Published in final edited form as:

Hear Res. 1991 February ; 51(2): 215–230.

Application of a commercially-manufactured Doppler-shift laser velocimeter to the measurement of basilar-membrane vibration*

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Abstract

A commercially-available laser Doppler-shift velocimeter has been coupled to a compound microscope equipped with ultra-long-working-distance objectives for the purpose of measuring basilar membrane vibrations in the chinchilla. The animal preparation is nearly identical to that used in our laboratory for similar measurements using the Mössbauer technique. The vibrometer head is mounted on the third tube of the microscope's trinocular head and its laser beam is focused on high-refractive-index glass microbeads (10–30 μm) previously dropped, through the perilymph of Scala tympani, on the basilar membrane. For equal sampling times, overall sensitivity of the laser velocimetry system is at least one order of magnitude greater than usually attained using the Mössbauer technique. However, the most important advantage of laser velocimetry vis-à-vis the Mössbauer technique is its linearity, which permits undistorted recording of signals over a wide velocity range. Thus, for example, we have measured basilar-membrane responses to clicks whose waveforms have dynamic ranges exceeding 60 dB.

Keywords

Laser Doppler-shift velocimetry; Laser vibrometry; Laser heterodyne interferometry; Basilar membrane; Cochlear mechanics

Introduction

Many methods have been used, with varying degrees of success, for the study of the submicroscopic vibrations of the basilar membrane of the mammalian cochlea. Such methods have included standard microscopy combined with stroboscopic illumination (Békésy, 1960) the Mössbauer technique (e.g., Johnstone and Boyle, 1967; Rhode, 1971; Sellick et al., 1982; Robles et al., 1986b), the use of capacitive probes (e.g., Wilson and Johnstone, 1975; Le Page and Johnstone, 1980; Le Page, 1987) laser 'fuzziness detection' (Kohlöffel, 1972) electronic speckle-pattern laser interferometry (Neisswander and Slettemoen, 1981), fiber-optic laser interferometry (Nokes et al., 1978; Albe et al., 1982), laser homodyne interferometry (Khanna and Leonard, 1982 and 1986) laser heterodyne interferometry (Khanna et al., 1989) and the fiber optic lever (Le Page, 1989). Among these techniques, it seems fair to state, the Mössbauer method has so far proved the most productive: its application pioneered the modern era of measurements of cochlear mechanics (Johnstone and Boyle, 1967), and it led to the discovery of basilar membrane nonlinearity (Rhode, 1971) and to the two series of reports in which, exceptionally, nonlinear, sensitive

*Portions of this paper were presented at the 119th. Meeting of the Acoustical Society of America (Ruggero and Rich, 1990a).

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and sharply frequency-tuned responses have been demonstrated in individual basilar membranes, namely in guinea pig (Sellick et al., 1982, 1983a and 1983b; Patuzzi and Sellick, 1983; Patuzzi et al., 1982, 1984a, and 1984b) and in chinchilla (Robles et al., 1986a, 1986b and 1989; Ruggero et al., 1986; Ruggero and Rich, 1990c). Nevertheless, the Mössbauer technique suffers from certain drawbacks, particularly the severely nonlinear nature of its transduction characteristic, which precludes accurate measurement of nonsinusoidal waveforms and restricts reliable measurements to a narrow range of velocities (Lynch et al., 1982), and the relatively long data-sampling times required by the probabilistic nature of gamma radiation. In addition, there is a possibility (Kliauga and Khanna, 1983), so far supported only by indirect observations (Sellick et al., 1982; Robles et al., 1986b), that the presence of a radioactive metal foil on the basilar membrane may cause significant tissue damage. In any case, even in the absence of damage due to radiation itself, the mere placement of the metal-foil radioactive source on the basilar membrane is fraught with difficulty and very often results in trauma.

Laser heterodyne interferometry is a potentially powerful replacement of the Mössbauer technique because it is free of the latter's main disadvantages: it is deterministic, has an essentially linear input-output characteristic, and does not involve ionizing radiation. Similar to the Mössbauer technique, laser heterodyne interferometry is a velocity-sensing method, in which the velocity of a target is derived from the Doppler shift of the frequency (energy) of photons reflected from the moving target. Previous applications of laser heterodyne interferometry to the measurement of mammalian-ear vibrations have entailed extensive ad-hoc engineering work (e.g., Buunen and Vlaming, 1981; Willemin et al., 1988 and 1989; see also Nokes et al., 1978). We describe here how a laser velocimeter, bought "off-the-shelf", has been coupled to a standard compound microscope to permit relatively rapid measurements of the velocity response of a basal site in the chinchilla basilar membrane. As a validation of the new method, we show initial results for tonal stimulation that match the frequency tuning previously demonstrated in chinchilla basilar membrane using the Mössbauer technique (Robles et al., 1986b). Finally, we present measurements of basilar membrane responses to clicks which could only be recorded with highly distorted waveforms when using the Mössbauer technique. An application of laser velocimetry similar to the present one has been independently developed by Nuttall et al. (1989, 1990, 1991).

Methods

Laser vibrometer hardware

A laser Doppler-shift fiber vibrometer system was purchased from Dantec Elektronik (Skovlunde, Denmark; see Acknowledgements). This system, which provides a voltage signal proportional to target velocity, consists of a 20 mW He-Ne laser (Spectra Physics 106-1) a vibrometer head (model 41X60, Fig. 1) and an electronic frequency tracker (model 55N20). The (red) monochromatic and coherent light beam produced by the laser is coupled into the vibrometer head via a 5-m. single-mode glass fiber. The vibrometer head both emits the light beam toward the target and receives, on the same optical axis, the light reflected from the target. The vibrometer head contains a Bragg (optoacoustic) cell, used to frequency-shift the reflected beam so as to allow measurement of zero mean velocity and detection of the velocity sign. By means of the optical heterodyning technique, the original (outgoing) beam and the reflected beams are compared. In general, the frequency of the reflected light is changed according to the velocity of the reflector; the frequency change (Doppler frequency shift) is a decrease when the reflector moves away from the detector and an increase when it moves toward the detector. The Doppler-shift detector, a pair of differentially-coupled photodiodes, generates an output electrical current containing the instantaneous Doppler frequency shift, which is a linear function of target velocity. This

current is fed to the frequency tracker, which generates a voltage output (1–10 V) proportional to target velocity. The frequency tracker has 7 sensitivity settings, ranging from 0.3164 mm/s/V to 316.4 mm/s/V in 10 dB steps. Selecting a higher sensitivity lowers the effective noise floor but also reduces the bandwidth and the maximum velocity measurable without distortion. A more detailed description of the principles of operation of the Dantec vibrometer has been published (Buchhave, 1975).

Vibrometer / microscope coupling

In order to measure the velocity of a restricted portion of the basilar membrane it is necessary to focus the laser beam onto a small spot and to visually observe its position. These goals are achieved by means of a standard compound microscope to which the vibrometer head is coupled (Fig. 1). A metallurgical microscope (Olympus BHMJ) was selected because in this type of microscope illumination is delivered to the target through the objective (since the cochlea must be observed via reflected, rather than transmitted, light) and focusing is done via translation of the objective (rather than motion of a stage, which would be inconvenient for our purposes). The microscope has been equipped with 5 × and 20 × ultralong working-distance objectives (Mitutoyo M Plan Apo 5 ×, N.A. 0.14, and 20 ×, N.A. 0.42). A very long working distance (20 mm with the 20 × objective) is necessary due to the geometry of the bulla and cochlea, which prevents placement of a microscope objective close to the basilar membrane. The microscope is attached to a sturdy stand equipped with a boom arm mounted on roller bearings and can be translated smoothly along the boom-arm axis and also rotated along three orthogonal axes. Both the microscope stand and the stereotaxic animal head-holder are mounted on a vibration-isolation cradle (Ehrenreich Photo-Optical Industries, Model 78240) which rests on a table within a sound-insulated room (Industrial Acoustics 1204A).

The vibrometer head is coupled to the microscope via a standard TV-camera adaptor (Olympus MTV-3) mounted on the third tube of the microscope's trinocular head (Fig. 1). The adaptor contains a lens which replaces the standard focusing lens normally supplied with the vibrometer head; together with the microscope objective, this lens focuses the laser beam onto the focal plane of the microscope. At present, the beam at the test target has a 30- μ m diameter. Although we hope, eventually, to reduce this diameter to 10 μ m, the currently-used microscope/vibrometer head-coupling yields satisfactory velocity recordings in in-vivo experimental situations. The (nominal) 20-mW laser actually produces a 12-mW beam, whose power is reduced by losses at the optic fiber and at the lenses, prisms and beam splitters of the vibrometer head; at the output of the vibrometer head it is 6 mW. This power is further reduced by the microscope optics to 1 mW as the beam exits the 20 × objective.

Preliminary animal preparation

In almost all respects, the animal surgery and cochlear manipulations for laser-vibrometry recordings from the chinchilla basilar membrane are identical to the procedures previously detailed for recordings in our laboratory using the Mössbauer technique (Robles et al., 1986b). The only methodological differences consist of attachment of the stereotaxic head-holder base to a 2-axis micropositioner (Newport 400) and the placement of glass microbeads, instead of a radioactive metal foil, on the basilar membrane. Glass microbeads were chosen as light reflectors because they reflect light incoming from a wide range of angles along the same optical axes of incidence. In contrast, using a flat mirror would require orienting the microscope objective at an angle perpendicular to the plane of the basilar membrane, which is a very difficult or impossible task. The glass beads (MO Sci Corp., Rolla, MO) were selected on the basis of their high index of refraction (2.1) and their size (10–30 μ m). An index of refraction substantially higher than that of standard glass (about 1.5) is necessary to maximize reflections of the laser beam at the interphase between

perilymph (refractive index about 1.3) and the glass bead. The size of the beads is a compromise between, on the one hand, providing a large reflective surface (maximizing reflected light) and, on the other, minimizing the mass load on the basilar membrane and maximizing the spatial resolution of measurements.

Chinchillas were initially anesthetized with a subcutaneous injection of ketamine hydrochloride (100 mg/kg). Additional anesthetic doses (sodium pentobarbital) were administered intraperitoneally at intervals throughout the experiment to abolish muscular responses to a strong pinch of a hind-paw. The chinchilla's body was partially enveloped with a servo-controlled heating pad to maintain rectal temperature at 38°C. The trachea was intubated but forced lung ventilation was used only rarely, when the animal was unable to breathe by itself. The left pinna was removed and the bony meatus was chipped away to obtain direct access to the tympanic membrane. The animal's skull was exposed over the entire left bulla and the posterior portion of the right bulla. Two metal pins, inserted into the right bulla, and a snout and maxillary clamp provided a solid attachment of the head to the stereotaxic head holder. The left bulla was widely opened to provide access to the cochlea and a clear path for the laser beam. A silver-ball electrode was placed on the round window and the tensor tympani muscle was routinely cut. A plastic speculum, the front end of an acoustic-stimulus delivery system (which includes two Beyer DT-48 earphones), was placed in contact with what remained of the left bony external meatus and sealed to it by means of ear-impression compound.

Cochlear surgery, acoustic system and data gathering

Upon completion of the foregoing preparations, sound pressure (amplitude and phase) was measured as a function of stimulus frequency near (within 2 mm of) the tympanic membrane by means of a precalibrated miniature microphone (Knowles 1785) equipped with a metal probe tube. Acoustic stimuli were produced by a computer-controlled digital arbitrary-waveform generator (Ruggero and Rich, 1983). The acoustic calibration (with a resolution of 10 Hz between 30 and 200 Hz, and 100 Hz between 200 and 24000 Hz) was stored electronically and used by the computer during the experiment to generate sinusoidal stimuli with specifiable sound pressure levels (SPL, re 0.0002 dyne/cm²) and phases. Immediately after the in-situ acoustic calibration, compound action potential (CAP) pseudo-thresholds (10 μ V) were measured by means of the round window electrode at 1/2 octave intervals in the frequency range 500–16000 Hz. These thresholds were subsequently compared with CAP thresholds measured throughout the experiment to assess the physiological state of the cochlea.

To gain access to the basilar membrane, the otic capsule was opened – under observation with an operation microscope (Zeiss Opmi 1, 25 $\times$ or 40 $\times$ magnification) – in the cochlear 'hook' region, overlying the Scala tympani and basilar membrane at a site 3.5 mm from the round window. A hole was made in the bony capsule by first deeply scoring a trapezoidal groove upon its surface and then pulling out the bony trapezoid in one piece, using a metal hook. Transient bleeding into the cochlea occurred commonly, but the resulting blood coagulum usually did not settle upon the basilar membrane in the area of interest but rather flowed apicalward and tended to remain near the perilymphatic surface. Any coagulum obstructing a clear view of the basilar membrane could often be removed without causing physiological damage, as judged by the CAP thresholds.

Placement of the glass microbeads was accomplished using either a metal probe or a glass pipette attached to a micromanipulator. In either case, the most important requirement for proper placement was that the basilar membrane adjacent to the hole in the otic capsule be on the horizontal plane. When the metal probe (tungsten wire, 100 μ m diameter) was used, a few microbeads were first attached to its tip by means of a sucrose solution, which was

allowed to dry. The scala tympani was then partially drained and the probe tip was rapidly lowered below the perilymph surface, attempting to fully wet the beads before they separated from the probe. When this was achieved, the microbeads gently settled on, and remained attached to, the basilar membrane. Alternatively, glass microbeads immersed in physiological saline were introduced by capillary action into a glass pipette (tip 70–100 μm in diameter), the tip of which was then submerged in the perilymph of the partially drained scala tympani. The beads were dislodged and allowed to fall onto the basilar membrane by applying positive air pressure, by mouth, into a plastic tube fitted to the back end of the glass pipette.

Positioning of the compound microscope with attached vibrometer head was carried out in four stages. In the first stage, the ‘best’ angle for observation with the operation microscope was noted and recorded. The ‘best’ angle was one which yielded a clear image of the target microbead(s), roughly centered within the edges of the otic capsule hole, and which minimized image distortions (due to the perilymph meniscus) and unwanted light reflections (from the perilymphatic surface or from the external surface of the otic capsule). In the second stage, the compound microscope (bearing the laser head) was oriented at the selected angle. While observing the target microbead under standard-light epi-illumination via the $5\times$ objective, the positions of the objective and animal platform were adjusted, using the microscope’s focusing knob and the 2-axis micropositioner, so as to bring the target microbead into sharp focus at the center of the field of view. In the third stage, the $5\times$ objective was replaced with the (parfocal) $20\times$ objective and the laser light was turned on, after covering the ocular eyepieces with green acetate sheets to protect the experimenters’ eyes from the red laser light. Finally, adjustments of the focus and the animal platform were repeated, first to obtain an intense and clear reflection of the laser beam off the glass/perilymph interphase and then to maximize the signal-to-noise ratio at the vibrometer electrical output. Such adjustments were repeated as required during data collection to compensate for small changes of the fluid meniscus at the perilymph/air interphase which changed the focus and/or optical position of the glass bead(s) and resulted in deterioration of the quality of the velocity signal.

The electrical output of the Doppler-frequency tracker is a voltage whose instantaneous value, in the range 1–10 V, is proportional to velocity. This signal was frequency filtered with a pass band of 26–15000 Hz before analog-to-digital conversion (maximum sampling rate of 40 kHz) under computer control. Except at intense stimulus levels, the velocity signal was usually buried in noise: with a tracker-sensitivity setting of 3.164 mm/s/V the RMS noise level was typically about 300 mV, equivalent to a velocity of 0.9 mm/s. To extract the velocity signal, the responses to 1000–4000 repetitions of identical stimuli were averaged together, thus improving the signal-to-noise level by 30–36 dB by reducing the effective noise levels to 5–9 mV, equivalent to 16–28 $\mu\text{m/s}$. Fourier transformation of the time waveform permitted further reductions of the effective noise level, depending on sampling time; with a sample time of 3 s, the effective noise floor was 3–5 $\mu\text{m/s}$.

Results

Responses to tone pips

For the sake of coherence and simplicity, we present in this paper data obtained from the basilar membrane of a single chinchilla (L13). In all qualitative and most quantitative respects, these basilar-membrane data are representative of those recorded with the laser vibrometer in several other relatively normal chinchilla cochleae. Fig. 2 shows basilar membrane velocity responses to tone pips with frequency ranging between 6 and 10 kHz, at an intensity of 50 dB SPL. Each waveform, the average of responses to 1024 repetitions of 3.8-ms tone pips presented every 25 ms, generally resembles that of the acoustic stimulus.

The largest response was evoked by 8-kHz tone pips, with responses diminishing monotonically toward higher and lower frequencies. A conspicuous feature of several waveforms is a ringing at their onset and/or offset. The ringing was presumably elicited by the click-like nature of the onset or offset stimulus ramps, which in this case were rather abrupt (0.58 ms rise/decay time between 10% and 90% of full height). When more gradual onset and offset ramps were used, the ringing was absent.

In order to study the frequency and level dependence of the responses to tone pips, Fourier transforms were computed from averaged velocity waveforms such as those of Fig. 2. The resulting peak-velocity amplitudes at the stimulus center frequency were then plotted against stimulus frequency as a family of iso-SPL contours (Fig. 3). Such plots have been previously named ‘response areas’ in the context of responses of cochlear afferent fibers to tones (e.g., Rose et al., 1971; Geisler et al., 1974). Indeed, the family of basilar-membrane iso-SPL curves depicted in Fig. 3 resembles the ‘response areas’ of high-characteristic-frequency cochlear afferents in several respects. At low stimulus levels, response bandwidth is restricted to a narrow range near 9 kHz (characteristic frequency, CF). At higher levels, the response bandwidth grows asymmetrically, encompassing a wider frequency range below CF than above. At CF, response growth with stimulus intensity is highly nonlinear: with a stimulus change of 77 dB, velocity amplitude rises by only some 17 dB (i.e., at a rate of 0.2 dB/dB). Response growth is also compressively nonlinear at 8 kHz over much of the intensity range. At 7 kHz, nonlinear growth occurs only at the highest stimulus levels, with linear growth at low and moderate levels. At 6 kHz and below, responses are quite linear, except for a few irregularities that probably reflect measurement errors. The different rates of response growth as a function of frequency cause a shift of the maximal response toward lower frequencies at the highest stimulus levels: whereas CF (i.e., the stimulus frequency yielding the largest response at low stimulus levels) was 9 kHz, at 80 dB SPL the largest response was evoked by 6-kHz tone pips. Nonlinear compressive response growth persisted at frequencies immediately above CF; at 11 kHz, a stimulus level change of 30 dB lead to a response growth no larger than 4 dB.

Noteworthy in Fig. 3 is the fact that seemingly reliable responses are shown for velocities as low as 4 $\mu\text{m/s}$. Considering that each data point represents less than 4 s of data, the sensitivity of measurement using the laser Doppler vibrometer appears to be between one and two orders of magnitude better than we previously attained using the Mössbauer technique in otherwise identical chinchilla basilar-membrane preparations. With the latter technique, the lower limit of usable responses was around 30 $\mu\text{m/s}$, with a sampling time of 100–200 s.

Because of the limited dynamic range of measurement achievable with the Mössbauer technique, basilar-membrane responses to tones recorded with that method have been usually displayed as iso-response curves. Therefore, to permit comparison of the present results using laser vibrometry to those previously obtained in our laboratory by means of the Mössbauer technique, isovelocity contours have been derived by logarithmic interpolation from the data shown in Fig. 3 and have been plotted in Fig. 4 (solid lines) for velocities of 0.1, 0.2 and 0.4 mm/s. As expected from the linear growth of responses with stimulus intensity at stimulus frequencies sufficiently lower than CF (< 6 kHz), the three isovelocity contours are spaced at these frequencies at vertical intervals of 6 dB. At frequencies near CF (8–11 kHz), on the other hand, the isovelocity contours are spread out over intervals much larger than 6 dB, in accordance with the compressive nonlinearity noted in Fig. 3. At 9 kHz ($\approx$ CF) the 0.1-mm/s curve is 17 dB more sensitive than the 0.2-mm/s curve, and the 0.2-mm/s curve is 25 dB more sensitive than the 0.4-mm/s curve. The tip-to-tail ratios (measured between CF and 2 kHz) are 78 dB, 68 dB and 54 dB, respectively, for the isovelocity curves at 0.1 mm/s, 0.2 mm/s and 0.4 mm/s. All three isovelocity contours are

sharply frequency-tuned but, in accordance with the iso-SPL contours of Fig. 3, the 0.1-mm/s curve has a higher Q_{10} (7.53; Q_{10} = CF divided by the bandwidth at 10 dB re CF level) than the 0.2-mm/s curve (Q_{10} of 6.22) or the 0.4-mm/s curve (Q_{10} of 4.36). In addition, there is a shift of CF toward a lower value (8 kHz) at higher velocities.

Comparison of the 0.1 mm/s iso-velocity curve with its Mössbauer counterpart (the average of recordings from five normal chinchilla cochleae at approximately the same basilar-membrane site; Robles et al., 1986b) indicates substantial similarities. The Mössbauer 0.1-mm/s curve has a CF of 8.35 kHz, a Q_{10} of 5.7, and a tip-to-tail ratio of 67 dB. The curve obtained by means of laser vibrometry is somewhat more sensitive and more sharply tuned, and has a larger tip-to-tail ratio than the Mössbauer average curve. These differences probably reflect slightly different physiologic states of the relevant experimental cochleae; however, it is not yet clear whether the differences are due to the measurement technique or to other factors. What is clear is that the use of laser vibrometry can yield tuning curves with properties generally considered to indicate relatively 'intact' or normal cochlear mechanical function and fully comparable to those obtained with the Mössbauer technique (Sellick et al., 1982; Robles et al., 1986b).

Responses to clicks

With sinusoidal stimulation, the Mössbauer technique permits the recording of response-related signals which, given an all-important assumption, can be used to compute the magnitude and phase of the underlying sinusoidal velocity responses in the range 0.03-1 mm/s. The assumption, of course, is that the basilar membrane response is actually sinusoidal. Therefore, within the limited practical confines of the dynamic range of the Mössbauer technique, this method yields iso-response measures fully equivalent to those obtainable with the (essentially linear) laser vibrometer. However, in the case of velocity signals that contain large variations in level, such as in responses to clicks and other transient stimuli, the limitations of the Mössbauer technique can preclude altogether the achievement of faithful recordings. An illustration of the difficulties inherent in measuring basilar-membrane responses to clicks with the Mössbauer technique is given in Fig. 5, taken from Robles et al. (1976). Panel (d) is a plot of the Breit-Wigner function, which relates gamma-photon rate to instantaneous velocity. It is apparent that, because the photon rate saturates at relatively low velocities, waveforms with high-velocity peaks will tend to be clipped. In addition, because in the Breit-Wigner function for the particular combination of source and absorber there is a trough coinciding almost precisely with zero velocity, the waveform will be full-wave rectified, causing an almost complete polarity ambiguity. Fig. 5 shows that, while the effect of full-wave rectification can be overcome in extracting the underlying velocity waveform from the gamma-photon counts, peak clipping is unavoidable at sufficiently high signal levels. As illustrated in Fig. 6, Doppler vibrometry does not suffer from equivalent limitations.

Fig. 6 shows a series of basilar-membrane velocity responses to rarefaction clicks presented at peak levels of 35–95 dB SPL, recorded with the laser vibrometer. In the left column, the velocity waveforms are displayed with uniform scaling. These waveforms should be compared to the photon-rate histograms in panel (a) of Fig. 5. In contrast to the Mössbauer histograms, the laser vibrometry signals are neither rectified nor peak-clipped and they therefore display, without need for further processing, the actual velocity waveforms of the basilar membrane. The basilar-membrane responses to clicks recorded with the laser vibrometer display nonlinearities which are appropriate counterparts of those evident in frequency-domain descriptions (Figs. 2–4): compressive growth of the maximal response with stimulus level, approximately-linear growth in the amplitude of the initial peaks (which have lower periodicity than later peaks) and highly compressive growth of later peaks.

These trends cause responses to more intense clicks to be skewed toward earlier times in comparison to lower-level responses.

To emphasize the level-dependent nonlinear aspects of the basilar membrane responses to clicks, the responses displayed on the left column of Fig. 6 have been normalized to click peak pressure and plotted on the right column. If the responses had grown linearly, the normalized waveforms for all stimulus levels should have been identical, except for expected poorer signal-to-noise ratios at lower click levels. In fact, however, waveforms are progressively smaller as a function of increasing click level. The nonlinear features of click responses recorded with laser vibrometry, which will be more fully described in another publication (Ruggero and Rich, 1990b), are qualitatively in agreement with trends first demonstrated by Robles et al. (1976) in their study using the Mössbauer technique. Results with the two techniques, however, differ markedly from a quantitative perspective: the present recordings are substantially more sensitive (i.e., the waveforms in Fig. 6 are larger at equivalent stimulus level) and they indicate much sharper frequency tuning (i.e., the responses in Fig. 6 last longer and have more detectable cycles of oscillation). The quantitative differences probably do not reflect inherent differences between the recording methods, but rather are a result of better physiological conditions in the present preparations.

Lability of the mechanical response

Starting with the work of Rhode (1973), studies using the Mössbauer technique have provided evidence, most convincingly by correlating compound action potential (CAP) thresholds with the vibratory response of the basilar membrane, that the mechanical function of the basilar membrane is related, probably causally, to the physiological state of the cochlea (Sellick et al., 1982; Robles et al., 1986b). In particular, it has been shown that initially sensitive and highly frequency-tuned responses to tones from basilar membranes in live cochleae become insensitive and poorly frequency tuned upon death of the animal (Sellick et al., 1982; Robles et al., 1986b). Figs. 7 and 8 show equivalent findings for the responses to clicks recorded with laser vibrometry.

Fig. 7 presents the waveforms of velocity responses to clicks at two intensity levels, recorded in one case (left) while the cochlea was still in a relatively good, albeit deteriorated, physiological state (CAP thresholds at CF elevated by 18 dB re the presurgical condition) and in the other (right) after the animal was killed with an overdose of sodium pentobarbital. It is clear that death was accompanied by striking changes in the basilar membrane response. The post-mortem maximal response at 95 dB is, in fact, substantially smaller than the in-vivo response to clicks 20 dB less intense. Even more striking than the effects of death on peak amplitude are those upon the duration of the oscillatory response: while in the 95-dB in-vivo recording small oscillations can be detected as late as 9 ms following click onset, the 'ringing' terminates in the corresponding postmortem response by 2 ms. It is noteworthy that while late response cycles are abolished by death, the initial response cycle is hardly altered. Less noticeable in the time-domain representation of responses to clicks is the linearization of response growth as a function of click level: whereas a 20 dB increment in click level was accompanied by an identical (20 dB) response growth in the basilar membrane of the dead chinchilla, the same increment in click level caused only a 1.7-fold increment of peak velocity in vivo (equivalent to 4.6 dB). Such contrasting changes in response magnitude with click level are more evident in their frequency-domain representation (Fig. 8).

Fig. 8 shows the velocity-magnitude frequency spectra obtained by Fourier transformation of the time-domain click responses depicted in Fig. 7. The spectra are presented as gain plots: i.e., with units of velocity per unit of peak pressure. In linear systems, responses expressed as gains are constant and independent of stimulus level. Comparison of the post-

mortem spectra of responses evoked by 75-dB and 95-dB clicks indicates that, within the limits of experimental error, the basilar-membrane response gains were constant, implying a linear velocity growth with stimulus intensity. This is in contrast with the spectra for the in-vivo responses. In agreement with the compressive nonlinearity previously noted in responses to tone pips, spectra of in-vivo responses to 75-dB and 95-dB clicks differ by 14–20 dB near CF, indicating that velocity responses at these frequencies hardly grew at all with stimulus intensity. Also in accordance with the previously noted frequency-selective nature of the compressive nonlinearity, it is apparent that responses approach linearity at frequencies sufficiently lower than CF.

The effect of death upon the gain spectra of responses to clicks may be succinctly described in the same terms that we and others (Robles et al., 1986b; Sellick et al., 1982) have used to describe equivalent changes in responses to tones: death or cochlear trauma causes a linearization of basilar-membrane responses and a frequency-dependent decrease in sensitivity near CF. Because the in-vivo responses are compressively nonlinear only at frequencies close to CF, the post-mortem linearization of responses implies a reduction of response sensitivity only at these near-CF frequencies, leaving intact the response sensitivity at frequencies lower than about 1/2 octave below CF. Thus, while the near-CF gains of in-vivo responses in Fig. 8 exceeded the post-mortem gains by 25 or 40 dB (depending on stimulus level), the gains at 5 kHz were hardly affected by death. Further, the effect of death is large for low-intensity stimuli and diminishes at high stimulus levels.

Discussion

The various techniques available for the study of basilar-membrane and middle-ear vibrations have been discussed by many investigators (e.g., Békésy, 1960; Köhllöffel, 1972; Wilson and Johnstone, 1975; Yates and Johnstone, 1979; Buunen and Vlaming, 1981; Khanna and Leonard, 1986; Willemin et al., 1988; Le Page, 1989; Peake, 1990; Rhode, 1990). Therefore, we shall comment here only on the comparative advantages of the Mössbauer and laser-velocimetry techniques as applied to the measurement of basilar-membrane motion. We are in an especially good position to concretely compare the performance of the Mössbauer and the laser-Doppler techniques because our laboratory appears to be the only one to date to have acquired experience in the application of both methodologies to the study of basilar-membrane preparations differing only in the vibration recording method. A summary of the similarities between the Mössbauer technique and laser Doppler-shift velocimetry, as used in our laboratory for the measurement of basilar-membrane vibration, and of their respective advantages and disadvantages is shown in Table I.

Similarities between the Mössbauer and laser velocimetry techniques

The Mössbauer technique and laser velocimetry are both based on the detection of the Doppler frequency shift caused by motion upon electromagnetic radiation: motion away from the detector causes a decrease in frequency or energy, while motion toward the detector causes an increase in frequency. In the case of the Mössbauer technique, the electromagnetic radiation is that of gamma photons with energy about 14.4 keV; in the case of laser velocimetry using a He-Ne laser, the radiation is red (wavelength of 633 nm) coherent visible light. A corollary of the velocity- (rather than displacement-) sensitivity of both techniques is that sensitivity to displacement becomes poorer as a function of decreasing frequency, at a rate of 20 dB per decade. This decrease in sensitivity with decreasing signal frequency is both advantageous and disadvantageous. The advantage is that unwanted (non-stimulus related) low-frequency basilar-membrane vibrations, such as those caused by respiration and heart beat, tend to be filtered out. The disadvantage is that measurement of low-frequency responses to sound and, particularly, the detection of

possible DC responses (Le Page, 1987 and 1989) is made more difficult, although not necessarily precluded (Nuttall et al., 1989, 1990, 1991).

Both the Mössbauer technique and the present application of laser velocimetry are invasive in that they require opening the otic capsule and placing foreign objects on the basilar membrane. In the case of the Mössbauer technique, the foreign object is a radioactive metal foil (50–100 μm square, 6- μm thick). In laser velocimetry, high-refractive-index glass microbeads are required to efficiently reflect the laser beam back to the microscope objective. Although it is apparently possible to obtain adequate laser light reflection from cochlear structures without need for artificial reflectors (Khanna et al., 1989), in our particular application of laser velocimetry it has proven impossible to carry out measurements without employing either the glass microbeads or a metal foil similar in size to that used for Mössbauer measurements. The microbeads are preferable to metal foil because they provide specular reflection over a wide range of incident angles.

A second way in which both the Mössbauer technique and laser velocimetry are invasive is that they involve exposing the cochlea to possibly deleterious electromagnetic radiation. [Additionally, both gamma radiation and intense laser light pose some minimal but finite risk to the experimenter.] In the case of the Mössbauer technique, a source of gamma radiation is placed on the basilar membrane. If intense enough, this source should surely cause tissue damage (Kliauga and Khanna, 1983). An experimental assessment of actual, rather than potential, damage of radioactive sources to the organ of Corti has not been carried out. What evidence exists is circumstantial, based on the progressive deterioration of initially healthy cochleae undergoing Mössbauer measurements (Sellick et al., 1982; Robles et al., 1986b). However, while these data are compatible with radiation-induced damage, they do not necessarily imply radiation-induced damage.

The use of laser light, similarly, could conceivably cause damage to the organ of Corti. Willemin et al. (1988) mention otherwise unpublished observations of their own that indicate damage to cochlear cells by high-intensity light. For lack of more tissue-specific data, the same authors assumed that the damage threshold for retinal injury (given as 0.5 W/cm²) also applies to cochlear hair cells. Such an assumption may be too conservative considering that retinal receptor cells are specialized to absorb visible light. Nevertheless, focusing even a low-power laser beam onto a small area can raise the light-power density to very high values, far in excess of 0.5 W/cm². In the present application of laser vibrometry, using a 1-mW laser beam (measured as it exits the microscope objective; see Methods) focused onto a spot with a 30- μm diameter, the power density at the basilar membrane should be 140 W/cm². Whether such light-power density actually damages the organ of Corti is not known. Although we have often found a progressive deterioration of responses in cochleae undergoing basilar-membrane measurements with laser vibrometry, it is not clear whether the deterioration was related to the intense laser light. It may be worthwhile to investigate this question by histological comparison of laser-exposed and control cochleae.

Advantages of the Mössbauer technique over laser velocimetry for measuring basilar-membrane vibration

Because of the high penetrance of gamma radiation, in applying the Mössbauer technique the relative positions of the radioactive metal-foil source and the absorber/detector combination are not critical. It is, in fact, possible to make useful measurements when objects opaque to visible light, possibly solid (such as bone), are interposed between the source and the absorber/detector. In contrast, laser velocimetry requires an optically transparent, unobstructed pathway to permit the light to travel from the microscope objective to the reflecting glass bead and back. For example, even a relatively small amount of blood coating the beads or suspended in the perilymph of Scala tympani can prevent useful

measurements. Similarly, the optical meniscus formed by the perilymph/ air interphase at the otic capsule hole can greatly affect the strength of the laser-beam reflection arriving at the microscope objective.

A further advantage of the Mössbauer technique over laser vibrometry is expense: the laser vibrometer and the compound microscope cost several times as much as the equipment required for applying the Mössbauer technique to the measurement of basilar-membrane vibrations.

Advantages of laser vibrometry over the Mössbauer technique for measuring basilar-membrane vibrations

The main advantage of laser vibrometry over the Mössbauer technique lies in the relative linearity of its input-output function, which contrasts with the severely nonlinear nature of the Mössbauer input-output characteristic (illustrated in Fig. 5). Because of the Mössbauer nonlinearity, it is very difficult to extract accurate instantaneous-velocity information from responses with known complex waveshape and perhaps impossible to do so for responses of arbitrary (unknown) wave-shape. As discussed above in the Results section on responses to clicks, even with an isomer shift substantially differing from zero velocity (in which case sufficiently-low velocity waveforms are reproduced relatively faithfully), the dynamic range is extremely constrained by the saturation at moderately-low velocities (see Fig. 5, panel d).

The linearity of the Dantec vibrometer is, of course, not perfect. Using two-tone stimuli in a test cavity (consisting of two earphones coupled acoustically and a speculum terminated by a rubber diaphragm), we have measured intermodulation distortion products as large as 50 dB below the level of the primary tones at relatively low vibrometer-signal levels (Robles et al., 1990). Since such vibrational distortion is larger than the acoustic intermodulation distortion measurable in the same cavity with a microphone, it is presumed to be generated by the vibrometer system. Further, harmonic and intermodulation distortion and spurious DC shifts sharply increase in magnitude when the output voltage of the frequency tracker exceeds 0.5 V peak. We have not yet been able to ascertain whether such nonlinearity is inherent in the vibrometer design or results from malfunction or misadjustment of the instrument.

The second major advantage of laser vibrometry over the Mössbauer technique is its greater sensitivity: laser vibrometry is between one and two orders of magnitude more sensitive than the Mössbauer technique. The relative insensitivity of the Mössbauer technique arises as a byproduct of the recording of probabilistic events, combined with practical limitations on the strength of radioactivity of the source and on sampling time. Because radioactive decay is a probabilistic point process, recording motion using the Mössbauer technique requires the usage of the same kinds of statistical averages involved in extracting information from trains of action potentials evoked by auditory stimuli: i.e., histograms are constructed that convert the point processes into quasi-analog versions. If both velocity-magnitude and phase are to be measured, one usually constructs period histograms; if timing information is not needed, then only the average rate need be recorded and sampling time may be shortened. In either case, it is necessary to sample for relatively long periods of time to reduce the variance and thus the noise floor of measurements to tolerable levels. In contrast, the laser velocimeter delivers an analog waveform which is proportional to the velocity of the target. In theory, the need for long sampling could be reduced by utilizing a sufficiently 'hot' radioactive source, yielding a very high average gamma-photon rate. In practice, however, it has not been possible (at least commercially) to anneal Co-57 to the rhodium matrix at a density greater than about 25 mCi/rmn². In any case, because of the toxic nature of ionizing radiation (discussed above), it is doubtful that increasing the level of radioactivity much above the values now typically used in basilar-membrane experiments would be useful.

The gamma-photon rate saturation, combined with the comparatively low velocity sensitivity (discussed above), conspire to produce a dynamic range of measurements which is orders of magnitude smaller for the Mössbauer technique than for laser vibrometry. For a sampling time of 100 s, using the 'period histogram' method for extracting magnitude and phase information, the approximate dynamic range of the Mössbauer technique is 30–1000 μm (i.e., about 30 dB). In contrast, with the same sampling time, Fourier transformation of averaged laser vibrometry waveforms yields a noise floor of less than 0.5 $\mu\text{m/s}$. We have not yet explored the upper limit of measurements for the vibrometer in our application, in which the very restricted depth of field of the microscope's objective must degrade severely the otherwise enormous dynamic range of the vibrometer itself (> 160 dB!). We have ascertained, however, that velocities at least as high as 10 mm/s can be reliably measured. Thus, the effective dynamic range in our application of laser vibrometry is larger than 86 dB.

A caveat is necessary in the measurement and interpretation of 'dynamic range'. As indicated in the Methods section, the Dantec frequency tracker provides 7 sensitivity ranges, with the higher sensitivity settings having both lower noise floors and lower ceilings of measurable velocity. Dynamic range, therefore, may be defined in terms of the noise floor and the maximum measurable velocity either at any single sensitivity setting or combining the lowest noise floor (from the highest-sensitivity setting) with the highest velocity ceiling (from the lowest sensitivity setting). Obviously, the 160-dB dynamic range claimed by Dantec for its vibrometer is based on the latter definition. In practice, however (e.g., when measuring responses to impulsive stimuli), the former (more restrictive) definition may be more useful. Under optimal conditions, the restrictively-defined dynamic range of our laser vibrometry application appears to amount to 60–70 dB. It is worth noticing that it is possible (albeit cumbersome) to combine velocity waveforms obtained with different frequency-tracker sensitivity settings into a single 'synthetic' waveform with extended dynamic range, perhaps approaching the 160-dB manufacturer's specification.

The third major advantage of laser vibrometry over the Mössbauer technique is the relative ease with which glass microbeads, in contrast to metal foils, can be placed on the basilar membrane. Because of the relatively low weight/surface area ratio of a metal foil, surface tension makes it difficult to submerge it in the perilymph; thus, releasing the metal foil on the perilymph surface often results in its floating away, instead of settling on the basilar membrane. In contrast, the spherical glass microbeads used in laser vibrometry have minimal surface area per unit weight and, additionally, they can be manipulated while suspended in a perilymph-like fluid within a pipette. Thus, they can be conveniently carried through the air/perilymph interphase and then expelled from the pipette, causing them to settle by gravity on the basilar membrane.

In conclusion: although until recently the Mössbauer technique remained the most effective for measuring basilar-membrane vibrations, the comparative advantages of laser Doppler-soft velocimetry make it now the method of choice for such measurements.

Acknowledgments

We thank Fred Nuttall for alerting us about his own work with a laser vibrometer and for generously spending much time on the telephone giving us advice and encouragement. We also thank Craig Goulbourne and Robert Suhoke (Dantec Electronics, Inc., 777 Corporate Drive, Mahwah, NJ 07430) and Ken Kilby (Leeds Precision Instruments, Inc., 801 Boone Ave. No., Minneapolis, MN 55427) for their help in coupling the vibrometer to the compound microscope and the Mo-Sci Corp. (Twitty Industrial Park, Mead Bldg./P.O. Box 2, Rolla, MO 65401) for the gift of glass microbeads. The laser vibrometer was purchased with grants from the Minnesota Medical Foundation and the Lions 5M Hearing Research Endowment. We were also supported by NIH (NIDCD) Grants DC-00110 and DC-00419.

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Fig. 1.

Diagram illustrating the manner of coupling between the head of the laser vibrometer and the compound microscope. The arrow heads indicate the direction of signal transmission. The dotted lines indicate lenses in the vibrometer head (3), the microscope and the microscope/vibrometer head adaptor (5). 1) Glass fiber carrying light from the laser to the vibrometer head. 2) Electrical signal from the vibrometer head to the frequency tracker. 3) Vibrometer head. 4) Bragg cell, photodiodes, prisms, etc. inside vibrometer head. 5) TV-camera adaptor: couples the vibrometer head to the third tube of the microscope trinocular head. 6) Microscope ocular: permits visual observation of target and laser-beam spot. 7) Mirrored prism: allows simultaneous visual observation of the target and laser beam

transmission from the vibrometer head to the target and back. 8) Half mirror: directs incident light from the standard epi-illuminator (not shown in Figure) toward the target, while allowing the laser beam to be transmitted from the vibrometer head to the target and back. 9) Focusing knob: translates objective. 10) Post: connects the microscope to a boom stand. 11) Ultra-long-working-distance 20× objective. 12) Green acetate filter: protects experimenter's eyes from laser light.

Fig. 2.

Basilar-membrane velocity responses to 50-dB SPL tone pips with frequency 6–10 kHz. Each waveform represents averaged responses to 1024 repetitions of identical stimuli presented every 25 ms. The abscissa indicates time, in ms, after electrical stimulus onset. All responses are identically scaled. The electrical tone pip stimuli consisted of tones modulated at onset and offset by waveforms specified by $\cos(\phi) + 1$ ($180^\circ < \phi < 360^\circ$ at onset, $0^\circ < \phi < 180^\circ$ at offset). Rise/fall time was 0.82 ms. The time between application of the electrical signal to the earphone and the beginning of the offset was 3 ms.

Fig. 3.

Isointensity contours for basilar membrane responses to tone pips, as a function of stimulus frequency. Tone pip characteristics were as specified in the legend for Fig. 2. Velocity amplitudes at the tone pip center frequencies (indicated in the logarithmic ordinate, with units of $\mu\text{m/s}$) were extracted by Fourier transformation of waveforms such as those of Fig. 2. The parameter is sound pressure level (re: 0.0002 dyn/cm^2).

Fig. 4.

Isoresponse contours ('tuning curves') for basilar-membrane velocity responses to tone pips recorded with the laser-vibrometer (solid lines) or using the Mössbauer technique (dashed line). The ordinate indicates the sound pressure level required, at any particular frequency, to elicit a given velocity amplitude (0.1, 0.2 or 0.4 mm/s, indicated as the parameter). For the laser vibrometry responses, sound pressure levels were interpolated logarithmically using the data of Fig. 3. For comparison, a 0.1-mm/s tuning curve is shown which is representative of results previously obtained in our laboratory using the Mössbauer technique at approximately equivalent basilar-membrane sites in normal chinchillas (Robles et al., 1986b).

Fig. 5. Illustration of the method used to extract velocity information from basilar membrane responses to clicks recorded with the Mössbauer technique (from Robles et al., 1976, with permission). Panel (a) indicates the number of gamma photons detected as a function of time from click onset. Panel (d) shows a plot of the Breit-Wigner equation, which relates photon counts to relative velocity between the Mössbauer source on the basilar membrane, and the absorber and detector. Application of the Breit-Wigner equation permits derivation of velocity curves that are full-wave rectified [panel (b)]. Full-wave rectification arises from the near-zero isomer shift of the particular source-absorber combination used. Panel (c) shows how the actual velocity waveform may be derived from the rectified waveform.

Fig. 6. Basilar membrane velocity responses to clicks, measured with laser vibrometry. Click intensity, expressed as peak pressure (re: 0.0002 dyne/cm^2), is indicated for each tracing. The acoustic-click waveshape, recorded in an artificial cavity by means of a microphone with a flat frequency response, is shown at the top of the right column. Waveforms in the left column, which are identically scaled, represent averaged responses to 2048 presentations of rarefaction clicks. Electrical click onset occurred at time zero. Upward deflections indicate velocity toward Scala vestibuli. The right column displays the same data after normalization to stimulus intensity: if the responses grew linearly with click level, all waveforms in the right column would be identical. In fact, because of compressive nonlinearity, higher-level normalized responses appear smaller than responses to less intense clicks.

Fig. 7.

The effect of death upon basilar-membrane responses to clicks. Each waveform is the average of 2048 responses. The left-column waveforms were recorded approximately 6 1/2 h after opening the otic capsule, while the cochlea was still in relatively good physiological condition. The waveforms on the right show basilar membrane responses several minutes after killing the chinchilla with an intravenous injection of sodium pentobarbital. Click peak pressures are indicated alongside each tracing. Note that response amplitudes in the live preparation change less with stimulus intensity than those in the dead chinchilla.

Fig. 8.

Frequency spectra of basilar-membrane velocity responses to clicks in a relatively healthy cochlea and in the same cochlea, post mortem. The frequency spectra were obtained by Fourier transformation of the time waveforms shown in Fig. 7. Spectra are shown for responses at 75 and 95 dB SPL for each of the healthy and dead preparations. Responses are much more sensitive and frequency selective in the live cochlea. Note that the frequency spectra, expressed in units of velocity per unit pressure, are strongly dependent on stimulus level in the live cochlea (and hence strongly nonlinear), while almost perfectly linear in the dead chinchilla.

TABLE I**SUMMARY COMPARISON OF THE MÖSSBAUER AND LASER VELOCIMETRY TECHNIQUES**

	Mössbauer technique	Laser Doppler-shift velocimetry
Similarities	Velocity detection	
	Invasive: a) requires opening of otic capsule b) requires placement of foreign object (metal foil or glass beads) on basilar membrane	
Advantages of Mössbauer technique	Non-critical positioning: little effect of opaque screening material between radioactive metal foil and detector/absorber	Critical positioning: requires straight and transparent optical path between glass beads and microscope objective
	Lower cost	Higher cost
Advantages of laser Doppler-shift velocimetry	Non-linear Probabilistic (pulse frequency)	Linear Deterministic (analog)
	Insensitive	Sensitive
	Narrow dynamic range (30–1000 $\mu\text{m/s}$)	Wide dynamic range (0.5– $\gg$ 10000 $\mu\text{m/s}$)
	Metal foil difficult to place on basilar membrane	Glass microbeads easily placed on basilar membrane