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Research Article

Using Gap-induced Inhibition of the Post-auricular Muscle Response as an Objective Measure of Tinnitus in Humans

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Abstract

A widely used method for detecting tinnitus in rodents is the gap pre-pulse inhibition of the acoustic startle (GPIAS). One variant uses the Preyer reflex to assess the startle response and a component of this can be measured as a small muscle potential generated by the post-auricular muscle reflex (PAMR). The question was whether the GPIAS method could also be used to identify tinnitus in humans using the PAMR response. We recruited 19 participants with chronic tinnitus and 18 age-matched controls, but 12 tinnitus participants were unable to contribute data to the final result due to hyperacusis or lack of a PAMR. A majority of those tinnitus participants with a detectable PAMR showed some evidence of GPIAS (71%, 5/7). In the control group, most showed a PAMR response (67%, 12/18) and most of these demonstrated GPIAS (67%, 8/12). Our stimulus parameters were not completely optimal for showing a PAMR response so, with further refinement it may be possible to use the PAMR response and GPIAS as an objective method for demonstrating tinnitus in humans.

Keywords: Gap Induced Pre-pulse Inhibition; Preyer Reflex; Acoustic Startle Reflex; Eyeblink Reflex; Humans; Personalised Medicine

Abbreviations

BBN: Broadband Noise; GPIAS: Gap Pre-pulse Inhibition of the Acoustic Startle; PAMR: Post-auricular Muscle Reflex; PPI: Pre-pulse Inhibition; TCHQ: Tinnitus Case History Questionnaire; TFI: Tinnitus Functional Index

Introduction

The tinnitus community has long called for an objective measure of tinnitus that can be assessed in both animals and humans [1], to assist in drug development and in claims for medical insurance. At present, there is no established objective measure of tinnitus in humans. Objective behavioural tests have been developed for animals, but they are generally not suitable for use in humans. An objective tinnitus measure, suitable for both humans and animals, could reduce the risk that a novel drug compound, which produces a significant change in animals, fails to have a demonstrable benefit in humans [2]. Establishing one would also allow confirmation of a tinnitus diagnosis; for example in situations where army veterans

need to establish the basis of their claims for tinnitus-related benefits [3], and could help to further the goals of personalised medicine for tinnitus patients.

Attempts have been made to identify biomarkers for tinnitus in humans based on recording spontaneous oscillations in cortical electroencephalographic (EEG) activity [4,5], but this appears to be unreliable [6] and there are some disparities between animals and humans [7]. Despite this, adapting methods successfully used in the animal model for use in humans appears to be a promising approach. One method is based on using the tinnitus to mask a brief gap in a background noise that has similar spectral properties to the tinnitus. Thus, attempts have been made in humans [8,9] to use GPIAS to identify tinnitus based on the method developed by Turner., *et al*. [10] for rats. For humans, the acoustic startle was measured using the eyeblink reflex, while in rodents the whole body startle was measured using a platform accelerometer [11]. Another alternative is to measure reductions in the cortical evoked potential produced by a gap in background noise before a startle pulse. This has been used in guinea pigs [12] and humans [13-15]. However, the usefulness of these techniques needs to be confirmed and the electrophysiological recordings are complicated procedures involving chronically implanted electrodes in guinea pigs and electroencephalographic recording in humans.

In small rodents the whole body startle reflex is relatively easy to measure [10], but in larger, less active mammals, such as the guinea pig, it habituates very rapidly and the pinna (Preyer) reflex is a more suitable way to measure the acoustic startle and detect the presence of tinnitus [16,17]. We have recently demonstrated a technique for measuring GPIAS in humans that involves measuring the post-auricular muscle reflex (PAMR) via an electrode placed on the skin immediately behind the ear [18]. Many humans cannot move their pinna, but the vestigial, post-auricular muscle potential acts as a surrogate for the pinna reflex that can be measured in animals with mobile pinnae. Using this method we have shown GPIAS in a control population of young adults who did not have any significant hearing loss [18]. The next step is to confirm whether a typical group of people with chronic tinnitus who commonly have age-related hearing loss would still show a reliable PAMR response. Here, we show that a PAMR response can be detected in some people with tinnitus and that this can be used to demonstrate GPIAS. We compared the size of the PAMR responses in such a group with those of an age-matched control group without tinnitus. We also used questionnaires, measured pure tone audiograms and performed gap detection tests to characterise the tinnitus and hearing of the participants. This allowed us to assess the potential effect of factors such as age, duration of tinnitus, hearing loss and gap detection ability in reducing performance in the GPIAS test.

This study evaluated two interdependent aims. The first was to test the possibility of using the PAMR response to measure GPIAS in a typical group of people with chronic tinnitus. The second was to assess whether the PAMR response can be used to detect evidence of tinnitus reducing the effectiveness of a gap in a background noise to inhibit a subsequent startle response. To do this, we measured GPIAS in participants using narrowband background noise centred on a frequency matched to their dominant tinnitus pitch and compared it to the responses obtained with a gap in noise centred at 1 kHz. We postulated that the gaps in the 1 kHz noise would be more effective at reducing the startle response than the gaps in noise centred at the tinnitus frequency since they are far away from the tinnitus pitch and correspond to normal hearing.

Materials and Methods

Participants

All participants were over 18 years old, fluent in English and had no severe health problems. Those with tinnitus experienced symptoms for longer than 6 months. Prior to testing, informed written consent was obtained from each participant. The studies were approved by the University of Nottingham, School of Medicine Ethics Committee (REF E14062016). Control participants not currently experiencing tinnitus (N = 30) were recruited in two groups through campus poster advertisements, social media and word-of-mouth. Tinnitus participants were recruited from the National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre participant database, through campus posters and via the British Society of Audiology Facebook page. Participants were paid a small inconvenience allowance and when necessary also travel expenses. A total of 19 tinnitus participants started the study. Four participants withdrew midway through the study after reporting hyperacusis and explaining that they were not comfortable listening to the experimental stimuli used to elicit the PAMR response. One withdrew completely during the audiogram testing and three withdrew after starting the PAMR testing (electrophysiological recording), but agreed to complete the gap recognition test. A flow diagram of the study is shown in figure 1.

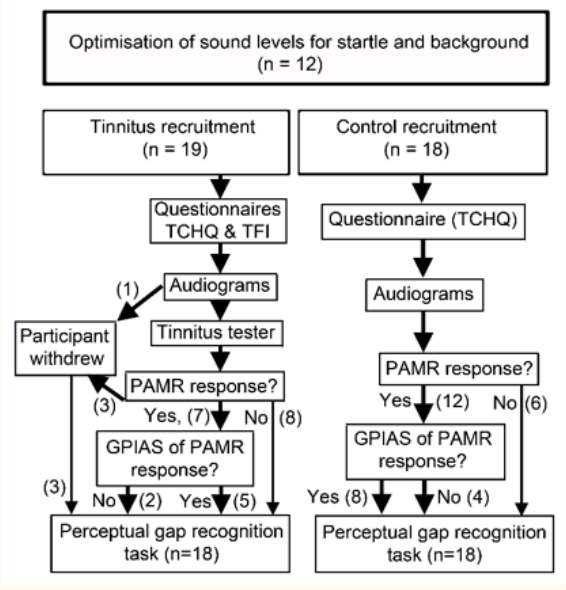

Figure 1: Flowchart of testing procedures. An initial control group were recruited for optimising the stimuli and then the main tinnitus group and a new set of age-matched controls were recruited for the main study. The tinnitus was characterised by using the tinnitus case history questionnaire (TCHQ), the tinnitus functional index (TFI) and the tinnitus tester programme. Audiograms were measured for both ears before placing electrodes and measuring PAMR responses and testing for GPIAS and then gap perception.

Tinnitus and hearing assessment

Participants completed a Tinnitus Case History Questionnaire [19] and Tinnitus Functional Index (TFI, [20]) to assess their tinnitus history and its severity. The TCHQ was used for collating descriptive information on the participant's tinnitus (onset, location, loudness, and characteristics), additional health problems associated with tinnitus (hyperacusis, hearing impairment, and headaches) and familial incidence of tinnitus. The TFI is a self-report measure consisting of eight subscales and classifies tinnitus symptom severity as a score out of 100 with higher scores reflecting a greater impact on daily functioning. After an otoscopic examination, hearing was assessed from 0.125 - 12 kHz in each ear separately according to the British Society of Audiology (2014) procedure with a Diagnostic Audiometer (GSI 16) in a sound proof booth.

Tinnitus tester

The tinnitus tester programme [21,22] was used to psychoacoustically assess the properties of a participant's tinnitus. The computer automated procedure initially allowed the participant to become accustomed to the concepts of pitch and loudness. It then assessed the perceived location of tinnitus (left, right, bilateral), the type of perception (hissing, ringing or tonal) and its loudness and frequency. Participants were asked to select one of three sounds that best characterised their tinnitus. The comparison sound for ringing tinnitus was a band-passed noise whose bandwidth was ± 5% of the 5 kHz centre frequency, for tonal tinnitus it was a 5-kHz pure tone and for hissing tinnitus it was a band-passed noise at ± 15% of the 5-kHz centre frequency, each measured at a previously determined sound level that was comfortable for that participant. All of the participants characterised their tinnitus as containing frequencies over a range of about 1 octave when asked to identify a dominant frequency for their tinnitus in the tinnitus tester; hence an octave-wide narrowband noise background was used in all the PAMR testing of tinnitus participants. Participants rated the similarity of their tinnitus to pure tones of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 kHz on a scale from 0 to 100. The frequency corresponding to the dominant tinnitus pitch was taken as that with the highest similarity score after repeating the test three times. This frequency was used to synthesise an individual narrowband noise background for the PAMR testing of each participant.

Acoustic stimulation

All stimuli (startle and background sounds) were created using Matlab software (version r2014b, Mathworks, Natick, MA, USA). Stimulation was monaurally to the right ear using ER-1 inserts (https://www.etymotic.com/auditory-research/insert-earphones-for-research/er1.html) unless the tinnitus was ascribed solely to the left ear, in which case it was presented to the left ear. Signals to the earphones were from a Tucker Davis Technologies RP2.1 (Alachua, FL, USA) interface, which provided digital signal processing for the headphone amplifier (HB7). As the tinnitus, perceived by each participant, differed in terms of its centre frequency and estimated bandwidth, it was important to use a background sound condition that would match their tinnitus as closely as possible. In this study, the background noise comprised either broadband noise (BBN), narrowband noise centred at 1 kHz (1 octave wide) or a one-octave wide noise centred on a frequency corresponding to the participant's own dominant tinnitus pitch. All three backgrounds were tested on each participant. For the control group, a narrowband background noise centred on 6 kHz (1 octave wide) was used as an alternative to the tinnitus specific background used in the tinnitus group. This frequency was chosen as it was the highest frequency that was not impacted by severe hearing loss in the control group's audiograms. In the initial stimu-

lus optimisation group, we used a 1 kHz tone as the background instead of noise.

We used a 20-ms broadband noise burst with 0.1 ms rise time presented at 105 dB SPL as the startle stimulus and the background noise (or tone) was presented at 70 dB SPL. "No-gap" trials consisted of the startle stimulus presented in the presence of a continuous background sound, while for "Gap" trials a silent gap of 20 ms (0.1 ms rise/fall times) was introduced into the background sound with the gap ending 50 ms before the startle (Figure 2). The duration of the gap was decided on after some preliminary work to study gap duration and GPIAS [18]. The inter-trial-interval was randomly varied from 18-22 seconds in order to minimise PAMR habituation and avoid anticipation of the startle stimulus [23]. A total of 60 "Gap" and 60 "No-gap" trials were presented to each participant for each background noise condition.

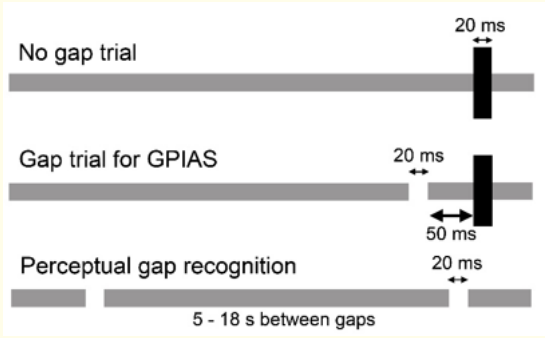

Figure 2: Acoustic stimuli. The stimulation protocol for No-gap and Gap trials compared to the gap recognition test. The grey band represents the background noise at 70 dB SPL that was either BBN, narrowband noise or a 1 kHz tone. The background sound was continuous except for the silent gaps and startle pulses (105 dB SPL) that were inserted into it. The same three background noise conditions used in the GPIAS test with the PAMR response were also used in the gap recognition task.

Participants were asked to listen to the trial using the BBN background condition first before their responses were recorded. This allowed the participant to ask any questions and to familiarise themselves with the task. For the perceptual gap recognition task, participants in each group were instructed to press a button each time they heard a silent gap and to refrain from pressing the button if they did not detect a gap. Responses were recorded as 'correct' if the button was pushed within 2s from the presentation of the gap.

PAMR recording procedures

Participants were seated in a sound and electrically shielded booth (IAC Acoustics, Winchester, UK). The PAMR responses were recorded using a BrainAmp DC system (BrainVision, Gilching, Germany) at a sampling rate of 2500 Hz (filters set at 0.1 - 250 Hz) with 10 mm cupped AgCl electrodes fitted to ensure that all impedances were below 10 kΩ. In most cases, the impedances were about 3 kΩ, but in some subjects, even after rubbing the skin with abrasive gel, the impedance was still 5 kΩ. The active PAMR electrode was placed behind the ipsilateral (usually right) ear, over the insertion of the muscle to the pinna. The reference electrode was on the tip of the ipsilateral pinna and the ground electrode was positioned at the centre of the forehead [18,24,25].

Participants were instructed to sit as motionless and quiet as possible with minimal head movement. The central position of the eyes was maintained by fixation on a black cross on the facing wall. To ensure comfort and maintain arousal level, subjects were permitted short breaks between recording sessions, which in total lasted approximately one hour including electrode placement and instruction in how to respond during different parts of the protocol.

Analysis

PAMR data were analysed using in-house custom software (Matlab version r2014b,) with the EEGLAB toolbox (SCCN, University of California, San Diego, USA). To exclude neurogenic potentials [26], the data were initially rectified and filtered using a bandpass filter (1 - 300 Hz [25]). Allowing for some variability in the latency of the responses we defined an analysis window from 10 - 30 ms after the stimulus pulse, for the PAMR [18,25]. Within this window, the peaks occurred with a variable latency and we therefore detected and aligned the peaks before averaging. To achieve this, the highest value of the predominant peak in each trial was set as the zero timepoint and the adjacent segment of trace (± 10 ms) was aligned, for all 60 trials, so that an adjusted waveform was obtained for each participant. This was then compared to the average aligned waveform of the greatest peak from a previous 2s of baseline trace, starting at 3s before the startle pulse. A response was only accepted as "real" if it exceeded 2.5 times the standard deviation of the mean of this baseline [18]. To compensate for different absolute response amplitudes responses were normalised before making inter-subject comparisons.

GPIAS of the PAMR was expressed as a percentage calculated using a ratio of the peak-to-baseline measure of the amplitudes for gap and no-gap trials, using the formula: 100-((mean PAMR amplitude gap trials/mean PAMR amplitude no-gap trials)*100). Mean PAMR amplitudes were non-normally distributed and so non-parametric Wilcoxon matched pairs signed rank tests were performed.

Results

Optimisation of sound levels for startle and background

We recruited a group of a 12 healthy controls (6 male, 6 female) from around the University campus. Their age ranged from 22 - 59 years (mean 35, S.D. 12.7). They did not report any hearing loss and none made use of a hearing aid. Electrodes were placed on the right ear and the stimulus presented to the right ear. In this optimisation study alone, the background sound was a 1 kHz tone at either 60 or 70 dB SPL. This was the same background as we had used in our previous study [18] and the participants reported it to be more pleasant than a noise background. Five different sound levels were used for the startle pulse using 5 dB steps between 85 and 105 dB SPL. At each combination of sound levels there were 20 gap trials and 20 no-gap trials. Two of the participants did not have detectable PAMR signals at any sound level combination and four only had a detectable PAMR response at the highest startle sound level (105 dB).

The mean PAMR amplitude for the remaining six participants when plotted against startle sound level is shown in figure 3A for a 70 dB SPL background tone. Despite the variability in the data, there is a clear trend showing increasing PAMR amplitude for increasing startle sound level. The effect of sound level on the degree of GPIAS as a function of sound level is shown in Figure 3B for background tone levels of 60 and 70 dB SPL. GPIAS is defined as a larger PAMR amplitude in the no-gap trials than the gap trials, measured as a positive percentage increase. When the gap trials produce a larger PAMR amplitude than the no-gap trials there is pre-pulse facilitation, which is plotted as a negative percentage value. With the background tone set at 60 dB SPL, the amount of GPIAS detected varied between trials and there were large shifts between facilitation at one pulse level and GPIAS at a different pulse level. However, with the background set at 70 dB SPL, there was much less variability in the amount of GPIAS and very little facilitation. At this background sound level the strongest GPIAS was obtained by the loudest startle pulse (105 dB SPL). Based on these results, it was decided to use a background of 70 dB SPL and a pulse of 105 dB SPL with future participants.

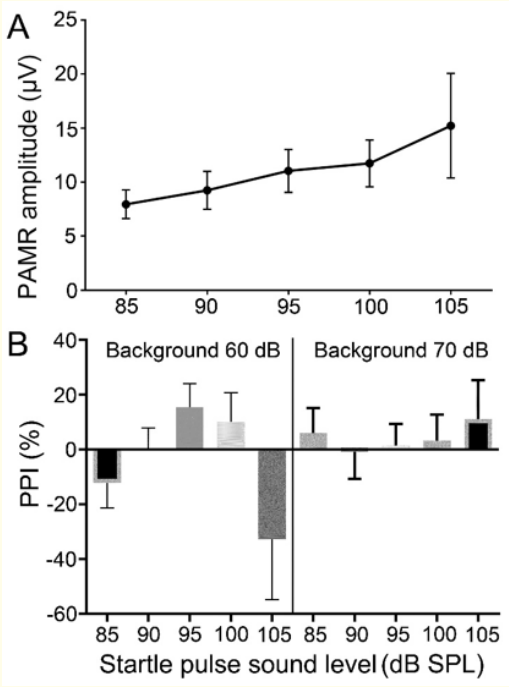

Figure 3: Effect of varying sound level on the PAMR and GPIAS responses. A With a background tone set at 70 dB SPL there is a monotonic increase in PAMR amplitude as the sound level of the startle pulse increases. B When the background tone is set at 60 dB SPL there is considerable variation in the amount of GPIAS or pre-pulse facilitation (negative numbers) when the sound level of the startle pulse is varied. With a 70 dB background there is more consistent GPIAS.

Description of tinnitus and control groups

The 19 people with tinnitus (4 female, 15 male) ranged in age from 23 - 74 years (mean 55, S.D. 16) and the duration of their symptoms ranged from 2 - 50 years (mean 19, S.D. 17). The severity of their tinnitus was assessed by the TFI and varied from 6 - 90 (mean 37, S.D. 22, maximum is 100) with a level of 90 indicating a debilitating impact on daily life. Participants reported a variety of different suspected aetiologies with 5 having an abrupt (idiopathic) onset, 14 a more gradual (idiopathic) onset, 5 a suspected somatosensory involvement and 1 pulsatile tinnitus of suspected vascular origin. The tinnitus was experienced bilaterally in 74% (14/19) of participants, but in six of these cases it was more annoying on one side (shown in bold) than the other. A smaller number (26%; 5/19) reported a unilateral tinnitus. Complete data was obtained from only 15 individuals.

The 18 control participants (10 male, 8 female) were recruited using purposive sampling and matched for age to the tinnitus group. None of them were currently experiencing tinnitus or had suffered from chronic tinnitus in the past. Their age ranged from 23 to 71 years (mean 50, S.D. 14).

The tinnitus tester programme was used to obtain an approximate value for the pitch and sound level of the tinnitus. Initially two trials were used to determine the equivalent sound level of the tinnitus and then a further three trials to determine the pitch. In both sets of trials, there was considerable variability between trials and the participant seldom felt there was an exact match between their experience of tinnitus and a presented sound. The tinnitus was often experienced as a broad-band hissing sound and in these cases the use of narrowband test sounds often meant that multiple pitches were assigned a high similarity rating. Despite this, we felt that just taking the frequency with the highest similarity rating was adequate to determine the centre frequency of the narrowband noise that we synthesised to mimic the tinnitus in the GPIAS test. Three of the participants had low-frequency tinnitus of up to 4 kHz while the remaining 15 had high-frequency tinnitus of 6 kHz or above (Table 1).

	Tinnitus group								Control group			
ID	**Age**	**Sex**	**Side**	**Quality**	**Duration (years)**	**TFI**	**Freq. (kHz)**	**% Gap Detect**	**ID**	**Age**	**Sex**	**% Gap Detect**
500	47	F	L, R	Hissing	24	42	8	100				
501	53	F	R	Hissing	2	40	6	95	601	47	M	90
502	65	M	L, **R**	Tonal	41	33	8	65	602	47	F	100
503	61	M	R	Tonal	50	36	12	95	603	71	F	95
504	72	M	L, **R**	Hissing	48	34	1	0	604	64	M	90
505	36	M	R	Tonal	6.5	28	4	90	605	25	M	95
506	50	M	**L**, R	Tonal	18	46	10	95	606	67	F	100
507	72	M	**L**, R	Hissing	?	12	6	100	607	61	M	80
508	63	M	L	Hissing	2	20	8	100	608	59	M	100
510	67	F	**L**, R	Tonal	40	64	6	90	609	55	F	90
511	47	F	L,R	Tonal	29	49	10	90	612	30	F	90
513	59	M	L,R	Ringing	20	44	7	90	613	50	F	100
514	70	M	L,R	Hissing	3	65	10	95	614	46	F	90
515	74	M	L,R	Tonal	30	7		?	615	59	M	100
516	57	M	L	Hissing	4	54	10	95	616	61	M	85
517	69	M	L,R	Tonal	5	6	2	85	617	46	F	100
518	28	M	L,R	Tonal	5	6	8	90	618	35	M	80
519	30	M	L,R	Tonal	4	90	12	?	619	55	M	95
520	23	M	L, **R**	Tonal	9	22	7	100	620	23	M	100

Table 1: Characteristics of tinnitus and control participants**.** Data was extracted from the TCHQ and TFI questionnaires, the tinnitus tester (frequency) and the perceptual gap detection test.

Comparison of audiograms in tinnitus and control groups

Many of the tinnitus participants had a significant degree of hearing loss and to quantify this we used pure tone audiometry for both ears. For most participants, the tinnitus was located either bilaterally or in the right ear and for consistency sounds were presented to the right ear except in the two participants where tinnitus was located on the left. The right audiograms are shown in figure 4 with panel A illustrating the audiograms for the seven participants who had a detectable PAMR response. The large square symbols on each trace indicate the pitch identified by the tinnitus tester. Two

of these participants had no significant hearing loss (506 and 518) while the other five had a range of mild to moderate hearing loss that peaked in each case at 8 kHz. In two of the cases (502 and 514) the threshold at the tinnitus frequency was slightly higher than the sound level of the background noise used in the GPIAS testing. Panel B shows the audiograms for the right ear in the tinnitus participants who did not show a PAMR response. Their audiograms were similar with two showing no hearing loss and nine showing mild to moderate hearing loss that peaked at 6 - 8 kHz. Panel C shows the audiograms for the right ear in the age-matched control group. There is a variety of degrees of hearing loss with the largest increases in threshold at 8 - 12 kHz. However, the mean hearing loss in the control group is less than that in the tinnitus group as shown in panel D. The mean threshold for the control group is at least 10 dB lower at frequencies above 3 kHz. The hearing loss was generally similar in each ear. Only one participant (ID, 503) had an asymmetric hearing loss where the difference between the pure-tone average for the two ears at 0.5 to 3 kHz was ≥ 15 dB.

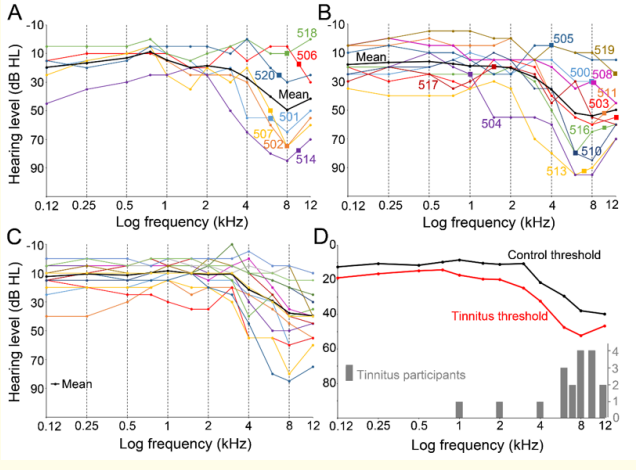

Figure 4: Audiograms for the right ear in tinnitus and control groups. A Audiograms for seven tinnitus participants who showed a detectable PAMR response. The large square symbols indicate the tinnitus frequency. B Audiograms for 11 tinnitus participants who did not show a detectable PAMR response. C Audiograms for 18 control participants. D Mean audiograms for the tinnitus and control groups along with a histogram showing the number of tinnitus participants who linked their dominant tinnitus pitch to a single-frequency tone.

Plotting the audiograms enabled the peak hearing loss to be related to the dominant tinnitus pitch identified by the tinnitus tester. The number of tinnitus participants with a tinnitus pitch matched to a particular frequency is shown by the histogram in the bottom right-hand corner of figure 4D. This illustrates that over the group as a whole, the tinnitus pitch was mainly located at the frequencies with the greatest threshold elevation. However, when the tinnitus pitch was compared to the hearing threshold for individuals as shown in figure 4A,B, only two individuals had a tinnitus pitch that corresponded to their highest hearing threshold (ID 502 and 519). In most people, the tinnitus pitch was slightly offset from the frequency of the peak hearing loss (the tinnitus pitch was below for nine, above for seven). This is consistent with our findings from a much larger cohort (n = 129) of tinnitus participants [27].

Confirmation that participants could hear the gaps in background noise

We wanted to confirm that all our participants were able to detect the brief (20 ms) gaps in the background noise (70 dB SPL). Perceptual gap detection ability was assessed by presenting 20 gaps in narrowband background noise centred at either the tinnitus frequency or 6 kHz (control subjects) and the values are shown in Table 1. Most participants did not have much difficulty in detecting the gaps. The main exception was one member of the tinnitus group (ID 504) who was unable to detect any of the gaps. The rest of the tinnitus group showed almost no impairment in their gap detection ability compared to the control group. In the tinnitus group (including ID 504), the mean % correct value was 87 (S.D. 24; range 0 - 100) and the corresponding values in the control group were 93 (S.D. 7; range 80 - 100). There was no significant difference in the means (Student's T-test p = 0.14).

PAMR response in tinnitus and control groups

The posterior auricular muscle is small compared to some of the surrounding muscles and the signal it generated was often difficult to detect even when using the method for aligning the peaks [18]. An example of the aligned peaks is shown in figure 5.

The measure used to indicate the size of the PAMR was the difference between the PAMR amplitude (mean peak of the aligned PAMR response) and the largest mean peak of the baseline, measured in μV. This was evaluated using data acquired during the GPIAS testing where startle pulses were superimposed on one of three types of background noise. The largest value was taken from these three background conditions and is shown in table 2. The values in bold indicate the responses that were significantly above the baseline electrical activity. There were significant PAMR responses in 7/15 of the tinnitus participants tested and 12/18 in the control group. The mean PAMR value for the tinnitus group was 1.8 (sd

1.2) and the range from 0 - 6.91. The mean PAMR value for the control group was 2.9 (sd 5.4) and the range from 0 - 20.95. Thus the PAMR values were generally smaller in the tinnitus group, but not significantly different (Student's t-test, p = 0.13).

The participants recruited in the tinnitus and control groups were generally older and had more hearing loss than the young healthy students usually recruited in previous studies. Thus we wanted to determine if age or hearing loss were factors in the relatively small number of participants who showed a detectable PAMR response (58%; 19/33) in the combined groups. We also wanted to check if chronic tinnitus itself might be a factor in reducing the number of participants with a significant PAMR response. First, we plotted the PAMR amplitude separately against the age of the participants, the mean bilateral hearing loss and the duration of chronic tinnitus. In each case, the regression line was calculated as shown in figure 6. There was no significant correlation between the PAMR responses and increased age (Figure 6A), increased hearing loss (Figure 6B) or increased duration of tinnitus (Figure 6C). However in each case, the trend line did have a negative slope. This suggests that there was an effect, but it was masked by the large variability in the amplitude of the PAMR response.

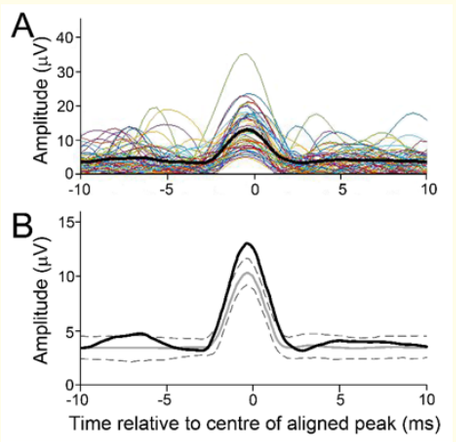

Figure 5: Comparison of the mean aligned PAMR response with the biggest mean background peak. A Mean waveform (thick black line) of 60 aligned PAMR responses to a startle pulse in participant 506. B The biggest mean, peak waveform for the baseline, taken from outside the acquisition window, is represented in solid grey with the grey dotted lines showing values at +/- 2.5 times the standard deviation of the baseline, plus the mean of the baseline.

		Tinnitus GPIAS			Control GPIAS				
ID	**PAMR (μV)**	**BBN**	**NB1 (control)**	**Tinnitus**	**ID**	**PAMR (μV)**	**BBN**	**NB1**	**NB6**
500	0.26				601	**4.9**	5.7	-	7.6
501	**1.4**	-	-3	-	602	**6.5**	**12.1**	**15.7**	-0.46
502	**2.4**	-	3.5	2	603	**0.9**	-	**10.8**	-
503	1.4				604	0			
504	0.13				605	0			
505	0				606	0			
506	**2.6**	**8.9**	1.8	1.7	607	**3.9**	-6.4	-	-
507	**6.9**	**10.9**	**10.5**	-8.3	608	0			
508	0.9				609	0			
510					612	0			
511	2.3				613	**1.3**	-	**11.7**	-
513					614	**3.9**	**8.8**	-	12.3
514	**3.2**	5.3	**22.3**	**12.9**	615	**0.9**	-	**15**	-
516	0.9				616	**0.8**	-	-	**7.4**
517	0				617	**20.9**	-12.8	3.8	7
518	**3**	-	-	**10.6**	618	**15.3**	-17.6	-17.2	-0.4
519					619	**6.1**	-5.9	-9.7	0.7
520	**3.8**	**19.2**	-	**13.3**	620	**3.3**	**32.7**	**20.9**	**19.3**

Table 2: PAMR and GPIAS results for tinnitus and control participants**.** The bold numbers for the PAMR values indicate those which are above the threshold for detection. The GPIAS values are in three columns for each group: the first with a broadband noise background (BBN), the second with a narrow band noise background centred at 1 kHz (NB1) and the third with a narrow band noise background centred either on the tinnitus frequency or 6 kHz (NB6). The bold numbers indicate where there was significant inhibition of the PAMR response (percentage decrease) and the negative numbers indicate pre-pulse facilitation. The blank boxes mean no measurement was made while the dash indicates a measurement was made but there was no detectable signal.

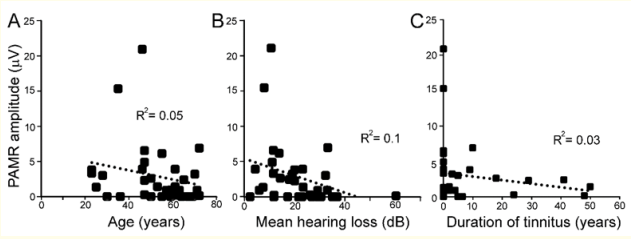

Figure 6: Relationship between PAMR amplitude and age, bilateral hearing loss or duration of tinnitus. The amplitude of the PAMR response from participants in the combined tinnitus and control groups was plotted against age (A), mean hearing loss (B) and duration of tinnitus (C). The regression lines indicated that there was a weak trend for the PAMR amplitude to decrease with each of these factors.

Next to confirm whether age, hearing loss and the presence of tinnitus affected the size of the PAMR response, the results were fitted to a general linear model using the PAMR amplitude as the dependent variable, with age (years) and hearing loss (mean PTA for both ears) as covariates, and tinnitus (yes or no) as a fixed factor. A full analysis, using the complete model with all interactions and main effects, yielded no significant effects. The overall interactions between tinnitus, age and audiogram with PAMR amplitude had a mean square of 5.765 ($p = 0.601$); the interaction between tinnitus and age with PAMR had a mean square of 1.741 and $p = 0.773$; the interaction between tinnitus and audiogram with PAMR gave a mean square of 1.11 ($p = 0.818$); while the interaction between age, audiogram and PAMR gave a mean square of 16.857 ($p = 0.374$). Subsequent analyses using a stepwise regression, reducing the model from three-way to two-way to no interactions (i.e. main effects only) still yielded no significant results. The main effect of age gave a mean square of 11.068 ($p = 0.47$); the main effect of audiogram gave a mean square of 20.558 ($p = 0.574$); while the main effect for tinnitus gave a mean square of 0.545 ($p = 0.872$). The results suggest that tinnitus, age and hearing loss do not have a major effect on the PAMR response.

Gap induced inhibition of the PAMR response

A main aim of the current study was to determine whether or not GPIAS was decreased when the background noise was centred on the tinnitus frequency compared to a background centred at 1 kHz. A corollary of any decrease of this sort in the tinnitus group should be that the GPIAS overall would be less effective in the tinnitus group than in the control group. GPIAS was calculated in the two groups from participants whom elicited a significant PAMR response (7 tinnitus; 12 control). The mean amplitude for the aligned peaks obtained from the gap and no-gap trials were measured and the percentage decrease (or increase) calculated. An example for the reduction in the PAMR response produced by a gap in the broadband noise background of participant ID 520 is shown in figure 7. Values for the amount of GPIAS in the tinnitus and control groups is shown in table 2. Significant values for GPIAS are shown as bold numbers while the negative numbers suggest gap induced facilitation. Only five (5/7) of the tinnitus group and eight (8/12) of the control group showed significant GPIAS with any of the backgrounds. Only one participant showed significant GPIAS at all background frequencies (control group, 620). Among the tinnitus participants there was no clear pattern indicating less GPIAS at the tinnitus frequency than for backgrounds centred on other frequencies. A one-way ANOVA was performed with GPIAS as the dependent variable and background condition as the independent variable. There was no significant effect of the background conditions in the tinnitus group ($F_{(2,12)} = 0.533$, $p = 0.6$).

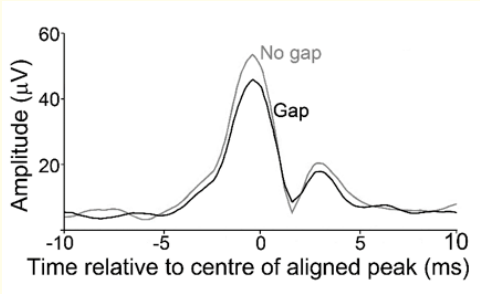

Figure 7: Change in PAMR amplitude resulting from GPIAS. The amplitude of the PAMR response for participant 520 was reduced by 19.2% by a gap inserted in the broad band noise background.

Among the control group there was similar variability and no clear indication of the GPIAS being stronger for one type of background compared to the others. When the results from the tinnitus and control groups were compared there was no evidence of a significant difference between them. A Fisher exact test was used to compare the results for the tinnitus group with a tinnitus centred background and the control group with a background centred on 6 kHz. The statistic value was 0.59 which is not significant. Thus, the results failed to support the hypothesis that GPIAS was reduced in the tinnitus group when the background was centred on the tinnitus frequency.

Discussion

Reliability of the PAMR and GPIAS responses in tinnitus participants

The main problem we faced was the variability of the PAMR response, which meant that less than half of the tinnitus participants tested (47%, 7/15) had a measurable response. The variability of the PAMR response between subjects is well known [28] and a variety of refinements have been described to increase its amplitude and reliability. Some of these involve simultaneous activation of other nuclei that control the muscles of the face and neck so that there is an overflow of neural input into the part of the facial nucleus controlling the posterior auricular muscle [29]. These methods include deflecting the eyes towards the side [25], smiling or changing head position [30] or opening the mouth (Meier-Ewert., *et al*. 1974). The ear of stimulation is also important in determining the size of the PAMR. We used ipsilateral stimulation of the ear on the side where the tinnitus was most dominant. However, stimulation of the contralateral ear generally gives a bigger PAMR response [30] and the response may become even bigger when binaural stimulation is used [31]. Subjective factors are also involved. The PAMR response is potentiated by emotional state [24] and affected by the state of arousal, so that falling asleep reduces the PAMR amplitude [31]. In future, a stronger PAMR response should be achieved if the recording electrodes are placed on the ear contralateral to the side where tinnitus is perceived so that a contralateral or binaural stimulus can be used. It should also be possible to obtain a more reliable PAMR response if the number of repetitions is increased to several hundred instead of 60 (as we used here) as long as the participant can remain alert [28,31].

One factor that might affect GPIAS strength in the tinnitus patients is their TFI score. However, we found little evidence of this. The mean TFI for the 19 tinnitus participants was 36.7 (Table 1) while that for the tinnitus participants who showed a PAMR response, but did not show any GPIAS at the tinnitus background frequency, was 32.75. The mean TFI for the participants who did show GPIAS at the tinnitus background was 31. These differences were not significant. The only indication of an effect of TFI was on the participants who withdrew because the stimuli were distressing (Table 2). Their mean TFI was 51.25. However even this difference was not significant because of the range of values (7 - 90).

Limitations of the GPIAS Technique

Of the initial 19 tinnitus participants, only five provided any GPIAS data and even that was incomplete as they only showed GPIAS at 1 or 2 of the 3 background conditions. Difficulties in showing any clear effect of tinnitus on this type of pre-pulse inhibition (PPI) were also found in studies using the eyeblink reflex [9]. One of these did show deficits in participants with tinnitus, but they were not restricted to background noise centred on the perceived tinnitus frequency and the underlying mechanisms were unclear [8]. In future, if GPIAS is to be developed as a method for testing tinnitus in humans, then methods will need to be refined to improve reliability. If a stronger PAMR response can be obtained then it should be useful to use more sophisticated statistical methods in calculating the GPIAS [32].

GPIAS is a type of PPI and a substantial amount of work has shown that the amount of PPI is also variable between individuals and in test-retest data from single individuals. The magnitude of the PPI can be altered by various factors such as gender [33], hormonal status (e.g. ovarian cycle), withdrawal from caffeine or nicotine, fatigue and medications [23] as well as attention [34]. There are also differences between individuals in normal brainstem circuitry that affect the strength of PPI in control subjects [35]. A further complication is that the startle response is subject to habituation depending on how frequently and often the stimulus is presented [36]. However one major factor in favour of using the PAMR to measure the acoustic startle response is that it is a pure acoustic pathway with a short latency and simpler pathway than the longer latency, multimodal pathways involved in the eyeblink reflex [18].

People with tinnitus have shown no significant increases in gap perception threshold when compared to age- and hearing-matched controls [37,38]. However, gap perception is a conscious process, whereas GPIAS may depend on a pre-attentive process involving sensorimotor gating in the brainstem [23,39]. There are likely important mechanistic differences between a perceptual gap detection task assessing temporal acuity [40] and GPIAS, which is commonly used as a test for tinnitus in animals and where a silent gap is used as standard [12]. Whatever is being detected by GPIAS it is now reasonably well-established that tinnitus does not fill in a silent gap inserted in a background noise during a perceptual gap recognition task, even when the background has been chosen to mimic the tinnitus percept as closely as possible [37,38,41]. We know from tinnitus matching studies that the tinnitus percept is usually relatively quiet (equivalent to about 20 dB, e.g. [42]) and so it is not realistic to expect the tinnitus to fill in a silent gap in a 60 or 70 dB SPL background. The eyeblink response is inhibited

by acoustic pre-pulses of less than 20 dB above background noise [43]. In future it may be better to test for tinnitus using a 20 dB reduction in the background noise rather than complete silence. Humans can detect a sudden decrease in the sound level of a background noise even when the decrease is only 20 dB SPL and this sensitivity may allow a more realistic GPIAS test of tinnitus to be developed in future [44].

Conclusion

This study evaluated two interdependent hypotheses. The first hypothesis was that it would be possible to use the PAMR response to measure GPIAS in a typical group of people with chronic tinnitus. The second and main hypothesis was that there would be a reduction in the amount of GPIAS in the tinnitus group when the background noise was matched to their tinnitus percept. We were able to confirm that some tinnitus participants did show GPIAS based on their PAMR response (first hypothesis). However, our optimisations did not produce a reliable PAMR response and this unreliability prevented an adequate test of the second hypothesis.

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Disclosure

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

Bibliography

1. Eggermont JJ. "Can Animal Models Contribute to Understanding Tinnitus Heterogeneity in Humans?" *Frontiers in Aging Neuroscience* 8 (2016).

2. Hall DA., *et al*. "A balanced randomised placebo controlled blinded phase IIa multi-centre study to investigate the efficacy and safety of AUT00063 versus placebo in subjective tinnitus: The QUIET-1 trial". *Hearing Research* 377 (2019): 153-166.

3. Saunders GH and Griest SE. "Hearing loss in veterans and the need for hearing loss prevention programs". *Noise Health* 11 (2009): 14-21.

4. van der Loo E., *et al*. "Tinnitus intensity dependent gamma oscillations of the contralateral auditory cortex". *Plos One* 4 (2009): e7396.

5. Weisz N., *et al*. "The neural code of auditory phantom perception". *Journal of Neuroscience Methods* 27 (2007): 1479-1484.

6. Adjamian P. "The application of electro- and magneto-encephalography in tinnitus research - methods and interpretations". *Frontiers in Neurology* (2014): 5.

7. Berger JI., *et al*. "Reductions in cortical alpha activity, enhancements in neural responses and impaired gap detection caused by sodium salicylate in awake guinea pigs". *European Journal of Neuroscience* 46.3 (2017): 398-409.

8. Fournier P and Hebert S. "Gap detection deficits in humans with tinnitus as assessed with the acoustic startle paradigm: Does tinnitus fill in the gap?" *Hearing Research* 295 (2013): 16-23.

9. Shadwick K and Sun W. "Acoustic startle reflex and pre-pulse inhibition in tinnitus patients". *Journal of Otology* 9 (2014): 141-145.

10. Turner JG., *et al*. "Gap detection deficits in rats with tinnitus: A potential novel screening tool". *Behavioral Neuroscience* 120 (2006): 188-195.

11. Gerum RC., *et al*. "Open (G) PIAS: An open-source solution for the construction of a high-precision acoustic startle response setup for tinnitus screening and threshold estimation in rodents". *Frontiers in Behavioral Neuroscience* 13 (2019): 140.

12. Berger JI., *et al*. "Gap-induced reductions of evoked potentials in the auditory cortex: A possible objective marker for the presence of tinnitus in animals". *Brain Research* 1679 (2018): 101-108.

13. Han JH., *et al*. "Objective measurement of subjective tinnitus using the acoustic change complex". *Plos One* 12 (2017).

14. Ku Y., *et al*. "The gap-prepulse inhibition deficit of the cortical N1-P2 complex in patients with tinnitus: The effect of gap duration". *Hearing Research* 348 (2017): 120-128.

15. Paul BT., *et al*. "Towards an objective test of chronic tinnitus: Properties of auditory cortical potentials evoked by silent gaps in tinnitus-like sounds". *Hearing Research* 366 (2018): 90-98.

16. Berger JI., *et al*. "A novel behavioural approach to detecting tinnitus in the guinea pig". *Journal of Neuroscience Methods* 213 (2013): 188-195.

17. Wu C., *et al*. "Increased Synchrony and Bursting of Dorsal Cochlear Nucleus Fusiform Cells Correlate with Tinnitus". *Journal of Neuroscience* 36 (2016): 2068-2073.

18. Wilson CA., *et al*. "Gap-induced inhibition of the post-auricular muscle response in humans and guinea pigs". *Hearing Research* 374 (2019): 13-23.

19. Langguth B., *et al*. "Consensus for tinnitus patient assessment and treatment outcome measurement". *Progress in Brain Research* 166 (2007): 525-536.

20. Meikle MB., *et al*. "The tinnitus functional index: development of a new clinical measure for chronic, intrusive tinnitus". *Ear Hearing* 33 (2012): 153-176.

21. Roberts LE., *et al*. "Residual inhibition functions in relation to tinnitus spectra and auditory threshold shift". *Acta Oto-Laryngology* 556 (2006): 27-33.

22. Roberts LE., *et al*. "Residual inhibition functions overlap tinnitus spectra and the region of auditory threshold shift". *Journal of the Association for Research in Otolaryngology* 9 (2008): 417-435.

23. Braff DL., *et al*. "Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies". *Psychopharmacology (Berlin)* 156 (2001): 234-258.

24. Benning SD., *et al*. "Emotional modulation of the post-auricular reflex". *Psychophysiology* 41 (2004): 426-432.

25. Patuzzi RB and O'Beirne GA. "Effects of eye rotation on the sound-evoked post-auricular muscle response (PAMR)". *Hearing Research* 138 (1999): 133-146.

26. Thornton ARD. "Use of Post-Auricular Muscle Responses". *Journal of Laryngology and Otology* 89 (1975): 997-1010.

27. Sereda M., *et al*. "Relationship between tinnitus pitch and edge of hearing loss in individuals with a narrow tinnitus bandwidth". *International Journal of Audiology* 54 (2015): 249-256.

28. O'Beirne GA and Patuzzi RB. "Basic properties of the sound-evoked post-auricular muscle response (PAMR)". *Hearing Research* 138 (1999): 115-132.

29. De Grandis D and Santoni P. "The Post-Auricular Response - a Single Motor Unit Study". *Electroencephalography and Clinical Neurophysiology* 50 (1980): 437-440.

30. Dus V and Wilson SJ. "Click-Evoked Post-Auricular Myogenic Response in Normal Subjects". *Electroencephalography and Clinical Neurophysiology* 39 (1975): 523-525.

31. Doubell TP., *et al*. "The effect of interaural timing on the posterior auricular muscle reflex in normal adult volunteers". *Plos One* (2018): 13.

32. Schilling A., *et al*. "A new statistical approach for the evaluation of gap-prepulse inhibition of the acoustic startle reflex (GPIAS) for tinnitus assessment". *Frontiers in Behavioral Neuroscience* 11 (2017): 198.

33. Aasen I., *et al*. "Sex effects in prepulse inhibition and facilitation of the acoustic startle response: implications for pharmacological and treatment studies". *Journal of Psychopharmacology* 19 (2005): 39-45.

34. Filion DL., *et al*. "Modification of the acoustic startle-reflex eyeblink: a tool for investigating early and late attentional processes". *Biological Psychology* 35 (1993): 185-200.

35. Swerdlow NR., *et al*. "Sensorimotor gating of the startle reflex: what we said 25 years ago, what has happened since then, and what comes next". *Journal of Psychopharmacology* 30 (2016): 1072-1081.

36. Cassella JV and Davis M. "Habituation, Prepulse Inhibition, Fear Conditioning, and Drug Modulation of the Acoustically Elicited Pinna Reflex in Rats". *Behavioural Neuroscience* 100 (1986): 39-44.

37. Campolo J., *et al*. "Does tinnitus "fill in" the silent gaps". *Noise and Health* 15 (2013): 398-405.

38. Boyen K., *et al*. "The Gap Detection Test: Can It Be Used to Diagnose Tinnitus?" *Ear and Hearing* 36 (2015): e138-e145.

39. Geyer MA. "The family of sensorimotor gating disorders: Comorbidities or diagnostic overlaps?" *Neurotoxicity Research* 10 (2006): 211-220.

40. Weible AP., *et al*. "Perceptual gap detection is mediated by gap termination responses in auditory cortex". *Current Biology* 24 (2014): 1447-1455.

41. Fournier P and Hebert S. "The gap-startle paradigm to assess auditory temporal processing: Bridging animal and human research". *Psychophysiology* 53 (2016): 759-766.

42. Moller AR. Textbook of Tinnitus, New York, Dordrecht, Heidelberg, London (2011).

43. Reiter LA and Ison JR. "Inhibition of the human eyeblink reflex: an evaluation of the sensitivity of the Wendt-Yerkes method for threshold detection". *Journal of Experimental Psychology and Human Perception Performance* 3 (1977): 325-336.

44. Peterson H and Blumenthal TD. "Efficacy of stimulus intensity increases and decreases as inhibitors of the acoustic startle response". *Psychophysiology* 55 (2018): e13266.