Neurobiology of Disease

Human Auditory Cortex Neurochemistry Reflects the Presence and Severity of Tinnitus

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It is not known why tinnitus occurs in some cases of hearing damage but not others. Abnormalities of excitation–inhibition balance could influence whether tinnitus develops and its severity if it does. Animal models of hearing damage, which also produce tinnitus based on behavioral evidence, have identified abnormalities of GABAergic inhibition, both cortically and subcortically. However, the precise relationships of GABA inhibitory changes to tinnitus itself, as opposed to other consequences of hearing damage, remain uncertain. Here, we used magnetic resonance spectroscopy to non-invasively quantify GABA in the left (LAC) and right (RAC) auditory cortices of a group of 14 patients with lateralized tinnitus (eight left ear) and 14 controls matched for age, sex, and hearing. We also explored the potential relationships with other brain metabolites (i.e., choline, *N*-acetylaspartate, and creatine). The presence of tinnitus was associated with a reduction in auditory cortex GABA concentration. Regardless of tinnitus laterality, *post hoc* testing indicated reductions that were significant in RAC and nonsignificant in LAC. Tinnitus severity and hearing loss were correlated positively with RAC choline but not GABA. We discuss the results in the context of current models of tinnitus and methodological constraints.

Key words: **auditory cortex; choline; GABA; MR spectroscopy; tinnitus**

Significance Statement

Permanently affecting one in seven adults, tinnitus lacks both widely effective treatments and adequate understanding of its brain mechanisms. Existing animal models represent tinnitus that may not be distinguishable from homeostatic responses to the auditory insults used to induce it. Human studies can be well controlled in this regard but are usually not (with few even matching control subjects for hearing loss) and are limited in scope as a result of relying solely on non-invasive recording techniques. Here, we exploit recent advances in non-invasive spectroscopic techniques to establish, in a human study tightly controlled for hearing loss and hyperacusis, that tinnitus is associated with a significant reduction in auditory cortex GABA concentration, which has implications for understanding and treatment of the condition.

Introduction

Persistent sensation of sound in one or both ears (tinnitus) commonly results from certain cases of peripheral auditory damage. Although initiated in the periphery, the importance of central mechanisms in tinnitus is highlighted by reduced spontaneous and sound-driven cochlear nerve firing, instances of exacerbation attributable to sectioning the cochlear nerve, and excessive activity in the central auditory pathway (Noreña and Farley, 2013). Although neuronal firing rates are usually elevated in noise trauma animal models, the time course and tonotopic dis-

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tribution of these may or may not match those of the tinnitus behavior (Eggermont, 2013). However, increased synchrony between neurons has been observed, in certain studies, to begin immediately after noise trauma (thus mirroring tinnitus onset; Noreña and Eggermont, 2003) and in the tonotopic region of noise trauma (Seki and Eggermont, 2003). Human tinnitus patients exhibit excessive neural synchrony, in the form of abnormal spontaneous electromagnetic field/potential oscillations in auditory cortex (Weisz et al., 2007; Adjamian et al., 2012; Tass et al., 2012) and increased responses to sound at various levels of the central auditory pathway (Lanting et al., 2008; Melcher et al., 2009; Gu et al., 2010). Although the exact role of these different types of activity in tinnitus pathogenesis is still debated (Sedley and Cunningham, 2013; De Ridder et al., 2015), they appear important and might plausibly be produced or enhanced by deficient inhibition in the central auditory pathway. If identified, reversing such inhibitory deficiency via pharmacological or other interventions could prove effective in treating tinnitus.

GABA systems in tinnitus

The inhibitory neurotransmitter GABA is instrumental in maintaining excitation–inhibition balances. From first principles, insufficient GABAergic inhibition could underlie tinnitus, or elevated inhibition could act to compensate for it. Changes could take the form of altered gross concentration, relative distribution in the tissue, receptor affinity, or receptor density. The only relevant study measuring GABA directly compared measurements from rats with behavioral evidence of chronic tinnitus, after noise exposure, with those from unexposed controls (Brozoski et al., 2012). Significant differences were found only at the level of the auditory thalamus, contralateral to the exposed ear, with exposed rats showing lower GABA concentrations. Studies using flavoprotein autofluorescence to measure the spatial spread of neural responses have found evidence of reduced GABAergic inhibition, associated with noise trauma animal tinnitus models, in the dorsal cochlear nucleus (Middleton et al., 2011) and auditory cortex (Llano et al., 2012). Chronic salicylate exposure (which causes tinnitus) has been found to result in reduced expression of glutamic acid decarboxylase (which converts glutamate to GABA) and reduced GABA receptor affinity in the inferior colliculus of rats (Bauer et al., 2000). Although indicative of GABAergic deficiency, these studies do not uniquely attribute observed abnormalities to tinnitus specifically, as opposed to other consequences of the auditory insults applied. Indirect evidence of the importance of GABA systems in tinnitus comes from a number of human and animal studies finding improvements in tinnitus measures after administration of drugs that increase GABA concentration (Brozoski et al., 2007) or have a GABAergic action (Han et al., 2012; Zheng et al., 2012). Furthermore, withdrawal from chronic benzodiazepine use is a potent inducer of tinnitus (Busto et al., 1986, 1988), suggesting a direct relationship between GABA systems and tinnitus.

Magnetic resonance spectroscopy in tinnitus patients

Magnetic resonance spectroscopy (MRS) is a non-invasive method capable of making brain neurochemical measurements in humans and has been proposed as a potentially useful tool for studying tinnitus (Cacace and Silver, 2007). We are aware of only one published report of MRS in tinnitus (Kilicarslan et al., 2014), which measured *N*-acetylaspartate (NAA) and creatine in acoustic neuroma patients but without studying other metabolites or a control group. Therefore, we applied the technique to the bilateral auditory cortices of a group of chronic tinnitus patients and age/hearing-matched controls to highlight abnormalities specifically linked to tinnitus. Given the *pre hoc* focus on GABA, we used methods optimized for GABA estimation.

Materials and Methods

Subjects. We studied a group of 14 patients (six females) with predominantly or entirely unilateral tinnitus (eight left, six right) and 14 controls (seven females) matched for age and hearing loss. Lateralized tinnitus was studied to assess hemispheric bias of any neurochemical changes in terms of left versus right and ipsilateral versus contralateral. Exactly half of each group had normal (<20 dB hearing loss) mean hearing thresholds in frequencies between 0.25 and 8 kHz so that the effects of hearing loss on cortical neurochemistry could be studied. Exclusion criteria included any neurological disorder other than tinnitus, the use of sedating or GABA acting medications, and contraindications to MRI.

Assessment of tinnitus phenomenology. The diagnosis of tinnitus was made based on a clinical history of persistent simple auditory percepts heard all or most of the time, when not being masked by environmental sounds, for a duration of >6 months. Controls were screened for the presence of tinnitus and excluded if they experienced any persistent sounds in either ear on a regular or permanent basis. Quantification of tinnitus severity was performed in three ways: (1) completion of the tinnitus handicap inventory (THI; Newman et al., 1996), which measures the effect of tinnitus rather than its loudness; (2) visual analog scoring (VAS) of subjective tinnitus loudness; and (3) VAS assessment of the distress caused by tinnitus. VAS measures were indicated on a continuous scale from 0 to 10, with 0 being no sound at all or no distress, and 10 being the loudest sound in existence or the most distress it is possible to experience. In a novel approach to disentangling chronic from acute influences on tinnitus (which might have different correlates), for each VAS, subjects provided four responses: (1) an "average" measure, indicating their response with respect to a typical or average day; (2) a "minimum" response, indicating their lowest score applicable over the past 3 months; (3) a "maximum" response, indicating their highest score applicable over the past 3 months; and (4) a "now" measure, indicating their response applicable at the time of the experiment. These measures were converted to two values to be used for the analysis of MRS data: (1) an "overall" score, which was simply the average response; and (2) a "current" score, derived from interpolation, onto a scale of 0–10, of the now score between the minimum and maximum responses, such that the minimum response was 0, the average response was 5, and the maximum response was 10. Hyperacusis was quantified in all subjects using the hyperacusis questionnaire (HQ; Khalfa et al., 2002). Audiological assessment for all subjects included pure tone audiometry (PTA) in 1 dB steps at frequencies of 0.25, 0.5, 1, 2, 4, 6, and 8 kHz. PTA was performed in ascending frequencies in the left and then the right ear, with three repetitions of each frequency in immediate succession, and the median value across the three repetitions was taken.

MRS acquisition. GABA has three distinct peaks in its magnetic resonance spectrum, but each of these coincides in frequency with the peak of a more abundant metabolite, necessitating the use of a specific GABA measurement technique to quantify its concentration (Puts and Edden, 2012; Mullins et al., 2014). We used the Mescher-Garwood proton resolved spectroscopy (MEGA-PRESS) technique (Mescher et al., 1998), in which alternate acquisitions are applied an editing pulse at 1.9 ppm, which modulates all three of the GABA peaks attributable to J coupling. Subtraction of the EDIT-OFF from EDIT-ON spectra thus allows separation of the GABA signal at 3 ppm from the overlying creatine signal. MEGA-PRESS spectra were acquired from each of the left (LAC) and right (RAC) auditory cortex of each subject on a Phillips Achieva 3 Tesla MRI scanner using an eight-channel head coil. Specific sequence parameters included the following: TR, 2000 ms; TE, 68 ms; 320 averages; acquisition bandwidth, 1000 Hz; scan duration, 11 min; sinc Gaussian editing pulse applied at 1.9 ppm (during EDIT-ON scans) and 7.5 ppm (during EDIT-OFF scans); voxel size, 45 (anteroposterior) × 32 (right to left) × 20 (foot to head) mm for both LAC and RAC; variable power radiofrequency pulses with optimized relaxation delays (VAPOR) water suppression (Tkác et al., 1999). Non-water-suppressed spectra were also

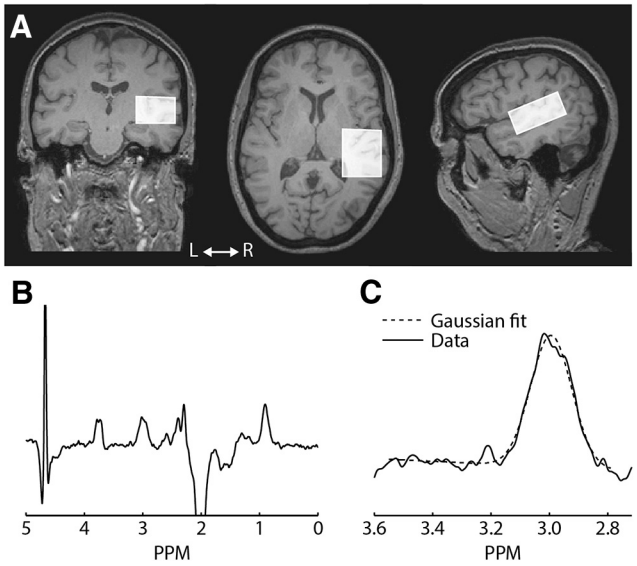

Figure 1. Example GABA spectrum acquisition from the RAC in one typical subject. ***A***, Orthogonal section view (neurological convention; L, left; R, right) of voxel placement. Voxels were placed parallel with and superiorly abutting the Sylvian fissure and were otherwise centered on Heschl's sulcus. This volume encompassed nearly all of Heschl's gyrus, including the primary auditory cortex, the planum temporale, the superior temporal sulcus, planum polare, and also small parts of the insula and middle temporal gyrus adjacent to these auditory regions. ***B***, Edited spectrum, including the GABA peak for quantification at 3 ppm. ***C***, Expanded view of ***B***, showing 3 ppm GABA peak and fitted Gaussian function used for quantification.

obtained from each auditory cortex (PRESS: TE, 68 ms; TR, 2000 ms; 10 averages). Previous T1w structural scans (3D MPRAGE; sagittal acquisition aligned with the anterior commissure–posterior commissure line; 1 mm isotropic resolution; matrix, 240 × 240 × 180; TR, 9.6 ms; TE, 4.6 ms; flip angle, 8°; sensitivity-encoded factor 2) were acquired to aid positioning of the MRS voxels in the LAC and RAC (for details, see Fig. 1). Macromolecular suppression editing was not performed to maximize the signal-to-noise ratio for GABA+ (i.e., GABA plus macromolecules) quantification using the MEGA-PRESS acquisition (Edden et al., 2012). To reduce sources of noise or bias, all subjects were requested to refrain from alcohol for 24 h before the study and caffeine on the day of the study, scans were performed at approximately the same time each morning, the scanner was not used after any high-demand sequences to prevent scanner frequency drifts that might have influenced the MEGA-PRESS acquisition (Harris et al., 2014), and LAC and RAC were scanned in random order. Subjects all listened to music or radio during the acquisition of the structural T1 image and voxel placement and had no acoustic input besides scanner noise during MRS acquisition.

Spectroscopy data analysis. GABA quantification was performed using the Gannet toolbox for MATLAB (Edden et al., 2014) and comprised the following steps: (1) alignment of each pair (EDIT-ON and EDIT-OFF) of spectra (Near et al., 2014); (2) subtraction of edited spectra, to yield GABA spectra, and averaging across acquisitions; and (3) fitting a Gaussian function to the GABA peak at 3 ppm and quantifying GABA based on the area under the curve. For one typical edited spectrum and Gaussian fit, see Figure 1, *B* and *C*, respectively. Water level was obtained from a Gaussian–Lorentzian fit to the non-water-suppressed data. Tissue fractions of gray matter (GM), white matter (WM), and CSF within the MRS voxels were calculated based on automated segmentation in SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/), using a volume mask generated from the voxel position in Gannet (Harris et al., 2015). The final measurement of interest, GABA concentration, was estimated relative to water amplitude, accounting for metabolite relaxation times and tissue fractions, as discussed by Gao et al. (2015). Choline, creatine, and NAA amplitudes were quantified from non-edited spectra only using the AMARES (Advanced Method for Accurate, Robust, and Efficient Spectral fitting of MRS data with use of prior knowledge) algorithm from jMRUI (Naressi et al., 2001), and similarly to GABA were converted to tissue concentrations by taking account of water level, tissue fractions, and known tissue metabolite relaxation times from the literature (Mlynárik et al., 2001; Ethofer et al., 2003). As part of the quality assurance routine, MRS fit quality was assessed by an experienced physicist; the spectra were rejected if the Gannet fit error was >10% (GABA measurements) or metabolite line width was >12 Hz (NAA, creatine, and choline).

Statistical analysis. Nonparametric statistics were used, namely the Friedman's test for main effects of group, with Wilcoxon's rank-sum test for *post hoc* group differences in individual hemispheres, and Spearman's rank correlation coefficients for correlations with tinnitus-related or other continuous variables. GABA was treated as the primary outcome measure and therefore was not subject to multiple comparison correction. Choline, creatine, and NAA were treated as exploratory and therefore subject to Bonferroni's correction. All statistical tests were two tailed.

Table 1. Metabolite concentrations for tinnitus and control groups

	LAC			RAC			
	Control (mM/L), $n = 14$	Tinnitus (mM/L), $n = 14$	p	Control (mM/L)	Tinnitus (mM/L)	p	Combined p
GABA	1.08 (0.24)	0.99 (0.13)	0.30	1.28 (0.12)	1.12 (0.10)	**0.018**	**0.015**
Choline	2.72 (0.60)	2.60 (0.38)		3.43 (0.82)	3.23 (0.71)		0.15
NAA	13.5 (2.17)	13.7 (1.64)		15.2 (2.22)	15.3 (1.47)		0.67
Creatine	8.03 (2.36)	7.76 (1.48)		9.75 (2.13)	9.61 (4.22)		0.21

When applicable, values are median (interquartile range). Combined p values are uncorrected (although GABA represents a primary hypothesis) and are based on Friedman's test, treating hemisphere as a blocking variable. Where these are significant, p values are shown for the LAC and RAC, which are based on Wilcoxon's rank-sum tests. Significant group differences are shown in bold.

Results

Spectroscopy results overview

Gaussian fit errors of the GABA spectra were all <10%, except for one subject in whom the LAC fit error was 15%. This spectrum was reacquired, with an equal fit error in the second acquisition, and the GABA values obtained from the two spectra were averaged. All line widths for non-GABA metabolites were <12 Hz. Table 1 shows all metabolite concentrations for tinnitus and control groups. It can be seen from this that all metabolites were less abundant in LAC than RAC by ~20%. The absence of any relative differences between metabolites suggests that this was an issue of decreased signal from a particular voxel location, as has been reported in phantom studies (Edden and Barker, 2007), as opposed to chemical shift misregistration; in this phenomenon, the spatial distance between the intended and sampled voxel locations varies for the different metabolites, thus producing varying degrees of signal loss (as opposed to the consistent 20% reduction we observed).

GABA spectroscopy results

Compared with controls, the tinnitus group showed GABA decreases in the LAC (median, 0.99 vs 1.08 mM/L) and RAC (median 1.12 vs 1.28 mM/L). Friedman's test showed a significant main effect of subject group ($p = 0.015$), and Wilcoxon's rank-sum tests found the difference to be significant in the RAC ($p = 0.018$) but not LAC ($p = 0.30$). The reduction in RAC GABA concentration in the tinnitus group remained significant ($p = 0.0308$) after excluding a control outlier from the analysis. These results are shown in Table 1 and Figure 2. There was no significant difference in RAC GABA concentration between the left and right ear tinnitus patients ($p = 0.85$). GABA concentrations showed no significant correlation with age, sex, hearing loss, hyperacusis, or any of the tinnitus severity measures.

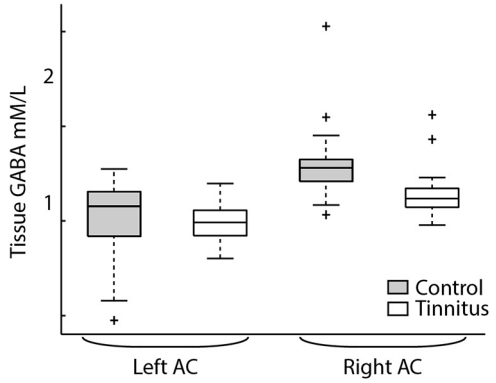

Figure 2. Auditory cortex GABA concentrations in the tinnitus and control groups. AC, Auditory cortex. Boxes indicate interquartile range, with horizontal line at the median, and whiskers indicate full range, barring outliers that are indicated with + signs. GABA was significantly reduced ($p < 0.05$) as a main effect of subject group (tinnitus vs control) and in the RAC. Results displayed here are also tabulated in Table 1.

Table 2. Subject characteristics of tinnitus and control groups

	Control	Tinnitus	Difference (*p*)
Group size (*n*)	14	14	
Age (years)	55.7 ± 10.6	53.7 ± 15.1	0.87
Sex (*n* females)	7 of 14	6 of 14	
Mean left-ear hearing loss (dB)	19.4 ± 14.4	18.8 ± 15.3	0.91
Mean right-ear hearing loss (dB)	17.4 ± 11.5	18.0 ± 20.2	0.58
Hyperacusis (HQ)	11.6 ± 5.7	13.6 ± 6.4	0.30
t duration (years)		9.4 ± 7.6	
Laterality (*n* left)		8 of 14	
THI		26.7 ± 13.2	
Overall loudness		3.9 ± 1.8	
Current loudness		4.3 ± 2.3	
Overall distress		3.1 ± 1.6	
Current distress		3.9 ± (2.2	

When applicable, values are indicate mean ± SD. *p* value is between the tinnitus and control groups based on Wilcoxon's rank-sum statistic.

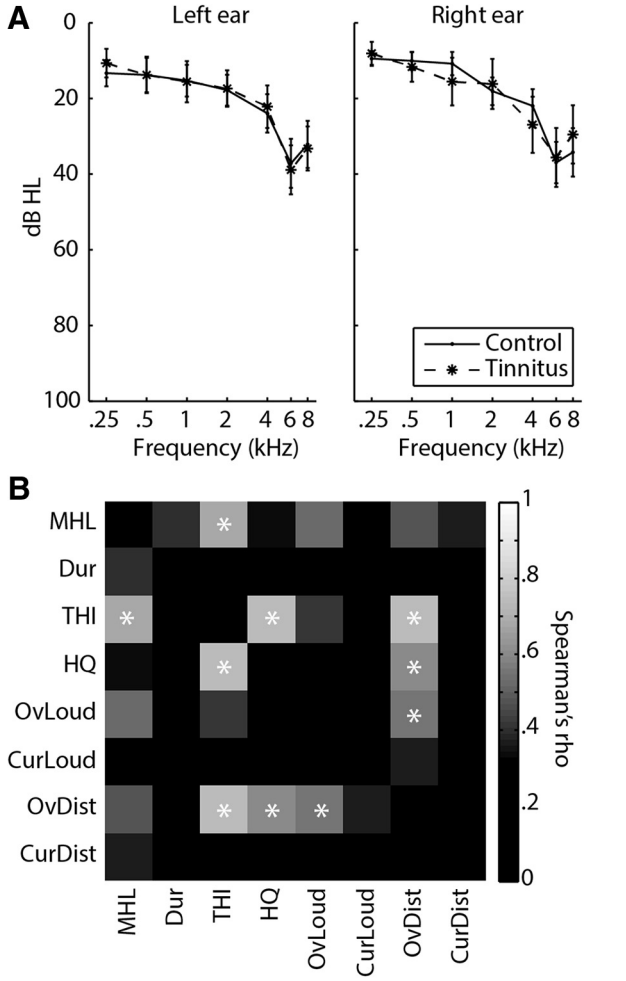

Figure 3. Hearing and tinnitus-related subject characteristics. ***A***, Mean pure tone hearing thresholds of the tinnitus and control groups. Error bars represent SEM. ***B***, Positive nonparametric correlations between hearing and tinnitus measures within the tinnitus group. Significant ($p < 0.05$ uncorrected) correlations are denoted by asterisks. No negative correlations close to significance were observed, hence only positive correlations are shown. MHL, Mean hearing loss (in decibels; across all frequencies shown in ***A***). Dur, Tinnitus duration (years); OvLoud, overall VAS loudness; CurLoud, current VAS loudness; OvDist, overall VAS tinnitus distress; CurDist, current VAS tinnitus distress.

Subject characteristics

Fourteen tinnitus patients and an equal number of matched controls were studied. No significant differences were present between groups in terms of age, hearing loss in either ear, or hyperacusis. There was one extra female subject in the control group compared with the tinnitus group. Mean tinnitus duration was 9.4 years (range, 2–29 years). Eight of the tinnitus patients had left-ear-predominant tinnitus compared with 6 right ear. Table 2 summarizes the group characteristics and Figure 3*A* the group hearing thresholds.

There were significant positive correlations between certain tinnitus measures, as illustrated in Figure 3*B*. Most notably, THI score correlated with hearing loss and hyperacusis, and VAS overall tinnitus distress score correlated positively with HQ, THI, and VAS overall tinnitus loudness scores. Based on these correlations and our relative interest in certain measures above others, we eliminated hyperacusis and overall VAS distress from additional analysis.

Structural brain changes

There were no (uncorrected) significant differences in GM, WM, or CSF concentrations in the LAC or RAC between the tinnitus and control groups. However, in the subject group as a whole, GM fraction correlated negatively with both age (LAC, $\rho = -0.57$, $p = 0.0016$; RAC, $\rho = -0.48$, $p = 0.009$) and hearing loss (LAC, $\rho = -0.59$, $p = 0.001$; RAC, $\rho = -0.49$, $p = 0.0078$), and CSF fraction correlated positively with age (LAC, $\rho = 0.52$, $p = 0.0047$; RAC, $\rho = 0.56$, $p = 0.0019$) and hearing loss (LAC, $\rho = 0.25$, $p = 0.20$; RAC, $\rho = 0.40$, $p = 0.033$).

Choline spectroscopy

Choline concentration was not significantly different between the tinnitus and control groups (see Table 1). RAC choline correlated with overall VAS tinnitus loudness ($\rho = 0.050$, $p = 0.069$ uncorrected), THI score ($\rho = 0.73$, $p = 0.034$ corrected), hearing loss in the subject group as a whole ($\rho = 0.62$, $p = 0.0055$ corrected), hearing loss in the tinnitus group ($\rho = 0.81$, $p = 0.0078$ corrected), and hearing loss in the control group ($\rho = 0.41$, $p = 0.14$ uncorrected). These correlations are illustrated in Figure 4. Because choline is present in higher concentrations in WM than GM (Rae, 2014) and tissue composition correlated with age and hearing loss, we repeated significant correlation analyses after partialing out age, hearing loss, and all tissue fractions from both RAC choline concentration and the subject variable of interest. It should be kept in mind that such extensive partialization removes a lot of variance from the data, so the same strength of correlation

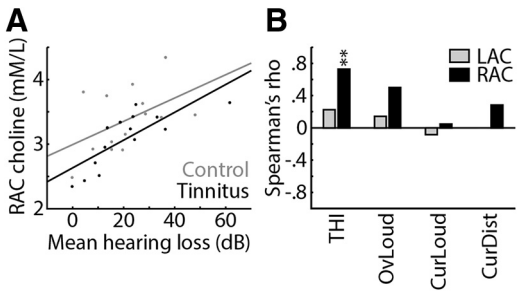

Figure 4. Correlations between choline concentration and subject variables. ***A***, Relationship between mean hearing loss and RAC choline concentration in control (gray) and tinnitus (black) groups. Individual dots denote individual subjects, and lines represent least squares best linear fits. ***B***, Spearman's rank correlation coefficients (ρ) between choline and subjective tinnitus variables in bilateral auditory cortices. OvLoud, VAS overall tinnitus loudness; CurLoud, VAS current tinnitus loudness; CurDist, VAS current tinnitus distress. $^{**}p < 0.01$ uncorrected.

cannot be expected. Nonetheless, these partial analyses showed that the correlation between hearing loss and choline in the whole subject group remained significant ($\rho = 0.39$, $p = 0.040$ uncorrected), and the majority of the correlation between choline and THI score remained ($\rho = 0.41$, $p = 0.15$ uncorrected).

NAA and creatine spectroscopy

There were no group-level differences between tinnitus and control groups for NAA or creatine, in either auditory cortex, even before Bonferroni's correction. Choline and NAA both reflect neuronal density (Miller et al., 1996); therefore, we tested whether there were similar correlations with hearing loss and THI scores with NAA, as there were for choline, which might indicate a structural basis to observed changes. NAA did not correlate significantly with either mean hearing loss ($p = 0.95$ uncorrected) or THI score ($p = 0.76$ uncorrected).

Discussion

We studied a group of tinnitus patients and controls matched for age, sex, hearing loss, and hyperacusis; thus, observed differences can reasonably be inferred to relate to tinnitus as opposed to any of these confounding factors. Because we did not perform macromolecule suppression, for reasons of preserving the signal-to-noise ratio and allowing greater robustness to subject motion, all GABA results should be considered as indicating GABA+ (i.e., GABA plus macromolecules). This means that, as with a large proportion of human MRS studies of GABA, there is a theoretical possibility that a proportion of the findings might relate to molecules other than GABA. MRS measurements were made from a large voxel spanning almost all of auditory cortex, encompassing primary and association areas, and including some areas of surrounding non-auditory cortex. Although the primary reason for using such a large sampling area was to provide a robust GABA measurement, it is likely to have been appropriate in tinnitus, in which ongoing abnormal neurophysiological signals have been found to occur across a correspondingly large anatomical area as opposed to just circumscribed parts of the auditory cortex (Sedley et al., 2015). However, the exact size and position of the voxel used could influence the sensitivity of our measurements to detecting changes in other factors that might correlate with neurotransmitter concentrations in more localized parts of the auditory cortex. Such a voxel size and placement issue might explain why we did not observe a relationship between GABA concentration and hearing loss, as was reported recently in association with age-related hearing loss (Gao et al., 2015). Specifically, voxels in the present study were placed to capture as much of auditory cortex as possible and as little of non-auditory areas, which meant that the primary auditory cortex was at the superior edge of the voxel and slightly anterior of center. Conversely, Gao et al. centered voxels on Heschl's gyrus and therefore on or near the primary auditory cortex. Thus, it is possible that their results were relatively more strongly influenced by the primary auditory cortex than those in the present study. However, our study had other methodological differences to this one, including studying undifferentiated hearing loss as opposed to specifically the age-related type, and it is not known whether hearing loss of different etiologies also differs in its neurochemical correlates. For all significant results, there were qualitatively similar findings in the LAC as in the RAC, but the findings were much stronger and only significant in the RAC. The 20% loss of signal observed in the LAC can be potentially explained by spatial effects influencing GABA MRS measurements (Edden and Barker, 2007). Thus, it is possible that in reality the observed neurochemical correlates of tinnitus are bilateral. However, we stress the importance of obtaining direct evidence rather than assuming this to be the case. Although there was a slight excess of left-ear-predominant tinnitus subjects (8 vs 6), there were no significant differences between left and right ear tinnitus even after markedly relaxing statistical thresholds, making tinnitus laterality a very unlikely explanation.

GABA in tinnitus

We have demonstrated, for the first time, an auditory cortex GABA deficit in human tinnitus subjects. Given the tight matching of the control group for age and hearing loss, this deficit can be specifically attributed to tinnitus itself. Presently, the implications of this finding for understanding tinnitus pathophysiology as a whole are uncertain, although in light of findings in animal tinnitus models (Middleton et al., 2011; Llano et al., 2012), it is likely that this GABA deficiency is responsible for excessive magnitude and lateral spread of cortical responses to thalamic stimulation. Therefore, GABA deficiency may underlie excessive sound-evoked (Gu et al., 2010) and spontaneous (Adjamian et al., 2012) activity that has been observed previously in tinnitus in humans, even after matching for hearing loss and hyperacusis. However, GABA systems may have a more complex role in tinnitus; a recent study found evidence of increased GABA-mediated tonic inhibition in the auditory thalami of rats with noise-induced tinnitus (Sametsky et al., 2015). This excess inhibition was in turn linked to abnormal burst firing of thalamic neurons, which has been argued to trigger abnormal cortical activity linked to tinnitus (Llinás et al., 1999). Thus, the net effect of GABA on tinnitus-linked cortical activity may vary according to such factors as anatomical location and receptor type. With regard to the present results, it is possible that GABA deficiency is a primary cause of tinnitus that, in conjunction with hearing loss, removes the necessary inhibition to prevent spontaneous activity in the auditory system from being perceived as tinnitus. In addition to a role in enhancing cortical responses to sounds or spontaneous subcortical activity, GABA deficiency in the auditory cortex might also act in other ways. Various brain structures outside the auditory system, including limbic, parietal, and prefrontal regions, have been implicated in tinnitus (Lockwood et al., 1998; Moazami-Goudarzi et al., 2010; Vanneste et al., 2010; De Ridder et al., 2013); together, these may mediate the entry of the tinnitus percept into conscious perception and mediate its cognitive and affective consequences. The nonprimary auditory cortex shows rich connectivity with many of these regions in

association with tinnitus (De Ridder et al., 2013; Sedley et al., 2015), and, although there is not yet direct evidence on the subject, it seems possible that GABA changes in the nonprimary auditory cortex might affect entry of the tinnitus signal into wider cortical networks. Because the present data cannot separate GABA changes in the primary from the nonprimary auditory cortex, this possibility should be considered. It is also plausible that GABA deficiency is the direct consequence of chronic stimulation (by tinnitus or its precursors originating subcortically) of the auditory cortex, but we are not aware of any evidence about the effect of chronic sensory stimulation on cortical GABA concentration that could address this possibility. It is also uncertain whether the GABA deficit is a cause or consequence of tinnitus. Although there was not a relationship between tinnitus duration and GABA concentration, which would have favored the GABA deficit being a consequence of tinnitus, this question remains open. Regardless of the exact origin and role of cortical GABA in tinnitus, it seems probable that it is a significant positive force in its pathogenesis, because reduced GABA concentration most likely indicates reduced GABAergic inhibition and therefore a relative excess of excitation in the auditory system. There remains the need for additional understanding of other aspects of GABA systems in tinnitus, such as receptor density, which presently only has limited evidence in humans (Shulman et al., 2000). As well as replicating the present findings, future studies of GABA systems in humans might involve combining GABA measurements using MRS with GABA receptor density measurements using positron emission tomography. In animals, a number of additional measures, such as glutamic acid decarboxylase expression and $GABA_A$ receptor subunit composition, might also be measured simultaneously. Studies might also examine both acute and chronic tinnitus to establish the temporal order of tinnitus phenomenology and GABA changes and also the effect of chronic physiological auditory stimulation on GABA systems.

Choline in tinnitus

Our results indicate that auditory cortex choline concentration correlates positively with both tinnitus severity (in terms of distress and possibly loudness) and hearing loss (particularly in the tinnitus group). Choline is strongly influenced by neuronal density (Miller et al., 1996), and GM loss (hence increased WM/GM ratio) increased with hearing loss, as reported previously (Husain et al., 2011). However, given the persistence of observed findings after statistically adjusting for the influence of GM and WH tissue fractions, it seems probable that choline relates directly to hearing loss and tinnitus. Because choline measured by MRS reflects increased neuronal membrane turnover (Rae, 2014), which may relate to plasticity (Gutiérrez-Fernández et al., 2012), and (although acetylcholine barely contributes to the choline signal) correlates strongly with local concentration of acetylcholine (Wang et al., 2008), we cannot presently determine which of these factors is perturbed as a function of hearing loss and tinnitus severity. Additional work to directly measure neuronal plasticity and acetylcholine is required to understand the relationship between choline and tinnitus.

In summary, we have specifically related the presence and severity of tinnitus, after eliminating confounding factors, to non-invasively measured metabolite concentrations in the auditory cortex. The finding of a GABA deficit in tinnitus patients is beyond a homeostatic response to hearing loss, underscores the importance of GABA systems in the pathophysiology of tinnitus, and may help to direct future treatments.

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