Neural Abnormalities Underlying Tinnitus and Hyperacusis

by

Jianwen Wendy Gu

Bachelor of Science in Physics, MIT, 2003
Master of Engineering in Biomedical Engineering, MIT, 2004

Submitted to the
Harvard-MIT Division of Health Sciences and Technology
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Health Sciences and Technology

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

June 2011

Author .. ....
Harvard-MIT Division of Health Sciences and Technology
May 17, 2011

Certified by .....
Jennifer R. Melcher, PhD
Associate Professor of Otology and Laryngology and HST
Thesis Supervisor

Accepted by ....
Ram Sasisekharan, PhD
Edward Hood Taplin Professor of HST and Biological Engineering
Director, Harvard-MIT Division of Health Sciences and Technology

Neural Abnormalities Underlying Tinnitus and Hyperacusis

by

Jianwen Wendy Gu

Submitted to the Harvard-MIT Division of Health Sciences and Technology
on May 17, 2011, in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Health Sciences and Technology

Abstract

Tinnitus, the ongoing perception of sound in the absence of a physical stimulus, and hyperacusis, the intolerance of sound intensities considered comfortable by most people, are two often co-occurring clinical conditions lacking effective treatments. This thesis identified neural correlates of these poorly understood disorders using functional magnetic resonance imaging (fMRI) and auditory brainstem responses (ABRs) to measure sound-evoked activity in the auditory pathway. Subjects with clinically normal hearing thresholds, with and without tinnitus, underwent fMRI or ABR testing and behavioral assessment of sound-level tolerance (SLT). The auditory midbrain, thalamus, and primary auditory cortex (PAC) showed elevated fMRI activation related to reduced SLT (i.e. hyperacusis). PAC, but not midbrain or thalamus, showed elevated fMRI activation related to tinnitus, perhaps reflecting undue attention to the auditory domain. In contrast to fMRI activation, ABRs showed relationships only to tinnitus, not SLT. Wave I of the ABR, which reflects auditory nerve activity, was reduced in tinnitus subjects, while wave V, reflecting input activity to the midbrain, was elevated. Wave I reduction in tinnitus subjects suggests that auditory nerve dysfunction apparent only above threshold is a factor in tinnitus. Because ABRs reflect activity in only one of multiple pathways from cochlear nucleus to midbrain, the wave V elevation implicates this particular pathway in tinnitus. The results directly link tinnitus and hyperacusis to hyperactivity within the central auditory system. Because fMRI and ABRs reflect different aspects of neural activity, the dependence of fMRI activation on SLT and ABR activity on tinnitus in the midbrain raises the possibility that tinnitus and hyperacusis arise in parallel from abnormal activity in separate brainstem pathways.

Thesis Supervisor: Jennifer R. Melcher, PhD
Title: Associate Professor of Otology and Laryngology and HST

Acknowledgements

My advisor Jennifer Melcher has been a wonderful mentor in every sense of the word: scientifically, professionally, and personally. My committee members have given me many helpful comments and suggestions: Charlie Liberman, Barb Herrmann, Joe Mandeville, and Bob Levine. Special thanks to Bob for recruiting his tinnitus patients to participate in my studies. I am very grateful to my co-workers, Barbara Kiang and Inge Knudson, for their kindness, support, and help. I have truly enjoyed their company. I thank Nelson Kiang for his advice and books, and for many interesting conversations. Chris Halpin taught me a lot about working with human subjects. Chris Shera gave me useful data analysis suggestions and let me borrow his oscilloscope for the ABR study. Previous members of the group, Eui-Cheol Nam, Dave Langers, and Elif Özdemir, helped me collect data for the fMRI study. The Eaton-Peabody Laboratory would not function nearly as well without the staff, and I am particularly grateful to Dianna Sands, Jess Cunha, Ish Stefanov, and Frank Cardarelli for effectively and efficiently addressing my questions and issues. John Guinan and Nik Francis graciously allowed me to use Chamber 3b for the ABR study. I thank the study participants, especially the tinnitus patients who knew that they would not benefit directly from participating but wanted to help others with the condition by supporting science. My previous advisors, Denny Freeman and A.J. Aranyosi, laid the foundation for my scientific growth and have continued to help me to this day. I am deeply grateful to my family and friends whose love and encouragement have sustained my spirit.

Contents

List of Figures

Chapter 1

Introduction

Tinnitus, the ongoing perception of sound in the absence of a physical stimulus, is a clinical condition that is rarely curable. People often describe their tinnitus as ringing, buzzing, chirping, or whooshing. Chronic tinnitus affects 5–15% of the general population [Heller, 2003]), and 1–3% of the general population suffers reduced quality of life as a result of tinnitus [Dobie, 2003]. Tinnitus is a common symptom of acoustic trauma, acoustic neuroma, and Menieres disease (Hoffman and Reed, 2004). Tinnitus is often associated with depression, anxiety, and sleep disruption [Dobie, 2004]. The prevalence of tinnitus increases with increasing hearing loss, and hearing loss increases with increasing age [Hoffman and Reed, 2004]. However, for a given hearing loss, younger people are more likely to have tinnitus [Hoffman and Reed, 2004], indicating that the relationship of tinnitus to hearing loss and age is complex. One of the mysteries of tinnitus is that although hearing loss is associated with tinnitus, people with clinically normal thresholds can have tinnitus and not everyone with hearing loss has tinnitus.

Hyperacusis, the intolerance of sound levels considered comfortable by most people, is a clinical condition that often accompanies tinnitus [Baguley, 2003]. One study estimated the prevalence of hyperacusis to be 8–9% in Swedish adults. Hyperacusis can be associated with depression, migraines, and Williams syndrome as well as other conditions [Katzenell and Segal, 2001].

Tinnitus and hyperacusis have been hypothesized to arise from excessive levels

of neural activity (commonly referred to as hyperactivity) in the central auditory pathway (Jastreboff and Hazell, 1993). No empirical evidence has linked neural hyperactivity to hyperacusis. However, neural hyperactivity has been demonstrated in both animal and human studies of tinnitus. For instance, elevated spontaneous activity has been found in the cochlear nucleus and inferior colliculus of animals showing behavioral evidence of tinnitus [Brozoski et al., 2002, Kaltenbach et al., 2004], [Brozoski et al., 2007, Bauer et al., 2008]. Elevated sound-evoked activity in the auditory cortex has been demonstrated in animals exposed to an ototoxic drug [Salvi et al., 2000]. Evidence for cortical reorganization, which has also been proposed to lead to tinnitus [Eggermont and Roberts, 2004] has been found in animals after acoustic trauma: cortical neurons that represented frequencies in the hearing loss region pre-trauma changed their tuning to represent frequencies near the border between hearing loss and normal thresholds after trauma [Eggermont and Komiya, 2000].

Few human studies comparing tinnitus patients to non-tinnitus control subjects have matched hearing thresholds between the two groups. Commonly, the tinnitus subjects had hearing loss while the non-tinnitus subjects had normal thresholds. Thus, the effects of tinnitus and hearing loss were impossible to disentangle. Of particular relevance to this thesis are: (1) One of the functional magnetic resonance imaging (fMRI) studies that controlled for hearing loss reported greater sound-evoked activation in the inferior colliculus of subjects with tinnitus than those without [Melcher et al., 2009]. (2) Three auditory brainstem response (ABR) studies that controlled for hearing loss, but reported conflicting results: greater sound-evoked activity by [Attias et al., 1996] and [Kehrle et al., 2008], but not [Attias et al., 1993].

Despite the close association between tinnitus and hyperacusis, none of the previous studies, human or animal, considered the effects of hyperacusis. Thus, whether the neural hyperactivity in previous reports was related to tinnitus, hyperacusis, or both is unknown. This thesis addresses this knowledge gap in the tinnitus field by using fMRI and ABRs to measure sound-evoked neural activity in people with and without tinnitus who also underwent behavioral testing to assess sound-level tolerance (SLT). Because the subjects in these studies did not have highly abnormal SLT

(i.e. hyperacusis), we refer to those who were less tolerant of sound level to have abnormal SLT, a milder condition than the severe hyperacusis that propels people to seek clinical help.

Functional MRI and ABRs are complementary neuroimaging techniques that enabled us to examine different aspects of neural activity: fMRI is sensitive to gross neural activity while ABRs reflect the activity of specific neuronal populations. The spatial resolution of fMRI is sufficient for capturing activity in the cochlear nucleus. While ABRs have poor spatial resolution, extensive work has been done relating the waves of the ABR to the locations of the neurons generating them. Therefore, we were able to measure sound-evoked activity in the central auditory pathway attributable to discrete structures and/or neuronal populations from auditory nerve to auditory cortex.

Chapter 2

Tinnitus, diminished sound-level tolerance, and elevated auditory activity in humans with clinically normal hearing sensitivity

2.1 Introduction

Subjective tinnitus, the ongoing perception of sound in the absence of any physical stimulus, is a common clinical condition lacking effective treatments. Severe hyperacusis, in which sound intensities considered comfortable by most people are unbearably loud [Baguley, 2003], can accompany tinnitus. Importantly, "hyperacusis" does not imply a better-than-normal threshold sensitivity to sound, nor is it the same as loudness recruitment, the abnormally rapid growth in perceived loudness with increasing sound intensity that occurs with hearing loss [Tyler et al., 2003]. Even people with clinically normal auditory thresholds can have hyperacusis, as was the case for participants in this study. Tinnitus and hyperacusis have been hypothesized to arise from "abnormal gain" within the auditory pathway. The presumptive neural gain results in abnormal perception: of sound without a physi-

cal stimulus (tinnitus) or of sound loudness (hyperacusis) [Levine and Kiang, 1995, Salvi et al., 2000]. Neural abnormalities suggestive of "abnormal gain" have been reported in animals showing behavioral evidence of tinnitus, and in humans with tinnitus [Arnold et al., 1996, Lockwood et al., 1998, Kaltenbach et al., 2004]. The defining characteristic of hyperacusis, reduced tolerance of sound on the basis of loudness, has yet to be linked to any neural abnormality. The present experiments build on the following finding demonstrated with fMRI: on average, tinnitus subjects show elevated responses to sound in the auditory midbrain compared to non-tinnitus controls [Lanting et al., 2008, Melcher et al., 2009]. Melcher et al. closely matched tinnitus and non-tinnitus subjects on major covariates of tinnitus: threshold sensitivity, depression, anxiety, and age, thus excluding the possibility that these variables, rather than tinnitus, accounted for the increased sound-evoked activity in the tinnitus group [Melcher et al., 2009]. The study did not, however, consider a remaining and potentially crucial variable: hyperacusis. Thus, it remains unclear whether the previously documented elevations in sound-evoked activation reflect tinnitus, hyperacusis, or both.

The possibility of a relationship between hyperacusis and the elevated sound-evoked activity seen in the midbrain of tinnitus subjects is raised because of (1) psychophysical data in normal subjects showing increases in perceived loudness with increasing sound intensity, repetition rate, or bandwidth and (2) fMRI data, also in normal subjects, showing increases in midbrain activation for similar parameter variations [Brittain, 1939, Pollack, 1951, Zwicker et al., 1957, Harms and Melcher, 2002, Hawley et al., 2005, Sigalovsky and Melcher, 2006] Importantly, the increases in loudness and activation cannot be entirely attributed to increases in sound energy: increasing the bandwidth of sound while holding total sound energy constant still increases perceived loudness and fMRI activation of the midbrain [Zwicker et al., 1957, Hawley et al., 2005]. This tendency in normal subjects leads to the following hypothesis: subjects abnormally intolerant of sound on the basis of loudness would have elevated activation to sound in the midbrain, and possibly other auditory centers. The present study tested this hypothesis, and more generally addressed whether

tinnitus, abnormal sound-level tolerance (SLT), or both contribute to the elevated activation to sound reported previously in tinnitus subjects.

This chapter was published as an article in the Journal of Neurophysiology and used in this thesis with permission granted by the American Physiological Society (doi:10.1152/jn.00226.2010).

2.2 Methods

2.2.1 Subjects

Twenty-seven subjects underwent behavioral testing followed by functional imaging. 13 subjects had tinnitus (age = 43 ± 3 (mean $\pm$ standard error); 10 male; 10 right-handed and one ambidextrous). The remaining 14 did not have tinnitus (age = 45 ± 3; 8 male; 13 right handed and one ambidextrous). Each subjects SLT was quantified as described below. Subjects had no known neurological disorders. Mean characteristics for tinnitus and non-tinnitus subjects with normal and abnormal SLT are given in Table B-1. Characteristics of individual subjects are given in Tables B-3–B-5. All but one of the tinnitus subjects (#10) was recruited through the Tinnitus Clinic at the Massachusetts Eye and Ear Infirmary (MEEI). The remaining tinnitus subject and all non-tinnitus subjects were recruited from advertisements in local newspapers and personal contacts. All of the subjects recruited through the Tinnitus Clinic reported tinnitus, not sound-level intolerance, as their primary complaint. Any abnormality of SLT was mild and not always self-recognized. This study was approved by the institutional committees on the participation of human subjects at the Massachusetts Institute of Technology, Massachusetts General Hospital, and MEEI. All subjects gave their written informed consent.

2.2.2 Hearing threshold

Subjects had normal pure tone thresholds ($\leq$ 25 dB HL) at octave intervals from 250 through 8000 Hz, except for two subjects (#72 and #85), both with tinnitus,

in whom the threshold at 8 kHz was 30–35 dB HL in one ear. While elevated high-frequency thresholds are not the norm in subjects of these ages (45 and 52 years), this finding is not uncommon clinically. Mean pure tone threshold for the tinnitus and non-tinnitus subjects differed by 3 dB or less at any given frequency (Figure A-1, top). Figure A-1, middle and bottom, shows mean thresholds for tinnitus and non-tinnitus subjects respectively, separating subjects into two SLT categories: normal and abnormal (categories defined below).

2.2.3 Measuring loudness discomfort level

The highest tolerable level of broadband noise, the stimulus used during imaging, was determined for each subject as follows [Cox et al., 1997]. The noise was presented over headphones (TDH-39P) for about 1 second at a time at progressively higher levels beginning with 35 dB SPL. The noise level was incremented in 5 dB steps until 115 dB SPL was reached or the subject indicated the stimulus was uncomfortably loud. For each sound level, subjects rated the perceived loudness on a numerical scale that ranged from 1 ("very soft") to 7 ("uncomfortably loud"). Prior to testing, subjects were read a slightly modified form of the instructions given by [Cox et al., 1997]. The instructions were as follows:

"The purpose of this test is to find your judgment of the loudness of different sounds. You will hear sounds that increase in volume. You must make a judgment about how loud these sounds are. Pretend you are listening to the radio. How loud would it be? We will stop when you say the sound is uncomfortably loud (number 7). Please hold out as long as you can before indicating that the sound is uncomfortably loud."

Each ear was tested four times over which subjects converged on a highly repeatable assignment of number to level. The sound levels deemed uncomfortable during the last two of the four tests were averaged to obtain the loudness discomfort level (LDL) for the tested ear. LDL differed between ears by 6 dB or less in all but three subjects and by only 9 dB in those cases. Therefore, LDL averaged between the two ears was used as the final LDL for a given subject. Note that this test of assigning

a numerical rating to sounds of increasing level was used because it provided a systematic and reliable way to reach the well-defined end point of the range when the sound level is "uncomfortably loud" (i.e., 7). The test does not, however, provide an accurate measure of absolute loudness since the range of numbers available for assignment is restricted. Thus, ratings below "7" are not considered further.

One subject with tinnitus (#28) declined to do the full LDL test because of concerns about exposure to high-level sounds. He underwent an abbreviated procedure that tested each ear twice instead of four times, began the testing at 55, rather than 35 dB SPL, and did not provide loudness ratings other than an indication of 7 (uncomfortable). Of the two measurements made for each ear, the higher level deemed uncomfortably loud was taken to be the LDL.

2.2.4 Assessment of behavioral tinnitus characteristics

In tinnitus subjects, tinnitus pitch, tinnitus loudness, minimum masking level (MML) and residual inhibition were assessed. The pitch of the tinnitus was the pure tone frequency from 250 to 8000 Hz deemed most similar in pitch to the tinnitus (determined with half-octave frequency resolution). Tinnitus loudness was determined by adjusting the level of monaural broadband noise to match the loudness of the tinnitus to within 5 dB. For subjects with unilateral tinnitus, the noise was presented to the tinnitus ear. For subjects with bilateral tinnitus, each ear was tested. MML was the lowest level of binaurally-presented broadband noise needed to completely mask the tinnitus. Tinnitus loudness and MML were expressed relative to the detection threshold of the broadband noise (that is, in dB sensation level (dB SL)). The test for residual inhibition established whether or not one minute of binaurally-presented broadband noise at 10 dB above MML resulted in complete tinnitus suppression for any length of time after the noise was turned off. In Table B-5, the "residual inhibition" column indicates whether or not complete tinnitus suppression occurred.

2.2.5 Questionnnaires

All subjects completed a handedness questionnaire [Oldfield, 1971] and inventories of depression and anxiety [Beck et al., 1988, Beck et al., 1961]. For the latter, higher scores indicate greater symptom severity; the maximum score is 62 (depression) and 63 (anxiety). All subjects also completed a questionnaire assessing SLT (SLTQ) [Tyler et al., 2003]. This questionnaire asked subjects to rate their agreement with the following three statements by assigning a number from 0 (strongly disagree) to 100 (completely agree): (1) Many everyday sounds are unbearably loud to me. (2) Sounds that others believe are moderately loud are too loud to me. (3) I hear very soft sounds that others with normal hearing do not hear (taken from the Hyperacusis Intake questionnaire of [Tyler et al., 2003]). A score on the SLTQ was calculated as one minus the following: the sum of the three responses, divided by the maximum sum of 300. Since the third question on the SLTQ differs from the first two in that it probes the perceived loudness of near-threshold sounds, we examined whether it had an undue influence on SLTQ score and on our eventual categorization of subjects into normal and abnormal SLT groups. Two analyses indicated it did not: (1) The response to the third question was correlated with the sum of the responses to the first two questions (Spearman $r = 0.4$, $p = 0.03$); (2) No subjects SLT category changed when question 3 was omitted from the calculation of the SLTQ score. (Note that in calculating the SLTQ score without question 3, the sum of the remaining two responses was divided by 200, not 300.) Subjects with tinnitus completed two additional questionnaires: one asking about the characteristics of their tinnitus (e.g., quality of percept, location) and a second assessing the effects of tinnitus on quality of life (the Tinnitus Reaction Questionnaire (TRQ) of [Wilson et al., 1991]). For the latter, higher scores indicate greater distress. The range of possible scores is 0 (no distress) to 104. The questionnaire data for each subject are given in Tables B-3–B-5.

2.2.6 Acoustic stimulation and visual task

During imaging, subjects were stimulated with 32-second segments of broadband continuous noise delivered binaurally at 50, 70, and 80 dB SPL. The periods of stimulation were separated by 36 or 38-second periods of no stimulation. Four alternations of stimulation/no stimulation comprised a single scanning run. Noise level was the same for all stimulation periods of a given run and was varied pseudorandomly across runs. The noise was low-pass filtered (10 kHz cutoff) and spectrally shaped to account for the frequency response of the insert earphones used for delivery of the sound stimulus (Sensimetrics Corp., Malden, MA). The noise spectrum measured on an artificial ear was flat to within 5 dB. In the scanner room, prior to imaging, detection threshold for the noise stimulus was measured separately for each ear to establish stimulus levels in dB SL. (The scanner coolant pump was turned off for these measurements as well as during the functional imaging described below.) Threshold ranged from 5 to 30 dB SPL (mean $\pm$ standard error for tinnitus subjects: 19 ± 2 dB SPL; non-tinnitus: 19 ± 2). Thus the 50, 70 and 80 dB SPL stimulation levels corresponded to 20–45, 40–65, 50–75 dB SL. Also, in the scanner room prior to imaging, subjects were presented with each stimulus level for the full 32-second duration to be used during imaging. Based on this exposure (longer than during the LDL test and in a different environment), eight subjects declined to be imaged with the 80 dB SPL stimulus. Subjects were instructed to watch a black cross in their field of view that briefly turned red at random intervals throughout each run (throughout both stimulation and no stimulation periods). They were further instructed to press a button whenever the color changed. The average interval between color changes was approximately 7 seconds, only short enough to prevent subjects from falling asleep.

2.2.7 Imaging

Subjects were imaged in a 3 Tesla scanner using a 12-channel head coil (Siemens Trio with Matrix head coil). In each imaging session: (1) T1-weighted, high-resolution anatomical images of the whole head were acquired (128 slices, thickness = 1.33 mm,

gap = 0.67 mm, in-plane resolution = 1 × 1 mm, TR = 2530 ms, TI = 1100 ms, TE = 3.39 ms). (2) The brain slices to be functionally imaged were selected based on the anatomical images. Ten parallel slices covered the brainstem, thalamus, and temporal lobe. The second posterior-most slice was positioned to intersect the inferior colliculi and cochlear nuclei. (3) Functional images were acquired using a blood oxygenation level dependent (BOLD) sequence (gradient echo, TE = 30 ms, flip = 90°, slice thickness = 6 mm, gap = 2 mm, in-plane resolution = 3.125 × 3.125 mm). Images of the ten selected slices were acquired, from posterior to anterior, in brief (< 1 s) clusters with a TR of approximately 8 s [Edmister et al., 1999, Hall et al., 1999]. The onset of stimulus presentation was delayed by 0, 2, 4, or 6 s relative to the first cluster of each run, so that the effective temporal sampling interval within the overall data set would be approximately 2 s. Typically, three runs were collected at each stimulus level, yielding 108 images per level. Functional image acquisition was synchronized to the subject's first pulse (measured using a pulse oximeter) following a minimum inter-image interval of 7.5 s, yielding a TR of approximately 8 s [Guimaraes et al., 1998].

2.2.8 Image processing

Image signal in the functional data was (1) corrected for slight movements of the head using SPM2, (2) corrected for drifts in amplitude over time, (3) normalized such that the time-average signal had the same (arbitrary) value for all runs
[Harms and Melcher, 2002]. The time series of images corresponding to functional runs at the same stimulus level were concatenated to form a single data set from which activation maps were derived by comparing image signal between sound on and off periods using an unpaired t-test (e.g., see Figure A-2A). A hemodynamic delay of 4 s was assumed in the assignment of images to the on and off periods. For subjects in whom two, rather than three runs were obtained (one or two stimulus levels in three subjects) or where four runs were obtained, the p-value result of the t-test was adjusted to what it would have been if the number of runs had been three. This adjustment was to multiply the t-score by the square root of the following: the number of images in three runs divided by the actual number of images collected.

Finally, for each stimulus level, the percent change in image signal was calculated on a voxel-by-voxel basis: percent signal change $= (S_{\text{on}} - S_{\text{off}})/[0.5(S_{\text{on}} + S_{\text{off}})] \times 100\%$ where S_{on} and S_{off} are the signal averaged over stimulus on and off periods, respectively.

2.2.9 Quantification of activation

Activation was quantified within regions of interest (ROIs) defined relative to gross anatomical landmarks. These ROIs included three subcortical structures, the inferior colliculus (IC), medial geniculate body (MGB) and cochlear nucleus (CN), localized as described in [Harms and Melcher, 2002] and [Hawley et al., 2005]. They also included the following cortical ROIs (Figure A-2B): (1) PAC, occupying the posterior medial two-thirds of Heschls gyrus (the more anterior one, or "first", when there are two) and defined to coincide with primary auditory cortex [Rademacher et al., 2001, Sigalovsky et al., 2006], (2) anterolateral Heschls gyrus (HGal), the remaining anterolateral third of first Heschls gyrus, coinciding with primary-like areas on the gyrus, (3) anteromedial area (AMA), the region anterior and medial to PAC and HGal; AMA also extended rostrally with a lateral limit corresponding to the medial edge of HGal at its rostral limit. (4) anterolateral area (ALA), the region lateral to AMA and anterior to HGal, (5) planum temporale (PT), defined as the region lateral and posterior to Heschls gyrus, but excluding any second Heschls gyrus (present on one side in 6 subjects).

Results of a previous cytoarchitectonic study of human superior temporal lobe guided the choice of cortical ROIs and their detailed definition. The study, by [Fullerton and Pandya, 2007], concluded that auditory cortex in humans has the same organizational plan as that of monkeys: a central core surrounded by medial and lateral belts. Further, they showed the relationship between cytoarchitectonically-defined core/belt areas and the gross anatomy of the human superior temporal lobe. These relationships were used to define AMA and PT so as to approximate the medial and lateral belt, respectively. PAC, HGal, and ALA correspond to the core.

Quantification of activation for each ROI was as follows. First, voxels showing a significant ($p \leq 0.01$) difference in image signal between stimulus on and off peri-

ods for at least one sound level were identified. Second, voxels with a percent signal change greater than 4% for any sound level were excluded because the response was considered artifactual. Third, activation was quantified by averaging percent signal change across the remaining voxels. Note that the voxels included in this average were the same for all stimulus levels. Finally, for each structure (IC, MGB, etc.), a single activation value was obtained by averaging the activation of the left and right ROIs for that structure. This averaging was deemed appropriate since systematic differences in percent change between the left and right hemispheres were not seen for any structure or for any subject group. In instances where percent change could only be determined for the ROI on one side (because of poor signal-to-noise on the other side; explained below), the activation from that one ROI was used instead of the average.

ROIs that failed to show activation at any sound level were handled in one of two ways depending on the most likely reason for the lack of activation: low percent signal change or poor signal-to-noise (SNR), where SNR was defined as the mean signal over time divided by the standard error of the mean. If at least two-thirds of the voxels comprising an ROI had a high enough SNR at a particular level to detect signal changes of 0.5% or less, and yet no activation was detected, percent signal change for the ROI was assigned a value of zero at that level. All other ROIs failing to activate were excluded from analysis since they could not be reasonably assigned a percent signal change value of zero. (Because the SNR of most voxels in the ROI was low, percent changes in excess of 0.5% were possible, but not detectable.) The number of excluded ROIs was as follows: PAC 0/54 hemispheres, HGal 8/54, PT 4/54, AMA 5/54, ALA 10/54, IC 2/54, MGB 23/54, CN 32/54). Since fewer than half the CNs produced usable data and firm conclusions could not be drawn from the remaining data, the CN will not be considered further in this report.

2.3 Results

2.3.1 Behavioral data: Measurements of sound-level tolerance

Subjects reported numerical loudness ratings that increased with increasing stimulus level: from 1 (very soft) to 7 (uncomfortably loud), or from 1 to 6 (loud, but okay) in 9 subjects for whom even the highest intensity stimulus possible with our equipment was not uncomfortable. These data provided one of the two measures of SLT used in this study: LDL (the sound level assigned a "7") or a lower bound on LDL in subjects whose highest numerical rating of loudness was "6". The second measure was SLTQ score. The two measures generally confirmed each other in that subjects with high LDLs had SLTQ scores near 1, indicating normal SLT by both measures, while those with lower LDLs tended to have lower SLTQ scores, indicating an intolerance of sound levels usually considered comfortable (Figure A-3; $r = 0.4$, $p = 0.05$; Spearman correlation).

2.3.2 Activation in the auditory midbrain and thalamus: Dependencies on sound-level tolerance, not tinnitus

Figure A-4 shows percent signal change to the 70 dB SPL sound stimulus plotted versus SLTQ score (left) and LDL (right), for the two subcortical structures that showed activation in a majority of subjects: IC and MGB (IC: 27 of 27 subjects; MGB: 19 of 27). The points in each panel correspond to individual subjects (tinnitus: filled circles, non-tinnitus: open circles). The dark solid line in each panel is a linear fit to the data while the lighter dotted lines each show what the fit would be if one of the data points were absent. There was a clear correlation between percent signal change and LDL and SLTQ score: percent change in IC and MGB increased with decreases in both of these measures. The fact that this trend is apparent in all of the dotted line fits to the data indicates that it is not reliant on any one data point. The correlation between percent signal change and SLTQ score was statistically significant in both IC

and MGB ($p = 0.02$ and 0.03, respectively; Spearman correlation) and the correlation between MGB activation and LDL was nearly so ($p = 0.06$; correlation coefficients and p-values are at the lower left of each panel in Figure A-4).

The dependence of percent signal change in the IC and MGB on SLT was confirmed by a complementary analysis that involved classifying tinnitus and non-tinnitus subjects into two SLT groups (normal and abnormal). Classification was based on LDL, SLTQ score, and whether or not subjects found the 80 dB SPL stimulus presented during scanning tolerable (see Figure A-4 and caption). Figure A-5 (top row, left) shows the average percent signal change at 70 dB SPL in the IC for each of the four resulting subject groups: tinnitus and abnormal SLT, non-tinnitus and abnormal SLT, tinnitus and normal SLT, non-tinnitus and normal SLT. Average percent change was greater for the two groups with abnormal SLT (Figure A-5, top, left panel: two left-most bars) than for the two with normal SLT (two right-most bars). A similar trend across groups was also apparent for MGB at 70 dB (Figure A-5, top, middle panel).

At 70 dB, the two groups with normal SLT, one with tinnitus and one without, showed comparable percent signal change in both the IC and MGB, suggesting that there was little or no effect of tinnitus on the sound-evoked activation levels of these structures. This was confirmed by a two-way ANOVA (tinnitus $\times$ SLT) showing a significant effect of SLT (IC: $p = 0.002$, MGB: $p = 0.004$) but not tinnitus ($p \leq 0.7$) and no interaction between these variables ($p \geq 0.4$).

Figure 5 (bottom left and middle) shows percent signal change in the IC and MGB for each subject group at the lower stimulus level of 50 dB SPL. In the IC, average activation showed a distribution across the four subject groups defined by tinnitus and SLT that was qualitatively similar to the distribution for 70 dB (Figure A-5, left, compare top and bottom rows). A two-way ANOVA of the 50 dB data showed a significant effect of SLT ($p = 0.03$) but not tinnitus ($p = 0.8$). The 50 dB data for MGB showed a distribution across subject groups that was qualitatively similar to the 70 dB data, but did not show statistically significant effects of SLT ($p = 0.2$, two-way ANOVA) or tinnitus ($p = 0.7$) likely due in part to the lower percent signal

changes produced generally by the 50 dB stimulus.

Even though eight of the subjects with abnormal SLT declined to be imaged with the 80 dB stimulus, activation at 80 dB SPL could still be compared between the two groups with normal SLT. Mean activation at 80 dB (Table B-2) was similar for these two groups in both the IC (1.0 vs 0.97%) and MGB (0.8 vs 0.7%) and did not differ significantly ($p \geq 0.8$; Wilcoxon rank-sum). Thus, the 80 dB data supported the data at lower levels in indicating no effect of tinnitus on sound-evoked activation levels in the IC and MGB.

To address whether the activation might depend on some variable correlated with SLT rather than depending on SLT per se, the following analyses were performed. The IC and MGB activation data at 50 dB and 70 dB were cross-correlated with each of the following: age, threshold for the continuous noise stimulus, score on the anxiety inventory, and score on the depression inventory. Cross-correlation with these variables was performed separately for each of the two structures and each stimulus level. While there was a non-significant tendency for IC activation at 70 dB to be correlated with age ($r = -0.34$; $p = 0.08$), none of the other cross-correlations showed even a trend ($p \geq 0.4$; Spearman correlation). (Note that p-value results of the correlations have not been corrected for multiple comparisons.) A three-way ANOVA (tinnitus $\times$ SLT category $\times$ age) also showed a tendency for IC activation at 70 dB to co-vary with age ($p = 0.07$; not corrected for multiple comparisons), but additionally confirmed a far more significant effect of SLT ($p = 0.002$) and none of tinnitus ($p = 0.9$). A three-way ANOVA was also performed on the activation data for each structure and stimulus level but replacing age with threshold, anxiety score, or depression score. The results again confirmed an effect of SLT, no effect of tinnitus, and, in most cases, no effect whatsoever of the third variable ($p \geq 0.1$; not corrected). The only exception was an effect of anxiety in the IC data. However, this effect (50 dB: $p = 0.05$; 70 dB: $p = 0.002$) was far less significant than that of SLT ($p = 0.005$ and $p = 0.00003$, respectively). The results of the analysis suggest that age and anxiety may have played some role in determining the magnitude of IC activation, but indicate that, among the factors tested, the dominant factor in the IC, and indeed

the only apparent factor in the MGB, was SLT. Note that SLT would have emerged as even more dominant if the p-value results of the analyses had been corrected for multiple comparisons (and hence increased), especially since the correction is only applicable to those variables not hypothesized, a priori, to be related to activation (that is, age, threshold, depression, anxiety, but not SLT, tinnitus).

2.3.3 Activation in auditory cortex: Dependencies on both sound-level tolerance and tinnitus

Figure A-6 shows percent signal change for the 70 dB SPL stimulus plotted versus SLTQ score (left column) and versus LDL (right column) for the five cortical areas, which together contained almost all of the activation on the superior temporal lobe (PAC, HGal, PT, AMA, ALA; defined in Figure A-2). As in the IC and MGB, percent signal change in the cortical areas tended to increase with decreasing LDL and with decreasing SLTQ score. Such trends were significant in three regions: PAC, which overlaps primary auditory cortex, HGal and ALA, which comprise primary-like cortical areas anterior to PAC. In all three regions, the Spearman correlation between percent signal change at 70 dB and one or both of the SLT measures was significant ($p \leq 0.05$; see lower left of each panel in Figure A-6).

In contrast to IC and MGB, some of the cortical areas showing an effect of SLT also showed an effect of tinnitus. Data for one region showing dependencies on both variables, PAC, can be seen in Figure A-5 (top right) where, for the 70 dB stimulus, percent signal change in the tinnitus, normal SLT group is close in height to that of the groups with abnormal SLT and greater than that of the group with neither tinnitus nor abnormal SLT. A two-way ANOVA (tinnitus x SLT) on PAC activation at 70 dB demonstrated a significant effect of tinnitus ($p = 0.02$) as well as SLT ($p = 0.006$; no significant interaction, $p = 0.09$). The 70 dB activation of HGal and PT showed average differences between subject groups that resembled those of PAC (Table B-2). However, only PAC showed statistically significant effects of tinnitus at 70 dB ($p \geq 0.7$ for the other cortical ROIs).

For the 50 dB stimulus, the dependence of PAC activation on tinnitus was even more apparent than at 70 dB, and one other cortical region, HGal, also showed significant effects of tinnitus. The dependence of PAC activation at 50 dB on tinnitus can be seen from Figure A-5 (bottom right) where the two groups with tinnitus showed similar percent changes to the 50 dB stimulus and greater percent changes than the two non-tinnitus groups. A two-way ANOVA showed a significant effect of tinnitus in PAC ($p = 0.006$), but not of SLT ($p = 0.4$; no interaction, $p = 0.8$). HGal, the region immediately anterolateral to PAC showed similar results: a significant effect of tinnitus ($p = 0.02$) and no effect of SLT ($p = 0.6$; no interaction,$p = 0.7$). Neither PAC nor HGal activation at 50 dB was significantly correlated with age, threshold for the continuous noise stimulus, anxiety ($p \geq 0.1$), or depression ($p = 0.08$; not corrected for multiple comparisons). And, a three-way ANOVA, tinnitus $\times$ SLT $\times$ age, sex, threshold, anxiety, or depression showed no significant effect of any of these potentially confounding variables (HGal: $p \geq 0.3$; PAC: $p \geq 0.2$; not corrected) while confirming a significant effect of tinnitus (HGal: $p \leq 0.03$; PAC: $p \leq 0.03$). These analyses indicate that tinnitus, more than any other variable considered, influenced PAC and HGal activation levels at 50 dB.

2.4 Discussion

Our subject cohort comprising people with and without tinnitus showed sound-evoked fMRI activation in the IC, MGB, and PAC that was significantly correlated with measures gauging subjects' SLT: LDL and SLTQ score. Importantly, the correlations were demonstrated in subjects with clinically normal hearing thresholds using the same physical stimulus levels in all subjects. Furthermore, the correlations could not be attributed to other factors potentially affecting the magnitude of fMRI activation, including stimulus threshold, depression, anxiety, and age. Thus, the results provide strong evidence for a relationship between the magnitude of sound-evoked fMRI activation in the auditory pathway and SLT. The nature of the relationship was as follows: activation from sound increased with decreasing SLT.

2.4.1 A physiological correlate of hyperacusis

The present results are, to our knowledge, the first to directly demonstrate a physiological correlate of abnormal SLT, that is, hyperacusis. Our results pertain specifically to people with mild hyperacusis since hyperacusis was never the primary complaint among the tinnitus patients recruited for this study and was self-recognized by only a few of the subjects who ultimately showed abnormal SLT under the controlled conditions of our testing. Interestingly, we had difficulty finding subjects with tinnitus and completely normal SLT as defined by our behavioral tests. While anecdotal, this observation nevertheless suggests the possibility that abnormal SLT, by which we mean hyperacusis ranging from mild to severe, may be more directly related to tinnitus than generally appreciated.

2.4.2 Re-interpretation of previous fMRI studies of tinnitus patients

While sound-evoked activation in the IC and MGB was correlated with SLT, it was not correlated with tinnitus. This result suggests that previous demonstrations of elevated sound-evoked fMRI activation in the IC of tinnitus subjects were likely related to abnormal perception of the sound stimulus, rather than the phantom perception of sound, that is, tinnitus [Lanting et al., 2008, Melcher et al., 2009]. We suspect that a sizable fraction of the tinnitus subjects in these previous studies had abnormal SLT, which would account for the previous findings of elevated activation in the subject groups with tinnitus.

2.4.3 Dependence of PAC activation on tinnitus: Possible role of attention

Activation in PAC resembled that of the IC and MGB in showing an effect of SLT, but also differed from the subcortical activation in two respects. First, PAC only showed a dependence on SLT at 70 dB; activation at the lower level of 50 dB showed

no such dependence. Thus, the activation enhancements seen subcortically at 50 dB were not simply reflected verbatim in cortex. The difference between subcortex and cortex could reflect a difference in the population neural coding of loudness, and/or a difference in the fraction of total neural activity allocated to coding loudness vs. other stimulus attributes, for instance. Second, PAC differed from the IC and MGB in showing a dependence of activation on tinnitus in addition to SLT. In particular, at both 50 and 70 dB, activation in the subject groups with tinnitus was elevated regardless of whether SLT was normal or abnormal. We can only speculate as to why this was the case, but one possibility is suggested by neuroimaging data showing that sound produces increased activation in auditory cortex during selective attention to the auditory domain [Woodruff et al., 1996, Degerman et al., 2006, Krumbholtz et al., 2007, Paltoglou et al., 2009]. Perhaps the increased cortical activation among those with tinnitus reflected sustained over-attention to the auditory domain that then resulted in enhanced responses to sound. In other words, the elevations in sound-evoked cortical activation that occurred with tinnitus irrespective of SLT might not reflect the tinnitus percept per se, but rather reflect attention drawn to the auditory domain by the presence of tinnitus. The absence of tinnitus-related effects in subcortical centers in our data is consistent with this attention-based hypothesis for the tinnitus-related effects in PAC since attentional state has a far subtler effect on subcortical compared to cortical auditory fMRI activation [Rinne et al., 2008].

But undue attention drawn to the auditory domain by the tinnitus percept is just one possible explanation for the tinnitus-related elevations in PAC activation. Others include the possibility that cause and effect are reversed such that the tinnitus percept results because PAC activity is enhanced by over-attention to the auditory domain (for instance, spontaneous activity is increased to the point that it is heard as sound). Or, enhancement of PAC activity and tinnitus perception might build on one another. The latter situation might occur in a manner suggested by the well-known "vicious cycle" of tinnitus and its hypothesized neurophysiological substrates (tinnitus causes distress; distress causes tinnitus [Jastreboff et al., 1996]). For instance, aberrant auditory activity that is not consciously perceived as sound might become

perceptible because it is assigned behavioral significance through what amounts to implicit aversive conditioning. The emergent percept then reinforces the aversive response, enhancing the aberrant activity and hence the percept, and so on.

2.4.4 Cortical activation dependencies on SLT and tinnitus: Core vs belt

While cortical activation dependencies on SLT and tinnitus were most evident in PAC, other cortical ROIs showed dependencies as well. Since the cortical ROIs of the present study were defined to follow the organization of primary auditory cortex into a core region of primary (PAC) and primary-like (HGal, ALA) areas and belt regions surrounding the core medially (AMA) and laterally (PT) [Kaas and Hackett, 2000] these dependencies can be examined with respect to the core/belt distinction. Within the core, HGal and ALA, in addition to PAC, showed dependencies on SLT, and in the case of HGal, a dependence on tinnitus. In contrast, ROIs corresponding to the belt (AMA, PT) showed no significant activation dependencies on SLT or tinnitus. Thus, any cortical dependencies on SLT and tinnitus appeared limited to the core of auditory cortex.

2.4.5 Relationship to animal work

The elevations in activation to sound seen in the present study are highly suggestive of abnormal gain within the auditory pathway. Previous electrophysiological data from animals showing activity elevations in inferior colliculus and/or cortex in response to pharmacological agents or auditory peripheral damage offer some clues as to how such a gain might arise [Salvi et al., 1990, Syka et al., 1994, Salvi et al., 2000]. Perhaps the most directly relevant animal work is that showing abnormally elevated responses to sound in the inferior colliculus of acoustically traumatized animals, a finding potentially relevant to the present study of subjects with normal audiograms because it was seen for sound frequencies at which threshold was normal (that is, for stimulus frequencies below the range of any hearing loss). The elevated responses in

animals have been postulated to arise as a result of reduced GABA-mediated inhibition, a possible mechanism by which the elevations in fMRI activation seen here might arise.

2.4.6 Relationship to previous studies relating auditory cortical activation and loudness

Two previous studies showing increased activation of auditory cortex with increased sound loudness as distinct from physical sound level warrant consideration in light of the present results. One study found that the amount of activation produced by a variety of pure and complex tone stimuli was better correlated with the calculated loudness of the sound stimuli than to their level [Hall et al., 2001]. The other showed that activation increased similarly with loudness, but not level for two subject groups, normal hearing listeners and hearing impaired listeners in whom loudness grew abnormally with stimulus level because of recruitment [Langers et al., 2007]. Both studies indicate that the degree to which auditory cortex activates is directly related to the perceived loudness of a sound stimulus. While the present results are somewhat different in that they demonstrate a correlation between activation and the upper limit of comfortable loudness, they can be seen to be consistent with a dependence of activation on loudness by noting the following: Having normal thresholds for detecting sound, as in the present study, but reaching uncomfortable loudness at a lower-than-normal sound level, implies the perception of greater-than-normal loudness for at least some sound levels below those deemed uncomfortable. Thus, the elevated cortical activation seen for a stimulus level below uncomfortable (70 dB SPL) in subjects with abnormal SLT (Figure A-5, top right panel) may reflect the fact that the perceived loudness of the sound evoking the activation was greater than normal. Given the relationship between loudness and auditory midbrain activation described in the introduction, the elevated inferior colliculus activation seen for 50 and 70 dB SPL in subjects with abnormal SLT may also reflect abnormal (that is, heightened) loudness perception.

2.4.7 Clinical implications

The present results provide an entrance to understanding and quantifying physiological effects of candidate treatments for tinnitus and hyperacusis, perhaps most particularly sound therapies designed to promote a reduction in central auditory gain [Davis et al., 2007, Noreña and Chéry-Croze, 2007]. Such therapies, which involve the delivery of controlled sounds in a prescribed manner, along with counseling, have been shown to ameliorate hyperacusis and reduce tinnitus severity as assessed from behavioral tests and questionnaire evaluations. Based on the present results, we hypothesize that such improvements coincide with reductions in sound-evoked fMRI activation in the auditory pathway. This hypothesis could be tested by comparing measurements of sound-evoked fMRI activation, like those of the present study, before and after administration of sound therapy.

A variety of clinical conditions are characterized by disordered perceptions that, like tinnitus and hyperacusis, have been hypothesized to arise from abnormal elevations in neural activity in the CNS. These include the phantom sensation of an amputated limb and chronic neuropathic pain, conditions analogous to tinnitus [Møller, 2007]. They also include conditions analogous to hyperacusis: the heightened sensitivity to light occurring with migraine and the lowered thresholds for stimulated pain (e.g., from pressure, thermal stimulation) occurring with chronic pain [Kosek et al., 1996]. In some of these cases of disordered perception, there is evidence indicating underlying elevations in neural activity [Cook et al., 2004], [Ambrosini and Shoenen, 2006]. These previous data combined with the present results reinforce proposals that the neurophysiology behind tinnitus and hyperacusis may not be entirely unique to the auditory system, but may rather have strong commonalities with the disordered perceptions characterizing other clinical conditions involving the visual and somatosensory domains.

Chapter 3

Threshold-matched tinnitus and non-tinnitus subjects differ in auditory nerve and brainstem function

3.1 Introduction

Tinnitus, the ongoing perception of sound in the absence of a physical stimulus, is a common clinical condition lacking effective treatments. Loss of hearing sensitivity is often associated with tinnitus [Shargorodsky et al., 2010], although the mechanistic relationship between tinnitus and hearing loss remains unclear. Clues to the mechanisms underlying the development of tinnitus come from animal studies showing that cochlear damage can lead to increased spontaneous and sound-evoked neural activity in the central auditory system (reviews: [Salvi et al., 2000, Kaltenbach, 2007]). The auditory brainstem, particularly the dorsal cochlear nucleus (DCN) and inferior colliculus (IC), is one region where elevated neural activity has been repeatedly reported in animals exposed to common tinnitus inducers (e.g. [Salvi et al., 1990, Kaltenbach and McCaslin, 1996]). Many studies suggest that re-

duced peripheral input to the brainstem leads to changes in the balance of excitation and inhibition at a synaptic level, which then results in heightened post-synaptic activity (e.g. [Suneja et al., 1998, Asako et al., 2005, Illing et al., 2005]). Furthermore, several studies have reported elevated activity in the CN and IC of sound-traumatized animals showing behavioral evidence of tinnitus [Brozoski et al., 2002, Kaltenbach et al., 2004, Brozoski et al., 2007, Bauer et al., 2008]. Despite the extensive animal work on brainstem abnormalities possibly related to tinnitus, relatively little is known about brainstem function in humans with tinnitus. In this paper, we examine brainstem function in people with tinnitus via the auditory brainstem response (ABR).

The present study was motivated by three previous results. The first comes from a functional magnetic resonance imaging (fMRI) study of tinnitus and the often concomitant condition hyperacusis, the intolerance of sound intensities considered comfortable by most people [Gu et al., 2010]. To include milder forms of hyperacusis that are not severe enough to be a clinical problem, we use abnormal sound-level tolerance (SLT) to refer to the reduced tolerance of sound level in people without better-than-normal hearing sensitivity. Gu et al. disentangled the effects of tinnitus and abnormal SLT by showing that elevated sound-evoked fMRI activation in the IC was related to abnormal SLT but not tinnitus. This finding raises the possibility that the elevated IC activation reported by previous fMRI studies of tinnitus [Lanting et al., 2008, Melcher et al., 2009] was actually related to abnormal SLT rather than tinnitus, thus highlighting the importance of considering SLT in studies of tinnitus. A second question motivated by this result is whether the ABR manifests SLT-related abnormalities similar to those seen with fMRI. Thus, we wanted to test whether the amplitude of wave V of the ABR, which is generated by neurons innervating and/or within the IC [Møller and Jannetta, 1982, Melcher and Kiang, 1996], of the ABR also shows elevations related to abnormal SLT.

The second set of results motivating the present study comes from previous ABR studies of tinnitus. There are many such studies, but three in particular that controlled for hearing sensitivity and were therefore able to distinguish the effects of

tinnitus from those of hearing loss: [Attias et al., 1996, Kehrle et al., 2008] found elevations in the ABR (wave III amplitude and wave III/I ratio, wave V/I amplitude ratio, respectively) in tinnitus patients compared to non-tinnitus subjects matched in hearing sensitivity, although [Attias et al., 1993] did not find any differences between the two groups. These results warrant follow up for two reasons: (1) the IC fMRI data raise the possibility that the previously reported amplitude elevations are related to SLT in addition to, or instead of, tinnitus; and (2) because hearing sensitivity was matched only up to 8 kHz between the tinnitus and non-tinnitus groups, and the click stimulus contained energy at higher frequencies, it cannot be completely excluded that high-frequency hearing loss (> 8 kHz) rather than tinnitus accounts for the ABR differences between groups seen by [Kehrle et al., 2008]. (This issue does not pertain to the other two studies because those subjects had hearing loss above 2 kHz.)

The third collection of findings that motivated the present study comes from recent animal work showing that sound exposure causing only a temporary threshold shift (not a permanent one) and leaving hair cells intact can, nevertheless, lead to substantial auditory nerve (AN) loss [Kujawa and Liberman, 2009, Kujawa et al., 2011, Lin et al., 2011]. This loss was evident in suprathreshold wave I amplitude, which increased more slowly with stimulus level in sound-traumatized animals [Kujawa and Liberman, 2009]. Wave I is generated by AN activity [Buchwald and Huang, 1975, Møller and Jannetta, 1981]; thus the amplitude of wave I is dependent on the number of intact AN fibers. Loss of AN fibers and/or decrease of AN activity can lead to reorganization of the central auditory system (review: [Irvine et al., 2000]), which has been hypothesized to lead to tinnitus [Eggermont and Roberts, 2004]. Furthermore, [Bauer et al., 2007] showed that AN loss, in the absence of hair cell loss, was associated with behavioral evidence of tinnitus in rats. Based on these results, we wanted to test whether tinnitus in people with clinically normal hearing sensitivity is related to AN damage manifest in wave I amplitude.

With these motivations, the present study revisited the question of whether there

are differences in ABR amplitude between subjects with tinnitus and those without, and further examined whether there are amplitude differences related to SLT. Tinnitus and non-tinnitus subjects were matched in hearing sensitivity through 14 kHz and in age. Because ABR amplitude is strongly sex-dependent [Jerger and Hall, 1980, Michalewski et al., 1980], all subjects were the same sex (male) to avoid sex-related, within-group variability in ABR amplitude, and thus increasing the likelihood of identifying subtle differences related to tinnitus or SLT. ABRs of the closely-matched tinnitus and non-tinnitus subjects were compared to identify any tinnitus- and SLT-related effects. Finally, to identify ABR abnormalities that might be present in both groups, ABRs were also measured in a cohort of male non-tinnitus subjects with even better thresholds (normal above as well as within the standard clinical frequency range).

Portions of this work were presented at the 33rd Annual Meeting of the Association for Research in Otolaryngology (2010) and the 4th Meeting of the Tinnitus Research Initiative (2009).

3.2 Methods

ABRs were measured in three subject groups: (1) tinnitus [15 subjects, age 42 $\pm$ 6 yr (mean $\pm$ standard deviation)], (2) non-tinnitus with similar age and thresholds to the tinnitus subjects (21 subjects, age 43 $\pm$ 7 yr), and (3) young non-tinnitus (11 subjects, age 23 $\pm$ 2 yr). All subjects were male. Each subject underwent a behavioral testing session and one to five ABR recording sessions. Characteristics of each subject group are given in Table B-6. Individual subject characteristics and tinnitus characteristics are provided in Tables B-8 and B-9. All but one of the tinnitus subjects (#213) was recruited through the Tinnitus Clinic at the Massachusetts Eye and Ear Infirmary (MEEI). The remaining tinnitus subject and all non-tinnitus subjects were recruited from advertisements in local newspapers and personal contacts. This study was approved by the institutional committees on the participation of human subjects at the Massachusetts Institute of Technology and MEEI. All subjects gave

their written informed consent.

3.2.1 Behavioral testing

During the behavioral testing session, thresholds to tones with frequencies from 125 Hz to through 16 kHz were measured. Loudness discomfort levels were assessed as described in [Gu et al., 2010]. In tinnitus subjects, tinnitus pitch, loudness, minimum masking level, and presence of residual inhibition were assessed as described in [Gu et al., 2010]. All subjects completed questionnaires assessing handedness [Oldfield, 1971], depression [Beck et al., 1961], anxiety [Beck et al., 1988], SLT [Tyler et al., 2003], and medication intake. Subjects with tinnitus completed two additional questionnaires: one assessing effects of tinnitus on quality of life (Tinnitus Reaction Questionnaire (TRQ) [Wilson et al., 1991]) and the other assessing tinnitus characteristics (e.g. quality of percept, location).

3.2.2 Electrode placement

Chlorided silver electrodes (Grass Technologies) were applied to the scalp with conducting cream (EC2, Grass Technologies), after first abrading the skin with conducting gel (Nuprep, Weaver and Co.). The eletrode sites were at three locations in the standard 10-20 system: vertex, F3 (left frontal), and F4 (right frontal). Electrodes were also attached to the earlobes. The electrode on the earlobe of the stimulated ear served as the reference electrode. An electrode placed on the forehead or neck served as ground. Electrode impedances were measured before, during breaks if any, and after ABR recording, and were maintained ≤ 7 kΩ throughout each session.

3.2.3 Stimuli

Stimuli were digitized at a rate of 20 kHz, generated using a DAQPad (National Instruments), and presented over headphones (Sennheiser, HDA-200). Stimuli were 100 s clicks (condensation) presented monaurally at 30, 50, 70, and 80 dB HL. The click spectrum, adjusted for the headphone frequency response measured using an

artificial ear (Larson Davis, AEC101), is shown in Figure A-7. For click levels of 50, 70, and 80 dB HL, broadband noise at 10, 30, and 40 dB HL, respectively, was presented to the opposite ear to mask any stimulation via acoustic cross talk [Levine, 1981]. 0 dB HL was estimated by averaging the click threshold of four subjects age 23–27 who had pure tone thresholds of $\leq$ 20 dB HL at standard audiometric frequencies. Six tinnitus, two non-tinnitus of similar age, and two young non-tinnitus subjects did not tolerate the 80 dB stimulus. Clicks were presented at a rate of 11 per second in four minute runs. The interval between click presentations was jittered by 10% (9 ms). Six runs were collected at 30 dB and three runs were collected at each of the higher stimulus levels, yielding 15,840 and 7,920 total click presentations per stimulus level, respectively. Stimulus level was constant throughout a run and varied pseudo-randomly across runs of a given session. For sessions in which both ears were tested, measurements for one ear were completed before taking measurements for the other. For all except three subjects (#129, #148, #168), both ears were tested. For these three subjects, only the left ear was tested because each of them declined to return for a second session to test the right ear.

3.2.4 Data acquisition

A preamplifier (Medusa, Tucker-Davis Technologies) was used to amplify (20× gain) and digitize (25 kHz sampling rate) the signals from the vertex, F3, and F4 electrodes, each referenced to the earlobe of the stimulated ear. The digitized signals were relayed to a base station that band-pass filtered the signals between 5 Hz and 5 kHz, amplified them 2000X, and converted them to analog signals. These analog signals, as well as the stimulus waveform from the DAQPad, were digitized at a rate of 25 kHz using a National Instruments board (CA-1000) and streamed to disk. Throughout each session, the signal outputs of the base station were monitored visually on an oscilloscope to assess signal quality and whether adjustments were needed to improve signal quality (e.g. asking the subject to relax or reattaching electrodes).

3.2.5 Data processing

Data processing was done using custom software in MATLAB (MathWorks). The stimulus waveform, recorded simultaneously with the signals from the electrodes, was used to identify times at which the stimulus was presented. For each level, segments of data (trials) encompassing 20 ms before to 20 ms after each stimulus presentation were extracted for analysis. The mean amplitude of the 20 ms prior to click presentation was subtracted from each segment. Each segment was low-pass filtered (2 kHz cutoff, Butterworth, fourth-order). Trials for which the standard deviation of the 20 ms prior to click presentation exceeded 8 V and/or the maximum amplitude after click presentation exceeded 30 V were rejected on the basis of being excessively noisy. These rejection criteria were chosen to reject the noisiest 10% of trials recorded from the first 10 subjects tested. A weighted average of the trials passing the criteria was computed, where the weighting for a trial was the reciprocal of the standard deviation of the 20 ms prior to click presentation divided by the sum of the reciprocal of the standard deviations for all accepted trials. To remove signal drift, a linear fit to the 10 ms prior to click presentation (the pre-stimulus baseline) was then subtracted from the average to yield a drift-corrected ABR waveform for each electrode, ear, level, and subject. Average waveforms with standard deviation of pre-stimulus baseline exceeding 0.03 V (30 dB) or 0.05 V (50, 70, 80 dB) were rejected (178 out of 999 waveforms). The criterion for 30 dB was more stringent because wave amplitudes were lowest at that level. Waveforms for which the fraction of negative data points in the waves I to III windows (window definition described below) exceeded 0.7 were also excluded on the basis of being excessively noisy (75 out of 999 waveforms). In summary, of the 999 average ABR waveforms, 253 were rejected on the basis on noisiness; the results are based on the remaining 746.

Time windows for the peaks of waves I, II, III, and V were defined from the average of the ABR waveforms for the young non-tinnitus subjects (Figure A-9, panel E). The amplitude of waves I and II was measured from peak to following trough, and amplitude of waves III and V was measured from pre-stimulus baseline to peak. In

cases where no peak was distinguishable [out of 746 waveforms; wave I: 54 (30 dB), 35 (50 dB), 1 (70 dB); wave II: 119 (30 dB), 83 (50 dB), 21 (70 dB), 9 (80 dB); wave III: 21 (30 dB), 12 (50 dB)], for waves I and II, the amplitude was set to 0 and the latency omitted; for wave III, the mean amplitude in the time window was used and the latency omitted. Wave V was always distinguishable.

For each subject, ear, and stimulus level, measurements of wave amplitude and latency did not vary systematically across the three electrode pairs: vertex-, F3-, and F4-earlobe. On average, variability in amplitude measurements across electrode pairs was on the order of variability in pre-stimulus baseline. On average, variability in latency measurements across electrode pairs was an order of magnitude less than ABR latencies of interest. Based on the similarity of measurements across electrode pairs, we averaged amplitude and latency data for a given wave across electrodes for each subject, ear, and stimulus level.

3.2.6 Stimulus artifact removal

Because the dependence of auditory nerve responses on stimulus level is different for condensation and rarefaction clicks [Peake and Kiang, 1962], the conventional method of alternating click stimulus polarity to cancel stimulus artifact in the across-trial average was not used to avoid complicating interpretation of the wave I data. As a consequence of using single-polarity clicks (condensation), the ABR waveforms at 70 and 80 dB were overlapped by a stimulus artifact; there was no appreciable artifact overlap at 30 and 50 dB because of the longer ABR latencies and lower stimulus levels. While twisting the electrode leads reduced the artifact, it did not eliminate it. Therefore, the artifact was computationally removed from the 70 and 80 dB waveforms by subtracting an estimate of the artifact alone. Estimates were obtained by measuring waveforms produced by click stimuli at electrodes applied to an inert sphere of conducting material (ground chicken). The electrode locations and contact impedances, as well as the headphone locations, were as they would be in an actual subject. A comparison of measurements at 30, 50, 70, and 80 dB confirmed that the artifact amplitude scaled linearly with level. Therefore, 80 dB measurements

were used to generate artifact estimates (one for twisted and one for untwisted leads) that were scaled and subtracted from the 70 and 80 dB waveforms, as illustrated in Figure A-8.

3.3 Results

3.3.1 Normal ABRs in young non-tinnitus subjects (all male)

Data for the ears of young non-tinnitus subjects are shown in Figure A-9. Panels A–D (white bars) show mean amplitude of waves I, II, III, and V, respectively, at the four click intensities presented. For each wave, amplitude increased with increasing click intensity and latency decreased (mean amplitude and latency data in Table B-2). Mean interpeak latencies for I-III, I-V, and III-V were within the range of those reported in previous studies for adults with clinically normal hearing [Stockard et al., 1979]. Mean pure tone thresholds for the ears of the subjects in panels A–D were less than 20 dB HL from 0.125 through 14 kHz (dashed curve and white dots in panel F).

3.3.2 Reduced ABR amplitudes in older non-tinnitus subjects (all male)

Figure A-9 also shows data for the ears of non-tinnitus subjects chosen for their similarity in age and threshold to the tinnitus subjects. On average, these subjects were twenty years older than the young non-tinnitus subjects, hence they are designated "older non-tinnitus subjects" in the figure. Most of these subjects had clinically normal thresholds ($\leq$ 25 dB from 250 through 8000 Hz), as reflected in the mean audiogram in panel F (solid line with gray dots). They did, however, have hearing loss at frequencies above the normal clinical range. Consistent with this high frequency hearing loss, which included frequencies within the spectrum of the click stimulus (Figure A-7), the ears of the older non-tinnitus subjects showed reduced amplitude for almost all waves and levels compared to the ears of the young non-tinnitus sub-

jects (gray bars panels A–D). This difference in amplitude was significant at all levels for wave I (rank-sum: $p \leq 0.02$, not corrected for multiple comparisons, panel A) and at two levels for wave II ($p \leq 0.04$, panel B) and V ($p \leq 0.05$, panel D). The reduced ABR amplitude of the older non-tinnitus subjects can also be seen in the grand average ABR waveforms at 70 dB for the two groups (panel E).

3.3.3 Reduced wave I amplitude but elevated wave V amplitude in tinnitus subjects (all male)

Figure A-10 compares tinnitus subjects to closely-matched subsets of the older non-tinnitus subjects. The tinnitus subjects providing data at each level were matched with a subset of older non-tinnitus subjects selected to achieve close age and audiometric matching (panel F). Despite the close matching, mean wave I amplitude was reduced in the ears of the tinnitus subjects for 50, 70, and 80 dB clicks (panel A). The reduction at 80 dB was significant (rank-sum: $p = 0.0007$, not corrected for multiple comparisons). Despite the reduction in wave I, wave II amplitude was similar for the two groups (panel B). Also, mean wave III and wave V amplitudes were elevated in the ears of the tinnitus subjects. The elevations for wave V at 80 dB and 30 dB were significant ($p \leq 0.05$). The trends at 70 dB in the bar plots can also be seen in the grand average ABR waveforms in panel E.

To determine whether there was a relationship between reduced wave I and elevated wave V and III, the amplitude of each of the later waves was plotted versus wave I amplitude (Figure A-11). The vertical line in each panel of Figure A-11 indicates mean wave I amplitude of the ears of the matched non-tinnitus subjects in the plot (gray dots, individual ears); the horizontal line indicates mean wave V (top row) and III (bottom row) amplitudes. While only wave V amplitude at 80 dB correlated significantly with wave I amplitude (Spearman: $p = 0.05, r = -0.39$; others, $p \geq 0.1$), tinnitus subjects (black dots, individual ears) tended to fall in the upper left quadrant of each panel and be absent from the lower right quadrant, meaning there was some tendency for subjects with smaller wave I amplitudes to also have larger wave V and

III amplitudes.

3.3.4 Elevated wave V/I and III/I amplitude ratios in tinnitus subjects

The tendency in Figure A-11 motivated an analysis of the following amplitude ratios: V/I and III/I (Figure A-12; individual ears indicated by dots, medians indicated by bars). The tinnitus subjects showed significantly greater V/I and III/I amplitude ratios at 80 and 70 dB compared to their matched non-tinnitus counterparts (rank-sum: $p \leq 0.04$, not corrected for multiple comparisons) and the young non-tinnitus subjects ($p \leq 0.0006$). The matched non-tinnitus subjects also showed significantly larger V/I and III/I ratios at 80 and 70 dB than the young non-tinnitus subjects ($p \leq 0.03$), though the difference was far less significant than the difference between the tinnitus and young-non tinnitus groups.

3.3.5 Effects of variables other than tinnitus

To assess whether variables other than tinnitus could account for the observed differences in ABR amplitude and V/I and III/I ratio between the tinnitus and matched non-tinnitus groups, we first looked for differences in possible confounding variables between the two groups. At all levels, the tinnitus and matched non-tinnitus subjects did not differ significantly in age (by design), click threshold (also by design), head size, or LDL (rank-sum: $p \geq 0.1$, not corrected for multiple comparisons). However, there were significant differences in depression and anxiety scores at 30, 50, and 70 dB ($p \leq 0.01$); and SLTQ score at 30 dB ($p = 0.05$). After identifying depression, anxiety, and sound-level tolerance to be potential confounding variables, we used a two-way ANOVA to determine the relative effect of tinnitus and each of these variables on every measure for which we found a significant difference between tinnitus and matched non-tinnitus subjects (wave I amplitude: 80 dB; wave V amplitude: 30, 80 dB; V/I, III/I ratio: 70, 80 dB). Most of these comparisons showed an insignificant effect of depression, anxiety, and sound-level tolerance as well as insignificant

interaction with tinnitus ($p \geq 0.2$). For V/I ratio at 70 dB, there was significant interaction between tinnitus and anxiety ($p = 0.05$), but a far more significant effect of tinnitus ($p = 0.003$) and no effect of anxiety alone ($p = 0.3$). Thus, any effects of anxiety were far less than those of tinnitus. Only two results raised the possibility that a factor other than tinnitus might have contributed on par with tinnitus to the ABR differences between tinnitus and matched non-tinnitus subjects: (1) A two-way ANOVA (tinnitus $\times$ depression) showed a significant effect of depression ($p = 0.03$) as well as tinnitus ($p = 0.02$) on wave I amplitude at 80 dB (no interaction, $p = 0.2$). (2) Another ANOVA showed a significant effect of depression on wave V amplitude at 80 dB ($p = 0.03$) and no effect of tinnitus ($p = 0.8$), but a significant interaction between tinnitus and depression ($p = 0.008$). Thus, analyses suggest that depression, in addition to tinnitus, may have been a factor in the wave I and wave V differences seen. To the extent that tinnitus and depression are linked [Dobie, 2004], their effects cannot be teased apart for these particular subjects.

3.3.6 Relation to tinnitus characteristics

To determine whether the tinnitus-related abnormalities we found were related to tinnitus pitch, loudness, minimum masking level (MML), or severity as assessed by the TRQ (Table B-9), we looked for correlations with wave I or V amplitude at 80 dB, wave V amplitude at 30 dB, and wave V/I and III/I amplitude ratios at 70 and 80 dB. There was a significant correlation between tinnitus loudness and wave I amplitude at 80 dB (Spearman: $p = 0.008$, $r = -0.75$), wave V amplitude at 30 dB ($p = 0.04$, $r = -0.46$) and wave V/I amplitude ratio at 80 dB ($p = 0.04$, $r = 0.62$). All other correlations were insignificant ($p \geq 0.2$). These correlations were likely to be spurious because the correlation with wave I amplitude at 80 dB resulted from the drop out of subjects due to rejected data and inability to tolerate 80 dB, the correlation with wave V/I amplitude ratio at 80 dB was attributed to the correlation with wave I amplitude rather than wave V amplitude, and the correlation with wave V amplitude at 30 dB did not qualitatively look convincing.

3.4 Discussion

We found reduced wave I amplitudes and elevated wave V amplitudes in tinnitus subjects compared to closely-matched non-tinnitus subjects. Consistent with this result, the V/I amplitude ratio was significantly greater in tinnitus subjects. The III/I amplitude ratio was also elevated in tinnitus subjects. These differences could not be attributed to other factors potentially affecting ABR amplitude, including age, sex, click threshold, head size, sound-level tolerance, and anxiety. However, depression might have played a role in determining the amplitudes of waves I and V at 80 dB. There was also a tendency for wave I amplitude in tinnitus subjects to grow more slowly at the highest sound levels tested.

3.4.1 Extent and pattern of auditory nerve dysfunction may be a factor in tinnitus

The reduced wave I amplitude in the tinnitus group indicates that tinnitus subjects had greater peripheral dysfunction than matched non-tinnitus subjects despite matched mean audiograms. This reduction was most pronounced at the highest stimulus levels, implicating dysfunction of AN fibers rather than hair cells. One possibility is that tinnitus subjects had greater diffuse loss of AN fibers, which may not be apparent in wave I amplitude near threshold but could lead to lower amplitudes above threshold due to fewer fibers contributing to the response [Kujawa and Liberman, 2009]. Another possibility is that tinnitus subjects had greater loss of AN fibers with low and medium spontaneous rates (SR), consistent with evidence that these fibers are particularly vulnerable to sound exposure [Lin et al., 2011]. Low and medium SR fibers have higher thresholds than high SR fibers [Liberman, 1978]; thus, their contribution to wave I amplitude would be apparent only at higher sound levels. These results suggest that the extent and pattern of AN dysfunction may be a factor in tinnitus. While hearing loss strongly correlates with tinnitus [Shargorodsky et al., 2010], not everyone with hearing loss develops tinnitus and people with clinically normal hearing, like most of the subjects in the present study, can develop tinnitus. Perhaps

tinnitus is more closely associated with the extent and pattern of AN dysfunction than with hearing thresholds.

The tinnitus and matched non-tinnitus subjects in our study had normal or near-normal pure tone thresholds up to 8 kHz, the highest frequency tested during a standard clinical evaluation. However, they had wave I amplitudes that were significantly lower than those of the young non-tinnitus subjects, demonstrating that a standard clinical audiogram can miss peripheral dysfunction. Thresholds for frequencies above 8 kHz, on the other hand, were indicative of peripheral dysfunction.

3.4.2 Spherical bushy cell pathway implicated in tinnitus

The elevated wave V amplitude and V/I and III/I amplitude ratios suggest that neural activity was abnormally amplified from AN to higher brainstem centers in tinnitus subjects. Previous human and animals studies suggest that in humans, neurons within the medial superior olive (MSO), lateral lemniscus (LL), and/or inferior colliculus are the generators of wave V; and neurons within the ventral cochlear nucleus (VCN) are the generators of wave III [Møller and Jannetta, 1982, Fullerton et al., 1987, Melcher and Kiang, 1996]. These neurons have been identified even more specifically in cat to be those within the spherical and globular bushy cell pathways originating in the VCN [Melcher and Kiang, 1996]. Based on anatomical comparisons across species [Irving and Harrison, 1967] and evidence that the globular bushy cell pathway is poorly represented in humans [Adams, 1986], Melcher and Kiang proposed that the human ABR is mainly generated by the spherical bushy cell pathway. While many studies have focused on the DCN as a contributor to tinnitus (review: [Kaltenbach, 2007]), relatively little attention has been paid to the VCN. Our results suggest that the VCN, particularly the spherical bushy cells, also plays a role in tinnitus.

3.4.3 Possible mechanisms underlying brainstem hyperactivity

Because ABR amplitudes are dependent on number of neurons, firing rate of neurons, and synchrony of firing across neurons, increases in any of these features could lead to elevated amplitudes. An increase in the number of neurons in the brainstem is unlikely, therefore we consider possible mechanisms leading to increased firing rate and synchrony.

One possible mechanism leading to increased firing rate is a shift of the balance of excitation and inhibition at a synaptic level in favor of excitation, which leads to increased post-synaptic activity. Support for this mechanism comes from studies in animals showing that acoustic trauma leads to degeneration and subsequent regrowth of synaptic endings on globular bushy cells in the VCN with a more complete recovery of excitatory endings compared to inhibitory endings [Kim et al., 2004].

Another possibility is that changes in neurotransmitter levels or number or composition of receptors leads to an overall loss of inhibition. Consistent with this mechanism are findings that sound trauma can lead to a reduction in number of functional glycine (inhibitory) receptors in DCN fusiform cells [Wang et al., 2009], and ototoxic deafening can lead to changes in relative distribution of glutamate (excitatory) transporters in the VCN and DCN [Zeng et al., 2009]. Also consistent with loss of inhibition are studies showing that age-related hearing loss is associated with decreased levels of glycine in the CN and GABA (inhibitory) in the IC (review: [Caspary et al., 2008]).

A possible mechanism leading to increased synchrony of neuronal firing is suggested by anatomical studies of dendritic trees of spherical and globular bushy cells in rodents and primates [Gómez-Nieto and Rubio, 2009, Gómez-Nieto and Rubio, 2011], and proposed by a model of tinnitus based in the VCN [Melcher, 2010]. Gómez-Nieto and Rubio showed that single AN terminals can synapse on multiple bushy cells and that bushy cells are connected to one another through gap junctions, allowing fast communication through electrotonic coupling. This arrangement could increase the

correlation of firing in bushy cells, which could enhance synchrony of AN activity in response to sound. However, this arrangement could also lead to abnormal correlations, and thus abnormally synchronous activity, in bushy cell firing both in the presence (sound-evoked AN activity) and absence (spontaneous AN activity only) of sound given certain patterns of AN loss [Melcher, 2010].

The complex circuitry of the brainstem ([Malmierca et al., 1996], reviews: [Oliver, 2000, Thompson and Schofield, 2000, Cant and Benson, 2003]) suggests many possible pathways by which hyperactivity could be mediated. One possibility is that loss of inhibition in the VCN spherical bushy cells leads to elevated activity in the VCN, which is then propagated to higher brainstem centers via direct projections to the MSO and LL; indirect projections to the IC and LL via the MSO; or indirect projections to the IC via the LL. Another possibility is that loss of inhibition in the VCN is accompanied by loss of inhibition in higher auditory centers, resulting in an overall loss of inhibition in the brainstem. Yet another possibility is that hyperactivity originates in the DCN. DCN fusiform cells, shown to have elevated spontaneous and sound-evoked activity in animals with behavioral evidence of tinnitus [Brozoski et al., 2002], project directly to the central nucleus of the IC (CNIC). CNIC neurons, in turn, project to VCN via the trapezoid body [Shore et al., 1991, Malmierca et al., 1996], suggesting a pathway by which DCN activity could modulate VCN activity. On the other hand, there are also projections from CNIC to DCN [Shore et al., 1991] and from VCN to DCN [Doucet and Ryugo, 1997], so VCN activity could just as likely modulate DCN activity. However, the human DCN is quite different from that of animals in terms of cell type and organization [Adams, 1986], so analogies drawn between human and animal DCN are speculative at best.

3.4.4 Comparison to previous ABR studies of tinnitus

Our results are consistent with previous studies showing tinnitus-related elevations in the ABR [Attias et al., 1996, Kehrle et al., 2008]. However, the specific abnormalities reported by these two studies differ from each other: Attias et al. found elevations in wave III amplitude and III/I amplitude ratio while Kehrle et al. found elevations in

V/I amplitude ratio. The other ABR study designed to distinguish between effects of tinnitus and hearing loss did not report any tinnitus-related abnormalities in the ABR [Attias et al., 1993]. However, we used the mean ABR amplitudes given in the paper to calculate the V-I and III-I amplitude ratios, which were both larger in the tinnitus group than in the non-tinnitus group, hinting at possible elevations. The discrepancies in the previous reports might be a consequence of high-pass ($\geq$ 100 Hz) filtering the ABR, which misses some of the neural activity contributing to waves III and V [Melcher, 1993].

3.4.5 Comparison of ABR and fMRI results: Tinnitus and abnormal SLT may be arise in parallel brainstem pathways

We did not find evidence of hyperacusis-related abnormalities in the ABR. In contrast to the dependence of fMRI activation in the IC on sound-level tolerance [Gu et al., 2010], there was no such relationship between SLT and wave V amplitude. On the other hand, wave V amplitude showed tinnitus-related elevations, which were not apparent in fMRI activation in the IC. These differences are likely a consequence of the inherent differences in the two techniques. The ABR is generated by specific neuronal populations, while fMRI activation is related to gross neural activity. Therefore, fMRI may not be sensitive to tinnitus-related activity if it is generated by a small proportion of neurons. Because the ABR reflects activity in only one of many parallel pathways from CN to IC, the hyperacusis-related hyperactivity in the IC seen with fMRI but not ABR raises the possibility that hyperacusis is mediated by pathways other than the one that the present ABR study has identified to mediate tinnitus. Parallel pathways originating in the CN have common inputs, which could explain why tinnitus and abnormal SLT often co-occur; but divergent outputs, which could explain why the two clinical conditions do not always occur together.

Chapter 4

Conclusion

This thesis identified neural correlates of tinnitus and abnormal sound-level tolerance: (1) elevated sound-evoked fMRI activation related to abnormal SLT in the inferior colliculus, medial geniculate body, and primary auditory cortex; (2) elevated fMRI activation related to tinnitus in the primary auditory cortex; (3) reduced ABR wave I amplitude (auditory nerve activity) related to tinnitus; and (4) elevated ABR wave III/I (cochlear nucleus activity normalized by auditory nerve activity) V/I (normalized medial superior olive and lateral lemniscus activity) amplitude ratios related to tinnitus.

Interestingly, input activity to the inferior colliculus as measured with ABRs was related to tinnitus while fMRI activation in the inferior colliculus was related to abnormal SLT. Because ABRs reflect the activity of neurons in only one of many pathways from the cochlear nucleus to the inferior colliculus, one possibility is that abnormal SLT arises in pathway(s) other than the one we identified with ABRs to be involved in tinnitus, the spherical bushy cell pathway. If the inferior colliculus neurons receiving inputs from the spherical bushy cell pathway were only a small proportion of neurons in the inferior colliculus, any tinnitus-related activity generated by these neurons could be missed by fMRI, which is sensitive only to gross neural activity. Brainstem pathways originating in the cochlear nucleus have common inputs (auditory nerve fibers) but divergent, parallel outputs. If tinnitus and hyperacusis (highly abnormal SLT) are indeed generated by parallel neural pathways, it could

account for why these two conditions often occur together, but not always.

4.1 Clinical implications

ABR amplitude and fMRI activation could potentially be used to objectively evaluate treatments for tinnitus and hyperacusis. For instance, we expect the wave V/I amplitude ratio to decrease after effective treatment of tinnitus, and the fMRI activation in the inferior colliculus to decrease after effective treatment of hyperacusis. However, we acknowledge current limitations of these measures: (1) There is considerable overlap among data from tinnitus subjects and non-tinnitus subjects as well as those with abnormal SLT and without. Thus, defining normal ranges of these measures and evaluating treatment efficacy in patients who are not markedly abnormal may not be possible. (2) Variability of these measurements within the same individual limits the size of treatment effects that can be seen. ABR amplitude in the same individual is less variable than fMRI activation, and is thus a more promising marker for tinnitus than is fMRI activation in the cortex.

The involvement of neurons within the spherical bushy cell pathway in tinnitus suggests potential targets for pharmacological treatments of tinnitus. For example, glycine is a major inhibitory neurotransmitter affecting spherical bushy cells in the ventral cochlear nucleus. If tinnitus were related to reduced levels of glycine and/or reduced ability of spherical bushy cells to utilize glycine, drugs that could specifically normalize glycine levels/utilization in spherical bushy cells could potentially alleviate tinnitus.

4.2 Future work

A logical continuation of this thesis is to investigate ABR amplitude and fMRI activation in tinnitus and hyperacusis patients with different patterns of hearing loss. The study participants in this thesis had clinically normal or near-normal hearing thresholds. Thus, they represent only a fraction of people who experience chronic

tinnitus. Whether similar neural abnormalities are present in tinnitus/hyperacusis populations with greater degrees of hearing loss is unknown.

Based on the possibility that tinnitus-related fMRI activation in the primary auditory cortex is an effect of attention, another interesting line of research is to investigate the role of attention in tinnitus. Do tinnitus patients attend more to the auditory domain than the visual domain? Can attentional training help patients cope with tinnitus, or perhaps lessen the tinnitus percept?

Another question worth pursuing is why are some people bothered by tinnitus and others not? Identifying neural abnormalities specifically related to distressing tinnitus could inform the development of treatments that help patients cope with tinnitus, which might be less difficult than developing treatments that eliminate tinnitus. One possibility is that neural systems involved in depression and anxiety also play a role in distressing tinnitus, and treating depression and anxiety would also alleviate distressing tinnitus.

Animal models of tinnitus have been helpful in suggesting possible mechanisms by which tinnitus could arise in humans. No animal models of hyperacusis have been tested, but recent methods involving gap detection have been proposed for assessing both tinnitus and hyperacusis in animals [Turner and Parrish, 2008]. If these methods prove to be successful, animal models could be used to investigate physiological mechanisms underlying the development of tinnitus and hyperacusis.

This thesis identified abnormalities related to tinnitus and hyperacusis throughout the auditory pathway from auditory nerve to auditory cortex in humans. The involvement of many auditory centers, and the likely involvement of non-auditory brain areas, illustrate the complexity of these two clinical conditions. Although tinnitus and hyperacusis are rarely curable at the present time, advances in the understanding of their physiology, including the contributions of this thesis, offer hope that eventually we will find effective treatments for these disorders.

Appendix A

Figures

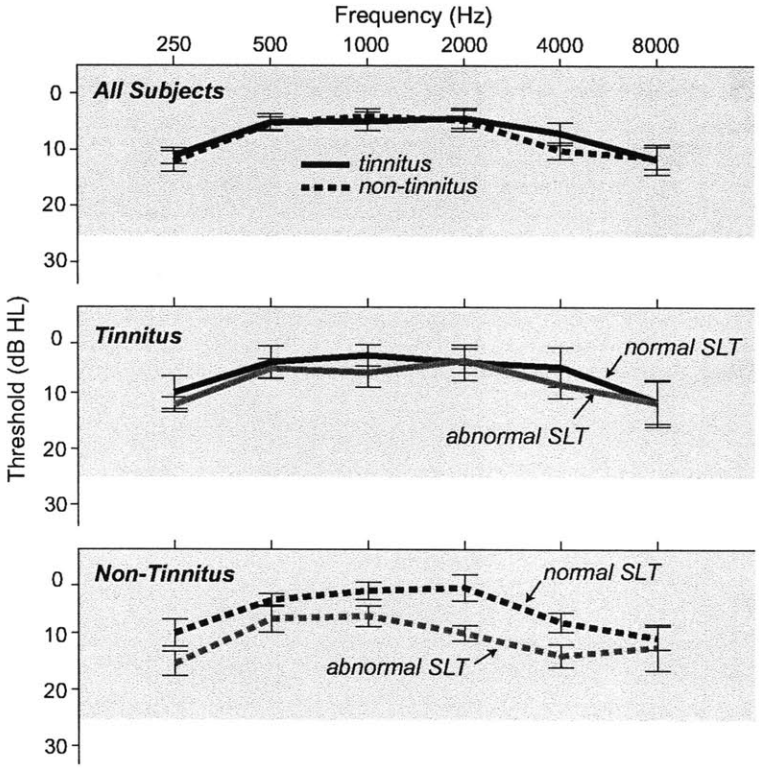

Figure A-1: *Mean pure tone thresholds for each subject group (fMRI).* Top: Tinnitus and non-tinnitus subjects. Middle: Tinnitus subjects divided according to SLT (normal or abnormal) as shown in Figure A-3. Bottom: Non-tinnitus subjects divided according to SLT. Bars indicate ± one SE. Gray shading indicates clinically normal threshold range.

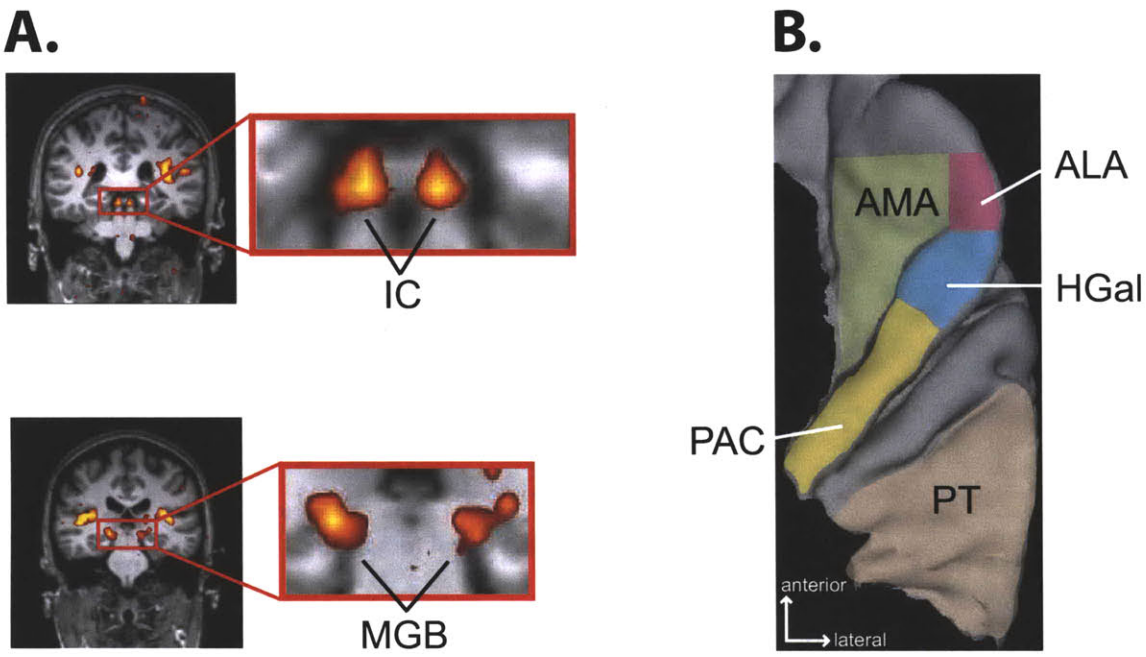

Figure A-2: *Regions of interest in fMRI analysis.* A: Subcortical ROIs. Location of the IC and MGB is illustrated by the images from one subject (#37). The IC and MGB are shown in enlargements of the rectangular area (red outline) in near-coronal images of the whole head. Sound-evoked activation (color) is localized to the inferior colliculi (top) and medial geniculate bodies (bottom). The significance of the activation is colorized on a red ($p = 0.01$) to yellow ($p = 2 \times 10^{-9}$) scale. The activation is superimposed on anatomical images obtained in the same imaging session (gray scale). B: Cortical ROIs. The location of each analyzed region is indicated on a reconstructed superior temporal lobe. PAC: primary auditory cortex, HGal: anterolateral Heschls gyrus, PT: planum temporale, ALA: anterolateral area, AMA: anteromedial area.

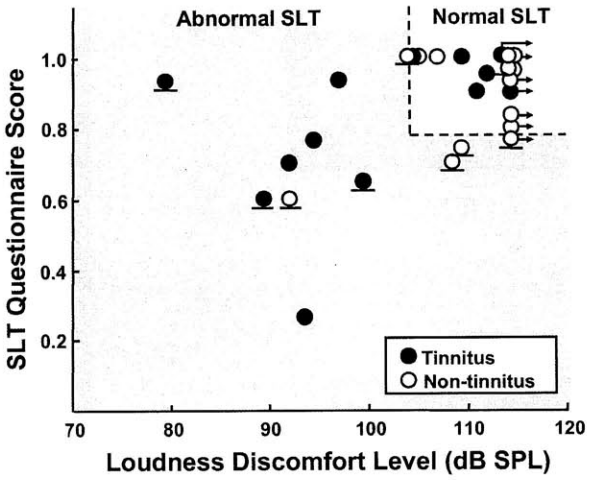

Figure A-3: *Classification of subjects by sound-level tolerance (fMRI).* Subjects were classified as having abnormal (gray shaded area) or normal (white at upper right) SLT based on LDL and SLTQ score. Each symbol corresponds to a subject. The division between normal and abnormal SLT regions was defined such that subjects unable to tolerate the 80 dB SPL stimulus during scanning (symbols with horizontal line below) would lie in the abnormal SLT region. Right-pointing arrows are placed next to the data for subjects who deemed no sound level "uncomfortable," including the maximum possible level produced by our testing equipment. In two cases where the maximum level was not deemed uncomfortable for one ear and a lower level was deemed "uncomfortable" for the other ear, the average of the maximum possible level and the level deemed "uncomfortable" was defined as the LDL and a right-pointing arrow was included.

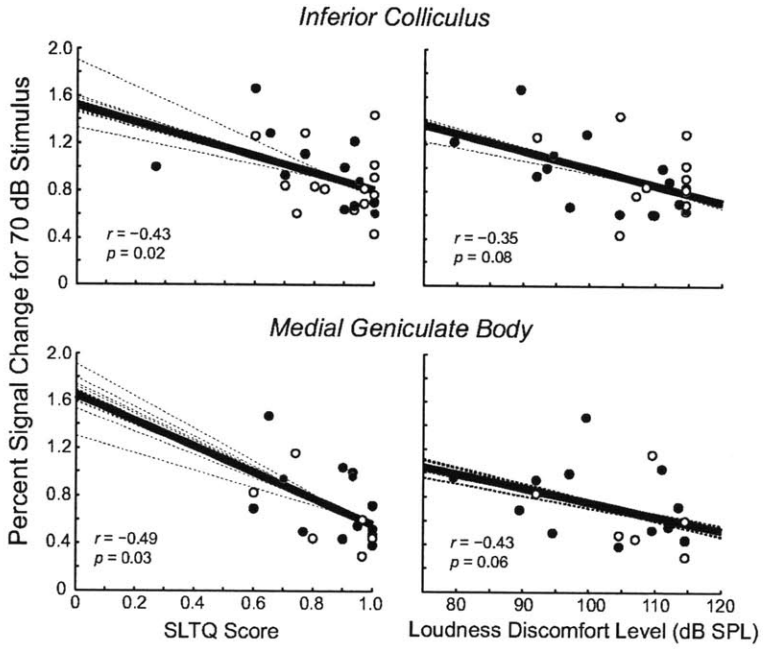

Figure A-4: *fMRI activation in the IC and MGB increased with decreasing SLTQ score (left) and LDL (right).* Stimulus level: 70 dB SPL. Each symbol in each panel corresponds to an individual tinnitus (filled symbol) or non-tinnitus (unfilled) subject. The solid line is a linear fit to all of the data. Spearmans coefficient and associated p-value are given in the lower left hand corner of each plot. Each dotted line is the result of a linear fit to all but one data point; a different data point was omitted for each line.

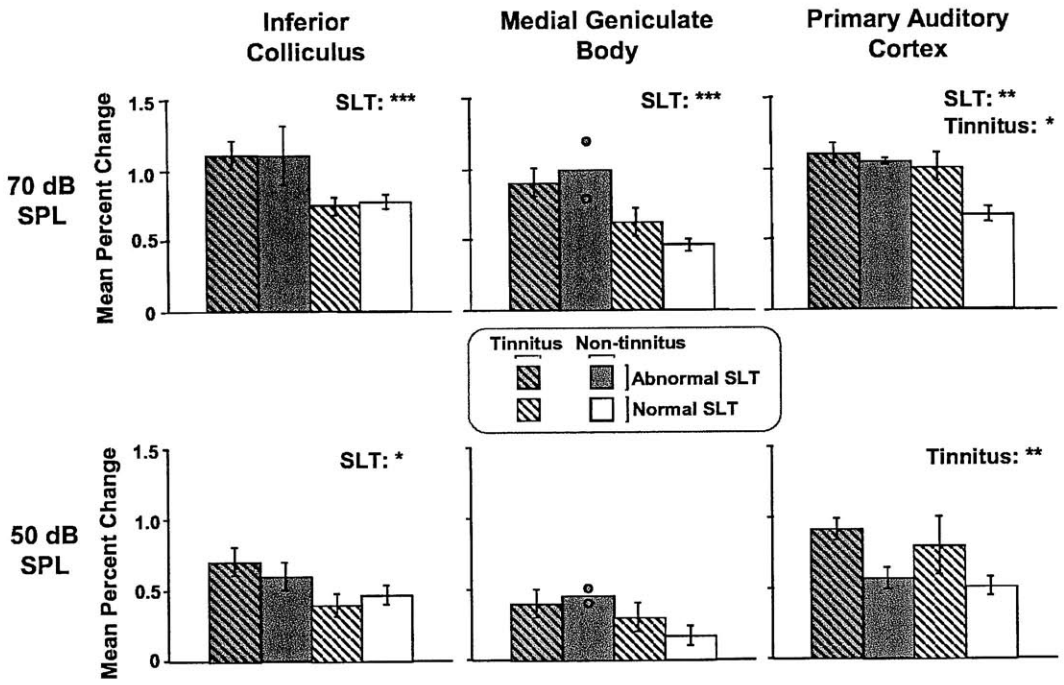

Figure A-5: *Auditory fMRI activation in subject groups defined by SLT and tinnitus.* Sound-evoked activation of the IC, MGB, and PAC showed a significant effect of SLT. PAC also showed a significant effect of tinnitus. The height of each bar indicates the mean percent signal change in response to 70 dB SPL (top row) or 50 dB SPL (bottom row) sound in IC (left column), MGB (middle column), or PAC (right column) for one of four subject groups defined based on SLT and tinnitus (see legend). Each subject contributed a single value to the mean. Error bars indicate $\pm$ one SE. For MGB in the non-tinnitus, abnormal SLT group, circles indicate individual subject data because only two subjects contributed to the mean. Results of a two-way ANOVA (tinnitus $\times$ SLT) are indicated at upper right of each panel as follows: $^{***}p \leq 0.005$, $^{**}p \leq 0.01$, $^{*}p \leq 0.05$.

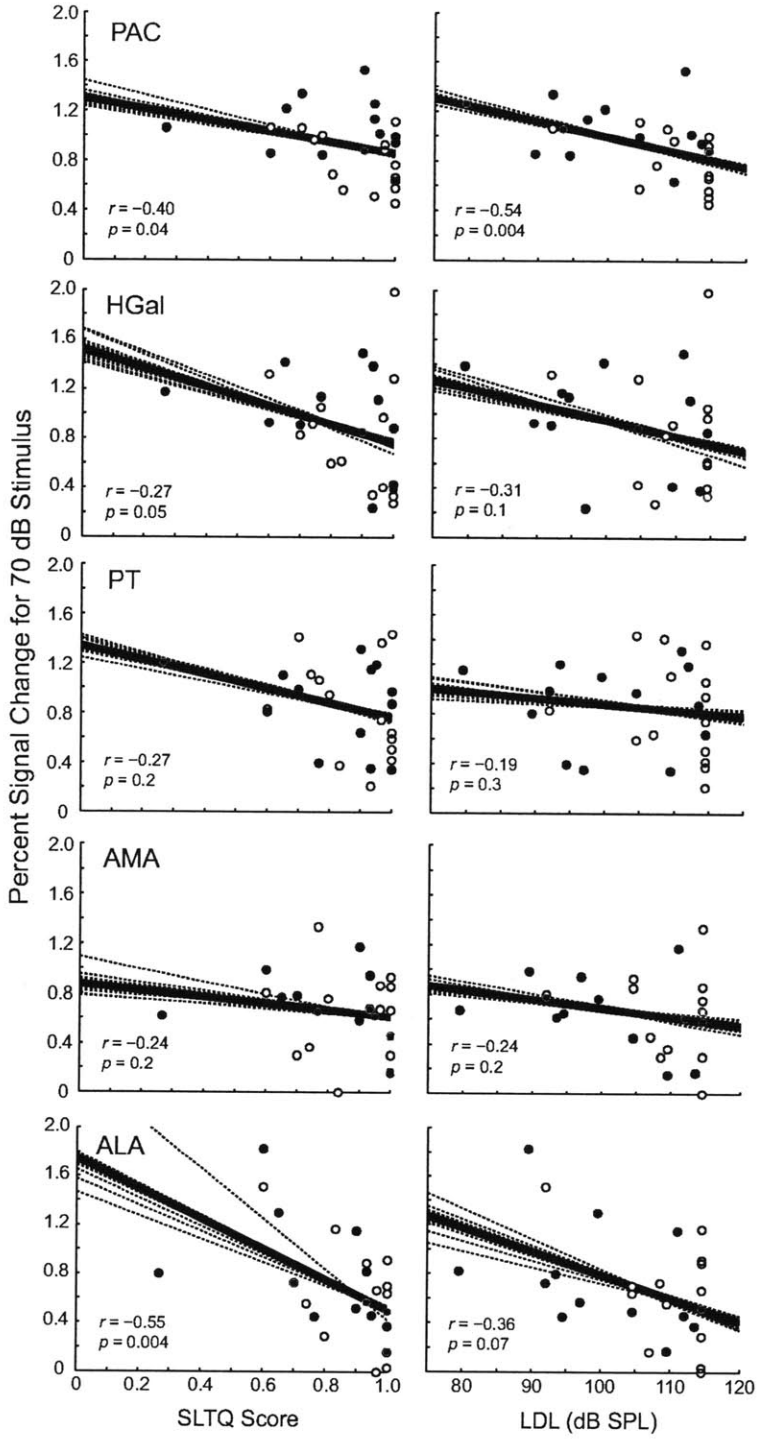

Figure A-6: *fMRI activation in auditory cortical regions tended to increase with decreasing SLTQ score (left column) and LDL (right).* Stimulus level: 70 dB SPL. See Figure A-6 caption for explanation of symbols and line fits.

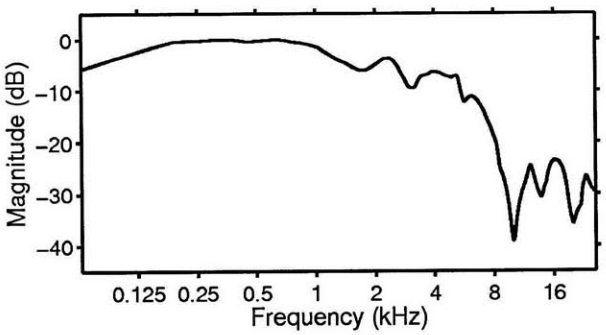

Figure A-7: *Spectrum of click stimulus for ABR measurements.*

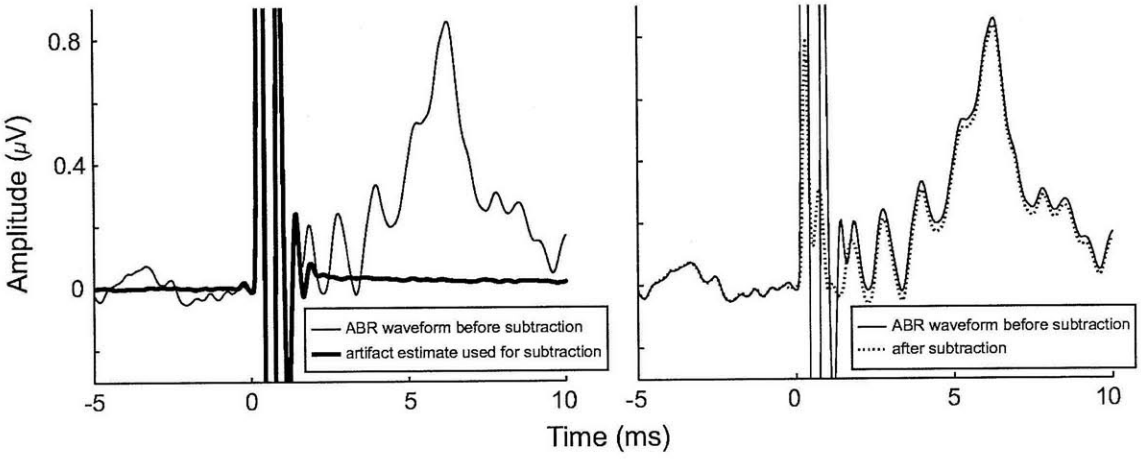

Figure A-8: *Subtraction of stimulus artifact from ABR waveform.* Typical example shown (subject #72, left ear, 80 dB).

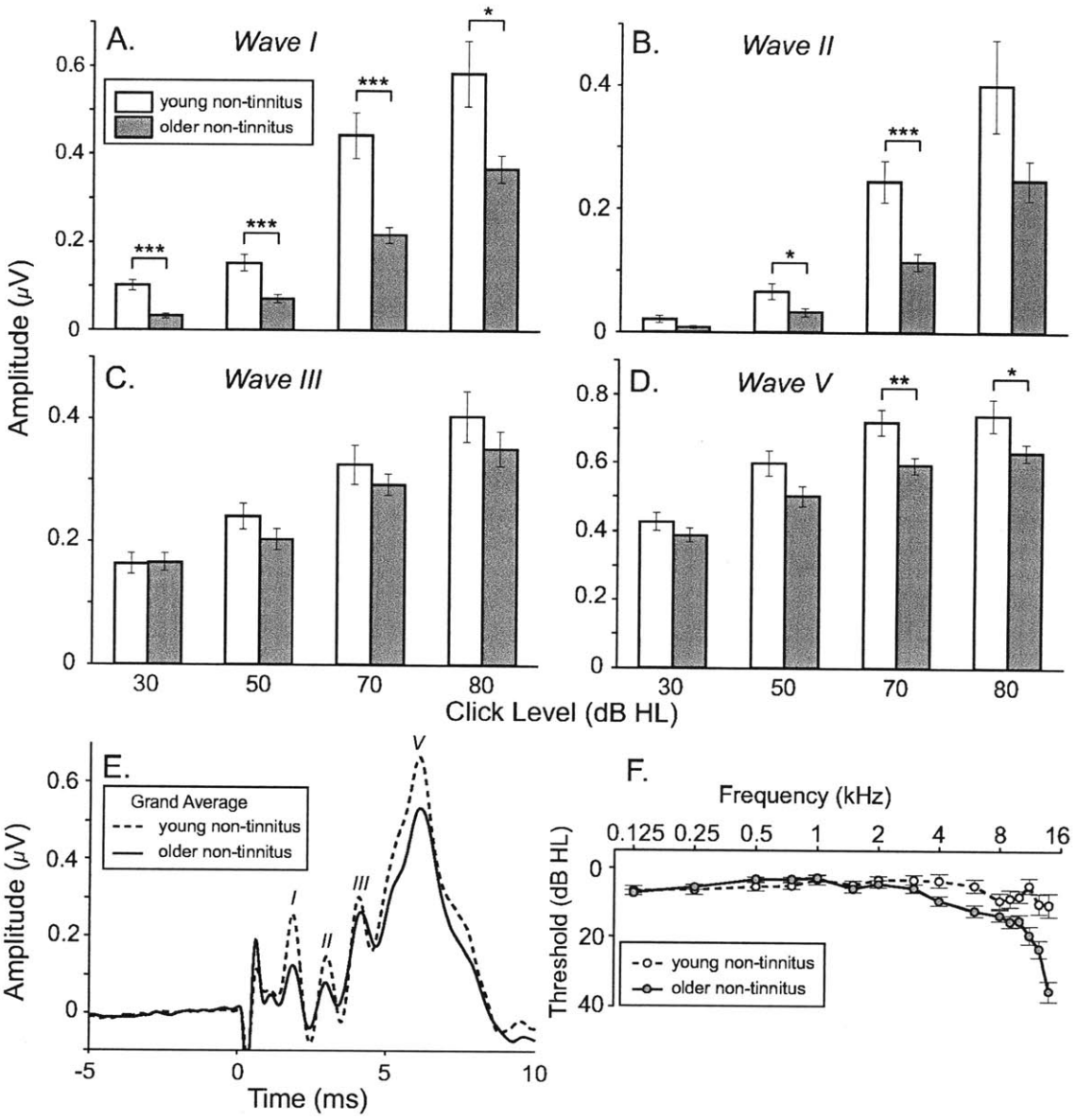

Figure A-9: *Reduced ABR amplitudes in older non-tinnitus subjects compared to young non-tinnitus subjects.* At each level, each ear of each subject was considered an individual data point. A–D: Values are mean ± SE. Rank-sum comparisons: $^{***}p \leq 0.001$, $^{**}p \leq 0.01$, $^{*}p \leq 0.05$. E: Mean ABR waveforms across all ears and electrodes. F: Mean pure tone thresholds ± SE. Older non-tinnitus subjects had high-frequency hearing loss.

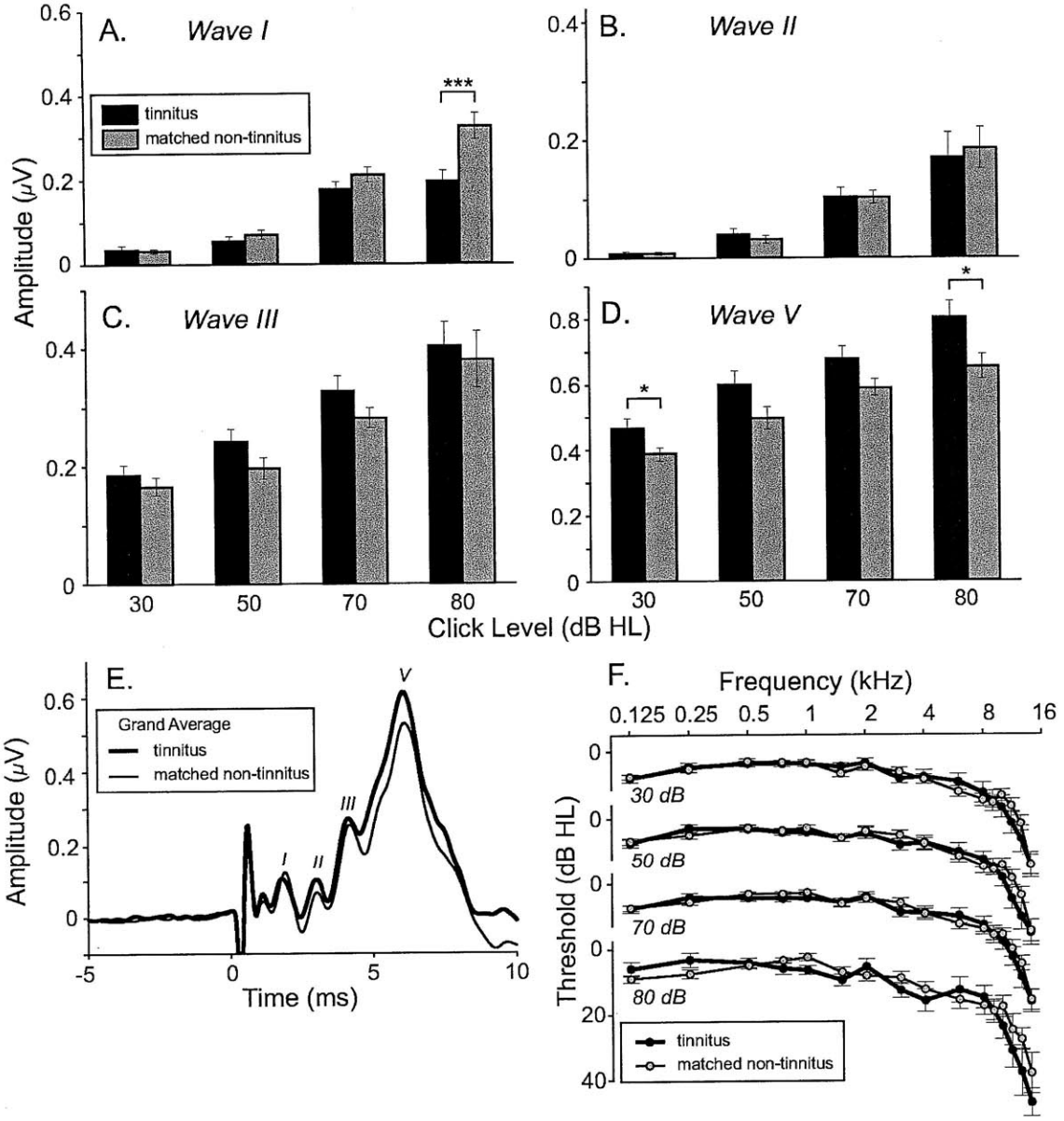

Figure A-10: *Reduced wave I amplitude but elevated wave V amplitude in tinnitus subjects compared to matched non-tinnitus subjects (ABR).* Matched non-tinnitus subjects are a subset of the older non-tinnitus subjects in Figure A-9. Same format as Figure A-9; see caption for Figure A-9.

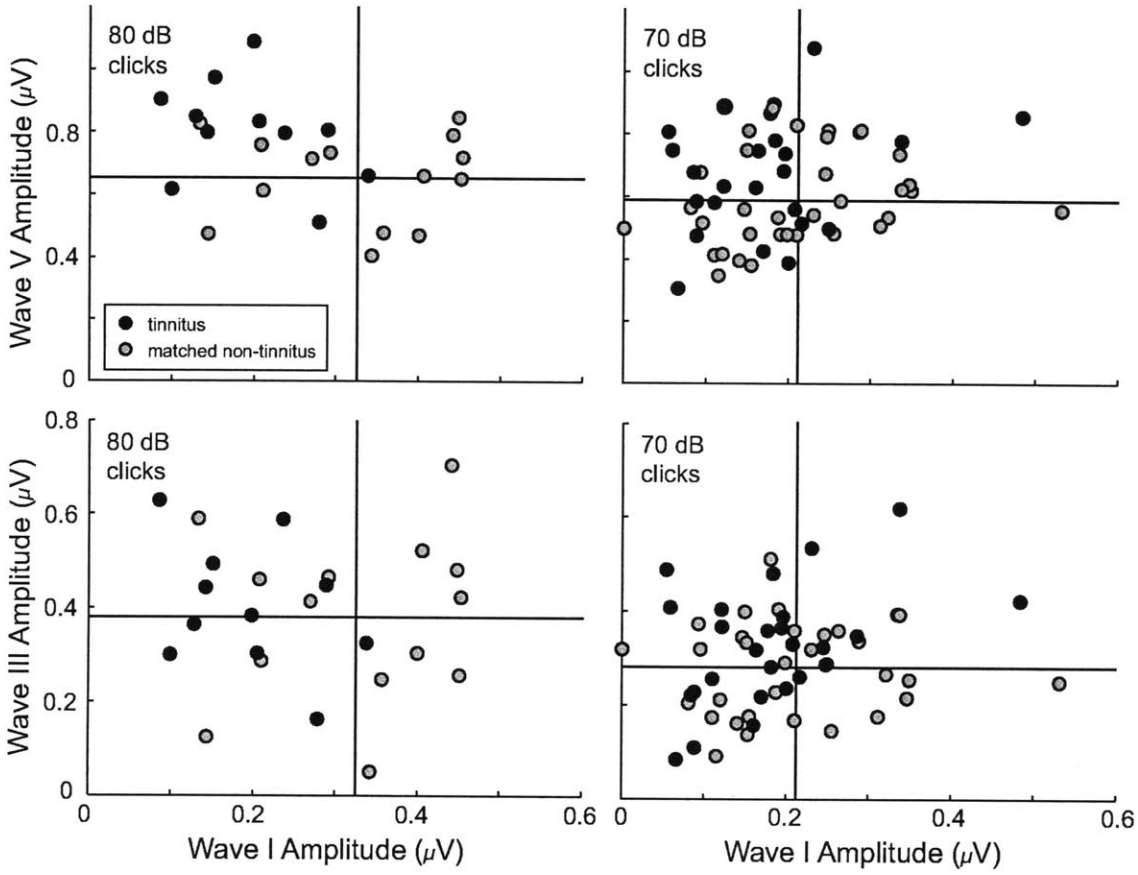

Figure A-11: *Individual data showing reduced wave I amplitude and elevated wave V amplitude in tinnitus subjects (ABR).* Tinnitus and matched non-tinnitus subjects same as those in Figure A-10. Dots represent individual ears. Vertical lines indicate mean wave I amplitudes of the matched non-tinnitus subjects; horizontal lines indicate mean wave V (top row) and III (bottom row) amplitudes.

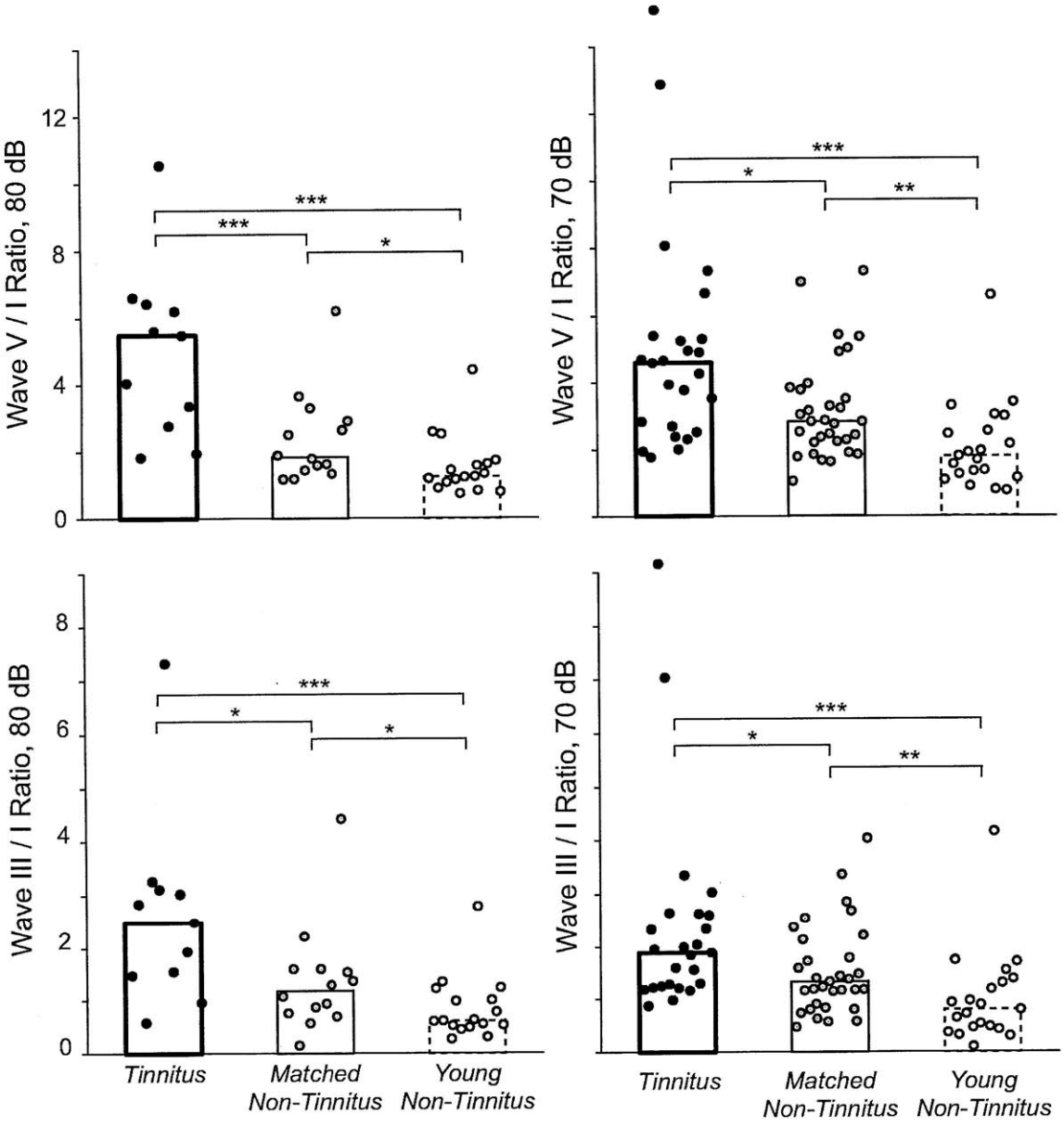

Figure A-12: *Wave V/I and III/I amplitude ratios were elevated in tinnitus subjects (ABR).* Tinnitus and matched non-tinnitus subjects same as those in Figures A-10 and A-11 with the exception of one matched non-tinnitus subject (#9) who had zero wave I amplitude at 70 dB; young non-tinnitus subjects same as those in Figure A-9. Dots represent individual ears. Bars indicate median. Rank-sum comparisons: $^{***}p \leq 0.001$, $^{**}p \leq 0.01$, $^{*}p \leq 0.05$.

Appendix B

Tables

	Tinnitus, Abnormal SLT	Non-Tinnitus, Abnormal SLT	Tinnitus, Normal SLT	Non-Tinnitus, Normal SLT
Number of subjects	7	5	6	9
Age[1]	41 ± 4	45 ± 5	45 ± 5	45 ± 4
Sex (% female)	43	60	0	33
Depression score[1]	10 ± 3	4 ± 2	10 ± 4	1.1 ± 0.4
Anxiety score[1]	8 ± 3	6 ± 4	6 ± 1	0.8 ± 0.3
TRQ score[1]	28 ± 6		30 ± 10	
LDL (dB SPL)[1]	92 ± 2	106 ± 4	111 ± 1	113 ± 1
SLTQ score[1]	0.69 ± 0.09	0.76 ± 0.07	0.96 ± 0.02	0.94 ± 0.03
Threshold for continuous noise (dB SPL)[1]	20 ± 3	23 ± 2	17 ± 3	16 ± 2

Mean ± one SEM[1]

Figure B-1: *Characteristics of each subject group (fMRI).*

Structure and Stimulus Level (dB SPL)	Tinnitus, Abnormal SLT n = 7	Non-Tinnitus, Abnormal SLT n = 5	Tinnitus, Normal SLT n = 6	Non-Tinnitus, Normal SLT n = 9
IC 50	0.7 ± 0.1	0.6 ± 0.1	0.39 ± 0.08	0.46 ± 0.07
IC 70	1.1 ± 0.1	1.1 ± 0.2	0.74 ± 0.07	0.77 ± 0.06
IC 80			1.0 ± 0.1	0.97 ± 0.05
MGB 50	0.4 ± 0.1 (n=6)[1]	0.45 (0.4, 0.5)[2]	0.3 ± 0.1	0.17 ± 0.07 (n=5)
MGB 70	0.9 ± 0.1 (n=6)	1.0 (0.8, 1.2)	0.6 ± 0.1	0.45 ± 0.05 (n=5)
MGB 80			0.8 ± 0.2	0.7 ± 0.1 (n=5)
PAC 50	0.91 ± 0.08	0.57 ± 0.08	0.8 ± 0.2	0.51 ± 0.07
PAC 70	1.10 ± 0.07	1.04 ± 0.03	1.0 ± 0.1	0.67 ± 0.05
PAC 80			1.0 ± 0.2	0.94 ± 0.09
HGal 50	0.6 ± 0.1	0.3 ± 0.2	0.6 ± 0.2	0.2 ± 0.1
HGal 70	1.0 ± 0.2	1.1 ± 0.1	0.9 ± 0.2	0.7 ± 0.2
HGal 80			1.0 ± 0.2	0.7 ± 0.1
PT 50	0.7 ± 0.1	0.8 ± 0.2	0.8 ± 0.2	0.6 ± 0.1
PT 70	0.9 ± 0.1	1.2 ± 0.1	0.9 ± 0.2	0.6 ± 0.1
PT 80			0.9 ± 0.3	0.87 ± 0.09
AMA 50	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
AMA 70	0.77 ± 0.05	0.7 ± 0.2	0.5 ± 0.2	0.58 ± 0.09
AMA 80			0.5 ± 0.1	0.73 ± 0.09 (n=8)[3]
ALA 50	0.5 ± 0.1	0.5 ± 0.3 (n=4)	0.33 ± 0.06	0.3 ± 0.2
ALA 70	0.9 ± 0.2	0.9 ± 0.2 (n=4)	0.5 ± 0.1	0.5 ± 0.1
ALA 80			0.51 ± 0.08	0.7 ± 0.2

[1]The number of subjects contributing to the mean is indicated whenever it is less than the total.
[2]Only two subjects contributed to the mean, so the data for each are given instead of an SEM.
[3]Anatomical ROI met criterion (see text) for being assigned a percent change of zero at 50 dB and 70 dB but not 80 dB for one subject.

Figure B-2: *Mean percent signal change (mean ± one SE) for each subject group structure, and stimulus level (fMRI).*

	Subject	Age	Sex	Handedness	Depression Score (max. 62)	Anxiety Score (max. 63)
non-tinnitus subjects	8	45	M	R	1	0
	9	49	M	R	0	0
	19	54	M	L & R	3	0
	20	46	F	R	1	1
	25	25	F	R	0	1
	37	33	M	R	3	2
	42	59	M	R	0	0
	46	45	M	R	0	0
	49	43	F	R	12	9
	51	47	F	R	0	0
	53	57	F	R	2	1
	55	45	M	R	0	2
	108	56	F	R	7	20
	114	28	M	R	1	0
tinnitus subjects	10	62	M	R	0	1
	22	37	F	R	0	0
	23	32	M	L & R	7	7
	28	23	M	R	21	1
	32	41	F	R	4	3
	72	45	M	L	9	7
	84	47	M	R	5	8
	85	52	M	L	0	2
	109	45	M	R	22	21
	111	36	M	R	21	6
	112	60	F	R	5	15
	116	43	M	R	13	10
	117	30	M	R	19	7

Figure B-3: *General subject characteristics (fMRI).*

	Subject	LDL (L, R; dB SPL)	ST Score	Tolerated 80 dB in Scanner
non-tinnitus subjects	8	105, 104	1.0	yes
	9	90, 94	0.6	no
	19	115, 114	1.0	yes
	20	>115, >114	1.0	yes
	25	>115, >114	0.8	yes
	37	>115, >114	1.0	yes
	42	>115, >114	0.9	yes
	46	>115, >114	1.0	yes
	49	>115, >114	0.8	no
	51	105, 104	1.0	no
	53	>115, >114	0.8	yes
	55	110, 104	1.0	yes
	108	105, 114	0.7	no
	114	108, 109	0.7	no
tinnitus subjects	10	105, 114	1.0	yes
	22	80, 79	0.9	no
	23	100, 99	0.7	no
	28	90, 89	0.6	no
	32	95, 94	0.8	yes
	72	90, 94	0.7	yes
	84	>115, >114	1.0	yes
	85	110, >114	1.0	yes
	109	100, 94	0.9	yes
	111	100, 109	1.0	yes
	112	93, 94	0.3	yes
	116	113, 114	1.0	yes
	117	108, 114	0.9	yes

Figure B-4: *Sound-tolerance characteristics (fMRI).*

Subject	Tinnitus Duration (yr)	Tinnitus Location	Tinnitus Quality	Tinnitus Pitch (kHz)[1]	Tinnitus Loudness (dB SL)[1]	MML (dB SL)	TRQ (max. 104)	Residual Inhibition
10	lifelong	in head, centered	ringing	(12, 12)	(20, 25)	(85, 85)[2]	0	n/a[2]
22	1	L	ringing	(750, n/a)	(15, n/a)	20	4	n/a
23	10	both ears, worse R	ringing	(8, >8)	(10, 20)	55	33	n/a
28	10	both ears, worse R	ringing	(1.5–2 , 2)[3]	(25, 45)	55	34	n/a
32	10	L	whining	(8, n/a)	(20, n/a)	45	33	n/a
72	13	in head, to R	ringing	(8, 8)	(5, 10)	20	43	no
84	4	both ears, equal	ringing	(>8, >8)	(10, 15)	(60, 70)[2]	29	no[2]
85	"many"	both ears, worse R	hissing, pulsing, tonal	(6, 6)	(5, 15–20)[3]	55	19	yes
109	10–15	both ears, worse L	ringing	(>8, 6–8)[3]	(30–35, 35–40)[3]	60	37	no
111	2	both ears, equal	tonal	(3, 3)	(20, 20)	15	15	no
112	8	in head, centered	multiple tones	(2, 1.5)	(15, 15)	45	10	no
116	18	both ears, equal	tonal, ringing	(6, 6)	(20, 10)	35	53	no
117	3	both ears, worse R	hissing, whistling	(>8, >8)	(15, 15)	35	69	no

[1](Left ear, Right ear)
[2]MML and residual inhibition were tested using monaural instead of binaural stimulation.
[3]Subject said pitch/loudness was between tested values.

Figure B-5: *Tinnitus characteristcs (fMRI).*

	Tinnitus, Abnormal SLT	Non-Tinnitus, Abnormal SLT	Tinnitus, Normal SLT	Non-Tinnitus, Normal SLT
Number of subjects	7	5	6	9
Age[1]	41 ± 4	45 ± 5	45 ± 5	45 ± 4
Sex (% female)	43	60	0	33
Depression score[1]	10 ± 3	4 ± 2	10 ± 4	1.1 ± 0.4
Anxiety score[1]	8 ± 3	6 ± 4	6 ± 1	0.8 ± 0.3
TRQ score[1]	28 ± 6		30 ± 10	
LDL (dB SPL)[1]	92 ± 2	106 ± 4	111 ± 1	113 ± 1
SLTQ score[1]	0.69 ± 0.09	0.76 ± 0.07	0.96 ± 0.02	0.94 ± 0.03
Threshold for continuous noise (dB SPL)[1]	20 ± 3	23 ± 2	17 ± 3	16 ± 2

Mean ± one SEM[1]

Figure B-6: *Characteristics of tinnitus and matched non-tinnitus subject groups (ABR).*

Wave	Click Level (dB HL)	*Young Non-Tinnitus*		*Matched Non-Tinnitus*		*Tinnitus*	
		Amplitude (μV)	Latency (ms)	Amplitude (μV)	Latency (ms)	Amplitude (μV)	Latency (ms)
I	30	0.10 ± 0.01 (20)	3.38 ± 0.06 (20)	0.032 ± 0.005 (32)	3.71 ± 0.08 (23)	0.03 ± 0.01 (20)	3.7 ± 0.1 (13)
	50	0.15 ± 0.02 (21)	2.47 ± 0.05 (21)	0.07 ± 0.01 (28)	2.54 ± 0.07 (24)	0.06 ± 0.01 (22)	2.6 ± 0.1 (17)
	70	0.44 ± 0.05 (21)	1.87 ± 0.03 (21)	0.21 ± 0.02 (33)	1.89 ± 0.03 (32)	0.18 ± 0.02 (25)	1.95 ± 0.05 (25)
	80	0.59 ± 0.07 (17)	1.72 ± 0.03 (17)	0.33 ± 0.03 (14)	1.71 ± 0.03 (14)	0.20 ± 0.03 (11)	1.81 ± 0.06 (11)
II	30	0.021 ± 0.006 (20)	4.42 ± 0.08 (10)	0.009 ± 0.002 (32)	4.50 ± 0.05 (14)	0.008 ± 0.004 (20)	4.37 ± 0.07 (6)
	50	0.07 ± 0.01 (21)	3.45 ± 0.06 (16)	0.031 ± 0.007 (28)	3.5 ± 0.1 (18)	0.04 ± 0.01 (22)	3.5 ± 0.1 (14)
	70	0.25 ± 0.03 (21)	3.05 ± 0.04 (20)	0.10 ± 0.01 (33)	3.02 ± 0.03 (30)	0.10 ± 0.02 (25)	3.07 ± 0.04 (22)
	80	0.40 ± 0.08 (17)	2.87 ± 0.05 (17)	0.19 ± 0.04 (14)	2.81 ± 0.07 (12)	0.17 ± 0.04 (11)	2.89 ± 0.06 (11)
III	30	0.16 ± 0.02 (20)	5.53 ± 0.05 (20)	0.17 ± 0.02 (32)	5.86 ± 0.07 (29)	0.19 ± 0.02 (20)	5.78 ± 0.09 (19)
	50	0.24 ± 0.02 (21)	4.62 ± 0.07 (20)	0.20 ± 0.02 (28)	4.74 ± 0.06 (28)	0.24 ± 0.02 (22)	4.76 ± 0.09 (21
	70	0.33 ± 0.03 (21)	4.11 ± 0.03 (21)	0.28 ± 0.02 (33)	4.15 ± 0.03 (33)	0.33 ± 0.03 (25)	4.19 ± 0.04 (25)
	80	0.40 ± 0.04 (17)	4.02 ± 0.04 (17)	0.38 ± 0.05 (14)	3.99 ± 0.05 (14)	0.40 ± 0.04 (11)	4.10 ± 0.07 (11)
V	30	0.43 ± 0.03 (20)	7.35 ± 0.07 (20)	0.39 ± 0.02 (32)	7.75 ± 0.09 (32)	0.47 ± 0.03 (20)	7.57 ± 0.08 (20
	50	0.60 ± 0.04 (21)	6.45 ± 0.05 (21)	0.50 ± 0.03 (28)	6.52 ± 0.05 (28)	0.60 ± 0.04 (22)	6.60 ± 0.06 (22)
	70	0.72 ± 0.04 (21)	6.09 ± 0.04 (21)	0.59 ± 0.03 (33)	6.19 ± 0.04 (33)	0.68 ± 0.04 (25)	6.09 ± 0.04 (25)
	80	0.74 ± 0.05 (17)	5.96 ± 0.06 (17)	0.65 ± 0.04 (14)	6.0 ± 0.1 (14)	0.80 ± 0.05 (11)	5.9 ± 0.1 (11)

Values are means ± SE. Numbers in parentheses are number of stimulated ears. Because latency could not be determined when wave amplitude was zero, the number of ears contributing to the mean is less for latency than for amplitude.

Figure B-7: *Mean amplitude and latency for each subject group, wave, and stimulus level (ABR).*

	Subject	Age	Sex	Handed-ness	Depression (max = 62)	Anxiety (max = 63)	LDL L, R (dB SPL)	SLTQ Score (max = 1)
Tinnitus	23	34	M	R	11	8	83, 89	0.3
	72	47	M	L	5	5	108, 114	0.8
	84	49	M	R	4	3	108, 114	0.07
	85	55	M	L	0	4	113, 114	1.0
	109	47	M	R	7	2	118, 114	1.0
	110	41	M	R	8	7	83, 79	0.5
	111	38	M	R	5	2	>118, >119	1.0
	116	44	M	R	12	16	>118, >119	1.0
	128	44	M	R	20	6	78, 79	0.2
	129	45	M	R	0	1	73, 79	1.0
	145	38	M	R	2	6	108, 104	0.7
	160	39	M	R	11	12	118, 109	1.0
	186	33	M	R	9	10	108, 114	0.4
	213	49	M	R	0	0	118, 119	0.8
	215	34	M	R	36	22	98, 94	0.4
Matched Non-Tinnitus	8	47	M	R	2	0	103, 104	0.9
	9	51	M	R	0	0	93, 94	0.8
	19	56	M	L&R	14	0	>118, >119	1.0
	46	47	M	R	0	0	>118, >119	1.0
	119	43	M	R	0	1	>118, 119	1.0
	125	50	M	R	0	0	98, 104	0.4
	135	35	M	R	4	7	>118, >119	0.5
	142	48	M	R	5	3	113, 114	0.9
	146	43	M	R	6	3	113, 114	1.0
	148	36	M	R	5	0	118, 109	0.6
	151	40	M	R	2	9	108, 114	0.9
	191	38	M	R	0	0	>118, >119	0.7
	200	36	M	R	0	0	103, 104	0.8
	203	53	M	R	2	1	118, >119	1.0
	205	38	M	R	0	0	108, 104	0.7
	206	46	M	R	0	0	98, 104	1.0
	208	39	M	L	0	0	93, 89	0.7
	210	45	M	R	0	0	103, 109	1.0
	211	48	M	R	0	0	118, 114	1.0
	229	33	M	R	3	8	103, 109	1.0
	230	34	M	R	0	0	113, 114	0.8
Young Non-Tinnitus	152	21	M	R	0	4	103, 104	0.7
	156	21	M	R	0	3	88, 99	0.8
	158	23	M	R	1	4	>118, >119	1.0
	168	22	M	L	0	2	98, 109	1.0
	169	25	M	R	1	2	98, 94	1.0
	176	24	M	R	3	1	118, >119	0.8
	181	24	M	R	0	0	>118, >119	1.0
	217	24	M	R	0	0	98, 104	0.8
	220	26	M	R	0	0	93, 104	0.8
	223	25	M	R	4	3	103, 104	1.0
	225	21	M	R	16	9	103, 109	0.7

Figure B-8: *General subject characteristics (ABR).*

Subject	Tinnitus Duration (yrs)	Tinnitus Location	Tinnitus Quality	Tinnitus Pitch L, R (kHz)	Tinnitus Loudness L, R (dB SL)	MML (dB SL)	TRQ (max = 104)	Residual Inhibition
23	13	both ears, worse in L	tonal	>8, >8	35, 40	45	33	yes
72	15	both ears, worse in R	ringing	>8, 8	25, 25	40	35	no
84	6	both ears, worse in R	ringing, tonal, humming	12, 12	20, 20	30	33	no
85	"many"	both ears, worse in R	crackling, hissing	12, 14	10, 20	50	18	yes
109	10–15	in head, center	ringing, tonal	10, 12	30, 30	45	7	no
110	3	both ears, worse in L	ringing, buzzing, hissing	1.5, 1.5	20, 10	20	61	yes
111	3	in head, center	ringing, tonal	2, 2	15, 15	20	8	no
116	19	both ears, equal	ringing	12, 12	15, 10	35	61	no
128	11	right ear	ringing	n/a, 6	n/a, 50	50	9	no
129	6	right ear	ringing	n/a, 2–3	n/a, 10–15	20	64	yes
145	1	in head, toward L	tonal	3.5, 3.5	0, 0	20	5	no
160	2	in head, center	hissing, electronic	10, 10	25, 15	50	14	no
186	16	both ears, worse in R	ringing, tonal	8, 2	35, 35	65	24	yes
213	"since teens"	both ears, equal	ringing	16, 16	0, 5	25	0	no
215	10	both ears, equal	shooshing	>16, >16	10, 10	>80	92	n/a

Figure B-9: *Tinnitus characteristics (ABR).*

Bibliography

[Adams, 1986] Adams, J. (1986). Neuronal morphology in the human cochlear nucleus. *Arch Otolaryngol Head Neck Surg*, 112:1253–1261.

[Ambrosini and Shoenen, 2006] Ambrosini, A. and Shoenen, J. (2006). Electrophysiological response patterns of primary sensory cortices in migraine. *J Headache Pain*, 7:377–388.

[Arnold et al., 1996] Arnold, W., Bartenstein, P., Oestreicher, E., Romer, W., and Schwaiger, M. (1996). Focal metabolic activation in the predominant left auditory cortex in patients suffering from tinnitus: A PET study with [^{18}F]deoxyglucose. *J Otorhinolaryngol*, 58:195–199.

[Asako et al., 2005] Asako, M., Holt, A., Griffith, R., Buras, E., and Altschuler, R. (2005). Deafness-related decreases in glycine-immunoreactive labeling in the rat cochlear nucleus. *J Neurosci Res*, 81:102–109.

[Attias et al., 1996] Attias, J., Pratt, H., Reshef, I., Bresloff, I., Horowitz, G., Polyakov, A., and Shemesh, Z. (1996). Detailed analysis of auditory brainstem responses in patients with noise-induced tinnitus. *Audiology*, 35:259–270.

[Attias et al., 1993] Attias, J., Urbach, D., Gold, S., and Shemesh, Z. (1993). Auditory event related potentials in chronic tinnitus patients with noise induced hearing loss. *Hear Res*, 71:106–113.

[Baguley, 2003] Baguley, D. (2003). Hyperacusis. *J Royal Soc Med*, 96:582–585.

[Bauer et al., 2007] Bauer, C., Brozoski, T., and Myers, K. (2007). Primary afferent degeneration as a cause of tinnitus. *Neurosci Res*, 85:1489–1498.

[Bauer et al., 2008] Bauer, C., Turner, J., Caspary, D., Myers, K., and Brozoski, T. (2008). Tinnitus and inferior colliculus activity in chinchillas related to three distinct patterns of cochlear trauma. *J Neurosci Res*, 86:2564–2578.

[Beck et al., 1988] Beck, A., Epstein, N., Brown, G., and Steer, R. (1988). An inventory for measuring clinical anxiety: Psychometric properties. *J Consult Clin Psychol*, 56:893–897.

[Beck et al., 1961] Beck, A., Ward, C., Mendelson, M., Mock, J., and Erbaugh, J. (1961). An inventory for measuring depression. *J Arch Gen Psychiatry*, 4:561–571.

[Brittain, 1939] Brittain, F. (1939). The loudness of continuous spectrum noise and its application to loudness measurements. *J Acoust Soc Am*, 11:113–117.

[Brozoski et al., 2002] Brozoski, T., Bauer, C., and Caspary, D. (2002). Elevated fusiform cell activity in the dorsal cochlear nucleus of chinchillas with psychophysical evidence of tinnitus. *J Neurosci*, 22:2383–2390.

[Brozoski et al., 2007] Brozoski, T., Ciobanu, L., and Bauer, C. (2007). Central neural activity in rats with tinnitus evaluated with manganese-enhanced magnetic resonance imaging (MEMRI). *Hear Res*, 228:168–179.

[Buchwald and Huang, 1975] Buchwald, J. and Huang, C. (1975). Far-field acoustic response: origins in the cat. *Science*, 189:382–384.

[Cant and Benson, 2003] Cant, N. and Benson, C. (2003). Parallel auditory pathways: projection patterns of the different neuronal populations in the dorsal and ventral cochlear nuclei. *Brain Res Bull*, 60:457–474.

[Caspary et al., 2008] Caspary, D., Ling, L., Turner, J., and Hughes, L. (2008). Inhibitory neurotransmission, plasticity and aging in the mammalian central auditory system. *J Exp Biol*, 211:1781–1791.

[Cook et al., 2004] Cook, D., Lange, G., Ciccone, D., Liu, W.-C., Steffener, J., and Natelson, B. (2004). Functional imaging of pain in patients with primary fibromyalgia. *J Rheumatol*, 31:364–378.

[Cox et al., 1997] Cox, R., Alexander, G., Taylor, I., and Gray, G. (1997). The contour test of loudness perception. *Ear Hearing*, 18:388–400.

[Davis et al., 2007] Davis, P., Paki, B., and Hanley, P. (2007). Neuromonics tinnitus treatment: Third clinical trial. *Ear Hearing*, 28:242–259.

[Degerman et al., 2006] Degerman, A., Rinne, T., Salmi, J., Salonen, O., and Alho, K. (2006). Selective attention to sound location or pitch studied with fMRI. *Brain Res*, 1077:123–134.

[Dobie, 2003] Dobie, R. (2003). Depression and tinnitus. *Otolaryngol Clin North Am*, 36:383–388.

[Dobie, 2004] Dobie, R. (2004). Overview: Suffering from tinnitus. In Snow, J., editor, *Tinnitus: Theory and Management*, pages 1–7. BC Decker Inc, London.

[Doucet and Ryugo, 1997] Doucet, J. and Ryugo, D. (1997). Projections from the ventral cochlear nucleus to the dorsal cochlear nucleus in rats. *J Comp Neurol*, 385:245–264.

[Edmister et al., 1999] Edmister, W., Talavage, T., Ledden, P., and Weisskoff, R. (1999). Improved auditory cortex imaging using clustered volume acquisitions. *Hum Brain Mapp*, 7:89–97.

[Eggermont and Komiya, 2000] Eggermont, J. and Komiya, H. (2000). Moderate noise trauma in juvenile cats results in profound cortical topographic map changes in adulthood. *Hear Res*, 142:89–101.

[Eggermont and Roberts, 2004] Eggermont, J. and Roberts, L. (2004). The neuroscience of tinnitus. *Trends Neurosci*, 27:676–682.

[Fullerton et al., 1987] Fullerton, B., Levine, R., Hosford-Dunn, H., and Kiang, N.-S. (1987). Comparison of cat and human brain-stem auditory evoked potentials. *Electroen Clin Neuro*, 66:547–570.

[Fullerton and Pandya, 2007] Fullerton, B. and Pandya, D. (2007). Architectonic analysis of the auditory-related areas of the superior temporal region in human brain. *J Comp Neurol*, 504:470–498.

[Gómez-Nieto and Rubio, 2009] Gómez-Nieto, R. and Rubio, M. (2009). A bushy cell network in the rat ventral cochlear nucleus. *J Comp Neurol*, 516:241–263.

[Gómez-Nieto and Rubio, 2011] Gómez-Nieto, R. and Rubio, M. (2011). Ultrastructure, synaptic organization, and molecular components of bushy cell networks in the anteroventral cochlear nucleus of the rhesus monkey. *Neuroscience*, 179:188–207.

[Gu et al., 2010] Gu, J., Halpin, C., Nam, E.-C., Levine, R., and Melcher, J. (2010). Tinnitus, diminished sound-level tolerance, and elevated auditory activity in humans with clinically normal hearing sensitivity. *J Neurophysiol*, 104:3361–3370.

[Guimaraes et al., 1998] Guimaraes, A., Melcher, J., Talavage, T., Baker, J., Ledden, P., Rosen, B., Kiang, N.-S., Fullerton, B., and Weisskoff, R. (1998). Imaging subcortical auditory activity in humans. *Hum Brain Mapp*, 6:33–41.

[Hall et al., 1999] Hall, D., Haggard, M., an dAR Palmer, M. A., Summerfield, A., Elliot, M., Gurney, E., and Bowtell, R. (1999). "Sparse" temporal sampling in auditory fMRI. *Hum Brain Mapp*, 7:213–223.

[Hall et al., 2001] Hall, D., Haggard, M., Summerfield, A., Akeroyd, M., and Palmer, A. (2001). Functional magnetic resonance imaging measurements of sound-level encoding in the absence of background scanner noise. *J Acoust Soc Am*, 109:1559–1570.

[Harms and Melcher, 2002] Harms, M. and Melcher, J. (2002). Sound repetition rate in the human auditory pathway: Representations in the waveshape and amplitude of fMRI activation. *J Neurophysiol*, 88:1433–1450.

[Hawley et al., 2005] Hawley, M., Melcher, J., and Fullerton, B. (2005). Effects of sound bandwidth on fMRI activation in human auditory brainstem nuclei. *Hear Res*, 204:101–110.

[Heller, 2003] Heller, A. (2003). Classification and epidemiology of tinnitus. *Otolaryngol Clin North Am*, 36:239–248.

[Hoffman and Reed, 2004] Hoffman, H. and Reed, G. (2004). Epidemiology of tinnitus. In Snow, J., editor, *Tinnitus: Theory and Management*, pages 16–41. BC Decker Inc, London.

[Illing et al., 2005] Illing, R., Kraus, K., and Meidinger, M. (2005). Reconnecting neuronal networks in the auditory brainstem following unilateral deafening. *Hear Res*, 206:185–199.

[Irvine et al., 2000] Irvine, D., Rajan, R., and McDermott, H. (2000). Injury-induced reorganization in adult auditory cortex and its perceptual consequences. *Hear Res*, 147:188–199.

[Irving and Harrison, 1967] Irving, R. and Harrison, J. (1967). The superior olivary complex and audition: a comparative study. *Comp Neurol*, 130:77–86.

[Jastreboff et al., 1996] Jastreboff, P., Gray, W., and Gold, S. (1996). Neurophysiological approach to tinnitus patients. *Am J Otol*, 17:236–240.

[Jerger and Hall, 1980] Jerger, J. and Hall, J. (1980). Effects of age and sex on auditory brainstem response. *Arch Otoloaryngol*, 106:387–391.

[Kaas and Hackett, 2000] Kaas, J. and Hackett, T. (2000). Subdivisions of auditory cortex and processing streams in primates. *Proc Natl Acad Sci*, 97:11793–11799.

[Kaltenbach, 2007] Kaltenbach, J. (2007). The dorsal cochlear nucleus as a contributor to tinnitus: mechanisms underlying induction of hyperactivity. *Prog Brain Res*, 166:89–106.

[Kaltenbach and McCaslin, 1996] Kaltenbach, J. and McCaslin, D. (1996). Increases in spontaneous activity in the dorsal cochlear nucleus following exposure to high intensity sound: a possible neural correlate of tinnitus. *Auditory Neurosci*, 3:57–78.

[Kaltenbach et al., 2004] Kaltenbach, J., Zhang, J., and Zacharekl, M. (2004). Neural correlates of tinnitus. In Snow, J., editor, *Tinnitus: Theory and Management*, pages 141–161. BC Decker Inc, London.

[Katzenell and Segal, 2001] Katzenell, U. and Segal, S. (2001). Hyperacusis: Review and clinical guidelines. *Otol Neurotol*, 22:321–327.

[Kehrle et al., 2008] Kehrle, H., Granjeiro, R., Sampaio, A., Bezerra, R., Almeida, V., and Oliveira, C. (2008). Comparison of auditory brainstem response results in normal-hearing patients with and without tinnitus. *Arch Otolaryngol Head Neck Surg*, 134:647–651.

[Kim et al., 2004] Kim, J., Gross, J., Morest, D., and Potashner, S. (2004). Quantitative study of degeneration and new growth of axons and synaptic endings in the chinchilla cochlear nucleus after acoustic overstimuluation. *J Neurosci Res*, 77:829–842.

[Kosek et al., 1996] Kosek, E., Ekholm, J., and Hansson, P. (1996). Sensory dysfunction in fibromyalgia patients with implications for pathogenic mechanisms. *Pain*, 68:375–383.

[Krumbholtz et al., 2007] Krumbholtz, K., Eickhoff, S., and Fink, G. (2007). Feature- and object-based attentional modulation in the human auditory "where" pathway. *J Cog Neurosci*, 19:1721–1733.

[Kujawa and Liberman, 2009] Kujawa, S. and Liberman, M. (2009). Adding insult to injury: Cochlear nerve degeneration after "temporary" noise-induced hearing loss. *J Neurosci*, 29:14077–14085.

[Kujawa et al., 2011] Kujawa, S., Micucci, S., and Liberman, M. (2011). Noise-induced primary neural degeneration: Effects of spectrum, duration, intensity and survival. *Assoc Res Otolaryngol*, 34:401.

[Langers et al., 2007] Langers, D., van Dijk, P., Schoenmaker, E., and Backes, W. (2007). fMRI activation in relation to sound intensity and loudness. *NeuroImage*, 35:709–718.

[Lanting et al., 2008] Lanting, C., de Kleine, E., Bartels, H., and van Dijk, P. (2008). Functional imaging of unilateral tinnitus using fMRI. *Acta Oto-Laryngol*, 128:415–421.

[Levine, 1981] Levine, R. (1981). Binaural interaction in brainstem potentials of human subjects. *Ann Neurol*, 9:384–393.

[Levine and Kiang, 1995] Levine, R. and Kiang, N.-S. (1995). A conversation about tinnitus. In Møller, A. and Vernon, J., editors, *Mechanisms of Tinnitus*, pages 149–161. Allyn and Bacon, Boston.

[Liberman, 1978] Liberman, M. (1978). Auditory-nerve responses from cats raised in a low-noise chamber. *J Acoust Soc Am*, 63:442–455.

[Lin et al., 2011] Lin, H., Furman, A., Kujawa, S., and Liberman, M. (2011). Noise-induced primary neural degeneration in guinea pig: Does vulnerability depend on spontaneous discharge rate? *Assoc Res Otolaryngol*, 34:400.

[Lockwood et al., 1998] Lockwood, A., Salvi, R., Coad, M., Towsley, M., Wack, D., and Murphy, B. (1998). The functional neuroanatomy of tinnitus. *Neurology*, 50:114–120.

[Malmierca et al., 1996] Malmierca, M., Le Beau, F., and Rees, A. (1996). The topographical organization of descending projections from the central nucleus of the inferior colliculus in guinea pig. *Hear Res*, 93:167–180.

[Melcher, 1993] Melcher, J. (1993). *The Cellular Generators of the Brainstem Auditory Evoked Potential.* PhD thesis, Massachusetts Institute of Technology.

[Melcher, 2010] Melcher, J. (2010). A model for tinnitus generation based in the ventral, not dorsal, cochlear nucleus. *Assoc Res Otolaryngol*, 33:242.

[Melcher and Kiang, 1996] Melcher, J. and Kiang, N.-S. (1996). Generators of the brainstem auditory evoked potential in cat III: Identified cell populations. *Hear Res*, 83:52–71.

[Melcher et al., 2009] Melcher, J., Levine, R., Bergevin, C., and Norris, B. (2009). The auditory midbrain of people with tinnitus: abnormal sound-evoked activity revisited. *Hear Res*, 257:63–74.

[Michalewski et al., 1980] Michalewski, H., Thompson, L., Patternson, J., Bowman, T., and Litzelman, D. (1980). Sex differences in the amplitudes and latencies of the human auditory brain stem potential. *Electroen Clin Neuro*, 48:351–356.

[Møller, 2007] Møller, A. (2007). Tinnitus and pain. *Prog Brain Res*, 166:47–53.

[Møller and Jannetta, 1981] Møller, A. and Jannetta, P. (1981). Compound action potentials recorded intracranially from the auditory nerve in man. *Exp Neurol*, 74:862–874.

[Møller and Jannetta, 1982] Møller, A. and Jannetta, P. (1982). Evoked potentials from the inferior colliculus in man. *Electroen Clin Neuro*, 53:612–620.

[Noreña and Chéry-Croze, 2007] Noreña, A. and Chéry-Croze, S. (2007). Enriched acoustic environment rescales auditory sensitivity. *NeuroReport*, 18:1251–1255.

[Oldfield, 1971] Oldfield, R. (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropyschologia*, 9:97–113.

[Oliver, 2000] Oliver, D. (2000). Ascending efferent projections of the superior olivary complex. *Microsc Res Techniq*, 51:355–363.

[Paltoglou et al., 2009] Paltoglou, A., Sumner, C., and Hall, D. (2009). Examining the role of frequency specificity in the enhancement and suppression of human cortical activity by auditory selective attention. *Hear Res*, 257:106–118.

[Peake and Kiang, 1962] Peake, W. and Kiang, N.-S. (1962). Cochlear responses to condensation and rarefaction clicks. *Biophys J*, 2:23–32.

[Pollack, 1951] Pollack, I. (1951). On the threshold and loudness of repeated bursts of noise. *J Acoust Soc Am*, 23:646–650.

[Rademacher et al., 2001] Rademacher, J., Morosan, P., Schormann, T., Schleicher, K., Freund, H., and Zilles, K. (2001). Probabilistic mapping and volume measurement of human primary auditory cortex. *NeuroImage*, 13:669–683.

[Rinne et al., 2008] Rinne, T., Balk, M., Koistinen, S., Autti, T., Alho, K., and Sams, M. (2008). Auditory selective attention modulates activation of human inferior colliculus. *J Neurophysiol*, 100:3323–3327.

[Salvi et al., 1990] Salvi, R., Saunders, S., Gratton, M., Arehole, S., and Powers, N. (1990). Enhanced response amplitudes in the inferior colliculus of the chinchilla following acoustic trauma. *Hear Res*, 50:245–258.

[Salvi et al., 2000] Salvi, R., Wang, J., and Ding, D. (2000). Auditory plasticity and hyperactivity following cochlear damage. *Hear Res*, 147:261–274.

[Shargorodsky et al., 2010] Shargorodsky, J., Curhan, G., and Farwell, W. (2010). Prevalence and characteristics of tinnitus among US adults. *Am J Med*, 123:711–718.

[Shore et al., 1991] Shore, S., Helfert, R., Bledsoe, Jr, S., Altschuler, R., and Godfrey, D. (1991). Descending projections to the dorsal and ventral divisions of the cochlear nucleus in guinea pig. *Hear Res*, 52:255–268.

[Sigalovsky et al., 2006] Sigalovsky, I., Fischl, B., and Melcher, J. (2006). Mapping an intrinsic MR property of gray matter in auditory cortex of living humans: A possible marker for primary cortex and hemispheric differences. *NeuroImage*, 32:1524–1537.

[Sigalovsky and Melcher, 2006] Sigalovsky, I. and Melcher, J. (2006). Effects of sound level on fMRI activation in human brainstem, thalamic and cortical centers. *Hear Res*, 215:67–76.

[Stockard et al., 1979] Stockard, J., Stockard, J., Westmoreland, B., and Corfits, J. (1979). Brainstem auditory-evoked responses: Normal variation as a function of stimulus and subject characteristics. *Arch Neurol*, 36:823–831.

[Suneja et al., 1998] Suneja, S., Potashner, S., and Benson, C. (1998). Plastic changes in glycine and GABA release and uptake in adult brain stem auditory nuclei after unilateral middle ear ossicle removal and cochlear ablation. *Exp Neurol*, 151:273–288.

[Syka et al., 1994] Syka, J., Rybalko, N., and Popelar, J. (1994). Enhancement of the auditory cortex evoked responses in awake guinea pigs after noise exposure. *Hear Res*, 78:158–168.

[Thompson and Schofield, 2000] Thompson, A. and Schofield, B. (2000). Afferent projections of the superior olivary complex. *Microsc Res Techniq*, 51:330–354.

[Turner and Parrish, 2008] Turner, J. and Parrish, J. (2008). Gap detection methods for assessing salicylate-induced tinnitus and hyperacusis in rats. *Am J Audiol Supp*, 17:S185–S192.

[Tyler et al., 2003] Tyler, R., Bergan, C., Preece, J., and Nagase, S. (2003). Audiologische messmethoden de hyperakusis. In Nelting, M., editor, *Hyperakusis*, pages 39–46. Georg Thieme Verlag, Suttgart.

[Wang et al., 2009] Wang, H., Brozoski, T., Turner, J., Ling, L., Parrish, J., Hughes, L., and Caspary, D. (2009). Plasticity at glycinergic synapses in dorsal cochlear nucleus of rats with behavioral evidence of tinnitus. *Neuroscience*, 164:747–759.

[Wilson et al., 1991] Wilson, P., Henry, J., Bowen, M., and Haralambous, G. (1991). Tinnitus reaction questionnaire: Psychometric properties of a measure of distress associated with tinnitus. *Speech Hear Res*, 34:197–201.

[Woodruff et al., 1996] Woodruff, P., Benson, R., Bandettini, P., Kwong, K., Howard, R., Talavage, T., Belliveau, J., and Rosen, B. (1996). Modulation of auditory and visual cortex by selective attention is modality-dependent. *Neuroreport*, 7:1909–1913.

[Zeng et al., 2009] Zeng, C., Nannapaneni, N., Zhou, J., Hughes, L., and Shore, S. (2009). Cochlear damage changes the distribution of vesicular glutamate transporters associated with auditory and nonauditory inputs to the cochlear nucleus. *J Neurosci*, 29:4210–4217.

[Zwicker et al., 1957] Zwicker, E., Flottorp, G., and Stevens, S. (1957). Critical band width in loudness summation. *J Acoust Soc Am*, 29:548–558.