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Frequency organization of the 40-Hz auditory steady-state response in normal hearing and in tinnitus

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We used the 40-Hz auditory steady-state response (SSR) to compare for the first time tonotopic frequency representations in the region of primary auditory cortex (PAC) between subjects with chronic tinnitus and hearing impairment and normal hearing controls. Frequency representations were measured in normal hearing ($n=17$) and tinnitus ($n=28$) subjects using eight carrier frequencies between 384 and 6561 Hz, each amplitude modulated (AM) at 40-Hz on trials of 3 min duration under passive attention. In normal hearing subjects, frequency gradients were observed in the medial–lateral, anterior–posterior, and inferior–superior axes, which were consistent with the orientation of Heschl's gyrus and with functional organization revealed by fMRI investigations. The frequency representation in the right hemisphere was ~5 mm anterior and ~7 mm lateral to that in the left hemisphere, corroborating with MEG measurements hemispheric asymmetries reported by cytoarchitectonic studies of the PAC and by MRI morphometry. In the left hemisphere, frequency gradients were inflected near 2 kHz in normal hearing subjects. These SSR frequency gradients were attenuated in both hemispheres in tinnitus subjects. Dipole power was also elevated in tinnitus, suggesting that more neurons were entrained synchronously by the AM envelope. These findings are consistent with animal experiments reporting altered tonotopy and changes in the response properties of auditory cortical neurons after hearing loss induced by noise exposure. Degraded frequency representations in tinnitus may reflect a loss of intracortical inhibition in deafferented frequency regions of the PAC after hearing injury.

Keywords: Tonotopic maps; Primary auditory cortex; Tinnitus; MEG; 40-Hz auditory steady-state response

Introduction

Tinnitus is an auditory phantom sensation usually accompanied by hearing loss consequent on noise exposure or the aging process. Tinnitus sensations are variable but typically consist of continuous tonal or hissing sounds with center frequencies of 3–8 kHz, which overlap the measured region of hearing impairment in the audiogram (Norena et al., 2002). Neural changes induced by noise exposure in this frequency region have been identified by animal models of hearing loss and include (1) increased spontaneous firing rates of neurons in auditory cortical and subcortical structures although not in auditory nerve fibers; (2) increased synchronization of the spontaneous activity of cortical neurons in the affected frequency region; and (3) a change in the frequency representation of auditory cortex, such that frequencies bordering the edge of hearing loss are over-represented in the cortical tonotopic map (for reviews, see Eggermont and Roberts, 2004; Kaltenbach, 2000; Irvine et al., 2000). Decreased inhibition in central auditory structures consequent on hearing injuries or damaged input from non-auditory pathways may underlie these effects, which point to a central rather than peripheral origin of tinnitus sensations. Positron emission tomography (PET) studies of human tinnitus sufferers have documented increased neural activity in subcortical auditory nuclei (Melcher et al., 2000), in limbic structures associated with emotion (Lockwood et al., 1998), and in the auditory cortex (Lockwood et al., 1998, 2001) where lemniscal and extralemniscal inputs converge and the sensation of tinnitus may be generated.

In principle, functional brain activity related to tinnitus should be expressed in auditory-evoked potentials (AEPs) and magnetic fields (AEFs), which reflect current sinks and sources generated by neural activity in the superficial neocortical laminae (Fishman et al., 2000). Studies comparing N1 transient responses of the AEP/AEF between tinnitus and normal hearing subjects have produced an inconsistent picture with some studies reporting increases in N1 amplitude and N1 slope/intensity functions in tinnitus for tones presented near or below the tinnitus frequency (Dietrich et al., 2001; Norena et al., 1999; Hoke et al., 1989; Weisz et al., in press), and other studies reporting either decreases in these variables at frequencies near the edge of the tinnitus pitch (P2/N1 intensity function at 2 kHz; Kadner et al., 2002) or no changes at all in N1 amplitude in tinnitus (Jacobson et al., 1991). Most of these studies have been guided by the idea that expansion

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of the cortical representation for edge frequencies after hearing loss modulates neural activity in cortical tonotopic map just below the edge of the deafferented region (edge of normal hearing in the audiogram). The expected modulations may be complex, however, with AEP enhancements predicted when edge frequencies are over-represented in the tonotopic map (Dietrich et al., 2001) and AEP diminution when surround inhibition is deployed from the enhanced representation to frequencies below the region of hearing impairment (Kadner et al., 2002).

A different approach to the study of neural correlates of tinnitus has investigated cortical tonotopic map reorganization in tinnitus sufferers. Following this approach, Mühlnickel et al. (1998) found that the cortical source of the N1m magnetic field evoked by the tinnitus frequency in tinnitus subjects was shifted on average by 2.7 mm from its location in the cortical place map of normal hearing controls. The extent of the deviation correlated the subjective degree of tinnitus, implicating map reorganization as a contributing factor. A broadened tuning response of neurons to spectral frequency in the tinnitus frequency region could explain these findings, if it is assumed that excitation of a larger population of neurons shifted the center of activation from its expected location in the cortical representation. Whether the deviations recorded by Mühlnickel et al. reflect changes in the PAC is uncertain, however, because the cortical generators of the N1m typically localize outside of the PAC (Picton et al., 1999; Bosnyak et al., 2004; Engelien et al., 2000) and may reflect interactions occurring between the PAC and belt regions of the auditory cortex. An alternative approach is to investigate auditory "middle latency responses" (MLRs) and the closely related 40-Hz "steady-state response" (SSR) in tinnitus subjects. MLRs generate a 19- to 30-ms waveform with a wave period of about 25 ms that localizes to the region of Heschl's gyrus (Schneider et al., 2002). Neural sources underlying the waveform appear to stabilize into an oscillating network with repetitive stimulation and summate to yield a prominent SSR when carrier frequencies are amplitude modulated (AM) near 40 Hz (Galambos et al., 1981; Gutschalk et al., 1999; Ross et al., 2005). Accordingly, the cortical sources of the 40-Hz SSR overlap those of the 19- to 30-ms MLR waveform and are tonotopically organized in the region of PAC (Pantev et al., 1996; Ross et al., 2000). Diesch et al. (2004) probed cortical representations in tinnitus subjects using 40-Hz SSRs at six carrier frequencies spanning the edge frequency of hearing loss in the subject's audiogram. SSR amplitude increased relative to previously published transfer functions relating SSR amplitude to carrier frequency (Ross et al., 2000) when carrier frequencies entered the tinnitus frequency region, suggesting altered neuronal excitability in this region. In addition, no evidence for a tonotopic gradient was found, pointing to possible map reorganization in tinnitus subjects. While the findings of Diesch et al. (2004) are suggestive, they could not be linked conclusively with tinnitus, because normal hearing controls were not tested. Gerken et al. (2001) studied MLRs in tinnitus and normal hearing subjects. In agreement with the suggestion of Diesch et al. (2004), Gerken et al. (2001) found evidence of enhanced MLRs in tinnitus compared to normal hearing controls. However, tonotopic organization was not investigated in this study.

The present experiment investigated tonotopic organization and neural dynamics in the PAC associated with tinnitus, in a large sample of tinnitus subjects and normal hearing controls. Our first goal was to compare frequency gradients measured by the SSR in normal hearing subjects with the results of fMRI studies of tonotopy (Wessinger et al., 2001; Schönwiesner et al., 2002; Formisano et al., 2003; Talavage et al., 2004) and to assess hemispheric differences in the 3D location of SSR maps in relation to cytoarchitectural (Rademacher et al., 2001) and morphometric (Penhune et al., 1996) studies of the PAC. Previous studies of tonotopic organization assessed by the magnetic SSR have not distinguished maps in the two hemispheres or considered their relation to functional and structural features of the PAC assessed by other brain imaging methods. Our second goal was to use our assessments of the frequency organization of the SSR in normal hearing subjects as a baseline for evaluating tonotopic reorganization in tinnitus and for detecting changes in the amplitude and phase of SSRs which could signal a loss of intracortical inhibition thought to underlie neural dynamics associated with this condition.

Materials and methods

Subjects

The subjects were 28 patients with tinnitus (18 female, 10 male, all right handed; mean age 50.3 years, range 22–73 years) and 17 control subjects reporting normal hearing and no tinnitus (14 female, 3 male, all right handed; mean age 36.8 years, range 20–63 years). Tinnitus subjects completed a questionnaire assessing their tinnitus sensations (ears affected, chronicity, pitch, quality, and loudness). Half of the tinnitus subjects reported bilateral tinnitus, 39.3% left ear only, and 10.7% right ear only. Tinnitus had been experienced for an average of 7.3 ± 8.6 years; most subjects described their tinnitus as chronic (82.1%) and the sensation as tonal or ringing (71.4%) or hissing (32.1%). The tinnitus sensation was described as high pitched by 50.0% of the subjects and as either medium pitched (42.9%) or low pitched (7.1%) by the remainder. All subjects gave written informed consent following procedures approved by the ethics committee of the University of Konstanz.

Stimulation

Subjects were stimulated binaurally via ear inserts with amplitude-modulated (AM) tones (39 Hz modulation frequency, called 40 Hz herein; modulation depth 100%) of different carrier frequencies. Eight AM carrier frequencies (384, 576, 864, 1296, 1944, 2916, 4374, or 6561 Hz) were presented separately in a random order on trials of 180 s duration. In order to assess hearing function and calibrate sound intensity, auditory detection thresholds were measured in the MEG system for each subject, ear, and AM stimulus prior to testing. Sound intensity was adjusted to +60 dB above hearing thresholds (60 dB sensation level, SL) within the limits achievable via flexible tubing in the MEG sound delivery system.

MEG recording

Recording was done with a 148-channel magnetometer (MAGNESTM 2500 WH, 4D Neuroimaging, San Diego, USA). The subjects lay supine in a comfortable position in a magnetically shielded room (Vakuumschmelze Hanau). They were instructed to lie still for 30 min and to fixate a point on the ceiling in order to keep eye movements to a minimum. Continuous data sets were recorded with a hardwired high-pass filter of 0.1 Hz and a sampling rate of 678.17 Hz (bandwidth 200 Hz) using real time noise reduction procedures.

Signal processing

Global noise was filtered from the MEG data by subtracting external, non-biological noise recorded by 11 MEG reference channels. The data were then filtered forward and backward with a digital band pass of 35–43 Hz (Butterworth) of order 2. One thousand epochs of 0.1795 s length (7 cycles of the 39-Hz modulation) were averaged for each subject. All epochs with a MEG level >3.5 pT between the minimum and maximum on one or more MEG channels were artefact rejected.

The 40-Hz SSR was analyzed in source space, which reduced 148 channels of magnetic flux to a single time series of dipole moment computed separately for each subject, stimulus, and hemisphere. Source localization was performed in a coordinate system called the headframe (see Fig. 2A, later). The headframe was defined by the two preauricular points LPA and RPA and the nasion. The center was half way between LPA and RPA. The anterior–posterior axis (y) pointed to the front through the center and the nasion. The medial lateral axis (x) was perpendicular to the anterior–posterior axis, going through the center and pointing to the LPA and RPA. The remaining axis (z) was perpendicular to the other two axes and pointed to the top of the subject's head.

A source model consisting of 2 regional sources (one in each hemisphere) was fitted with BESA® (Brain Electric Source Analysis http://www.besa.de) to all sampling points in the 0.1795-s epoch including all channels. Regional sources were not constrained to be symmetrical between the hemispheres. A regional source applied to MEG data uses two orthogonal dipoles to model MEG field patterns. The 3D location and orientation of each regional source were determined iteratively to maximize goodness of fit with the observed data. The resulting 3D coordinates of regional sources determined for each carrier frequency were used to assess tonotopic frequency organization (defined as a systematic ordering of cortical sources for each carrier frequency with respect to one or more spatial locations in the representation) for each subject and hemisphere. Regional sources were converted to single equivalent current dipoles to estimate source strength as dipole moment (q, evaluated herein as q^2 or dipole power). Single equivalent dipoles were co-registered on the average brain of BESA to verify their location in the auditory cortex. It should be noted that by using a two source model in each hemisphere and click trains of 800 ms duration under conditions of attention (a button press was required to signal changes in AM rate), Gutschalk et al. (1999) described a weaker secondary generator contributing to the SSR in approximately half their subjects, which they speculated may reflect communication with auditory belt areas and/or be related to attention. We were unable to objectively identify a stable second generator in our procedure where trains of 40-Hz stimulation of 3 min duration were presented under passive attention. We therefore opted for a single regional source in each hemisphere that permitted an identical analysis for all subjects and is the practice followed in most studies of SSR localization.

Statistical analysis

Repeated measures analyses of variance (ANOVAs) employing the variables group (tinnitus versus controls), hemisphere, and carrier frequency were applied to dipole power (q^2), phase, and the source coordinates x, y, and z determined for each subject. All analyses were conducted using the general linear model of Statistica (version 6.0) adopting where appropriate multivariate tests for effects involving the repeated measures variable carrier frequency to obviate assumptions of sphericity and compound symmetry. Contrasts were performed using the least significant difference test, including preplanned comparisons of the four lowest with the four highest stimulus frequencies to assess frequency gradients (tonotopic organization) for each coordinate in the control and tinnitus groups separately. Supplementary ANOVAs contrasted subjects with bilateral and left ear tinnitus with one another and with normal hearing controls. We did not contrast unilateral right ear tinnitus with the other tinnitus groups, owing to the small number of subjects with this tinnitus type ($n=3$). Repeated measures ANOVAs were also used to evaluate effects of group, ear, and stimulus frequency on auditory thresholds and on sound intensity levels for each subject. Significance level was set at $p=0.05$ (two-tailed).

To assess the amplitude and phase of the SSR for each carrier frequency, hemisphere, and subject, we calculated the discrete Fourier transform (DFT) of the time series of the dipole moment of each regional source. SSR amplitude was measured as the complex modulus (magnitude), and SSR phase as the phase angle, of the DFT at 39 Hz (the AM rate of the steady-state stimulus). Phase variability was assessed as the circular variance S_0 of the phase angles ($S_0=1-R$, where R is the mean resultant length). The Rayleigh test (Mardia and Jupp, 2000) was applied to R to assess the uniformity of SSR phase over trials within subjects. R assumes the value 1.0 when phase is invariant across trials, and 0.0 when phase is random (no SSR is present).

Results

Hearing function

Prior to the session, hearing thresholds were measured in the MEG by attenuating the sound signal until subjects reported they could no longer detect the stimulus (see Fig. 1A). Attenuation levels were lower (hearing thresholds higher) in tinnitus subjects than in controls at all frequencies, pointing to partial hearing impairments in the tinnitus group (main effect of group $F(1,39)=9.29$, $p<0.005$). An effect of frequency was also found ($F(7,33)=80.1$, $p=0.0000$) , which reflected at least in part the sensitivity curve for the human ear known to peak at sound pressure levels near 1–2 kHz (Moore, 1989). Overall, attenuation factors were slightly higher in the right compared to the left ear ($F(1,39)=5.16$, $p=0.029$), which may reflect idiosyncrasies of the sound delivery system.

Sound intensity was subsequently set at 60 dB above threshold (60 dB SL) in order to equate perceived loudness across ears, stimuli, and subjects. Although intensities of 60 dB SL were achieved for a majority of subjects (75.3% over all frequencies, groups, and ears), exceptions were more common among tinnitus subjects (31.7%) than in normal hearing controls (11.2%) and at the highest (53.7%) and lowest (32.9%) frequencies compared to 1296 Hz (7.3%). Exceptions occurred because, owing to elevated thresholds, a sound of the magnitude 60 dB SL could not be delivered by the sound system. In order to estimate what the subjects actually heard (input to the auditory cortex), we calculated sound intensity with respect to threshold for each subject in the tinnitus and normal hearing groups. The results are given for each

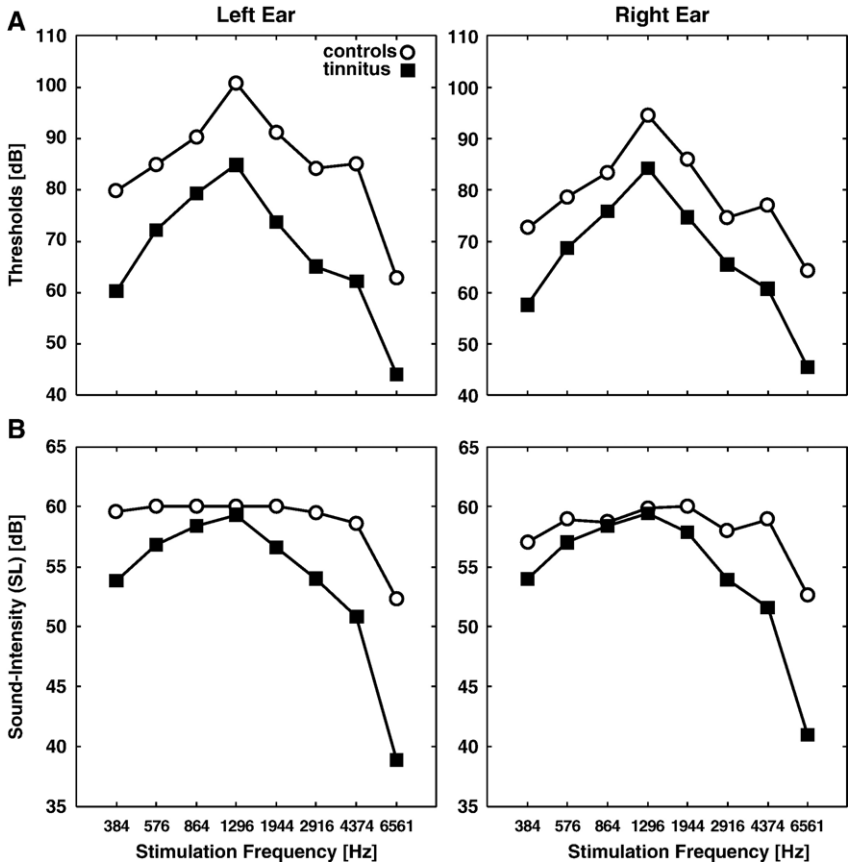


Fig. 1. Hearing status. (A) Sound detection thresholds (level of attenuation to threshold) are shown for each carrier frequency and ear in the tinnitus and normal hearing groups. (B) Sound intensity at each carrier frequency measured with respect to sensation level in the tinnitus and normal hearing groups. Sound intensity was 60 dB HL except where the required sound pressures were limited by tubing in the MEG sound delivery system.

frequency in Fig. 1B. Except for the highest frequency, sound intensity was comparable across frequencies at ~60 dB SL in the control subjects. Sound intensities were lower in the tinnitus subjects than in the normal hearing group at high and low frequencies, and of similar intensity in the two groups in the middle frequency range (576–1944 Hz). The disparity between the two groups was larger for the two highest frequencies than for two lowest ones ($F(1,39)=11.8$, $p=0.0014$), reflecting relatively greater high frequency than low frequency hearing loss in the tinnitus group. Sound intensity was similar for the two ears in each group with no significant effects attributable to this variable. Overall, these results suggest that sound intensities (SL) were comparable for tinnitus and control subjects in the middle frequency range. Outside of this range, weaker stimuli may have been experienced at low frequencies (about −5 dB) and at higher frequencies (up to about −20 dB at 6561 Hz) by tinnitus subjects, notwithstanding the possibility of auditory recruitment in these subjects. In control subjects, sound intensity was similar across frequencies with some attenuation at 6561 Hz.

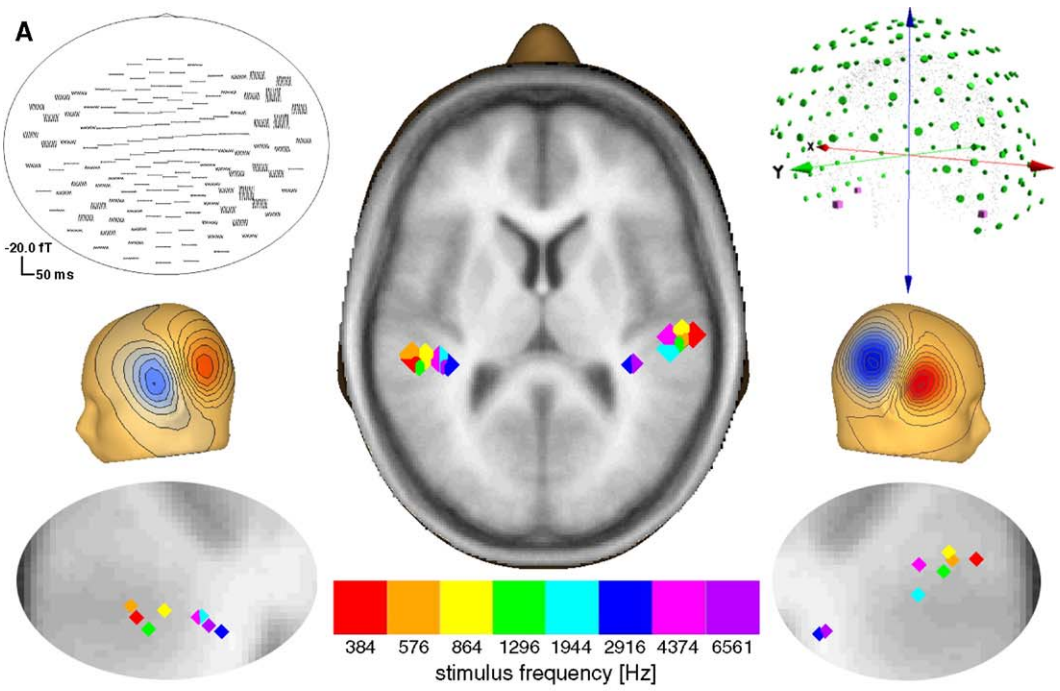
Source model

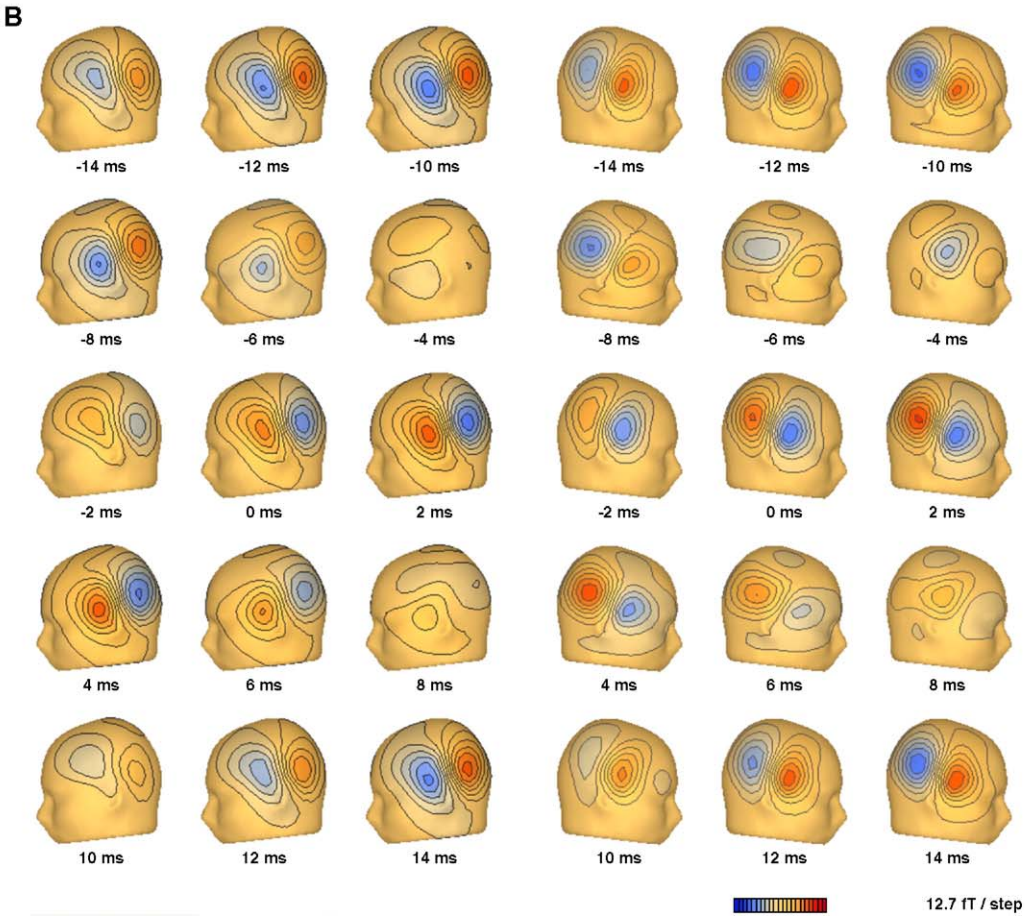
The results of source modeling in a representative normal hearing subject are shown in Fig. 2A. Cortical sources determined for each carrier frequency are superimposed on the average brain of BESA in the middle panel (axial view) to depict the frequency representation in relation to Heschl's gyrus in each hemisphere. A hemispheric asymmetry can be seen with the frequency representation in the right hemisphere residing somewhat anterior and lateral to the representation in the left hemisphere. The time domain SSR waveform (unmodeled data) is shown at each sensor location in the upper left panel of Fig. 2A, and the pattern of magnetic flux at the field maximum in each hemisphere in the two middle panels. In Fig. 2B magnetic flux patterns are shown at successive time points over one steady-state stimulation cycle in each hemisphere. Goodness of fit typically exceeded 90% at field maxima in this and other subjects in both the normal hearing and tinnitus groups.

Frequency organization

Medial lateral coordinates

Source locations obtained in the medial lateral axis (x) are shown for each group, hemisphere, and stimulus frequency in the upper panels of Fig. 3. Overall, a shallow gradient was found in which cortical sources for higher frequencies localized medial to those for low frequencies, giving a main effect of frequency $F(1,37)=2.94$, $p=0.015$. When the tinnitus and control groups were examined separately, preplanned contrasts found that the four lowest and highest frequencies differed for control subjects ($p=0.029$) but not in the tinnitus group ($p=0.301$). A main effect


A
-20.0 fT
50 ms
X
Y
384 576 864 1296 1944 2916 4374 6561
stimulus frequency [Hz]


B
-14 ms
-12 ms
-10 ms
-14 ms
-12 ms
-10 ms
-8 ms
-6 ms
-4 ms
-8 ms
-6 ms
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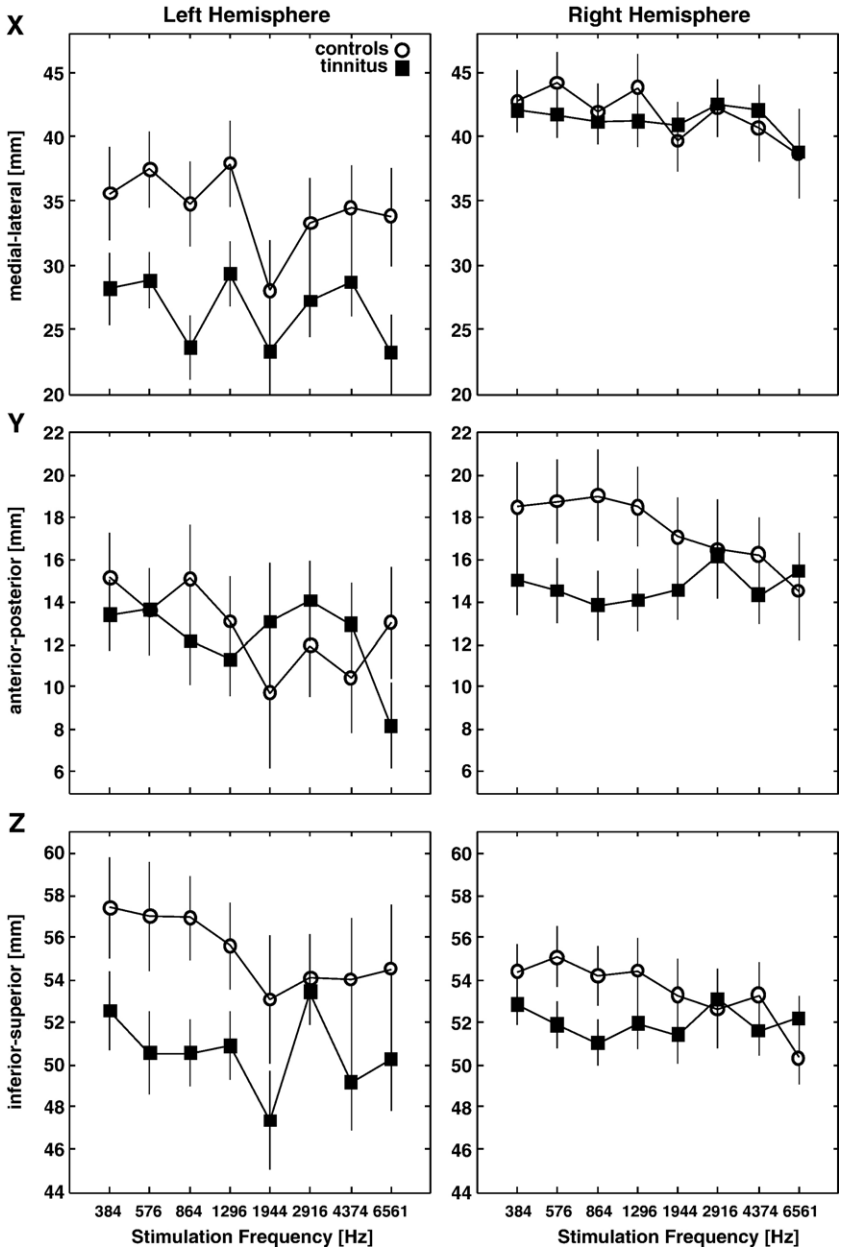

Fig. 3. Tonotopic organization. 3D coordinates (medial–lateral x, anterior–posterior y, and inferior–superior z) are shown for regional sources at each carrier frequency and hemisphere in the tinnitus and normal hearing groups. Bars depict ± 1 standard error.

of hemisphere was also found ($F(1,43)=97.89$, $p=0.0000$), and an interaction of hemisphere with group ($F(1,43)=11.05$, $p=0.002$). Fig. 3 shows that cortical sources for all frequencies were more lateral in the right compared to the left hemisphere. Although present in both groups, the hemispheric asymmetry was more pronounced in the tinnitus group, owing to a more medial map for this group in the left hemisphere compared to control subjects.

Subsequent analyses compared subjects reporting bilateral tinnitus and unilateral (left ear) tinnitus separately with control subjects. In both analyses, cortical sources were again more lateral

Fig. 2. Source modeling in a representative normal hearing subject. (A) Axial view of the tonotopic representation is shown in the center panel. Regional sources determined for each frequency are superimposed on the average brain of BESA. Source locations in the right hemisphere are anterior and medial to those of the left hemisphere, in agreement with the group data. An expanded frequency representation is shown for each hemisphere in the lower left and right panels. The distance between the endpoints in the frequency representation for this subject is 13.9 mm in the left hemisphere and 23.8 mm in the right hemisphere. The upper left and right panels show the time domain SSR at 1296 Hz and the sensor array with the coordinate system, respectively. The middle left and right panels show the pattern of magnetic flux at 1296 Hz at their amplitude maxima in the left and right hemispheres. (B) Magnetic flux patterns at 1296 Hz are shown at 2-ms time steps in each hemisphere over one steady-state stimulation cycle.

in the right hemisphere than in the left hemisphere for all frequencies (main effects of hemisphere $p=0.000$ in each case). When the two types of tinnitus were compared directly, a main effect of type of tinnitus was found ($F(1,21)=4.86$, $p=0.039$), which was due to cortical sources being more medial in each hemisphere in unilateral compared to bilateral cases. Contrasts of x-coordinates collapsed over the four highest and lowest frequencies were not significant in either tinnitus group, indicating that tonotopy was not present in the medial–lateral axis in this condition.

Anterior–posterior coordinates

Anterior–posterior source coordinates (y) are shown for each group, hemisphere, and stimulus frequency in the middle panels of Fig. 3. These results reveal a tendency for high frequencies to localize posterior to those of lower frequencies, particularly in the control group. By comparison, this tonotopy was not strongly expressed in the tinnitus group, particularly in the right hemisphere. Although the main effect of frequency was not significant, a three-way interaction of group, frequency, and hemisphere was found ($F(7,37)=2.53$, $p=0.031$), which reflected frequency representations overlapping between controls and tinnitus subjects more so in the left hemisphere than in the right hemisphere (Fig. 3). Preplanned contrasts of the four lowest and highest frequencies differed significantly in both hemispheres in the control group ($p=0.015$ in each case) but not in the tinnitus condition. Fig. 3 also shows that cortical sources in the right hemisphere were anterior to those in the left hemisphere at all frequencies, in both groups (main effect of hemisphere $F(1,43)=22.07$, $p=0.0000$).

Comparison of bilateral and unilateral left ear tinnitus cases to control subjects separately found main effects of hemisphere ($p=0.006$ or better), which reflected sources being more anterior in the right hemisphere than the left hemisphere in each case. The hemispheric effect remained significant when the two tinnitus types were compared directly ($F(1,147)=4.32$, $p=0.049$), but no effects attributable to type of tinnitus were found.

Inferior–superior coordinates

Inferior–superior source coordinates (z) are shown in lower panels of Fig. 3 for each group, frequency, and hemisphere. A trend for high frequencies to localize inferior to low frequencies can be seen in both hemispheres in the control but not the tinnitus group. Cortical sources also tended to be more inferior in the tinnitus group compared to the control group at most frequencies. These trends approached but did not reach significance (main effects of frequency and group $p=0.071$ and 0.078, respectively). However, when the four highest and lowest frequencies were compared, a tonotopic organization with high frequencies inferior to low frequencies was found ($F(1,43)=7.72$, $p=0.008$) to which the control group contributed ($F(1,43)=8.83$, $p=0.005$) but the tinnitus group did not ($F(1,43)=0.49$, $p=0.48$). Fig. 3 suggests that tonotopy was absent in the tinnitus group.

These findings were characteristic of bilateral tinnitus as well as unilateral left ear tinnitus (no significant differences were found when the two types were compared directly). The main effect of frequency (inferior–superior tonotopy) reached significance when bilateral cases were compared with controls ($F(7,196)=2.96$, $p=0.017$) and came close when unilateral tinnitus was compared with controls ($F(7,175)=2.73$, $p=0.061$), owing in both cases to the presence of a frequency gradient mainly in the control subjects.

Normalized map

In most subjects, Heschl's gyrus is oriented anterolaterally to posteriomedially, with an inferior tilt where the gyrus convolutes near its medial extent (Morosan et al., 2001; Rademacher et al., 2001). Because of this geometry and significant anatomical variation between subjects, the 3D axis that best expresses tonotopy could vary across subjects in the 3D coordinate system used to describe functional organization. In addition, between-subject variability in the maximum spatial extent of tonotopic representations (mean averaged across 3D coordinates 22.7 mm in the left hemisphere, 12.6 mm in the right hemisphere, $p<0.0005$) may blur tonotopic maps when source coordinates are averaged across subjects for a given axis. To reduce the possible influence of between-subject variability in these factors, we normalized the 3D representation for each subject and hemisphere by assigning the value 1.0 to (i) the frequency most laterally situated in the medial–lateral axis, (ii) the frequency most anterior in the anterior–posterior axis, and (iii) to the frequency most superior in the superior–inferior axis, and the value 0.0 to the frequencies situated at the opposite end of these coordinates. Intermediate frequencies were represented as a proportion of the distance between these end points on each axis. Normalization (which removed the effects of hemisphere in the medial–lateral and anterior–posterior coordinates shown in Fig. 3) accentuated tonotopic frequency gradients in each 3D coordinate. Main effects of frequency were now found for every 3D coordinate ($p=0.029$, 0.048, and 0.027 for coordinates x, y, and z, respectively) in which contrasts of the four lowest and highest frequencies were significant in each coordinate for control subjects ($p=0.012$ or better) but for the y-coordinate only in tinnitus ($p=0.003$). The results are collapsed across coordinates in Fig. 4A (upper panels of the figure) where normalized maps are depicted as a single gradient extending from an anteriolateral–superior endpoint (normalized value 1.0, upper left) to a posteriomedial–inferior endpoint (normalized value 0.0, lower right), separately for each group and hemisphere. Analysis of these data revealed main effects of group ($F(1,43)=5.35$, $p=0.025$) and frequency ($F(7,37)=4.35$, $p=0.001$) as well as interactions of group with frequency ($F(7,37)=2.62$, $p=0.026$) and of frequency with hemisphere ($F(7,37)=3.00$, $p=0.013$). Preplanned comparisons conducted for the right hemisphere showed that normalized source positions differed between the four lowest and highest frequencies in control subjects ($F(1,43)=20.86$, $p=0.00004$) but not in the tinnitus group ($F<1$), giving a main effect of frequency ($F(7,37)=2.88$, $p=0.017$) and group × frequency interaction ($F(7,37)=2.60$, $p=0.027$) for this hemisphere. A similar group pattern can be seen in the left hemisphere where a main effect of frequency ($F(7,37)=4.88$) and group × frequency interaction ($F(7,37)$ 2.48, $p=0.034$) was again found. However, in this hemisphere the tonotopic gradient was inflected at 1944 Hz in control subjects, with low and high frequency sources shifting toward end points in the tonotopic representation from this frequency in all coordinates (also seen in the 3D coordinates of Fig. 3 in the control group). This hemispheric difference gave rise to an interaction of frequency with hemisphere, which was significant when tested in control subjects ($F(7,10)=6.29$, $p=0.005$) but not in the tinnitus group. A main effect of frequency was also found in the control group when tested alone in the left hemisphere ($F(7,10)=3.70$, $p=0.030$), indicating that frequency differences were present in this hemisphere in control subjects. The frequency 1944 Hz differed from each of its low frequency neighbors ($p=0.011$ or better) but not from its high

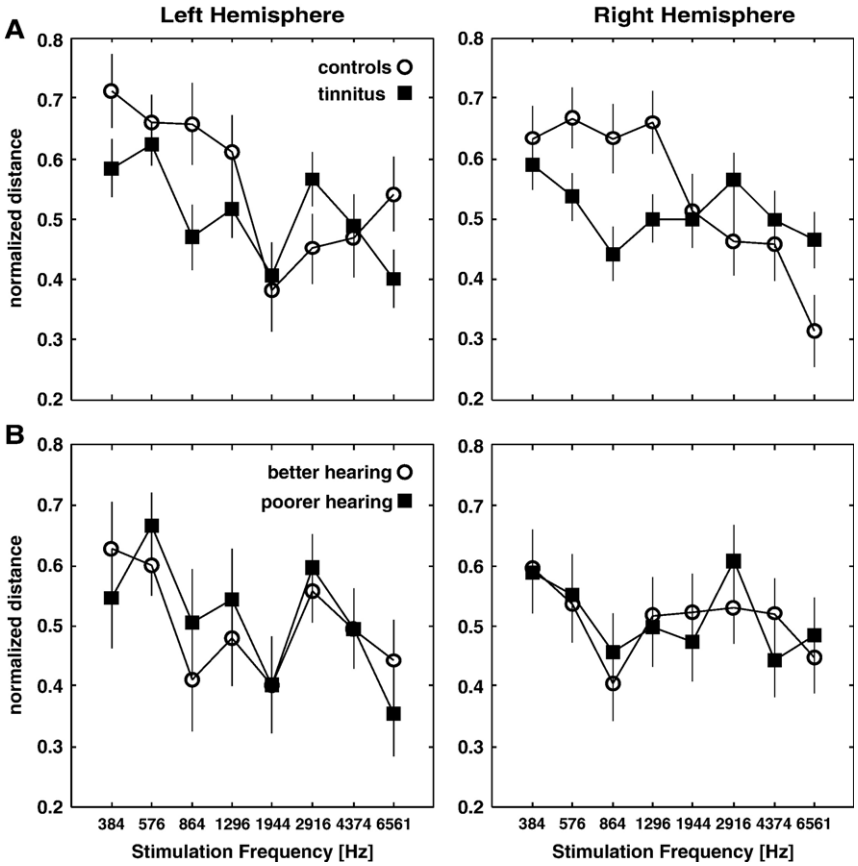

Fig. 4. Normalized tonotopic gradients averaged over 3D coordinates. Bars depict ± 1 standard error. (A) Normalized gradients are contrasted between the control and tinnitus groups in each hemisphere. (B) Normalized gradients are contrasted between better-hearing and poorer-hearing tinnitus subgroups formed by a median split applied to mean hearing function. The subgroups also differed in age (mean difference 19.4 years) and sound pressure levels of auditory stimulation consequent on their hearing impairment (mean difference 14.7 dB SPL).

frequency neighbors, although the comparison with 6561 Hz approached significance ($p=0.085$). These results point to a shallow reversal or flattening of the high frequency representation in the left hemisphere of control subjects, which was not seen in the right hemisphere of these subjects.

The normalized gradients of Fig. 4A were also compared between left ear and bilateral tinnitus in an ANOVA including the two tinnitus types. No effects attributable to type of tinnitus were observed. However, a main effect of frequency ($F(7,15)=3.34$, $p=0.023$) was found which reflected a shallow normalized gradient with no effect of hemisphere (see Fig. 4A). Post hoc contrasts collapsed over hemispheres indicated that source position did not differ among the five frequencies above 864 Hz (minimum $p=0.21$) although these sources when contrasted as a group were shifted with respect to the two frequencies below 864 Hz ($F(1,21)=7.52$, $p=0.012$). These findings suggest a flattening of the 3D gradient in tinnitus subjects at frequencies above about 1 kHz.

We also evaluated qualitatively the extent to which the effects of hemisphere and group found in the averaged normalized data of Fig. 4A were characteristic of individual subjects in the normal hearing and tinnitus groups. For this purpose, the results for each individual normal hearing and tinnitus subject were plotted separately and overlaid within the two groups. The differences in tonotopic organization seen in the averaged data of Fig. 4A between the hemispheres of normal hearing subjects, and between the normal hearing and tinnitus groups, could be visualized in the resulting overlays. However, substantial variability was found in the spatial ordering of the 8 carrier frequencies within subjects in each group. When normal hearing subjects were considered, the local minimum seen at 1944 Hz in Fig. 4A for the left hemisphere was observed in 7 of 18 subjects, and at the adjoining frequencies of 1296 or 2916 Hz in 4 additional subjects. In the right hemisphere, the local minimum was expressed at 6561 Hz in 7 of 18 subjects and at the adjoining frequency of 4374 Hz in two more subjects. Hence, the local minima seen in the left and right hemispheres in Fig. 4A in the normal hearing group were characteristic of 61% of subjects (11/18) in the left hemisphere and 50% of subjects (9/18) in the right hemisphere. Among the 28 tinnitus subjects, local maxima corresponding to the group trend of Fig. 4A were observed at 384 Hz or 576 Hz in 13 of 28 subjects (46%) in left hemisphere and 9 of 28 subjects (32%) in the right hemisphere, with the remaining subjects (a majority of cases) showing local maxima distributed widely across other carrier frequencies. These observations set into relief a wide range of individual variability underling the averaged results presented in Fig. 4A in both the normal hearing and tinnitus groups.

Dipole power and SSR phase

The strength of cortical activations measured as dipole power (q^2) is presented in Fig. 5 for each group, hemisphere, and stimulus

frequency. Overall, dipole power decreased with increasing stimulus frequency ($F(7,301)=3.83$, $p=0.011$), corroborating the previously observed dependence of the amplitude of the SSR on this variable (Ross et al., 2000). A main effect of hemisphere was also found ($F(1,43)=13.16$, $p=0.0008$), which reflected elevated responding in the left hemisphere. Fig. 5 shows that dipole power was greater in the tinnitus group compared to control subjects at all stimulus frequencies in the left hemisphere, and at most frequencies in the right hemisphere. The group difference yielded a main effect, which approached significance ($F(1,43)=2.89$, $p=0.096$) notwithstanding the fact that somewhat weaker sound stimuli were delivered to tinnitus subjects (Fig. 1B). When the three frequencies on which the tinnitus and control groups were least well matched for sound intensity (SL) were discarded (384, 4374, and 6561 Hz), the group difference gave $F(1,43)=3.95$, $p=0.053$.

The group difference portrayed in Fig. 5 was amplified in subjects reporting unilateral left ear tinnitus. In these subjects, the main effect of group (left tinnitus versus controls) was significant ($F(1,25)=6.285$, $p=0.019$), as was the main effect of hemisphere ($F(1,25)=16.21$, $p=0.0005$) and the interaction of hemisphere with group ($F(1,25)=6.10$, $p=0.020$). Left sided tinnitus subjects showed greater dipole power than control subjects at all frequencies in the left hemisphere and at all but the two highest frequencies in the right hemisphere. A main effect of frequency was also found in this analysis ($F(7,175)=3.68$, $p=0.001$) reflecting smaller dipole moments with increasing frequency. When unilateral and bilateral cases were compared directly dipole power was greater in unilateral tinnitus (main effect of tinnitus type $F(1,147)=4.353$, $p=0.049$) particularly in the left hemisphere (type × hemisphere interaction $f(1,21)=5.92$, $p=0.039$). Bilateral subjects did not differ when compared with normal hearing controls, although dipole power tended to be larger in bilateral tinnitus than in controls for the middle frequencies where sound intensities were more closely matched (minimum $p=0.066$, 1944 Hz).

The mean phase of the SSR at each frequency is shown with respect to 0-delay in Fig. 6A. In both the control and tinnitus groups, mean phase lengthened from 384 Hz to 864 Hz and shortened thereafter up to 6561 Hz. This pattern gave a main effect of frequency ($F(7,37)=5.24$, $p<0.0004$), which remained significant when control and tinnitus subjects were evaluated separately ($p=0.004$ and 0.0001, respectively). Phase variability across trials within subjects measured as vector length (R0) is shown in Fig. 6B, which includes levels of significance up to $p<0.00001$ for this measure assessed by the Rayleigh test. In control subjects, vector length stabilized above 0.85 at frequencies up to 1296 Hz, indicating a high degree of synchronization of the SSR with the AM envelope (reduced variability) within subjects at these frequencies. At higher frequencies vector length decreased, indicating an increase in phase variability in the normal hearing subjects. In the tinnitus group phase variability was somewhat greater than in the control group at low frequencies and changed less at higher frequencies in comparison to controls. Thus, phase variability appeared to be more constrained over the frequency range in the tinnitus group. Bilateral and unilateral (left ear) tinnitus subjects did not differ in these measures.

Effects of sound intensity, hearing status, and age

It will be recalled that sound intensity was presented at 60 dB with respect to thresholds measured for each carrier frequency, subject, ear, in the MEG sound delivery system (60 dB SL), in order to achieve comparable input from peripheral to central auditory structures in the tinnitus and control groups despite the presence of hearing impairment in tinnitus subjects. This sound intensity was achieved for all but the highest frequency in normal hearing subjects, and in the middle frequency ranges in the tinnitus group but not at the frequency extremes in this group owing to their hearing loss and limitations of the sound delivery system (see Fig. 1B). We assessed the effect of sound intensity on 3D source coordinates (the normalized data of Fig. 4A) and on dipole power and phase by calculating correlations between sound intensity (SL) and these variables for each carrier frequency and hemisphere. Sound intensity did not correlate with the normalized source coordinates or with dipole power when the tinnitus and control groups were examined separately or when the groups were combined (overall mean $r=0.06$ and 0.02 for the normalized coordinates and dipole power, respectively). However, a weak significant correlation of SL with phase was obtained within both groups (overall mean $r=0.15$, $t(15)=3.05$, $p=0.008$), which reflected a tendency for SSR phase to advance at higher SL sounds.

Presenting sounds at 60 dB SL necessarily meant that the absolute levels of sound pressure delivered to the ears were likely

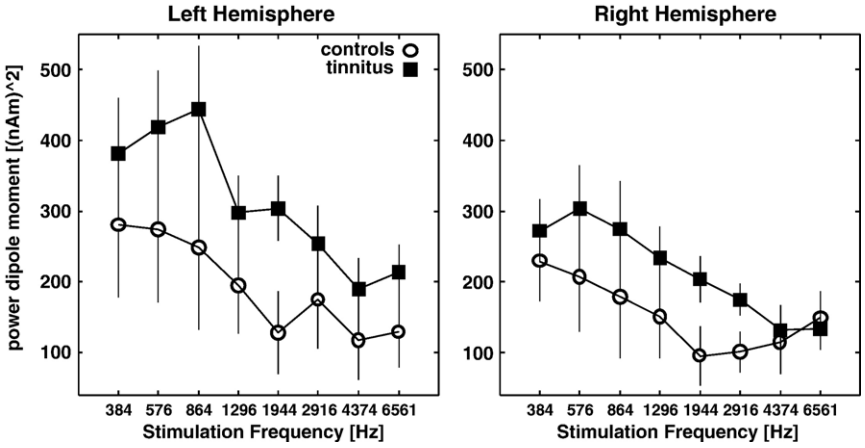

Fig. 5. Dipole power is shown for regional sources at each frequency, hemisphere, and group. Bars depict ±1 standard error.

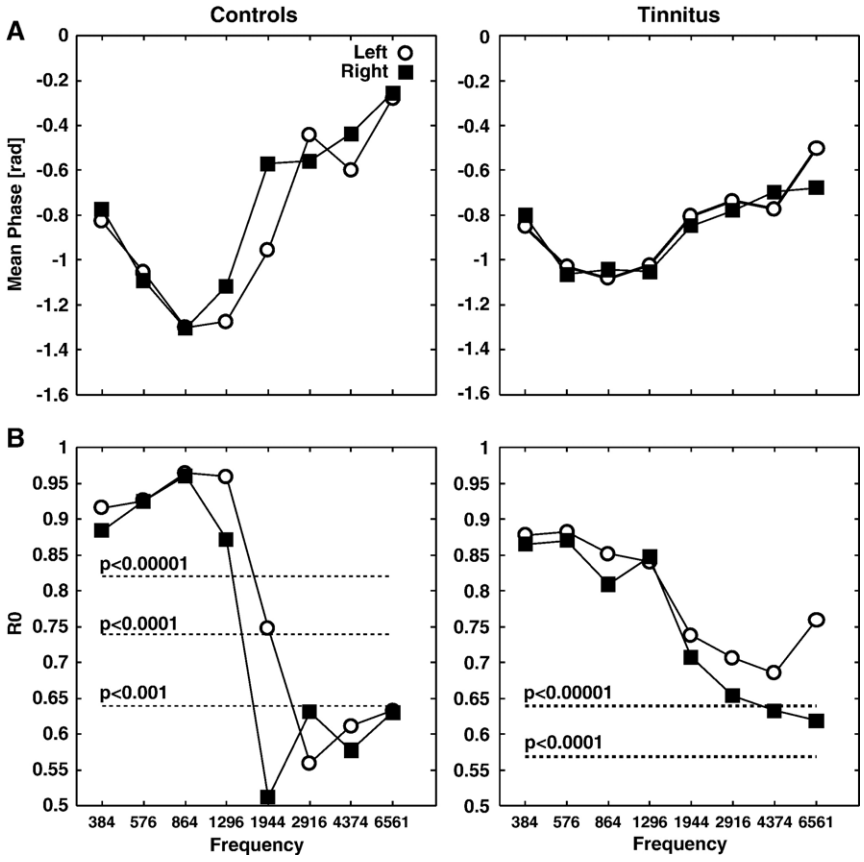

Fig. 6. Dipole phase and phase variability. (A) Mean dipole phase with respect to 0-delay is depicted for regional sources for each frequency, hemisphere, and group. (B) Phase variability (R0) and levels of significance for the Rayleigh test. Phase variability is zero when R0=1.0.

to be lower for normal hearing control subjects than for tinnitus subjects who experienced hearing loss as well as tinnitus. It should also be noted that although the ages of our tinnitus and control subjects overlapped (22–73 years versus 20–63 years, respectively), tinnitus subjects were on average 13.5 years older than control subjects (50.3 and 36.8 years, respectively; $t(41)=2.85$, $p=0.007$). In order to examine the effect of sound pressure level (SPL), hearing loss, and age on frequency gradients in tinnitus, we used a median split to divide our total tinnitus sample into two subgroups differing with respect to hearing function. Sounds between 55 and 60 dB SL were achieved in the better-hearing group and between 38 and 54 dB SL in the poorer-hearing group; SPL was 14.7 dB higher in the latter condition ($t(25)=7.67$, $p=0.0000$) when averaged across sound frequencies. As expected, poorer-hearing tinnitus subjects were also on average 19.4 years older than better-hearing tinnitus subjects ($t(25)=4.19$, $p=0.0003$). Normalized frequency gradients collapsed over the 3D coordinates are shown for these subgroups in Fig. 4B. Although the subgroups differed with respect to hearing function, SPL, and age, the frequency gradients of the two subgroups were similar, showing generally flat gradients above 864 Hz in both hemispheres (the pattern found the combined tinnitus groups in Fig. 4A). This pattern of normalized 3D shifts below but not above 864 Hz in the two tinnitus subgroups gave a main effect of frequency ($F(7,19)=5.16$, $p=0.002$), but the main effect of subgroup and interactions involving this variable were not significant (all F's<1.0). These results suggest that the frequency gradients observed in the tinnitus subgroups were associated with the presence of tinnitus and were not affected by differences in age, degree of hearing impairment, or the absolute levels of sound pressure presented to the two tinnitus subgroups.

We also compared SPL and age between our better-hearing tinnitus subgroup and normal hearing control subjects. The SPLs presented to our better-hearing tinnitus subjects were similar to those presented to control subjects (overall mean difference 3.1 dB, $p=0.37$) but were slightly higher for sounds above 1296 Hz presented to the left ear (mean difference 3.8 dB for these sounds, group × ear interaction $F(7,20)=2.71$, $p=0.038$) reflecting a small high frequency hearing loss favoring this ear. The mean age of the two groups did not differ (36.8±3.7 years for controls and 40.9± 3.8 years for better-hearing tinnitus subjects, $t<1.0$). Because these SPL and age differences between the groups were small, but one group experienced tinnitus and the other did not, we contrasted their normalized 3D gradients with ANOVA. Main effects of group ($F(1,29)=5.91$, $p=0.021$) and frequency ($F(7,23=0.015$) were found, and also an interaction of frequency and hemisphere ($F(7,23)=3.02$, $p=0.021$). These effects reflected 3D representations that were more anterolateral–superior (normalized distance >0.6) for low frequencies (384–1296 Hz) in control subjects than in tinnitus, and a frequency representation that inflected around 1944 Hz especially in the left hemisphere (these effects can be visualized by comparing the control and better-hearing tinnitus groups between Figs. 4A and B). Interactions of group with frequency approached but not reach significance in the left

($p=0.16$) or right ($p=0.082$) hemispheres, although the pattern obtained was similar to that shown in Fig. 4A for the combined tinnitus sample compared to normal hearing subjects. These results concur with those above in suggesting that SSR frequency representations in tinnitus were associated with the presence of tinnitus and not with differences in SPL, age, or the degree of hearing impairment between tinnitus and control groups.

Tinnitus duration, loudness, and pitch

Subjects reported the duration of their tinnitus in years, its loudness on a 4-point scale (barely audible to unbearably loud), and its pitch (high, medium, low) on their questionnaires. To assess the relation of these tinnitus variables to changes in tonotopy, we subtracted the mean tonotopic gradient observed in the control group in Fig. 4A (normalized data) from the tonotopic gradients observed for each tinnitus subject, separately for the two hemispheres. The root mean square deviation across frequencies was then determined for each tinnitus subject in each hemisphere, giving a measure of deviation from normal tonotopic structure in each hemisphere for each tinnitus case. This measure did not correlate significantly with tinnitus loudness, duration, or pitch in either hemisphere or when the hemispheres were combined. We also calculated correlations relating tinnitus pitch, duration, and loudness to dipole power for each frequency and hemisphere, separately in the tinnitus group. Only one of 48 correlations reached significance (for loudness, −0.46 at 1944 Hz in the left hemisphere). Correlations among the tinnitus properties duration, loudness, ear, and pitch, and of these variables with age, also did not reach significance.

Discussion

We assessed auditory cortical function using the 40-Hz SSR whose generators have been localized by source modeling and by intracerebral recordings of contributing middle latency responses to the region of Heschl's gyrus where the tonotopic maps of PAC are found. We obtained neuromagnetic evidence for tonotopic frequency representations in normal hearing subjects that were diminished or absent in subjects experiencing chronic tinnitus. We discuss the relationship of SSR frequency representations in normal hearing subjects to tonotopic organization assessed by other functional brain imaging methods and consider how deviant SSR frequency representations could be produced in tinnitus.

Frequency organization of the SSR in normal hearing subjects

Tonotopic gradients arise as a consequence of the place coding of sound by the basilar membrane and are prominent features of an aggregation of auditory fields generally referred to as the PAC or auditory core. This region of the auditory cortex occupies the posteriomedial extent of Heschl's gyrus and is characterized by densely packed neurons, a high degree of myelination, and a large input layer (IV) innervated by thalamocortical afferents, as well as by functional properties including neurons with receptive fields sharply tuned to spectral frequency and the presence of tonotopic gradients across the cortical surface. At least two subdivisions of the auditory core have been distinguished independently by several investigators (labeled areas AI and R by Hackett et al., 2001, approximating areas Kalt and Kam respectively in the model of Galaburda and Sanides, 1980, or areas Te1.0 and Te1.1 in the model of Morosan et al., 2001) on the basis of architectonic properties (fiber density, myelination, and Ach expression) and by reversing tonotopic maps sharing a low frequency border. Surrounding the auditory core are several auditory belt and parabelt regions, which are reciprocally connected with each other and with the core region in the area of the superior temporal gyrus. Although PAC defined cytoarchitectonically occupies the posterior–medial extent of HG, covering about 2/3 of the total surface area of the gyrus, the borders of PAC do not align closely with the boundaries of HG visualized in magnetic resonance images or determined by anatomical studies. Rademacher et al. (2001) estimated from cytoarchitectonic mapping that when the volume of HG is taken as equal to the volume of PAC, an error as large as 100% is introduced in about 40% of hemispheres. Considerable variation also exists between subjects in the size of HG, its orientation, and the degree of convolution. In about 40% of subjects, an intermediate sulcus divides the surface of HG into two distinct subregions with similar functional and cytoarchitectonic properties.

While this variability precludes a precise mapping of PAC onto HG for individual subjects, recent brain imaging studies point to several regularities that appear to characterize HG and PAC functionally and structurally at the level of groups of subjects. Notwithstanding individual variations that can be striking (Rademacher et al., 2001; Figs. 2 and 3), HG is oriented in an anterior–lateral to posterior–medial direction in both cerebral hemispheres in the typical brain. In addition, recent anatomical data show that the borders of PAC and HG in the right hemisphere are situated anterior with respect to their borders in the left hemisphere, and laterally on the right compared to the left. Rademacher et al. (2001) observed an anterior displacement of PAC of ~7 mm in the right hemisphere compared to the left hemisphere, and a lateral displacement on the right side in the range of ~5 mm, in a postmortem investigation of 27 brains; these estimates are in agreement with MRI morphometry of HG reported by Penhune et al. (1996). A further generalization (in this case from functional brain imaging) concerns frequency gradients in the medial–lateral and anterior–posterior planes. Reversing tonotopic maps are a defining property of distinctive auditory fields in the auditory core, and evidence for at least two maps sharing a low frequency border, possibly distinguishing areas RI and Al, and up to four additional progressions of frequency sensitivity in the superior temporal gyrus, have been reported in fMRI investigations of tonotopy in the human brain (Schönwiesner et al., 2002; Formisano et al., 2003; Talavage et al., 2000, 2004). However, an additional finding of fMRI investigations is that neurons situated near the anterolateral extent of the auditory cortex appear to be predominantly selective for low frequencies, whereas neurons situated near the posterior–medial extent of HG appear to respond to both high and low frequencies, with high frequencies dominating (Schönwiesner et al., 2002; Petkov et al., 2004; Talavage et al., 2004, their Fig. 7C). As a consequence of this tuning, fMRI studies have consistently reported a medial–lateral frequency gradient spanning the extent of HG, with high frequencies producing comparatively stronger activations posterior–medially and low frequency stimuli comparatively stronger activations anterolaterally (Wessinger et al., 2001; Engelien et al., 2002; Schönwiesner et al., 2002; Petkov et al., 2004; Talavage et al., 2000). This functional organization may result from several

converging factors including the relative strengths of cortical activations produced by high and low frequency stimulation in different tonotopic zones, contributions arising from activity in belt and parabelt regions which may not be strongly tonotopic, and anatomical regularities that are expressed at the group level despite significant variation among subjects.

Our findings, which were based on neuromagnetic imaging of the 40-Hz SSR known to localize to the region of the auditory core, corroborated these results from anatomical and fMRI studies in the following respects, in our sample of normal hearing subjects. (1) When the medial–lateral coordinate was considered for all subjects, main effects of frequency pointing to a shallow gradient were found before and after normalization of the data, with cortical sources activated by high frequencies residing medial to those activated by low frequencies. Subsequent comparison of the four highest and lowest frequencies reached significance in the normalized and unnormalized data when control subjects were considered alone, but not in the tinnitus group. This medial–lateral frequency organization in normal hearing subjects concurs with the fMRI findings cited above and with results reported for normal hearing subjects in studies by Pantev et al. (1996), Ross et al. (2000), and Weisz et al. (2004a), which assessed tonotopic organization with the magnetically recorded 40-Hz SSR. (2) Main effects of frequency were also found in the anterior–posterior and inferior–superior axes when our normal hearing subjects were analyzed as a group. Evidence for an anterior–posterior gradient corroborates a recent MEG study by Weisz et al. (2004b) whereas evidence for an inferior–superior gradient has not previously been reported by studies using MEG. These gradients reflected high frequency sources, which were posterior and inferior to sources activated by low frequency stimulation in each hemisphere. Frequency effects in the anterior–posterior coordinate are congruent with the anterolateral to posteriomedial orientation of HG in most brains. Evidence for a frequency gradient in the inferior–superior coordinate may reflect a mapping of cortical sources for high frequency stimulation onto the posterior–medial convexity of HG. This region of HG tends to lie inferior to its anterolateral aspect in a significant number of subjects and in some cases is convoluted in a manner which could be consistent with an inferior mapping of high frequency sources in MEG data (Rademacher et al., 2001, Fig. 3). (3) Hemispheric differences in the anatomy of HG and PAC revealed by anatomical studies were also corroborated by the SSR frequency representations we measured with MEG. Frequency representations in the right hemisphere were shifted anterior to those in the left hemisphere (Fig. 3, *y*-coordinate), and laterally in the right hemisphere compared to the left (Fig. 3, *x*-coordinate), in both groups of subjects. When measured across frequencies in normal hearing subjects, the magnitude of these shifts (right hemisphere minus the left) averaged 4.6 mm in the anterior direction and 7.3 mm laterally; these values correspond well with distances for PAC calculated by Rademacher et al. (2001) from postmortem investigations and for HG by Penhune et al. (1996) from MRI images. As far as we are aware, these results from neuromagnetic imaging of the auditory core with the 40-Hz SSR are the first functional data to corroborate hemispheric differences in the location of PAC estimated from anatomical and cytoarchitectonic imaging.

We imaged frequency representations by modeling steady-state field patterns with regional sources that represented centers of cortical activation evoked by the stimulus. Studies that have coregistered SSR sources on individual MRI images (Pantev et al., 1996; Schneider et al., 2002) or used intracortical electrodes to localize auditory middle latency networks that contribute to SSR generation (Celesia, 1976; Godey et al., 2001; Liégeois-Chauvel et al., 1993) point to generators of the SSR residing in the region of Heschl's gyrus where PAC is found. This approach entails some limitations for studies of SSR tonotopy that should be noted. Neurophysiological and fMRI studies cited above suggest that multiple frequency representations are found in the posteriomedial region of Heschl's gyrus, each receiving input of possibly varying densities from parallel thalamocortical pathways. This picture implies that steady-state stimuli should active multiple tonotopic zones, such that SSR representations reflect a blending of discrete cochleotopic activations although activation from one cochleotopic region might dominate the response pattern. A further limitation is that although SSR generators localize to the auditory core region, we cannot discount the possibility of contributions arising from belt regions that may be activated by thalamocortical or cortical-cortical pathways. Gutschalk et al. (1999) described two generators contributing to the SSR in each hemisphere, a strong one localizing to the auditory core in all subjects, and a second comparatively weaker source present in about 50% of their subjects that was situated laterally in the direction of the auditory belt and possibly sensitive to attention. Although we were unable to objectively isolate a second source in our data where unattended trains of 3 min duration were used, SSR frequency maps could be influenced by activity in regions of the auditory belt that are specialized for processing acoustic features associated with different frequency bands (Schönwiesner et al., 2002), provided that activity in these regions generates a spectral signature in the 40-Hz range. In this regard, it may be noteworthy that the frequency representation of the SSR was inflected above 1944 Hz in the left hemisphere but not in the right hemisphere of our normal hearing subjects, and spanned a larger cortical distance in the left than the right hemisphere. The frequency representation on the left, which does not align in any obvious way with functional maps observed for this hemisphere in the fMRI data of Talavage et al. (2004), could be interpreted to suggest (i) distinctive frequency regions biased for processing of low and high frequency sounds in the left hemisphere, (ii) a frequency gradient changing direction on the cortical surface in this hemisphere, or (iii) the dominant pattern of gyral folding in this region notwithstanding anatomical variation that is large (Rademacher et al., 2001). These considerations, plus the high degree of individual variability in our SSR frequency maps, call for caution in equating SSR frequency maps with continuous tonotopic (cochleotopic) gradients. It may be more appropriate to speak instead of the frequency organization of the SSR (a term we have used extensively in this paper) rather than of its tonotopic organization. Our results suggest that there is a frequency organization of the SSR in normal hearing subjects, and that this organization differs between the two hemispheres.

Frequency organization of the SSR in tinnitus

A second major finding of our study is that the frequency organization of the SSR found in normal hearing subjects is altered in tinnitus. In the unnormalized data of Fig. 3 significant main effects or interactions involving frequency were found for each coordinate, which reflected in each instance contrasts of the four highest and lowest frequencies achieving significance in

normal hearing subjects but not in the tinnitus group. When the tonotopic gradients were normalized, main effects of frequency were observed for all three coordinates in which contrasts of the four highest and lowest frequencies were significant for each coordinate for normal hearing subjects but only for the *y*-coordinate in the tinnitus group. When the normalized maps were collapsed across coordinates (Fig. 4A), a difference was found between the hemispheres of control subjects in which the frequency representation reversed around 1944 Hz on the left side compared to the right. In contrast, in tinnitus subjects the frequency representation did not differ between the hemispheres. Instead, a shallow normalized gradient was observed in each hemisphere with no detectable systematic change in source position at frequencies above 864 Hz. These findings point to a disruption of the frequency organization of the auditory core region in tinnitus.

Disturbances of tonotopy associated with tinnitus may reflect neural dynamics that underlie this condition. Altered tonotopy is seen in animal studies in which frequency-selective hearing loss is induced by traumatic noise exposure. Following noise exposure in cats, neurons in the damaged region begin to express the frequency tuning of their less affected neighbors, producing a flattening of the tonotopic gradient at frequencies above about 8 kHz in this species (Irvine et al., 2000). Map reorganization may reflect an unmasking either of lateral connections in the affected frequency region of the cortical place map (Eggermont and Roberts, 2004) or of normally silent thalamocortical projections from nearby unaffected frequency regions of the auditory thalamus after hearing injury (Norena et al., 2002), or both. Similar phenomena occurring in human tinnitus cases would be expected to blur frequency gradients in the region of hearing impairment. In this respect, it is noteworthy that our tinnitus subjects showed elevated auditory thresholds compared to normal hearing subjects when tested in the MEG (Fig. 1A) indicating that some degree of hearing impairment was present. Elevation was seen at all frequencies but was more pronounced at the higher frequencies (Fig. 1B) suggesting presbyacusis (15 of our 28 tinnitus cases were over 50 years of age). Correspondingly, SSR frequency gradients were diminished in our tinnitus subjects compared to normal hearing controls. In Fig. 4, frequency gradients appeared to be flattened in tinnitus at frequencies above 864 Hz giving a shape that might be considered to resemble cortical map reorganization seen in animal studies of hearing loss. Although present in both hemispheres, differences in the SSR frequency representation between the tinnitus and control groups appeared to be more pronounced in the right hemisphere (Fig. 4A). The frequency representation observed in normal hearing subjects was less complex in this hemisphere, which may have aided in setting the group difference into relief.

In addition to map changes, animal models of hearing loss have documented increases in the spontaneous firing rate of neurons in the auditory cortex and dorsal cochlear nucleus occurring after noise trauma, and increases in neural synchrony in the reorganized cortical region (Eggermont and Roberts, 2004). Because activity in the auditory nerve typically diminishes after noise trauma, these phenomena appear to reflect reduced inhibition in cortical and subcortical structures consequent on damage to the cochlea. Changes in spontaneous activity in the primary auditory cortex have been found inside as well as outside of the damaged cortical region, whereas increases in neural synchrony are confined to the damaged cortical area (Norena and Eggermont, 2003) and may thus underlie tinnitus sensations that typically localize to the region of hearing loss in the audiogram (Norena et al., 2002; Roberts et al., in press). It is not known precisely how these response properties of auditory neurons relate to the magnetically recorded SSR. However, because the constraints of intracortical inhibition are reduced in tinnitus, our results showing enhancement of dipole power in the tinnitus group (Fig. 5) may mean that more neurons may have been available for recruitment by the amplitude modulation envelope in this condition. Gerken et al. (2001) similarly found that auditory MLRs (which appear to contribute to the generation of 40-Hz SSRs) are enhanced in tinnitus. In our study, dipole power decreased with carrier frequency in tinnitus and control subjects, in accordance with previously documented properties of the SSR (Ross et al., 2000), but was larger in tinnitus subjects at most frequencies including 576 Hz to 1944 Hz where the two groups were closely matched for sound intensity (SL). SSR phase (Fig. 6A) and phase variability (Fig. 6B) were also affected by carrier frequency in both groups. Effects of frequency on SSR phase are broadly congruent with those documented previously in normal hearing subjects over the range of 250–4000 Hz (Ross et al., 2000) and are believed to reflect at least in part delays in propagation of the travelling wave along the basilar membrane (Rodriguez et al., 1986). Effects of carrier frequency on SSR phase and phase variability appeared to be diminished in tinnitus compared to control subjects, which could arise from altered cochlear function or because a larger population of neurons was activated by the steady-state stimulus at each frequency in the tinnitus group.

The above accounts assign a major role to changes in the tuning properties and functional activity of auditory neurons which occur in the auditory core region consequent on hearing loss associated with tinnitus. However, input to the auditory core from disinhibited auditory belt areas and other brain regions affected in tinnitus (Kaltenbach, 2000) may also have contributed to changes in the SSR observed in the tinnitus group. In particular, neurons in auditory belt regions are targeted by thalamocortical afferents (Hackett et al., 2001) and are interconnected with neurons in the auditory core. In the left hemisphere, neurons in the auditory core possess properties (a high degree of myelination and broad spectral tuning) that appear to be specialized for representing the temporal properties of acoustic stimuli (Zatorre et al., 2002), including amplitude modulation envelopes for speech sounds that may be represented in auditory belt regions. Input from disinhibited representations in belt areas to the temporally specialized auditory core of the left hemisphere induced by our AM stimuli might explain why tonotopic representations were shifted medially in this hemisphere in tinnitus compared to control subjects (Fig. 3a), and why dipole moment was enhanced in the left hemisphere in unilateral left ear tinnitus cases. The latter result (ipsilateral cortical activation in tinnitus) contrasts with contralateral activations of auditory cortex reported by PET studies in which unilateral tinnitus was induced by discrete oral facial movements (Lockwood et al., 1998). Contributions from the auditory belt may have influenced the results of Mühlnickel et al. (1998) who found that deviations from tonotopy correlated with the subjective degree of tinnitus when tonotopic gradients were measured by the N1m-evoked field. The cortical generators of this response are differentiable from those of the SSR and localize to the region of the auditory belt (Pantev et al., 1993; Engelien et

al., 2000; Bosnyak et al., 2004). Our failure to observe relationships between SSR variables and properties of the tinnitus sensation (loudness, pitch, chronicity) may imply that neural activity in the belt areas is important to the perception of tinnitus. Alternatively, our questionnaire assessments may not have been sufficiently detailed to relate behavioral and brain imaging data at the level of individual subjects.

Tinnitus is more likely to be experienced by older than by younger people, and most (if not all) cases are associated with some degree of hearing impairment. The presence of hearing impairment also means that sound pressure levels will differ between tinnitus and normal hearing groups, when sound intensity is adjusted with respect to individual thresholds in order to deliver comparable input to central auditory pathways. In the present study, we presented stimuli at 60 dB above threshold (SL) for each subject, an intensity that is in the middle of the SSR intensity/amplitude characteristic (Ross et al., 2000) and believed to favor tonotopic organization that is best expressed at low to moderate sound intensities (Phillips et al., 1994). Sound pressure levels delivered to the ears were thus on average 10.2 dB higher across frequencies in the tinnitus group compared normal hearing controls, owing to hearing impairment in the former condition. Because tinnitus, hearing loss, sound pressure levels, and age tend to be confounded, it is conceivable that frequency gradients seen in tinnitus subjects do not relate to the presence of tinnitus but to one or more of these associated changes. We therefore assessed the possible contribution of group differences in age, hearing impairment, and sound pressure levels (SPL) to frequency gradients in tinnitus by comparing these gradients between subgroups of better and poorer-hearing tinnitus subjects that were created by applying a median split to their hearing function averaged over all frequencies. The two tinnitus subgroups correspondingly differed in the sound pressure levels they received (mean SPL difference 14.7 dB) and their age (mean difference 19.4 years) as well as in hearing status. Despite these substantial differences in age and SPL, the SSR frequency representations observed in the two subgroups were similar, showing in both cases no consistent differences among frequencies above 864 Hz in either hemisphere (Fig. 4B). We also contrasted frequency gradients between the better-hearing tinnitus subgroup and normal hearing controls. In the former group, hearing impairment was observed only at frequencies above 1296 kHz presented to the left ear, and group differences in SPL at these frequencies were small (3.8 dB). The two groups did not differ significantly in age. Although the two groups thus received stimuli of similar SPL and were of similar age, the group difference in their SSR frequency representations was generally well preserved and similar to that seen when tinnitus subjects as a whole were contrasted with normal hearing controls. Overall, these results suggest that the altered frequency gradients that we observed in tinnitus were associated with the experience of tinnitus and not with age differences between tinnitus subjects and normal hearing controls, or differences in the degree of hearing impairment and associated sound pressure levels within the tinnitus group. The small hearing impairment detected in our better-hearing tinnitus group suggests that low levels of hearing loss not normally considered to be significant in clinical audiometry can set the stage for changes in intracortical inhibition, spontaneous neural firing rates, and neural synchrony that may underlie the sensation of tinnitus (Eggermont and Roberts, 2004).

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