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Potential Audiological and MRI Markers of Tinnitus

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Abstract

Background: Subjective tinnitus, or ringing sensation in the ear, is a common disorder with no accepted objective diagnostic markers.

Purpose: The purpose of this study was to identify possible objective markers of tinnitus by combining audiological and imaging-based techniques.

Research Design: Case-control studies.

Study Sample: Twenty adults drawn from our audiology clinic served as participants. The tinnitus group consisted of ten participants with chronic bilateral constant tinnitus, and the control group consisted of ten participants with no history of tinnitus. Each participant with tinnitus was closely matched with a control participant on the basis of age, gender, and hearing thresholds.

Data Collection and Analyses: Data acquisition focused on systematic administration and evaluation of various audiological tests, including auditory-evoked potentials (AEP) and otoacoustic emissions, and magnetic resonance imaging (MRI) tests. A total of 14 objective test measures (predictors) obtained from audiological and MRI tests were subjected to statistical analyses to identify the best predictors of tinnitus group membership. The least absolute shrinkage and selection operator technique for feature extraction, supplemented by the leave-one-out cross-validation technique, were used to extract the best predictors. This approach provided a conservative model that was highly regularized with its error within 1 standard error of the minimum.

Results: The model selected increased frontal cortex (FC) functional MRI activity to pure tones matching their respective tinnitus pitch, and augmented AEP wave N_1 amplitude growth in the tinnitus group as the top two predictors of tinnitus group membership. These findings suggest that the amplified responses to acoustic signals and hyperactivity in attention regions of the brain may be a result of overattention among individuals that experience chronic tinnitus.

Conclusions: These results suggest that increased functional MRI activity in the FC to sounds and augmented N_1 amplitude growth may potentially be the objective diagnostic indicators of tinnitus. However, due to the small sample size and lack of subgroups within the tinnitus population in this study, more research is needed before generalizing these findings.

Key Words: auditory-evoked potentials, magnetic resonance imaging, predictors, tinnitus

Abbreviations: ABR = auditory brainstem response; AEP = auditory-evoked potentials; ALR = auditory late response; ART = acoustic reflex threshold; BBN = broadband noise; BOLD = blood-oxygen–level-dependent; CBF = cerebral blood flow; DPOAE = distortion product otoacoustic emissions; EPI = echo-planar imaging; FC = frontal cortex; fcMRI = functional connectivity MRI; FMPT = frequency modulated pure tone; fMRI = functional MRI; FOV = field-of-view; FWE = family-wise error rate; LASSO = Least Absolute Shrinkage

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and Selection Operator; LDL = loudness discomfort level; MRI = magnetic resonance imaging; OAE = otoacoustic emissions; nHL = normal hearing level; TE = echo time; THI = Tinnitus Handicap Inventory; TR = repetition time; TRQ = Tinnitus Reaction Questionnaire; vmPFC = ventromedial prefrontal cortex

INTRODUCTION

Subjective tinnitus is described as the perception of ringing in the ears, heard only by the listener, in the absence of an external acoustic signal. Approximately 40–50 million Americans suffer from this disorder. Among this group, about 20 million people struggle with burdensome chronic tinnitus, while 2 million have extreme and debilitating tinnitus (American Tinnitus Association, 2016). Tinnitus is a complex disorder, stemming from a variety of etiological factors and exhibiting various degrees of associated stress. Tinnitus is often accompanied with hearing loss and hyperacusis. However, hearing loss or hyperacusis is not necessary for tinnitus to be present (Eggermont and Roberts, 2004; Eggermont, 2013; 2015). It is believed that though peripheral abnormalities in the cochlea may trigger the onset of tinnitus, it is the central mechanisms that play an important role in the maintenance of tinnitus (Adjamian et al, 2009). However, our basic understanding of the underlying neurological mechanisms involved in tinnitus is still lacking (Leaver et al, 2011; 2012).

Numerous studies involving humans, animal models—including our own in vitro model (Wu et al, 2011)—and computer-generated models have been conducted to understand the phenomenon of tinnitus, identify its biologic or neural correlates, and explore treatment possibilities (Henry et al, 2013; Schaette, 2014; Szczepek et al, 2014). Several studies have positively linked tinnitus to excess auditory activation in auditory and nonauditory regions in the brain (Lockwood et al, 1998; Melcher et al, 2000; 2009; Kaltenbach et al, 2002; Noreña and Eggermont, 2003; Lanting et al, 2009; Haller et al, 2010). Other possible underlying mechanisms of tinnitus discussed so far include intensification of burst firings, increased neural synchrony (Noreña and Eggermont, 2003), and cortical tonotopic reorganization (Muhlnickel et al, 1998; Seki and Eggermont, 2003; Eggermont, 2006).

Clinically, the presence of tinnitus and its impact on daily life are assessed using subjective outcome measures such as questionnaires. For example, the Tinnitus Handicap Inventory (THI; Newman et al, 1996) and Tinnitus Reaction Questionnaire (TRQ; Wilson et al, 1991) are widely used in clinical practice, as well as in research, to assess tinnitus-related distress or severity of tinnitus. Currently there are no objective audiological or nonaudiological tests for diagnosing tinnitus; hence, the presence of tinnitus is established based on subjective reports by the tinnitus sufferer (Henry et al, 2013; Szczepek et al, 2014). Legitimacy of such diagnosis can always be challenged, thus making the diagnosis extremely challenging for audiologists. In this study, our central hypothesis is that measures of physiological activity of the auditory system, that is, otoacoustic emissions (OAE) and auditory-evoked potentials (AEPs), as well as magnetic resonance imaging (MRI)–based blood-oxygen-level-dependent (BOLD) functional MRI (fMRI) response to auditory stimulus, regional baseline cerebral blood flow (CBF), and resting state functional connectivity patterns among relevant brain regions may serve as objective markers of tinnitus. These markers can be measured with audiological and MRI techniques.

Several studies have reported abnormal auditory brainstem responses (ABRs, a subtest of AEPs) in tinnitus patients such as delayed ABR; absolute latencies for peaks I, III, and V; prolongation of interpeak latency for peaks I–III, III–V, and I–V; as well as significant enhancement of peak V/I amplitude ratio (Shulman and Seitz, 1981; Maurizi et al, 1985; Ikner and Hassen, 1990; Lemaire and Beutter, 1995; Kehrle et al, 2008; Liu and Chen, 2012; Knipper et al, 2013). Contrary to these findings, some studies have found normal ABR results in tinnitus patients (Barnea et al, 1990; McKee and Stephens, 1992). Furthermore, Schaette and McAlpine (2011) showed mixed results, that is, in participants with tinnitus with normal hearing, the amplitude of ABR wave I was reduced, but the amplitude of ABR wave V was normal. They argued that their results provided direct physiological evidence of reduced neural output from the cochlea leading to renormalization of response amplitude in higher centers of the brainstem.

Auditory late response (ALR) is another subtest of AEPs which characterizes auditory-evoked potentials originating primarily from primary and secondary auditory cortex, with additional sources in the frontal lobes (Picton et al, 1999; Santos Filha and Matas, 2010). Among the ALRs, wave N_1 has been studied more frequently (Zhang et al, 2011). Lee et al (2007) reported significant differences in the intensity dependency of amplitude N_1 in tinnitus patients. Their tinnitus patients responded less to increased sound intensity and were more inclined to weaker intensity dependence. Jacobson et al (1996) showed increased latencies for N_1 and P_2 waves in their participants with tinnitus. In addition to N_1 and P_2 waves, Santos Filha and Matas (2010) found that their tinnitus group had increased latencies for peaks P300, and increased mean N_1 amplitude. Attias et al (1993) showed no difference in latencies of N_1, P_2, or P300, but did find increased amplitudes of all three waves in participants with tinnitus. Although studies so far have given us conflicting information, they have

nevertheless supported the notion that tinnitus modulates activity at the auditory subcortical and cortical levels. However, due to heterogeneity in participant groups and methodology, there is no consensus among researchers regarding the use of AEPs as diagnostic indicators of tinnitus.

Brain imaging, in particular MRI measures, can provide spatially specific examinations of the brain's structure, function, and physiology. Consequently, these tools are ideal for identification of markers of tinnitus. Importantly, these techniques can be performed in a noninvasive manner, and if validated, can be translatable to the clinic for screening and monitoring applications. It is also important to note that brain imaging results are highly objective and are minimally dependent on the examiner or patient. Yet, brain imaging in tinnitus patients is an under-studied area.

To provide a substantial improvement in our understanding of tinnitus and thereby identify the most sensitive markers, a multimodal imaging approach is needed. Specifically, it is desirable to conduct a systematic study to examine multiple domains of brain function, such as spontaneous and selected auditory stimulus-driven brain activity, resting CBF, and connectivity among brain regions. Such a multimodal approach can integrate synergistic information across modalities and obtain a comprehensive understanding of this debilitating condition. Unfortunately, most of the literature to date has typically focused on a limited scope of brain function. Hence, the purpose of this study was to explore potential objective markers of tinnitus using audiological and MRI-based measures. Our hypothesis was that regardless of hearing thresholds, significantly altered audiological and MRI measures are exhibited in tinnitus patients, and these measures can be used as predictors of tinnitus group membership.

METHODS

Participants

Prior to any evaluation, all participants were required to read and sign the Institutional Review Board consent forms approved by the University of North Texas and the University of Texas Southwestern Medical Center. The tinnitus group consisted of ten participants with varying degrees of hearing, constant bilateral tinnitus for at least 6 mo, and no history of anxiety or depression. The remaining ten participants formed the control group consisting of individuals with no history of tinnitus, anxiety, or depression. Participants that were unable to tolerate 95 dB SPL acoustic signals in the MRI scanner or those with anxiety symptoms during scanning were not included in the study.

The participants were chosen so that each participant with tinnitus could be paired with a control participant after carefully matching for age, gender, and hearing thresholds. All participants were first seen at the University of North Texas Tinnitus and Hyperacusis Clinic for audiological and tinnitus evaluation. Following this, MRI testing was conducted at the Advanced Imaging Research Center on the campus of University of Texas Southwestern Medical Center. None of the participants selected for this study had pacemakers or any type of implanted prosthesis.

Audiological Assessment

Audiological testing procedures involved in this study have been discussed in our previous article (Gopal et al, 2015). Briefly, the audiological test battery consisted of the following procedures: case history, otoscopic examination, pure-tone audiometry, immittance audiometry, loudness discomfort level (LDL) testing at 500, 1000, and 4000 Hz, OAE testing which included spontaneous emissions and distortion product otoacoustic emissions (DPOAE), and AEP, which included ABRs and ALRs. The tinnitus evaluation conducted on the tinnitus group included the THI and TRQ questionnaires and tinnitus pitch identification using the two-alternative forced-choice paradigm (Vernon and Meikle, 2003). For the tinnitus pitch matching process, groups of two pure tones were presented alternatively, and the participant was asked to indicate which tone, one or two, best represented the pitch of their tinnitus. Based on the participant's response, the tone pairs were bracketed upward or downward, and the participant was again asked to indicate the tone that best represented their tinnitus. Subsequent tone pairs progressively reduced in difference until the participant was able to determine the frequency that matched their tinnitus pitch. The tinnitus pitch identified during this procedure was used to create the frequency-modulated pure-tone (FMPT) signal that was used during fMRI testing of participants with tinnitus and their respective matched-control participants.

AEP testing was conducted using a calibrated ICS CHARTR EP system (Taastrup, Denmark). For evoked potential recordings, gold cup electrodes were positioned at high forehead (active), right and left ear lobes (reference), and low forehead (ground). Electrode impedances were kept below 5 kΩ, with interelectrode readings within 1 kΩ. AEP recordings were obtained for each ear separately, but data were collapsed across ears for analyses. ABR recordings were obtained for rarefaction clicks (100 μsec) delivered through inserts at a repetition rate of 21.1/sec. A bandpass filter setting of 100–3000 Hz was used, and responses were averaged for a minimum of 1,500 runs. Every run was repeated at least twice. Variables used in the data analyses included latency and amplitude measures of ABR wave V. Stimuli for ALR recordings consisted of 1000 Hz tone bursts presented at a rate of 1.1/sec. The bandpass filters were set between

1 and 30 Hz, and the window was 400 msec. Each intensity level was repeated at least twice, and a minimum of 200 artifact-free trials were averaged. Latency and amplitude measures for ALR wave N_1 were obtained.

MRI

MRI Protocol

MRI scans were performed on a 3T Philips Achieva system (Philips Medical Systems, Best, The Netherlands). An eight-channel receive-only head coil was used for all MRI scans. Foam padding was used to stabilize and prevent head movement during the scans. Three complementary MRI techniques were used in the study: BOLD fMRI response to acoustic signals (broadband noise [BBN] and FMPT), baseline CBF measured with arterial-spin-labeling MRI, and resting state functional connectivity MRI (fcMRI).

fMRI brain activations were measured using an auditory task that included FMPT stimulus at the pitch of tinnitus experienced by the patient, and BBN. Details of the procedures have been published earlier (Gopal et al, 2015). The frequency of the FMPT for each participant pair was determined by the tinnitus-match pitch obtained from the tinnitus patient during their tinnitus evaluation. Thresholds were obtained for BBN and FMPT signals on the day of scanning in the scanner room. The acoustic stimuli for fMRI testing were presented at suprathreshold levels (20–50 dB SL) dictated by the individual's threshold at the frequency of FMPT, with participants within each pair receiving the same sensation level. Participants were required to press a button each time they heard the acoustic stimulus. All participants performed the task in the scanner while (BOLD) MRI images were acquired using a sparse temporal sampling technique (Gaab et al, 2003) with the following parameters: repetition time/echo time (TR/TE) = 10,000/30 msec, flip angle = 70°, field-of-view (FOV) = 220 × 220, matrix = 64 × 64, whole brain coverage with 39 slices, 4 mm thick, duration = 5 min. The sparse sampling technique (Figure 1) used a long TR of 10 sec, during which magnetic resonance image acquisition only took place in 2 sec. The timing placement of the acquisition period is such that each TR begins with a 4-sec acoustic signal block (BBN or FMPT), followed by 2 sec of silence, 2 sec of scanner acquisition, and 2 sec of silence. This acquisition scheme is less efficient compared to the conventional, continuous image collection methods, as only 20% (2 sec of 10 sec) of the total time available is actually used for data collection. However, the advantage of this design is that hemodynamic response function induced by acoustic stimulus is acquired by the scanner at its peak, and the hemodynamic response induced by the scanner noise (of the acquisition of the previous image volume) has returned to baseline. The participants were instructed to press a button whenever they heard the BBN/FMPT. BOLD fMRI images were acquired for 5 min while BBN (or FMPT) stimuli were presented. Another set of BOLD images were acquired without the acoustic stimuli, for statistical comparison. To obtain sufficient power to detect BOLD activations, the fMRI task was performed five times in the scanner.

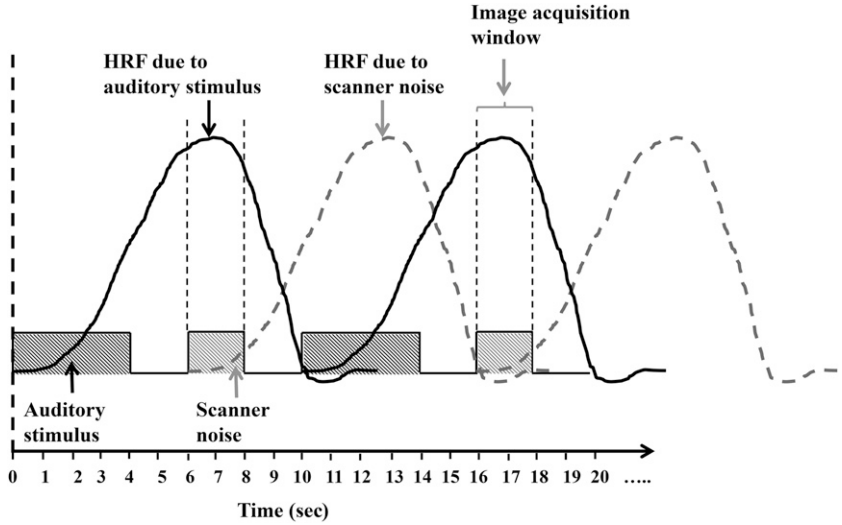

Figure 1. Schematic diagram of the sparse sampling acquisition technique used in this study. The acquisition uses a TR of 10 sec. The key intention of this design is to acquire the hemodynamic response (HRF) induced by the auditory stimuli while the scanner-noise-generated hemodynamic response is minimal. As illustrated in the diagram, the auditory stimulus is present during time 0–4 sec; its BOLD response will peak at time 6–8 sec. The image acquisition noise during 6–8 sec will in turn generate a BOLD response which will peak at 12–14 sec. Thus, the data acquisition is sampling the hemodynamic response induced by the auditory stimulus without any contamination due to the noise generated by the scanner during data acquisition.

Resting CBF was measured using a pseudo-continuous arterial-spin-labeling technique with a gradient-balanced scheme (Aslan and Lu, 2010). Forty control and label pairs of images were acquired using an echo-planar imaging (EPI) acquisition using the following parameters: TR/TE = 4,250/14 msec, label duration = 1,650 msec, post-label delay = 1,525 msec, FOV = 240 × 240, matrix = 80 × 80, 29 slices, 5 mm thick, duration = 5 min 45 sec.

Functional connectivity between right and left auditory cortices was measured with a BOLD EPI sequence, while the participants fixated on a crosshair. The MRI pulse sequence parameters were TR/TE = 2000/25 msec, flip angle = 80°, FOV = 220 × 220, matrix = 64 × 64, 43 slices, 3.5 mm thick, duration = 5 min.

MRI Data Analysis

fMRI data were analyzed using SPM5 (Wellcome Trust Center for NeuroImaging, London, UK), and custom MATLAB (MathWorks Inc., Natick, MA) routines. Details of the analyses have been described in an earlier article (Gopal et al, 2015). Briefly, individual participant's fMRI images were realigned to remove the effect of motion during image acquisition and then transformed to Montreal Neurological Institute template space (voxel size 2 × 2 × 2 mm^3). Images were then smoothed with a Gaussian kernel, full-width-half-maximum of 6 mm^3, to help improve signal-to-noise ratio. Brain activations to the auditory task were detected for each participant using standard general linear model analysis. Voxel-wise whole brain comparison between the control and tinnitus groups was performed using a paired t test.

A CBF map was obtained from pseudo-continuous arterial-spin-labeling MRI using a perfusion kinetic model comparable to the model described by Chalela et al (2000) and Wang et al (2003). CBF was first obtained in the units of mL/100 g/min. To minimize the effect of CBF modulation by global factors, for example, breathing pattern or caffeine consumption, and focus on regional CBF, the CBF map was normalized by the whole brain mean CBF to obtain a relative CBF map.

For processing the functional connectivity data, we used the Xu et al (2011) procedures. Data analyses included the Analysis of Functional Neuroimages (National Institutes of Health, Bethesda, MD) and our in-house MATLAB scripts (Tung et al, 2013). Images were realigned to correct for motion during image acquisition and then transformed to Montreal Neurological Institute template space. Patient motion and white matter time course were regressed out, and the data were band-pass filtered between 0.01 and 0.1 Hz. Functional connectivity analyses in this study primarily focused on the connectivity of right and left auditory cortices. These regions were defined anatomically using a brain atlas provided by the software WFU Pickatlas (Maldjian et al, 2003), and BOLD time series within the regions of interest were averaged. Cross-correlation coefficient between right and left auditory cortex was calculated to signify the "connectivity" between them. Voxel-wise analysis results (using seed-based or independent component analysis–based methods) as well as brain network properties will complicate the current article, which already includes AEP and multiple MRI modalities, and thus will be described in a future report.

Statistical Analyses for Identification of Predictors

The intent of this study was to explore potential objective markers of tinnitus by identifying the best predictors of tinnitus group membership. Several behavioral tests were incorporated in the audiological test battery to obtain a better understanding of group characteristics, but these behavioral test measures were not included in the statistical analysis for identification of predictors. It should be mentioned that even though THI and TRQ scores have been thought to impact MRI measures and vary considerably within our tinnitus group, they could not be included as potential predictors, since the scores are zero for the control group. Furthermore, due to the constraints of the small sample size used in this study, it was not feasible to include all of the objective test measures, such as all peak latencies and amplitudes, interpeak latencies, and interaural latency differences, for further statistical analyses. Hence, based on the information obtained from existing literature, and from the findings of our earlier publication (Gopal et al, 2015), only a limited number of imperative physiological measures/variables were selected. These variables encompass the primary measures representative of several levels of the primary auditory nervous system and its association areas, and reflect the evoked electrophysiological activity of the auditory system (AEPs) and measures of brain regions accessible using MRI.

Even with this selection process, we were faced with a statistical challenge due to potential strong multicollinearity and overfitting problems. There is not an entirely satisfactory solution to this challenge, but we have performed statistical analyses based on what we believe is the best approach. We implemented the Least Absolute Shrinkage and Selection Operator (LASSO) technique for feature extraction (Tibshirani, 1996), and supplemented it with the leave-one-out cross-validation technique to extract the best predictors of tinnitus group membership. The LASSO is a shrinkage and selection method for regression models (Tibshirani, 1996), originally applied to ordinary least squares regression. The LASSO is best described as a constraint on the sum of the absolute values of the model parameters, where this sum has a specified constant as an upper bound. Compared to ordinary least squares parameter estimates, the estimates obtained using the LASSO

are generally more accurate and some parameters will be shrunk toward zero, allowing for better interpretation of the model and identification of those covariates most strongly associated with the outcome. One obvious advantage of LASSO over conventional model selection methods, such as stepwise regression, is a clear hierarchy of the predictors in the procedure. Once a variable enters the model, it never leaves the model as the constraint is relaxed. This simple constraint may be applied to an ordinary logistic regression, which yields LASSO logistic regression. The assumptions for LASSO logistic regression are the same as ordinary logistic regression, which essentially assumes that the logit model is valid in estimating the probabilities of the binary outcome. The primary appeal of LASSO logistic regression is its ability to select an appropriate set of predictor variables when there is a large number of potential predictors relative to the sample size, and there is no distributional assumption to make to apply LASSO.

In LASSO, each of the predictor variables enter the model in order of importance. There are two standard methods to select the top predictors to be included in the final model: one that corresponds to the value of λ that gives the minimum mean cross-validated error and the other that gives the most regularized model such that error is within 1 standard error of the minimum. The second approach is more conservative and selects fewer predictors. Due to the relatively small sample size and large number of predictors in our study, it was appropriate to use the more conservative most regularized model approach.

Since the approach is data driven and no statistical test is involved in the process, a formal power analysis is not possible. However, as mentioned above, the most regularized model used to select the predictors is conservative by design and only selects the predictors that have high predictive ability with regard to the outcome. In this approach, the sum of the magnitudes of the predictor-coefficients is constrained to be no greater than a given value which determines the number of parameters retained in the model. For our study dataset, we used a logistic regression model with tinnitus status (present/absent binary) as the response and the objective measures as predictors. In the context of logistic regression, the objective function for the penalized logistic regression uses the negative binomial log-likelihood, and is

$$\min_{(\beta_0,\beta)\in\mathbb{R}^{p+1}} -\left[\frac{1}{N}\sum_{i=1}^{N} y_i\cdot(\beta_0+x_i^T\beta)-\log\left(1+e^{(\beta_0+x_i^T\beta)}\right)\right] +\lambda\left[(1-\alpha)\|\beta\|_2^2\Big/2+\alpha\|\beta\|_1\right].$$

The parameter λ is the penalty on the number of parameters in the model (higher λ corresponds to fewer parameters).

RESULTS

The participants in the tinnitus group (n = 10) and control group (n = 10) were matched for gender, age, and hearing thresholds. Each group consisted of five male and five female participants.

Audiological Results

Table 1 summarizes the demographic information of the participants. The tinnitus group did not differ significantly from the control group in age ($p = 0.53, p_{corr} = 0.97$) or average air-conduction pure-tone thresholds ($p = 0.10$, $p_{corr} = 0.87$), demonstrating good matching between the two groups. Figure 2 depicts the average pure-tone air-conduction audiograms for tinnitus and control groups.

All participants exhibited normal otoscopic results and bilateral type A tympanograms. In the case history form, all ten participants with tinnitus indicated bilateral constant tinnitus for more than 6 mo. The participants in the control group had no complaints of tinnitus. Six participants with tinnitus and three control participants stated that some external sounds that others can normally tolerate were uncomfortable. Seven participants in the tinnitus group and four participants in the control had a history of noise exposure. All participants in the tinnitus group were right handed. In the control group, one participant was ambidextrous and the remaining were right handed.

Ipsilateral and contralateral acoustic reflex thresholds (ART) varied from normal to absent, but were consistent with their respective pure-tone thresholds. Table 2

Table 1. Basic Demographic Information, Noise Exposure History, and Average Air Conduction Pure-Tone Thresholds in Tinnitus and Control Groups

Group	Mean Age (yr)	Gender	Handedness	History of Noise Exposure	Pure-Tone Average (0.25–8 kHz) dB HL
Tinnitus (n = 10)	48.9 ± 16.1	5 M	Right (n = 10)	7 of 10	20.7 ± 14.9 (R)
		5 F			20.8 ± 12.9 (L)
Control (n = 10)	49.8 ± 17.9	5 M	Right (n = 9)	4 of 10	17.2 ± 13.7 (R)
		5 F	Ambidextrous (n = 1)		18.8 ± 15.2(L)

Note: F = female; L = left ear; M = male; R = right ear.

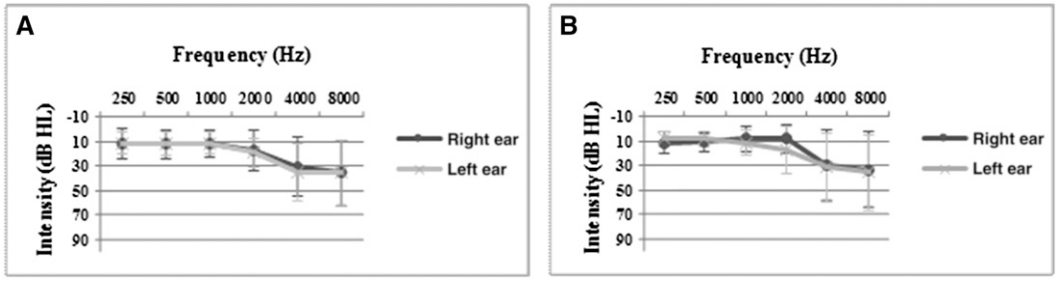

Figure 2. Mean and standard deviation pure-tone air-conduction thresholds in (A) tinnitus and (B) control groups.

depicts the average ARTs for both groups. The LDLs, averaged across 0.5, 1, and 4 kHz, showed higher levels for the control group, indicating greater tolerance to loud acoustic stimuli. Spontaneous OAEs were present in three control participants and one participant with tinnitus. DPOAEs evoked by 65/55 dB HL tone pairs at the F2/F1 ratio of 1.2:1 indicated signal-to-noise ratios that ranged from 1 to 19 dB SPL. The average overall strength of DPOAEs is shown in Table 2.

Tinnitus evaluation: All participants included in the tinnitus group had a history of bilateral tinnitus for a duration ranging from 6 mo to 49 yr. Table 3 depicts the duration of tinnitus, tinnitus pitch, THI scores, and TRQ scores for all participants in the tinnitus group. The tinnitus pitch information obtained during the audiological evaluation was later used in fMRI testing. The THI scores among the participants with tinnitus ranged from 10 to 94, and the TRQ scores ranged from 2 to 80, thus indicating diverse reactions and varied levels of distress related to their constant bilateral tinnitus. Four participants in the tinnitus group exhibited slight distress, one had mild distress, one moderate, three severe, and one catastrophic distress. As expected, all participants in the control group indicated a score of 0 on THI and TRQ questionnaires.

AEP Tests

The latency and amplitude values from ABR recordings on all participants were consistent with their hearing thresholds. The group means for ABR wave V latency and amplitude measured in response to 80 dB normal hearing level (nHL) clicks, and mean amplitude growth of ABR wave V from 60 to 80 dB nHL clicks are shown in Table 4. ALR peak N_1 latency and amplitude values along with the amplitude growth of wave N_1 between 60 and 80 dB nHL are shown in Table 5 (data collapsed across ears). We used t tests applying a significance level set at an unadjusted $p = 0.007$ (two-tailed), thus controlling for multiple testing by the Bonferroni multiple-significance t test correction (equivalent to adjusted $p = 0.05$). No significant differences on any of the auditory-evoked measures were observed between the two groups.

MRI Results

The tinnitus pitch information (Table 3) obtained from each of the participants with tinnitus was used in the creation of the FMPT signal that was used during sound-evoked fMRI testing of participants with tinnitus as well as their respective matched-control participants. fMRI recordings were obtained for both FMPT as well as BBN signals. All participants reported that they were able to hear the FMPT and BBN signals without difficulty during fMRI recording, but did not report hearing their tinnitus during that time. As shown in Figure 3, the whole brain voxel-wise comparison between the groups for the FMPT stimuli revealed that the tinnitus group had higher brain activity in the frontal lobe compared to the control group. These regions included superior frontal gyrus (BA 9), superior medial frontal and middle frontal gyrus; all part of the attention network in the frontal lobe. The control group did not show any regions in the brain with increased activity compared to the tinnitus patients. These results indicate that the tinnitus group has increased activity in the attention network, suggesting hyper-attentiveness to pure-tone sounds, when the tone is played at the same frequency of tinnitus. To obtain percent signal change in these regions for each group, a mask was created using

Table 2. Average ARTs, LDLs, and DPOAEs in Tinnitus and Control Groups

Group	Ipsi ART–R/L (dB HL)	Contra ART–R/L (dB HL)	LDL–R/L (dB HL)	DPOAE–R/L (dB SPL)
Tinnitus	90.9 ± 6.1	93.9 ± 8.4	92.3 ± 8.7	6.9 ± 6.8
M ± SD	93.8 ± 7.1	94.1 ± 9.8	93.4 ± 7.6	4.9 ± 6.4
Control	91.5 ± 4.7	94.8 ± 5.6	100.3 ± 6.9	8.3 ± 4.6
M ± SD	93.1 ± 6.4	94.2 ± 4.5	99.4 ± 9.5	7.8 ± 6.9

Note: Contra = contralateral; Ipsi = ipsilateral; L = left ear; M = mean; R = right ear; SD = standard deviation.

Table 3. Tinnitus Group Gender Distribution, Tinnitus Duration, Tinnitus Pitch, and Questionnaire Scores

Pair	Gender	Tinnitus Duration	Tinnitus Pitch (Hz)	THI Score	TRQ Score
1	F	5 yr	3000	14	13
2	F	7 mo	4000	14	30
3	M	45 yr	8000	94	80
4	F	10 mo	8000	40	23
5	M	11 yr	1000	62	60
6	M	3 yr	4000	60	43
7	F	9 yr	2000	18	11
8	M	6 mo	8000	10	2
9	M	49 yr	3000	12	2
10	F	5 yr	250	68	60

Note: F = female; M = male.

the regions shown in Figure 3 and was applied to each individual participant's FMPT minus silence contrast data. As depicted in Figure 4A, the control group suppressed these regions when they heard the tone stimulus with a $-0.03 \pm 0.01\%$ signal change. The tinnitus group, however, did not suppress these regions, instead they activated these regions with a $0.06 \pm 0.02\%$ signal change; groups were found to be significantly different at $p < 0.001$. When the mask was applied to the BBN–silence contrast (Figure 4B), the tinnitus group showed a larger percent signal change ($0.07 \pm 0.03\%$) compared to the control group (0.006 ± 0.02). The groups were significantly different at a $p < 0.05$ threshold. These results showed increased activity in the frontal lobe in tinnitus patients to both FMPT and BBN signals, suggesting that participants with tinnitus are more attentive to sounds.

It was postulated that tinnitus patients have abnormal function in the auditory cortex, but no significant differences were seen in the auditory cortex in the whole brain voxel-wise analysis, so an anatomic mask of the auditory cortex was created and applied to the fMRI data. It was found that the participants with tinnitus showed 25% lower signal change ($0.38 \pm 0.07\%$) compared to control participants ($0.50 \pm 0.07\%$), in response to FMPT stimuli, as seen in Figure 5A. The groups were significantly different at $p < 0.05$. In response to the BBN stimuli, the tinnitus group showed a 16% lower signal change ($0.63 \pm 0.11\%$) compared to the control group ($0.74 \pm 0.07\%$), as shown in Figure 5B. This suggests that the auditory cortex in tinnitus patients may be desensitized to sound due to the constant tinnitus that these patients experience.

While an fMRI signal examines task-related (e.g., by pure tone) neural activity, it does not provide an assessment of baseline brain activity. To examine the baseline function, we collected resting-state CBF data in these participants, and the auditory cortex mask was applied to baseline CBF data. It was found that baseline relative CBF was lower in the tinnitus group ($1.26 \pm 0.06\%$) compared to the control group ($1.38 \pm 0.05\%$, $p < 0.05$) as shown in Figure 6A. When whole brain voxel-wise data were compared between groups, it was found that baseline CBF in participants with tinnitus was indeed lower in the auditory cortex bilaterally compared to control participants, as shown in Figure 6B. When frontal cortex (FC) mask was applied to baseline CBF data, the relative baseline CBF was 17% higher in the tinnitus group (Figure 7), although the difference was not significant ($p > 0.05$). These data may perhaps indicate diminished or abnormal baseline brain function in the auditory and frontal cortices in the tinnitus group.

For the resting state fcMRI data, the mean correlation coefficient between right and left auditory cortices was higher in the tinnitus group (0.82 ± 0.09) compared to the control group (0.70 ± 0.21), but the difference was not significant ($p > 0.06$).

Statistical Analyses

Based on the abovementioned audiological and MRI findings, we selected the 14 most promising objective test measures, shown in Table 6, for the membership prediction analysis. The LASSO technique for feature

Table 4. Mean ABR Wave V Latency and Amplitude at 80 dB nHL, and Mean Amplitude Growth of ABR Wave V from 60 to 80 dB nHL in Tinnitus and Control Groups

Group	Mean ABR Wave V Latency (msec)	Mean ABR Wave V Amplitude (μV)	Mean ABR Wave V Amplitude Growth (μV)
Tinnitus-R	5.74 ± 0.30	0.45 ± 0.22	0.20 ± 0.17
Tinnitus-L	5.77 ± 0.41	0.41 ± 0.12	0.15 ± 0.11
Control-R	5.80 ± 0.34	0.40 ± 0.18	0.18 ± 0.12
Control-L	5.87 ± 0.40	0.31 ± 0.14	0.10 ± 0.09

Note: L = left ear; R = right ear.

Table 5. Mean Group Data for ALR Wave N_1 Latency and Amplitude Scores at 80 dB nHL, and Mean Amplitude Growth of ALR Wave N_1 from 60 to 80 dB nHL

Group	Mean ALR Wave N_1 Latency (msec)	Mean ALR Wave N_1 Amplitude (μV)	Mean ALR Wave N_1 Amplitude Growth (μV)
Tinnitus-R	95.6 ± 11.1	5.6 ± 2.4	1.4 ± 1.5
Tinnitus-L	96.0 ± 12.8	5.1 ± 2.3	1.6 ± 0.8
Control-R	100.5 ± 8.7	4.2 ± 2.3	0.2 ± 0.3
Control-L	97.3 ± 9.2	4.2 ± 2.6	0.6 ± 1.2

Note: L = left ear; R = right ear.

extraction and the leave-one-out (20-fold) cross-validation for LASSO extracted the best predictors of tinnitus group membership and provided the optimal number of predictors (from a possible 14 measures/predictors) with robust estimates of the coefficients. This approach gave us the most regularized model, with its error within 1 standard error of the minimum.

In LASSO, the predictor variables entered the model in the order of importance as shown in Figure 8. In Figure 9, only the first six predictors entering the model are depicted for the sake of clarity. The more conservative and most regularized model approach that we used selected the two predictors shown below, with their corresponding coefficients and the order in which they enter the model as the penalty is decreased.

V3 12.9997529 (FC tone–silence)
V14 0.2347410 (ALR peak N_1 amp growth)

These results indicate that the increased FC fMRI activity to pure tones matching their respective tinnitus pitch (V3), and increased AEP wave N1 amplitude growth in the tinnitus group (V14) are the top two predictors of tinnitus group membership. These findings suggest that the amplified responses to acoustic signals leading to hyperactivity in attention regions of the brain may be the underlying diagnostic indicators of tinnitus. As mentioned earlier, subjective scores from THI and TRQ questionnaires were not included as potential predictors, since the scores are zero for the control group. Although these scores varied considerably within the tinnitus group, the correlations between the THI and TRQ scores with V3–fMRI FC activity for the tone–silence variable (the top predictor of the tinnitus group), were low, that is, 0.051 (p = 0.89) and 0.21 (p = 0.55), respectively, both statistically nonsignificant.

Figure 10 depicts the misclassification errors for various values of log (λ) using the leave-one-out cross-validation technique. Leave-one-out cross-validation involves using one observation as the validation set and the remaining observations as the training set. This is repeated on all 20 ways to cut the original sample on a validation set of 1 observation and a training set of 19 observations, which gives an estimate of the misclassification rate as shown by the red dots. A 95% confidence interval for the estimated misclassification rate is also provided (error bars). The two selected λs are indicated by the vertical dotted lines. The left dotted line corresponds to the values of log(λ) for minimum mean cross-validated error, which selected the top six predictors. The right dotted line corresponds to the most conservative regularized model that chose the top two predictors V3 and V14.

DISCUSSION

Traditionally THI and TRQ questionnaires have been used as key test measures to differentiate tinnitus from nontinnitus patients. As pointed out earlier, this study focused on identifying potential objective

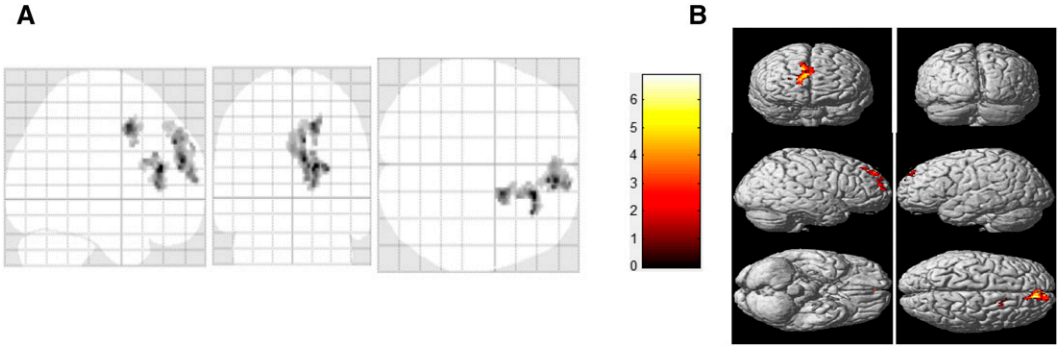

Figure 3. Whole brain voxel-wise results indicating regions where the tinnitus group shows hyperactivity compared to the control group, in response to the FMPT stimulus, significant at family-wise error rate (FWE)-corrected p = 0.005. (A) Glass brain view shows that hyperactive regions are present only in the frontal lobe. (B) Activations rendered on a single participant brain for better visualization.

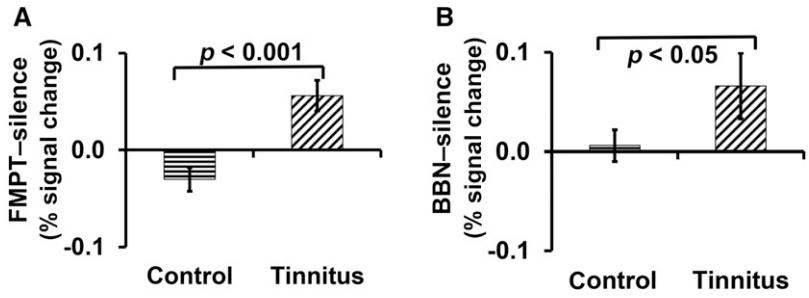

Figure 4. fMRI percent signal change in the frontal lobe region (regions shown in Figure 3A). (A) The control group showed suppression of activity in the frontal lobe region, while the tinnitus group showed increased activity; groups are significantly different at $p < 0.001$. (B) The tinnitus group showed increased activity in the frontal lobe region compared to the control group in response to BBN; groups are significantly different at $p < 0.05$.

measures/predictors of tinnitus group membership by incorporating the findings from multimodal objective test procedures. The measures we used to separate the tinnitus group from the nontinnitus group were based on these questionnaires, with the THI and TRQ scores being zero in all of our nontinnitus patients. Thus, the THI/TRQ questionnaires served as the classifying variables and determined the group membership and were not used as possible predictors. We acknowledge the findings from earlier studies that indicate a high correlation between fMRI activity and THI/TRQ scores; however, in our study we found that the correlations between the THI and TRQ scores with V3–fMRI FC activity for tone–silence (the top predictor) in the tinnitus group were low and statistically nonsignificant.

The results of this study support our proposition that audiological and MRI markers are attainable in participants with tinnitus, and can be used in differentiating individuals with and without tinnitus. In this study, the participants in the tinnitus group were relatively diverse in terms of history of noise exposure, tinnitus-related stress, duration of tinnitus, tinnitus pitch, and hearing thresholds. Yet with pairwise matching of participants with tinnitus with control participants for age, gender, and hearing thresholds, the study revealed that multimodal markers (audiological and MRI) of tinnitus are present.

In the present study, we observed that ABR wave V and ALR wave N_1 amplitudes were higher for the tinnitus group compared to the control group. This is comparable to the findings of Gu et al (2012) who reported elevated activity in the auditory brainstem among tinnitus patients. However, mere amplitude measures may be inadequate to show subtle differences between the groups. Hence, based on the positive findings from our earlier studies (Gopal et al, 2004; 2015), we examined the intensity-dependent amplitude functions for ABR wave V and ALR wave N_1 as potential predictor variables. As our current study included participants with various levels of hearing thresholds, we chose to measure the amplitude growth at relatively high intensity levels to obtain repeatable waveforms from all participants. LASSO regression results identified the amplitude growth of N_1 as one of the top two predictors of tinnitus group membership.

The results revealed that the tinnitus group characteristically exhibited an augmented intensity-amplitude function. This augmentation seen in the tinnitus group for ALR wave N_1 elicited with a 1000-Hz tone burst stimulus occurred even in participants with tinnitus whose tinnitus pitch was below 1000 Hz (n = 1) or above 1000 Hz (n = 8). The increase in neural firing from the introduction of the external signal (1000-Hz tone burst) in conjunction with the presumed pathological

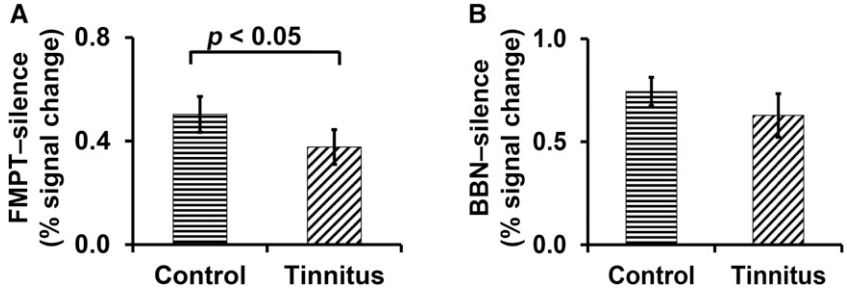

Figure 5. fMRI percent signal change in the auditory cortex. (A) In response to FMPT stimulus, the tinnitus group showed 25% less activation compared to the control group; the groups are significantly different at $p < 0.05$. (B) In response to BBN stimulus, the tinnitus group showed 16% less activity compared to the control group.

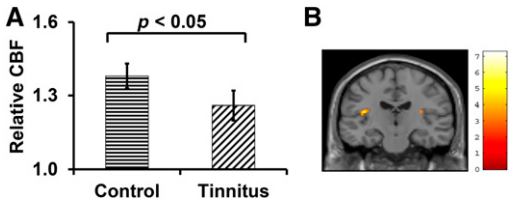

Figure 6. Relative baseline CBF results from the auditory cortex mask. (A) The tinnitus group showed 8% lower baseline CBF compared to the control group; groups were significantly different at $p < 0.05$. (B) Whole brain voxel-wise comparison between the groups showed that the tinnitus group had a lower baseline CBF in the auditory cortex bilaterally compared to the control group.

increase in the spontaneous firing of central auditory neurons in tinnitus patients may be sufficiently strong to induce significant augmentation in and around their tinnitus pitch. The same external signal when presented to the matched-control participants without tinnitus did not result in augmentation to the same degree. An earlier study (Kadner et al, 2002) had reported that tinnitus had an interesting impact on the intensity dependence (or intensity-dependent amplitude function) of the ALR N100 potential in a frequency-specific manner. They contended that the tinnitus-related activity produced an increase in firing rate of neurons in the areas around their tinnitus pitch, and caused inhibition of neighboring regions via lateral inhibitory mechanisms. However, we attribute the augmentation seen in our participants with tinnitus, regardless of the tinnitus pitch, to altered gain regulation in the central auditory system as proposed by earlier studies (Arnold et al, 1996; Lockwood et al, 1998; Kaltenbach et al, 2004).

Multiple generators have been reported for ALR wave N_1, including the temporal, frontal, and limbic lobes (Giard et al, 1994; Anderer et al, 1998; Picton et al, 1999; Gallinat et al, 2002; Rosburg et al, 2005). The contribution of the primary and secondary auditory cortex in the generation of wave N_1 and their association with attention to the stimulus source have been

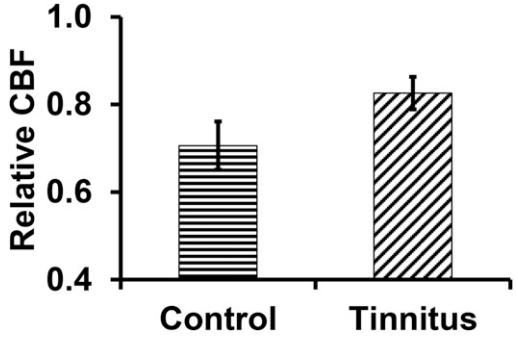

Figure 7. Relative baseline CBF results from the FC mask. The tinnitus group showed 17% higher baseline CBF compared to the control group; however, the difference was not significant ($p > 0.05$).

Table 6. The 14 Objective Variables Selected for LASSO Analysis

Variable/Predictor	Test Measure
V1	fMRI auditory cortex (tone–silence)
V2	fMRI auditory cortex (noise–silence)
V3	fMRI FC (tone–silence)
V4	fMRI FC (noise–silence)
V5	Auditory cortex CBF
V6	FC CBF
V7	fcMRI Auditory cortex right and left
V8	DPOAE
V9	ABR peak V latency
V10	ABR peak V amplitude
V11	ABR peak V amplitude growth
V12	ALR peak N_1 latency
V13	ALR peak N_1 amplitude
V14	ALR peak N_1 amplitude growth

widely recognized (Santos Filha and Matas, 2010). Walpurger et al (2003) reported a less distinct habituation of the N_1 amplitude in tinnitus patients and attributed it to the failure on the part of the tinnitus patients to properly habituate to auditory stimuli. The intensity dependency or response augmentation of wave N_1 is considered an index of central serotonergic activity, associating higher steepness of the N_1 amplitude to lower central serotonin activity (Cartocci et al, 2012). In our earlier study (Gopal et al, 2004), we showed that unmedicated clinically depressed individuals with low levels of serotonin exhibited a significantly larger growth of N_1 amplitude compared to the normal control group. Earlier literature has discussed a common pathophysiology underlying tinnitus and depression (Langguth et al, 2011; De Ridder et al, 2013). The augmented N_1 amplitude suggestive of abnormal gain, seen in this present study with nondepressed participants, may perhaps reflect the gain changes in cortical activity secondary to subtle serotonin modulation. Yet another possibility discussed in the literature for abnormal gain in the auditory pathways is reduced GABA-mediated inhibition (Gu et al, 2010). Further discussion of the complex pathophysiology of tinnitus as it relates to serotonin or GABA is beyond the scope of this study. Nevertheless, the augmented amplitude growth function may well be an electrophysiological marker for the tinnitus condition. It must be noted that our analysis did not choose ABR amplitude growth function as a significant predictor of tinnitus group membership, which may indicate that the amplitude growth is more pronounced at the cortical level, reflecting the modulatory effects of tinnitus on cortical auditory and attention areas of the brain.

The neuroimaging data from fMRI recordings indicated a lesser signal change between silence and external acoustic signals (FMPT and BBN) in the auditory cortex for the tinnitus group compared to the control

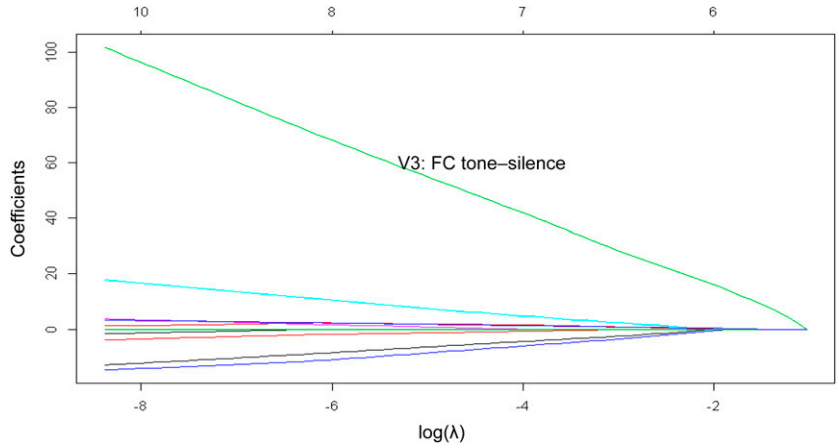

Figure 8. The LASSO logistic regression curve with the ten most dominant predictors. It shows the path of its coefficient against log(λ) as λ varies. It is evident that V3 (FC tone–silence) enters the model very early and strongly dominates the other predictors. The dominance of V3 makes it hard to identify the progression of any other predictor and the order in which it enters the model. The *x* axis above indicates the number of nonzero coefficients at the current log(λ).

group. This suggests that the auditory cortex in tinnitus patients may perhaps be desensitized to sound due to their constant tinnitus. Contrary to the above findings, the external acoustic signals exhibited a greater increase in FC activity in the tinnitus group compared to the control group. Several studies in the past have shown the involvement of the prefrontal cortex in tinnitus and have implicated attention as a factor in the development and maintenance of tinnitus (Schlee et al, 2009; Heeren et al, 2014; Husain, 2016), although specific attention-related neural changes have not been identified (Roberts et al, 2013). Additionally, the frontal lobe is known to be associated with conscious processing of tinnitus signals (Lanting et al, 2009; De Ridder et al, 2013), which may stem from strong interactions between auditory and FC regions. A number of studies have revealed the role of attention in tinnitus in various ways, for example, by demonstrating disruption in the allocation of attention to nonauditory stimuli, and by showing reduction of tinnitus with habituation training and cognitive distraction (Burton et al, 2012).

Our findings demonstrating increased activity in the tinnitus group predominantly in the FC (normally associated with attention and emotion) are comparable with previous studies (Mirz et al, 1999; 2000). Roberts et al (2013) argued that tinnitus may be the result of an interaction of the aberrant engagement of top-down attention and abnormal bottom-up attention. Using anatomical MRI, Leaver et al (2012) reported that participants with chronic tinnitus had reduced gray matter in ventromedial prefrontal cortex (vmPFC), and increased gyrification of dorsomedial prefrontal cortex. This effect was not correlated with tinnitus distress exhibited by their participants. They concluded that the neural systems related to tinnitus perception are distinct from those affected by tinnitus distress, mood disorders, and noise sensitivity. Results of this study concur with the above inference as our tinnitus group showed a wide range of tinnitus severity scores, but

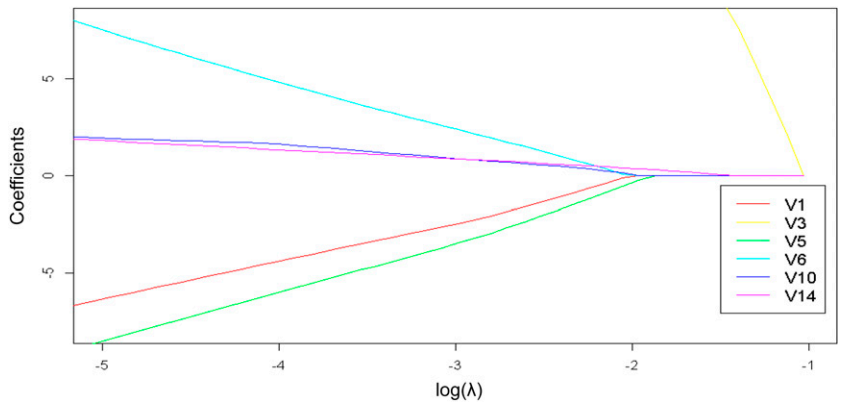

Figure 9. Same as Figure 8 except only the first six predictors that enter the model are depicted for the sake of clarity. Again, it is evident that V3 (FC tone–silence) enters the model very early followed by V14, V5, V1, V6, and V10, respectively.

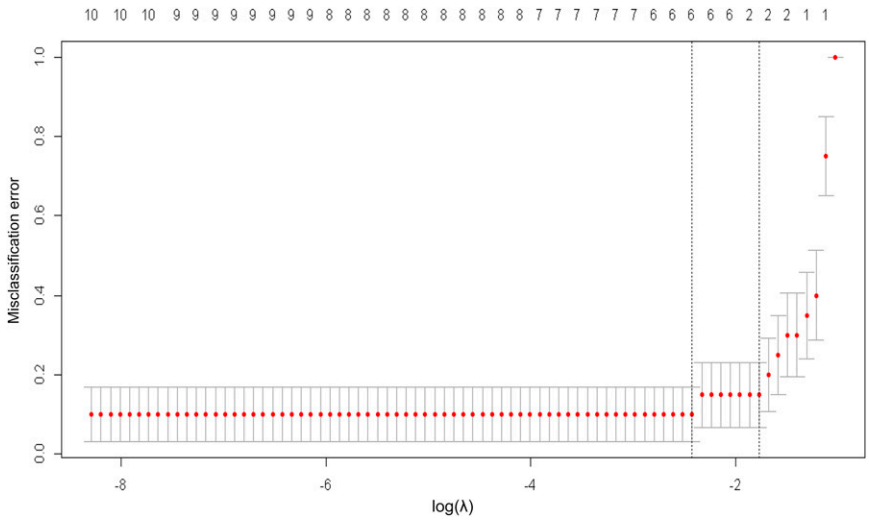

Figure 10. The cross-validation curve (red dotted line), and upper and lower standard deviation curves along the λ sequence (error bars). The *x* axis depicts the penalty log(λ) and the *y* axis depicts the misclassification error for the chosen value of log(λ) in the *x* axis. The *x* axis on the top indicates the number of predictors at the current log(λ). The two vertical dotted lines correspond to the values of log(λ) for minimum mean cross-validated error (left) and the conservative most regularized model (right). We used the most regularized model which selected the top two predictors as evident from the figure.

the scores were not correlated with the FC activity, our top predictor of tinnitus group membership. Whole brain voxel-wise results were considered significant at an family-wise error rate (FWE)-corrected *p* value of 0.005. This is a very stringent threshold, so we tested the same group comparison results at an FWE-corrected *p* value of 0.05. BOLD percent signal changes to the FMPT and white noise stimuli were extracted from the clusters that were significantly activated at each of the two *p* values. Following this, cross correlation coefficients were determined for percent signal change obtained at each *p* value. Results indicated a correlation coefficient of >0.99 for both control participants and participants with tinnitus. So, we decided to report the FWE-corrected *p* value of 0.005.

Furthermore, the vmPFC may be related to the perception of tinnitus and the gating mechanism as discussed by Rauschecker et al (2010). Seydell-Greenwald et al (2012) validated the model of Rauschecker et al (2010) by demonstrating that tinnitus patients had larger BOLD responses in vmPFC during auditory tasks compared to control participants. This is thought to be due to "vmPFC overdrive" to compensate for not achieving the desired regulating influence on other centers, especially the auditory centers. In this study, the regions in the FC associated with increased activity in response to acoustic signals were superior frontal gyrus, superior medial frontal and middle frontal gyrus—all parts of the attention network. This reflects persistent overattention drawn to the tinnitus, or in our case to tinnitus-like signals (FMPT) as participants with tinnitus did not report experiencing tinnitus during the task, and suggests increased activity in a spatially extensive population of neurons in the FC.

Our results are amenable with the recent findings from Heeren et al (2014), where the authors argued that participants with tinnitus suffer impairments in executive control (which involves prefrontal brain areas) that is necessary to focus attention on task-relevant information while inhibiting task-irrelevant stimuli. Additionally, in our previous case study (Gopal et al, 2015) we found evidence at the therapeutic level. In that study, the participants with tinnitus exhibited significant changes in audiological and fMRI measures following antioxidant treatment. While on the antioxidant acetyl-L-carnitine, the participant's amplitude growth for ABR wave V from 40 to 80 dB nHL was less steep, and the fMRI measures showed less brain activation for both FMPT and BBN signals. Most importantly, all of these changes were accompanied by the participant's report that she barely noticed her tinnitus and had less tinnitus-related stress (also seen from her THI score) while on the antioxidant.

In this study, the resting state CBF was found to be lower in the auditory cortex and higher in the FC in the tinnitus group compared to the control group. Hyperactivity in the FC may have resulted in an increase in its resting CBF, owing to the mechanism of neurovascular coupling. This study also found that the functional connectivity between the right and left auditory cortices in the tinnitus group was higher than that in the control group. These results are comparable to the findings from the Chen et al (2015) study, which showed increased interhemispheric voxel-mirrored homotopic connectivity in chronic tinnitus patients in several brain areas, including the middle temporal gyrus. However, the LASSO regression analysis did not identify

resting state CBF or fcMRI variables as our top predictors. Our current findings do not include fcMRI analyses of auditory cortex with other brain areas associated with emotional and attentional networks, thus we are currently unable to comment any further on the connectivity issue.

Thus our study outcomes are supportive of earlier findings of abnormal central gain in participants with tinnitus (Arnold et al, 1996; Lockwood et al, 1998; Kaltenbach et al, 2004), as reflected by the augmented amplitude growth of the ALR wave N_1. Our results are also supportive of the modified neural activity concept involving attention in tinnitus patients (Roberts et al, 2012; 2013; Paul et al, 2014), depicted as increased activity in FC. These changes can be attributed to neural circuitry changes secondary to the existence and perception of tinnitus. Thus the upregulation of attention networks may perhaps be the central characteristic of tinnitus patients, displayed as sound-induced elevated brain activation. The interpretation of our findings is that augmentation of ALR wave N_1 secondary to acoustic stimulation represents an overall surge of excitatory postsynaptic activity. The increase in BOLD signals in the FC regions upon acoustic stimulation represents a surge of overall synaptic activity in those areas. Areas of the FC are vital for attentional processes, and most models of attention consider the FC a central hub that interacts with modality-specific regions such as auditory cortices for auditory stimuli (Heinrichs-Graham et al, 2014). The involvement of the FC in our findings is not surprising as it is an important part of the network involved in the top-down control of attention that can bias sensory processing of information that is behaviorally relevant (Rossi et al, 2009).

The results of this study support our hypothesis that there are auditory and MRI-based measures that are predictive of tinnitus group membership. Our contention is that the hyper-attentiveness to pure-tone stimulus matching the tinnitus pitch, which results in increased activity in FC attention networks, is perhaps the most important marker of tinnitus. Increased activity in the FC stemming from its amplified response to acoustic signals may be the underlying component responsible for differentiating participants with tinnitus from participants without tinnitus. The study offers a renewed view of auditory electrophysiological and MRI-based profiles of tinnitus-related activity, and provides ample evidence for designing a larger scale study. Due to the small number of participants and a multitude of test measures, we were hesitant to add more test measures to the LASSO analyses such as fcMRI measures between auditory cortex and emotional centers of the brain. Forthcoming articles will discuss additional fcMRI data in more detail. Furthermore, implementing a bilateral auditory stimulation protocol for fMRI recordings, and combining right and left brain regions for analysis purposes could have masked some of the hemisphere-specific differences. The small sample size and the lack of subgroups may perhaps have contributed toward a lack of significance in other test measures. Although this study may preclude generalization, it emphasizes the need for expansion to a larger participant pool with appropriate subgrouping of tinnitus patients, and inclusion of additional findings such as fcMRI measures between auditory cortex and the limbic areas.

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