeks after induction [68]. Hamsters exposed to a 10 kHz tone at 110 dB SPL for 4 h exhibited tinnitus symptoms within one day after exposure [62] indicating the possibility of an almost immediately tinnitus onset after acoustic trauma.

The changes after acoustic trauma at the different stages of the ascending auditory pathway are manifold and complex. Within hours after an acoustic trauma, the spontaneous neuronal activity in the primary auditory cortex (A1) of the cat increases in the frequency region below the damage [113]. This increase presumably originates from a loss of inhibition from the cortical regions representing frequencies of the cochlear damage. Weeks after an acoustic trauma, the tonotopic map of A1 reorganizes so that there are no neurons with characteristic frequencies above the frequency of the traumatizing stimulus [114]. In parallel, the activity in the auditory cortex becomes more synchronous after acoustic trauma [113]. Neurons in the inferior colliculus exhibit increased spontaneous firing rates after an acoustic trauma [60]. In the dorsal cochlear nucleus (DCN) an acoustic trauma induces an increase in spontaneous activity which correlated with the strength of the behavioral tinnitus evidence [63] and specifically in fusiform cells [59]. However, DCN ablation does not change the psychophysical indicators of tinnitus [21].

3.3. The Role of Anesthesia. While salicylate can be administered for tinnitus induction in awake animals, it is usually anesthetized for tinnitus induction through acoustic trauma. The anesthesia is either injectable (very often a combination of ketamine and xylazine, or pentobarbital) or an inhalable one (usually isoflurane). How different anesthetics influence the development of hearing loss and tinnitus after acoustic trauma is largely unknown. However, isoflurane has been shown to diminish the amplitude and duration of temporary tinnitus after a short exposure to loud sounds [25]. Under pentobarbital, isoflurane, or halothane anesthesia noise-induced hearing loss in mice is less (62.5 dB, 45.5 dB, 39.3 dB threshold increase, respectively) compared to the unanesthetized control group (77.5 dB threshold increase) [115]. In addition, the influence of anesthesia on any electrophysiological recordings has to be taken into account, as anesthesia influences the receptive fields and the spontaneous activity of the rat auditory cortex [116].

3.4. Summary Tinnitus Induction. The advantages of a pharmacological induction of tinnitus with salicylate are the following. Salicylate has a fast onset and is metabolized within hours to days. It can be tested in human subjects as well as in animal models. Salicylate administration can be locally confined either to the cochlea [50, 117] or to specific brain structures and systemic administration is possible without anesthesia. The drawbacks are a presumed multitude of mechanisms giving rise to tinnitus, a lackof specificity interms of the locus of action, tinnitus pitch (0.9 to 14.5 kHz [118]), and relevance for the human pathology since in humans it is usually triggered by noise trauma. Furthermore, salicylate does not induce chronic tinnitus as it recedes when the intake is stopped. These aspects hinder the identification

of neuronal substrates involved in the pathogenesis and maintenance of human tinnitus by means of salicylate.

One advantage of inducing tinnitus through acoustic trauma is the possibility to induce unilateral tinnitus, allowing the animal to serve potentially as its own control as done in some studies (e.g., Turner et al. [55]). However, one has to keep in mind that the ascending auditory pathway is characterized by significant binaural projections on every stage. Even if the tinnitus is perceived unilaterally, it is manifest in contra- and ipsilateral instances. Therefore, real controls (i.e., animals not exposed to noise as done by Turner et al. [55]) are required as well. Another advantage of tinnitus induction by acoustic trauma is the fact that this is most likely the most common form observed in human patients [1]. One of the biggest uncertainties when inducing tinnitus through an acoustic trauma is the resulting percentage of animals exhibiting tinnitus in behavioral tests. These numbers vary significantly in the literature, according to Knipper et al. [119] from 30% to 80%.

Ultimately, the choice of how to induce tinnitus in a behavioral study depends on the research question and which form of tinnitus will be studied. It has to be kept in mind that an acoustic trauma and drugs induce tinnitus through different mechanisms [120] and that both methods have certain methodological constrains (e.g., that an acoustic trauma very often has to be applied under anesthesia, depending on local animal welfare regulations).

4. Behavioral Models for Assessing Tinnitus in Animals

Diagnosis of subjective tinnitus in human patients relies almost exclusively on the self-report as there is no external sound source present and it manifests itself only in the neuronal activity of subject's brain. There are some noninvasive approaches that provide potentially objective measures for subjective tinnitus by means of functional magnetic resonance tomography, electroencephalography, magnetoencephalography, and positron emission tomography [121]. However, at present none of these methods is applied routinely for diagnosing tinnitus and it is unknown whether the observed effects are directly caused by tinnitus or by the emotional stress usually accompanying severe tinnitus. This challenge of diagnosing tinnitus poses a supreme obstacle for developing an animal model with behavioral evidence of tinnitus. Nevertheless, a reliable behavioral assessment of tinnitus in the animal model is essential for understanding the pathology and the development of therapies. In typical behavioral tests performed in sensory physiology, the presence of a stimulus has to be detected or stimuli have to be discriminated and the animal's decision is indicated by a nose poke or a lever press. The absence of a stimulus usually requires no specific response, as seen in go/nogo paradigms [122]. A continuous phantom percept like tinnitus hardly fits into such a framework of psychophysical experiments, as it is assumed to abolish the notion of silence [105]. Since the first publications by Jastreboff et al. [6, 7] 25 years ago, a number of different behavioral paradigms for addressing this issue have been developed. Any behavioral assessment of tinnitus has to consider the confounding influences of possible hearing loss (after noise trauma) and hyperacusis accompanying tinnitus induction. Furthermore, an ideal test for tinnitus in animals would be closely modeled on tinnitus tests performed in humans and might even be applicable to humans as well.

4.1. Conditioned Avoidance Paradigms. Jastreboff et al. [6, 7] used a standard learning technique, the Pavlovian conditioned response suppression by the induction of fear [123]. Water-restricted animals were exposed to a constant background noise (approximately 40 dB SPL) during which they were allowed to collect water from a drinking tube. The conditioned stimulus (CS) was the offset of the background noise for 30 s. The behavioral readout was the ratio of licks during the CS compared to the number of licks in the period preceding the silent gap (suppression ratio). During suppression training the CS periods were terminated with an inevitable foot shock as unconditioned stimulus (US). This led to the extinction of licking during the CS. The training was continued until the suppression ratio was below 0.2. Next, animals were injected with salicylate in order to induce a phantom sound that was assumed to fill out the silent gap of the CS. During the testing there was no foot shock (US) and the response suppression extinguished over time. In salicylate-treated animals the response suppression extinguished within 2 days, while it took saline-injected animals 4 days until the response suppression was extinguished. The faster extinction time course in salicylate-treated animals has been interpreted as an indicator of tinnitus as the animals did not perceive the silent gaps (CS) anymore. The most important control of this study was a group of animals that received salicylate before the suppression training. These animals associated the tinnitus perceived during the silent gaps with the foot shock. Consequently, during the testing sessions, when no foot shock was given, the animals stopped licking during the silent gaps as they associated the tinnitus with punishment and the extinction took longer. This rules out the possibility that salicylate by itself changed the behavior in some ways (e.g., increased thirst, altered impulsivity). Hearing loss after salicylate administration as an explanation for the faster extinction was ruled out since reducing the amplitude of the continuous noise by 20 dB did not lead to a faster extinction.

Heffner and Harrington [61] modified this conditioned response procedure and tested hamsters for tinnitus. They aimed at a protocol that allows to measure behavioral indicators of tinnitus in individual animals. The basic paradigm again consisted of a broadband noise during which the animals were allowed to drink (safe signal) and silence during which the animals had to stop drinking. In training, the animal was shocked if it contacted a water spout during a silent period. Animals were trained for 32–35 sessions in order to achieve a performance above 70%. Performance was calculated as the average percentage of time the animal contacted the spout during noise and was not in contact during silence. The tinnitus was induced by a pure tone

acoustic trauma (10 kHz, 124 dB SPL for 4 h) applied to the left ear. During test sessions (5 days after acoustic trauma), there was no shock when the animal contacted the spout during silent periods. As in Jastreboff et al. [6, 7] the time course of the extinction of the response suppression during silent periods was indicative of the perceived phantom sound. Animals receiving a pure tone trauma were more likely to drink during silent periods compared to a control group. This difference was visible in performance scores of individual animals as well. However, the variability was quite big and there was a certain overlap in the distributions of performance scores of the control group and the one that received a trauma.

Similar conditioned suppression paradigms have been used in other studies as well (e.g., Zheng et al. [44]). The main advantage of their approach is that it can be applied easily to larger numbers of animals since the training period is quite short. Different tinnitus induction protocols have been proven to be effective with such paradigms which allow pitch and amplitude of the tinnitus to be characterized. Its major drawback is a relatively short period for actually assessing the tinnitus. Since the indication for tinnitus is the time course of suppression extinction (no foot shock), only short time spans (days) can be monitored and a more detailed analysis of the tinnitus over time is impossible.

Bauer and Brozoski [37] published an operant conditioning approach for measuring tinnitus in the animal model (rats). Here, subjects were trained to lever-press in order to receive a food reward when an auditory test stimulus was present (60 dB SPL broadband noise or pure tones). During silent periods, the animals had to stop lever pressing. A running index of lever press behavior was computed for windows of 1 min length. If the animals kept lever pressing in the silent periods, they were punished with a foot shock if they met or exceeded a certain criterion of the running index. In the testing sessions, pure tones of different frequencies and amplitudes were presented as well as silent gaps. Lever pressing during pure tone presentation was not punished; however, pressing during silent gaps was still punished. The discrimination functions (pure tones and silence) of animals receiving an acoustic trauma (noise centered at 16 kHz, 1 octave bandwidth) and unexposed control animals (or animals with a simulated hearing loss through ear plugs) differed significantly. This has been interpreted as an indicator for tinnitus as the traumatized animals could not differentiate between test tones and real silent gaps which were "filled" with the phantom sound. Since the behavioral contingencies were the same during testing and training, it was possible to measure the tinnitus induced by noise trauma over extended periods (up to 17 months). Additionally, the tinnitus properties (pitch, loudness) could be measured in detail, as Bauer and Brozoski [37] identified the tinnitus pitch at 20 kHz. The downside of this approach is that it requires careful training and can take extended periods of time for the animals to reach criterion before the actual testing takes place.

A slightly different approach was published by Lobarinas et al. [11]. Rats were put on a food restriction schedule and received a food pellet in regular intervals. This scheduled food intake induced polydipsia leading to a constant licking for water between the food deliveries. Sound stimuli were paired with a foot shock and silence periods were the "safe signal" for drinking. The behavioral readout is the number of licks during silent periods. Animals perceiving a phantom sound are expected to lick less during quiet periods as they try to avoid a foot shock. The motivation to develop such a schedule-induced polydipsia avoidance conditioning paradigm was to assess tinnitus in individual animals and over extended periods of time. IIn order to achieve a performance of >90% of licks during quite periods the animals were trained for 2-3 weeks. Another study confirmed the sensitivity of this test for tinnitus by measuring it with different paradigms as well [19]. Lobarinas et al. [11] were able to monitor salicylate-induced tinnitus and recovery over 40 sessions.

4.2. Positive Reinforcement Paradigms. An operant paradigm with positive reinforcement has been proposed by Rüttiger et al. [16] which reduced the need for punishment through foot shocks to a minimum. Again, a continuous noise was a safe signal for the rat to access one of two water spouts in order to receive a reward (3% sucrose in water). The rat had to switch from one spout to the other in order to collect a reward. If the animal accessed one spout during a silent period, no reward was delivered and a foot shock is applied. During testing for tinnitus, there was no reward and no punishment during the silent gaps. In order to still get useful behavioral responses, even before testing for tinnitus, only a percentage of switches between reward spouts were rewarded. This prolonged the time to extinction of the discriminative behavior between noise and silent gaps. It should be emphasized that the foot shock in this study was quite weak and avoidable and the behavior of the animals was most likely driven by the reward value of the sugar water itself. The reinforced behavior was activity (alternating between spouts). Tinnitus was induced with an injection of salicylate (350 mg/kg bodyweight) after the animals achieved a certain performance level (12 to 15 sessions before administration). Testing took place immediately after tinnitus induction in order to characterize the immediate effects of salicylate. The behavioral indicator was the ratio between number of reward spout access during noise and during silence, divided by the ratio between noise duration and silence duration. After salicylate treatment, the number of access to the reward spouts during silent periods increased relative to the access during noise presentation. This paradigm has been used in a couple of follow-up studies, where the tinnitus was induced through an acoustic trauma, emphasizing its robustness and applicability to a wider range of tinnitus models [42, 51, 119].

Another paradigm using only mild electric shocks and positive reinforcement was published by Heffner and Koay [62]. Here, hamsters received a unilateral acoustic trauma and were trained to localize a sound source (left or right) in order to collect a reward at that side. Responses to the wrong side were shocked. During training, sound trials were interleaved with a few silent trials (catch trials) which were not punished or rewarded. These trials served as an indicator for the animal's side preference. After the acoustic trauma, the side preference shifted to the side where the trauma

was applied. This was interpreted as a result of a phantom sound perceived by the animals, as they were trained to go to the side where a stimulus was localized. In summary, the operant conditioning paradigms described here usually require a very careful and time-consuming training of the animals. However, this is compensated by the possibility to test animals repeatedly and over extended periods.

One very recently published paradigm does not apply any aversive stimulus at all but only positive reinforcement through food pellets [47]. Here, the rats had to press one lever in the presence of a sound (tone lever) and press another lever in the absence of sound (0 Hz lever). After treatment with salicylate (75, 150, 300, or 450 mg/kg body weight) or exposure to intense sounds (140 dB SPL at 4 kHz for 4 hours) the animals exhibited an increased number of "tone lever" presses in the absence of any sound. This increase was ascribed to the presence of the tinnitus phantom sound. Again, the extensive training required (2-3 months) by this paradigm is balanced by the possibility to test animals over extended periods.

A navigation approach was pursued by Guitton and Dudai [39]. Here, the rats had to swim in a water T-maze and find a hidden platform. The platform was in one of the two arms of the maze if a tone was presented and in the other arm, when no tone was presented. Two measures were taken for quantifying the sound perception of the animal: time spend in one arm of the maze and percentage of correct choices. After 3 days of training the animals reached the correct arm in 80% of the cases within an average time of 4 s. After an acoustic trauma approximately half of the rats (12 out of 26) behaved as if they perceived in tone even when there was no sound present (measured as an increased time spent in the arm associated with the tone).

4.3. Gap Startle Reflex Paradigm. During the last years, a completely different and objective paradigm was established for measuring tinnitus in laboratory animals. It is based on the acoustic startle reflex or response (ASR) which is a very rapid contraction of skeletal muscles following the presentation of acoustic stimulus with high intensity [124]. The central pathway for this startle response is well described and involves only three synapses. The cochlear input is relayed through the brainstem to the pedunculopontine tegmental nucleus and the nucleus reticularis pontis caudalis which initiates the startle response [105]. The amplitude of this response is modulated by many factors like fear potentiation and sensitization. In particular it can be reduced by a preceding stimulus or silent gap in a continuous background noise. The basic idea for tinnitus detection is that a phantom sound can mask these gaps. In animals experiencing tinnitus, the acoustic startle reflex is not diminished even when preceded by a gap. This concept was first tested and published by Turner et al. [8] as a new approach to efficiently test for tinnitus in the animal model. To this end rats received an acoustic trauma (unilateral 16 kHz octave-band noise at 116 dB SPL, under anesthesia). Next, animals were placed in a testing chamber where a continuous background noise was presented (centered at 10 or 16 kHz or broadband noise, 60 dB SPL). The animal's response was measured as force applied to a Piezo transducer in the floor of the chamber. The startle stimulus was a 115 dB SPL noise burst for 20 ms. Half of the startle stimuli were preceded by a 50 ms gap in background noise which would reduce the startle amplitude in naïve animals. Animals receiving an acoustic trauma exhibit less inhibition of the startle response when it was preceded by a gap compared to controls. However, this was only the case when the background noise was centered at 10 kHz and not at 16 kHz or for broad band noise. This result confirmed the previously characterized tinnitus pitch at 10 kHz which was determined by an operant conditioning paradigm [8]. Hearing loss was ruled out as possible explanation for this effect as a simulated unilateral hearing loss (ear plugs) did not change the inhibition of the startle response by a preceding gap.

This paradigm or some derivatives (e.g., measuring the Preyer reflex in guinea pigs by Berger et al. [67]) were adopted by many research groups [11, 20, 23, 46, 48, 49, 52, 53, 64, 65, 68, 69] because they offer a number of advantages. The main benefit for experimentalists is that it is a fast method in terms of training and testing. No training beyond test chamber adaptation is required and testing can take place in less than one hour, allowing high-throughput screening which is not possible with more complex conditioned behavioral paradigms. Additionally, the animals do not have to be on a restricted food or water schedule and the neuronal circuitry giving rise to the startle response is well described. Finally, this is a fairly objective measurement as the reflex is only to a certain degree modulated by top-down processes [125]. However, a number of issues have to be taken into account when considering a gap startle paradigm for assessing tinnitus in animal models. First, it is unknown whether in human tinnitus patients gaps are "filled" with the phantom percept. In the light of transferability of results from the animal model to humans, this is a major drawback and has been only very recently addressed by Fournier and Hébert [126]. This study explicitly tested gap inhibition of a startle response (eye blink) in tinnitus patients (high-pitched) in order to compare it to animal studies. The key finding was that tinnitus patients exhibited a similar change of startle response amplitude when preceded by a gap as the traumatized animals did in the studies mentioned above. Despite some differences in the results compared to the study by Turner et al. [8] (e.g., gap deficits occurred at high- and low-frequency background noise in humans but not in the animal study) this is evidence that the gap startle paradigm could be a valid model for studying tinnitus and that it measures manifestations of a phantom sound comparable to the one observed humans.

One objection put forward regarding the gap startle paradigm is its reflex nature and that it does not necessarily involve the auditory cortex. It has been shown that ablation of auditory cortex in mice does not change the gap startle response after one month compared to a control group. However, one day after cortex ablation there were differences, indicating a temporary modulatory effect of auditory cortex on activity in the brain stem circuitry that gives rise to the startle response [81]. Other studies in rats [127, 128] lesioning or deactivating the auditory cortex found changes for certain gap durations. Thus, the role of auditory cortex in the gap

startle paradigm still remains to be elucidated. It has been hypothesized that the neural substrate of tinnitus involves an increase in spontaneous activity, an increase in neuronal synchrony, and a reorganization of the tonotopic map in auditory cortex [105, 120]. Testing this hypothesis ideally requires a behavioral paradigm, which necessarily involves the auditory cortex and not only a brain stem circuit. It has been shown that tinnitus patients and healthy subjects can detect gaps typically used in gap startle paradigms with similar performance [129]. This result indicates that changes in gap startle paradigms do not automatically mean that higher processing of these stimuli is impaired in tinnitus patients. Lobarinas et al. [49] put forward the potential influence of hearing loss on the gap startle response and tackle this concern twofold in a dedicated study: first, by optimizing the startle stimulus so that it was outside the range of the hearing loss and second, by substituting the broad band noise startle stimulus with a rapid air puff to the animal's back which cannot be subject to hearing loss. In particular, the air puff approach preserved the startle response, even after conductive hearing loss. However, its operational reliability for measuring tinnitus remains to be proven.

5. Summary

The ultimate benchmark for any animal model measuring subjective tinnitus is comparability to the human patient. Any researcher starting to model tinnitus in laboratory animals has to make a decision regarding the species, the method of tinnitus induction, and the behavioral test. The current review provides an overview over the most commonly used methods and approaches.

The most important criteria for choosing a certain species is its hearing range, its aptitude for behavioral studies and the availability of genetically modified strains. These strains allow the recording and manipulation of specific types of neurons revealing their role in tinnitus. The behavioral differences between the commonly used species are a source of uncertainty. The majority of studies discussed here were done in rats, considered to be well suited for behavioral testing even with more difficult sensory decision making paradigms [130]. Another advantage of the rat as an experimental model for studying the neuronal circuitry underlying tinnitus is the possibility to implant electrode arrays with high channel counts and perform chronic recordings in awake [131] and behaving animals (e.g., Otazu and Zador [132]). The disadvantage of the rat as a model is its high-frequency hearing range, which differs significantly from the human one. Still, it remains unclear so far if these differences in hearing rage are significant for the pathogenesis, perception, and potential therapy of tinnitus. Additionally, there are only a limited number of genetically modified rat strains available. However, this last factor is certainly changing in the future as more and more recombinase-driver rat lines are developed (e.g., [133]) and the establishment of the potentially universally applicable CRISPR genome-editing technique [134], which has already been applied successfully in cynomolgus monkey (*Macaca fascicularis*) [135].

The tinnitus induction protocol should model the human pathogenesis. For the majority of human cases, an acoustic trauma-induced hearing loss is suspected. This favors a tinnitus induction through acoustic trauma over a pharmacological induction. On the other hand, an induction through salicylate has the advantage of fast onset of tinnitus and its reversibility. This allows a behavioral setting that can be controlled for tinnitus related behavioral peculiarities of individual animals. Furthermore, salicylate can be applied locally which allows to study tinnitus-related changes at different stages of the auditory processing hierarchy. Whichever method is used, the accompanying hearing loss and hyperacusis have to be taken into account for interpreting the results. However, to disentangle tinnitus and hyperacusis is very challenging as they are comorbid. Very recently, it has been demonstrated that mice exposed to "neuropathic" noise displayed a hyperresponsivity to acoustic startle stimuli. At the same time the gap detection deficits (measured as prepulse inhibition of the startle response) were limited to certain gap-stimulus latencies which cannot be explained by the presence of a phantom sound which should fill the gap for all latencies [107] and which therefore has be interpreted as a potential indicator of hyperacusis.

The behavioral approaches testing for subjective tinnitus presented here include paradigms using reflexes, Pavlovian conditioning, and operant conditioning. Tinnitus in humans is a conscious percept which involves the auditory cortex [120]. It is usually measured through sensory decision making tests which can be applied over extended periods. A behavioral test for laboratory animals should be shaped along these aspects, in particular the cortical involvement and extended testing period. Additionally, such a test should only require limited training periods in order to achieve a high throughput. For conditioned responses the auditory cortex is not essential, as a cortical ablation does not prevent an animal from a classical conditioning response to simple tones [105]. However, more complex tones (e.g., frequency modulated tones) necessarily require a functional auditory cortex for discrimination [136]. More complex operant conditioning tasks most likely rely on an intact auditory cortex [105]. This has to be balanced with the usually more time consuming training protocols required for operant conditioning paradigms. For the conditioning paradigms introduced here, an involvement of the auditory cortex has not been shown yet, leaving an explanatory gap between the observed behavior and its neuronal substrate. Furthermore, modulation of the tinnitus percept through higher cognitive functions as demonstrated in humans (e.g., attention [137]) has been ignored in animal studies so far, most likely due to a lack of behavioral paradigms allowing the manipulation of these functions. However, a comprehensive animal model should ideally take this factor into account as well.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

Polarity Specific Suppression Effects of Transcranial Direct Current Stimulation for Tinnitus

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Tinnitus is the perception of a sound in the absence of an external auditory stimulus and affects 10–15% of the Western population. Previous studies have demonstrated the therapeutic effect of anodal transcranial direct current stimulation (tDCS) over the left auditory cortex on tinnitus loudness, but the effect of this presumed excitatory stimulation contradicts with the underlying pathophysiological model of tinnitus. Therefore, we included 175 patients with chronic tinnitus to study polarity specific effects of a single tDCS session over the auditory cortex (39 anodal, 136 cathodal). To assess the effect of treatment, we used the numeric rating scale for tinnitus loudness and annoyance. Statistical analysis demonstrated a significant main effect for tinnitus loudness and annoyance, but for tinnitus annoyance anodal stimulation has a significantly more pronounced effect than cathodal stimulation. We hypothesize that the suppressive effect of tDCS on tinnitus loudness may be attributed to a disrupting effect of ongoing neural hyperactivity, independent of the inhibitory or excitatory effects and that the reduction of annoyance may be induced by influencing adjacent or functionally connected brain areas involved in the tinnitus related distress network. Further research is required to explain why only anodal stimulation has a suppressive effect on tinnitus annoyance.

1. Introduction

Tinnitus is a common perception of a tone, hissing, or buzzing sound in the absence of an external auditory stimulus and is therefore also described as a phantom sound [1]. It is continuously present in about 10–15% of the Western population [2], of which 2–4% perceives severe interference with their quality of life [3] as it is often associated with symptoms such as annoyance [4], anxiety [5], depression [5], and sleep disturbances [6]. In elderly patients, the prevalence percentages can even rise up to 33% [7, 8], most likely due to the higher prevalence of hearing impairment [9].

Although no consensus has been reached about the neurophysiological model of tinnitus, it is proposed that tinnitus is related to either auditory deafferentation [10–15] or a deficit in noise cancelling [16, 17] or a combination of both [18]. Moreover, tinnitus has been hypothesized to be the expression of a thalamocortical dysrhythmia, in which there is a constant (pathologic) coupled theta-gamma band activity (theta: 4–7 Hz, gamma > 30 Hz) due to hyperpolarization of specific thalamic nuclei [19]. In normal circumstances, incoming auditory stimuli induce a transient gamma band activity [20] in a restricted area [21], which binds by nesting on theta activity [22, 23], that is, a transient coupling between a high- and low-frequency band of ongoing electrical activity in the human brain [22]. This is mediated by a shift of alpha activity towards high-frequency gamma band oscillations [20]. In a deafferented state, neural activity is shifted towards theta band activity [24] which in turn leads to a decreased lateral inhibition mediated by γ-amino butyric acid [25] and results in a persistent and thus pathological gamma activity of the neighboring neurons, also known as the

"edge effect" [19, 25]. This sustained gamma band activity present in temporal areas is related to tinnitus as observed by quantitative electroencephalography (qEEG) [26] and magnetoelectroencephalography (MEG) [12, 19, 25]. The coupled presence of theta and gamma activity in tinnitus has also been demonstrated by recordings from an implanted electrode overlying the auditory cortex in a tinnitus patient [27]. Furthermore, this theta-gamma coupled activity is maximal at the area of blood oxygenation level dependent (BOLD) activation evoked by tinnitus-matched sound presentation in the magnetic resonance imaging (MRI) scanner [27], suggesting that the BOLD signal can demonstrate pathological tinnitus related activity in the auditory cortex [28]. Moreover, a positive correlation has been demonstrated between the amount of gamma band activity in the auditory cortex and the perceived tinnitus loudness in the contralateral auditory cortex [29].

The involvement of the auditory cortex cannot only be concluded from neuroimaging and electrophysiological measurements but it can also be claimed from the results of both invasive and noninvasive neuromodulation studies. Extradural stimulation of the secondary auditory cortex by a fMRI-guided neuronavigated electrode implant, based on BOLD activation evoked by tinnitus-matched sound [30–32], could partially or completely suppress tinnitus in 67% of the patients who perceived a tinnitus suppressive effect by transcranial magnetic stimulation (TMS) [33]. Several studies explored the therapeutic effect of TMS over the temporoparietal cortex in the treatment of tinnitus demonstrating that repetitive high frequency TMS, that is, increasing cortical excitability, causes a tinnitus suppression effect in about 50% of the patients [34–37]. Interestingly, both single [38, 39] and repetitive sessions [40–44] of low-frequency TMS over the temporoparietal cortex have been successful in the treatment of tinnitus as well. Another noninvasive neuromodulation technique applied in the treatment of tinnitus is transcranial direct current stimulation (tDCS). tDCS is applied by two surface electrodes, one anode and one cathode, of which one or two are placed over the scalp. Although a part of the applied current will be shunted by scalp tissue, a substantial part reaches the brain [45]. It has been demonstrated that anodal direct current stimulation induces depolarization of the underlying neurons, while cathodal stimulation leads to hyperpolarization [46], mainly by influencing the resting membrane potential. Combining this with the above-mentioned alterations in neural activity observed in tinnitus patients leads to the suggestion that the cathode overlying the auditory cortex should exert a tinnitus suppressing effect while the anode should have a potentially tinnitus worsening effect. However, Fregni et al. could obtain a transient suppression on tinnitus loudness using anodal tDCS over the left temporoparietal cortex [36]. These results were replicated by Garin et al. [47], albeit different stimulation parameters were used. It should be noticed that these tDCS results are rather paradoxical to the previously proposed model of tinnitus, whereas tinnitus is related to neural hyperactivity of the auditory cortex. Therefore, we retrospectively looked at our data in 175 tinnitus patients in which the effects of a single session of anodal or cathodal tDCS over the auditory cortex was evaluated both for tinnitus loudness and annoyance.

2. Methods

2.1. Subjects. 175 patients (116 male, 59 female) with chronic tinnitus (>1 year) received auditory cortex tDCS (Table 1). The mean age of the patients was 48.46 years (Sd = 13.27) and the mean tinnitus duration was 5.56 years (Sd = 6.82). All patients underwent a single session of tDCS performed in the treatment of tinnitus at the Tinnitus Research Initiative (TRI), Antwerp. Of these 175 patients, 43 received tDCS with an intensity of 1.5 mA, while 132 patients received tDCS of 2.0 mA. The applied stimulation intensity and the side of stimulation were chosen randomly. Individuals with pulsatile tinnitus, Ménière disease, otosclerosis, chronic headache, and neurological disorders such as brain tumors and individuals being treated for mental disorders were not included in the study in order to obtain a homogeneous sample. Therefore, all patients included for this study firstly underwent a complete audiological, ENT, and neurological investigation. In addition, several technical investigations were performed including MRI of the brain. Collection of the data was under approval of IRB UZA OGA85. All patients gave an informed consent.

2.2. Transcranial Direct Current Stimulation. For the application of tDCS, a pair of electrodes with a surface of 35 cm^2 was placed in saline-soaked sponges. Both electrodes, one anode and one cathode, were connected to a battery, which delivers a constant current with a maximum output of 10 mA (Neuroconn; http://www.neuroconn.de/). The stimulation was applied for 20 minutes with a current intensity of 1.5 or 2.0 mA in a quiet room. These stimulation parameters are considered to be safe and without any significant side effects [48, 49]. The anodal or cathodal electrode was placed over the left or right auditory cortex, that is, T3 or T4 of the International 10/20 Electroencephalogram System, respectively, while the reference electrode was placed on the contralateral arm. An advantage of placing the reference electrode extracephalic is that interference from the reference electrode can be avoided [50], contrary to most previous studies in which they made use of a bicephalic electrode positioning.

2.3. Evaluation. A numeric rating scale (NRS) for tinnitus loudness ("How loud do you perceive your tinnitus? 0: no tinnitus and 10: as loud as imaginable") and annoyance ("How annoying is your tinnitus? 0: not annoying and 10: extremely annoying") was asked before and directly after tDCS stimulation.

2.4. Statistical Analysis. Calculations were performed using SPSS 22 software package. A repeated measure ANOVA was conducted with tinnitus loudness pre- and posttreatment as within-subjects variable and stimulation parameter (cathodal versus anodal stimulation) and location (left versus right auditory cortex) as between-subjects variables for

Table 1: Patient characteristics and stimulation parameters.

	Stimulation intensity	
	1.5 mA	2.0 mA
Age (years)	48.37 ± 15.73	48.49 ± 12.44
Gender (female/male)	19/24	40/92
Tinnitus laterality (left/right/bilateral)	17/4/22	29/19/84
NRS tinnitus loudness	6.41 ± 1.37	6.42 ± 1.76
NRS tinnitus annoyance	5.83 ± 1.69	6.22 ± 1.82
Stimulation (anodal/cathodal)	7/36	32/100
Anodal stimulation: location (left/right)	2/5	16/16
Cathodal stimulation: location (left/right)	22/14	44/56

the group receiving 1.5 mA tDCS. Likewise, a repeated measure ANOVA was performed with tinnitus annoyance pre- and posttreatment as within-subjects variable and stimulation parameter (cathodal versus anodal stimulation) and location (left versus right auditory cortex) as between-subjects variables for the group receiving 1.5 mA tDCS. Both analyses were repeated for the group of patients receiving 2.0 mA tDCS.

To further interpret the interaction effect, we conducted a simple contrast analysis. This latter method has the advantage that a specific contrast can be compared within the full model, without separating the groups (stimulation, location) into different independent statistical tests excluding part of the variance. Although the repeated measures ANOVA was conducted as a whole model (including the different main effects as well as the interaction effects), we report the results in different subheadings for reasons of clarity.

In addition, a post hoc analysis was performed for the 2.0 mA group to control for tinnitus lateralization as only in this group significant results could be obtained. Therefore, a repeated measures ANOVA was performed with the pre- and posttreatment as within-subjects variable and stimulation (anodal versus cathodal auditory cortex stimulation) as between-subjects variable for both loudness and annoyance while we controlled for tinnitus lateralization (left-sided, right-sided, or bilateral tinnitus). This is necessary as it could be assumed that the effect of treatment is influenced by tinnitus laterality and therefore, depending on the side of the tinnitus, stimulation should be applied over a specific side or with a specific polarity to gain a therapeutic effect.

3. Results

3.1. 1.5 mA tDCS. A repeated measures ANOVA with the pre- and posttreatment as within-subjects variable and stimulation (anodal versus cathodal auditory cortex stimulation) and location (left versus right auditory cortex) as between-subjects variables for both loudness and annoyance was performed for the patients receiving tDCS with an intensity of 1.5 mA. This analysis revealed no significant effect for tinnitus loudness or annoyance (see Figure 1) and no significant interaction effect could be demonstrated with polarity. Moreover, no additional main or interaction effects could be demonstrated for tinnitus loudness or annoyance in the patients receiving 1.5 mA. In addition, when defining tDCS responders as those patients having a difference in NRS loudness or annoyance greater than zero when comparing pre- to poststimulation NRS scores, only 3 out of 43 patients experienced a suppressive effect of tDCS on tinnitus loudness, while 2 patients experienced a suppressive effect on tinnitus related annoyance. Responders were only present in the group of patients receiving cathodal stimulation.

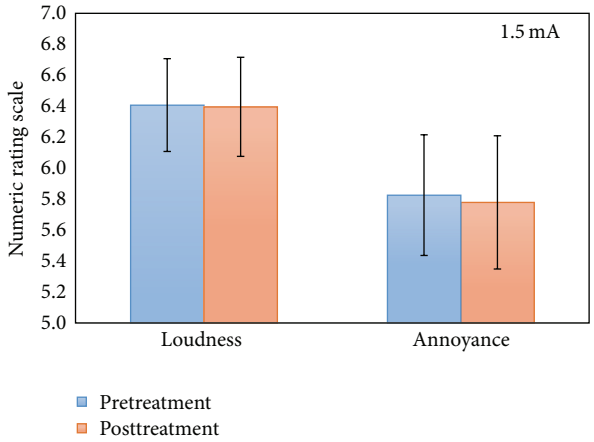

Figure 1: NRS loudness and annoyance pre- and posttreatment for the 1.5 mA group.

3.2. 2.0 mA tDCS

3.2.1. Pre- versus Posttreatment. A repeated measures ANOVA with the pre- and posttreatment as within-subjects variable and stimulation (anodal versus cathodal auditory cortex stimulation) and location (left versus right auditory cortex) as between-subjects variables for both loudness and annoyance was performed for the patients receiving tDCS with an intensity of 2.0 mA. This analysis yielded a significant treatment effect for tinnitus loudness ($F(1, 130) = 15.90$, $P < .001$) indicating that after the treatment session (M = 6.11, Sd = 1.78) tinnitus patients had a decrease of their tinnitus loudness in comparison to pretreatment (M = 6.42, Sd = 1.76) (see Figure 2), although only 23 patients out of 132 experienced a tinnitus suppressing effect.

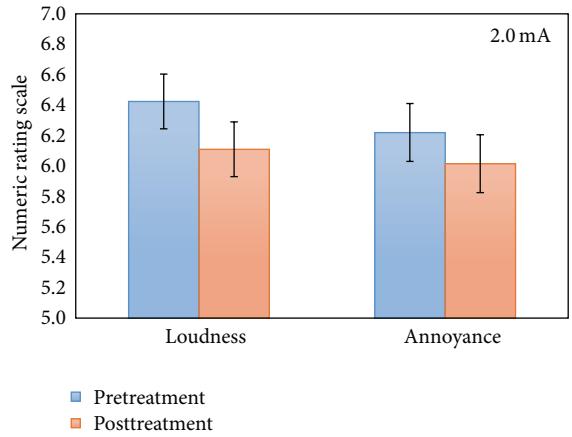

Figure 2: NRS loudness and annoyance pre- and posttreatment for the 2.0 mA group.

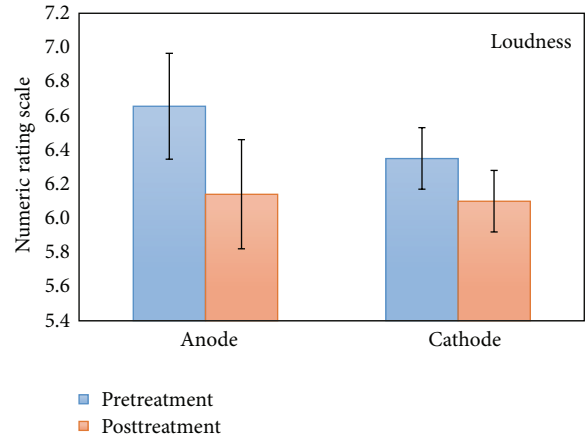

Figure 3: NRS loudness pre- and posttreatment (anodal and cathodal) for the 2.0 mA group.

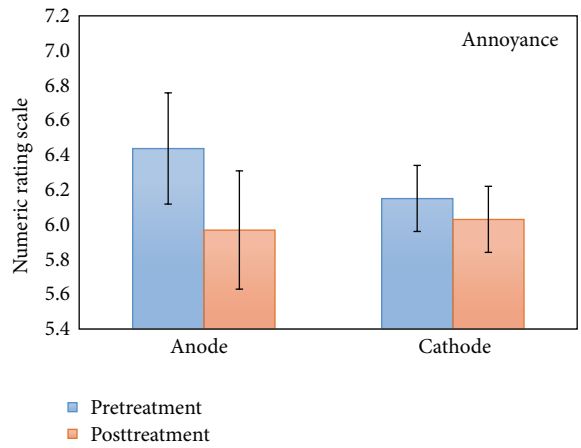

Figure 4: NRS annoyance pre- and posttreatment (anodal and cathodal) for the 2.0 mA group.

Of these responders, 10 received anodal stimulation, while 13 received cathodal tDCS. In addition, a significant decrease ($F(1, 130) = 13.79$, $P < .001$) of tinnitus annoyance was observed when posttreatment scores (M = 6.02, Sd = 1.89) were compared to pretreatment scores (M = 6.22, Sd = 1.82) (see Figure 2), while only 15 patients perceived a reduction of tinnitus related annoyance. 9 Of these patients received anodal tDCS and 6 were given cathodal stimulation.

3.2.2. Pre- versus Posttreatment Dependence on Anodal or Cathodal Stimulation. This repeated measures ANOVA with pre- and posttreatment as within-subjects variable and stimulation (anodal versus cathodal auditory cortex stimulation) and location (left versus right auditory cortex) as between-subjects variable demonstrated no significant interaction effect between pre- and posttreatment for loudness and stimulation polarity (see Figure 3), although a significant interaction effect between polarity and pre- and posttreatment measurement was observed for tinnitus annoyance ($F(1, 130) = 3.98$, $P < .05$). A simple contrast analysis revealed that for tinnitus annoyance there was a significant effect for anodal stimulation when comparing prestimulation (M = 6.44, Sd = 1.58) to poststimulation (M = 5.97, Sd = 1.69) ($F(1, 130) = 10.56$, $P = .001$), but no significant effect was obtained for cathodal stimulation between pre- (M = 6.15,Sd = 1.89) and poststimulation (M = 6.03, Sd = 1.95) (see Figure 4).

3.2.3. Other Main and Interaction Effects. Our repeated measures ANOVA revealed no significant effect for the two-way interaction between treatment (pre versus post) and location (left versus right auditory cortex) or for the three-way interaction between treatment (pre versus post), stimulation (anodal versus cathodal), and location (left versus right auditory cortex) for tinnitus loudness or annoyance.

In addition, no significant effects were demonstrated for loudness or annoyance for the between-subjects variables stimulation (anodal versus cathodal) and location (left versus right auditory cortex). Furthermore, no significant effect was obtained for the two-way interaction between stimulation (anodal versus cathodal) and location (left versus right auditory cortex) for tinnitus loudness or annoyance.

3.2.4. Controlling for Tinnitus Lateralization. A repeated measures ANOVA with pre- and posttreatment as within-subjects variable and stimulation (anodal versus cathodal stimulation) as between-subjects variable for loudness was performed, while we controlled for tinnitus lateralization (left-sided, right-sided, or bilateral tinnitus). This analysis revealed no significant effect for tinnitus lateralization or an interaction effect between treatment (pre versus post) and tinnitus lateralization. Moreover, no significant interaction effect could be demonstrated between pre- and posttreatment and stimulation (anodal versus cathodal).

The same analysis was performed for tinnitus related annoyance, demonstrating that there was no significant effect for tinnitus lateralization or interaction effect between treatment (pre versus post) and tinnitus lateralization. However, a significant interaction effect between pre- and posttreatment

and stimulation (anodal versus cathodal) for the 2 mA condition remained ($F(1, 130) = 4.11$, $P < 0.05$).

4. Discussion

Our results demonstrate an overall significant suppressive effect of tDCS applied over the auditory cortex for tinnitus loudness and annoyance, but only when tDCS was applied with an intensity of 2 mA. This overall effect on tinnitus annoyance was however likely mediated by the specific polarity of stimulation. That is, a significantly more pronounced effect was demonstrated for anodal than for cathodal stimulation when the electrode was placed over the auditory cortex, irrespective of whether stimulation was applied over the left or right auditory cortex.

Currently, only limited studies have been performed in which single session tDCS has been applied over the auditory cortex. Both Fregni et al. [36] and Garin et al. [47] could obtain a significant reduction of tinnitus loudness when anodal stimulation was applied over the left temporoparietal area, but not when the cathode was placed over the left temporoparietal cortex. However, it has to be mentioned that 6 out of 20 patients reported a reduction of their tinnitus loudness with cathodal stimulation, even though results were not statistically significant in the experiment of Garin et al. In addition to the positive effects on tinnitus loudness, neither anodal nor cathodal stimulation could induce a significant reduction on tinnitus discomfort [47]. The most plausible explanation for the different results compared to our study, that is, a significant reduction of tinnitus loudness independent of polarity and a suppressive effect on annoyance mainly mediated by anodal stimulation, is the difference in stimulation parameters. Both Fregni and Garin used tDCS with an intensity of only 1 mA and this with duration of 3 minutes in the study of Fregni, while the study of Garin as well as our own applied tDCS for 20 minutes. Based on these observations and our negative results for both tinnitus loudness and annoyance when tDCS was applied with an intensity of 1.5 mA, we may suggest that tDCS intensity is a decisive parameter and that cathodal stimulation might require a higher stimulation intensity to gain equally pronounced effects as anodal stimulation. Recently, Shekhawat et al. performed anodal stimulation over the left temporoparietal cortex and revealed that 2.0 mA was the more effective stimulation parameter when compared to an intensity of 1.0 mA [51].

Although the effects of tDCS on tinnitus loudness are more pronounced when a higher stimulation intensity is used, the effects of tDCS on tinnitus loudness are not influenced by polarity or by the side of stimulation. If we look at the pathophysiological model of tinnitus, one of the most consistent findings is the constant presence of pathological gamma activity in the auditory cortex demonstrated with both MEG [12, 19, 25] and qEEG [26], as well as on implanted electrodes [27]. Moreover, a strong positive correlation has been found between gamma oscillations in the contralateral auditory cortex and tinnitus intensity [29]. Because cathodal stimulation has been shown to have an inhibitory effect, it seems plausible that cathodal stimulation should induce the most pronounced effect as it can counteract this pathologic hyperactivity, but based on our results and the results of the above mentioned studies, other mechanisms should be considered. One possible explanation is that both cathodal and anodal stimulation have a disrupting effect on ongoing network activity, independent of their inhibitory or excitatory effect. Moreover, anodal stimulation may decrease pathologic hyperactivity of surrounding brain areas, by either competitive or inhibitory effects [36]. An interesting remark we need to make is that, in a recent study, in which cathodal tDCS of 1.0 and 2.0 mA was applied over the motor cortex in healthy subjects, reversed effects were obtained. More precisely, application of 2.0 mA cathodal tDCS resulted in cortical excitability enhancement instead of inhibition, similar to the results obtained with 2.0 mA anodal tDCS [52]. They suggest that the reversed effects are possibly due to the dependency of the direction of plasticity from the amount of neuronal calcium influx caused by the stimulation or that the resulting neuronal excitability change is determined by the axonal orientation relative to the electric field vector.

Besides the suppressive effect of tDCS on tinnitus loudness, a decrease of tinnitus related annoyance could be identified. Moreover, a significant interaction effect could be demonstrated between overall treatment and stimulation polarity with further analysis revealing that only anodal stimulation has a significant effect on annoyance. The amount of annoyance correlates with an alpha network consisting of the amygdala-anterior cingulate cortex-insula-parahippocampus-dorsolateral prefrontal cortex (DLPFC) [53, 54] and annoyance in tinnitus patients is related to the alpha and beta activity in the dorsal anterior cingulate cortex [53, 54]. Performing single-session, bilateral tDCS with the anode placed over the right DLPFC and the cathode over the left DLPFC induces a suppressive effect on annoyance [55, 56]. Similarly, repeated sessions of bifrontal tDCS could induce a small clinical effect on tinnitus discomfort [57]. Interesting results as the DLPFC has been shown to be involved in depression [58, 59], anxiety, and the affective component of pain [60], as well as in the processing of aversive auditory stimuli [61] and the sensory and emotional aspects of tinnitus [10, 53]. Most likely, our stimulation design does not only influence the underlying auditory cortex but also adjacent and functionally connected brain regions, for example the DLPFC. As we made use of an extracephalic reference electrode, the applied electric current will show a more widespread distribution than when bicephalic electrode positions are used [62]. This might as well explain why Garin et al. [47] could not find a significant effect on tinnitus discomfort, besides the lower current intensities used, as they positioned the reference electrode on the right scalp. But although we suggest that tDCS targeting the auditory cortex may influence the tinnitus related distress network, we cannot yet explain why only anodal stimulation, and this independently of the side of stimulation, leads to a decrease in annoyance. However, we should notice that recently a correlation between tinnitus distress and grey matter volume in bilateral auditory areas using voxel based morphometry (VBM) was identified [63], suggesting that stimulation of

the auditory cortex may have a direct influence on tinnitus related annoyance as well, analogous to what has been seen in implants on the auditory cortex for tinnitus suppression [31, 33].

Some limitations of this study should be noted. Firstly, although we included 175 patients, only 43 patients received tDCS with an intensity of 1.5 mA and of the remaining 132 patients with 2.0 mA tDCS, only 32 patients received anodal stimulation while 100 patients received cathodal stimulation. The reason for this unequal distribution is that this was a retrospective study. Furthermore, it was not a placebo controlled study, but it has been previously demonstrated that sham stimulation applied over the left temporal lobe does not induce a significant effect [36] and the observation that no significant results could be obtained with 1.5 mA supports the fact that our results are not likely to be due to a placebo effect. Moreover, the main scope of our study was to explore the different effects of anodal and cathodal stimulation, rather than the therapeutic effect of tDCS in tinnitus per se.

In conclusion, we observed an overall suppressive effect for tDCS applied over the auditory cortex on tinnitus loudness and annoyance when performed with an intensity of 2.0 mA, but in contrast to previous tDCS studies the effect on tinnitus loudness was independent of polarity. For tinnitus annoyance on the other hand, a significant influence of stimulation polarity could be demonstrated, with a more pronounced effect for anodal than cathodal stimulation. Based on these observations, we suggest that reduction of tinnitus intensity may be caused by a disrupting effect on ongoing hyperactivity in the auditory cortex and functionally related brain areas, independent of polarity. Moreover, we hypothesize that auditory cortex stimulation may influence the tinnitus related distress network, but further research has to be performed to reveal why only anodal stimulation, independent of the side of stimulation, is capable of reducing annoyance.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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