

Local mechanical stimulation of the hearing organ by laser irradiation

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Light produces force when interacting with matter. Such radiation pressure may be used to accelerate small objects along the beam path of a laser. Here, we demonstrate that a moderately powerful laser can deliver enough force to locally stimulate the hearing organ, in the absence of conventional sound. Damped mechanical oscillations are observed following brief laser pulses, implying that

the organ of Corti is locally resonant. This new method will be helpful for probing the mechanical properties of the hearing organ, which have crucial importance for the ear's ability to detect sound. *NeuroReport* 17:33–37 © 2006 Lippincott Williams & Wilkins.

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Introduction

The organ of Corti (OoC) of the inner ear is a remarkably sensitive frequency analyzer. Each frequency component in the incoming sound causes maximum vibration at a different place within the hearing organ. Vibrations are converted into electrical signals by the sensory hair cells. For this process to work, each segment of the OoC must have the right stiffness, mass and damping (reviewed in Ref. [1]). In addition, neighboring segments interact to some degree with each other and with the surrounding fluid. Thus, the mechanical properties and interactions between different segments of the OoC have crucial importance for the hearing process.

The OoC rests on the basilar membrane. The mechanical properties of the basilar membrane have been investigated by pushing it with stiff probes [2–5]. This method produced data of fundamental importance, but it is limited to relatively large, low-frequency displacements. Another method is to slowly change the pressure in scala tympani while measuring the resulting motions within the organ [6]. This produced a more integrated understanding of cellular interactions, but only for low frequencies. Thus, new methods are needed.

Laser light can accelerate small objects along the beam path [7]. If a 1 W laser beam is focused to a 5- μ m spot on a perfectly reflecting surface, a pressure of \sim 300 Pa is generated [8]. The reflectivity of the OoC is around 0.03% [9]. Thus, the same beam would create a pressure of 0.09 Pa if aimed at the OoC. This equals 73 dB sound

pressure level, a relatively loud sound. The comparison, however, has limited applicability because natural sounds stimulate the entire OoC, not a 5- μ m spot. Furthermore, sound travels through the middle ear, which causes a frequency-dependent pressure gain of up to 40 dB [10]. Nonetheless, these considerations suggest that a moderately powerful laser may provide sufficient force to move the OoC.

Here, we demonstrate that laser light can evoke mechanical and electrical responses from the cochlea. Such localized stimulation may permit non-contact measurements of local mechanical properties and interactions among different segments of the OoC, in a sensitive cochlea operating close to its normal physiological condition.

Methods

Two different approaches were used. Low-frequency responses from the cochlear apex were probed in an in-vitro preparation of the guinea pig cochlea. Our experimental setup for measuring vibration in the high-frequency regions of the cochlea is adapted for the small size of a gerbil. Therefore, gerbils were used for basal turn experiments. The relevant institutional review board approved all experimental procedures.

Gerbil preparation

Young gerbils were anesthetized by the intraperitoneal injection of ketamine (30 mg/kg) followed by intramuscular

xylazine (5 mg/kg). A heating pad maintained constant body temperature. The animal was placed in a head holder and the bulla exposed as described previously [11,12]. The round window membrane was carefully peeled off and replaced with a glass coverslip to ensure that the optical interface remained constant, which is important for good interferometric measurements. A gold-coated glass bead was placed on the basilar membrane. The bead served as a reflector, used when measuring basilar membrane vibrations. Vibrations were measured using a commercial laser vibrometer (OFV3000S, Polytec Inc., Waldbronn, Germany). The measurement beam was focused on the bead using a custom-built microscope with a $20\times$, NA 0.4 lens (Mitutoyo Inc., Kawasaki, Japan). Cochlear sensitivity was assessed by measuring the compound action potential of the auditory nerve.

Guinea pig temporal bone preparation

The temporal bone from young guinea pigs was isolated and maintained *in vitro* by perfusion with oxygenated tissue culture medium [13,14]. The middle ear ossicles were intact, so sound stimulation occurred through the normal route. A small window in the bone of the apical turn gave access to the low-frequency regions of the OoC. A $2\text{M}\Omega$ glass microelectrode was advanced until it penetrated Reissner's membrane. Cochlear microphonic potentials were measured using a standard amplifier (IX-1, Dagan Instruments, Minneapolis, Minnesota, USA). A $40\times$, NA 0.8 water immersion lens was used for visualizing the OoC and for delivering laser light.

Laser stimulation

A 1.3 W laser diode (HHL, High Power Devices Inc., North Brunswick, New Jersey, USA), driven by a Tektronix LDC220 controller (Tektronix, Munich, Germany), was used to generate the light that provided the mechanical stimulus to the OoC. The wavelength was 813 nm, which is minimally damaging to cells [15]. Optical power was modulated by applying a square voltage to the analog modulation input of the controller, producing a laser pulse with a microsecond rise time. In interferometric measurements, this laser pulse was focused on the side of the measurement beam, to provide stimulation of one cochlear segment while recording mechanical responses from an adjacent segment. To avoid damage caused by tissue heating, 50- μs laser pulses were used, separated by 500 ms. Responses were averaged 100–300 times.

In a highly focused beam, small refractive objects will be trapped at the focal spot [16]. Therefore, the laser was not tightly focused. Most of the effects described here are due to the reflection, absorption, or some mixture of the two, of laser light by the structures of the OoC. A small part of the force may also originate from refraction of the beam as it passes through the OoC.

The motion of a thin piece of aluminum film held in air was used as a control (Fig. 1). A 50- μs laser pulse caused an initial large transient followed by a decaying oscillation. Thus, this laser could move small, relatively stiff objects. In contrast, no motion was recorded from a thick block of aluminum with similar reflectivity, demonstrating that the back-reflected infrared light from the metal surface had no adverse effect on the performance of the interferometer.

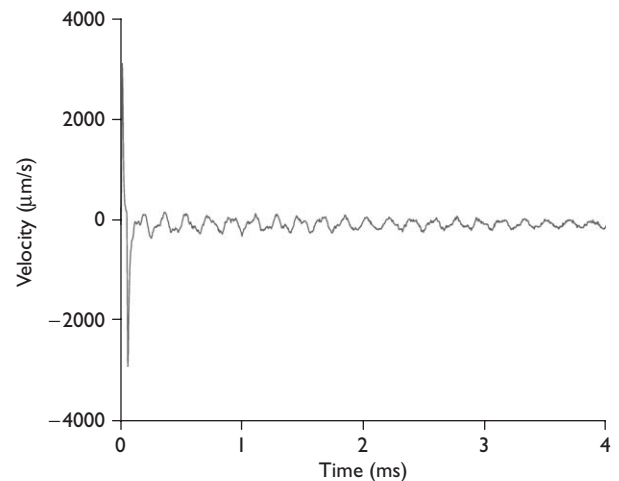


Fig. 1 Movement induced by infrared light. The laser was aimed at a piece of aluminum film, the motion of which was recorded by the interferometer. Note that the pulse caused very rapid initial motion followed by a decaying oscillation.

By aiming the laser at a photodiode, we verified that the pulse shape followed the signal applied to the modulation input of the controller. Fourier transformation of these recordings showed that the optical pulse had a flat spectrum up to 30 kHz in the case of 50- μs pulses. This is appropriate for basal turn experiments, because the basal turn is sensitive to stimulus frequencies above 10 kHz. For apical turn recordings, a pulse length of 1 ms was used. This gave significantly higher power at frequencies appropriate for the apical turn.

Results

Live animal data

The mechanical response from the basilar membrane took the form of an oscillating motion that decayed to zero in approximately 500 μs (Fig. 2a). The oscillations depended on the peak power of the pulse, with smaller responses at lower power levels. Extensive surgery is necessary to expose the basilar membrane. The inner ear is a delicate mechanoreceptor and surgical trauma frequently causes hearing loss. No laser-evoked mechanical responses could be recorded from animals that had suffered such hearing loss (assessed by measuring auditory nerve compound action potentials). This suggests that mechanical responses induced by these laser pulses are so small that active amplification by the cochlear mechanism is necessary for them to reach a size that can be measured with current technology.

Figure 2b shows the response to a broadband acoustic stimulus in a different animal. The motion generated by this click-like sound shows some similarities to its laser-evoked counterpart, but pronounced differences are also observed. In both cases, the highest amplitude was found soon after the onset, the amplitude decaying thereafter. For acoustic stimulation, there was an initial delay. This delay comprises the travel time in the ear canal, the middle ear and on the basilar membrane itself. Delays were absent from the laser pulse-evoked response, which appeared close to instantaneous.

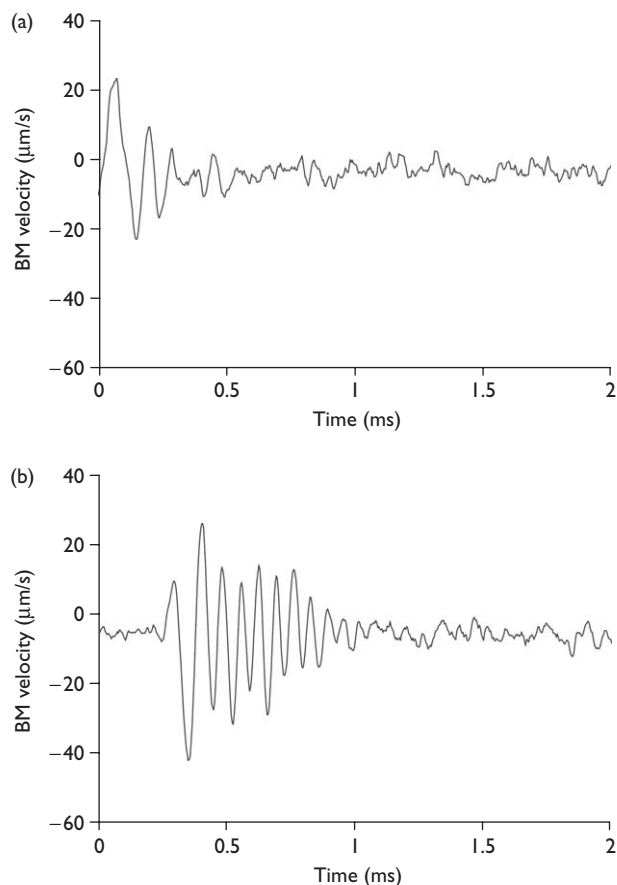


Fig. 2 (a) Basilar membrane (BM) motion induced by a 50- μs laser pulse delivered at $t=0$. (b) Basilar membrane velocity following delivery of a 50- μs acoustic click at $t=0$.

Although both stimuli were 50 μs long, the acoustical response had a much longer duration. Possibly, this is caused by dispersion. Low frequencies travel fast to a given place on the basilar membrane; higher frequencies are delayed progressively more as frequency increases. The response to an acoustic click therefore starts at a low frequency, the instantaneous frequency increasing thereafter. This is a property of travelling wave motion that is not expected for a strictly local stimulus such as the laser pulse used here. This may in part explain the shorter duration of the light-evoked response.

Light-evoked responses appeared vulnerable. Repeated exposure caused a decline in cochlear sensitivity and an inability to record additional mechanical responses.

Temporal bone data

In isolated temporal bones, laser pulses were directed on Hensen cells or on the reticular lamina. A glass micro-electrode was used to record stimulus-evoked potentials (the cochlear microphonic). Figure 3a shows a plot of response magnitude as a function of stimulus frequency, obtained by sweeping the acoustic stimulus from 60 to 500 Hz. The peak amplitude was found at 150 Hz; amplitudes declined in a relatively symmetric fashion on both sides of the peak.

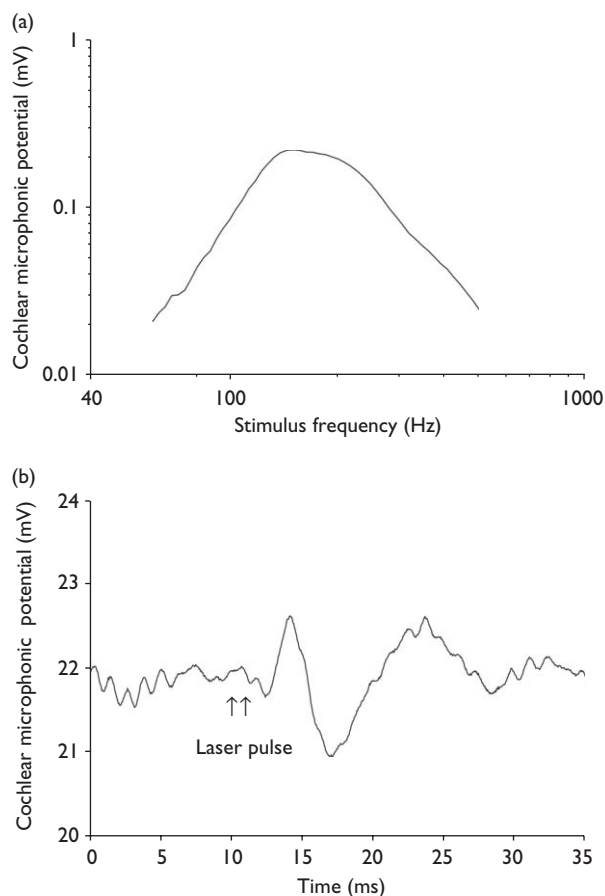


Fig. 3 (a) Cochlear microphonic potential tuning curve at 90 dB sound pressure level. (b) Laser-evoked response from the same preparation. The onset and offset of the pulse is denoted by the two arrows.

Figure 3b shows the laser pulse-evoked response. The 1-ms stimulus triggered an oscillation with approximately 15 ms duration and peak-to-peak amplitude around 2 μV . Fourier transformation showed a broad peak close to the best frequency of the sound-evoked response. Sound-evoked cochlear microphonics were almost 100 times larger at 90 dB sound pressure level. Interferometric recordings were also attempted in the cochlear apex, but the mechanical responses were too small to be measured with this technique. This may be a result of the interferometer's declining sensitivity at low frequencies.

The laser was also directed on structures outside the OoC. When aimed at the bone surrounding the cochlea, no electrical responses were recorded.

The responses shown in Fig. 3b also appeared vulnerable. Repeated exposure abolished the response. Tuning curves acquired after such repeated exposures had substantially reduced amplitudes.

Laser-induced artifacts (other responses)

The laser was also aimed at the ossicles of the middle ear, while recording the compound action potential of the auditory nerve. Large amplitude compound action potentials, resembling those evoked by acoustic clicks, were found. It may appear that these responses were caused by the motion of the middle ear ossicles induced by radiation

pressure. However, identical results were obtained when the laser was aimed at the animal's bony bulla. Adding a drop of water on the bony surface largely eliminated these responses, as did aiming the laser at the soft tissues surrounding the ear. A likely cause for these confounding effects is that absorption of laser light by a hard object such as bone generates sound because of rapid local heating.

Laser-induced electrical potentials were found when the laser was aimed directly at an Ag/AgCl electrode, but such potentials had characteristics quite different from the ones shown in Fig. 3b. To exclude this type of contamination, the electrode was placed outside the field of view of the objective lens used to deliver the laser pulse.

Great care was taken to eliminate the above artifacts when laser pulse-induced responses were measured from the cochlear partition. The fluid that surrounds the OoC provides cooling, and the structures have very low absorbance at the wavelengths used here. Both these factors will limit temperature changes induced by the laser.

Discussion

The goal here was to develop a method for non-contact force application to the OoC. Several reasons may be given for why this is desirable. First, it may permit investigations of mechanical parameters such as stiffness, damping and the degree of coupling between adjacent sections of the organ. These mechanical properties underlie responses to sound at all stimulus intensities. Lateral interactions between adjacent cells may also contribute to the high sensitivity and frequency selectivity of the cochlea [17,18].

Second, the ear has the capacity to emit sound [19]. Such emissions are an important tool for diagnosing auditory disorders, but the mechanisms underlying their production are unclear. A local excitation may help to differentiate between theories that presume otoacoustic emissions to arise through global coherent reflections [20] versus those that attribute emissions to mechanical activity by a restricted group of hair cells at one site within the cochlea.

It is important to ask whether these goals were fulfilled. Intense laser light did generate measurable motion in the high-frequency regions of the cochlea. In the apex, motion was large enough to produce electrical potentials, but too small to be measured with present techniques. Thus, we conclude that moderately powerful lasers can indeed produce forces that are large enough to move the OoC. Further studies are necessary to address the questions above. The most severe limitation of the technique appears to be the propensity for causing cellular damage. This limits the number of measurements that can be performed in a single preparation. Using shorter pulses at a lower repetition frequency may ameliorate this problem.

An essential finding is that the mechanical and electrical responses last much longer than the laser pulse itself. Following the pulse, damped oscillations were seen. These had a frequency appropriate for the location under study. This suggests that the OoC is locally resonant. Such localized resonance is often assumed to contribute to the travelling wave motion found in the cochlea, but to our knowledge, it has not been measured previously.

Clinical implications

High power lasers are used during middle ear surgery to ablate the hard bone surrounding the cochlea. Wavelengths

are chosen to maximize absorption by bone; tissue heating is minimized by repeated delivery of brief pulses. Lasers can cause hearing loss (e.g. [21]). Our experiments suggest that the OoC is sensitive to intense light. Laser beams are known to generate free radicals when they hit cells [15]. Such radicals are a known cause of damage to auditory sensory cells (e.g. [22]). Thus, any light reaching the OoC after ablating bone may cause hearing loss. Furthermore, absorption of laser light generated sounds loud enough to cause high-amplitude compound action potentials. Such sounds may also contribute to laser-induced surgical damage.

Conclusion

Moderately intense lasers may be used to move the OoC. This method may address fundamental questions in auditory physiology, such as the active mechanical properties of the hearing organ and the mechanisms that produce otoacoustic emissions. Results indicate that the hearing organ is locally resonant when this mode of stimulation is used.

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References

- Lighthill J. Biomechanics of hearing sensitivity. *J Vibrot Acoust* 1991; **113**:1–13.
- von Békésy G. *Experiments in hearing*. New York: McGraw-Hill; 1960.
- Gummer AW, Johnstone BM, Armstrong NJ. Direct measurement of basilar membrane stiffness in the guinea pig. *J Acoust Soc Am* 1981; **70**:1298–1309.
- Olson ES, Mountain DC. Probing the cochlear partition's micro-mechanical properties with measurements of radial and longitudinal stiffness variations. In: Duifhuis H, Horst JW, van Dijk P, van Netten SM, editors. *Biophysics of hair cell sensory systems*. Singapore: World Scientific; 1993. pp. 280–287.
- Naidu RC, Mountain DC. Measurements of the stiffness map challenge a basic tenet of cochlear theories. *Hear Res* 1998; **124**:124–131.
- Fridberger A, Boutet de Monvel J, Ulfendahl M. Internal shearing within the hearing organ evoked by basilar membrane motion. *J Neurosci* 2002; **22**:9850–9857.
- Ashkin A. Acceleration and trapping of particles by radiation pressure. *Phys Rev Lett* 1970; **24**:156–159.
- Hecht E. *Optics*. Reading: Addison-Wesley; 1987.
- Khanna SM, Willemin J-F, Ulfendahl M. Measurements of optical reflectivity in cells of the inner ear. *Acta Otolaryngol Suppl* 1989; **467**:69–75.
- Dancer A, Franke R. Intracochlear and extracochlear sound pressure measurements. *Hear Res* 1980; **2**:191–205.
- Ren T. Longitudinal pattern of basilar membrane vibration in the sensitive cochlea. *Proc Natl Acad Sci USA* 2002; **99**:17101–17106.
- Ren T. Reverse propagation of sound in the gerbil cochlea. *Nat Neurosci* 2004; **7**:333–334.
- Ulfendahl M, Khanna SM, Fridberger A, Flock Å, Flock B, Jäger W. Mechanical response characteristics of the hearing organ in the low-frequency regions of the cochlea. *J Neurophysiol* 1996; **76**:3850–3862.
- Fridberger A, Boutet de Monvel J. Sound-induced differential motion within the hearing organ. *Nat Neurosci* 2003; **6**:446–448.
- Neuman KC, Chadd EH, Liou GF, Bergman K, Block SM. Characterization of photodamage to *Escherichia coli* in optical traps. *Biophys J* 1999; **77**:2856–2863.
- Ashkin A, Dziedzic JM, Yamane T. Optical trapping and manipulation of single cells using infrared laser beams. *Nature* 1987; **330**:769–771.
- Geisler CD, Sang C. A cochlear model using feedforward outer-hair-cell forces. *Hear Res* 1995; **86**:132–146.

18. Steele CR, Lim KM. Cochlear model with three-dimensional fluid, inner sulcus and feed-forward mechanism. *Audiol Neurootol* 1999; **4**:197–203.
19. Kemp DT. Stimulated acoustic emissions from within the human auditory system. *J Acoust Soc Am* 1978; **64**:1386–1391.
20. Shera CA. Mammalian spontaneous otoacoustic emissions are amplitude-stabilized cochlear standing waves. *J Acoust Soc Am* 2003; **114**:244–262.
21. Kiefer J, Tillein J, Ye Q, Klinke R, Gstoettner W. Application of carbon dioxide and erbium: yttrium–aluminum–garnet lasers in inner ear surgery: an experimental study. *Otol Neurotol* 2004; **25**:400–409.
22. Duan M, Agerman K, Ernfors P, Canlon B. Complementary roles of neurotrophin 3 and a *N*-methyl-D-aspartate antagonist in the protection of noise and aminoglycoside-induced ototoxicity. *Proc Natl Acad Sci USA* 2000; **97**:7597–7602.