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Spontaneous cellular vibrations in the guinea-pig temporal-bone preparation

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Abstract

Mechanical vibrations of Hensen cells were measured with a laser-heterodyne interferometer in the guinea-pig temporal-bone preparation without the application of an external acoustic stimulus. Smoothed periodograms (spectral-density estimates *v.* frequency) were constructed from the velocity *v.* time waveforms recorded from individual cells. Several peaks were seen in the periodograms at levels as high as 10 dB above the noise floor, indicating the presence of spontaneous vibrations. The frequencies at which the peaks were located differed in different preparations, indicating that the observed peaks were not caused by the presence of ambient noise or ambient vibrations. Furthermore, vibrations were seen only in fresh preparations. The tuning curves of cells from which spontaneous vibrations were measured (determined by applying an external stimulus to the ear) had single principal peaks. Several peaks in the periodogram were found to be located within the principal-peak region of the tuning curve. The spontaneous response does not arise from noise filtered through the tuning curve which would have a single peak. We propose that these spontaneous vibrations originate at the outer hair cells and are the source of spontaneous otoacoustic emissions in the ear.

Introduction

Spontaneous otoacoustic emissions are weak, narrowband acoustic signals emitted from the ear in the absence of an external stimulus. The presence of these emissions has been established in man and in a variety of animals (Kemp, 1979, 1981; Wit and Ritsma, 1980; Zurek, 1985; Plinkert *et al.*, 1990; van Dijk and Wit, 1990). Although they have been observed at higher frequencies, these emissions are most frequently seen in the 2-kHz region. Their amplitude is generally less than 20 dB SPL.

Kemp (1981) postulated that otoacoustic emissions may be related to the active amplification process in the cochlea when the positive-feedback mechanism has gone out-of-control. Zenner (1987) suggested that otoacoustic emissions result from active mechanical processes in the outer hair cells of the cochlea. It has also been proposed that otoacoustic emissions may be related to the motile properties of outer hair cells. Spontaneous mechanical fluctuations have previously been seen in isolated turtle cochlear ciliary bundles, but not from the reticular lamina of these cells (Crawford and Fettiplace, 1985).

In the region of the third turn of the guinea-pig cochlea where our vibration measurements were made, Hensen cells display tuning with characteristic frequencies in the range between 500 and 1000 Hz. In addition to this a.c. tuning, the lengths of these cells change when sound is applied to the ear. This length-change response is sharply tuned (Brundin *et al.*, 1991), as has been demonstrated in the isolated-cell preparation (Brundin *et al.*, 1989). At a given place in the cochlea, the characteristic frequency of the displacement response and the a.c. tuning is the same (Brundin *et al.*, 1991),

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but the displacement response is more sharply tuned.

In view of these observations, it has been important to ascertain whether sensory cells vibrate spontaneously. To make this determination, we measured the time-course of the spontaneous velocity of Hensen cells in the third turn of a fresh guinea-pig temporal-bone preparation with no acoustic stimulus applied to the ear (Keilson *et al.*, 1993). It is known that Hensen cells are tightly coupled to outer hair cells (ITER, 1989) so that the vibrations of outer hair cells will be seen at Hensen cells.

The use of a non-parametric signal-analysis scheme was necessary to detect whatever natural frequency components were present in the velocity waveform (Keilson *et al.*, 1993). The smoothed (and averaged smoothed) periodogram was used to provide a reduced-variance estimate of the spectrum. In this paper we examine (1) the stability of the observed cellular vibrations (2) the trade-off between the confidence interval and bandwidth (frequency resolution) of the velocity spectral density estimate and (3) the relation between the tuning curve and the velocity spectral density estimate.

Methods

The general method of recording cellular vibrations in the cochlea has been described in detail elsewhere (ITER, 1989). The measurements reported in this paper were made in the third turn of the guinea-pig temporal-bone preparation. The preparation was immersed in a plexiglass tank filled with tissue-culture medium (MEM) through which oxygen was bubbled. An objective lens (Olympus 20X) with a custom-made dipping cone was utilized for viewing the cochlea with the optical sectioning microscope and for measuring the cellular vibrations with the heterodyne interferometer. The interferometer can measure the vibration of an object on which the microscope is focused, provided that the carrier-to-noise ratio is sufficiently high (ITER, 1989). The 2 μm-diameter laser spot on the object, seen through the microscope eyepiece, can be placed on a selected cell by moving the temporal bone with an x-y-z micropositioning system (ITER, 1989).

To measure the frequency tuning curve, sound was applied to the ear via a plastic tube connected to an acoustic driver. A flexible plastic probe tube, placed with its tip within 1 mm of the tympanic membrane, was used to measure sound pressure levels. The sinusoidal electrical signal applied to the acoustic driver was generated using a 386-based computer system coupled to a 16-bit D/A converter. The interferometer response was passed through an anti-aliasing filter, a 16-bit D/A converter, and then stored in the hard disk of the computer system. Details of the digital signal-processing system have been described earlier (ITER, 1989).

The smallest vibration amplitudes can be measured with the interferometer only when the carrier-to-noise ratio is high. This situation prevails when the microscope is focused on a Hensen cell because of its high reflectivity. In fact, it has so far only been possible to detect spontaneous vibrations at Hensen cells.

The interferometer utilized optical sectioning. The light reaching the photodetector originates principally from a volume of tissue about 2 μm in diameter and 10 μm in depth. The vibrations were therefore recorded from small regions of single cells.

With the interferometer focused on a selected Hensen cell, mechanical vibrations were measured with and without the application of an acoustic stimulus. The data samples were obtained at 40-μs intervals. In the presence of the stimulus, the response was averaged 25 times and placed in a buffer consisting of 1024 bins. For measuring the spontaneous response, the interferometer output was stored in 4096 bins. A set of successive (unaveraged) responses was obtained. In later experiments, longer sequences of the unaveraged response were stored in $128 \times 1024 = 131{,}072$ bins, each 40μs long.

Results

The details of the signal-processing technique are described elsewhere (Keilson *et al.*, 1993). In our implementation of periodogram analysis, the data are first subjected to an FFT and the squared magnitude of the complex frequency components is determined. The result is then inverse-transformed into the time domain, whereupon the temporal data may be properly regarded as an estimate of the autocovariance function. A temporal smoothing or window function (e.g. a Bartlett window) is then applied to this time function which, when transformed into the frequency domain, becomes the reduced variance estimate of the spectral density (Schwartz and Shaw, 1975). We collected long streams of data, as well as successive multiple sets of spontaneous vibration data. This enabled us to further reduce the variance of the spectral estimate by averaging the smoothed periodograms.

A single smoothed periodogram of spontaneous velocity vibrations of a Hensen cell is shown in Fig. 1(a). Averaging 25 smoothed periodograms substantially reduces (improves) the confidence interval, as shown in Fig. 1(b). Three peaks in the velocity spectral density estimate can now be clearly seen around 574, 732 (the largest peak),

and 952 Hz. Two low-frequency peaks are also observed at 128 and 201 Hz. Six sets of such averaged periodograms, taken successively, show excellent repeatability of the data. Indeed, the intra-record variability is far less than that seen among different preparations. The frequencies of the peaks were stable over as long a period as the data were collected (over some 25 s in this case). As

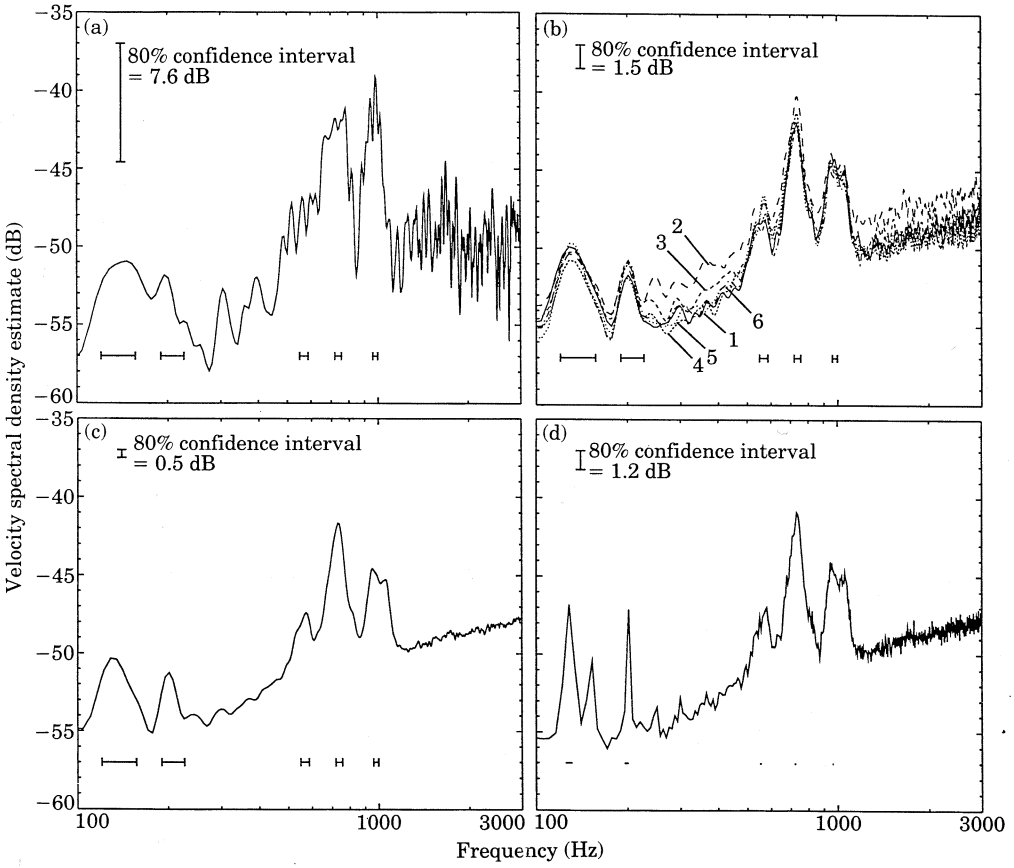

Fig. 1. (a) A single smoothed periodogram provides a spectral density estimate for the spontaneous vibration velocity of a Hensen cell. The estimate is provided in dB with a reference level of 1 cm²/s². The number of bins is N = *3072, each with a width of 40μs. A Bartlett window with L = 1024 bins was used, and the remaining 1024 bins were zero-padded. The bandwidth, representing the frequency resolution associated with this window, is 37 Hz. Data tapering was incorporated. The 80% confidence interval for the estimate is 7.6 dB; several spectral peaks between 100 and 200 Hz and between 600 and 1200 Hz appear to be present. (b) Averaging of the smoothed periodogram provides a further reduction in the variance. In this case, 25 single smoothed periodograms, such as the one represented in (a), have been averaged together. Six successive sets of 25 averages are shown. The bandwidth is again 37 Hz but the 80% confidence interval is reduced to 1.5 dB from 7.6 dB. The six data sets show very similar behaviour, indicating that the same kind of spontaneous vibrations are present in each of them. (c) The confidence interval can be further reduced by averaging all of the periodograms together. In this case, 174 single smoothed periodograms, such as the one represented in (a), have been averaged. The bandwidth is still 37 Hz but the 80% confidence interval is now reduced to 0.5 dB. Five spectral peaks can be clearly discerned: these are centred at 128, 201, 574, 732, and 952 Hz. The three highest-frequency peaks are in the vicinity of the characteristic frequency of the cell, which is 659 Hz. (d) An improvement in the frequency resolution can be attained by averaging the 174 single smoothed periodograms (each with* N = *4096 bins, no zero padding) using a rectangular window, with L = 2048 bins and data tapering. Comparing this with the Bartlett-window averaging shown in (c), the bandwidth is seen to be reduced from 37 to 6 Hz, but at the expense of the confidence interval which is increased from 0.5 to 1.2 dB. In the vicinity of the characteristic frequency of the cell, the increased frequency resolution does not result in a narrowing of the spectral peaks; this indicates that the widths of these peaks are true values and are not limited by the signal processing. In contrast, the number of peaks at lower frequencies is increased from two to three with the improved frequency resolution.*

shown in Fig. 1(c), averaging 174 periodograms further smooths the data, with five unambiguous peaks present.

To determine if the 37-Hz bandwidth in Figs 1(a,b,c) limits the frequency resolution of the peaks, we alter the signal processing to reduce it to 6 Hz in Fig. 1(d). The shapes of the peaks at 574, 732, and 952 Hz are essentially the same as observed with 37-Hz resolution. However, the low-frequency peaks become far sharper, and three of them are resolved instead of the two that are present in Fig. 1(c). A given set of data can be analysed either to minimize the confidence interval or the bandwidth. The trade-off between the two is illustrated in Fig. 2.

The relationship between the tuning curve and the spectral density estimate of the velocity vibration is shown in Fig. 3. The three peaks of the spontaneous vibration are located under the main peak of the tuning curve. However, the largest peak of the spectral density estimate, which lies at 732 Hz, does not coincide precisely with the peak of the tuning curve, which is at 659 Hz. The low frequencies of the spontaneous vibration are located under the tail of the tuning curve, between 100 and 200 Hz.

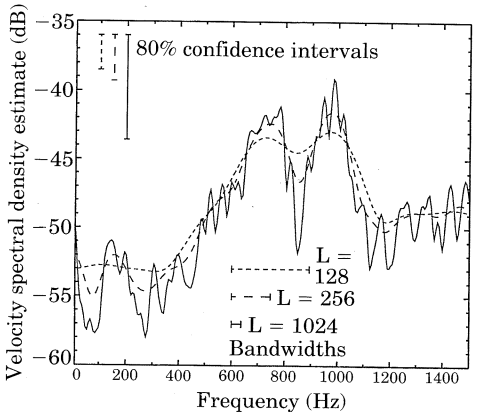

Fig. 2. Bandwidth and confidence interval of the velocity spectral density estimate can be traded off against each other by suitable construction of the periodogram. The data are the same as those analysed in Fig. 1(a). We again make use of a single smoothed periodogram with a Bartlett window, N = 3072 bins, zero padding of 1024 bins, and data tapering, but now the truncation point L is altered from 128 bins (------), to 256 bins (– – –), to 1024 bins (——). As L increases, the concomitant decrease in bandwidth, and increase in confidence interval, is evident. The solid curve (L = 1024) is the same as that shown in Fig. 1(a), except that frequency is now displayed on a linear scale.

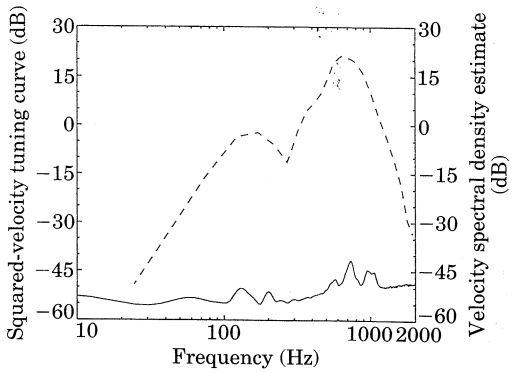

Fig. 3. Squared-velocity tuning curve (– – –) collected with a tone at a level of 105 dB SPL and spontaneous velocity spectral density estimate (——) for the Hensen cell shown in Figs 1 and 2. The solid curve is identical to that shown in Fig. 1(c). The peak of the tuning curve lies about 65 dB above the peak of the spectral density estimate. The peaks of the spontaneous velocity vibrations lie under those of the tuning curve.

Discussion

The smoothed-periodogram method of spectral analysis is particularly useful for revealing hidden periodicities, such as those shown in Figs 1(c, d). Multiple narrow spectral components are evident in the spontaneous response of some Hensen cells that are located in the third turn of the guinea-pig cochlea.

The periodogram reveals multiple peaks, with amplitudes that substantially exceed background noise levels. These peaks are located within the frequency region of principal tuning of the cell, but they are far sharper. The spontaneous vibrations in the periodogram therefore do not arise from filtered noise (which would have a single peak). Furthermore, these peaks cannot arise from external sources, such as mechanical vibrations of the components, or sound leaking in from outside the chamber, since they are present only in fresh preparations and their frequencies differ in different preparations.

We believe that the origin of these spontaneous vibrations is at the outer hair cells. We have been unable to measure the vibrations directly from these cells because their optical reflectivity is low (ITER, 1989).

Our results indicate that some sensory cells in the cochlea vibrate spontaneously; these vibrations are likely to be related to otoacoustic emissions. It is therefore of substantial interest to compare the characteristics of the spontaneous cellular vibrations we have measured with those of spontaneous otoacoustic emissions (SOAE). (a) In our experiments, the spontaneous vibrations were

measured in the guinea-pig temporal-bone preparation. The SOAE have been observed from guinea-pig ears by Evans *et al.* (1981) and by Ohyama *et al.* (1991, 1992). (b) Our periodograms show multiple peaks of different strengths. Multiple-peaked SOAE are common (Probst *et al.*, 1991). (c) In the region of the third turn where our measurements were made, characteristic frequencies range from 500 to approximately 1000 Hz. The spectral components of the spontaneous cellular vibrations also lie within this range. Indeed, SOAE observed in the ear canal are also usually in the 1–2 kHz region (Zurek, 1985; Ohyama *et al.*, 1991). One reason for the preference for this frequency range may be that the reverse coupling between the malleus and the tympanic membrane is poor at high frequencies. Since the SOAE are only about 15 dB above the noise floor, a loss of even 10 dB in coupling would be sufficient to make them unobservable. (d) The noise floor of our periodogram measurements (with 174 averages and a bandwidth of 37 Hz) is equivalent to about −4 dB SPL; the largest peak of the spontaneous vibrations is equivalent to about +7 dB SPL. These values are comparable with those of SOAE (Zurek, 1985). (e) Spontaneous cellular vibrations were observed in about 25% of the preparations we examined and the width of the largest peak was approximately 90 Hz. Spontaneous otoacoustic emissions in guinea pigs were observed by Ohyama (1991, 1992) in 21% of the animals tested. The maximum level of emissions was 26.8 dB SPL, and the average level was 12.1 dB SPL. The width of the spectrum at a point −6 dB with respect to the peak was in the range 30–50 Hz. Our cellular-vibration observations are therefore comparable with the SOAE reported by Ohyama.

In summary, we have found that the temporal-bone preparation, together with laser-heterodyne interferometry and a non-parametric signal-analysis scheme, has made it possible to study extremely small vibrations directly at the cellular level. These studies may elucidate the mechanisms underlying SOAE.

Acknowledgements

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