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The Auditory Midbrain of People with Tinnitus: Abnormal Sound-Evoked Activity Revisited

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Abstract

Sound-evoked fMRI activation of the inferior colliculi (IC) was compared between tinnitus and non-tinnitus subjects matched in threshold (normal), age, depression, and anxiety. Subjects were stimulated with broadband sound in an “on/off” fMRI paradigm with and without on-going sound from the scanner coolant pump.

(1) With pump sounds off, the tinnitus group showed greater stimulus-evoked activation of the IC than the non-tinnitus group, suggesting abnormal gain within the auditory pathway of tinnitus subjects.

(2) Having pump sounds on reduced activation in the tinnitus, but not the non-tinnitus group. This result suggests response saturation in tinnitus subjects, possibly occurring because abnormal gain increased response amplitude to an upper limit.

(3) In contrast to Melcher et al. (2000), the ratio of activation between right and left IC did not differ significantly between tinnitus and non-tinnitus subjects or in a manner dependent on tinnitus laterality. However, new data from subjects imaged previously by Melcher et al. suggest a possible tinnitus subgroup with abnormally asymmetric function of the IC.

The present and previous data together suggest elevated responses to sound in the IC are common among those with tinnitus and normal thresholds, while abnormally asymmetric activation is not, even among those with lateralized tinnitus.

Keywords

hyperacusis; midbrain; phantom sensation; fMRI; neuroimaging

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Introduction

A large number of neurophysiologic studies have been directed at understanding the phenomenon of tinnitus, a usually untreatable clinical condition defined by the phantom perception of sound (e.g., see Hoke et al., 1991; Lockwood et al., 2004; Adjajian et al., 2009; Lanting et al., 2009). Collectively, this work has taken a wide variety of approaches, using different experimental modalities (evoked potentials, neuromagnetic recordings, positron emission tomography - PET, functional magnetic resonance imaging - fMRI) and experimental paradigms involving, for instance, the modulation of tinnitus via jaw maneuvers, sound stimulation, or drug administration. This diversity suggests it may be possible to measure brain abnormalities related to tinnitus in many different ways. However, it also provides little opportunity for corroboration.

Somewhat disconcerting is the fact that the only two sets of studies to use similar experimental modalities and paradigms yielded divergent results. One set of divergent findings was seen in neuromagnetic recordings of N100/P200 in tinnitus subjects (Pantev et al., 1989; Hoke et al., 1991; Jacobson et al., 1991; Colding-Jørgensen et al., 1992; Shiomi et al., 1997). The other, and the focus of the present study, was seen in the sound-evoked activation of the auditory midbrain using fMRI (Melcher et al., 2000, 1999; Smits et al., 2007; Lanting et al., 2008). These latter studies, including one from our group, compared sound-evoked activation of the auditory pathway between tinnitus subjects and non-tinnitus controls. With this experimental paradigm, Melcher et al. (2000) reported an abnormal asymmetry of fMRI activation produced by sound in the inferior colliculi of people with symmetric hearing thresholds and tinnitus lateralized to one ear. Additional data using the same methodology and showing the same result were reported in the proceedings of a conference by Melcher et al., (1999). Smits et al. (2007) subsequently described a similar asymmetry of activation in the colliculi as well as an asymmetry of activation in auditory thalamus and cortex of subjects with lateralized tinnitus. Lanting et al. (2008) found no asymmetry of activation in subjects with lateralized tinnitus and instead reported greater amplitude of activation in the inferior colliculi of tinnitus subjects as compared to non-tinnitus controls.

The inconsistency of findings across studies echoes our own experience after publishing our 2000/1999 papers. We began imaging patients with lateralized tinnitus fully expecting to see abnormally asymmetric activation in the inferior colliculi. However, we did not, at least not to the extent seen in the original report. What followed were experiments re-examining the midbrain activation of tinnitus subjects. These experiments, which help explain some of the discrepancies among the published papers, are the topic of the present report.

The new experiments presented here again involved measuring fMRI activation produced by sound in the inferior colliculi of tinnitus and non-tinnitus subjects, but addressed the possibility that the tinnitus and non-tinnitus subjects tested by Melcher et al. (2000, 1999) were not sufficiently well matched in respects other than tinnitus. Inadequate matching could lead to differences in activation between groups that are not tinnitus-related. Therefore, the present experiments compared subject groups that were even more closely matched than before: in pure-tone threshold (not just symmetry of threshold as in Melcher et al.), age, and emotional state.

The experiments also addressed the possibility that divergent results might be obtained from people with seemingly similar behavioral tinnitus characteristics (e.g., tinnitus lateralized to one ear). The point is that people who all describe their tinnitus in the same way may nevertheless have different physiological bases for their tinnitus that manifest in different ways in fMRI studies. If so, then perhaps the asymmetry of activation seen by Melcher et al.

(1999, 2000) in subjects with lateralized tinnitus was not a spurious result, but instead a product of the particular subjects tested, that is subjects who happened to manifest an asymmetry of brain function that is not uniformly present in people with lateralized tinnitus. To examine this possibility, three of the 10 tinnitus subjects previously studied by Melcher et al. 2000, 1999, as well as one control subject, were tested again here to provide a basis of direct comparison between studies.

A final issue addressed by the present study is whether the acoustic conditions during scanning might be crucial to whether or not differences in sound-evoked activation are seen between tinnitus and non-tinnitus subjects. In fact, Lanting et al. (2008) proposed that differences in acoustic background might be *the* crucial factor in accounting for the disparities among published results. Here, most of our data were acquired using the same imaging paradigm as Melcher et al. (2000), which entailed imaging a single brain slice, rather than multiple slices, to reduce the sounds produced by the scanner gradient coils during image acquisition. However, some data were also collected while controlling the imaging sounds in a different way that involves imaging multiple slices in brief clusters spaced by long quiet periods (Edmister et al., 1999; Hall et al., 1999; the method used by Lanting et al.). In addition, we systematically examined the effects of the second major acoustic background sound in the imaging environment, the scanner coolant pump. In some scanners, the pump is on continuously. Here, the pump was turned off for some measurements and kept on for others, the latter being the situation in the study of Melcher et al. (1999, 2000).

The results of the present study indicate that sound does indeed produce abnormal activation of the auditory midbrain of tinnitus subjects. They also highlight how critical it is to pay close attention to the precise methodology of fMRI studies of tinnitus.

Methods

Subjects

A total of 32 paid volunteers were imaged. 12 had tinnitus. All were right-handed. Written informed consent was obtained from each subject prior to testing. Ten of the 12 tinnitus subjects were recruited through the Tinnitus Clinic at the Massachusetts Eye and Ear Infirmary or a tinnitus support group. All remaining subjects (tinnitus and non-tinnitus) were recruited through personal contacts and ads in a university newspaper. All procedures were approved by human studies committees at the Massachusetts Eye and Ear Infirmary, Massachusetts General Hospital, and Massachusetts Institute of Technology.

All of the non-tinnitus subjects and 10 of the 12 tinnitus subjects had pure tone thresholds of ≤ 25 dB HL at five out of six of the standard audiometric frequencies from 250 Hz through 8 kHz and ≤ 35 dB HL at the remaining frequency. The two remaining tinnitus subjects had high frequency hearing loss. Both ears of both subjects had pure tone thresholds of 25 dB HL or less at 4 kHz and below and 8 kHz thresholds of 60 – 85 dB HL.

Of the 10 tinnitus subjects without hearing loss, seven were tested for spontaneous otoacoustic emissions. In the two subjects with emissions, the frequency and side(s) of the emissions did not match the tinnitus percept.

Behavioral assessments

All subjects completed standard inventories of depression (Beck et al., 1961) and anxiety (Beck et al., 1988). Tinnitus subjects also completed a questionnaire asking about various tinnitus characteristics (e.g., location, pitch, loudness) as well as the Tinnitus Reaction Questionnaire (TRQ), an indicator of tinnitus-related distress (Wilson et al., 1991).

The following were assessed in each tinnitus subject using previously described methods (Melcher et al., 2000; Levine, 2004): (1) the pure tone frequency best matching the pitch of the tinnitus (“pitch match”), (2) the level of a tone at the pitch match frequency that best matches the loudness of the tinnitus (tinnitus loudness, dB re sensation level (SL)), (3) minimum masking level (MML), the lowest level of binaural, broadband noise that fully masked the tinnitus, (4) residual inhibition, the complete suppression of tinnitus for any period of time following the termination of one minute of binaural broadband noise presented 10 dB above MML (5) any modulation of the tinnitus percept by maneuvers of the head and neck. The characteristics of non-tinnitus and tinnitus subjects are summarized in Table 1 and Table 2. A more detailed history of subjects #8 and #10 may be found in Levine (1999) where they are cases 1 and 4, respectively.

Acoustic stimulation

The acoustic stimulus was broadband continuous noise presented binaurally at 50 – 55 dB SL (approximately 75 dB SPL). Threshold (that is, 0 dB SL) was measured in the scanner room (not during imaging) separately for each ear and in two conditions: with the scanner coolant pump off and with it on. The continuous noise stimulus was alternately turned on for 30 s and off for 30 s in a standard fMRI block paradigm. Four on/off repetitions comprised a scanning “run”.

Stimuli were generated by a digital-to-analog board (running under LabVIEW), amplified, and fed to a pair of audio piezoelectric transducers housed in earmuffs (custom-built by GEC Marconi, Inc.) that attenuated the scanner sounds by approximately 30 dB (Ravicz and Melcher, 2001).

Acoustic conditions during imaging

Extraneous sounds produced by the scanner came from two sources (Ravicz et al., 2000) and were handled in several ways. One source was the scanner gradient coils, which produced sound each time an image was acquired. In experiments using single-slice acquisition, the gradient sound could be heard as a “beep” occurring approximately every 2 seconds. The beep was approximately 100 ms in duration. Its spectrum was dominated by a peak at 1.0 kHz. In experiments using clustered volume acquisition, the gradient sounds consisted of a more prolonged beep (duration approx. 1 s) occurring approximately every 8 s. The beep spectrum was dominated by a peak at 1.4 kHz. The A-weighted sound level from the gradient coils was approximately 55 dB SPL (single-slice) or 65 dB SPL (clustered) at the ear (that is, under the earmuffs). These levels are a time-average over 30 s. They were calculated from measurements of earmuff attenuation and scanner sound levels measured outside the earmuffs (as described in Hawley et al., 2005).

The scanner coolant pump was the second source of scanner-generated sound. The effects of this sound on activation were examined explicitly by turning the pump off for some scanning runs and leaving it on throughout others. Note that when the pump was on for a given run, it (and the background sound it produced) was on continuously and thus during both stimulus on and off periods. 2 – 3 runs of data were collected in a given pump condition (on or off). Most, but not all, subjects were studied during both pump conditions. A complete accounting of the data obtained in each subject is given in Table 3.

To test whether having the pump off for isolated runs might affect image quality, we compared two measures between the pump on and off conditions: (1) image signal level in the inferior colliculi and (2) signal-to-noise. Neither measure differed systematically between conditions. We therefore conclude that having the pump on vs. off only affected the sensory experience of the subject, not the function of the scanner.

The sound produced by the pump was dominated by frequencies below approximately 200 Hz and cyclically varied in amplitude with a period of approximately 0.8 s. Figure 1 shows the spectrum of pump sounds calculated over one cycle. The A-weighted level of the pump sounds was approximately 45 dB SPL at the ear in both of the scanners used in this study. This level is a time average over several seconds that reflects the attenuation of the earmuff. The pump sound produced, on average, a 2 dB upward shift in behavioral threshold for the continuous noise stimulus.

Task during imaging

Throughout each scanning run, tinnitus subjects continuously indicated any changes in the loudness of their tinnitus by turning a hand-held knob controlling the illumination of a row of ten lights (visible through a mirror mounted on the imaging coil). More (fewer) lights were illuminated with increasing (decreasing) tinnitus loudness. Non-tinnitus subjects controlled the same lights, but they were instructed to illuminate five lights when the noise stimulus was on and none when the stimulus was off.

The task of reporting tinnitus loudness during scanning was adopted in case there proved to be marked differences among subjects in how loudness varied over a scanning run, as this would present an opportunity to understand any relationship between the perceived loudness of tinnitus and activation. However, all but three subjects showed the same pattern of changes in tinnitus loudness (and did so whether or not the background sound from the coolant pump was on). The variation was a reduction in tinnitus loudness during the stimulus on periods of each run and a recovery of tinnitus during the off periods. The three subjects not showing this pattern instead showed (1) an inconsistent increase in tinnitus loudness during the stimulus (subj. 10), (2) no variation in tinnitus loudness (subj. 35), or (3) complete tinnitus suppression throughout the scanning run (subj. 16). Unfortunately, no firm conclusions could be drawn about the relationship between activation and tinnitus loudness from these three subjects since they (1) showed very different patterns of tinnitus loudness variation (so their data could not be pooled for comparison with the others) and (2) showed activation data that was neither exactly “average” nor remarkably different from the other data. The results of the task will not be considered further.

Imaging

Imaging sessions using single-slice acquisition were conducted on a 1.5 Tesla scanner (Siemens Sonata) while those using clustered acquisition were conducted on a 3 Tesla scanner (Siemens Trio). Each imaging session included the following:

1. Contiguous sagittal images of the whole head were acquired.
2. Using the sagittal images, the brain slice(s) to be functionally imaged was selected. For single-slice acquisition, a single near-coronal slice was imaged that passed through the inferior colliculi and posterior aspect of Heschl's gyri. For clustered acquisition, 11 near-coronal slices were imaged. The second to most posterior intersected the inferior colliculi. The ordering of acquired slices was from posterior to anterior.
3. T1- or T2-weighted, high-resolution anatomical images were acquired of the selected brain slices for subsequent overlay of the functional data (in-plane resolution = 0.8×0.8 mm; thickness matched that of functional images).

Functional images were obtained using a blood oxygenation level-dependent (BOLD) sequence: asymmetric spin echo for sessions using single-slice acquisition (echo time (TE) = 70 ms; offset = -25 ms; slice thickness = 6 mm; in-plane resolution: 3.1×3.1 mm), gradient echo for sessions using clustered acquisition (TE = 30 ms; flip angle = 90 degrees; slice

thickness = 4 mm; 1.3 mm gap; in-plane resolution = 3.1 – 3.1 mm). A cardiac gating method was used to improve the detection of activation in the inferior colliculi (Guimaraes et al., 1998). In sessions imaging a single slice, images were acquired in synchrony with every other QRS complex in the subject's electrocardiogram, resulting in an interimage interval (time of repetition, TR) of approximately 2 s. Fluctuations in heart rate led to variations in TR that resulted in image-to-image variations in image signal strength (i.e., a "T1 effect"). Image signal was corrected to account for these variations using measurements of TR. In multislice sessions, acquisition of the 11 selected slices was synchronized to the first QRS complex following a minimum inter-image interval of 7.5 s, yielding a TR of approximately 8 s. This TR was long enough that T1 effects were negligible and no correction was necessary.

Analysis

The functional data were analyzed as described in detail previously (Harms and Melcher, 2002). In brief, the processing was as follows: (1) The functional images were corrected for slight movements of the head that may have occurred over the course of the imaging session by rotating and translating the images within the imaging plane. (2) Image signal versus time for each voxel and run was corrected for linear and quadratic drifts in signal amplitude over time. (3) Image signal was normalized such that the time-average signal had the same (arbitrary) value for all voxels and runs. (4) The time-series of images corresponding to individual functional runs were concatenated to form a single data set for each paradigm.

Spatial maps of activation were derived as follows. First, each image was assigned to either a "stimulus on" or "off" period assuming that changes in image signal were delayed by 2 s relative to stimulus onset and offset. Activation maps were created by comparing image signal during stimulus "on" and "off" periods on a voxel-by-voxel basis using an unpaired t-test.

Activation was quantified within regions of interest (ROIs) corresponding to the left and right inferior colliculi. The circular borders of the inferior colliculi could be seen directly in the anatomical images. For the lowest p-value voxel within each of these circular regions, activation was quantified in terms of the percent change in image signal.

$$\text{Percent Signal Change} = (S_{\text{on}} - S_{\text{off}}) / ((S_{\text{on}} + S_{\text{off}}) / 2)$$

where S_{on} is the signal averaged over stimulus "on" periods, and S_{off} is the average over the "off" periods.

Comparisons of tinnitus and non-tinnitus subjects were conducted separately for the single-slice and clustered acquisition data because different scanners and pulse sequences were used during these two types of acquisition, factors that are known to influence activation (Friedman et al., 2006; Meindl et al., 2008).

Results

Broadband noise stimulus, background sound from coolant pump off

Figure 2A, B illustrates how the inferior colliculi of tinnitus and non-tinnitus subjects responded to continuous broadband noise presented binaurally in the absence of the on-going background sound from the scanner coolant pump. Both the activation maps (Fig. 2A) and the plot below them (2B) show data for individual subjects. For 3 subjects studied twice (#8, 13, 19), data are shown separately for each session. The magnitude of activation, measured as the percent change in image signal between sound on and off conditions, ranged from approximately 0.5 to 1.6% across subjects. Mean percent signal change for the tinnitus and non-tinnitus subjects respectively was 1.0 and 0.8% for the data acquired using single-slice

acquisition (filled circles) and 1.4 and 0.9% for clustered volume acquisition (diagonally-shaded circles). Thus, on average, activation in tinnitus subjects was greater than in non-tinnitus subjects (single slice acquisition: $p = 0.03$; clustered volume acquisition: $p = 0.03$, Wilcoxon-Mann-Whitney test). (The two “single slice” data points for subjects 13 and 8 were averaged for the purposes of the statistical test to avoid artificial inflation of the degrees of freedom.) For reference, note that a 20 dB increase in stimulus level in normal, non-tinnitus subjects produces an approximately 1.2-fold increase in percent signal change in the inferior colliculus (Sigalovsky and Melcher, 2006), which is comparable to the increase in mean percent change between non-tinnitus and tinnitus subjects (single-slice: 1.2-fold, clustered: 1.5). The elevation of stimulus-evoked activation seen in tinnitus subjects is illustrated by the activation maps, which correspond to subjects with activation levels near the mean for their respective groups and whose data points are indicated by asterisks in Figure 2B.

The tinnitus and non-tinnitus subject groups compared in Figure 2B were closely matched in multiple respects. At any given frequency from 250 through 8000 Hz, mean pure tone threshold showed no significant difference between groups ($p > 0.2$, t-test; subjects studied with single-slice and clustered acquisition compared separately; Figure 2C). Average threshold for the continuous noise stimulus differed by less than 3 dB between the tinnitus and non-tinnitus groups. Tinnitus and non-tinnitus subjects studied with single-slice acquisition did not differ significantly in age, score on the depression inventory or score on the anxiety inventory ($p > 0.1$, t-test; compare two left-most data columns in Table 4). The same was true of subjects studied with clustered acquisition ($p > 0.25$, t-test; compare two right-most columns of Table 4). The sex of subjects was not closely matched for the clustered acquisition data, but was for the single-slice data, making it unlikely that the greater activation in tinnitus subjects is an artifact of subject sex. The generally close matching between groups in respects other than tinnitus strongly suggests that the elevated activation in tinnitus subjects was intimately related to the fact that the subjects had tinnitus.

Broadband noise stimulation, effect of background sound from the coolant pump

Figure 3 compares activation data acquired with the pump on to data acquired with the pump off for the subset of subjects from Figure 2 studied in both conditions (all of the subjects studied with clustered acquisition plus 5 tinnitus and 9 non-tinnitus subjects studied with single-slice acquisition). The “pump on” data were acquired in the same manner as the “pump off” data except for the presence of background sound from the pump throughout both “on” and “off” periods of the stimulation paradigm. For each subject in Figure 3, the activation produced by the sound stimulus during the pump off and on conditions is plotted with filled circles joined by a line, which is black in cases where activation was reduced by having the pump on and gray in cases where activation was unchanged or increased. (Note that the gray circles joined by lines correspond to the two subjects with elevated high-frequency thresholds considered separately below.)

Figure 3 illustrates two points. First, all but two of the tinnitus subjects showed reduced activation during the pump on condition whereas the number of non-tinnitus subjects showing a reduction equaled that showing an increase. The difference in activation between pump off and on conditions in tinnitus subjects was significantly greater than in non-tinnitus subjects ($p = 0.02$; Wilcoxon-Mann-Whitney test). Second, tinnitus and non-tinnitus subjects showed little overall difference in activation to the sound stimulus during the pump on condition. In sum, having the pump on suppressed sound stimulus-evoked activation in the inferior colliculi of tinnitus subjects thus eliminating any difference in the magnitude of activation relative to non-tinnitus controls. This effect is almost certainly due to the sensory effects of the pump since having the pump off had no effect on image quality (see Methods) and the effect on activation occurred differentially between the tinnitus and non-tinnitus groups. Finally, the difference in

activation between the pump on and off conditions cannot be attributed to a difference in effective stimulation level because: (1) the stimulus level used in each condition was set relative to stimulus threshold for that condition and, (2) stimulus threshold (and hence stimulation level) differed between conditions, on average, by approximately 2 dB, which is unlikely to account for the difference in activation seen for tinnitus subjects between pump conditions (Sigalovsky and Melcher, 2006).

In considering the effect of the pump on activation, it is important to recall that fMRI activation reflects a change in brain activity, not absolute levels of activity. With the pump off, the change is an increase in activity in response to the sound stimulus over and above any baseline activity produced endogenously or by background sounds from the scanner or subject (e.g., from breathing). The situation is the same with the pump on, except that any baseline activity also includes activity evoked by the pump sounds. The fact that tinnitus subjects generally showed less increase in activity (that is, stimulus-evoked activation) when the pump was on suggests that the increase may have been limited by a saturation mechanism (see Discussion). This interpretation is consistent with the fact that, before turning the pump on (that is, with pump off), activation magnitude in many of the tinnitus subjects was already approaching the upper limit of midbrain responses (1.5 – 2 %). That the activation difference between pump off and on conditions varied considerably across subjects - tinnitus, and non-tinnitus - might reflect inter-subject variability in the activity levels at which any saturation comes into play. Alternatively, it may reflect the inherent variability of activation magnitude that can occur even within subjects, as illustrated by tinnitus subject #8 and, more strikingly, by non-tinnitus subject #13 in Figure 2B. Note that the difference between the two measurements for subject #19, also shown in Figure 2B, may reflect the different scanner field strengths and image acquisition methods used in the two instances, in addition to any intra-subject variability.

Comparison of activation between sides

To compare the symmetry of activation in tinnitus vs. non-tinnitus subjects, an activation ratio (right inferior colliculus divided by left) was calculated for each subject and condition (pump off or on). Consider first the pump off condition (Figure 4, left). There was some tendency for tinnitus subjects to show a greater activation ratio than non-tinnitus subjects in the clustered acquisition data (diagonally shaded circles; mean $\pm$ standard deviation: 1.48 $\pm$ 0.74 (tinnitus), 0.97 $\pm$ 0.37 (non-tinnitus); $p = 0.02$, Wilcoxon-Mann-Whitney). While statistically significant, this difference should be treated with some caution given the amount of data (from 3 tinnitus, 4 non-tinnitus subjects), the lack of significant difference in activation ratio in the single-slice acquisition data of tinnitus and non-tinnitus subjects (black circles in Figure 4, left; $p = 0.12$) and, the potential for substantial intra-subject variability in the activation ratio measure, which is illustrated by the ratios for subject #8 for two different sessions in the single-slice data of Figure 4, left (black circles).

In contrast to the “pump off” condition, the pump on condition showed no tendency toward differing activation ratios between tinnitus and non-tinnitus subjects (Figure 4, middle). In this latter condition, the activation ratio for tinnitus and non-tinnitus subjects was comparable in mean (tinnitus: 1.11; non-tinnitus: 0.99), standard deviation (tinnitus: 0.32; non-tinnitus: 0.36), and range (tinnitus: 0.77 – 1.44; non-tinnitus: 0.60 – 1.48 (excluding one outlier at 0.30)).

Comparison with previous data of Melcher and colleagues

Activation ratios from Melcher et al. (1999, 2000) are shown in Figure 4 (right) for comparison with ratios obtained in the present study. These previous data were acquired with the pump on and resemble the “pump off” data of the present study (Figure 4, left). Both tend to show greater activation ratios for tinnitus subjects than for non-tinnitus subjects. However, they differ in that any average tendency for tinnitus subjects to differ from non-tinnitus subjects in the

combined data from clustered and single-slice acquisition is not statistically significant in the present study - nor is it related to the side of the tinnitus percept. Concerning the latter point, in the tinnitus data of Melcher et al., all of the activation ratios above 1.2 occurred in subjects with tinnitus localized exclusively, or mainly to the right ear while in the present study, the two highest activation ratios (two highest black points in Figure 4, left) occurred in subjects with opposite tinnitus locations (left ear for the top point, right ear for subj. #8). Even though the Melcher et al. data were acquired with coolant pump on and, the pump sounds were similar to those of the present study (in level, spectral content and amplitude modulation; Ravicz et al., 2000), the Melcher et al. data better resemble the present “pump off” data than the “pump on” data. This suggests that acoustic background conditions may not be a dominant factor in determining whether or not a given tinnitus subject will display a strong asymmetry of activation in the inferior colliculi.

In an attempt to better understand the previous Melcher et al data, three tinnitus subjects and one non-tinnitus subject tested by Melcher et al. were also tested in the present study. Questionnaire responses for the tinnitus subjects indicated no change in tinnitus quality or location in the interim and little or no change in the annoyance of the tinnitus or effects of tinnitus on concentration or sleep. The data for this limited sample of subjects provide further indication that intra-subject variability in the absolute magnitude of the activation ratio can be considerable (compare especially the data for subj. #8, 19 within and across panels in Figure 4). Despite this variability, however, there are repeatable trends: In six separate instances, the activation ratio for the non-tinnitus subject (#13) was near the mean for non-tinnitus subjects whereas the activation ratio for tinnitus subjects #8 and #16 always exceeded the mean for non-tinnitus subjects (6 and 4 occurrences, respectively). The data for subj. 8 are especially notable because this person was studied five times (one time in two conditions, pump on and off), always showing an activation ratio above the non-tinnitus mean.

Data from two tinnitus subjects with symmetric, high frequency hearing loss

In addition to the subjects presented above, we also imaged two tinnitus subjects with high-frequency hearing loss (described in Methods). These subjects were imaged using single-slice acquisition and therefore will be compared only to subjects studied with that acquisition method. Activation for the tinnitus subjects with high-frequency hearing loss was similar to that of the other tinnitus subjects studied in the following respects: (1) activation during the pump off condition (percent change = 1.26, 1.29, respectively) fell well above the mean for non-tinnitus subjects (0.84), and (2) this elevated activation during the “pump off” condition was reduced when the pump was on (gray filled circles connected by black lines in Figure 3, left panel). Thus, the data suggest that the activation of tinnitus subjects with elevated high-frequency thresholds shows the same abnormalities as the activation of tinnitus subjects with normal thresholds.

Discussion

The two main findings of the present study and their implications may be summarized as follows:

First, activation of the inferior colliculi in response to sound was, on average, elevated in tinnitus subjects with normal thresholds compared to a control group without tinnitus. Because of the close matching between the tinnitus and non-tinnitus groups, this result cannot be readily attributed to any of the following potentially confounding variables: pure tone threshold, age, sex, depression or anxiety. This first result suggests the presence of abnormal gain within the auditory pathway that is intimately related to tinnitus.

Second, the elevated activation evoked by the sound stimulus in the inferior colliculi of subjects in the tinnitus group was not apparent in the presence of on-going sound from the scanner coolant pump because activation in tinnitus subjects tended to be reduced by the pump sounds. While the effect of the pump could theoretically result from one or both of the sensory stimuli the pump produces, sound and vibration, in the following discussion we assume that the on-going 45 dBA sound from the pump is most likely responsible for the pump's effect, acknowledging that vibrations cannot be ruled out as a factor. As explained in more detail below, the reduction of stimulus-evoked activation in tinnitus subjects by pump sounds may reflect saturation of the neural response to the stimulus and/or saturation of the hemodynamic change that results from the neural response and produces the signal measured with fMRI. Importantly, the putative saturation mechanism itself may be entirely normal in tinnitus and manifest itself only because of the abnormally elevated responses in tinnitus subjects.

A heuristic model incorporating the results

Figure 5 presents an hypothesis to explain the results of the present study. Panels A and C of Figure 5 depict neural activity and fMRI activation in the inferior colliculus of a non-tinnitus and a tinnitus subject with the pump off. While fMRI measures the hemodynamic response to neural activity (largely synaptic), rather than neural activity directly, we assume for the moment that neural activity and hemodynamic response are proportional to one another and are thus interchangeable for present purposes. The stacked bars to the left in Figure 5A and 5C indicate levels of neural activity - activity evoked by the sound stimulus (solid black) and baseline (or spontaneous) activity occurring even in the absence of sound (diagonal shading). Since the latter is present during both stimulus "on" and "off" periods, it is not registered as fMRI activation, which reflects the difference in neural activity between periods of sound stimulation and periods of no stimulation (black, Figure 5A, right). Therefore, fMRI activation specifically reflects the change in activity produced by the sound stimulus. The situation for the tinnitus subject in Figure 5C is the same as for the non-tinnitus subject in 5A, except that the activity evoked by the sound stimulus is elevated in comparison. Thus, fMRI activation is also elevated.

Panels B and D in Figure 5 incorporate the effect of on-going sound from the pump. Here, the pump sound is assumed to produce its own activity (gray shading), which for the moment is assumed equal in tinnitus and non-tinnitus subjects (but see discussion below). Since the pump was on continuously, this activity is present during both stimulus "on" and "off" periods, just like the baseline activity (diagonal shading). In the non-tinnitus subject (Figure 5B), activity evoked by the sound stimulus (solid black) is the same as in Figure 5A and simply adds to the baseline and pump-evoked activity. Since the difference in activity between stimulus "on" and "off" periods is unchanged by the pump, fMRI activation for the non-tinnitus subject is also unchanged, that is, it is the same as in Figure 5A. This lack of difference is consistent with the results for non-tinnitus subjects in Figure 3, showing no systematic change in fMRI activation in non-tinnitus subjects when the pump was turned on.

For the tinnitus subject in Figure 5D, there is also baseline activity, pump-evoked activity and activity evoked by the sound stimulus, which would be as great as in Figure 5C except that either (1) total neural activity reaches an hypothesized maximum (i.e., saturates; Figure 5D, left) or, (2) the hemodynamic response to the neural activity reaches maximum (Figure 5D, right; i.e., is unable to increase in proportion to neural activity as assumed in Figure 5A–C). Note that the saturation depicted in Figure 5D is "hard" meaning that neural activity (or hemodynamic response) grows independently of absolute activity (or response) levels until a maximum limit is reached. However, a compressive saturation in which neural activity (or hemodynamic response) grows more and more slowly as absolute activity (or response) level increases would also be consistent with the data since this too would place a limit on the amount of activity (or hemodynamic response) resulting for a given sound input. As a result of the

putative saturation (neural or hemodynamic, hard or compressive), the difference in activity between stimulus “on” and “off” periods is less than in Figure 5C. As in the data of Figure 3, fMRI activation for the tinnitus subject is reduced in Figure 5D (with pump on) compared to Figure 5C (pump off).

While the saturation in Figure 5D occurs solely because the neural response to sound in tinnitus subjects is already nearing its maximum or is producing a close to maximal hemodynamic response (see Figure 5C), an additional factor could enhance the effects of saturation, namely an elevated neural response to the coolant pump sound. Such an elevation might occur if the same neural gain that leads to the elevated response to the sound stimulus also amplifies the neural response to the coolant pump sound. If the response to the pump sound were indeed elevated in tinnitus subjects, the gray bars in Figure 5D would be larger and the suppression of fMRI activation during the pump on condition would be accentuated. Indeed, with a sufficiently large response to the coolant pump sound, it would theoretically be possible to see no difference in fMRI activation between tinnitus and non-tinnitus subjects, or perhaps less activation in tinnitus subjects - result that is the opposite of that seen in the quieter, pump off condition. This realization leads to a crucial point that is applicable to any scanner-generated sounds that elicit a neural response during the fMRI measurement (from the coolant pump or the scanner gradients): the activity produced by those sounds may change the magnitude of any activation differences between tinnitus and non-tinnitus subjects, reverse the sign of the difference and, even prevent an underlying difference from being detected.

While Melcher et al. (2000) also used a saturation model to explain the abnormal midbrain activation seen in their study it differs from the saturation in tinnitus subjects depicted in Figure 5D. Specifically, in Melcher et al. (2000), a maximum level of neural activity was reached because of increased baseline activity corresponding to tinnitus. Here, however, baseline activity is assumed to be the same for tinnitus and non-tinnitus subjects and, the saturation in Figure 5D occurs solely because of the enhanced response to sound in tinnitus subjects. Including elevated baseline activity corresponding to tinnitus (that is, increasing the height of the diagonally shaded bars in Figures 5C and 5D) would not change the trends in fMRI activation depicted in Figure 5 unless it were so great that it drove stimulus-evoked neural activity in Figure 5C past the brink of saturation. Barring this, elevated baseline activity related to tinnitus would only enhance the trends of Figure 5 by causing a further reduction in fMRI activation in Figure 5D compared to Figure 5C. A last, important point, though, is that elevated baseline activity related to tinnitus is not needed to account for the present data. In other words, the present study neither supports nor refutes hypotheses that the tinnitus percept arises from abnormally high spontaneous activity levels in the auditory pathway (c.f., Kaltenbach et al., 2004).

Enhancement of sound-evoked activity in tinnitus subjects: one possible mechanism

It is worth considering whether the elevated responses to sound seen here in tinnitus subjects might be related in mechanism to previously reported elevations in sound-evoked activity of the inferior colliculi of animals with cochlear damage (Salvi et al., 1990, 2000). Even though the present study's subjects had normal thresholds according to standard clinical criteria, it may nevertheless be appropriate to compare their data with those of animals with cochlear damage for two reasons. First, normal behavioral thresholds at the standard audiometric frequencies assessed in the present study (250 – 8000 Hz) does not exclude the possibility of cochlear pathology (Kujawa and Liberman, 2006; Job et al., 2007). Also, the fact that average threshold for our subjects was below 0 dB HL is suggestive of some cochlear abnormality. Second, the elevations in sound-evoked activity in animals were seen for stimulus frequencies at which threshold was normal e.g., for a 1 kHz tone approximately one octave below the edge of a high frequency loss. The elevated sound-evoked responses in the animal work were

hypothesized to arise from a loss of gamma-amino-butyric acid-mediated inhibition of inferior colliculus neurons (Gerken, 1996; Salvi et al., 2000), a mechanism that perhaps contributes to the elevated sound-evoked fMRI activation seen in our tinnitus subjects.

Minimizing acoustic background sounds during scanning is especially crucial for fMRI studies of tinnitus

The effect of the pump sounds on stimulus-evoked activation in tinnitus subjects directly illustrates the importance of controlling the acoustic background during fMRI studies of tinnitus – both the acoustic background sounds produced in concert with image acquisition and those produced by the scanner coolant pump. The fact that having the pump on or off had no systematic effect on the activation of non-tinnitus subjects, in contrast to the situation for tinnitus subjects, illustrates that approaches adequate for reducing background scanner sounds during auditory fMRI studies of normal subjects, may not be adequate for studies of tinnitus.

A comparison of the single-slice and clustered acquisition data in Figure 2B reinforces this last point. While non-tinnitus subjects showed comparable levels of stimulus-evoked activation with the two acquisition methods, tinnitus subjects did not. Their activation was less with single-slice acquisition, which, in contrast to the clustered acquisition paradigm, did not have long quiet periods between image acquisitions to help prevent effects of background scanner sounds on stimulus-evoked activation. The lower activation for tinnitus subjects during single-slice acquisition suggests there may have already been some suppression of stimulus-evoked activation during single-slice acquisition, even with the pump off. This suppression in tinnitus subjects contrasts with previous data in normal subjects showing no difference in sound-evoked activation measured with the same single-slice acquisition protocol used here and with a protocol having even less potential for acoustic contamination than the clustered acquisition protocol of the present study (A single slice was imaged every 8 seconds. Hawley et al., 2005).

The present results regarding the effects of background scanner noise are especially important in light of reports suggesting that clustered volume acquisition with only a 3-second quiet period between image clusters, instead of the usual 8-plus seconds needed to avoid noise effects (Edmister et al., 1999; Hall et al., 2000), is adequate for auditory fMRI of clinical populations, including patients with tinnitus (Kovacs et al., 2006; Smits et al., 2007). The shorter duration quiet period of the suggested imaging protocol makes it possible to acquire more data in a given amount of time, and may therefore be an excellent choice for clinical protocols aimed at efficiently localizing auditory, speech and language areas in tumor patients, for instance. However, when assessments of the magnitude of activation are crucial, as is the case with tinnitus, abbreviated approaches to scanner noise control such as that proposed by Kovacs et al. could yield attenuated responses akin to those demonstrated here during the pump on condition or during single-slice acquisition. In brief, using short-cuts to reduce scanner background sounds is ill-advised in studies of tinnitus, at least until we better understand the underlying pathophysiology of this condition.

Comparison with previous fMRI studies of tinnitus

Since Lanting and colleagues (2008) measured sound-evoked activation in tinnitus and non-tinnitus subjects using a model of scanner that automatically switches the coolant pump off during scanning (Philips Intera, Philips Medical Systems), their data should be compared to the "pump off" data of the present study. Our data and the data of Lanting et al. converge in indicating an elevation of sound-evoked fMRI activation in the auditory midbrain of people with tinnitus. This convergence is especially noteworthy because it is the first instance of reliable replication in the imaging literature on tinnitus. Although the tinnitus subjects studied by Lanting et al. tended to have poorer high-frequency hearing thresholds than their non-

tinnitus controls, it is unlikely that the difference in hearing sensitivity was a major factor in their results, especially since the present study's data for two tinnitus subjects with high-frequency hearing loss (similar to that of Lanting et al.'s subjects) fell near the mean of the data for tinnitus subjects with no loss. The fact that the present study used binaural noise stimulation whereas Lanting et al. used monaural stimulation with a different broadband sound suggests that the occurrence of elevated responses in people with tinnitus is not highly specific to the laterality or exact type of stimulating sound.

Lanting et al. (2008) also reported results for auditory cortex, but noted no significant difference in the amplitude of sound-evoked activation between tinnitus and non-tinnitus subjects. However, the fact that the tinnitus subjects were, on average, 18 years older than the non-tinnitus subjects might have bearing on this observation. There are reports that cortical fMRI activation to a given stimulus declines with age (D'Esposito et al., 1999). If a similar age effect resulted in reduced activation in the (older) tinnitus subjects of Lanting et al., it would have counterbalanced any increase in sound-evoked cortical activation related to tinnitus. Such a counterbalancing might explain why Lanting and colleagues did not find increased auditory cortical activation in tinnitus subjects, whereas recent data in tinnitus and non-tinnitus subjects closely matched in age do show increased sound-evoked activation in the auditory cortex of tinnitus subjects. (Gu et al., 2008.)

The suggestion of counterbalanced age and tinnitus-related effects in cortex raises the question of whether such a counterbalancing could have influenced the inferior colliculus data of Lanting et al. To our knowledge, there are no published tests for age effects on inferior colliculus activation. However, an analysis of the inferior colliculus activation from non-tinnitus subjects in the present study showed no correlation with age (single-slice acquisition data: $p = 0.2$; Spearman correlation). This lack of correlation suggests that the age-related changes in activation apparent in cortex do not extend to the auditory midbrain and that the differences in inferior colliculus activation between tinnitus and non-tinnitus subjects reported by Lanting et al. were not influenced by the age difference between subject groups.

In contrast to the fMRI data of Melcher et al. (2000, 1999), tinnitus subjects overall did not consistently show a strong asymmetry of sound-evoked activation in the inferior colliculi. And, in the few instances of strongly asymmetric activation, the direction of the asymmetry was not systematically related to the location of the tinnitus percept. While the present data did not show the asymmetry emphasized by Melcher et al. (2000), it did manifest suppression of sound-evoked activation proposed by Melcher et al. (2000) to underlie the previously-reported asymmetry.

While it may be tempting to attribute the presence of the previous asymmetry to the noisier acoustic background during scanning (the pump was on and the peak scanner noise levels produced during image acquisition were approximately 10 dB greater in Melcher et al., 2000), this explanation is not consistent with the present study in which the few instances of highly asymmetric activation occurred during the condition with the least acoustic background (that is, with the pump off). It would seem that acoustic background conditions may be a factor influencing the symmetry of activation, but cannot by itself account for the presence of a strong asymmetry in the Melcher et al. studies and relative absence of asymmetry in the present study.

Given the data at hand, there would seem to be two possible interpretations of the distinct activation asymmetry reported by Melcher et al. (2000) for tinnitus subjects. One is that it was a chance occurrence. The other is that it reflects an as yet unidentified characteristic of the particular subjects sampled, a characteristic that might be related to tinnitus. The reason for suggesting the latter possibility is two-fold: (1) The tinnitus subjects we have studied most extensively (#8, 16) repeatedly showed an asymmetry in the same direction over multiple test

sessions. The probability of this repetition occurring by chance is fairly low - 0.016 and 0.063 for subjects #8 and #16, respectively (assuming that an asymmetry in one or the other direction is equally probable). (2) A case study of subject #8 suggests a link between the asymmetry and tinnitus. The study involved imaging sound-evoked activation of the colliculi before, during and after complete tinnitus suppression with intravenous lidocaine (Melcher et al., 1999; Levine and Melcher, 2000). The asymmetry before lidocaine delivery was eliminated during complete tinnitus suppression and then subsequently recurred along with the tinnitus. The coordinated changes in activation and tinnitus percept suggest a close coupling between activation asymmetry and tinnitus percept. Sprinkled throughout the neuroimaging literature are reports of activation asymmetries in the auditory pathway of tinnitus subjects (Arnold et al., 1996; Lockwood et al., 1998; Melcher et al., 2000). These findings raise the possibility that there is a tinnitus subgroup with asymmetric activation of their auditory pathway.

Using binaural stimulation and the same model scanner as Lanting et al., Smits et al. (2007) found in subjects with lateralized tinnitus an asymmetry of sound-evoked fMRI activation akin to that reported by Melcher et al. (greater activation ipsilateral to the tinnitus percept during binaural stimulation). However, the minimal description of the hearing thresholds of the tinnitus subjects leaves room for the possibility that hearing loss rather than tinnitus might have accounted for the measured activation asymmetry. The only information given about hearing is threshold at the tinnitus pitch in the tinnitus ear. If hearing were worse on the dominant side of the tinnitus, as is generally the case (Nuttall et al., 2004), it could result in the reported asymmetry of activation for binaural stimulation as follows: Input to the central auditory system on the side with poorer hearing would be reduced compared to the side with better hearing, resulting in lower activation in the contralateral inferior colliculus, medial geniculate body and auditory cortex as compared to their ipsilateral homologues. A contralateral reduction is hypothesized because fMRI activation to monaural sound is primarily contralateral to the sound in the auditory midbrain, thalamus and cortex (Melcher et al., 2000; Krumboltz et al., 2005). In this scenario, binaural stimulation would produce the reported asymmetry in activation because of asymmetrical hearing loss, not tinnitus.

Elevated sound-evoked activity in the central auditory system: Is it specifically related to tinnitus?

The fact that the tinnitus and non-tinnitus subject groups of the present study were closely matched rules out the possibility that most factors commonly correlated with tinnitus account for the elevated activation from sound seen in the inferior colliculi of tinnitus subjects. However, it still remains possible that the elevated activation in tinnitus subjects was less related to the fact that these people chronically hear a phantom sound (that is, tinnitus) than to some other variable that was not matched between groups. Perhaps the most obvious candidate for such a variable is hyperacusis, a condition often accompanying tinnitus in which sound levels comfortable to most people are perceived to be uncomfortably loud.

A reason for suggesting that hyperacusis, in particular, might be related to the elevated sound-evoked midbrain activation in tinnitus subjects follows from psychophysical and fMRI data in normal, non-tinnitus subjects, which show increases in loudness and inferior colliculus activation, respectively, with increasing sound intensity, repetition rate, or bandwidth (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, respectively). Because of this relationship between loudness and activation level, we hypothesize that the amplified loudness of hyperacusis may be reflected in elevated activation of the inferior colliculus. It may also be

reflected in elevated activation in auditory cortex, since this area also shows correlations between sound-evoked activation and loudness (as distinct from sound energy) in normal and hearing-impaired listeners (Hall et al., 2001; Langers et al., 2007).

With the nature of the midbrain activation abnormalities in tinnitus subjects on firmer ground, an important next step will be determining the extent to which the elevated sound-evoked activation of tinnitus subjects relates to tinnitus vs. the hyperacusis that can accompany it.

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Figure 1.

Spectrum of sound produced by the coolant pump measured in the two scanners used in this study: 1.5 T Siemens Sonata (black curve) and 3 T Siemens Trio (gray). The measurement of coolant pump sound was made from a microphone positioned where a subject's ear would be during scanning. The attenuation provided by the earmuffs worn by subjects has not been taken into account. In both scanners, the sound from the coolant pump varied in amplitude cyclically with a period of 0.83 s. The spectra were calculated over one period. The methods for measuring and analyzing the scanner pump sounds are given in Ravicz et al., 2000.

Figure 2.

The inferior colliculi of tinnitus subjects showed abnormally high sound-evoked activation when the scanner coolant pump (and the acoustic noise it produces) was off. The sound stimulus was continuous, broadband noise (binaural, 50–55 dB SL). **A:** Enlarged images of the inferior colliculi in one tinnitus and one non-tinnitus subject (corresponding to circles near asterisks in **B**). A map of activation (color) produced by the continuous noise stimulus is overlaid on a T1-weighted anatomical image (grayscale) obtained in the same imaging session. The color scale in the activation maps indicates the significance of the difference in image signal between stimulus on and off periods according to a t-test (uncorrected for multiple comparisons) (blue: $p = 0.01$; yellow: 2×10^{-9}). Both the activation maps (in-plane resolution of 3.1×3.1 mm)

and the anatomical images (1.5×1.5 mm) have been interpolated for these displays. **B:** Percent signal change in the inferior colliculi of each tinnitus and non-tinnitus subject studied during the “pump off” condition. Each circle indicates percent change averaged between the left and right inferior colliculi of a given subject. Data taken with single-slice and clustered acquisition are represented by solid and diagonally-shaded circles, respectively. For the three subjects imaged twice (8, 13, 19), data are plotted separately for the two sessions. Overlapping circles have been displaced horizontally so they can be seen. **C:** Mean pure tone thresholds for the tinnitus and non-tinnitus subjects in (B). The mean is shown separately for subjects imaged with single-slice (solid lines) and clustered (dotted lines) acquisition. Subjects imaged twice with single-slice acquisition (8, 13) are included once in the averages. The one subject imaged with both single-slice and clustered acquisition (19) is included in both averages.

Figure 3.

The addition of pump sounds to the acoustic environment usually reduced stimulus-evoked activation in tinnitus subjects (left) but not in non-tinnitus subjects (right). Circles indicate percent signal change in the inferior colliculi (average of left and right) measured with the pump off (left in each panel) or on (right). Each pair of circles joined by a line corresponds to a given subject. The joining line is black when activation was reduced during the pump on condition. It is gray when activation was increased or did not change. The circles are black for subjects with normal hearing thresholds and gray-filled for the two tinnitus subjects with high-frequency hearing loss. One tinnitus subject studied with both single-slice and clustered acquisition is represented twice in this figure, but was represented only once (by the average) in calculating the statistical results in the text.

Figure 4.

Activation ratio in the midbrain of individual subjects in the present study (left: pump off; middle: pump on) and in the previous Melcher et al. studies (right; only subjects meeting the audiometric criteria of the present study are included). Each point indicates percent signal change in the right inferior colliculus divided by that in the left for a given subject and test session. Numbers next to some of the data points label the data for the four subjects studied both here and by Melcher et al. (8, 13, 16, 19). Data obtained using single-slice and clustered acquisition are indicated by filled and diagonally-shaded circles, respectively.

Figure 5.

An hypothesis explaining the two main results of the present study: (1) elevated fMRI activation to a sound stimulus in tinnitus subjects, (2) suppression of stimulus-evoked fMRI activation in tinnitus subjects when there is on-going background sound from the scanner coolant pump. **(A)** Neural activity (left) and fMRI activation (right) for a non-tinnitus subject and pump off. At left, the bars indicate the levels of baseline (diagonal shading) and stimulus-evoked neural activity (solid black), while the horizontal lines indicate a theoretical maximum amount of neural activity. At right, fMRI activation is depicted as the difference in neural activity between conditions. It is actually the difference in hemodynamic response to the neural activity of the two conditions, but hemodynamic response and neural activity are assumed to be directly proportional to one another in this panel and so are interchangeable. Thus, hemodynamic response is not shown. The dashed, horizontal lines on the axes for fMRI activation are provided to facilitate comparison of fMRI activation levels across panels (A – D). **(B)** Neural activity (left) and fMRI activation (right) for a non-tinnitus subject and pump on. Gray shading indicates neural activity evoked by the pump sounds. **(C)** Same as (A) but for a tinnitus subject. The neural activity evoked by sound is elevated compared to the non-tinnitus subject in (A), so fMRI activation is elevated also. **(D)** fMRI activation in the tinnitus subject is reduced by the pump (reduced compared to (C)) because either neural activity (left) or hemodynamic response (right) saturates at a maximum level. Note that the activity evoked by the pump sound (gray shading) in (D) is assumed to be the same as for the non-tinnitus subject in (B). If one were to instead make the equally reasonable assumption that neural activity evoked by the pump sound is elevated in the tinnitus subject by the same mechanisms that elevate the response to the sound stimulus, the suppression of fMRI activation in (D) (compared to (C)) would be greater than that shown.

Table 1

General Characteristics of Subjects

Non-Tinnitus	Subject	Age	Sex	Depression	Anxiety
	7	42	f	6	2
	9	41	m	0	0
	12	39	m	2	3
	13	39, 39	f	2, 2	4, 4
	20	59	f	2	2
	21	48	m	3	1
	24	37	f	0	1
	25	40	m	n/a	n/a
	26	47	f	3	7
	27	48	f	6	3
	31	53	f	0	7
	33	57	m	7	0
	40	50	f	0	1
	44	56	f	0	1

Non-Tinnitus	Subject	Age	Sex	Depression	Anxiety
	48	45	f	6	4
	49	45	m	17	7
	50	49	f	7	0
	51	45	f	0	1
	56	64	f	8	2
	62	43	m	1	1
Tinnitus	8	59, 61	f	5, n/a	9,4
	10	48	m	5	8.5
	11	45	f	3	10
	14	48	m	19	25
	16	54	f	1	6
	18	50,50	m	6,3	4,10
	19	57,58	m	1,0	3,1
	35	43	f	8	2
	43	24	f	6	3

Non-Tinnitus	Subject	Age	Sex	Depression	Anxiety
	55	48	m	0	0
	57	53	m	2	0
	60	48	m	1	1

For subjects imaged twice, (#8, 13, 18, 19) there are separate age, depression and anxiety entries for the two sessions.
n/a - not available

Table 2

Tinnitus Characteristics

Subject	Duration of Tinnitus	Incident triggering tinnitus	Tinnitus Quality	Tinnitus Location	Tinnitus Pitch (kHz)	Tinnitus Loudness (dB SL)	MML (dB SL)	Residual Inhibition	Head/neck maneuvers alter tinnitus?	TRQ
8	6, 8 yrs.	lesser occipital n. block	ringing	rt ear	10	7	47	no	n/a	21, 23
10	> 5 yrs.	tooth abscess	pure tone	lft ear	n/a	n/a	n/a	n/a	n/a	22
11	5 yrs.*	viral illness	ringing/pulse	both ears, rt worse	5.2	4	18	no	yes	42
14	5 mos.	auto accident	roaring/popping	both ears, rt worse	n/a	n/a	n/a	n/a	no	76
16	>6 yrs.	pertussis	hissing/ringing	both ears, rt worse	n/a	n/a	n/a	yes	n/a	16
18	<6 mos.	rt. nasopharynx abrasion	tonal hissing	rt ear	3.5	3	65	yes	yes	57, 43
19	>20 yrs.	none	high pitched ringing	both ears	12.5	10	82	n/a	yes	0, 0
35	11 yrs.**	none/dive into water	tonal/ringing	both ears, rt worse	1.0	52	50	no	yes	28
43	8 mos.	auto accident	shrieking, squealing	both ears, rt worse	1.1	23	25	no	yes	28
55	23 yrs.	none	hissing	in head to lft	3.3	38	64	no	yes	23
57	n/a	n/a	buzzing/ringing	in head to rt	3.3	28	32	yes	n/a	19
60	1 yr.*	none	hissing	lft ear	11.9	8	22	no	n/a	2

* Subjects 11 and 60 reported having had intermittent tinnitus for one year prior to developing constant tinnitus. Duration of tinnitus indicates duration of constant tinnitus.

** Subject 35 had low-level tinnitus with no obvious trigger for 10 years that then became louder after diving into water. For subjects imaged twice (#8, 18, 19): Tinnitus duration is given for each session (#8) or applies to both sessions; TRQ scores are given for each session; The remaining characteristics were unchanged between sessions (quality, location) or were only measured for one session or the other.

n/a - not available

Table 3

Data obtained in each subject (indicated by an “X”)

Non-Tinnitus	Single-Slice Acquisition		Clustered Acquisition	
	Pump Off	Pump On	Pump Off	Pump On
Subjects	7	X		
	9	X		
	12	X		
	13	X		
	13	X		X
	20			X
	21			X
	24			X
	25			X
	26	X		X
	27	X		X
	31	X		X
	33	X		X
	40	X		X

	Single-Slice Acquisition		Clustered Acquisition	
	Pump Off	Pump On	Pump Off	Pump On
Non-Tinnitus				
44	X	X		
48	X	X		
49	X	X		
50			X	X
51			X	X
56			X	X
62			X	X
Tinnitus Subjects				
8	X			
8	X	X		
10	X			
11	X			
14	X	X		
16	X	X		
18		X		

Non-Tinnitus	Single-Slice Acquisition		Clustered Acquisition	
	Pump Off	Pump On	Pump Off	Pump On
18		X		
19	X	X		
19			X	X
35	X	X		
43	X	X		
55	X	X		
57			X	X
60			X	X

Table 4Subject characteristics: mean $\pm$ SEM for each group

	single-slice		clustered	
	tinnitus	non-tinnitus	tinnitus	non-tinnitus
age (yrs)	47.1 $\pm$ 4.5	46.8 $\pm$ 1.8	53.0 $\pm$ 2.9	50.2 $\pm$ 4.7
percent female	71	67	0	75
depression score	4.0 $\pm$ 1.1	4.1 $\pm$ 1.4	1.0 $\pm$ 0.6	4.0 $\pm$ 2.0
anxiety score	5.3 $\pm$ 1.3	3.2 $\pm$ 0.8	0.7 $\pm$ 0.3	1.0 $\pm$ 0.4