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Evidence for differential modulation of primary and nonprimary auditory cortex by forward masking in tinnitus

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ABSTRACT

It has been proposed that tinnitus is generated by aberrant neural activity that develops among neurons in tonotopic of regions of primary auditory cortex (A1) affected by hearing loss, which is also the frequency region where tinnitus percepts localize (Eggermont and Roberts 2004; Roberts et al., 2010, 2013). These models suggest (1) that differences between tinnitus and control groups of similar age and audiometric function should depend on whether A1 is probed in tinnitus frequency region (TFR) or below it, and (2) that brain responses evoked from A1 should track changes in the tinnitus percept when residual inhibition (RI) is induced by forward masking. We tested these predictions by measuring (128-channel EEG) the sound-evoked 40-Hz auditory steady-state response (ASSR) known to localize tonotopically to neural sources in A1. For comparison the N1 transient response localizing to distributed neural sources in nonprimary cortex (A2) was also studied. When tested under baseline conditions where tinnitus subjects would have heard their tinnitus, ASSR responses were larger in a tinnitus group than in controls when evoked by 500 Hz probes while the reverse was true for tinnitus and control groups tested with 5 kHz probes, confirming frequency-dependent group differences in this measure. On subsequent trials where RI was induced by masking (narrow band noise centered at 5 kHz), ASSR amplitude increased in the tinnitus group probed at 5 kHz but not in the tinnitus group probed at 500 Hz. When collapsed into a single sample tinnitus subjects reporting comparatively greater RI depth and duration showed comparatively larger ASSR increases after masking regardless of probe frequency. Effects of masking on ASSR amplitude in the control groups were completely reversed from those in the tinnitus groups, with no change seen to 5 kHz probes but ASSR increases to 500 Hz probes even though the masking sound contained no energy at 500 Hz (an “off-frequency” masking effect). In contrast to these findings for the ASSR, N1 amplitude was larger in tinnitus than control groups at both probe frequencies under baseline conditions, decreased after masking in all conditions, and did not relate to RI. These results suggest that aberrant neural activity occurring in the TFR of A1 underlies tinnitus and its modulation during RI. They indicate further that while neural changes occur in A2 in tinnitus, these changes do not reflect the tinnitus percept. Models for tinnitus and forward masking are described that integrate these findings within a common framework.

Abbreviations: A1, Primary Auditory Cortex; A2, Nonprimary Auditory Cortex; AM, Amplitude Modulated; ASSR, Auditory Steady-State Response; BPN, Band Pass Noise; CF, Center Frequency; EEG, Electroencephalogram; M, Masking Condition; MEG, Magnetoencephalography; NM, No Masking Condition; N1, N1 Transient Response; RI, Residual Inhibition; TFR, Tinnitus Frequency Region; THQ, Tinnitus Handicap Questionnaire

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1. Introduction

Most cases of persistent tinnitus are associated with hearing loss expressed either in the audiogram or detected by more sensitive measures. When subjects with audiometric hearing loss are asked to rate several sound frequencies for similarity to their tinnitus, similarity judgments typically commence near the edge of normal hearing in the audiogram and increase in proportion with the depth of hearing loss, comprising a tinnitus frequency region (TFR) spanning the hearing impaired region (Noreña et al., 2002; Roberts et al., 2006). Band-pass masking sounds that produce a brief forward suppression of tinnitus (called "residual inhibition" or RI) do so optimally in proportion to the extent to which their center frequencies (CFs) are also in the same frequency region (Roberts et al., 2008; Roberts, 2010). These psychoacoustic findings, which describe tinnitus associated with audiometric notches as well as sloping hearing loss (reviewed by Eggermont and Roberts, 2014), suggest that aberrant neural processes taking place in the hearing loss region of central auditory structures contribute to tinnitus while disrupting these processes with a masker suppresses it. Tinnitus appearing with a clinically normal audiogram (these cases constituting a minority of tinnitus cases) may not represent exceptions to this principle. Electrophysiological (Schaette and McAlpine, 2011; Gu et al., 2012) and psychoacoustic (Hébert et al., 2013) evidence suggests that such cases may involve damage to high threshold auditory nerve fibers (ANFs) not detected by the audiogram. The high-threshold ANFs most vulnerable to damage by noise exposure (Furman et al., 2013) or to deterioration with aging (Sergeyenko et al., 2013) are those with high frequency tuning (Kujawa and Liberman, 2009), which is consistent with the percepts reported in audiometrically normal tinnitus (Roberts et al., 2008; Schaette and McAlpine, 2011). Cochlear factors may also explain why not all individuals with high frequency hearing loss detected by the audiogram develop tinnitus (Tan et al., 2013). High threshold ANFs with high frequency tuning could be better preserved in such individuals, although this question has not been extensively studied.

Neural changes produced by putative tinnitus-inducing noise trauma in animals include (i) increased spontaneous firing of neurons in cortical (Noreña and Eggermont, 2003, 2006) and subcortical (Bauer et al., 2008 Brozoski et al., 2002; Kaltenbach et al., 2004; Mulders and Robertson, 2011; Vogler et al., 2014; Koehler and Shore, 2013a,b; Kalappa et al., 2014) auditory structures; (ii) increased synchronous activity among neurons in tonotopic regions of primary auditory cortex (A1) affected by hearing loss (Noreña and Eggermont, 2003; Seki and Eggermont, 2003; Engineer et al., 2011); (iii) reduced inhibition in the auditory cortex (Yang et al., 2011); (iv) increased gain in deafferented central auditory pathways (Engineer et al., 2011; Kalappa et al., 2014; Stefanescu, in press); and (v) shifts in the tuning preferences of auditory cortical neurons such that sound frequencies near the edge of normal hearing come to be overrepresented in the cortical tonotopic map (Robertson and Irvine, 1989; Rajan et al., 1993; Noreña and Eggermont, 2003). Behavioral and functional imaging studies of human tinnitus sufferers have corroborated increased gain in central pathways (Hébert et al., 2013; Gu et al., 2012; Schaette and McAlpine, 2011), reduced inhibition in the auditory cortex (Diesch et al., 2010b), and cortical map reorganization in A1, the latter at least when hearing loss is present (Wienbruch et al., 2006). Auditory cortical regions known to be sensitive to attention (Paltoglou et al., 2009) also appear to be persistently activated in humans experiencing tinnitus (Lanting et al., 2009; Gu et al., 2010; Roberts et al., 2013), which may explain deficits in the modulation of attention observed in such subjects (Cuny et al., 2004; Paul et al., 2014). Magnetoencephalography (MEG) studies have observed increased slow (<4 Hz; Weisz et al., 2005, 2007; Adjamian et al., 2012) and alpha (8–12 Hz; Weisz et al., 2005, 2007) oscillations in the auditory cortex of tinnitus subjects, as well as increased gamma oscillations (>40 Hz; Weisz et al., 2007) that may reflect changes in synchronous neural network activity associated with tinnitus percepts. Of the numerous neural changes reviewed here, hypersynchrony occurring in the TFR of A1 has been proposed by some models (Eggermont and Roberts, 2004; Roberts et al., 2013; also see Weisz et al., 2007) to be the proximal neural source of tinnitus. Another potential correlate (increased spontaneous firing) has been observed to occur below as well as within the hearing loss region of A1 in animals exposed to noise trauma, while increased synchronous activity is confined largely to the hearing loss region, which is where tinnitus percepts localize in humans.

In contrast to the aforementioned studies which have examined neural changes believed to accompany the experience of tinnitus, the experiment reported in this paper examined neural changes that occur when tinnitus is suppressed during RI. To achieve this aim, we contrasted sound-evoked brain activity between a baseline condition in which tinnitus sufferers experienced their tinnitus with that observed during a brief period of tinnitus suppression (RI) induced by exposure to an appropriate masking sound. Control subjects without tinnitus, matched as closely as possible in age and audiometric function to the tinnitus subjects, were also tested to determine whether the neural changes observed after masking were unique to individuals experiencing tinnitus. Brain activity was probed in tinnitus and in RI by recording the brain response evoked by a 40-Hz amplitude-modulated (AM) sound using either a carrier frequency of 5 kHz (in the TFR of the tinnitus subjects) or 500 Hz (well below this region) with 128-channel electroencephalography (EEG). We extracted from the EEG the 40-Hz auditory steady-state response (ASSR) known to localize to neural sources in A1 (Godey et al., 2001; Bidet-Caulet et al.,. 2007) and the transient N1 response known to localize to distributed sources in the region of the auditory parabelt (called here nonprimary auditory cortex, A2). ASSR sources show a coarse but consistent low-frequency anterolateral, high-frequency posteromedial tonotopic organization (Pantev et al., 1996; Wienbruch et al., 2006; Gander et al., 2010a) that reflects the summation of extracellular field potentials across two cochleotopic maps with strong low-frequency anterolateral and high-frequency posteromedial activations in Heschl's gyrus (Langers et al., 2012). In contrast, N1 sources localize to distributed and cytoarchitectonically heterogeneous regions of A2 (Godey et al., 2001) where tonotopy is lacking or not strongly expressed (Schreiner and Cynader, 1984; Langers et al., 2007; Lütkenhöner et al., 2003). N1 sources appear to integrate sound information over a wide frequency range to form auditory objects and link these objects with inputs from other brain regions in support of adaptive behaviour.

In the present study, these differing properties of ASSR and N1 responses were used to evaluate whether aberrant neural activity occurring specifically in the TFR of A1 underlies the tinnitus percept, as proposed by neural synchrony models of tinnitus (Eggermont and Roberts, 2004; Roberts et al., 2013). If ASSRs are modulated by the presence of neural changes in A1 related to tinnitus, these models predict that differences in the ASSR between tinnitus and control groups under baseline conditions should depend on whether the carrier frequency of the probe stimulus is in the TFR (5 kHz) or below it (500 Hz). Furthermore, changes observed in ASSR responses evoked by 5 kHz probes after forward masking should relate to RI depth and duration in the tinnitus subjects. These results are not expected for N1 owing to the different functional organization of N1 sources outside of the auditory core region. In the following we report experimental findings relating to these hypotheses. Within the limits of our test,

the results confirm that aberrant neural activity occurring in or projecting to the tinnitus (hearing loss) region of A1 is involved in the generation of tinnitus and its modulation during RI.

2. Materials and methods

Excerpts of the present data have been summarized in previous reports (Roberts, 2010; Roberts et al., 2013). The full data set is presented and analysed here in its entirety for the first time.

2.1. Subjects

Two groups of individuals experiencing chronic tinnitus were studied, along with two further groups of control subjects without tinnitus but of an age similar to the tinnitus groups and with similar hearing function. Auditory cortical representations were probed with either a 500 Hz 40-Hz AM sound below the TFR (these groups labeled Tinn/500 Hz and Cont/500 Hz) or with a 5 kHz 40-Hz AM sound in the TFR of the tinnitus subjects (these groups labeled Tinn/5 kHz and Cont/5 kHz), giving four independent groups overall. Subjects with tinnitus were recruited by advertisements in the local newspaper, from the otolaryngology clinic at McMaster University Medical Center, or from our laboratory archive. Eight of the total of 30 tinnitus subjects tested participated in the earlier study of Roberts et al. (2008). Controls were recruited from family and friends of the tinnitus subjects or from the local community. Controls reported no history of tinnitus or ear diseases. Informed consent was obtained in accordance with procedures approved by ethics committees at McMaster University. Subjects were reimbursed for their parking fees and received an honorarium of $50 for EEG measurement. The number of subjects tested in each group and their age and gender are given Table 1. Also reported in Table 1 are the hearing thresholds of each group at 500, 1000, and 5000 Hz, the stimulus levels they received during EEG testing (see below), and, where applicable, the properties of their tinnitus and RI.

Tinnitus subjects completed a structured interview, an audiogram, and a psychoacoustic assessment of their tinnitus in a preliminary session administered one to three weeks prior to the main study. A self-directed, computer based tool (the Tinnitus Tester of Roberts et al., 2008) determined the ear of the tinnitus, its loudness, frequency spectrum, and approximate bandwidth (tonal, ringing, or hissing). Residual inhibition (RI) functions were determined by a similar tool (the RI Tester, Roberts et al., 2008) that assessed the change in tinnitus loudness experienced after listening for 30 s to one of 11 band limited masking sounds differing in center frequency (CF; 500–12000 Hz) and white noise. Subjects rated RI depth on a scale ranging from −5.0 (tinnitus elimination) through zero (no change) to +5 (tinnitus increase); they then pressed a button indicating when tinnitus had recovered, giving a measure of RI duration. Control subjects completed a preliminary session identical to that of tinnitus subjects except for omission of the psychoacoustic assessments of tinnitus. The audiogram was measured from 125 Hz to 16 kHz for all subjects using a GSI-61 clinical audiometer with Telephonics TDH-50P (0.125–8.0 kHz) and Sennheiser HDA 200 (8.0–16 kHz) headphones (pulsed-tone method). The mean audiogram is contrasted between the tinnitus and control groups collapsed over probe condition in Fig. 1a. The mean tinnitus spectrum and RI function determined for the tinnitus subjects (N = 30) are given in Fig. 1b, where a similarity judgement (likeness rating) of ≥40 in the tinnitus spectrum signifies a sound in the TFR (Roberts et al., 2008). The results of Fig. 1b are in agreement with those reported by Roberts et al. (2008) for a larger sample of 59 subjects with bilateral tinnitus. It can be seen that the 5 kHz 40-Hz AM stimulus probed a frequency region of moderate threshold shift where tinnitus frequencies were experienced by the tinnitus subjects while the 500 Hz 40-Hz AM stimulus probed a region where hearing thresholds were normal and sound frequencies did not correspond to the tinnitus percept.

2.2. Auditory stimuli and task

Probe stimuli were 500 ms in duration and were amplitude-modulated with a 40.96 Hz sinusoid (called 40 Hz herein, 100% modulation depth, onset and offset following the modulation wave; see Fig. 2a). The stimuli were delivered in blocks of 12 stimuli with each stimulus in the block separated by an inter-stimulus interval (ISI) of 2.0 s offset to onset (see Fig. 2b). Twenty blocks of probe stimuli were delivered in each of two successive conditions, first a no-masking condition (NM, tinnitus baseline) in which tinnitus subjects would have experienced their tinnitus, and then in a forward masking condition (M) in which subjects were expected to experience a degree of RI. In the M condition (illustrated in Fig. 2b), each block of probes was preceded by a masking sound of 30 s duration. The masker (band-pass filtered noise, CF 5 kHz, bandwidth ±15% @ −10 dB, called a 5 kHz BPN masker herein; see Fig. 2c) was that found by Roberts et al. (2008) to produce an average tinnitus reduction of 24% of scale. In the present study this masker produced an average RI depth of 26.4% of scale with RI depth varying between subjects from a maximum tinnitus suppression of −4.90 (−5.0 denoting tinnitus elimination) to 0.97 (tinnitus increase; see Table 1) in concurrence with the results of Roberts et al. (2008). The first probe stimulus in each block of 12 stimuli commenced 2 s after masker offset. The time interval between maskers was 60 s offset to onset, allowing recovery from tinnitus suppression. The probe stimuli were delivered during the first 30.5 s of this interval, which covered the duration of RI (mean 15.1 s, Table 1) reported by the subjects for the 5 kHz BPN masker during the determination of their RI functions prior to the main experiment. The NM condition was identical to the M condition, except that during the NM condition the masker was switched off. The NM condition was administered first followed by the M condition after a brief pause of about 5 min; this order was adopted to ensure that tinnitus was experienced in the NM condition. Each condition lasted about 35 min giving a recording session of about 70 min exclusive of the time required for application of the electrodes and sound calibration.

Sound stimuli were generated by a digital signal processor (Tucker Davis RP2.1) and presented binaurally via ear inserts (Etymotic Research ER-2). Sound levels were determined by requiring each subject to adjust the perceived loudness of the probe stimuli and the 5 kHz BPN masker to match the perceived loudness of a 1000 Hz pure tone presented at 65 dB above each subject's measured 1000 Hz threshold (65 dB SL). The frequency of 1000 Hz was chosen as the standard for matching, assuming that hearing thresholds would be in the range of normal hearing (<20 dB HL) at this frequency for most subjects. This assumption was met for 54 of our 60 subjects, although the 6 exceptions were tinnitus subjects. Audiometric thresholds at 500 Hz, 1000 Hz, and 5000 Hz, and the sound levels of the probe and masking stimuli presented to each group, are reported in Table 1. It should be noted that the procedure used here for determining the level of the probe stimuli differs from the common practice of adding a fixed sound level (typically 65 dB) to the thresholds measured for the probe stimuli used on a task. The current procedure was adopted to ensure that the sounds would be of approximately equal perceived loudness across our four groups, notwithstanding the use of two different carrier frequencies, the presence of high frequency threshold shifts in the tinnitus and control groups, and the possibility of abnormal loudness growth (hyperacusis) in the tinnitus subjects (Hébert et al., 2013). Effects of small group differences in thresholds and sound levels will be

Table 1
Subject Characteristics and Properties of their Tinnitus and RI.

	Group			
	Tinn/500 Hz	Tinn/5 kHz	Cont/500 Hz	Cont/5 k
Number (male)	16 (12)	14 (8)	15 (9)	15(5)
Mean Age (SD)	53.8 (19.7)	55.3 (15.2)	43.9 (17.9)	54.1 (15.1)
Age range (years)	18–79	29–75	18–71	20–70
Threshold @ 500 Hz dB HL mean (SD)	8.4 (8.9)	12.3 (7.1)	4.3 (6.4)	7.0 (5.3)
Threshold @ 1000 Hz dB HL mean (SD)	12.0 (10.2)	14.4 (10.1)	5.2 (7.1)	5.7 (6.4)
Threshold @ 5000 Hz dB HL mean (SD)	31.5 (19.3)	33.0 (21.5)	19.7 (21.5)	21.3 (20.2)
Probe stimulus level dB SPL mean (SD)	75.4 (4.7)	69.1 (6.5)	70.3 (4.4)	60.8 (7.3)
Probe stimulus level dB SL mean (SD)	67.0 (8.5)	36.0 (22.9)	66.0 (8.0)	39.6 (18.1)
Masker level (dB SPL) mean (SD)	60.9 (5.9)	69.1 (6.2)	57.5 (5.0)	60.6 (6.9)
Tinnitus ear				
Bilateral	14	13		
Left	1	0		
Right	1	1		
Tinnitus duration in years mean (SD)	6.4 (6.5)	15.0 (6.7)		
Tinnitus Loudness Rating Borg Scale mean (SD)	44.2 (23.6)	44.6 (18.7)		
Tinnitus Loudness Match@1 kHz 65 dB SL mean dB (SD)	45.6 (15.8)	38.1 (18.7)		
THQ Score (total) mean (SD)	26.0 (22.0)	26.6 (14.9)		
RI Depth Rating @ 5 kHz (max −5.0) mean (SD)	−1.10 (1.5)	−1.54 (1.9)		
RI Depth Range (poorest to best; max −5.0)	0.53 to −3.90	0.97 to −4.90		
RI Duration @ 5 kHz (sec) mean (SD)	11.8 (14.1)	18.9 (11.2)		

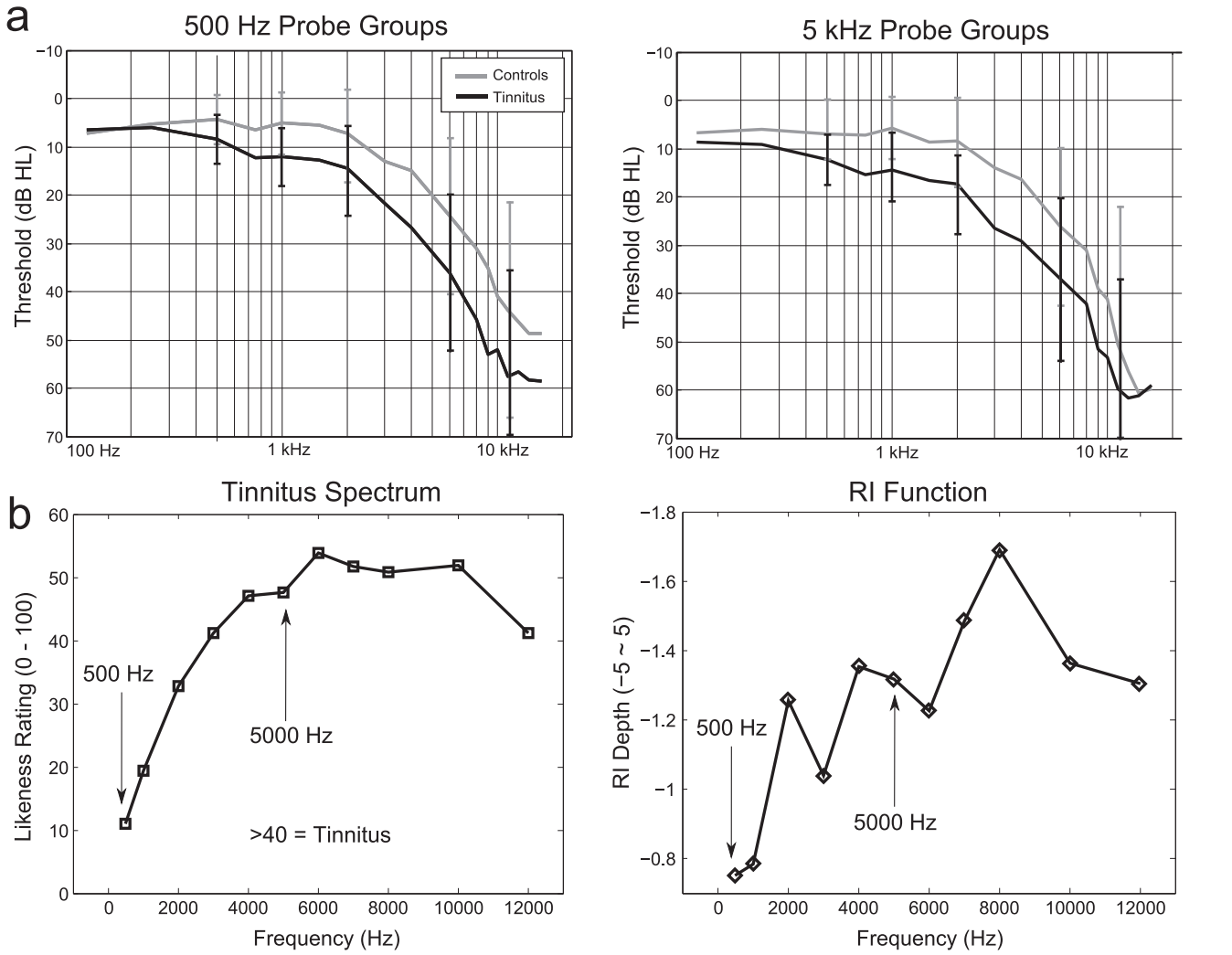

Fig. 1. (a) Audiometric thresholds (left and right ears averaged) for control and tinnitus groups probed at 500 Hz and 5 kHz. Confidence limits (95%) are shown for the audiometric frequencies 500 Hz, 1 kHz, 6 kHz, and 11.2 kHz. (b) Tinnitus spectrum (left panel) and RI function (right panel) for the tinnitus subjects (Tinn/500 Hz and Tinn/5 kHz groups combined). Arrows in the left panel denote 500 Hz and 5 kHz in the tinnitus spectrum measured in the preliminary session. Arrows in the right panel denote RI depth induced BPN maskers with CFs of 500 Hz and 5 kHz during RI testing in the preliminary session. The 5 kHz BPN masker was subsequently used to induce RI in the main study.

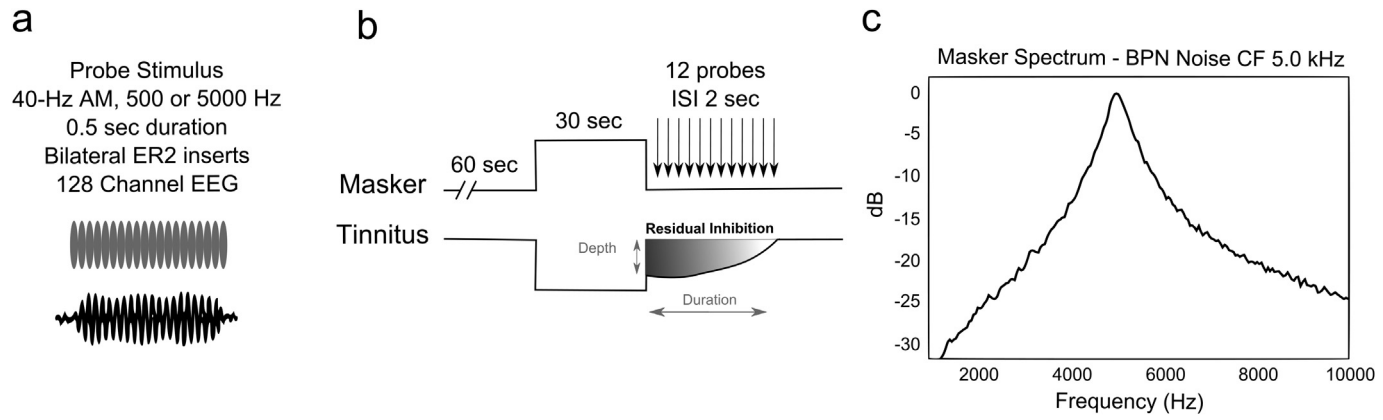

Fig. 2. (a) 40-Hz AM probe stimulus and time domain ASSR waveform. (b) Stimulus procedure for the masking condition. The no-masking condition was identical except the masking sound was switched off. (c) Spectrum of the 5 kHz BPN masker.

evaluated in the results section. It may be noted here that although tinnitus subjects adjusted probe intensity to higher sound pressure levels (mean 72.5 dB SPL) than did controls (mean 65.6 dB SPL, $p < 0.001$), probe intensity calculated with respect to audiometric thresholds measured for each subject (dB SL) did not differ between tinnitus and control groups at either probe frequency or when sound intensity was averaged over the two probe frequencies (52.5 dB SL versus 52.8 dB SL for the tinnitus and control groups respectively, $p = 0.962$). The probe intensities determined by sound level matching remained below the limit of our sound delivery system (90 dB SPL) for all subjects (no ceiling effects were encountered at either probe frequency).

2.3. Electrophysiological recording

The EEG was sampled at 2048 Hz (filtered DC to 417 Hz) using a 128-channel Biosemi ActiveTwo amplifier (Cortech Solutions, Wilmington, NC). The locations of the electrodes in the array were digitized for each participant (Polhemus Fastrak) prior to recording. EEG data were stored as continuous data files referenced to the vertex electrode. EEG responses to probe stimuli (128 channels) were epoched to include 200 ms pre- and post-stimulus baselines.

2.4. Signal processing (unmodeled data)

2.4.1. 40 Hz auditory steady state response

EEG responses for ~90% of trials (rejecting trials with amplitude changes >100 μV, indicative of artifacts) were averaged for analysis of the ASSR, and filtered 35–45 Hz (zero phase) after conversion to average reference. Using MATLAB (Mathworks Inc, Natick MA) the 128-channel data for each participant during the stimulus interval 100–500 ms (this interval covering the ASSR and excluding the transient gamma band response) were collapsed into a two-pulse wide waveform and its scalp topography determined. Grand averages of these two-pulse waveforms and their scalp topography are shown for the 500 Hz and 5 kHz probes separately in Fig. 3a, collapsed over the tinnitus and control groups. These topographies and waveforms are similar to those we have observed previously when probing control and tinnitus subjects with 500 Hz and 5 kHz 40-Hz AM stimuli (Roberts et al., 2012; Paul et al., 2014) and normal hearing subjects with 2 kHz 40-Hz AM stimuli (Gander et al., 2010a, 2010b). Following practices adopted in these previous studies, a Fourier transform was applied to the two-pulse waveforms for each subject. ASSR amplitude and phase were recorded for the 40-Hz component at the Fz electrode where the ASSR typically reached its amplitude maximum (bold trace, Fig. 3a).

We also examined the stability of time-locking between the 40-Hz ASSR response and stimulus waveforms using EEGLab (Delorme and Makeig, 2004). For this purpose, single trials for each subject were filtered 35–45 Hz (zero-phase) over the −50 to 550 ms stimulus epoch. The ASSR recorded at the Fz electrode was then convolved using a Morlet wavelet (7 wave cycles) moving in 1 Hz steps over the frequency band. A phase locking value (PLV; Delorme and Makeig, 2004) was calculated for the 40-Hz component and averaged across the 100–500 ms stimulus interval to depict the variability of 40 Hz phase on each trial for each subject and condition.

2.4.2. Transient responses

EEG responses for ~75% of trials (rejecting trials with amplitude changes >150 μV) were used for analysis of transient responses. The data were averaged and interpolated to the 81-channel "reference free" average reference montage of BESA using each participant's digitized electrode array. Subsequent filtering (0.2–20 Hz, zero phase) using custom routines written in MATLAB extracted the N1 transient response which was recorded as the peak negative amplitude (and corresponding latency) for the window 85–140 ms post-stimulus at electrode Fz where transient responses typically reached their amplitude maxima. The grand averaged scalp topography of N1 and the corresponding time-domain waveforms at each electrode are shown separately for the 500 Hz and 5 kHz probes in Fig. 3b. The transient responses P1 (30–85 ms), P2 (140–230 ms), N2 (250–350 ms) were also measured. P2 amplitude was larger when evoked by 500 Hz compared to 5 kHz probes ($p < 0.0001$) with a similar trend for P1 amplitude ($p = 0.063$), but no further effects were found for P1, P2, or N2. These responses are not considered further herein.

2.5. Signal processing (source space)

For N1, source models were constructed by fitting two symmetrical regional sources (one for each hemisphere) to the grand averaged waveforms (128 channel montage) for each of the eight experimental conditions (tinnitus/control × two carrier frequencies × UM/M), giving a source model for each condition. Residual variance of the eight source models ranged from 0.58% to 2.13% (mean 1.06%). The source models were then used as spatial filters through which the data of each subject in each condition were passed. For each subject the orientation of the regional source whose 3D location was fixed by the group model was recalculated so that one of three vectors accounted for most of the variance. Dipole power associated with this vector was extracted for each subject as a measure of source strength. Residual variance of the

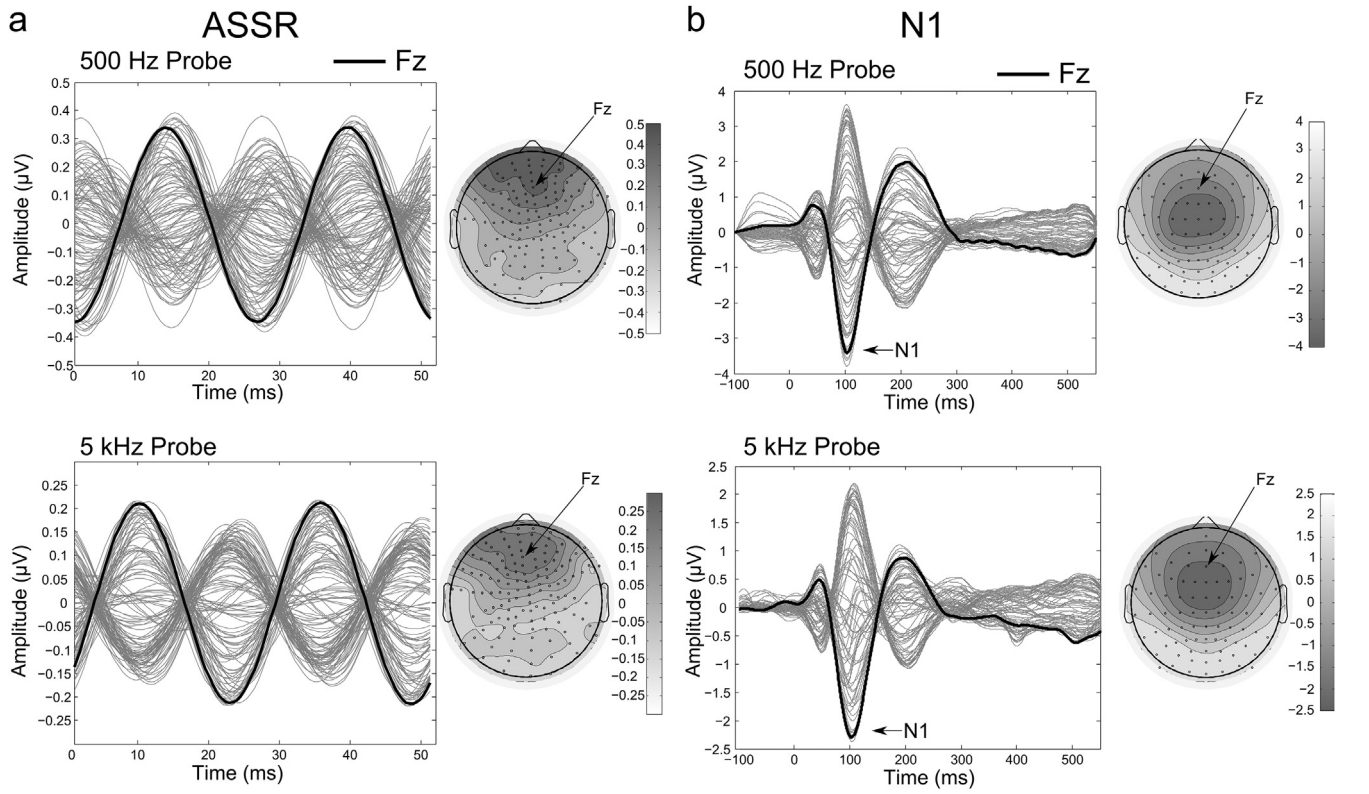

Fig. 3. (a) Grand average scalp topography and time-domain 2-pulse average (128 channel EEG) for the ASSR evoked by 500 Hz probes (top) and 5 kHz probes (bottom). (b) Grand average scalp topography and time domain waveform for the transient response evoked by 500 Hz probes (top) and 5 kHz probes (bottom). Transient responses P1, N1, and P2 can be seen (81 channel reference-free montage of BESA). In panels (a) and (b) the Fz electrode is highlighted in black in the time-domain waveforms.

individually filtered data averaging 10.4% and 10.5% for control and tinnitus subjects, respectively. The results of this analysis agreed closely with that of the unmodeled data and are reported briefly in the results section.

The same approach was applied to model the ASSR waveform, using the group averaged two-pulse waveforms of the 128-channel data for each condition. The residual variance of the eight source models was notably larger than for N1, averaging 9.92% over the four control conditions (UM/M by probe frequency) and 20.1% for the corresponding tinnitus conditions (a difference that was significant, $p = 0.016$). The residual variance of the spatially filtered individual subjects was larger still, averaging 33.0% across the 30 control subjects and 30.0% across the tinnitus subjects, with only 10 subjects in the total sample of 60 subjects returning residual variances <10%. Notwithstanding that the source models only approximated the ASSR waveform, ANOVA applied to the source data revealed larger ASSR amplitude at 500 Hz than 5 kHz ($p = 0.002$) and in the right hemisphere compared to the left hemisphere ($p = 0.016$) consistent with results reported for the ASSR recorded by magnetoencephalography (Ross et al., 2000). Interactions involving hemisphere were not significant, but the three way interaction of group, probe frequency, and masking condition came close ($p = 0.059$). In the results section it will be seen that this interaction appeared at improved levels of significance in the more robust unmodeled data at electrode Fz. Herein we focus on the analyses of the unmodeled data at Fz, where the ASSR typically reached its amplitude maximum (Fig. 3a).

2.6. Statistical analyses

ANOVAS with one within-subject factor (NM/M) and two between-subject factors (tinnitus/control, probe frequency 500 Hz/5 kHz) were conducted using the General Linear Model of Statistica (version 6.0). Significant main effects and interactions were evaluated by Least Significance Difference (LSD) tests or by one-sample t-tests when assessing masking effects with respect to zero. Further details with respect to statistical tests will be reported in the results section. Significance level was set at 0.05 (two-tailed) for all analyses with *p*−values returned by Statistica reported herein.

3. Results

The overall findings for N1 and ASSR amplitude in the eight conditions of the experiment (tinnitus/control, NM/M, 500 Hz/5 kHz) are summarized in Fig. 4. The amplitude of both responses was larger at 500 Hz than 5 kHz in the tinnitus and control groups in the M and NM conditions, yielding significant main effects of probe frequency for N1 amplitude ($F(1, 56) = 40.1$, $p < 0.00001$) and ASSR amplitude ($F(1, 56) = 10.5$, $p < 0.002$). Further inspection of the N1 results presented in the upper panel shows that N1 was larger in the tinnitus groups than the control groups at both probe frequencies before and after masking (main effect of tinnitus/control $F(1, 56) = 5.04$, $p = 0.029$; interaction of probe frequency and group $p = 0.781$). Masking reduced N1 amplitude by a similar amount in all groups (main effect of masking $F(1, 56) = 56.0$, $p < 0.00001$), with no group differences in the magnitude of this effect (three-way interaction $p = 0.493$).

Effects of group (tinnitus/control) and masking (NM/M) on ASSR amplitude were more complex. Before masking where subjects would have heard their tinnitus (baseline), larger ASSRs were observed in the tinnitus group compared to controls when probed at 500 Hz, but the reverse was observed when the groups were probed at 5 kHz. This result can be seen by comparing the lower left ASSR results in each panel of Fig. 4 without masking at each probe

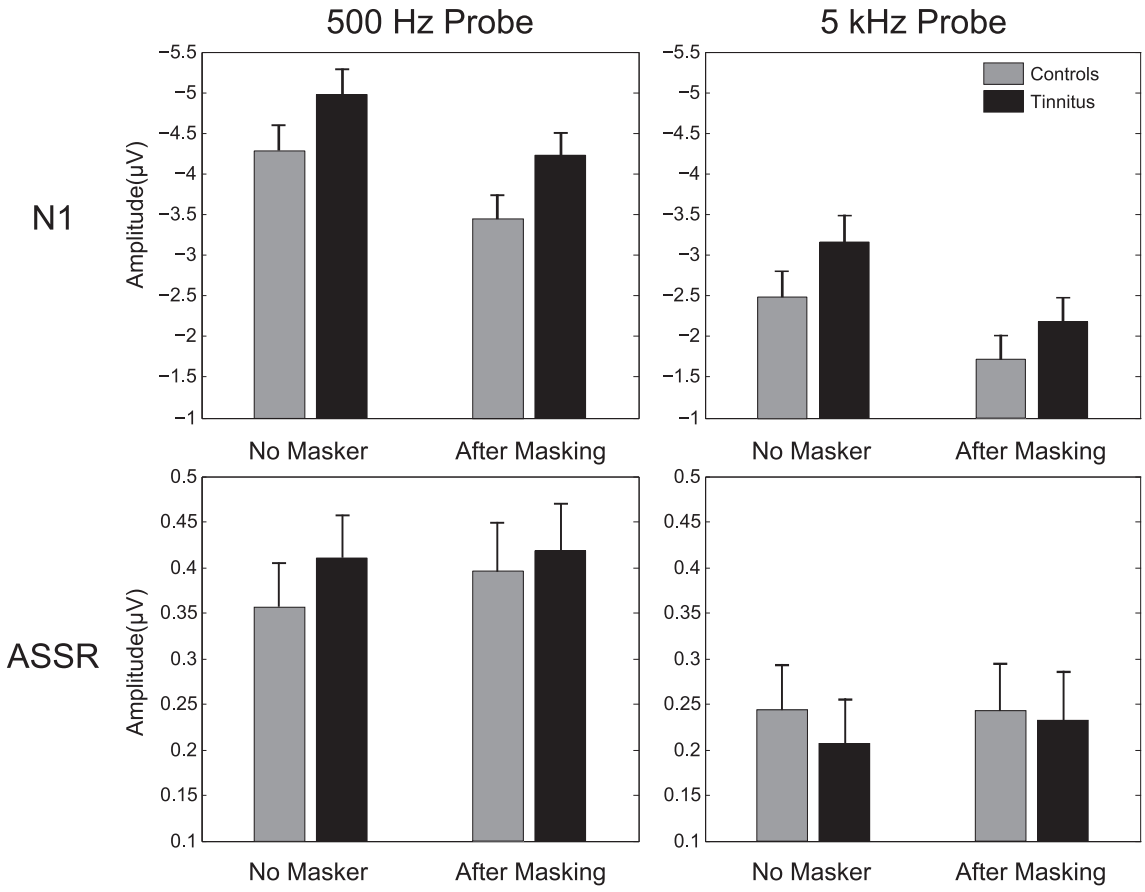

Fig. 4. Group averaged N1 and ASSR amplitude before and after masking in control (grey) and tinnitus (black) groups tested at 500 Hz and 5 kHz. Error bars are 1 between-subject standard error.

frequency. Overall, masking increased ASSR amplitude (main effect of masking $F(1, 56) = 6.62$, $p = 0.023$). However, further inspection of Fig. 4 shows that masking increased ASSR amplitude to 5 kHz probes in the tinnitus group (compare the black bars, lower right panel) and to 500 Hz probes in controls (compare the grey bars, lower left panel), but had no effect in the two remaining conditions. This pattern of within subject-changes proved to be significant (see later) and gave rise to a significant interaction of group, probe, and masking, $F(1, 56) = 4.65$, $p = 0.035$. As a consequence of this interaction, group differences in ASSR amplitude disappeared after masking (compare the black and grey bars after masking in Fig. 4, at each carrier frequency). We evaluate these results including the interaction in greater detail in the following section. It is convenient to consider the effects of masking on N1 and ASSR amplitude first.

3.1. Effects of masking

The differential effect of masking on N1 and ASSR responses is portrayed in Fig. 5a for the group averaged data and in Fig. 5b for individual subjects in each group. In this figure response amplitude after masking (where tinnitus subjects experienced a degree of RI) has been subtracted from that before masking (where tinnitus subjects would have experienced their tinnitus). As shown in Fig. 5a (upper panel), masking reduced N1 amplitude ($p < 0.00001$) in all conditions, with no difference in the magnitude of the masking effect between groups or frequencies (as reported above). Fig. 5b shows that this result was highly consistent across individual subjects in each group. When the four groups were evaluated singly the masking effect differed from zero in all cases (minimum $t(14) = 2.37$, $p < 0.033$, Cont/5 kHz group). Hence, as shown earlier in Fig. 4, the difference in N1 amplitude between the tinnitus and control groups was fully expressed before as well as after masking.

In contrast, the effect of masking on ASSR amplitude (shown in the lower row of Fig. 5) differed depending on group (tinnitus/control) and probe frequency. When masking effects were assessed with respect to zero, ASSR amplitude increased after masking in the Tinn/5 kHz group ($t(14) = 3.28$, $p < 0.006$) and in the Cont/500 Hz group ($t(14) = 2.92$, $p = 0.011$), but had no effect in the two remaining conditions, yielding the group by frequency interaction reported above for the results of Fig. 4 ($p = 0.035$). Inspection of the individual data shown in Fig. 5b for the ASSR shows that the direction of masking effects was balanced in the Tinn/500 Hz and Cont/5 kHz groups whereas in the Tinn/5 kHz and Cont/500 Hz groups there was a strong bias toward ASSR increases. As a consequence of this differential masking effect, group differences in ASSR amplitude that were observed prior to masking in Fig. 4 (tinnitus versus control) were abolished at both frequencies after masking (Fs < 1 for effects involving group after masking).

It is instructive to consider the sources of variability contributing to ASSR amplitude after masking, where a contribution of tinnitus was no longer detected. One contributor is the effect of carrier frequency described previously; subjects probed at 500 Hz expressed larger ASSRs than subjects probed at 5 kHz ($p < 0.002$). A second source of variability consisted of large individual differences in ASSR amplitude. These differences are shown in Fig. 6a where ASSR amplitude is correlated across the NM and M conditions separately for the 500 Hz and 5 kHz groups (tinnitus and control groups combined). At 500 Hz the ratio of the largest to the smallest

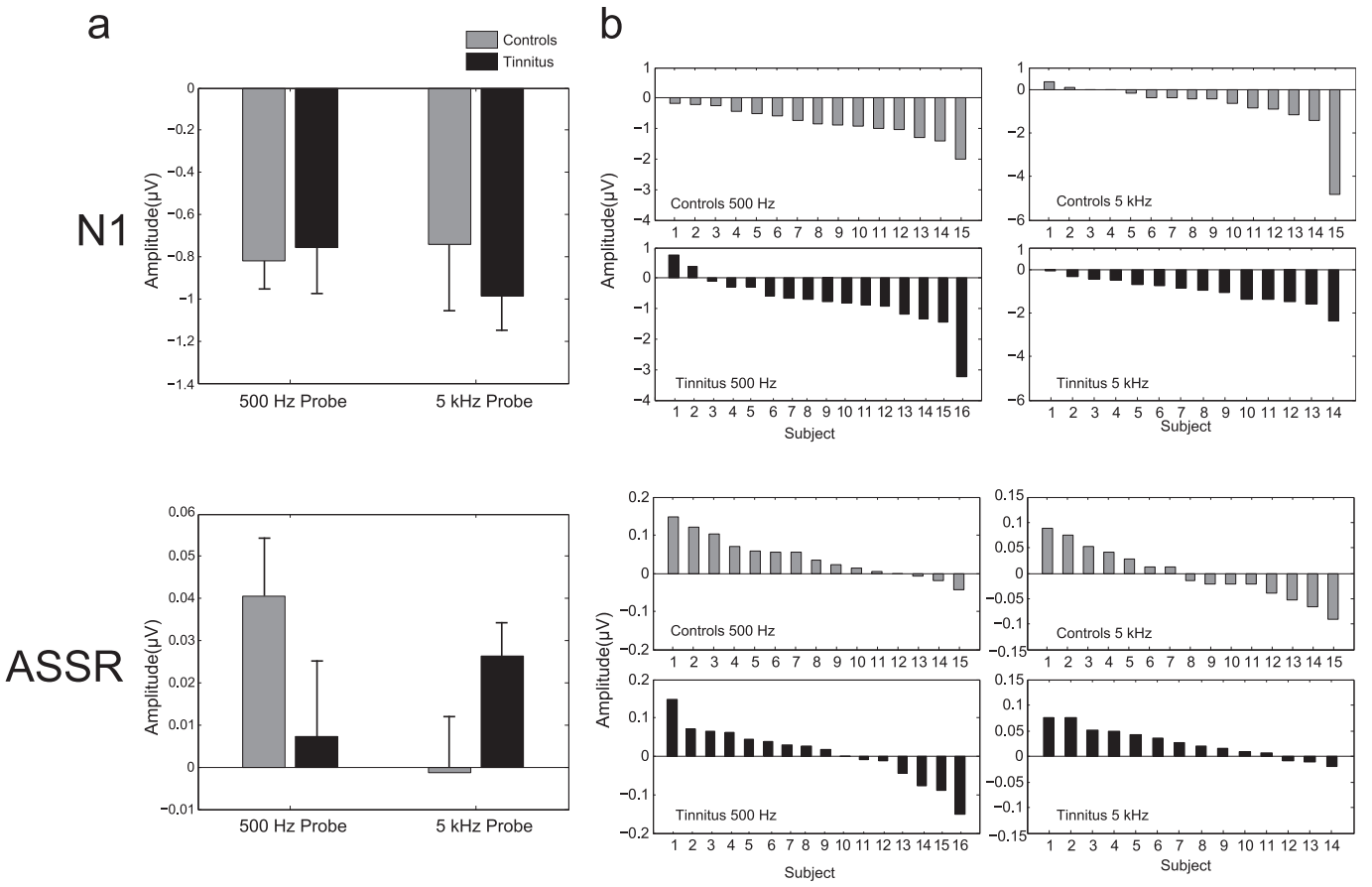

Fig. 5. Masking effects (response amplitude after masking minus amplitude before masking) are shown for each group (N1 upper row, ASSR lower row). (a) Group averaged masking effects on N1 amplitude (upper panel) and ASSR amplitude (lower row). Error bars are 1 within-subject standard error. (b) Masking effects of individual subjects. For N1 (upper row) almost every subject showed a decrease in N1 amplitude after masking. ASSR masking effects (lower row) were distributed bidirectionally among subjects in groups Cont/5 kHz and Tinn/500 Hz, whereas in groups Cont/500HZ and Tinn/5 kHz most subjects showed ASSR increases.

ASSR amplitude was 15.08, revealing large individual differences in this measure (Fig. 6a). However, the between-subject correlation across masking conditions was $r = 0.961$ ($p < 0.000$) indicating that these differences were stable. The results at 5 kHz were similar (ratio 21.53, $r = 0.963$ $p < 0.00001$). Fig. 6b presents typical results from an independent study (Roberts et al., 2012) where subjects were probed at 5 kHz in two EEG sessions separated by about 6 days. These data returned a ratio 18.9 and $r = 0.90$ ($p < 0.0001$), indicating that individual differences in ASSR amplitude while large are stable across days and reapplication of the recording sensors. Individual differences in ASSR amplitude likely reflect the summation of electrical fields generated by ASSR sources of idiosyncratic orientation across tonotopic maps sharing a common low frequency border situated laterally in Heschl's gyrus (Kaas and Hackett, 2000; Langers et al., 2007; Wienbruch et al., 2006). In the current study the maximum difference attributable to individual variability in ASSR generators at 500 Hz (0.837 μV) was 13.3 times greater than the contribution arising from the presence tinnitus at this frequency (0.063 μV; the corresponding ratio at 5 kHz was 17.3). These results underscore the challenge of detecting between-group differences in ASSR amplitude attributable to the presence of tinnitus against a background of variability arising from idiosyncratic anatomical factors.

Two approaches were adopted to reduce the contribution of individual differences to ASSR amplitude measured prior to masking (baseline), where individuals with tinnitus would have heard their tinnitus. Both approaches capitalized on the result that effects attributable to the presence or absence of tinnitus were not present in ASSR amplitude after masking, while individual differences in ASSR amplitude were fully expressed in this condition. In the first analysis, ASSR amplitude after masking was included as a covariate in a separate analysis of ASSR amplitude in the NM (baseline) condition. The results are shown in Fig. 7. Effects of carrier frequency and of individual differences in ASSR amplitude are removed in this analysis, since both sources were present in the covariate (this is why the means are called "adjusted means" in the figure, a determination made by Statistica 6.0). The results of Fig. 7 corroborate those of Fig. 4 but with greater statistical power. Main effects of carrier frequency ($p = 0.791$) and tinnitus ($p = 0.574$) were not significant, but the interaction of these variables was, $F(1,55) = 6.25$, $p = 0.015$. LSD contrasts revealed larger ASSR amplitude in tinnitus than in controls at 500 Hz ($p = 0.004$) and the reverse at 5 kHz ($p = 0.045$), such that both effects contributed to the significant interaction. The second method for reducing the contribution of source variability to ASSR amplitude represented ASSR amplitude prior to masking as a proportion of ASSR amplitude after masking. The results were similar, yielding an interaction of carrier frequency and tinnitus of $F(1, 56) = 8.77$, $p < 0.004$ and LSD contrasts comparable to those reported above.

We conducted a similar analysis of between subject variability in N1 amplitude. Test-retest correlations for N1 amplitude corresponding to Fig. 5a–c were $r = 0.880$, 0.595, and 0.598 (all p's < 0.05) and largest to smallest ratios 2.39, 6.62, and 4.50, respectively. Thus, although N1 amplitude showed stable

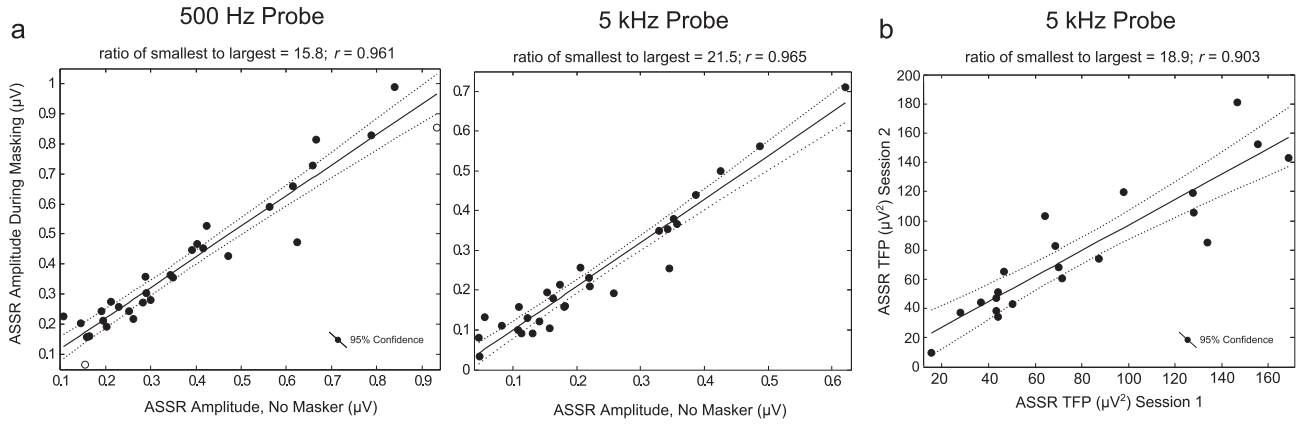

Fig. 6. (a) Within-session correlations between ASSR amplitude in the no-masking and masking conditions for 500 Hz probes and 5 kHz probes. (b) Correlation between ASSR amplitude in two sessions separated by about 4 days (data from Roberts et al., 2012). ASSR amplitude is measured as total field power (TFP) at 40 Hz.

individual differences, these differences were less repeatable and not as extreme as those observed for ASSR amplitude. However, as reported above, main effects of tinnitus ($p = 0.042$) and carrier frequency ($p < 0.00001$) on N1 amplitude were fully preserved after masking (see Fig. 4). This meant that using N1 amplitude after masking as a covariate removed contributions arising from both factors to N1 amplitude prior to masking, leaving no significant effects of group or probe frequency on N1 amplitude prior to masking. The same limitation applied to representing N1 amplitude prior to masking as a proportion of N1 amplitude after masking; analysis of this ratio found no effects attributable to probe frequency or the tinnitus/control condition. However, when N1 amplitude in the no-masking condition was analyzed without a covariate or without representation by a ratio, main effects were found for condition (tinnitus/control, $F(1, 56) = 7.25$, $p = 0.038$) and probe frequency ($F(1, 56) = 30.3$, $p < 0.00001$) with no interaction between the variables ($F < 1$; see Fig. 4). Source analyses of N1 amplitude yielded highly similar results at comparable levels of significance, concurring with the analyses of the unmodeled data.

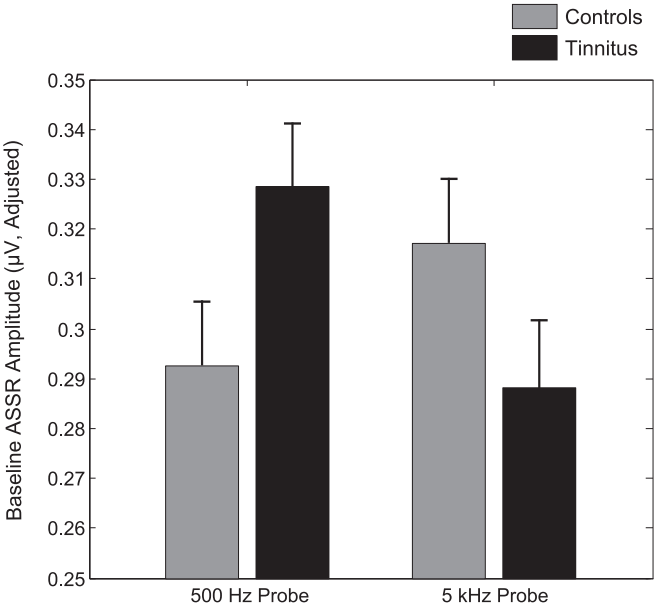

Fig. 7. ASSR amplitude during the no-masking (baseline) condition in tinnitus and control subjects probed at 500 Hz and 5 kHz. Effects of probe frequency and individual differences in anatomical ASSR generators are removed by using ASSR amplitude after masking as a covariate. Error bars are 1 between-subject standard error.

3.2. ASSR phase locking value

When averaged over trials ASSR amplitude reflects the number neurons phase locking to the AM stimulus on each trial (more neurons giving a larger response amplitude) as well as the stability with which the ASSR waveform time-locked to the stimulus over trials. To estimate the stability of time locking, we calculated Phase Locking Value (PLV; Delorme and Makeig, 2004) for the M and NM conditions for each subject. Like ASSR amplitude PLV was a stable individual trait when correlated between the NM and M conditions ($r = 0.862$ and 0.951 for 500 Hz and 5 kHz respectively, $p < 0.0001$ in each case). PLV was also higher at 500 Hz than 5 kHz ($F(1,56) = 6.85$, $p < 0.011$) as was ASSR amplitude, and correlated with ASSR amplitude between subjects within each group giving $r = 0.670$ ($p < 0.0001$) for the combined sample. The latter result suggests that while PLV contributed to ASSR amplitude explaining up to 44.9% of its variance (coefficient of determination r^2), ASSR amplitude reflected more than this factor. Other findings are consistent with this interpretation. The correlation between ASSR amplitude in the NM and M conditions remained significant at both probe frequencies when PLV was partialed out ($r_p = 0.944$ and 0.887 at 500 Hz and 5 kHz respectively, UM PLV used as the covariate). It will be recalled that masking increased ASSR amplitude at 5 kHz in tinnitus subjects and at 500 Hz in controls (Fig. 5); in contrast, masking had no significant effect on PLV in any group. In addition, in the NM condition where subjects would have heard their tinnitus, ASSR amplitude in tinnitus was larger than in controls at 500 Hz but smaller than in controls at 5 kHz, particularly when individual differences arising from idiosyncratic ASSR generators were removed by covariate analysis (Fig. 7). Parallel analyses of PLV reveal no significant differences between tinnitus and control groups at either frequency. These results suggest that the frequency specific-effects shown on ASSR amplitude in Figs. 5 and 7 primarily reflected changes in the number of neurons phase locking to the AM envelope in the various conditions rather than changes in the stability of phase locking over trials.

3.3. Effects of age, hearing loss, and sound level on brain responses

Three analyses were conducted involving the variables age, audiometric hearing loss, and probe sound level, and their relationship to brain responses, in the tinnitus and control groups. The first analysis assessed differences in age, audiometric hearing loss, and probe sound level between tinnitus and control groups tested at each carrier frequency. Although the mean age of subjects in the control/500 group (43.9 years) was 10.5 years younger than the

three remaining groups (mean 54.4 years, see Table 1), the range of ages was similar across the groups, and differences in mean age were not significant for any group contrast (overall main effect of age $p = 0.24$). Audiometric thresholds were elevated commencing above 2 kHz in all groups although somewhat more so in the tinnitus groups compared to controls (Fig. 1a), yielding a main effect of frequency ($F(14,784) = 99.6$, $p < 0.00001$) and of group (tinnitus/control $F(1,56) = 5.24$, $p = 0.026$) and no other effects. Thresholds were higher in tinnitus subjects than controls at the frequency used for sound level matching (1 kHz, mean group difference 7.8 dB, $p < 0.0009$) as well as the two probe frequencies of 500 Hz (group difference 4.7 dB, $p < 0.013$) and 5 kHz (group difference 11.8 dB, $p < 0.031$). The group difference in 1 kHz thresholds may have contributed to the observation seen in Table 1 that probe intensity was adjusted to somewhat higher absolute sound pressure levels by tinnitus subjects (72.3 dB SPL) than by controls (65.6 dB SPL; group difference 6.7 dB, $p < 0.0005$). However, when sound intensity was calculated as dB SL which took into account threshold shifts for individual subjects, a different picture emerged. Probe frequency had a large effect on sound level represented by this metric ($F(1, 56) = 51.4$, $p < 0.0001$); subjects receiving the 5 kHz 40-Hz AM probe matched their probe to the 1 kHz standard sound at a lower sensation level (37.8 dB SL) than did subjects receiving the 500 Hz 40-Hz AM sound (66.5 dB SL), indicating that the former stimulus was perceptually more salient at a constant SPL. Probe intensity determined by sound level matching and calculated as dB SL was near identical between the tinnitus and control groups at each frequency, with neither the main effect of group nor the interaction with frequency approaching significance ($Fs < 1$; see Table 1). These results suggest that the matching procedure equated the perceived loudness of the probe stimuli across the four conditions, including between tinnitus and control groups at each probe frequency.

Because the tinnitus and control groups were closely but not perfectly matched for age, audiometric thresholds, and probe intensity (dB SL and SPL), a second analysis examined the relationship of these variables to ASSR and N1 amplitude in the no-masking (baseline) condition. Because probe frequency had a large effect on the amplitude of the brain responses, correlations with the brain responses were calculated (i) for each of the four groups separately, (ii) when the tinnitus and control groups were collapsed to give a larger sample tested at each probe frequency, and then (iii) for the combined sample of 60 subjects. Age and probe SL or SPL did not correlate significantly with baseline ASSR amplitude within any of the above mentioned groupings. The same was true for baseline N1 amplitude, with the exception that the amplitude of this response (a negative-going ERP) increased with probe SL ($r = -0.517$, $p = 0.000$) and SPL ($r = -0.382$, $p = 0.003$) when calculated for all groups combined. Audiometric thresholds (tested separately at 500 Hz, 1 kHz, 5 kHz, and averaged 4–11.2 kHz) did not correlate with baseline N1 amplitude within any of the four groups tested separately. The same was true of ASSR amplitude with one exception, which was that baseline ASSR amplitude was larger when thresholds at 1 kHz were elevated in the Tinn/500 Hz group ($r = 0.676$, $p = 0.004$) and when all subjects tested at 500 Hz were combined ($r = 0.531$, $p = 0.002$). No threshold measure correlated with baseline ASSR or N1 amplitude for subjects tested at 5 kHz. To evaluate further whether age, audiometric thresholds, and probe level may have contributed to the group differences in baseline ASSR and N1 amplitude reported above, the ANOVAs conducted previously for these responses were repeated adding age, thresholds, and probe levels (dB SL and dB SPL) as covariates. The interaction of condition (tinnitus/control) and probe frequency (500 Hz/5 kHz) reported in Fig. 7 for baseline ASSR amplitude was fully preserved in this analysis, which returned a condition by probe frequency interaction of $F(1,51) = 6.78$, $p = 0.011$. The results for N1 amplitude were similar. ANOVA including age and probe levels as covariates returned main effects for condition (tinnitus/control) of $F(1,51) = 4.24$, $p = 0.044$ and for probe frequency ($F(1,51) = 6.43$, $p = 0.014$) with no interaction between the variables. These results did not change when audiometric thresholds (500 Hz, 1 kHz, 5 kHz, 4–11.2 kHz) were used as covariates.

The loudness of the 5 kHz BPN masker was also adjusted by the subjects using the same standard sound (1 kHz 65 dB SL) used for determining probe level. In Table 1 it can be seen that masker intensity was adjusted to a somewhat lower SPL in the 500 Hz than the 5 kHz conditions (difference = 5.70 dB SPL, main effect of frequency $p < 0.0005$) and to a somewhat higher SPL in the tinnitus groups compared to controls (difference 6.0 dB SPL, main effect of tinnitus/control $p < 0.0003$; the interaction of the factors was not significant in ANOVA). We therefore examined the relationship of masker intensity (measured as dB SPL and dB SL) to ASSR and N1 amplitude measured after masking as well as to the effects of masking on these responses (masking minus baseline). No correlations reached significance (range $r = -0.110$ to $r = 0.179$, all p's > 0.17).

Taken together, the analyses of this section indicate that effects of tinnitus/control and probe frequency on baseline ASSR and N1 responses, and effects of masking on these responses, could not be attributed to variations in probe or masker level which in the current procedure were of comparable perceived loudness across the groups.

3.4. Relationship of brain responses to properties of tinnitus

The first of several analyses in this group looked at the relation of baseline ASSR and N1 responses to several attributes of tinnitus. The results are reported in Table 2 for the Tinn/500 Hz and Tinn/5 kHz groups separately and for the combined sample. Tinnitus loudness determined by loudness matching correlated with ASSR amplitude in the tinnitus/500 Hz group ($r = 0.571$) and in the combined sample ($r = 0.369$), associating louder tinnitus with larger ASSR responses. No other attribute of tinnitus correlated with ASSR amplitude evoked by probes of either frequency. N1 amplitude did not correlate significantly with any measure of tinnitus loudness. In the Tinn/500 Hz group larger N1 responses were associated with increasing years of tinnitus ($r = -0.662$) and with tinnitus of wide bandwidth ($r = 0.519$, see below for definition of bandwidth), but these relationships were not consistent in the Tinn/5 kHz group and did not hold for the combined sample.

A second analysis examined the relationship of effects of masking on ASSR and N1 responses to properties of RI. The results are reported in Table 3 for the Tinn/500 Hz and Tinn/5 kHz groups separately and for the combined sample. In the Tinn/500 Hz group larger ASSR masking effects were associated with greater RI duration ($r = 0.516$, $p < 0.05$). This relation persisted in the combined tinnitus sample ($r = 0.423$, $p = 0.02$), probably because an RI of long duration covered more of the interval during which the 12 probes were delivered than did a brief RI. Similarly, RI depth tended to be associated with larger ASSR masking effects in the two tinnitus groups, with this relationship approaching significance in the combined sample ($r = -0.298$, $p = 0.110$). N1 masking effects in the Tinn/5 kHz group showed the opposite relation to RI, these masking effects being larger when RI depth was poorer ($r = 0.539$, $p < 0.05$) and RI duration shorter ($r = -0.526$, $p < 0.053$) in this group. However this relation did not hold for N1 masking effects in the Tinn/500 Hz group or in the total sample. These results suggest that masking effects on ASSR amplitude were more consistently related to RI than were masking effects on N1 amplitude. The results were analyzed further, as follows.

Table 2
Relationship of baseline ASSR and N1 amplitude to properties of tinnitus.

	Baseline ASSR amplitude	Baseline N1 amplitude
Group Tinn/500 Hz		
Years of tinnitus	0.297	−0.662**
Tinnitus bandwidth	−0.076	0.519*
THQ (total score)	0.151	0.445
Borg CR 100 loudness	0.433	0.278
Loudness match 1 kHz	0.571*	0.168
Group Tinn/5 kHz		
Years of tinnitus	−0.512	0.549*
Tinnitus bandwidth	0.367	−0.312
THQ (total score)	−0.403	−0.019
Borg CR100 loudness	−0.022	−0.041
Loudness match 1 kHz	−0.108	−0.495
All Tinnitus Subjects		
Years of tinnitus	−0.223	0.211
Tinnitus bandwidth	−0.026	0.243
THQ (total score)	0.011	0.252
Borg CR100 loudness	0.268	0.140
Loudness match 1 kHz	0.369*	−0.226

**P < 0.01.
*P < 0.05.

Reports of RI depth and RI duration proffered by the subjects during RI testing were intended to assess different attributes of the same percept (forward suppression of tinnitus after masking). Consistent with this assumption, RI depth and RI duration were correlated with one another in the Tinn/500 Hz group ($r = -0.789$, $p < 0.0001$) and the Tinn/5 kHz group ($r = -0.469$, $p = 0.091$) as well as in the combined sample ($r = -0.632$, $p < 0.0001$) revealing deeper RI associated with longer RI duration. Consequently, in a third analysis we utilized this relationship to identify two clusters of subjects in the combined sample of tinnitus subjects, one cluster displaying good RI and the other cluster poor RI, respectively, in RI depth and RI duration (see Fig. 8a). All subjects in cluster 1 (n = 6) reported RI depth exceeding −3.0 (a reduction of 60% of scale) with some subjects reporting near tinnitus elimination (RI rating of −5.0); all of these subjects reported RI duration exceeding 15 s and some notably longer. In contrast, all subjects in cluster 2 reported RI depth of −1.0 (20% of scale) or less, with some subjects reporting tinnitus increases; all but two of these subjects reported brief RI durations <15 s. ASSR and N1 amplitude in the baseline condition (NM) and after masking (M) are shown for both clusters in Fig. 8b. ANOVA of ASSR amplitude returned a significant interaction of cluster and masking condition ($F(1, 20) = 4.57$, $p = 0.044$) and no other effects, indicating that ASSR amplitude increased after masking in the cluster displaying good RI ($t = 5.07$, $p = 0.003$) but not in the cluster reporting poor RI ($t = -0.517$, $p = 0.613$). These results indicate that ASSR increases observed after masking were not induced solely by the presence of the masker but depended on the extent to which RI was experienced; the experience of the masker alone was not sufficient to increase ASSR amplitude. ANOVA of N1 amplitude gave a main effect attributable to masking ($F(1, 20) = 19.11$, $p = 0.0002$) but no other effects, indicating that unlike ASSR masking effects, N1 masking effects did not depend on the experience of RI. The results reported here were robust with regard to how cluster 2 was defined; for example, defining poor RI as all subjects reporting a duration <10 s or <15 s, or deleting the two subjects who can be seen in Fig. 8a to have reported poor RI depth but duration >15 s, did not alter either outcome.[2] However, it should be noted that subjects in cluster 1 were divided equally between the Tinn/500 Hz and Tinn/5 kHz groups, with three subjects in each group showing ASSR increases after masking. Thus, while at the group level masking with a 5 kHz BPN sound increased ASSR amplitude to a 5 kHz probe but not ASSR amplitude evoked by a 500 Hz probe (Fig. 5), strong RI expressed by individual subjects was accompanied by increased ASSR amplitude at both carrier frequencies. Because we could not (within the limits of our procedures) test the same subjects within sessions at both frequencies, we cannot say whether subjects with deep ASSR masking effects at 5 kHz would have shown large effects at 500 Hz as well (and vice versa). However, the current findings support this possibility.

Table 3
Relationship of ASSR and N1 masking effects to residual inhibition duration and depth.

	ASSR masking effect	N1 masking effect
Group Tinn/500 Hz		
RI Duration	0.516**	−0.059
RI Depth	−0.362	−0.379
Group Tinn/5 kHz		
RI Duration	0.079	−0.526*
RI Depth	−0.223	0.539**
All Tinnitus Subjects		
RI Duration	0.423**	−0.163
RI Depth	−0.298†	−0.007

**P < 0.05.
*P < 0.06.
†p ≤ 0.11.

A final analysis looked at how ASSR and N1 masking effects related to auditory thresholds, and whether masking effects on the responses related to features of the tinnitus spectrum. Effects of masking on the responses did not correlate with auditory thresholds at 500 Hz, 1 kHz, 5 kHz, or thresholds averaged between 4 kHz and 11.2 kHz, in the control and tinnitus group separately or in the combined sample. ASSR masking effects increased with the peak frequency reported by the subject in their tinnitus spectrum in the Tinn/5 kHz group ($r = 0.564$, $p = 0.036$). Although the peak of the tinnitus spectrum is known to shift toward higher frequencies when audiometric thresholds are comparatively better (Roberts et al., 2008; also see below), the relationship of ASSR masking effects to the spectrum peak was unaltered in the Tinn/5 kHz group when mean thresholds between 4 kHz and 11.2 kHz were partialed out ($r = 0.58$, $p = 0.037$). In contrast, ASSR masking effects did not correlate with the peak of the tinnitus spectrum in the Tinn/500 Hz group nor did N1 masking effects correlate with the tinnitus spectrum in any tinnitus group or in the combined tinnitus sample.

3.5. Relationships among age, tinnitus bandwidth, RI depth and duration, and the tinnitus spectrum

These analyses examined relationships among age and several properties of tinnitus, collapsing all tinnitus subjects into a single group. In the preliminary session tinnitus bandwidth (tonal, ringing, and hissing tinnitus, coded as 3, 2, and 1, respectively) was determined by the Tinnitus Tester software of Roberts et al. (2008) which asked subjects to choose one of three 5 kHz sounds with bandwidths (−10 dB, Fig. 2c) of 0%, ±5% and ±15%, respectively, to

[2] Two reviewers asked whether the ASSR masking effect related to RI depth when only those ASSR responses that occurred during the time interval of RI were analysed. The results while in the expected direction were not significant (maximum r = −0.436, p = 0.388, for subjects in Cluster 1 showing the deepest RI). This analysis suffered from a poor signal-to-noise ratio for ASSR responses near the noise level when RI was brief (few probes were available for study), and, unlike the analysis of Fig. 8, eliminated subjects reporting no RI. Fig. 8 which included all the subjects and data showed that the presence of the masker alone was not sufficient to deliver a masking effect on ASSR amplitude, but that the experience of some degree of RI depth and duration was needed.

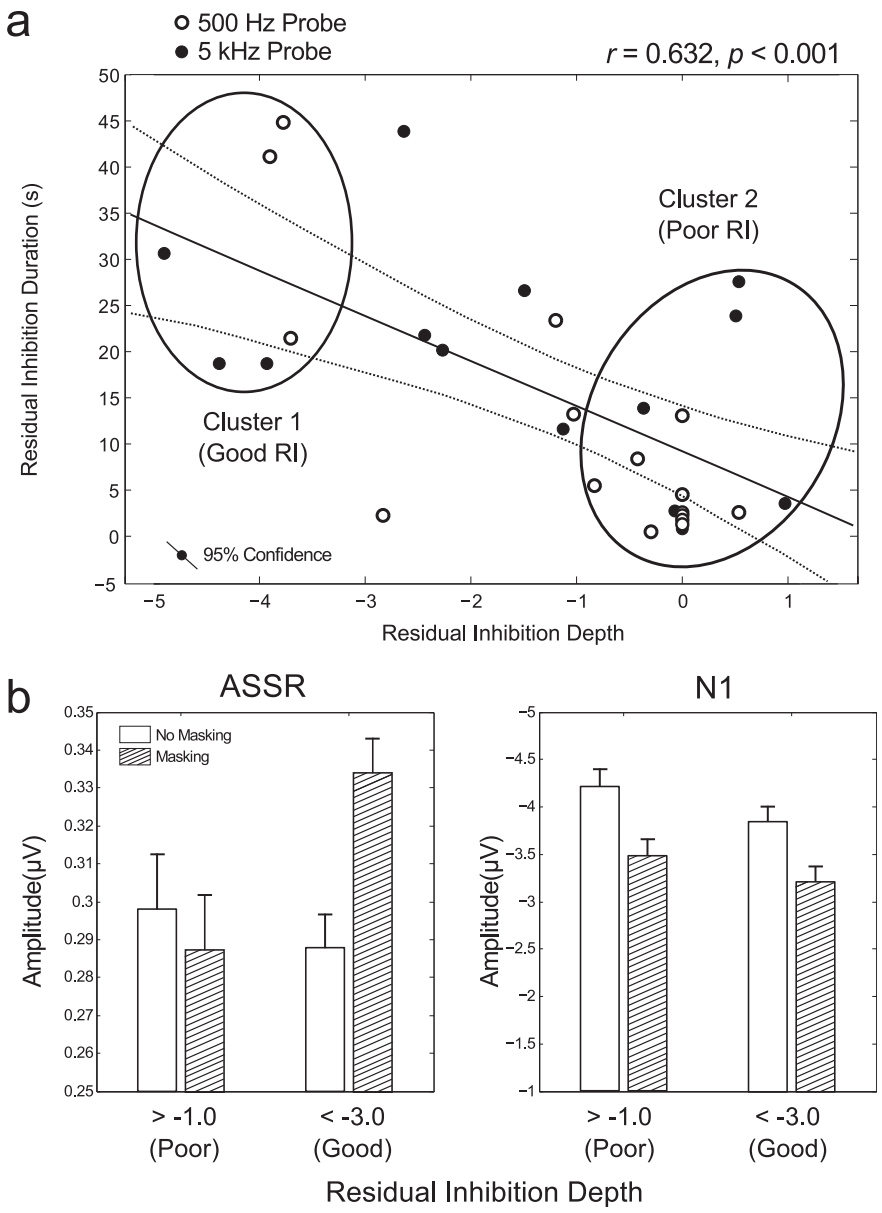

Fig. 8. (a) Relationship of RI depth and RI duration. Subjects in Cluster 1 displayed comparatively good RI (RI depth <−3.0) and in Cluster 2 comparatively poor RI (RI depth >−1.0). Filled circles identify tinnitus subjects probed with a 5 kHz sound and open circles tinnitus subjects probed with a 500 Hz sound. (b) ASSR and N1 responses for Cluster 1 and Cluster 2 in the baseline condition (NM) and after masking (M). Error bars are 1 within-subject standard error (reflecting within-subject masking effects).

represent their tinnitus. Age correlated negatively with bandwidth ($r = -0.46$, $p < 0.05$), indicating that younger subjects were more likely than older subjects to report a tinnitus of narrow bandwidth. All tinnitus subjects under age 40 ($n = 7$) reported a tonal rather than ringing or hissing tinnitus. RI depth was also poorer for younger subjects than for older subjects ($r = -0.49$, $p < 0.05$) and for tinnitus of narrow bandwidth ($r = 0.40$, $p < 0.05$). The sound frequency associated with the peak likeness rating in the tinnitus spectrum was higher for subjects with comparatively better hearing (smaller threshold shifts between 4 and 11.2 kHz; $r = -0.417$, $p = 0.022$). The average RI depth produced by the 5 kHz BPN masker (a reduction of 26.4% of scale) was similar to that reported by Roberts et al. (2008) for subjects tested with this masking sound (24.0%), as was the range of individual differences in RI depth reported in Table 1 (from near tinnitus elimination to some increases after masking). These findings corroborate those reported by Roberts et al. (2008) for a larger sample of 59 subjects with bilateral tinnitus tested with the same methods, and indicate that a comparable sample of tinnitus cases was studied here.

4. Discussion

We compared sound-evoked brain activity measured in subjects experiencing tinnitus under baseline conditions with that of subjects of similar age and hearing function who reported not having tinnitus. The presence of tinnitus was corroborated by psychoacoustic measurements which revealed a TFR covering the hearing loss region typical of that previously established for tinnitus sufferers. In the same session we subsequently examined how brain activity changed when tinnitus and control subjects were exposed to a forward masking procedure that was known from prior measurements to induce a variable degree of RI in the tinnitus subjects.

During baseline the 40-Hz ASSR (a response known to localize to A1) was larger in tinnitus than control subjects when evoked by a 500 Hz sound below the TFR. This difference reversed between tinnitus and control groups in which the ASSR was evoked by 5 kHz sound in the TFR, revealing frequency-dependent group differences in this response during the tinnitus baseline. Effects of forward masking on the ASSR also differed between the two probe frequencies and the tinnitus and control subjects. ASSR amplitude increased after masking in the tinnitus group probed with a 5 kHz sound, whereas ASSR amplitude did not change in the tinnitus group probed at 500 Hz. However, when tinnitus subjects with deep RI were contrasted to those with poor RI regardless of probe frequency, deep RI was associated with larger ASSR increases evoked by 500 Hz probes as well as by 5 kHz probes. These ASSR changes in the tinnitus subjects appear to have reflected changes in neural activities related to tinnitus, because they were not observed in control groups that were similar in age and degree of hearing loss compared to the tinnitus subjects. The masking effects seen in the control groups (ASSR increases to 500 Hz probes after masking and no change to 5 kHz probes) were reversed from those seen in the tinnitus groups. In contrast to these findings for the ASSR, the N1 transient response (this response known to localize to neural sources in A2) was larger in tinnitus than control subjects at both probe frequencies during the tinnitus baseline. Masking reduced N1 amplitude in all conditions with no relationship to RI depth or duration in the tinnitus subjects.

These results suggest that models that link tinnitus with aberrant neural activity occurring in or projecting to the hearing loss (TFR) of A1 will be required to explain the ASSR responses we observed in our tinnitus groups probed at different frequencies during baseline and during RI. Furthermore, although our baseline N1 results point to altered activity in A2 in tinnitus, the failure of N1 masking effects to relate to RI suggests that this activity does not reflect the tinnitus percept but some other process associated with tinnitus. The off-frequency masking effects that we observed at 500 Hz for the ASSR in controls and for N1 in tinnitus and control groups probed at this frequency also require explanation. In the following sections, we first develop explanations for our results that appear to integrate our ASSR findings in baseline and after masking within a common framework. Our N1 findings are then considered in terms of this framework. In two final sections previous studies of electrophysiological imaging in tinnitus and RI are briefly reviewed.

4.1. ASSR responses in tinnitus and RI

In developing an explanation for our ASSR findings at the level of mechanisms, it is convenient to consider first the effects of masking in our control subjects where high frequency hearing loss was present but neural changes relating to tinnitus presumably were not. Perhaps surprisingly, masking with a BNP15 noise masker (CF 5 kHz) increased ASSR amplitude in the Cont/500Hz group even though the masker contained no energy at the 500 Hz probe frequency. In contrast, masking had no effect on ASSR amplitude in the Cont/5 kHz group where the 5 kHz probes matched the CF of the masker. In the latter control group some subjects showed ASSR increases and others ASSR decreases after masking, such that at the group level no change was detected. While these results may seem counter-intuitive, an explanation of them is suggested by animal studies that have investigated the effects of auditory stimuli on neural responses using forward suppression paradigms. In normal hearing animals, presentation of a sound of a few seconds duration evokes a forward suppression of spontaneous neural activity in A1 neurons tuned to the stimulation frequency that lasts a few hundred milliseconds after sound offset (Wehr and Zador, 2005). Because post-synaptic intracellular inhibitory currents persist only 50–100 ms after stimulus offset, mechanisms that affect communication across synapses are thought to contribute most of the effect (Wehr and Zador, 2005). Galazyuk et al. (2014) lengthened the duration of the stimulus to 30 s and observed forward suppression in A1 neurons persisting for 30 s after sound offset, suggesting a scaling of suppression to the duration of masking. In what may be a striking amplification of this principle in normal hearing cats, Noreña et al. (2006) and Pienkowski et al. (2011) found that exposure for 12–24 h/day for 5–16 weeks to moderate-level asynchronous 4–20 kHz tone pips produced a forward suppression of neural responses to the exposure frequencies in A1 that lasted up to several weeks after sound cessation (the time frame of their measurements). This effect was observed to develop within two days in cats that were exposed continuously to tone pips in a pair of third-octave bands centered at 4 and 16 kHz (Pienkowski et al., 2011). Important for the current findings, forward suppression of spontaneous activity in the exposure band was attended by an increase in neural responsiveness (disinhibition) for frequencies regions above and below the exposure frequencies, possibly as a result of release from lateral inhibition, relative to that in the exposure band where responsiveness was reduced compared to that observed in unexposed control cats (Pienkowski and Eggermont, 2009, 2012). Increased responsiveness to frequencies outside of the exposure band (an “off-frequency” masking effect) compared to that within the band after masking resembles what we observed in our Cont/500 Hz and Cont/5 kHz groups, respectively, after 30 s of masking. Off-frequency effects of masking in our control subjects may have been further modulated by changes in the balance of excitation and inhibition in A1 neurons accompanying high frequency hearing loss, which was present in the control groups. When testing animals with high frequency hearing loss induced by noise trauma, Scholl and Wehr (2008) observed shifts in the balance of excitation and inhibition favoring increased excitation covering a region 2–3 octaves below the CF of neurons tuned approximately to the cut-off frequency of the ABR audiogram. For our control subjects showing an audiometric edge at about 2 kHz, the frequency region of increased responsiveness would have encompassed the 500 Hz probe sound. A shift in responsiveness in this region could have amplified the effect of off-frequency forward masking on ASSR amplitude observed in the Cont/500 Hz group.

Because the threshold shifts seen above 2 kHz in our control and tinnitus subjects were similar and overlapping, and because the groups tested at each probe frequency received the same masking and probe sounds, effects of high frequency hearing loss might have been expected to affect our tinnitus and control groups similarly. However the masking effects observed in ASSR amplitude for the Tinn/500 Hz and Tinn/5 kHz groups (no change and an increase in ASSR amplitude, respectively) were reversed from those observed in the Cont/500 Hz and Cont/5 kHz groups (an increase and no change in ASSR amplitude, respectively). It is unlikely that small threshold differences between groups averaging 4.7–11.8 dB over the frequency range 500 Hz to 5 kHz accounted for these different ASSR masking effects, since these effects did not correlate with auditory thresholds at 500 Hz, 1 kHz, 5 kHz, or 4–11.2 kHz in any condition or when the groups were combined. Alternatively, group differences may have reflected neural changes that have been reported in tinnitus subjects, which may have been consequent on cochlear neuropathy not expressed in the audiogram (Kujawa and Liberman, 2009; Plack et al., 2014). What are these neural changes, and how might they have interacted differently with ASSR responses in tinnitus compared to control subjects before and after masking?

One candidate activity, tonotopic map reorganization, has been documented by neuromagnetic imaging of tinnitus patients with hearing loss. Reorganization was observed as a shift in the 3D

location of the cortical sources of ASSR responses evoked by pure tones above ~2 kHz to spatially overlapping sites in the tonotopic frequency region where tinnitus percepts typically localize (Wienbruch et al., 2006). This result implies a loss of frequency selectivity for the affected neurons such that these neurons are now driven by a wider bandwidth of sounds than previously. In animal studies of cortical reorganization, a complete reorganization of preferred tuning frequencies from the impaired region of A1 to the audiometric edge has been observed in cases of moderate to profound cochlear damage (e.g., Rajan et al., 1993; Seki and Eggermont, 2002). In mild to moderate cases of cochlear damage which may be more applicable to our subjects, animal studies show cases of partial shifts in tuning towards the audiometric edge and broadened tuning bandwidth, in addition to some A1 neurons that are dually tuned to both their original preferred frequency and lower frequencies near audiometric edge (Rajan et al., 1993; Seki and Eggermont, 2002). Such neurons might explain the increase in baseline ASSR amplitude we observed in tinnitus relative to control subjects at 500 Hz if their modified bandwidth is sufficiently wide. Alternatively, Langers et al. (2012) suggested that map reorganization may be more closely related to audiometric hearing loss than to tinnitus, since in their study map reorganization measured by fMRI was not detected in a tinnitus group compared to a control group both of which had audiometrically normal hearing. If this hypothesis is accepted, we might have expected effects arising from map reorganization to have been similar in our tinnitus and control groups, given that similar high frequency threshold shifts were present in both groups. It is also not apparent how tonotopic map changes accompanying tinnitus with hearing loss can explain the different frequency-dependent masking effects we observed on ASSR amplitude in our tinnitus and control groups. These masking effects did not correlate with any audiometric variable that might have been expected to affect map organization.

These considerations suggest that a conjunction of other neural correlates of tinnitus, namely, disinhibition of auditory attention networks in tinnitus (Cuny et al., 2004; Gu et al., 2010; Roberts et al., 2013), increased spontaneous activity in auditory pathways (Noreña and Eggermont, 2003; Kaltenbach et al., 2004; Vogler et al., 2014; Koehler and Shore, 2013a), and increased neural synchrony in the hearing loss (TFR) of auditory cortex (Noreña and Eggermont, 2003; Engineer et al., 2011), may give a better account of the present results. Disinhibition of auditory attention networks in tinnitus could explain larger ASSR responses evoked by 500 Hz probes during the tinnitus baseline in tinnitus subjects compared to controls probed at this frequency, if this disinhibition is frequency nonspecific. Increased ASSR responses in the Tinn/500 Hz group during baseline may also have mitigated against an off-frequency ASSR masking effect appearing in this group compared to the Cont/500Hz group. However, disinhibition of cortical networks by auditory attention (or some other mechanism) in tinnitus subjects does not appear to explain the results at 5 kHz, where ASSR responses were smaller in tinnitus subjects than in controls. Masking subsequently shifted ASSR amplitude evoked by 5 kHz probes in tinnitus subjects toward control levels. In this respect it is relevant that at 5 kHz (in the TFR) aberrant neural activity related to tinnitus would have been present in tinnitus subjects but not in controls at this frequency. Increased spontaneous activity and neural synchrony are prime candidates for such aberrant activity, particularly increased neural synchrony which in noise exposed cats is confined to the hearing loss region (Noreña and Eggermont, 2003) where in humans the tinnitus frequencies lie (Noreña et al., 2002; Roberts et al., 2006, 2008; Sereda et al., 2011). Synchronous activity among neurons is a likely code for auditory percepts, since in normal hearing subjects phase locking is a probable mechanism by which the auditory system detects sound information conveyed by auditory nerve fibers that are inherently spontaneously active (Eggermont, 1990). In our subjects hypersynchronous neural activity supported by spike-timing dependent plasticity operating in the TFR (Eggermont and Roberts, 2004) could have reduced baseline ASSR amplitude evoked by 5 kHz probes, by reducing the number of neurons available to phase lock with the 40-Hz AM envelope. Masking with the 5 kHz BPN sound may have suppressed hypersynchronous activity, rendering more neurons available for phase locking and giving a masking effect on ASSR amplitude evoked by 5 kHz probes in tinnitus subjects. The same mechanism may have generated RI, which correlated with ASSR increases when RI was strong.

While the foregoing account proposes that mechanisms operating in A1 are responsible for the ASSR increase after masking at 5 kHz, in principle the effect could alternatively have been projected to ASSR sources in A1 from changes that occurred in the auditory midbrain after forward masking. Working with a salicylate model of tinnitus in rats, Liu and Chen (2015) observed an increase in the amplitude of ABR waves II and IV but not ABR wave I after forward masking, which occurred in the frequency region where hearing loss and behavioral evidence of tinnitus were present. This result was attributed to suppression of spontaneous neural activity in this frequency region by forward masking, which may have enhanced sound-driven responses generated in midbrain nuclei. It should be noted that the duration of the masking stimulus (~5 ms) and the time delay between the masker and the probes (~20 ms) were far shorter in the Liu and Chen (2015) study than in the present research, and the animals had been treated with salicylate. Nonetheless the suppressive effect of masking sounds on spontaneous activity proposed by Liu and Chen (2015) could scale with exposure duration as suggested above. This mechanism might also be implemented at different levels of the auditory pathway from midbrain to cortex. If so, our findings suggest that at the level of the cortex its effect is to suppress tinnitus-related neural activity in the TFR, giving RI.

4.2. Integrating the findings into a common framework

Models for tinnitus and masking based on these considerations are presented in Fig. 9a and b, respectively, which may give a coherent explanation of the ASSR results and other findings related to tinnitus. The mechanism for tinnitus described here (Fig. 9a, adapted from Roberts et al., 2013) assumes that one role of the auditory cortex is to predict its sensory state. When prediction fails in normal hearing, cortical neurons are disinhibited by activation of cholinergic projections from the basal forebrain. This effect, which makes neurons more sensitive to their afferent inputs (an effect ascribed to auditory attention; Sarter et al., 2005), subsides as a more accurate representation of the auditory scene is constructed from input provided by intact auditory pathways. However, in tinnitus the disparity persists, because neural activity coding for the tinnitus sound (this activity represented in and read out from memory) is not corroborated by input arriving from the damaged cochlea. The outcome is a persistent shift in the balance of excitation and inhibition toward excitation of auditory cortical neurons in A1 and A2. Evidence for persistent activation of auditory attention networks (Cuny et al., 2004; Gu et al., 2010; Paul et al., 2014) and for disinhibition of auditory neurons (Yang et al., 2011; Diesch et al., 2010b) has been reported in tinnitus, although the source of these effects has not been established.

The mechanism proposed in Fig. 9a sets the stage for the effects of masking which are addressed in Fig. 9b. In Fig. 9b the perceptual disparity perceived by tinnitus subjects has shifted the balance of excitation and inhibition in A1 toward excitation in these subjects compared to controls, in the baseline condition (left panels, Fig. 9b).

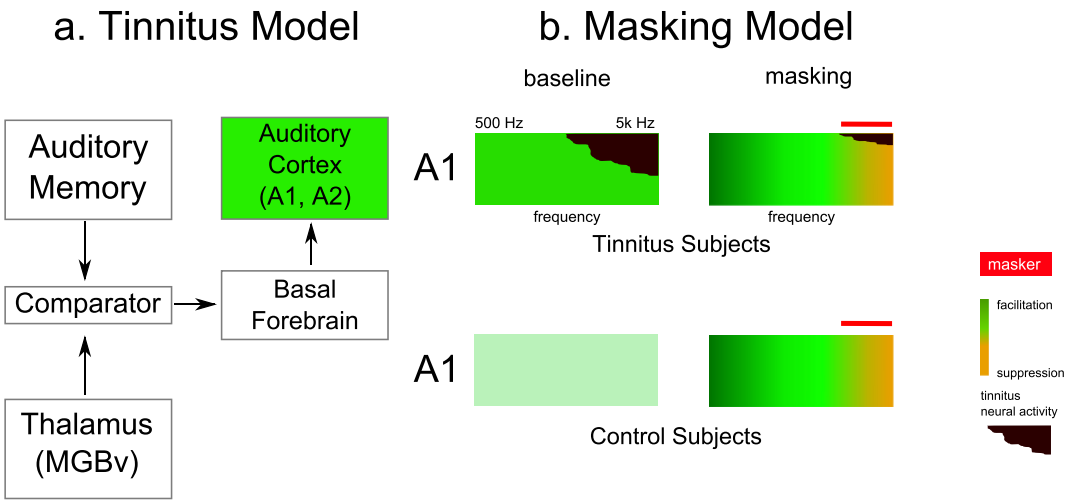

Fig. 9. Models of tinnitus and forward masking in individuals with and without tinnitus applied to the ASSR. (a) Model of Tinnitus (adapted from Roberts et al., 2013). Aberrant synchronous neural activity consequent on deafferentation underlies the tinnitus percept and is stored in auditory memory. The disparity between predicted and obtained input from the damaged cochlea disinhibits neural activity in A1 and A2 via the basal forebrain cholinergic system (auditory attention). (b) Model of Forward Masking. The balance of excitation and inhibition is shown for A1 where the sources of the ASSR are found. In control subjects without tinnitus this balance is tipped toward excitation under baseline conditions (lower left panel, light green). Forward masking in controls gives a weak suppression of driven responses in the 5 kHz exposure band with a stronger shift toward facilitation off-frequency at 500 Hz (dark green, lower right panel). In tinnitus, A1 is disinhibited in baseline more so than in controls (upper panel, left). Hypersynchronous neural activity in the TFR gives the tinnitus percept and interferes with driven responses, reducing baseline ASSR amplitude at 5 kHz. However, if a masker of sufficient intensity and spectral content is presented, matching the frequencies in the tinnitus, tinnitus-related neural synchrony is disrupted inducing deep RI (upper panel, right). ASSR amplitude now increases toward control levels at 5 kHz as more neurons are available to phase lock with the 40-Hz AM stimulus. Elimination of the tinnitus-related activity shifts the balance of excitation and inhibition toward that of control subjects when RI is deep, enabling off-frequency facilitation of the ASSR at 500 Hz in these tinnitus subjects. If the intensity or frequency content of the masker are not optimal, tinnitus-related neural activity is not disrupted, RI is shallow, and ASSRs evoked on- and off-frequency are changed little by masking. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ASSR responses evoked by probes presented to the 500 region of A1 are therefore larger in tinnitus than in controls during baseline. However, tinnitus-related hypersynchrony is present in the 5 kHz region (the TFR) subjects but absent in controls, which reduces ASSR amplitude evoked by 5 kHz probes presented to the tinnitus subjects during baseline. Masking changes these dynamics differently in tinnitus and control subjects (Fig. 9b, right panels). In controls masking produces a suppression of spontaneous (but not necessarily driven) activity in the 5 kHz region, followed by disinhibition in the 500 Hz region giving an off-frequency masking effect. In tinnitus a masking sound of sufficient intensity and spectral content suppresses spontaneous activity and tinnitus-related hypersynchrony in the TFR, which enables an increase in ASSR amplitude from baseline at 5 kHz toward control levels while delivering RI. Deep RI evoked by the 5 kHz BPN masker reflects an improved correspondence between predicted and obtained auditory inputs and has the consequence of shifting the balance of excitation and inhibition in A1 of the tinnitus subjects in the direction of controls. When deep RI is present, an off-frequency masking effect may be also expressed at 500 Hz in the tinnitus subjects as was seen in controls at 500 Hz. Thus while ASSR increases may occur to probes at 500 Hz as well as 5 kHz in tinnitus subjects when RI is deep, the mechanisms underlying these increases may be different.

Evidence supporting the models of tinnitus and masking of Fig. 9 is found in the human and animal studies cited above, although the latter model in particular extrapolates from animal data and requires further testing. For example, Fig. 9b suggests that mirror-symmetric off-frequency masking effects should be observed in control subjects for ASSRs evoked by 500 Hz and 5 kHz 40-Hz AM probes after exposure to a BPN maskers with a CF of 500 Hz compared to 5 kHz. To our knowledge, off-frequency masking effects of this novel type expressed in brain responses have not been previously investigated in normal hearing subjects. It may also be the case that effects of masking on ASSR amplitude observed in our Tinn/5 kHz group might not appear when tinnitus subjects are probed at 5 kHz after masking with a BPN 500 Hz sound, because there is no hypersynchrony to be disrupted in the 500 Hz region. Off-frequency disinhibition induced by this masker may have little effect on tinnitus-related activity persisting in the already disinhibited TFR, which could explain why such maskers deliver poor RI (Roberts et al., 2008; also see Fig. 1b earlier). The models of Fig. 9 appear able to address our ASSR results, but what can be said about N1?

4.3. N1 responses in tinnitus and RI

In striking contrast to the ASSR, no frequency-specific differences were found between tinnitus and control groups for N1 in baseline or after masking. Instead, N1 amplitude was larger in tinnitus groups compared to controls at both probe frequencies. This result implies that neural changes occur in A2 in tinnitus, even though these changes while possibly including common elements are not identical in A1 and A2. A factor common to both regions may be disinhibition of neural responses in tinnitus by auditory attention. Disinhibition occurs over wide regions of auditory cortex including A2 when normal hearing subjects perform auditory attention tasks (Paltoglou et al., 2009), and appears to occur in A2 in individuals with tinnitus as well (Paul et al., 2014). In principle this mechanism (proposed by the tinnitus model of Fig. 9a) could explain larger N1 responses in the baseline condition at both probe frequencies, although reports of enhanced N1 responses in tinnitus are inconsistent (see below). Because auditory attention networks appear to be disinhibited in A1 as well as A2 in normal hearing individuals performing attention tasks (Paltoglou et al., 2009; Gander et al., 2010a,b) and in individuals with tinnitus (Gu et al., 2010), this mechanism may also explain larger ASSR responses evoked by 500 Hz probes in tinnitus subjects compared to controls during baseline. The model of Fig. 9b proposes that disinhibition extends to the TFR region of A1 in tinnitus subjects. However, ASSR

amplitude evoked by 5 kHz probes presented to the TFR is reduced in these subjects owing to the presence of hypersynchrony in this region.

This interpretation may be successful in explaining frequency-specific effects appearing in ASSR and not N1 amplitude, but it does not explain why N1 was reduced equally in all groups after masking. Reduced N1 amplitude evoked by 5 kHz probes could have been expected, since the 5 kHz BPN masker may have adapted A2 neurons tuned to this frequency. But this explanation requires extension if it is to account for reduced N1 amplitude evoked by 500 Hz probes in the Tinn/500Hz and Cont/500Hz groups. In this respect it may be important that N1 sources localize outside of Heschl's gyrus to lateral aspects of the superior temporal gyrus (Godey et al., 2001; Lütkenhöner and Steinsträter, 1998), are weakly or not tonotopic (Lütkenhöner et al., 2003), and appear to reflect contributions arising from several regions of nonprimary cortex. Nonprimary regions exhibit a heterogeneous cytoarchitectonic structure (Schreiner and Cynader, 1984; Langers et al., 2007) in which layer II/III pyramidal neurons receive inputs from diverse regions of the brain and in turn form intrinsic contacts that are more distal than in A1 where links are made in more localized modules (Tardif and Clarke, 2001; Schreiner and Weiner, 2007). The tuning curves of neurons in A2 are broad, such that they integrate inputs from several frequency regions of A1 as well as inputs from other sources. Hence it is possible that adaptation consequent on A2 neurons being driven by a 5 kHz BPN sound may have down-regulated (adapted) the sensitivity of synapses tuned to 500 Hz as well, preserving the input–output function of the neuron within a prescribed dynamic range for a brief time period after masking (Turrigiano and Nelson, 2004; Pozo and Goda, 2010). Off-frequency adaptation may have depended on our use of band limited noise rather than a pure tone as a masking sound, since pure tones have been reported to yield narrow-band frequency-specific N1 adaptation effects (Butler, 1968; Brattico et al., 2003). Noise would have depressed more synaptic inputs on A2 neurons than would have a pure tone. Regardless of the mechanism at work, our 500 Hz N1 data suggest that off-frequency enhancement is weak in A2 compared to that expressed in A1 where lateral inhibition is generally considered to be strong.

4.4. Previous studies of ASSR and N1 responses in tinnitus and RI

Previous studies comparing ASSR and N1 responses between tinnitus and control groups under baseline conditions are partially consistent with our ASSR results but frequently inconsistent with regard to our findings for N1. Wienbruch et al. (2006) observed significantly larger ASSR responses in subjects with persistent bilateral tinnitus compared to controls for sound frequencies below 2 kHz, which is below the TFR for most tinnitus subjects. For sounds above 2 kHz the group difference while in the same direction diminished to statistical insignificance, suggesting a frequency-dependence albeit weaker than that shown here in Fig. 7. Diesch et al. (2010a) observed descriptively larger ASSRs in tinnitus than control subjects at sound frequencies which were determined individually for each subject to equal to the tinnitus frequency, the audiometric edge frequency, and frequency 1–1/2 octaves below the edge frequency; however, the group difference was not reported to be statistically significant at these frequencies. In another study Diesch et al. (2010b) presented three carrier frequencies, each AM at a different AM rate, either singly or in various combinations to tinnitus and control subjects. The three carrier frequencies were below the audiometric edge, at the audiometric edge, and at the putative tinnitus frequency (individualized for each subject). The main finding of this study was that in controls ASSR amplitude was larger when a frequency was presented singly than in combination, whereas in tinnitus this effect was lost suggesting a deficit in lateral inhibition in the tinnitus group (a result consistent with disinhibition in A1). Overall there was no difference in ASSR amplitude between the tinnitus and control groups. However, for controls ASSR amplitude was larger for the sub-edge compared to the other frequencies, which did not happen for tinnitus; instead, the ASSR response to the tinnitus frequency was larger than the sub-edge response suggesting facilitation of ASSR responses at higher sound frequencies in tinnitus. When the data of this study were analyzed for N1 amplitude (Diesch et al., 2012), N1 amplitude decreased with increasing carrier frequency in both groups but did not differ significantly between the groups (a finding that was interpreted to suggest that ASSR responses in tinnitus do not reflect attention). Overall, prior research comparing N1 responses between tinnitus and normal hearing subjects have produced an unclear picture with some studies reporting increases in N1 amplitude or N1 loudness growth functions in tinnitus subjects compared to controls for tones presented near the edge of the tinnitus (hearing loss) region (Dietrich et al., 2001; Noreña et al., 1999; Hoke et al., 1989), while other studies have reported either decreases in these variables at frequencies in the TFR (Sereda et al., 2013) or below or near the audiometric edge (Kadner et al., 2002; Lee et al., 2007), or no changes at all in N1 amplitude in tinnitus subjects compared to controls (Jacobson et al., 1991; Jacobson and McCaslin, 2003; Diesch et al., 2012; Sereda et al., 2013).

At this time it is not possible to specify which of many procedural variables might have contributed to inconsistent previous results regarding baseline ASSR and N1 responses in tinnitus. However, several aspects of our procedures may be important for the findings we obtained. First, our tinnitus and control groups were relatively well matched for the presence of high frequency threshold shift, such that effects attributable to this factor would have been similar in the two types of subject. Second, rather than individualize sound frequencies on the basis of the putative tinnitus pitch (a practice that results in different sound frequencies being presented to different subjects), all of our subjects were presented with either a 500 Hz sound known to be well below the TFR of the tinnitus subjects or a 5 kHz sound known to be well within it. Both sounds were well removed from the audiometric edge where interactions among map reorganization, lateral inhibition, and spectral contrasts may be complex. Third, sound levels for the probes and the masker were determined by having each subject adjust the loudness of the stimuli to equal the perceived loudness of a 1 kHz tone presented at 65 dB SL. This procedure may have removed or attenuated the contribution of hyperacusis consequent on increased gain in auditory pathways which has been reported for tinnitus subjects (Schaette and McAlpine, 2011; Gu et al., 2012; Hébert et al., 2013). This in turn may have set effects attributable to tinnitus-related neural activity into relief. Finally, an adventitious outcome of the present study was that after masking differences in ASSR amplitude between the tinnitus and control groups were absent. This allowed us to factor out from the baseline data prior to masking the obscuring effect of individual differences in ASSR amplitude which while highly reliable (reflecting anatomical attributes of ASSR generators) can be very large.

Notwithstanding the possible relevance of these factors, a limitation affecting future experiments may be that neural changes with opposing effects on stimulus-driven brain responses related to tinnitus may make group comparisons difficult. In an elaboration of the interpretation proposed for the present data, Paul et al. (2014) suggested that hypersynchrony focused in the TFR of A1 may also drive neural activations non-tonotopically in A2. This elaboration was able to explain why ASSR and N1 responses evoked by a 5 kHz sound and N1 evoked by a 500 Hz sound were not modulated by top-down attention in tinnitus subjects, while ASSR responses to a

500 Hz sound showed a normal attention effect in the tinnitus group (all responses were modulated by attention in controls). If this hypothesis is correct, enhancement of N1 amplitude in tinnitus would be expected to depend on the interaction of driven activity from the TFR of A1 distributed across A2 (this effect reducing N1 amplitude) and disinhibition in this region related to tinnitus (this effect increasing the response). The same interaction would be expected to affect ASSR responses evoked in the TFR of tonotopically organized A1, but less so below the TFR. The presence of possible opposing effects on brain responses and of large individual differences in baseline responding could lead to variable outcomes when comparisons are made between tinnitus and control groups. However, within-subject comparisons such as those reported by Diesch et al. (2010b) pointing to reduced inhibition in tinnitus subjects compared to controls, or differences in the response of such groups to forward masking (Fig. 5 here), may be more robust results from electrophysiological imaging of tinnitus subjects.

4.5. Other electrophysiological responses in tinnitus and RI

Following a different approach to RI, Kahlbrock and Weisz (2008) compared auditory oscillatory brain activity measured by MEG between a group of tinnitus subjects who experienced RI after masking and control subjects without tinnitus who did not. RI was accompanied by a suppression of spontaneous oscillatory activity in the delta band (1.3–4.0 Hz) while no change was seen in controls, linking delta suppression with suppression of tinnitus (also see Adjamian et al., 2012). Because increased delta activity has been observed in tinnitus and other deafferentation syndromes (Weisz et al., 2005; De Jongh et al., 2003; Meinzer et al., 2004; Vieth et al., 1996) and has been linked by one report with increased gamma oscillations in the auditory cortex of tinnitus sufferers (Weisz et al., 2007), Kahlbrock and Weisz suggested that suppression of delta activity may be a prerequisite for attenuation of the tinnitus sensation after masking. The hypothesis advanced by Kahlbrock and Weisz (2008) to explain RI (normalization of neural network activity in auditory cortex by masking) is similar to the hypothesis proposed here (suppression of hypersynchrony in the TFR) to explain the increase in ASSR amplitude evoked by 5 kHz probes after masking in the tinnitus group probed at this frequency but not in their controls. One widely cited neural synchrony model of tinnitus has attributed oscillatory activity in the TFR to hyperpolarization of thalamic nuclei consequent on deafferentation of auditory pathways (Llinás et al., 2005; Kalappa et al., 2014). Hyperpolarization could explain reduced sound-evoked functional connectivity between auditory cortical and subcortical structures which has been reported in tinnitus sufferers compared to controls (Boyen et al., 2014; Lanting et al., 2014).

Notwithstanding reduced functional connectivity in tinnitus, the coticofugal output of A1 neurons affected by deafferentation may be sufficient to distribute to nonauditory brain regions yielding neural changes there. Functional imaging has confirmed that neural changes associated with RI extend beyond the auditory regions that were investigated here with ASSR and N1 responses. Osaki et al. (2005) observed changes in metabolic activity measured by positron emission tomography in the temporal gyrus and cerebellum during RI that were not observed in control subjects after masking. This finding aligns with evidence for distributed brain network activity associated with tinnitus reviewed by Husain and Schmidt (2014) and Vanneste and De Ridder (2012). When using MEG to examine oscillatory activity during epochs of RI, Sedley et al. (2012) found increased delta and gamma activity associated with increased tinnitus; however, when oscillatory activity was measured during epochs of residual excitation (reported by 4 of 17 tinnitus patients; cf. Fig. 8a here), increased tinnitus perception was associated with a decrease in gamma and no changes in the strength of delta oscillations. Significant oscillatory power changes were also identified in a variety of cortical regions (default mode network, cerebellum, insula and anterior temporal lobe) that were highly variable across the subjects in terms of the frequency bands involved and the direction of the power change associated with reports of increased tinnitus after masking. At present the extent of nonauditory electrophysiological changes in tinnitus appears to be considerable although their mechanisms are not well understood. The strength of tinnitus-related neural activity distributing from the TFR of A1 to nonauditory regions may be important in determining whether access is gained to brain networks believed to be underlie consciousness awareness (De Ridder et al., 2011).

5. Summary and conclusions

We compared sound-evoked brain activity (the 40-Hz ASSR localizing to cortical sources in A1 and N1 to sources in A2) in subjects experiencing tinnitus with that of subjects of similar age and hearing function who reported not having tinnitus, under baseline conditions in which the tinnitus subjects experienced their tinnitus. Subjects in separate groups were probed either with a 5 kHz 40-Hz AM sound known to be in the TFR of the tinnitus subjects or a 500 Hz 40-Hz AM sound known to be below the TFR of the tinnitus subjects. Subsequently in same session we examined how brain activity changed when all subjects were exposed to a forward masking procedure known to induce RI in the tinnitus groups. In the baseline condition ASSR amplitude (extracted from 128-channel EEG) was larger in the tinnitus group tested with 500 Hz probes compared to controls while the reverse was true for the tinnitus group tested with 5 kHz probes, revealing frequency-dependent group differences in this response. In contrast, frequency dependence was not observed for N1 which was larger in the tinnitus groups than in controls at both probe frequencies. In the control groups masking had no effect on ASSR amplitude evoked by 5 kHz probes but increased ASSR amplitude evoked by 500 Hz probes even though the masking sound (band pass noise, CF 5 kHz) contained no energy at 500 Hz (an off-frequency masking effect). In the tinnitus groups the effects of masking on the ASSR were reversed, revealing increased ASSR amplitude evoked by 5 kHz probes after masking and little change in ASSR amplitude evoked by 500 Hz probes. Across all tinnitus subjects larger ASSR masking effects at both frequencies (ASSR increases) were associated with greater RI depth and duration. In contrast to these effects of masking on the ASSR, N1 amplitude was reduced by masking at both probe frequencies equally in all groups and did not relate to RI depth or duration in the tinnitus subjects.

These results support the view that aberrant neural activity occurring in or projecting to the tinnitus frequency (hearing loss) region A1 is involved in the generation tinnitus and its modulation during RI. They suggest further that neural responsiveness is increased in A2 without frequency specificity in tinnitus, but that neural changes occurring in this region do not relate to RI. The findings appear to be explicable by models of tinnitus and forward masking (Fig. 9) that entail the following principles, for which support to varying degrees can be found in the relevant human and animal literature. (1) Cortical neurons in A1 and A2 are disinhibited in tinnitus by auditory attention or some other mechanism. (2) Aberrant synchronous activity is forged among A1 neurons affected by hearing loss (hypersynchrony) and codes for the tinnitus sound. (3) Suppression of hypersynchrony by masking induces RI and promotes neural phase locking to the 40-Hz AM sound, increasing ASSR amplitude when RI is deep. (4) Masking enhances driven responses for A1 neurons tuned to frequencies below the exposure

band in normal hearing individuals, and in individuals with tinnitus as well, when tinnitus is suppressed during RI (off-frequency masking effects). (4) Effects of forward masking in A2 are different from those in A1, reflecting the specialization of A2 neurons for multisensory integration and the relative lack of tonotopic organization in this region.

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