Otoacoustic emissions at different click intensities: invariant and subject-dependent features

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Otoacoustic emissions at different click intensities: invariant and subject-dependent features. J Appl Physiol 95: 2299–2305, 2003. First published August 22, 2003; 10.1152/japplphysiol.00667.2003.—A study of click-evoked otoacoustic emissions (CEOAEs) elicited at stimulation intensities from 35 to >80 dB was carried out by recurrence quantification analysis on signals from both normal and hearing-impaired subjects. In normal subjects, a clear scaling of determinism with increasing stimulation intensity was observed in the click intensity range from 41 to 89 dB. Outside that range and, in particular, above its upper end, subject-dependent features appeared in the form of different maximal determinism levels. A comparative analysis of responses from hearing-impaired subjects with conductive hearing losses and sensorineural hearing losses suggested that the principal contributor to this behavior is the middle ear and allowed us to discriminate the two pathologies solely on the basis of CEOAEs. These observations are consistent with a simple phenomenological model of the auditory periphery in which different functional modules are sequentially recruited at increasing stimulus intensities, with a consequent rise in CEOAE coherence.

hearing physiology; click-evoked otoacoustic emissions; recurrence quantification analysis; determinism

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quantify the dynamic features of CEOAEs in a somewhat unexpectedly broad range of conditions. As for hearing-impaired subjects, in particular, we show that the measure of determinism is actually able to discriminate between conductive hearing losses (CHL) and sensorineural hearing losses (SHL), corresponding to middle and inner ear disorders, respectively. Besides the physiopathological implications, the possible clinical use of such a finding seems also relevant.

METHODS

CEOAE Recording Protocols

The CEOAE signals were collected in the Audiology Department of the University of Ferrara, Italy. They were responses to click stimuli with a relatively flat acoustic spectrum for frequencies between 0.5 and 5 kHz, according to a nonlinear stimulation protocol (12, 13). Each stimulus sequence contains four clicks: three clicks of positive polarity followed by a fourth click with inverse polarity and intensity equal to the sum of the previous three. The CEOAEs were recorded in a sound-attenuated booth by using an Oto dynamics ILO-292 system running software version 5.6 with standard adult ILO probes. In the study of normal ears, CEOAEs were collected from nine adult female subjects (age range: 26.8 ± 7.1 yr) chosen on the basis of the absence of 1) any pathophysiological objective signs of clinical relevance, and 2) any systematic pharmacological treatment within 3 mo from the acquisition of the signals. Sixteen responses were collected for each subject, corresponding to levels of click stimuli ranging from 35 to >80 dB. The intensity of the stimuli was monotonically decreased down from the maximum level in discrete steps of 3-dB sound pressure level. The time elapsed between exposures to different stimuli was, on the average, 1 min (time necessary for the operator to set the new stimulus settings on the ILO-292 system). In this context, there was no hysteresis in the recorded signals. Responses were high-pass filtered at 500 Hz. Every accepted response was the average of at least 260 individual responses for each subject.

The same recording setup was adopted for the collection of the transient-evoked otoacoustic emission signals aiming to compare normal ears and ears with hearing deficits; for each subject, at least four transient-evoked otoacoustic emission responses were recorded, elicited by click stimuli with intensity of 50, 65, 70, and 80 dB, respectively. Forty-nine subjects were tested, and a total of 224 responses (108 from the left and 116 from the right ear) were recorded. The subjects were subdivided into three classes: normal (10 cases), SHL (21 cases), and CHL (18 cases).

RQA and Determinism

At difference with classic methods of signal analysis (e.g., Fourier), RQA is not limited by specific requirements on signals, like stationarity or length, and, compared with other semiempirical techniques [e.g., wavelets (24)], it has the virtue of a relatively simple implementation and intuitive meaning. The RQA procedure projects a signal into a multidimensional space by means of an embedding matrix having as columns the lagged copies of the original signal by a fixed delay. On this matrix, RQA identifies time correlations that cannot be observed in one dimension. The first step of the analysis is the computation of the Euclidean distance between every row pair in the embedding matrix, to work out a distance matrix and visualize it in the form of a recurrence plot (6, 9, 23). In this plot, any pair of rows whose Euclidean distance falls below a user-defined threshold (radius) marks a recurrence, and the corresponding point is darkened. Finally, RQA works out a number of variables describing the nonlinear dynamic features of the signal, which proved very useful in several contexts, ranging from physiology to molecular biophysics (5, 10, 11, 17, 25, 26).

The RQA variables used in this study are defined as follows: Rec (percentage recurrence) is the density of recurrent points in recurrence plots; Det (%determinism) is the fraction of recurrent points that occur in lines parallel to the main diagonal; Ent (entropy) is computed by the application of the Shannon formalism to the length distribution of determinis- tics. Besides their operational definition, all of the RQA variables are endowed with a specific meaning. In particular, Rec is a measure of both periodic and autosimilar features in the signal, Det indicates the degree of structuring of the phase space or of the system, i.e., of regions in which the system lies for longer times than expected by chance alone, and Ent is linked to the richness of deterministic structuring (23).

The Rec, Det, and Ent were calculated with the following choice of working parameters: lag (delay in the embedding procedure) = 1; embedding dimension (number of elements in the rows of the embedding matrix) = 10; radius = 15; and line (minimum number of consecutive, recurrent points scored as deterministic) = 8. This choice derives from our previous experience in applying RQA to CEOAEs (27, 28), and, in all cases, the results were checked for their robustness against alternative choices. The radius is expressed as a percentage of the maximum Euclidean distance between rows, to make variance and amplitude independent of the observed dynamic features of the signal. This is relevant in consideration of the known large differences in amplitude induced by different stimulation intensities (14).

Statistical Analyses

Principal component analysis. Principal component analysis (PCA) is a quite common statistical technique (3) whose aim is to project a multivariate data set into a space of orthogonal axes, called principal components, selected, one after the other, on the basis of the maximal variance explained in the space of the original variables. The presence of correlations between the original variables allows for the reduction of dimensionality of the data set in the new space without noticeable loss of information. Because the principal components are, by construction, orthogonal to each other, a clear-cut separation of the different and independent features characterizing the data set is made possible. In other words, going from the original variables to a principal component space, any statistically significant observation made on any axis points to a truly autonomous effect. We applied PCA on the space spanned by the RQA variables to estimate the subject-dependent features of CEOAEs.

Linear discriminant analysis. Linear discriminant analysis (LDA) is a supervised pattern recognition method whose aim is to find the best linear discrimination between two (or more) groups of statistical units defined into a multivariate space.

In the case of two a priori defined A and B groups, LDA generates a linear function of the "symptoms" variables $X_1 - X_n$ of the form

$$Y = \sum A_i X_i$$

1 The programs for RQA are available from http://homepages. luc.edu/~cwebber/ in self-extracting file format (RQA62.EXE).
so that the plane defined by Eq. 1 separates the elements pertaining to the A and B groups as neatly as possible (e.g., all the A group elements are above the plane, and all the B group elements are below).

**Inferential statistics.** To generate statistical inferences from independent CEOAE observations, only one ear per subject was considered in the analysis. Statistical comparisons of Det in CEOAE signals were performed by means of Student’s t-test, ANOVA, and LDA.

**RESULTS**

Figure 1 shows CEOAE responses elicited by low (35 dB; A) and high (80 dB; B) stimulation intensities, with the latter being in the region of signal saturation. In clinical studies (12, 13), CEOAE responses are evoked by high-intensity stimuli (>65 dB), and the hearing function is evaluated in terms of the waveform reproducibility. This is indicated by the so-called Repro variable, which corresponds to an estimate, on a 0–100 scale, of the Pearson correlation between two CEOAE digitized waveforms (Fig. 1, A and B) recorded in alternating sampling times. For the 35-dB stimulus, the resulting signals are barely distinguishable from the baseline noise, and the two waveforms are quite poorly correlated (Repro = 4.4 at 35 dB, compared with Repro = 99.1 at 80 dB). A pictorial view of the chaotic nature of the signals elicited by low-stimulation intensities is provided in Fig. 1A, inset. As shown in Fig. 1, when going from a very-low-stimulus intensity (A) to the CEOAE-saturating phase (B), the signal increases both in amplitude and in organization: an irregular and noisy pattern (see also inset) changes into a markedly oscillatory behavior. It should be noted that, by virtue of RQA, attention may exclusively focus on the variation in the signal ordering on increasing the stimulation intensity, and any change in variance and amplitude is ruled out.

Figure 2 reports the changes of the deterministic character (Det) of CEOAEs from normal subjects, at click intensities from 35 to 80 dB.

For all subjects, three different phases can be distinguished in the Det changes: a first phase (A) corresponds to a subthreshold stimulation; a second phase (B), corresponding to a steady increase in Det, and a third phase (C), where Det does not change with the increasing stimulus intensity. During phase A, the signals appear quite noisy, whereas phase C corresponds to quasi-harmonic, extremely ordered signals (see also Fig. 1). The boundaries of phase B were estimated according to the following procedure: first, for each subject and for each possible couple of click intensities, we fitted the Det values (shown as averages...
in Fig. 2) to straight lines; second, we chose the click intensity couple maximizing the average slope of the straight lines, namely 41 and 59 dB. The corresponding linear fitting of Det for each subject is reported in Table 1.

The clear linear relations (scaling) between Det and stimulus intensity, observed for all subjects in the intermediate phase (B), do not allow us, under our conditions, to distinguish among individuals in that stimulus intensity range, which is at odds with the other two phases (see below). This points to a relative invariance of phase B, even in the presence of relatively large deviations in slopes (0.91–2.50), and suggests the presence of similar self-organization mechanisms in all individuals, possibly based on the sequential involvement of different portions of the auditory periphery. A previous study (28) indicated that, in the saturation phase, the CEOAEs show a marked individual character and act as a sort of “auditory fingerprint” of each individual. To contrast the common trend observed in the scaling phase (B) with the individual character of the saturation phase (C), first we generated, for each phase, the space of the RQA variables: Rec, Det, and Ent. Then the matrix, which has the signals as rows and the corresponding RQA variables as columns, was subjected to a PCA. The percentage of total variability explained by the first two principal components (PC1 and PC2) was 85.42% for PC1 and 9.23% for PC2, corresponding to an almost complete RQA description of the signals.

To highlight differences (if any) in the discrimination ability of phases B and C, independent analyses were carried out for the two phases. Then the signals were projected onto a principal component space derived from a previous set of 78 responses of normal, adult subjects, recorded (under identical conditions) at high-stimulus intensity (28). The lack of subject-related features in Fig. 2 suggests the same type of scaling of Det in all subjects, even with some differences in the slope due to factors related to biological and measurement variability. This general resemblance points to a similar mechanism of progressive involvement of the corresponding structures in the auditory system by stimuli of increasing intensity. This phenomenon could be analogous, in a way, to the progressive magnetization of paramagnetic materials subject to a static, magnetic field.

Given the presence in the plateau phase of markedly individual specific features (28), it is important to validate whether these features can be evidenced even in the subthreshold phase, because that may reflect some morphoanatomic, stimulus-independent peculiarity of individual systems. A statistical confirmation of the latter conjecture lies in the significant correspondence in the relative positions of single signals in the A and C phases. This link was demonstrated by 1) computing all of the differences in determinism between any of the 9 × 8/2 = 36 possible couples of the nine signals, both by 10.22±0.33.6 on July 14, 2017 http://jap.physiology.org/ Downloaded from

Table 1. Scaling of determinism with stimulation intensity

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Slope (Reciprocal dB)</th>
<th>Intercept</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>1.40</td>
<td>−14.43</td>
<td>0.649</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>1.78</td>
<td>−20.25</td>
<td>0.890</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>0.91</td>
<td>4.31</td>
<td>0.834</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>1.29</td>
<td>−15.91</td>
<td>0.628</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>2.50</td>
<td>−62.08</td>
<td>0.865</td>
</tr>
<tr>
<td>6</td>
<td>23</td>
<td>1.77</td>
<td>−41.86</td>
<td>0.843</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>1.82</td>
<td>−20.01</td>
<td>0.713</td>
</tr>
<tr>
<td>8</td>
<td>21</td>
<td>2.23</td>
<td>−47.99</td>
<td>0.863</td>
</tr>
<tr>
<td>9</td>
<td>38</td>
<td>2.27</td>
<td>−51.74</td>
<td>0.878</td>
</tr>
</tbody>
</table>

The % determinism (Det) values (shown as averages in Fig. 2) were fitted to straight lines for each subject in the B region in Fig. 2, according to the expression Det = slope × dB + intercept. The regression parameters and \( R^2 \) values are listed in columns 3–5, respectively. Notice the absence of any correlation between slope values and subject age (Pearson’s \( r = 0.33 \)) and the similar quality of the linear fitting indicated by the \( R^2 \) values.
The difference in Det between the two groups of pathological [sensorineural hearing loss (SHL) and conductive hearing loss (CHL)] signals studied in this work (row 1), as well as between each of 2 pathologies and the control group (rows 2 and 3). In all cases, differences were estimated at each of the 4 studied click intensities (50, 65, 70, and 80 dB) by t-test (columns 2–5) and by repeated-measures ANOVA (column 6). In parentheses, the significance (P) is reported.

This is true at all of the studied stimulation intensities, namely 50, 65, 70, and 80 dB. Table 2 also indicates that 1) signals from the CHL class, compared with the normal group for the amount of Det, were significantly different; 2) a repeated-measures ANOVA computed over all of the four intensities indicates a marked effect of decreased determinism, passing from normal to CHL; and 3) no statistically significant difference between the normal and the SHL groups was scored by both t-test and ANOVA run under identical conditions. Thus the possible hypothesis that only SHL, being directly related to the function of the hair cells, could significantly change the CEOAE dynamic properties and make them distinguishable from normal responses was contradicted, and the role played by the middle ear in determining the overall shape of CEOAEs was emphasized. The combined influence of OHCs and middle ear on the CEOAE dynamic features is confirmed by representing, in a principal component space, the information in the whole set of RQA variables for the two groups of pathological signals under different stimulation regimes.

In Fig. 4, the clustering in a reference PC1-PC2 plane (identical to the one in Fig. 3) of CEOAEs from normal and pathological subjects, according to the same procedure applied to the data in Fig. 3, is reported for the two extreme stimulation conditions: 50 and 80 dB. The results reported in Table 2 indicate that this is true at all of the studied stimulation intensities, namely 50, 65, 70, and 80 dB. Table 2 also indicates that 1) signals from the CHL class, compared with the normal group for the amount of Det, were significantly different; 2) a repeated-measures ANOVA computed over all of the four intensities indicates a marked effect of decreased determinism, passing from normal to CHL; and 3) no statistically significant difference between the normal and the SHL groups was scored by both t-test and ANOVA run under identical conditions. Thus the possible hypothesis that only SHL, being directly related to the function of the hair cells, could significantly change the CEOAE dynamic properties and make them distinguishable from normal responses was contradicted, and the role played by the middle ear in determining the overall shape of CEOAEs was emphasized. The combined influence of OHCs and middle ear on the CEOAE dynamic features is confirmed by representing, in a principal component space, the information in the whole set of RQA variables for the two groups of pathological signals under different stimulation regimes.

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(A) and 80 dB (B). Under both conditions, the data show that signals of the CHL class, in contrast to SHL, fall clearly outside the region of physiological variability of healthy subjects, and LDA scored 84 and 90% correctness in separating CHL from SHL signals at 50 and 80 dB, respectively. As a consequence, discrimination between CHL and SHL is possible in both cases, although it is easier at the higher stimulation regime.

DISCUSSION

In clinical applications, CEOAEs are usually studied at stimulation intensities of 80 dB, which is a “response saturation” region (see Fig. 2), where any decrease in the Repro variable, namely the Pearson correlation between the A and B waveforms, can be directly associated with a decreased response intensity and hence to a sort of hearing deficit. At lower stimulation intensities, the less favorable signal-to-noise ratio induces an overall decrease of Repro values and makes the study of response signals difficult. In addition, at these stimulus levels, it is difficult to evaluate any physiological and mechanical factors on the global performance of the auditory system (1, 21).

We have tackled this problem by taking advantage of the remarkable independence of the RQA from changes in signal amplitudes, as illustrated in Fig. 5. The figure shows, in contrast to Repro, the much lower sensitivity to click intensity of Rec, the basic RQA variable. Thus the relatively stable trend of Rec allows the estimation of any significant change in the Det (see METHODS and Fig. 2) at stimulation levels that are usually out of the range of clinical and basic investigations.

In previous papers (27, 28), our laboratory could demonstrate that, at high-stimulation intensity, there appears a marked degree of individuality in both adult and newborn normoacoustic subjects, producing distinguishable CEOAEs in terms of RQA variables. In the present work, we explored a click intensity region well below the response saturation for normoacoustic and hearing-impaired subjects. In the former case, our observations can be summarized as follows.

1) There is a progressive increase in Det of CEOAE signals scaling with the increasing click intensity, in a subject-invariant fashion. However, in different subjects, different plateaus are reached starting at −60-dB stimulation intensity, and such differences correlate with those estimated at very low intensity.

2) There is a clear separation between common and subject-dependent portions in the dynamic features of CEOAEs, as outlined by statistical analysis.

A plausible interpretation is that the common portion reflects the recruitment of an increasing number of OHCs: in other words, we postulate that the increasing determinism corresponds to an increased synchronization of the active elements processing the acoustical stimuli, whereas the subject-dependent portion may be related to specific features of the passive, conductive section of the auditory system.

In an audiological clinical context, the term “recruitment” indicates the copresence of hyperacusis (super-sensitivity to normal sounds) and fuzzy (poor frequency discrimination) hearing, namely the most common symptom of neurosensorial hearing loss. In fact, assuming that 1) each hair cell belongs to a functional unit sensitive to specific frequency band and contributing to the intensity perception by a “unit” loudness, and 2) in case of malfunction of some cells the adjacent ones are “recruited” for an extra workload added to their own one, numerous phenomena of this sort produce an exceedingly loud and noisy sound (and speech) perception. To account for the higher determinism in CEOAE responses at higher stimulation intensities, we actually used recruitment in a broader sense, namely to indicate an increasing number of physiologically coordinated modules. Although this assumption seems fully justified by the analogy to similar situations occurring in many other systems, particularly in the context of motor unit activation (8), it leaves unclarified, however, the basic question concerning the identification and location of the involved modules in the organ of Corti.

In the case of CEOAE responses from hearing-impaired subjects, the pathological events involving different regions of the auditory system were helpful to confirm in the middle ear the origin of the individual features previously reported for normal hearing responses (28). Only the CHLs (related to the middle ear structures) were clearly distinguishable from the control group in a global analysis of CEOAE determinism at all of the considered stimulation intensities. The SHLs (related to the inner ear structures) were responsible for relatively minor changes in the signals, mainly in the scaling region (B region in Fig. 2). This point is more evident by examining the data from Fig. 4, in which the differences between the SHL, CHL, and controls are represented in a principal component space and appear to be more significant at a stimulation level of 80 than at 50 dB.

An immediate use of these results and, in particular, the unequivocal distinction between SHL and CHL could be envisaged in clinical applications. In fact, if SHL and CHL can, in principle, be discriminated also by the Repro variable, the information on the signal dynamics extracted through the RQA variables would appear more reliable because 1) it is less affected by noise and/or possible instrumental flaws over a wide range of stimulation intensities, and 2) it is solidly rooted on the global morphoanatomic features of the auditory system.

DISCLOSURES

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