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Using the auditory steady-state response to record response amplitude curves. A possible fast objective method for diagnosing dead regions

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Cochlear dead regions (DRs) can be diagnosed by recording psychophysical tuning curves (PTCs). PTC recording methods are time consuming and cannot be applied to infants. We propose, using the auditory steady-state response, to record response amplitude curves by development of the method that could be used to diagnose and define DRs in infants whilst they are asleep.

1 Introduction

Behavioral pure-tone hearing thresholds are usually used to determine the appropriate way to manage a hearing loss and to set the gain of a hearing aid. However, the pure-tone thresholds are not always a complete representation of an individual's hearing function (Halpin et al. 1994). It is possible that a pure-tone threshold obtained in hearing-impaired subjects may not represent the cochlear function at the corresponding characteristic frequency because of the test tone being detected in an area of the cochlea normally maximally activated by frequencies lower or higher than the test signal frequency. This phenomenon, known as off-frequency listening, can occur due to poorly functioning areas of the cochlea, i.e. dead regions (DRs) (Moore et al. 2000). Dead regions are thought to be areas in the cochlea where the inner hair cells are absent or so poorly functioning that sounds are more efficiently detected off-frequency (Moore et al. 2000). The prevalence of DRs in subjects with a severe or profound hearing loss has been found to be over 50 % (Vinay et al. 2007). DRs can be diagnosed using behavioral response masking techniques, such as the threshold equalizing noise (TEN) or psychophysical tuning curve (PTC) tests. This paper proposes an objective method of diagnosing DRs using the auditory steady-state response (ASSR).

The psychophysical tuning curve is generally accepted as the gold standard for DR diagnosis in adults (Moore 2001, 2004). The PTC test determines the ipsilateral masking level required to prevent the detection of the test signal (Moore 1978). The signal is fixed in frequency and level and presented with an ipsilateral masker with variable frequency and level. The level of masker required to prevent the detection of the signal is plotted against masker frequency. The frequency at which the masker is most efficient is known as the PTC-tip frequency, and occurs at the frequency-point on the basilar membrane where the signal is maximally detected (Moore 1978). In normally hearing subjects the PTC-tips are at or near to the signal frequency, although an upward frequency shift can occur due to suppression of the test signal during simultaneous masking (Moore 1978). The PTC-tips are shifted away from the signal frequency when the signal falls within a DR. This occurs as the signal is more efficiently detected in less impaired off-frequency regions of the cochlea (Moore et al. 2001). Behavioral measurements of the classical PTC can take up to two hours to complete (Kluk et al. 2005, 2006; Sek et al. 2005). An alternative fast PTC method that reduces test time can be used to obtain PTCs (Sek et al. 2005). The fast PTC method sweeps a masking noise across the masker-frequency range whilst its level is automatically adjusted according to the subject's response to the signal (Sek et al. 2005). Sek et al. (2005) compared the results of the fast PTC method to classical PTC testing and concluded that both the classical and fast PTC methods produced PTC-tips shifted away from a signal frequency inside a DR. However, as neither of these behavioral PTC DR diagnosis tests can be applied to infants, a fast objective method is required to enable the early detection of DRs in infants with hearing loss.

Objective hearing assessment methods can be used in subjects who are unable to perform a behavioral test. Electrophysiological tuning curves (ETCs) are an objective equivalent to PTCs. Klein and Mills (1981) recorded ETCs using auditory brainstem response (ABR) in place of the behavioral masked thresholds used in PTCs. The ABR masked threshold was defined as the level of masker required to prevent the presence of wave I and wave V on the recorded ABR waveform. The ABR masked-threshold testing was performed at multiple masker centre frequencies in a similar manner to behavioral PTCs. The ABR method was time consuming as, in common with the PTC method, thresholds are determined at each masker frequency and this involves recordings at multiple levels of the masker. The duration of the test is an important factor when performing electrophysiological testing in infants, as the test must be conducted whilst they sleep to ensure that the average EEG-noise level is minimal.

The ASSR is a form of envelope-following evoked potential that is obtained by recording the EEG across an active (vertex or high forehead) and reference (ipsilateral mastoid or nape of the neck) electrode and can be used to estimate subjects' auditory thresholds (Galambos et al. 1981). ASSRs can be evoked by continuous modulated pure tones with carrier frequencies set to the frequency of interest. The modulation can be applied to the carrier tone's amplitude (AM), frequency (FM) or a simultaneous mix of both (MM) (Picton et al. 2003). It is possible to record ASSRs at multiple test frequencies and in both ears simultaneously (Picton et al. 2002).

Markessis et al. (2009) estimated ETC-tip frequencies in normally hearing subjects by recording ASSR thresholds in the presence of masking noise. They found that the tips of the ASSR ETCs were often shifted to frequencies higher than the signal, which they referred to as detuning. Markessis et al. (2009) found no significant difference in detuning between the ASSR measurement and behavioral PTCs obtained using the same modulated ASSR signal used for the ETC measurement. They hypothesized that the upward tip shift effect of the ETCs was not caused by the use of modulated tones, which have a wider bandwidth than pure tone signals, but was due to cochlear non-linearities such as two-tone inhibition and

suppression which can also occur in behavioral PTCs as first shown by Moore (1978). Since this method requires high masker levels when the masker centre frequency differs from the signal frequency, it would not be practical to use in people with significant hearing loss. The method proposed by Markessis et al. (2009) could therefore be of limited clinical use in hearing-impaired subjects as the masking levels required could potentially exceed one or more of the following: maximum headphone output, maximum comfortable listening levels and safe signal level limits. These difficulties can also occur in behavioral PTC measurements (Stelmachowicz et al. 1984). Additionally, Markessis et al. (2009) ETC recordings took an average of 150 minutes for each 10-point tuning curve. This test time is likely longer than is feasible for an infant recording session.

An alternative approach that records the changes in electrophysiological response amplitude, or latency, using fixed level signal and maskers was first suggested by Folsom (1984) using the ABR. This approach was taken by Ross et al. (2003) and Herdman et al. (2002) who examined the masking effect of noise on the amplitude of the ASSR. Ross et al. (2003) examined the frequency specificity of the 40-Hz ASSR response in six normally-hearing adult subjects. They generated response amplitude curves (RACs) by recording the ASSR amplitudes in the presence of an interfering masker. The ASSR signals were amplitude modulated and maskers were pure tones and narrow-band noises. In each test the ASSR stimuli and masking levels remained constant whilst the response amplitude was recorded at multiple masker centre frequencies. This method requires shorter ASSR recording times compared to the threshold-based masking methods since it requires only one recording at each masker frequency. The Ross et al. (2003) frequency-specificity curves were derived from fitting a curve to the ASSR amplitudes versus masker center frequency. Ross et al. (2003) recorded ASSRs for 40-Hz modulated stimuli, which give higher ASSR amplitudes compared to the 80-Hz range stimuli in awake normally hearing adults (Cohen et al. 1991). However, the 40-Hz response is poorly recorded in sleeping infants (Levi et al. 1993) and so would not be suitable for use as an infant DR test. Herdman et al. (2002) also recorded masked ASSR amplitudes. They presented single and multiple ASSR stimuli in the presence of an interfering high-pass noise (HPN) with cut-off frequencies in octave steps from 250 Hz to 16 kHz presented at a constant spectrum level. The results of Herdman et al. (2002) indicated that the response was masked to an insignificant amplitude when the cut-off frequency was equal to the ASSR stimulus carrier frequency. It was not possible to determine the precise cut-off frequency needed to mask the response as insufficient intermediate frequencies were tested. These studies suggest the possibility of using an ASSR-amplitude-reduction method to record RACs which are related to ETCs. Since a RAC method does not involve threshold measurements, it could require less test time and lower masking levels when compared to the threshold based methods.

This study aims to investigate the feasibility of estimating RAC-tip frequencies by recording changes in the ASSR amplitudes in the presence of maskers with variable center frequencies presented at constant signal level.

2 Methods

2.1 Participants

Ten male and ten female normally-hearing adults, aged 18 to 49 years, were recruited from staff and students at the University of Manchester. This study received ethical approval from University of Manchester School of Psychological Science Ethics Board.

All subjects were normally hearing with air conduction thresholds ≤ 20 dB HL at standard audiometric frequencies (250 Hz to 8 kHz) and had no history of otological or neurological pathology. The subjects received financial compensation for their time.

2.2 Stimuli

The ASSR stimuli were presented through ER3A (Etymotic Research Incorporated, Grove Village-Illinois) insert earphones to the right ears of the subjects. The stimuli were digitally generated using a MATLAB (The Mathworks Incorporated, Natick-Massachusetts) program with a sample rate of 32 kHz. The ASSR test stimulus was an exponentially amplitude modulated (AMEXP) 2-kHz carrier tone (see Figure 1), generated using the following equation: $s(i) = a(\sin(2\pi ti)) * (2m_a((1 + \sin(2\pi f_m ti))/2)^N - 0.5) + 1)$ where i is the digital buffer sample point, t the time period for each sample point, m_a the modulation depth, f_m the modulation frequency and f_c the carrier frequency (John et al. 2002). The amplitude modulation depth was set to 100%. AMEXP stimuli have a wider signal bandwidth than AM alone but elicit higher ASSR amplitudes at low sensation levels compared to AM, maximizing the ratio of response amplitude to EEG noise (John et al. 2002). The final target population for the RAC-diagnostic test is sleeping infants. Therefore to keep the test conditions similar to those required in infants the ASSR modulation frequency was set to 95-Hz as 95-Hz responses have been shown to be successfully recorded in neonates (Rance et al. 2006). Additionally, Cohen et al. (1991) found that the response amplitude peaks near to this modulation rate in adults.

The swept-masking method used a narrow band of masking noise whose center frequency was continuously adjusted over a 16-second sweep (8 seconds upwards and 8 seconds downwards) between 1 kHz and 4 kHz with a logarithmic rate of change to attribute equal test time in the octave above and below the test signal frequency. The fixed-masking method used 8 separate narrow band maskers with center frequencies of 1000, 1500, 1800, 2000, 2200, 2500, 3000 and 3500 Hz. The swept masker was generated at equal overall RMS level to the ASSR tones and was calibrated as described below. The masker bandwidth (for both fixed and swept maskers) was set to the equivalent rectangular bandwidth (ERB) of the auditory filter centered at 2 kHz which is 240 Hz (Moore 1995). In the swept method the ASSR signal was fixed throughout the recording whilst the masker centre frequency was swept. Whereas, in the fixed method the signal and masker centre frequencies were fixed throughout each of the 8 individual recordings, each with a differing masker centre frequency.

The stimuli (masker and signal) were generated in two separate channels which were presented via the two external input channels of a GSI61 (Grason-Stadler, Eden Prairie-Minnesota) audiometer. In the swept-masker method the MASTER (Rotman Research Institute, Toronto-Canada) system converted both the masker and test stimulus to analogue output via a National Instruments DAQ Card 6062E (National Instruments Corporation, Austin-Texas). In the fixed-masker method the MASTER system generated the test stimulus and the masker was generated by a Lynx One sound card in a separate desktop PC. This method was used to reduce overall test time as the MASTER ASSR system requires up to one minute to process imported stimuli and eight such imports would have been necessary for each fixed-method RAC. The two channels were mixed in the audiometer and presented simultaneously via the insert earphone. This arrangement allowed setting the levels of masker and signal independently. The sound output levels of each of the channels were calibrated to ensure that the sound level corresponded to the displayed output level on the audiometer dial in dB SPL. Calibration was performed using a GRAS type 26AC .25" (G.R.A.S. Sound and Vibration A/S, Holte-Denmark) microphone connected to Agilent 35670A Dynamic Signal Analyzer (Agilent Technologies UK Ltd, Wokingham, United Kingdom) in octave analysis mode via a GRAS IEC711 coupler (G.R.A.S. Sound and Vibration A/S, Holte-Denmark). In this experiment all stimuli were calibrated in dB SPL.

2.3 Procedures

Subjects attended two 2-hour sessions, which were conducted on separate days. The subjects' skin was prepared using Nuprep (Weaver and Company, Aurora-Colorado) abrasive cleaner and single use electrodes were attached to the vertex (active), right collarbone (ground) and nape of the neck (reference) just below the hair line using TEN20 (Weaver and Company, Aurora-Colorado) EEG conductive paste. All electrode-impedance pairs were checked and re-applied until they were $\leq 4 \text{ k}\Omega$. The recordings took place in a darkened sound-treated booth and the subject was asked to relax and, if possible, sleep. The subjects' state of attention was subjectively monitored by listening for signs of sleeping and by asking them afterwards whether they slept.

The signal level was set to 50 dB above each subjects' 2-kHz pure tone threshold obtained using standard audiometric procedures (BSA 2004). The SPL levels were calculated by adding 50 dB to the subjects' pure tone thresholds converted from HL to SPL using the reference equivalent sound pressure level (RETSPL) for the insert earphone and IEC711 coupler combination. The signal levels ranged from 52 to 72 dB SPL. The RAC testing was performed at 0 dB signal-to-masker ratio as it was found to be optimal in our preliminary test parameter investigation study. The subjects' ASSR amplitudes were recorded without masking at the beginning of each test session. The EEG rejection level was determined by initially setting the epoch rejection level to 20 μV and increasing it in 5 μV steps until less than 20% of the epochs were rejected. Artifact rejection was used to reduce the possible effect of EEG artifact increasing or decreasing the recorded response amplitude. In the first test session half of the subjects were presented with swept method followed by the fixed method and this order was reversed in the remaining half. The test method order was reversed between the two recording sessions for each subject. This was necessary since there may have been a tendency to become more relaxed as test time progressed as it is known that subject state affects the EEG noise levels (Cohen et al. 1991).

The EEG was digitally sampled and recorded using the MASTER ASSR system with a Grass LP511 AC amplifier (Grass Technologies, Astro-Med, Inc. Product Group, Rhode Island, USA). The EEG amplifier was set to 10,000 times amplification with a high-pass filter of 30 Hz and low-pass filter of 300 Hz. The EEG sampling rate was set to 1000 Hz and 1000 samples per epoch were recorded. Stimulus artifact generated by aliasing of the voltages elicited on the EEG wires from the insert earphones was avoided by the use of -24 dB/octave 300 Hz low-pass filtering in the LP511 EEG amplifier (Picton et al. 2004). No stimulus artifact was recognized in a no-subject-test condition with a sound output level of 120 dB SPL.

In the swept method the MASTER artifact rejection system was turned off. This was necessary as the individual epochs throughout the 16 second sweep contain a different masker center frequency and are not interchangeable. The raw EEG data was saved for analysis in MATLAB as the MASTER ASSR system software does not contain any suitable analysis methods for the swept-masker stimuli.

2.4 Data Analysis

The detectability of the ASSR can be improved using a variance-weighted averaging method (John et al. 2001; Picton et al. 2003). The ASSR variance weighting method filters the EEG data around the expected response frequency (including adjacent frequencies used for response significance determination). The variances of the raw filtered EEG data across each epoch of each sweep are calculated. The averaging process weights individual epoch positions of each sweep according to their variance. Epochs with high variance (higher EEG noise levels) are given less weight in the averaging. Thus, the effect of noise is reduced. In

our analysis each sweep contained sixteen one second epochs. We used this method of averaging as our preliminary data showed that variance-weighted averaging could reduce the negative effect that EEG noise had on the response amplitudes. The raw EEG sample data was imported into MATLAB, band-pass filtered with cut-off frequencies 5 Hz above and below the stimulus modulation frequency and averaged using variance weighting over 120 sweeps in the swept method (32 minutes) and eight separate test runs of 15 sweeps in the fixed method (32 minutes plus additional time for repeated recordings of noise rejected epochs). In the fixed method the RACs were generated by plotting the response amplitude in each of the 8 test runs against the masker center frequency for that test run. In the swept method the signal, masker and response were time locked. The signal and masker were both presented by the MASTER ASSR system and hardware locked to the same DA/AD clock ratios and therefore it was possible to calculate the masker centre frequency at any point during the 16 second sweep. The averaging process produced one 16-second sweep per test run which was then converted into the frequency domain using a 1000 sample (1 second duration) FFT in 700 overlapping segments separated by 20 ms. The response amplitudes of the upward and downward direction of sweep were combined to produce an overall of 350 overlapping segments. The swept method averaging process is displayed in Figure 2. The upper panel of the figure shows the swept masker centre frequency (x-axis) against the time position in the 16 second sweep (y-axis). The points plotted on the upper and lower 8 second parts of the stimuli represent example individual EEG time analysis points. The masker frequency within each one-second FFT response analysis point passes through a range of smoothly swept centre frequencies. It was not possible to pinpoint the response with a higher degree of temporal resolution by reducing the number of points in the response FFT analysis. The higher temporal resolution was not possible as data from our earlier test parameter investigation study revealed that using an FFT size of at least 1000 points (1 second of test time) was required to adequately separate the response from the EEG noise in adjacent FFT frequency bins. Thus, each plotted point in the final RAC analysis represented the response amplitude over one second of test time in each sweep direction. The masker frequency in each analysis point was derived from the masker centre frequency at the mid-position of each one second analysis window. The masker signal passed through each frequency point within its frequency range twice within each sweep as the masker signal was swept first upward in frequency over 8 seconds and then downward in frequency over 8 seconds. The marked positions on the figure indicate examples of the combination of the upward and downward sweep direction analysis windows at the following positions: start of sweep at 1st second and 16th second (1094 Hz masker centre frequency), middle position 3.5 seconds and 11.5 seconds, (2000 Hz masker centre frequency) and end of sweep 7th and 15th seconds (3668 Hz masker centre frequency). The arrows, in Figure 2, represent the response averaging and phase correction process used to generate the RAC by combining the upward and downward sweep directions into a single curve as shown in the lower panel of the figure. Thus, the FFT energy at the modulation frequency (the ASSR amplitude) was plotted against the centre frequency of the swept narrow-band noise masker for each position in the combined upward and downward sweep. As each analysis point was offset in time from the start of the sweep, the phase of the response in each segment was corrected to ensure the final phase of the response remained constant relative to the stimulus phase. The sweep directions were combined by vector averaging the phase corrected response in the two sweep directions. The phase correction was necessary as the stimulus was continuously presented across each 16-second sweep and therefore the phase of the modulation, and the response, differed between the upward and downward analysis segments. This occurred as analysis point the upward sweep position increased by 20ms of test time whereas in the downward the starting place decreased by 20ms. The period of the modulation was 10.5 ms (1 / 95 Hz) whereas the step time was 20ms. The difference between the period of the modulation and the step time resulted in the relative phase difference of the upward and downward sweep response points across the sweep. Figure 3 demonstrates this phase

difference effect. Figure 3 is a representation of the 95 Hz modulation frequency of the ASSR signal and response. The left panel shows the modulation envelope at the start of the sweep (upward) and the right panel shows the modulation envelope at the end of the sweep (downward). The first analysis points are in phase (0th and 16th second start points), but the second analysis points are out of phase. Therefore, the response phases at each analysis point were corrected by calculating relative phase at each analysis start point and correcting the difference in relative phase between the upward and downward analysis points. The energy in the FFT frequency bins ± 4 Hz (modulation-frequency FFT bin ± 4 1-Hz wide FFT bins) for the swept method, and ± 3.75 Hz (modulation-frequency FFT bin ± 60 FFT bins) for the fixed method was averaged to calculate the EEG noise floor in each test run. In each test, the response amplitude required for the response to be considered significantly higher than the EEG-noise was calculated using the 1% level F-Ratio.

The RAC tip frequencies were estimated by using rounded exponential (ROEX) curve fitting (Patterson et al. 1982). The ROEX curves were fitted using the MATLAB non-linear fitting function using the ROEX curve equation: $W(g) = t + ((1 - r) (1 + pg)e^{-pg} + r)$ where $g = |f - f_c| / f_c$, p is the upper and lower slope, r the dynamic range, t the tip amplitude and f_c the tip frequency of the RAC. The fitting start parameters were as follows: t = minimum recorded amplitude, f_c = masker frequency at the minimum recorded amplitude, $p = 10$, $r_{upper} = r_{lower} = \text{maximum recorded amplitude} - \text{minimum recorded amplitude}$. The goodness of fit was assessed by calculating the r^2 of each fitted curve. The curves were fitted with two r (dynamic range) parameters, one above the tip and one below the tip. This was necessary as in some cases the RACs had a differing dynamic range for the frequencies above and below the estimated tip (see Figure 4, right panel and Appendix Table 1, Supplemental Digital Content 1). Fitting the RAC curves with two r parameters generated fits with higher r^2 values. Additionally, to improve fitting, the r (dynamic range) parameter values, used by the fitting program, were constrained to an upper limit of the starting r value plus 20% and a lower limit of the starting r value minus 20%. This was necessary as in some cases, when unconstrained, the fitting function returned fits with invalid r values. In extreme cases r values higher than 1000 occurred which were fitted with very low p values. In other cases lower than expected r values occurred which had poorer fits than in the constrained condition. An example of the fit obtained both with and without r -value constraints is shown in Figure 4, left panel.

The mean RAC shape differences between repetitions of each test type were compared as a measure of repeatability within each of the two masking methods. The response amplitudes in each RAC were normalized relative to the mean amplitude of each RAC and the root mean squared (RMS) differences between the normalized amplitudes of the two test runs at 8 frequency points were calculated. In the fixed-masking method the amplitude at each masker center frequency was considered. In the swept-masker method 8 amplitude points at identical masker frequencies to the fixed-masker method were considered. The dynamic range of each RAC was calculated as the difference between the maximum and minimum response amplitude recorded in each RAC. The mean dynamic range of the two test runs in each subject was calculated and the RMS difference between the two test runs as the percentage of the mean dynamic range was also determined to help quantify the size of the difference between the test runs.

3 Results

The RACs were plotted by calculating the response amplitude for each position as a percentage of the not-masked response amplitude recorded in the same test session. This enabled comparison of RACs between recordings and subjects. The raw recorded RACs for both masking methods are shown in Figure 5. The mean RACs across subjects and sessions

(solid line) for the swept and fixed methods are shown in Figure 6. The ROEX curve fit for the mean RAC is shown as a dashed line and the mean ± 1 standard deviation as dotted line for the swept method and as error bars for the fixed method.

The mean estimated RAC-tip frequencies, RAC-tip frequency differences between test runs and the RMS amplitude difference of repeat recordings within each method are shown in Appendix Tables 2 and 3, Supplemental Digital Content 1 and their respective curve fitting parameters are shown in Appendix Tables 4 and 5, Supplemental Digital Content 1. The correlation between response amplitudes in the two recordings for each subject in the swept and fixed methods was calculated and shown in the results Appendix Tables 2 and 3, Supplemental Digital Content 1. Swept-method and fixed-method example RACs are presented in Figure 7 and Figure 8, respectively. In these two figures, the *absolute* response amplitude in nV (rather than the *relative* response amplitude as shown in Figure 5) is plotted against masker centre frequency. The upper panels of Figure 7 show two RACs and their ROEX fits with high r^2 values (0.98 and 0.89). The two RACs shown appear to be of a visually acceptable shape. The upper left panel represents an RAC where the recorded response amplitude significantly exceeded the EEG noise (1% F-Ratio) for all masker frequencies (all such curves are marked in bold on Appendix Tables 2 to 5, Supplemental Digital Content 1). Whereas the upper right panel displays an example RAC where the recorded response amplitude did not reach the 1% significance level for part of the curve. The lower panels of Figure 7 display the two swept method RACs recorded in subject 3 and their ROEX fits. The bottom right panel shows the RAC recorded in the first session with ROEX fit r^2 value of 0.57 (lowest from all swept method recordings), while the bottom left panel shows the RAC recorded in the second session with a high r^2 value of 0.82. The swept-method RACs for subject 3, as shown in the bottom panels of Figure 7, when plotted using absolute response amplitude both appear flatter (lower amplitude dynamic range) compared to the RACs in the upper panels of Figure 7. The apparent lower dynamic range occurred as the unmasked response amplitudes for subject 3 were lower compared to the other two example curve fits shown. The effect the masker on subjects with lower unmasked response amplitudes was less in the absolute sense but similar in the relative sense as demonstrated when the RACs were plotted with the relative response amplitudes as in Figure 5. Additionally, the curve fitting examples revealed that visual inspection of the ROEX fits for both subject 3 swept-method recordings were poor, but this was not apparent from the r^2 fitting parameter obtained for the second RAC test run ($r^2 = 0.82$). This suggests that the r^2 fitting parameter is not always a suitable measure of goodness of fit. The left panel of Figure 8 shows an example of fixed-method RAC with a high r^2 value (0.99) and the right panel displays an example of fixed-method RAC with a low r^2 value (0.58). The RAC in the right panel of Figure 8 had the highest recorded background EEG noise (22 nV) which was reflected in the poor shape of the raw data points and associated ROEX fit.

In twelve subjects the recorded RMS amplitude (RAC shape) difference between test runs in the swept-method was less than 10nV. Visual inspection of these results (see Figure 5), shows RACs that appear to be an acceptable tuning curve shape and are similar in both recording sessions (with the exception of subject 3 as shown in Figure 7). In all of the swept-method recordings the correlation between test run amplitudes $r > 0.50$ ($p < 0.01$) with the exception of subject 1 where $r = 0.27$, subject 11 $r = 0.47$, subject 14 $r = 0.37$ and subject 20 $r = 0.46$. Subjects 1, 11, 14 and 20 also had the highest percentage swept method RMS differences. In the fixed method the correlation of amplitudes between test runs was significant in only 8 out of the 20 subjects compared with all 20 subjects in the swept method. The difference in number of subjects having correlation significance was likely to be due to the smaller number of data points in the fixed method (8 frequencies) compared with 350 (overlapping segments) in the swept method. The mean RAC-tip frequency was

2250 Hz for the swept and 2239 Hz for the fixed method. The expected RAC-tip was 2000 Hz since the ASSR stimuli carrier frequency was set to 2000 Hz.

In the swept method the test time was fixed at 120 sweeps (32 minutes). The fixed method had a variable test time of 15 sweeps for each of the 8 recordings required per RAC, plus additional time for rejected epochs. The mean number of rejected epoch in each fixed method RAC was 129, giving a mean total test time of 35 minutes per fixed method RAC.

The mean absolute tip difference between repeated estimated RAC-tip frequencies for the fixed and swept methods was 116 Hz and 158 Hz, respectively. Repeatability of RAC-tip estimation, RAC measurements, and agreement between the swept and fixed methods was assessed using the methods proposed by Bland and Altman (1986). They provide a method of plotting results from two different test methods measuring the same clinical factor and repeat measures of an identical test method to determine agreement and repeatability. This is achieved by considering the measured value difference that would be considered of clinical significance, for example, causing a change in treatment or treatment outcome. In order to determine the acceptability of clinical tests the repeatability coefficient and limits of agreement are compared with the clinically significant value. The limits of agreement are an estimate of the likely range of difference in measured values between two test types calculated from the mean difference of the sample plus and minus twice its standard deviation. The repeatability coefficient is an estimate of the likely worst case difference in same test method repeated measured values. The repeatability coefficient is estimated by calculating the sample differences which are the differences between the first and second measured value in each subject. The repeatability coefficient is then calculated from twice the standard deviation of the differences (standard deviation calculated using an assumed mean difference of zero). Clinical tests agree to an acceptable level if the maximum expected disagreement calculated from the two test methods (the repeatability coefficient) is less than a test value difference that would be of clinical significance. Similarly, if the repeatability coefficient is less than a clinically significant value then the test is acceptable for clinical use.

Figure 9 shows repeatability plots for the fixed and swept methods. The test agreement plot between swept- and fixed- method estimated RAC-tips is shown in Figure 10. In the repeatability and method agreement plots the difference in measurement (here, the first tip frequency minus the second tip frequency (Y-axis)) is plotted against mean measurement (here, the mean of the two measurements (X-axis)) for each subject. The upper and lower lines show the repeatability limits (Figure 9), or agreement limits for the first test run (Figure 10), with error bars representing their 95% confidence interval calculated from the 95% point on the t-distribution for (n-1, 95% two tailed) of the standard error of $\pm 2\sigma$ estimated by $(3\sigma / n)$ (Bland and Altman 1986). The estimated repeatability coefficients, calculated using an assumed mean difference of zero, were 389 Hz for the swept method and 342 Hz for the fixed method. The limits of agreement between swept- and fixed-method estimated RAC-tips (fixed-swept) were -355 Hz to 432 Hz with a mean difference of +39 Hz. However, due to the small sample size and high variance of the estimated RAC-tip frequencies the 95% confidence ranges of the repeatability and agreement coefficients are high at approximately 400 Hz (20% of the measured value). The estimated repeatability coefficient for the 10 subjects where the recorded EEG noise was below 10nV in both recording sessions of the swept method was 422 Hz, indicating that excluding results due to high EEG noise did not reduce (improve) the repeatability coefficient of the RAC-tip estimation.

In ASSR threshold estimation using the MASTER system the presence or absence of a response is determined by its significance above the background EEG noise floor. The

recorded ASSR amplitude was significantly above the EEG noise floor ($p < 0.01$) at all masker frequencies in both swept method test runs in eight subjects, and in five of these eight subjects in both fixed method test runs. The individual recordings where the response amplitude remained significantly above the EEG noise floor ($p < 0.01$) for all analyzed masker frequencies are indicated by use of bold font in Appendix Tables 2 to 5, Supplemental Digital Content 1. In all other recordings (shown in normal typeface on the tables) the response amplitude was significantly above the noise floor ($p < 0.01$) when the masker frequency was far from the stimulus carrier frequency but fell below significance as the masker frequency approached the stimulus frequency.

The RAC recordings were affected by the background EEG noise level. The recorded EEG noise levels in the RAC recordings are shown in Appendix Table 6, Supplemental Digital Content 1. Stevens et al. (2009) recommend disregarding ASSR threshold estimation measurements where the recorded noise level is higher than 10nV. In 51 out of 80 RAC recordings the recorded EEG noise levels were below this criterion.

4 Discussion

These results demonstrate that it is possible to record RACs by measuring the reduction in amplitude of the ASSR in the presence of narrow-band masking noise with variable center frequency and a fixed sound pressure level. We investigated two methods of varying the masking noise. In the first method the masker center frequency was continuously swept over a 32 minute recording time for each of the two repetitions. In the second method eight separate recordings with different fixed masker center frequencies were recorded with a mean test time of 35 minutes per RAC. There was no significant difference in RAC-shape repeatability between the two methods, but the subjects reported it was easier to relax during the swept-method recordings as there were no sudden changes in masker frequency.

Thus within 32 minutes we can obtain an objective estimate of the tuning curve at one particular frequency in one ear. This is much faster than the time required to obtain a tuning curve on the basis of estimating the thresholds for recording the response at different frequencies of noise since several (at least 2 and likely 3 or 4 recordings must be made to bracket the threshold level) and these recordings must be averaged sufficiently to be sure that no response is recorded at the sub-threshold levels. It takes longer to demonstrate that a response is not there than to measure a response amplitude.

The response curves we estimated were constructed on the basis of the change in amplitude of the response with the changing frequency of the masking noise. The curves track the response along one intensity level on the tuning curve. The resultant data plots are likely related to the tuning curves constructed on the basis of threshold data but the closeness of the relationship is not known and we do not know how robust the relationship might be in pathological cases.

4.1 Repeatability

The repeatability of behavioral PTC testing has yet to be fully established. Sek et al. (2005) performed repeated recordings of fast-PTCs in normally hearing adults but they did not calculate repeatability coefficients and the published data is limited to repetitions in three subjects. Malicka et al. (2009) recorded PTCs in normally hearing school aged children and found that the mean *absolute* tip difference was approximately 13% of the measured tip frequency for 1-kHz fast-PTC recordings and approximately 10% for 4-kHz fast-PTC recordings, indicating similar accuracy to our objective method where the mean absolute tip difference was found to be 158-Hz (8% of the 2-kHz signal). Further work is needed to establish the accuracy of both behavioral and objective tip estimation. In cases where a

suspected DR covers an extensive range of frequencies, for example, starting within the high-frequency range amplified by a conventional hearing aid and extending to all frequencies above that point, the accuracy of PTC-tip and RAC-tip estimation must be sufficient to confirm the presence of a DR by pinpointing the DR boundary within an acceptable accuracy. This could be achieved by multiple PTC testing with frequencies outside and inside the suspected DR. The necessity to record RACs at multiple signal frequencies would increase test time. However, it may be possible to record RACs from multiple signal frequencies simultaneously as it is possible to record ASSR from simultaneous multiple stimuli separated by octave intervals (John et al. 1998).

The repeatability coefficients of the estimated RAC-tip frequencies that we recorded in the swept method were high at approximately 20% of the measured value (twice SD of differences, 389 Hz). In this study the RAC test signal was limited to one test frequency and therefore additional work is needed to determine the normative expected range of RAC-tip frequencies for other audiometric test frequencies. The range of normative tip frequencies that we recorded were wide but, as previously discussed above, multiple measurements around the suspected DR boundary and well into the DR could enable DR diagnosis. Furthermore, the range of fast method PTC-tips recorded from a 4-kHz stimuli in children was 3770 Hz to 4760 Hz for ascending masker direction and 3345 Hz to 4915 Hz for descending masker direction indicating a wide range of expected normal PTC-tip frequencies from behavioral testing (Malicka et al. 2009). Presently, repeatability coefficients for current behavioral (PTC) methods have not been fully studied and so it is not possible to directly compare our estimated repeatability coefficient from objective testing with that of behavioral PTC methods. Since, in general, hearing assessment and hearing aid gain calculation is performed at octave intervals it is unlikely that it will prove necessary to estimate a DR boundary within an octave band. Furthermore, additional research into the effect DRs have on hearing aid outcomes and the best clinical practice methods to rehabilitating subjects with DRs is required. Presently, widely used hearing aid prescription formulae do not distinguish gain settings for subjects with or without DRs (Dillon 2001) and so the DR boundary estimation accuracy required to set a hearing aid has yet to be established. However, Moore and Alcantara (2001) stated that the current PTC test methods are able to diagnose the presence of a DR providing the test frequency is more than 500 Hz from the DR boundary. Therefore, a repeatability coefficient of less than 500 Hz is required to distinguish subjects with and without a DR, providing the test frequency condition is met. The repeatability coefficient of our proposed swept RAC method is within this limit (389 Hz).

4.2 Tip frequency shift

The mean estimated RAC-tip was higher than the expected frequency of 2-kHz. This has previously been reported by other experimenters using the ASSR and has been referred to as “detuning” (Markessis et al. 2009). The detuning effect has been shown to occur in behavioral PTC testing, using standard pure tone and AM signals and in electrophysiological ASSR ETC recordings (Markessis et al. 2009; Moore 1978). However, the upward tip frequency shift tendency found in PTCs (behavioral) recorded using simultaneous masking was not found when using forward masking (Moore 1978). The tip shifts were likely to have been due to cochlear non-linearities (Moore 1978). It is possible that the position of the tip is influenced by cochlear nonlinearities as simultaneous maskers are used in both behavioral and electrophysiological tuning curve methods. It is not possible to eliminate nonlinear effects, such as suppression, by the use of forward masking in our method, as the ASSR requires a continuous stimulus. In our experiment testing was limited to normally hearing subjects and at a level of 50 dB SL. Cochlear suppression is dependant on outer hair cell compressive basilar membrane function (Ruggero et al. 1992). If suppression is the reason

for the detuning it is possible that in hearing-impaired subjects (depending upon the severity of hearing loss) it would not occur, or occur to a lesser degree, either due to the high signal levels (where suppression effects are reduced) or due to poorly functioning outer hair cells (Ruggero et al. 1992).

A further possible explanation for the upward tip shift is suggested by the work of Folsom (1984) who found an upward shift in tip frequency for RACs recorded using the ABR. In Folsom's experiments the upward tip shift was found for 1-kHz filtered click stimuli presented at 60 dB above the subjects behavioral threshold (SL), but was not found for stimuli presented at 40 dB SL. This difference was attributed to the basal spread of excitation (upward frequency spread) of the higher level signal. In the present study we recorded RACs at 50 dB above subjects pure tone threshold. It is therefore possible that the upward tip shift we recorded may not occur at lower signal levels in normally hearing subject.

The fact that we recorded RACs at constant SL rather than SPL resulted in different dB SPL levels for each subject within a 20 dB range. We used constant SL in order to match the requirements of recordings in hearing impaired subjects. Recordings in hearing impaired subjects would require constant SL as constant SPL would not be appropriate due to threshold differences depending on the level of hearing impairment. We could not record RACs at dB SPL levels likely to be required in subjects with suspected DRs, for example with severe hearing loss, as such dB SPL levels would exceed safety and comfort levels of our normally hearing subjects. It is likely that in hearing impaired subjects the recorded RACs would be broader, as tuning is broader at higher signal levels and in impaired ears (Moore 2007). It is also possible that differences in recorded RAC shape and bandwidth between subjects in our present study occurred due to the different dB SPL levels used. A further point for discussion is that, as discussed above, previous studies that recorded masking profiles of the ABR revealed upward shifted tip frequencies at higher levels due to spread of excitation towards the basal regions of the cochlea. It is therefore possible in our present study that spread of excitation occurred to a greater degree in subjects where higher levels were used, thus leading to higher tip frequencies in those subjects. It could have been possible to eliminate this confounding factor by recording at constant dB SPL. There was no correlation between signal level and the mean tip frequency of two recordings in each subject ($r = 0.37$, $p = 0.11$) in our dataset. However, this factor may have been hidden by the effect of the accuracy RAC tip frequency dominating the differences in the tip frequency that may have been present due to signal level.

4.3 Effect of EEG noise

In many of the test sessions the recorded EEG-noise levels were above the recommended 10nV limit set by the recommended procedures for threshold estimation with ASSR (Stevens et al. 2009). In some cases throughout all recording sessions, subjects had high EEG-noise levels.

If the recommended 10nV EEG-noise level were adopted for this test in clinical use the data we recorded in normally hearing adults showed that in many cases repeat recordings would be required. The positive correlation between EEG-noise (mean noise of two recordings) and the RMS curve differences (shape difference) (fixed method $r = 0.83$, $p = 0.00$; swept-method $r = 0.66$, $p = 0.00$) indicate that reducing EEG-noise is an important factor to manage in the recording. It could be possible to apply our new RAC-tip estimation method during subject sedation as the ASSR has been found to be recordable in sedated hearing-impaired infants (Roberson et al. 2003) and thus reduce the EEG noise floor (Cohen et al. 1991). However, our results showed that there was no improvement in the repeatability coefficient of RAC-tip estimation when RACs which were recorded when the EEG-noise higher than

the 10nV level where excluded from analysis. The validity of the curve depends on the signal-to-noise ratio of the recording and thus is also affected by the amplitude of the response. It is possible that some of the noisy subjects had larger responses and that this compensated for their increased noise levels.

However, it is possible that transient noise across a sweep distorted the RAC shape since the response we measure is signal plus noise amplitude. In the swept method it was not possible to use on-the-fly noise rejection. Some evidence that high transient EEG noise levels can adversely affect the swept-method RAC shape comes from comparing the normal averaging to the variance-weighted averaging. In some cases RAC shapes were improved when variance-weighted averaging was used. Subjects 18 and 20 were chosen to highlight the least and greatest difference in RAC shape with and without variance-weighted averaging as shown in Figure 11. In subject 18 variance weighting has little visible effect upon the RAC shape or tip position, whereas in subject 20 the variance weighting improves the shape and tip-position of the RAC, indicating that there must have been higher levels of variability of EEG noise throughout this test run or high level transient noise peaks which possibly could have been eliminated with on-the-fly noise rejection were it possible with our test equipment. In future it would be desirable to use further customized software that could reject an entire sweep if any individual epoch within the sweep contained an EEG sample above the EEG-noise cut-off level but this would increase test time. The currently available software only allowed EEG rejection at the epoch level which in the case of the swept method would have lead to the swept masking noise becoming desynchronized with the recorded EEG as the epochs were not interchangeable.

4.4 Method advantages

Our new proposed method offers two advantages compared to the RAC masking level threshold-based methods of Markessis et al. (2009), who recorded ETCs using the ASSR, and Klein and Mills (1981), who recorded ETCs using the ABR. Firstly, since the method is not threshold based single test runs are sufficient at one signal-to-masker level ratio to estimate RAC-tip frequency leading to an overall test time of 32 minutes per RAC. Therefore, the swept and fixed methods we propose both offer test time advantage compared to the Klein and Mills (1981) method that took at least two hours per ETC and the Markessis et al. (2009) method that took an average of 150 minutes per 10 point ETC. Secondly, lower overall test sound pressure levels are required when compared to both current ETC and PTC methods which could enable the test to be performed in subjects with a high severity of hearing loss. Further research is needed to validate the method in hearing-impaired subjects with and without DRs to ensure it is able to accurately diagnose them.

5 Conclusions

This study demonstrates that it is possible to record RACs by measuring the reduction of ASSR amplitude in narrow-band masking noise. An RAC at a single test frequency can be recorded in approximately 32 minutes whilst the subjects relax or sleep. It is important to ensure that the subject recording conditions are conducive to low EEG-noise as higher noise correlates with poorer RAC-shape repeatability. The range of estimated RAC-tip frequencies for a 2-kHz signal in normally hearing subjects, calculated from the mean estimated tip frequency and the repeatability coefficient of the swept method, lies between 1861 Hz to 2639 Hz. If similar results are found in hearing-impaired subjects this could be adequate accuracy to enable the diagnosis of a DR with a boundary one octave away from the test signal. DR diagnosis could be confirmed by further RAC recordings over a range of test signal frequencies on both frequency sides of the possible DR boundary. This current study was limited to a 2-kHz test signal and so further studies are also required, recording RACs from a wide range of signal frequencies to further support the use of this test. Additionally,

work is needed to further define the accuracy, by examining the repeatability coefficient within a smaller confidence interval (larger sample size) and the method must also be validated in hearing-impaired adult subjects with and without DRs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Spectral content of 2-kHz 95-Hz exponentially modulated ASSR signal. Y-axis shown in digital sample units, signal generated with overall digital amplitude of 10.

Figure 2.

Schematic illustration of swept method averaging process. Upper panel shows the signal (grey line) and masker (black line) centre frequency plotted against test time in each sweep repetition. Lower panel represents the resultant RAC. Dashed arrows show the averaging process of the points of the EEG-waveform obtained for the upward and downward sweep direction.

Figure 3.

Illustration of modulation envelope phase difference between the averaged upward and downward sweep direction analysis. Plots represent the 95 Hz modulation envelope. X-axis shows the time position in the sweep relative to the start of the sweep (Left panel) and end of the sweep (Right panel). Vertical lines show the first and second analysis points which were 0 ms and 20 ms on the left panel, 0 and -20 ms on the right panel.

Figure 4.

Examples of ROEX curve fitting to RAC data. Left panel, example ROEX curve fitting with r parameters constrained (solid line) and unconstrained (dashed line). Right panel, example ROEX curve fitting with r parameters (r_{upper} and r_{lower}) separate for frequencies above and below the fitted tip (solid line) and with one single r parameter for all masker frequencies (dashed line). ROEX parameters as shown in Appendix Table 1, Supplemental Digital Content 1.

Figure 5.

Individual RACs recorded for the fixed and swept method for each subject in two sessions. Solid line shows the first test run recorded using the swept masking method. The filled circles represent the first test run recorded using the fixed masking method. The dashed line and unfilled circles are the second test run swept and fixed test method, respectively. Y-axis shows the response amplitude as a percentage of the not-masked amplitude recorded in the same test run as each RAC.

Figure 6.

Gross mean RACs recorded in 20 subjects from two recording sessions for the fixed (left panel) and swept (right panel) masking method. Solid line is the mean relative amplitude, dashed line shows the ROEX curve fit of the mean. The mean amplitude ± 1 SD is shown as the upper and lower dotted lines in the right panel and as error bars in the left panel.

Figure 7.

Example of RACs recorded using the swept masker method. Solid lighter line shows the unmasked response amplitude recorded in the same test session as the RAC. Dashed lighter line shows the amplitude below which the response was not considered significantly higher than the background EEG-noise. Darker solid curve shows the response amplitudes, darker dashed line shows the ROEX curve fit to the response amplitudes.

Figure 8.

Example RACs recorded using the fixed masker method. Solid lighter line shows the unmasked response amplitude recorded in the same test session as the RAC. Dashed lighter line with data points marked as 'X' shows the amplitude below which the response was not considered significantly higher than the background EEG-noise. Filled circular data points show the response amplitudes for each masker frequency, darker dashed line shows the ROEX curve fit to the response amplitudes.

Figure 9.

Fixed and swept method RAC-tip estimation recording repeatability. Data points show the mean estimated RAC-tip frequency (x-axis) vs. the difference in tip frequency between two test runs (y-axis). The upper and lower dashed lines show the assumed mean difference $\pm$ the repeatability coefficient. The error bars show the 95% confidence limits for the repeatability coefficient.

Figure 10.

Fixed and swept masking method RAC-tip estimation agreement for the first test run of each method. Data points show the mean estimated RAC tip from the two methods in the first recording (x-axis) vs. the difference in tip frequency between the two recording methods in the first test session. The upper and lower dashed lines show the mean tip difference $\pm$ the calculated limits of agreement. The error bars show the 95% confidence limits for the calculated limits of agreement.

Figure 11. Swept method RACs for subject 18 (left) and 20 (right). RAC curve produced from identical EEG data with (solid line) and without (dashed line) variance weighted averaging. The axes are as for Figure 5.