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Tinnitus as a Symptom of Cochlear Synaptopathy? A Study of Auditory Brainstem Responses

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Tinnitus and Cochlear Synaptopathy

Sammanfattning

Syfte. Syftet med denna studie är att studera sambandet mellan kokleär synaptopati och tinnitus, och därigenom eventuellt finna ett fysiologiskt korrelat att behandla. **Metod.** 29 deltagare med och utan tinnitus genomgick hörselundersökning, inklusive taluppfattbarhetsmätning och elektrokokleografi. Extraherade data slogs sedan samman med redan befintliga data över 423 deltagare från STOP:s tinnitusprojekt. Deltagarna delades upp i studiegrupper med bilateral konstant eller tillfällig tinnitus och en kontrollgrupp. Deltagare med nedsatt hörsel exkluderades från analysen. Data analyserades för gruppskillnader gällande amplitud och latenstid för alla ABR-vågor, samt tal i brus-prestation. **Resultat.** En tendens till lägre våg I-amplitud observerades mellan gruppen med tillfällig tinnitus och kontrollgruppen. Gruppen med konstant tinnitus hade lägre våg V-amplitud än kontrollgruppen. Absoluta latenstider för våg II till våg V var signifikant högre för gruppen med konstant tinnitus jämfört med både gruppen med tillfällig tinnitus och kontrollgruppen. **Slutsatser.** Studien bekräftar tidigare studiers mätresultat avseende latenstider men ger inget stöd åt kokleär synaptopati. Resultaten antyder att tillfällig och konstant tinnitus utgör olika undergrupper av tinnitus, med olika fysiologiska korrelat och bakomliggande orsaker. Ytterligare studier med större deltagarantal är nödvändiga för att tydligare särskilja undergrupper av tinnitus och för att bekräfta betydelsen av att ta hänsyn till hörtrösklar i högfrekvensområdet (>8 kHz).

Sökord: tinnitus, elektrokokleografi, ABR, synaptopati

Abstract

Purpose. The purpose of the present study is to study the relationship between cochlear synaptopathy and tinnitus. **Method.** 29 participants with and without tinnitus were tested for hearing, including speech in noise and electrocochleography. Extracted data were then merged with existing data of 423 participants from the STOP cohort. These were divided into cases, with either permanent or occasional bilateral tinnitus, and controls. Participants with impaired hearing were excluded from analysis. The data were analysed for intergroup differences on amplitude and latency for all ABR waves, as well as speech in noise performance. **Results.** A trend in lower wave I amplitude was observed in the occasional tinnitus group. The permanent tinnitus group had lower wave V amplitude than controls. Latencies for waves II through V were greater in the permanent tinnitus group compared to both occasional tinnitus and control groups. **Conclusions.** The study confirms latency findings of previous studies but finds no support for cochlear synaptopathy. Results suggest that occasional and permanent tinnitus are different subtypes of tinnitus, with different physiological correlates and underlying mechanisms. Further studies with larger sample sizes are needed to better differentiate tinnitus subtypes and to confirm the benefit of studying high-frequency thresholds (> 8 kHz).

Keywords: tinnitus, electrocochleography, ABR, synaptopathy

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Background

The term "tinnitus" stems from the Latin "tinnire", meaning "to ring", and dates back more than 2,000 years (Morgenstern, 2005). Despite its etymology, tinnitus is nowadays defined as "the perception of a phantom sound in the absence of a corresponding acoustic stimulus" (Schaette & Kempter, 2009, p.3042), and can be perceived as a constant ringing or buzzing sound, or more or less intermittent clicking or pulsating sounds. The more rare clicking and pulsatile tinnitus forms are generally understood to have their causes in damaged or abnormal tissue, such as palatile myoclonus and glomus tumours, however, the cause of the most common forms of tinnitus, i.e. ringing or buzzing, is still largely unknown (Chen et al., 2015; Jero & Salmi, 2000; Sismanis, 1998). An estimate 10-15% of the Swedish population suffers from some form of tinnitus, and roughly 2% so much so that they feel severely disabled in their everyday life (Johansson & Arlinger, 2003). The reported prevalence around the world varies greatly, from as little as 5% to as much as 43%, depending on geography, methodology and how tinnitus is defined (McCormack, Edmondson-Jones, Somerset & Hall, 2016).

As the causes of tinnitus have been so poorly understood, readily available treatments tend to focus on reducing the symptoms and helping the patient cope, rather than curing the underlying issue. Tinnitus retraining therapy and cognitive-behavioural therapy have been most successful at helping patients cope, but effectiveness depends on a number a factors, such as age, hearing thresholds, and tinnitus intensity (Hatanaka, Ariizumi & Kitamura, 2008; Koizumi, Nishimura, Sakaguchi, Okamoto & Hosoi, 2009; Theodoroff, Schuette, Griest & Henry, 2014; Marks et al., 2018). Hearing aids and tinnitus masking programs can help distract the patient from consciously noticing their tinnitus, but their effectiveness varies greatly between patients (Suzuki, Suzuki, Yonamine, Onishi & Penido, 2016; Oz et al., 2013).

Theories on Tinnitus

Engineer et al. (2011) have suggested that repeated vagus nerve stimulation in tandem with pure tone stimulation can reverse the pathological neural activity that arises after tinnitus inducing noise exposure, and showed that rats who were given this treatment stopped exhibiting tinnitus like symptoms for as long as several weeks post treatment. Marks et al. (2018) have similarly suggested that bimodal stimulation, consisting of tone bursts and transcutaneous electrical stimulation of the neck, can reduce neural synchrony and spontaneous activity correlated with tinnitus. This treatment was tested on both guinea pigs and human subjects, resulting in reduced physiological tinnitus correlates in the guinea pigs, and reduced tinnitus loudness and reported intrusiveness in the human subjects. De Ridder et al. (2010) have suggested that a specific type of tinnitus is caused by micro-vascular compression of cranial nerves VII and VIII. By surgically decompressing the cranial nerves of 20 tinnitus subjects, they found that half reported reduced tinnitus loudness. Half of the subjects also reported reduced intrusiveness, but the other half reported heightened intrusiveness. An analysis of tinnitus onset and duration revealed that decompression has effect for a subgroup of patients only if performed before the end of the fourth year of tinnitus duration.

A recent theory put forth to explain the more common "ringing tinnitus" proposes that inner hair cell destruction lies at the root of the symptom through enhanced central auditory gain (Salvi et al., 2017). Salvi et al.'s experiments in mice showed that a loss of up to 80% of inner hair cells still allows for good auditory brainstem thresholds in quiet, albeit with exceptionally poor thresholds in noisy environments. It was also shown that the auditory cortex had abnormally high activity post hair cell destruction. Salvi et al. concluded that, with the inner hair cells compromised, the brain compensates for the missing auditory input by increasing central gain. This increase in central gain, they theorised, could then be expected to result in enhanced recruitment, and thereby increased sound sensitivity and tinnitus. A theory proposed by Kujawa and Liberman (2009) suggests,

however, that it's not the destruction of the inner hair cells that's the main cause, but rather the destruction of the synapses that connect inner hair cells to the auditory nerve, a process known as cochlear synaptopathy.

Animal Studies on Cochlear Synaptopathy

Kujawa and Liberman (2009) first showed that mice subjected to prolonged levels of noise exposure intense enough to produce temporary threshold shifts (TTS), without permanently damaging or functionally affecting any hair cells, also resulted in a permanent loss of up to 60% of ribbon synapses that connect the inner hair cells to the auditory nerve. Despite normal distortion product otoacoustic emission (DPOAE) results, the noise exposure resulted in reduced wave I auditory brainstem response (ABR) amplitudes, suggesting a hidden hearing loss. Follow-up studies have shown that cochlear synaptopathy occurs immediately after exposure, and that such exposure can accelerate cochlear ageing (Fernandez, Jeffers, Lall, Liberman & Kujawa, 2015; Liberman & Liberman, 2015). Furman, Kujawa and Liberman (2013) could also show that cochlear synaptopathy exclusively targets high-threshold auditory nerve fibres with low to medium spontaneous firing rates. Other experiments have suggested that there could be a relationship between tinnitus and cochlear synaptopathy, but there is insufficient data to draw any solid conclusions at this time (Hickox & Liberman, 2014; Rüttiger et al., 2013). A few studies have suggested that post-exposure application of the protein neurotrophin-3, which is naturally present in both the peripheral and central nervous system to encourage neural growth, can reduce hearing loss and possibly regenerate lost synapses (Kujawa & Liberman, 2015; Sly et al., 2016).

Histological assessment of the synapses in mice have been performed post-mortem, as today's imaging technique so requires, which has made comparable studies on humans problematic. Promising attempts to find functional equivalence between human and rodent synaptology has been made recently (Liberman & Kujawa, 2017), and similar results to those found in noise exposed mice have been identified in rhesus monkeys (Valero et al., 2017), further suggesting functional equivalence in humans.

To summarise, animal studies have shown that 1) noise exposure sufficiently intense to produce TTS can result in cochlear synaptopathy, 2) cochlear synaptopathy is immediate following noise exposure and permanent, 3) cochlear synaptopathy can lead to accelerated cochlear ageing/degeneration, 4) cochlear synaptopathy specifically targets high-threshold low-firing rate fibres, 5) cochlear synaptopathy can be detected in ABR wave I, 6) there might be a correlation between cochlear synaptopathy and tinnitus, suggest that 7) it might be possible to reverse the effects of cochlear synaptopathy with medical intervention, and that 8) humans and rodents have comparable synaptology.

Auditory Brainstem Response

As the use of ABR for detecting cochlear synaptopathy has been successful in rodent studies, and it appears humans and rodents have comparable synaptology, it stands to reason that ABR could be used to detect cochlear synaptopathy in human subjects, as well. ABR is a well-established tool for diagnosing retrocochlear hearing impairments and is regularly used to complement psychoacoustic hearing measurements objectively (Matthis & Samii, 1997; Warren, 1989). Wave amplitudes are analysed for general signs of brainstem disorders, but a lower than normal wave I amplitude specifically has been linked to poorer performance in speech in noise tests (Bramhall, Ong, Ko & Parker, 2015; Liberman, Epstein, Cleveland, Wang & Maison, 2016). Although not reliable enough to estimate hearing thresholds by itself, ABR can also be used to differentiate between conductive and sensorineural hearing loss. Conductive hearing loss typically

results in a prolongation of all wave latencies, whereas sensorineural hearing loss typically only delays the later wave latencies (Winston & Stoner, 2013).

Functional relationship between ABR waves and the auditory pathway. The five ABR waves roughly correspond to the major encoding and processing nuclei of the ascending auditory pathway in humans. Wave I represents activity in the auditory nerve, in which the incoming auditory signal is broken down into different components for subsequent analysis. Wave II and wave III represent activity in the ventral and dorsal cochlear nuclei, in which the components are enhanced and prepared for analysis. The ventral cochlear nucleus sends sound identity specific information directly onward to the inferior colliculus, while the dorsal cochlear nucleus sends sound localisation specific information onward to the superior olivary complex. Wave IV represents activity in the superior olivary complex, which compares auditory input from both ears to localise the sound source in the horizontal plane. Wave V represents activity in the ventral and dorsal nuclei of the lateral lemniscus, which is where information from the ventral and dorsal cochlear nuclei is collected for a final check-up before reaching the inferior colliculus, as well as the inferior colliculus itself. The ventral nucleus extracts temporal patterns for complex sounds and analyses inter-frequency harmonic relationships, while the dorsal nucleus further enhances the localisation information. The inferior colliculus, then, is where all this information, sound identity and localisation, converges and integrates into a uniform signal anew (Pickles, 2013).

Previous Studies on the Relationship between ABR and Tinnitus

Milloy, Fournier, Benoit, Noreña and Koravand (2017) recently performed a systematic review over articles that studied the relationship between ABR results and tinnitus, out of which only five measured wave I amplitudes. Two articles found significantly lower wave I amplitudes in subjects with tinnitus (Gu, Herrmann, Levine & Melcher, 2012; Schaette & McAlpine, 2011) two articles found no difference between groups (Attias, Urbach, Gold & Shemesh, 1993; Attias et al., 1996), and one article found a tendency of higher wave I amplitude in subjects with tinnitus (Gilles et al., 2016). Several of the reviewed articles also studied latencies of waves I, III and V. Three articles found significantly larger latencies for wave I (Gu et al., 2012; Kehrle et al., 2008; Kehrle, Sampaio, Granjeiro, De Oliveira & Oliveira, 2016), with another two articles reporting similar tendencies (Ikner & Hassen, 1990; Singh, Munjal & Panda, 2011). Three articles found significantly larger latencies for wave III (Gu et al., 2012; Kehrle et al., 2008; 2016), with one other article reporting a similar tendency (Ikner & Hassen, 1990). Four articles found significantly larger latencies for wave V (Cartocci et al., 2012; Ikner & Hassen, 1990; Kehrle et al., 2008; 2016).

Many articles were excluded from analysis due to poor sample size or too varying methodology, which makes it difficult to draw any conclusions from the results at this time. There is very little data on how individual wave latencies are best interpreted but Kehrle et al. (2008) speculate that a prolongation of wave I reflects decreased synaptic processing, and that a prolongation of higher waves reflect decreased neural conduction speeds.

To summarise, human studies suggest that 1) there might be a correlation between reduced ABR wave I amplitude and tinnitus, and that 2) there might be a correlation between higher ABR waves I, III and V latencies and tinnitus.

Purpose

The purpose of the present study was to compare subjective and objective audiometric data between human subjects with and without tinnitus. These data were expected to show signs of cochlear synaptopathy in individuals with tinnitus but otherwise normal hearing (up to 16 kHz), more specifically lower ABR wave I amplitude and worse performance in a speech in noise test. The present study has a very large sample size, which should allow for strict inclusion and

exclusion criteria, as well as fully matched controls, making the results well suited for generalisation. If tinnitus can be shown to have a clearly demarcated physiological cause then this should make treatment of tinnitus a more tangible goal, either through means of surgical intervention or aimed medication.

Four hypotheses are postulated: 1) individuals with tinnitus show lower ABR wave I amplitude values compared to controls, 2) controlling for normal hearing up to 16 kHz, ABR wave I amplitude differences increase, 3) individuals with and without tinnitus show comparable amplitude and latency values for ABR waves II through V, and 4) speech in noise performance is significantly worse in the tinnitus group compared to controls.

Method

Participants

All participants were recruited through the Swedish Tinnitus Outreach Project at the Karolinska Institute in Stockholm, Sweden, and included as either cases (permanent or occasional tinnitus, n = 292) or controls (with no tinnitus, n = 160) based on self-reported tinnitus at the time of testing. Out of the 452 recruited participants, 423 were tested at Karolinska Institute in Stockholm, Sweden, between Aug 22 2016 and July 12 2017, and 29 were tested at Lund University in Lund, Sweden, between Jan 15 2018 and February 28 2018. The same testing procedure and materials were used at both sites. 316 participants were excluded from analysis due to hearing loss (defined as > 20 dB HL at any frequency up to 8 kHz) or missing ABR data. Another 5 participants were excluded for having unilateral tinnitus, leaving a total of 23 participants with permanent tinnitus, 38 with occasional tinnitus, and 70 controls with no tinnitus. Participants with hearing loss or unilateral tinnitus were excluded to reduce the number of influencing factors besides tinnitus. Both conductive and sensorineural hearing loss can affect ABR amplitudes and latencies, and unilateral tinnitus is often considered a case of special diagnostic interest, as it can be caused by tumours on the acoustic nerve. The exclusion process is presented in a flowchart in **Figure 1**. All participants had normal outer and middle ear status, confirmed by otoscopy and immitance tympanometry.

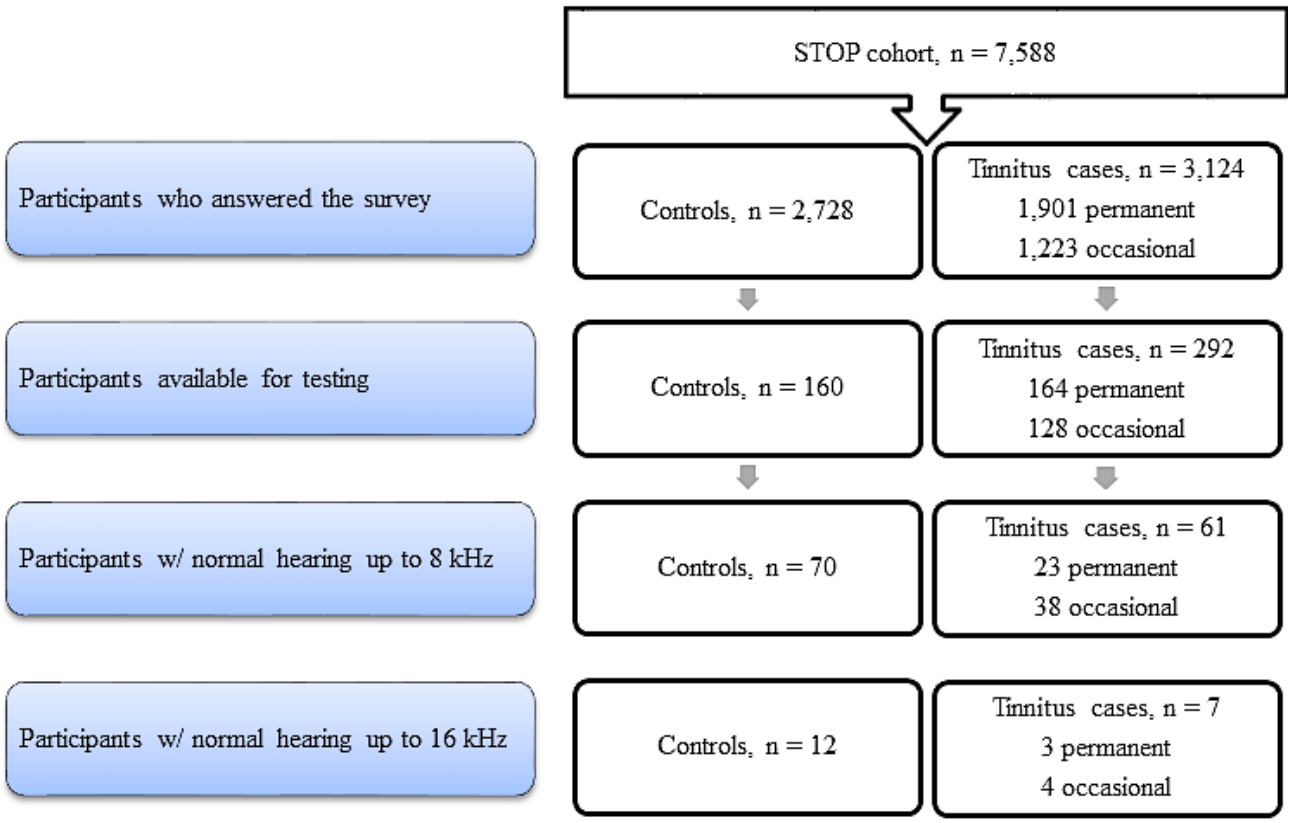

Figure 1. Flowchart of the participant exclusion process.

Testing Procedure and Materials

The project has been approved by the local ethics committee "Regionala etikprövningsnämnden" in Stockholm (2015/2129-31/1). The database project and server are coordinated and located at the department of Physiology and Pharmacology of the Karolinska Institute, Sweden. Testing procedure for both groups consisted of immitance tympanometry, DPOAE, pure tone audiometry (PTA) using the fixed frequency Békésy method, speech in noise testing, measurement of loudness discomfort levels (LDL), and electrocochleography (ECoG). ECoG was used instead of conventional ABR only because it is slightly easier to achieve low resistance in reference electrodes when placed directly in the ear canal; measurement and results are fully comparable to ABR. Participants in the study group also underwent a tinnitus matching step, where the frequency, loudness, minimum masking level and residual inhibition potential of the participants' tinnitus were measured. All these tests were performed to gather data for the STOP project, but only PTA, speech in noise and ECoG results were analysed in the current study.

Immitance audiometry was carried out with the Madsen OtoFlex 100 and resulted in either a pass (A type response) or a fail. DPOAE was carried out with the Capella 2 Madsen/Otometrics, using Etymotic research 10-D probes. PTA, speech in noise testing, LDL testing, and tinnitus matching was carried out with the Madsen Astera 2 audiometer with Sennheiser HDA200 high-frequency headphones and Radioear B71W bone conduction headphones. PTA was measured using the fixed frequency Békésy method over frequencies 125 Hz to 16 kHz. Audiometric configurations were considered normal if no frequency response was higher than 20 dB HL. Speech in noise testing used a speech weighted, continuous noise overlaid with consonant-vocal-consonant (CVC) words (50 per ear) at +6 dB SNR. Responses were scored both for correctly identified whole words, the word recognition score (WS), and correctly identified partial words or phonemes, the phoneme recognition score (PS). LDL testing was carried out by initially presenting pure tones at a

comfortable level (60 dB HL, unless hyperacusis was suspected, in which case the intensity was lowered as considered appropriate) and raising the intensity by 5 dB at a time until the participant asked to stop or the maximum intensity level for that frequency was reached. ECoG responses were measured using an Otometrics ICS Chartr EP200 with the included insert headphones and cables. Two disposable tiptrodes were placed on the high and low forehead, with insert headphones functioning as reference. Stimulus was 100 μs at 90 dB nHL, with synchronous masking at 50 dB nHL, at a rate of 9.1 clicks/s. Stimulus was filtered with high pass at 100 Hz and low pass at 1,500 Hz, and presented twice for each ear with 2,000 repetitions and alternating polarity. Wave amplitudes were measured from peak to following trough.

Data Analysis

ABR measurements, including wave I, wave II, wave III, wave IV, and wave V amplitudes and absolute latencies, were averaged and compared between cases with constant tinnitus, cases with occasional tinnitus and controls without tinnitus. A test of normality showed that half of the tested variables varied significantly from normality (Shapiro-Wilk, $p < .05$). For this reason statistical analysis was carried out with the non-parametric independent sample Kruskal-Wallis test with Steel-Dwass post hoc. To see if high-frequency hearing thresholds had an impact on ABR results, the same groups were further filtered for normal hearing (i.e. ≤ 20 dB HL) up to 16 kHz and compared again across ABR wave amplitudes and latencies (see **Figure 1** for sample size). Excluding all participants without normal high-frequency hearing, only 1 of 10 tested variables varied significantly from normality, so for these data statistical analysis was carried out with a parametric ANOVA test with Tukey post hoc.

All groups (filtered by normal hearing up to 8 kHz and 16 kHz) were also compared with regards to speech in noise performance. WS did not vary significantly from normality, and was therefore analysed with a parametric ANOVA test. PS, however, did vary significantly from normality, and was therefore analysed with the non-parametric independent sample Kruskal-Wallis test with Steel-Dwass post hoc.

Results

Auditory Brainstem Response

Analysis of ABR results for participants with normal hearing up to 8 kHz showed significant differences between groups for wave II latency, $H(2) = 7.459$, $p = .024$; wave III latency, $H(2) = 10.833$, $p = .004$; and wave V latency, $H(2) = 12.146$, $p = .002$. The results of the post hoc analysis are presented in **Table 1**. Post hoc of amplitude values revealed that the permanent tinnitus group had lower wave V amplitude compared to controls ($p = .047$). Additionally, a trend in lower wave I amplitude was observed in the occasional tinnitus group compared to controls, albeit not significant ($p = .097$). Post hoc of latency values revealed that the permanent tinnitus group had greater wave II ($p = .040$), wave III ($p = .002$), wave IV ($p = .040$), and wave V ($p = .001$) latency values compared to controls, and greater wave III ($p = .034$) and wave V ($p = .022$) values compared to the occasional tinnitus group.

Analysis of ABR results for participants with normal hearing up to 16 kHz showed significant differences between groups for wave II latency, $F(2, 18) = 3.661$, $p = .049$, and wave III latency, $F(2, 18) = 5.578$, $p = .015$. The results of the post hoc analysis are presented in **Table 2**. Post hoc of amplitude values didn't reveal any difference between cases and controls. Post hoc of latency values, however, showed that the occasional tinnitus group had lower wave II latency compared to controls ($p = .039$), and that the permanent tinnitus group had greater wave III latency compared to both the occasional tinnitus group ($p = .020$) and controls ($p = .019$).

Table 1. ABR wave differences between groups, normal hearing up to 8 kHz.

Amplitude			Mean Diff.	Std. Error	*p*	Latency			Mean Diff.	Std. Error	*p*
Wave I	Occ	Con	-13.013	6.311	.098	Wave I	Occ	Con	2.964	6.310	.886
	Per	Con	-.809	6.486	.992		Per	Con	11.755	6.485	.165
	Per	Occ	6.142	4.689	.390		Per	Occ	5.060	4.689	.527
Wave II	Occ	Con	-.122	6.309	1.000	Wave II	Occ	Con	.345	6.311	.998
	Per	Con	-4.101	6.485	.802		Per	Con	15.770	6.487	.040*
	Per	Occ	-3.280	4.686	.764		Per	Occ	10.434	4.689	.067
Wave III	Occ	Con	-9.626	6.307	.279	Wave III	Occ	Con	.372	6.307	.998
	Per	Con	-2.397	6.486	.928		Per	Con	21.921	6.486	.002*
	Per	Occ	4.618	4.636	.579		Per	Occ	11.563	4.637	.034*
Wave IV	Occ	Con	5.130	5.692	.640	Wave IV	Occ	Con	-.025	5.701	1.000
	Per	Con	3.918	5.805	.778		Per	Con	14.117	5.814	.040*
	Per	Occ	.123	4.260	1.000		Per	Occ	7.609	4.263	.175
Wave V	Occ	Con	-4.121	6.310	.791	Wave V	Occ	Con	5.705	6.311	.638
	Per	Con	-15.336	6.486	.047*		Per	Con	22.961	6.487	.001*
	Per	Occ	-7.154	4.690	.279		Per	Occ	12.458	4.690	.022*

Note: Steel-Dwass post hoc results based on a Kruskal-Wallis test of ABR wave amplitudes and latencies for “permanent”, “occasional” and “control” groups. Asterisks (*) mark significant differences.

Table 2. ABR wave differences between groups, normal hearing up to 16 kHz.

Amplitude			Mean Diff.	Std. Error	*p*	Latency			Mean Diff.	Std. Error	*p*
Wave I	Occ	Con	-.076	.079	.612	Wave I	Occ	Con	-.052	.039	.396
	Per	Con	-.013	.088	.989		Per	Con	.023	.043	.859
	Per	Occ	.063	.105	.820		Per	Occ	.075	.051	.337
Wave II	Occ	Con	.007	.035	.975	Wave II	Occ	Con	-.130	.048	.039*
	Per	Con	-.022	.039	.846		Per	Con	-.031	.054	.832
	Per	Occ	-.029	.047	.807		Per	Occ	.099	.064	.293
Wave III	Occ	Con	-.006	.061	.995	Wave III	Occ	Con	-.040	.066	.821
	Per	Con	-.063	.068	.631		Per	Con	.227	.074	.019*
	Per	Occ	-.057	.080	.761		Per	Occ	.267	.088	.020*
Wave IV	Occ	Con	-.044	.042	.566	Wave IV	Occ	Con	-.013	.141	.995
	Per	Con	.003	.042	.998		Per	Con	.243	.141	.235
	Per	Occ	.047	.055	.682		Per	Occ	.257	.183	.371
Wave V	Occ	Con	.014	.060	.970	Wave V	Occ	Con	-.066	.101	.792
	Per	Con	-.096	.067	.343		Per	Con	.170	.113	.315
	Per	Occ	-.110	.079	.365		Per	Occ	.236	.133	.212

Note: Tukey post hoc results based on an ANOVA test of ABR wave amplitudes and latencies for “permanent”, “occasional” and “control” groups. Asterisks (*) mark significant differences.

Speech in Noise

Speech in noise performance was scored for both word recognition, WS, and phoneme recognition, PS. Both these scores for participants with normal hearing up to 8 kHz and 16 kHz are presented in **Table 3** and **Table 4**, respectively. Analysis of WS didn't reveal any difference between cases and controls, either for participants with normal hearing up to 8 kHz, $F(2, 128) = 1.827$, $p = .165$ or up to 16 kHz, $F(2, 16) = .351$, $p = .709$. Similarly, no difference was found when analysing PS between cases and controls, with normal hearing up to 8 kHz, $H(2) = 4.123$, $p = .127$, or up to 16 kHz, $H(2) = .693$, $p = .707$.

Table 3. Word recognition score differences, filtered by normal hearing thresholds.

		Sum of Squares	*df*	Mean Square	*F*	*p*
≤ 20 dB HL, 8 kHz	Between Groups	83.528	2	41.764	1.827	.165
	Within Groups	2925.312	128	22.854		
	Total	3008.840	130			
≤ 20 dB HL, 16 kHz	Between Groups	20.048	2	10.024	.351	.709
	Within Groups	456.583	16	28.536		
	Total	476.632	18			

Note: ANOVA test result of word recognition score differences between tinnitus and control groups.

Table 4. Phoneme recognition score differences, filtered by normal hearing thresholds.

	≤ 20 dB HL, 8 kHz	≤ 20 dB HL, 16 kHz
Kruskal-Wallis *H*	4.123	.693
df	2	2
p	.127	.707

Note: Kruskal-Wallis test results of phoneme recognition score differences between tinnitus and control groups.

Discussion

Four hypotheses were postulated: 1) individuals with tinnitus show lower wave I amplitude values compared to controls, 2) controlling for normal hearing up to 16 kHz, wave I amplitude differences increase, 3) individuals with and without tinnitus show comparable amplitude and latency values for waves II through V, and 4) speech in noise performance is significantly worse in the tinnitus group compared to controls. With the results found in this study, all four hypotheses are rejected. Most notably, these results disagree with previous studies that found a correlation between tinnitus and ABR amplitudes (Gu et al., 2012; Schaette & McAlpine, 2011). However, a trend for lower wave I amplitude was observed in the occasional tinnitus group when filtered for normal hearing up to 8 kHz. Although this study did have a very large sample size, considering the clinical standard of determining normal hearing up to 8 kHz, only a handful of individuals from each group had normal hearing up to 16 kHz. This was unfortunately not enough to demonstrate a potential advantage of controlling for normal hearing at higher frequencies. This trend for lower wave I amplitude should be further studied in a future study with a more carefully matched sample group, as it could either indicate a difference between tinnitus and non-tinnitus groups or be the result of high-frequency hearing loss.

The present study lends support to several previous studies that have found greater wave latencies in individuals with tinnitus (Gu et al., 2012; Kehrle et al., 2008; 2016; Ikner & Hassen,

1990; Singh, Munjal & Panda, 2011; Cartocci et al., 2012). An increase in wave I latency might be a result of synaptopathy, however, waves III and V correspond with nuclei much farther up the auditory pathway. Exactly how these latencies should be interpreted remains to be seen, but the nuclei related to waves III and V are both responsible for sound identification and localisation – attributes that become increasingly difficult to pinpoint with poor speech in noise performance. It's possible that they represent different subtypes of tinnitus and thus require different forms of treatment. It's also possible that they exist as a logical extension of the prolonged wave I latency, and will be treated alongside it.

Additionally, the present study can show that wave latencies vary depending on tinnitus type – in this case permanent and occasional tinnitus. This suggests a physiological difference between permanent and occasional tinnitus, which, to the author's knowledge, has not previously been shown. What this suggests is that permanent and occasional tinnitus belong to different subtypes of tinnitus, and that these subtypes have different aetiology. Occasional tinnitus might, for example, be caused by stress, muscle contractions and similar temporary or situational triggers, and not permanent physiological damage to the auditory system. It could also be the first stage of permanent tinnitus in development. Exposing oneself to repeated TTS might push the cochlear structures bit by bit, making them more vulnerable to noise, until a threshold is reached and tinnitus becomes permanent.

The decision to focus on bilateral tinnitus was made to minimise the spread of aetiology, thus increasing the likelihood that what was tested was in fact cochlear synaptopathy. Ongoing analyses of the STOP cohort suggest that a major contributing aetiological factor for bilateral tinnitus is noise exposure, whereas approximately 1 in 2 cases of one-sided tinnitus can't be traced back to any specific cause. Furthermore, one-sided tinnitus has been linked to e.g. tumours, which, it stands to reason, might or might not affect ABR measurements depending on its specific location along the auditory pathway. Finally, considering noise exposure and natural ageing being the leading causes of cochlear synaptopathy, it should be more likely to affect both ears equally.

Speech in noise performance was expected to correlate with ABR wave I amplitude. As no difference in wave amplitude was found between groups, save for a trend, it is not surprising to see that there was no difference in speech in noise performance either. In relation to tinnitus, speech in noise performance is not directly of interest, but as a lower ABR wave I amplitude has been found to correlate with both cochlear synaptopathy and speech in noise performance, it's important to confirm these findings to make sure we're measuring the same processes; finding one correlation without the other could indicate that there's an issue with the methods used.

Neurotrophin-3 has been shown to reduce hearing loss immediately following noise exposure in animal studies and appears to be a candidate for synapse regeneration. If this can be confirmed in future studies, this would mark the first step towards a reliable medical treatment for noise induced tinnitus. Some types of tinnitus have been found sensitive to other types of treatment, such as vascular decompression and bimodal stimulation, and if tinnitus can be clearly divided into different subtypes then it should also become easier to describe an appropriate and effective treatment, where available.

Methodological Limitations

For this study, ECoG was used instead of ABR, which has the added benefit of using insert headphones as reference electrodes, making it easier to achieve low impedance values. However, as long as similarly low impedance values are achieved with ABR, the results are fully comparable. As was shown by Furman et al. (2013), cochlear synaptopathy affects high-threshold fibres exclusively, meaning they require a high-intensity stimulus to activate. For this reason, a click stimulus of 90 dB nHL was used. This stimulus level proved too intense for several participants,

most notably those with tinnitus, who elected to skip the ECoG measurement completely. This reliance on high-intensity stimuli to study a group of people with a suspected predisposition to sound sensitivity is problematic. For the moment, ABR data seems to be the best tool for evaluating cochlear synaptopathy, however, so the only workaround is to recruit more participants and focus on those with little to no sound sensitivity.

Although data was collected by different people at different sites, all data was organised and analysed by only one person. Having one person responsible for all data increases the risk of unobserved mistakes and data loss, either through technical issues or human error. ABR measurements are especially sensitive to subjective interpretation and should preferably be analysed and discussed by two audiologists. As only one person handled all analysis and data organisation, it's possible that this lack of oversight might have contributed to type I and type II errors. Although having only one person responsible for analysis and data organisation should always be avoided, potential errors in this study specifically could have been minimised by employing a test-retest analysis to check for consistency.

The present study did not exclude participants based on how they experience their tinnitus, i.e. as tonal or buzzing, as barely noticeable or severely intrusive, or how long it’s been since tinnitus onset. It would be interesting to expand on the present study, with a larger sample size and even stricter inclusion criteria, to properly demarcate possible synaptopathy induced tinnitus from other forms of tinnitus. It might also be beneficial to do the opposite and have very wide inclusion criteria, but carefully match and compare different factors in search of both narrow and broad patterns. As tinnitus doesn’t always make itself known overnight, however, it can be difficult to pinpoint cases with only noise-induced tinnitus.

Conclusion and Clinical Relevance

This study followed several previous studies in comparing ABR data between normal hearing individuals with tinnitus and individuals without. Where this study stands out is the large sample size of participants with normal hearing up to 8 kHz. Results obtained confirm previous smaller-scale studies on ABR wave latencies, but fail to confirm previous studies on ABR wave amplitude and cochlear synaptopathy. Additionally, the results seem to differentiate between permanent and occasional tinnitus, suggesting different subtypes and aetiologies.

In order to treat tinnitus effectively, it is first and foremost necessary to demarcate one type of tinnitus, with one specific cause, from another, with a wholly different cause. In this and many previous studies, the focus has been on permanent, noise-induced ringing or buzzing tinnitus and ears with damaged ribbon synapses but intact inner hair cells. If this correlation can be shown to constitute one type of tinnitus with a specific cause then it would open the door for e.g. neurotrophin-3 based treatment. It would also lay a foundation for identifying other types of tinnitus and their causes by the process of elimination.

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