Published in final edited form as:

Hear Res. 2011 December ; 282(1-2): 289–302. doi:10.1016/j.heares.2011.06.006.

Infrared neural stimulation: beam path in the guinea pig cochlea

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Abstract

It has been demonstrated INS can be utilized to stimulate spiral ganglion cells in the cochlea. Although neural stimulation can be achieved without direct contact of the radiation source and the tissue, the presence of fluids or bone between the target structure and the radiation source may lead to absorption or scattering of the radiation, which may limit the efficacy of INS. The present study demonstrates the neural structures in the radiation beam path that can be stimulated. Histological reconstructions and microCT of guinea pig cochleae stimulated with an infrared laser suggest that the orientation of the beam from the optical fiber determined the site of stimulation in the cochlea. Best frequencies of the INS-evoked neural responses obtained from the central nucleus of the inferior colliculus matched the histological sites in the spiral ganglion.

Keywords

optical stimulation; laser; hearing; cochlea; histology; beam path; guinea pig

Introduction

The goal for neuroprostheses is to restore neural function to the fidelity of a healthy system. Contemporary neural prostheses, including cochlear implants, achieve this via electrical stimulation of the remaining neurons. Despite the research advances and clinical implementation of neuroprostheses, there are several challenges that face neurostimulation (see Grill, Norman, and Bellamkonda (2009) for a recent review of neuroprosthesis challenges). One of the main challenges for neuroprostheses is achieving spatially and temporally selective stimulation. Several strategies have been used to increase the spatial selectivity of electrical stimulation over a conventional monopolar stimulation paradigm. Multipolar electrode configuration can increase the selectivity of stimulation. However,

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more power is required to generate threshold responses (Kral et al., 1998; Bierer and Middlebrooks, 2002; Snyder et al., 2004b). Cuff electrodes have been successfully used in several chronic animal and human studies to stimulate motor neurons and the optic nerve (Veraart et al., 1993; Veraart et al., 1998; Polasek et al., 2009). However, drawbacks of this method include nerve damage from the cuff (Larsen et al., 1998; Grill and Mortimer, 2000). Penetrating electrodes also allow for more spatially focused stimulation by inserting the stimulating source into the nerve tissue (Middlebrooks and Snyder, 2007). By compromising the structure and integrity of the nerve, penetrating electrodes can cause inflammation, edema and, subsequently, impair neural function (Bowman and Erickson, 1985; Lefurge et al., 1991). INS can confine the stimulated volume of tissue and may be another means of increasing spatial selectivity of neural stimulation. Another advantage of INS is the lack of an electrochemical junction. The fluid interface between the active electrode and the tissue, which is not needed for INS, is a common location for device failure (Brummer et al., 1983).

Recently, the efficacy of pulsed INS of the auditory nerve has been evaluated in different animal models. Pulses of IR can stimulate the cochlea without direct contact between the stimulating source and the target neurons (Izzo et al., 2006; Izzo et al., 2007; Richter et al., 2011). Although neural stimulation with IR appears more spatially selective than electrical stimulation, the method also has its limitations. Tissue between the radiation source and the target neurons can be a confounding factor for stimulation. For example, the fluids in the perilymphatic space can absorb the radiation, and blood vessels and the modiolar bone can absorb or scatter the radiation. At the wavelength used for INS (1862 nm), most of the incident radiant energy is absorbed by water in the tissue and is dissipated as heat. This heat may result in tissue damage. Although in soft tissues the effect is much less than absorption at this wavelength, scattering will increase the spot size of the incident radiation at the target neurons. Hence, the structures may attenuate the incident radiation via absorption and/or scattering.

In order to explore the feasibility of INS in the fluid-filled cochlea with its spiral, conical structure, it is imperative to determine the structures along the optical path between the stimulating source and the target neurons. Also, knowing the structures that are stimulated - hair cells, neural cell bodies and/or axons - will aid characterizing the radiation-tissue interaction.

The present study explored the optical path of INS in the guinea pig cochlea. A pulsed infrared laser was used to stimulate guinea pig cochleae. Recordings from the central nucleus of the inferior colliculus (ICC), using multi-channel electrodes, were used to determine the best frequency of the stimulation. After completing the ICC recordings, the orientation of the stimulating optical fiber within the cochlea and the radiation beam path was determined by intentionally ablating the tissue along the beam path. Histological reconstructions of the cochleae then showed the trajectory of the IR in the cochlea. In a second series of experiments the optical fiber was cemented in place at the conclusion of the experiments and the orientation of the optical fiber in the cochlea and the possible beam path were determined using microCT. Physiologic responses from the ICC were mapped onto the histological reconstructions, using a frequency-place map of the guinea pig cochlea obtained from the literature. The locations of INS were compared with different orientations of the stimulating fiber (different optical beam paths) in the cochlea. In addition, the likely path of the radiation beam was imaged in a hemicochlea preparation using thermochromic ink.

Methods

Guinea pigs (200–600 g) of either sex were used in the experiments. Care and use of animals were carried out within guidelines of the NIH Guide for the Care and Use of Laboratory

Animals were approved by the Animal Care and Use Committee of Northwestern University.

Animal anesthesia

Animals were anesthetized with urethane (1.3 g/kg i.p. in 20% sterile PBS) following the approach used by Palmer et al., (1996). Urethane injections were supplemented with ketamine (44 mg/kg) and xylazine (5 mg/kg) at the beginning of surgical procedures. To reduce bronchial secretions, atropine sulfate (0.04 mg/kg) was administered at the beginning of the experiment. Anesthesia in all guinea pigs was maintained by supplements of ketamine (44 mg/kg) and xylazine (5 mg/kg) along with saline solution (0.5 ml). Depth of anesthesia was assessed every 15 minutes with a paw withdrawal reflex. Core body temperature was maintained at 38°C with a thermostatically controlled heating pad.

After the animals were anesthetized, a tracheotomy was performed and a plastic tube was inserted into the trachea to facilitate breathing. The animals were ventilated on oxygen throughout the length of the experiment.

Acoustically evoked compound action potentials to monitor cochlear function

To document cochlear function during the experiments, acoustically evoked CAPs were recorded from each cochlea. The cochlea was accessed by a surgical bullotomy. A "C"-shaped skin incision was made behind the left ear lobe and the cervicoauricular muscles were removed by blunt dissection. The left cartilaginous outer ear canal was exposed and cut to insert a hollow ear bar into the ear canal. The hollow ear bar on the left side and a solid ear bar on the right side were used to fix the head in a stereotactic head holder (Stoelting, Kiel, WI). The hollow bar allowed acoustic stimulation of the left ear, and the solid ear bar blocked the right ear canal. The left bulla was exposed and opened approximately 2×3 mm with a motorized drill. For the CAP measurements a silver ball electrode was placed on the round window.

For the measurements, tone bursts of 12 ms in duration, including a 1 ms rise and fall time, were presented at a rate of 4 Hz. CAP thresholds were determined by comparing a threshold criterion ($A_2=30 \mu V$) with the peak-to-peak voltage (A_1) measured from the round window membrane electrode in a time window of 6 ms duration that began with the onset of the tone burst and contained the CAP. To reduce the contribution of cochlear microphonics, responses to 32 consecutive tone-burst presentations delivered in opposite phases were averaged. Moreover, the overall noise of the recordings was reduced by bandpass filtering the response using a preamplifier and filter (ISO 80, World Precision Instruments, Sarasota, FL), with the highpass filter cutoff frequency set to 300 Hz and the lowpass filter cutoff frequency set to 3000 Hz. The amplifier gain was set to 60 dB. CAP thresholds were determined for a frequency range of five octaves with a resolution of three steps per octave. The highest frequency was 50 kHz.

Placement and data acquisition with the multi-channel inferior colliculus electrode array

The right temporalis muscle was reflected, and an approximate 5×5 mm opening was drilled in the right parietal bone, just dorsal to the parietal/temporal suture and rostral to the tentorium. A small incision in the dura mater was made and the 16-contact silicon-substrate, thin-film multichannel penetrating electrode array (A1×16–5mm–100–177, NeuroNexus Technologies, Ann Arbor, MI) was advanced through the occipital cortex. Each electrode contact has a surface area of $177 \mu m^2$ and the contacts are separated by 100 μm . The shank of the array was 50 μm thick and 5 mm in length. Given the length of the array, no aspiration of the cortex was necessary to place the electrode in the ICC. The electrode was inserted into the ICC on a dorsolateral to ventromedial trajectory at an approximately 40-

degree angle off the parasagittal plane in the coronal plane using a 3D-micromanipulator attached to the frame of the stereotactic head holder (Stoelting, Kiel, WI). With this trajectory, the multichannel electrode passed through the ICC approximately orthogonal to its iso-frequency laminae (Merzenich and Reid, 1974; Snyder et al., 1990; Smith and Delgutte, 2007; Snyder et al., 2008). The exposed skull and dura mater were covered and protected from dehydration.

Neural activity was simultaneously recorded at the 16 contacts at 40 kHz sampling rate, with a 16-bit analog/digital (A/D) input conversion. The bandpass filter range for the neural recordings was 0.1–8 kHz. Activity at each electrode contact was analyzed in real time and time points, at which action potentials occurred from either single neurons or neuron clusters, were recorded for all 16 electrode contacts.

After the initial placement of the distal tip of the electrode into the ICC, the electrode was advanced while acoustic tone pips were presented to the left ear via the hollow ear bar. Proper placement of the electrode was determined when neural responses from the distal contact of the array could be stimulated with a tone burst between 16–25 kHz and the most superficial contact could be stimulated with a 1–2 kHz tone burst. In some instances the electrode was advanced several times into the ICC before the optimal placement was achieved. After placing the multichannel electrode array, the best frequencies at each electrode contact were identified. The stimulus frequency was decreased by 3 steps/octave, over 5 octaves, beginning at 32 kHz. The sound level, at each frequency, was attenuated from 0 to 80 dB SPL in steps of 10 dB SPL. While presenting the acoustic stimuli, evoked neural responses were simultaneously recorded at the 16 contacts of the electrode array inserted in the ICC. Acoustic stimuli were presented at a rate of 4 Hz. In the guinea pig, the 1.5 mm distance from the most proximal to the most distal recording site on the electrode allowed simultaneous recording of responses from neurons sensitive to pure tone frequencies spanning approximately 4.5 octaves. Other groups have obtained a similar frequency mapping along the electrode array in the guinea pig ICC (Snyder et al., 2004a; Snyder et al., 2008). According to published frequency-place maps of the guinea pig cochlea (von Békésy, 1960; Robertson, 1984; Tsuji and Liberman, 1997), the frequency range that is monitored with the multi-channel electrode corresponds to a basal section of the guinea pig cochlea (see also Discussion).

After acquiring the acoustic thresholds and determining the placement of the inferior colliculus electrode, the cochlea was intentionally damaged. Fluids were absorbed from the cochlea by placing paper wicks at the cochleostomy and subsequently 50 μ l freshly-prepared, warmed neomycin (20 mM in Ringer's lactate) was infused through the cochleostomy into scala tympani. Following the injection of the drug, no CAPs could be evoked with acoustical stimuli above 15 kHz. CAP threshold elevation was progressive thereafter. Animals were used for INS if the CAP threshold elevation was more than 40 dB for frequencies below 8 kHz.

Infrared neural stimulation

A cochleostomy was created in the basal turn of the cochlea with a 0.5 mm Buckingham footplate hand drill (Richards Manufacturing Co., Memphis, TN) approximately 0.5 mm from the bony rim of the round window. After the cochleostomy was made into the basal cochlear turn, a 200 μ m diameter optical fiber (P200-5-VIS-NIR, Ocean Optics, Dunedin, FL) was inserted through the opening of the cochlear wall. The optical fiber was coupled to a diode laser (Capella, Lockheed Martin Aculight Corp., Bothell, WA) and mounted to a micromanipulator to ensure consistent orientation during stimulation. The radiation wavelength was 1862 nm, the pulse duration was 100 μ s, and the pulse repetition rate was 10 Hz. The pulse energy of the laser was controlled directly by varying the current to the

laser diode. The energy per pulse was measured in air at the tip of the optical fiber with the J50LP-1A energy sensor (Coherent, Santa Clara, CA). It ranged between 0 and 127 $\mu\text{J}/\text{pulse}$ at 100 μs pulse duration.

It is not possible to directly measure the radiant energy delivered to the target structures. The energy at the target of stimulation depends on (1) the distance between the tip of the optical fiber and the neural structure and (2) any absorber(s) or scatterer(s) in the optical path. Radiant energy values, which are reported for this study, were measured at the tip of the optical fiber in air. The penetration depth for the lasing wavelength in water is about 0.8 mm (e.g. Hale and Querry, 1973). Penetration depth is defined as the distance over which the incident energy is reduced to $1/e$, or $\sim 37\%$ of its original value.

Data analysis

Raster plots showing the neural activity after the stimulus presentation at a given stimulus level were plotted for 400 infrared pulse repetitions. Raster plots were generated for each radiant energy tested. In addition to the raster plots, PSTHs were calculated from the neural activity. Each PSTH showed the number of action potentials that occurred during a 0.1 ms time interval at increasing times after the stimulus.

From the distribution of the activity at the different energy levels, a discrimination index or d' (*d-prime*), was calculated using receiver-operating characteristics, or ROC, analysis (Green and Swets, 1966). Neural responses to 20 trials of a given stimulus level and 20 trials of the next higher level were used to obtain distributions of spike counts. These distributions were then subjected to ROC analysis to compute discriminability. For two Gaussian-like distributions with different means of μ_1 , μ_2 and a common variance σ^2 , d' is the distance between the means in units of standard deviation. The larger the d' value, the more separated the distributions are. The area under the ROC curve, obtained from two distributions (Green and Swets, 1966; Middlebrooks and Snyder, 2007), gave the probability of correct discrimination, which expressed as a standard deviate (z-score) and multiplied by $\sqrt{2}$ provides d' . This procedure was repeated for each successive pair of stimulus levels to obtain cumulative d' . The cumulative d' -values for neural activity measured on each electrode contact at different energy levels were used to generate contour plots. The contour plots represent spatial tuning curves of the cochlear INS and were generated for each experiment. The spatial tuning curves were then compared against the histological reconstructions (see below) of the same cochleae.

Reconstruction of the beam path in the cochlea – histology

After optical stimulation was performed and ICC responses recorded, the optical fiber was removed. Fluids were absorbed from the cochlea by placing paper wicks at the cochleostomy. The optical fiber was replaced by a hollow waveguide (BeamPath[®] OTO-S, OmniGuide[®] Inc., Cambridge, MA) coupled to a CO₂ laser operating at 10.6 μm (Sharplan 20C, Lumenis[®], Santa Clara, CA). The hollow waveguide had an inner diameter of 250 μm and a spot size of 210, 260, 360, and 460 μm at 0, 1, 2, and 3 mm distance from the tip of the fiber (OmniGuide, M-1504, Rev. 0, Cambridge, MA). The hollow waveguide was placed on the same micromanipulator to match the orientation of the stimulating optical fiber. Once the hollow waveguide was aligned through the cochleostomy, the cochlea was irradiated with the CO₂ laser to intentionally ablate the tissue along the trajectory of the waveguide. A single pulse was delivered at 15 W laser power setting. Typical transmission values measured for 100 ms-duration pulses at the 15 W power setting were 414 ± 46 mJ (average $\pm$ standard deviation).

Animals were euthanized and the cochleae were harvested and subsequently fixed with paraformaldehyde (4% in PBS solution). To decalcify the cochleae, they were placed in 10% EDTA for 7 days. Initially, two whole mount cochleae were used to determine the optical path. The bony cochlear wall was carefully dissected away to visualize internal structures, while maintaining parts of the basilar membrane and the bony modiolus. Images were taken with a CCD camera (FE-280, Olympus America Inc., Lombard, IL) and a Zeiss operating microscope for analysis. Illustrations were created using Photoshop (CS3, Adobe Systems Incorporated, Ottawa, ON).

For histological reconstruction, the cochleae were embedded in plastic and subsequently sectioned. The cochleae were rinsed in phosphate buffer three times for 15 minutes each and dehydrated in a graded 6-step acetone series (25%, 50%, 75%, 90%, 100% and 100%). Cochleae were embedded in Araldite-Epoxy Resin (7:1 Acetone:Resin, 1:1 Acetone:Resin, 1:7 Acetone:Resin, three times pure Resin). The plastic was cured for 12 hours in an oven at 60°C.

Sections were cut perpendicular to the modiolus at 5 µm thickness on an ultramicrotome (LKB 8800 Ultratome III, Stockholm-Bromma, Sweden) and were placed on glass slides. The sections were stained with 1% toluidine blue (Sigma Aldrich, St Louis, MO) and 1% sodium tetraborate solution (1:20). Images of each tenth section were captured with a microscope (Leica DMRB, Buffalo Grove, IL) equipped with a Spot Insight Color camera (SPOT™ Imaging Solutions, Sterling Heights, MI) at 2.5×. Images were analyzed for carbonization and for tissue damage (determined by the histological appearance of the ablated tissue).

Three-dimensional reconstructions of the cochlea were created using image manipulation software (Photoshop CS3, Adobe Systems Incorporated, Ottawa, ON) and Igor Pro 6 (WaveMetrics Inc., Lake Oswego, OR). The reconstruction was started from the first image in which the inner pillar foot was observed. Consecutive images were imported in Photoshop to create a stack. To align the images, the opacity of the second image was decreased to 60%; the two images were superimposed and oriented by aligning corresponding tissue landmarks. Opacity was subsequently restored to 100%. Consecutive images were added and aligned resulting in a stack. From each image of the stack, the x and y coordinates were recorded for multiple reference points (inner pillar foot, spiral ganglion cells, cochleostomy, and carbonized tissue). The z-coordinates were calculated according to the thickness of the sections (5 µm each). In Igor Pro, these points were used to generate a 3-D reconstruction (Figure 3D).

To compare the neural responses and the histology, we mapped the neural response onto our cochlear reconstructions. From the spatial tuning curves, we determined the best frequencies at stimulation threshold and the frequency band for the maximum stimulus level tested. The extension of the frequency band along the reconstructed cochlea and the location of the best frequency was determined by converting the frequency data into a length measure using published frequency-place maps and 3D reconstructions of the guinea pig cochlea (von Békésy, 1960; Müller, 1996; Tsuji and Liberman, 1997; Hofman et al., 2009). The stimulation sites obtained from neural recordings and the corresponding location of the spiral ganglion cells obtained from histology were then plotted on the reconstructions.

Reconstruction of the beam path in the cochlea – microCT

X-ray computed tomography (CT) provides an accurate map of the variations in X-ray absorption within an object, regardless of whether there is a well-defined substructure of different phases or there are slowly varying density gradients. However, CT has a limited spatial resolution. A better resolution can be obtained with high-resolution microCT that can

image objects with at least 50–100 μm spatial resolution. MicroCT has been used previously to image the cochlea (Vogel, 1999; Rau et al., 2006; Lareida et al., 2009; Richter et al., 2009; Glueckert et al., 2011; Smith et al., 2011). This imaging method uses individual radiographs or projections that were captured from different viewing directions to reconstruct the internal structure of the object. In the present experiments the method was used to determine the internal bony structures of the guinea pig cochlea and the location of the optical fiber that was inserted through a cochleostomy into the cochlea. The method is non-invasive. A detailed description of the Methods is provided in Stock (2009).

Prior to scanning the cochleae using the MicroCT-40 system (Scanco Medical, Wayne, PA), the optical fiber was cemented in place after the final physiological measurement. Approximately 1 ml of dental acrylic was prepared in a small glass container and was aspirated with a 1 ml syringe. A 20-gauge needle was attached to the syringe and the dental acrylic was then carefully injected into the bulla of the guinea pig. Care was taken not to move the optical fiber during the procedure. After the acrylic had cured, the animals were euthanized and the bullae were removed from the skull and were placed into paraformaldehyde (4% RL) for a minimum of 24 hours. The cochleae were transferred and washed in RL and subsequently were placed in a plastic tube for scanning. The cochleae were imaged with the microCT operated at 45 kV tube voltage, 88 μA tube current and 300 ms integration time for each projection. A complete description of the scanner can be found elsewhere (Scanco Medical Web site, www.scanco.ch). Each of the 120–300 contiguous slices was reconstructed on $1,024 \times 1,024$ grid with 30 μm isotropic volume elements (voxels). The reconstructed slices were imported into ImageJ (<http://rsbweb.nih.gov/ij/>) using a plugin to load the Scanco files (Kunzelmann, <http://www.dent.med.uni-muenchen.de/~kkunzelm/exponent-0.96.3/index.php?section=49>) and were converted into a stack of TIFF files. The images were segmented using the Amira Software (Visage Imaging, San Diego, CA). A representation in three dimensions was created for scala tympani, spiral ganglion, cochlear nerve and optical fiber.

Imaging with temperature sensitive dyes

Thermochromic ink (5K0XX31D1204 – thermochromic UV screen, Chromatic Technologies Inc., Colorado Springs, CO) was used to image the beam path in the guinea pig hemicochlea preparations. The thermochromic ink has a transition temperature of 31 $^{\circ}\text{C}$, below which it is black, and becomes colorless or diminishes its color intensity (gray) when the temperature exceeds the nominal change temperature. The color changes can be used to determine the temperature change. The ink was dissolved in 1% solution of agarose (Sigma-Aldrich, St Louis, MO) to make a ~10% ink solution. Since the ink-agarose gel is primarily composed of water, IR from the laser is absorbed and causes an increase in temperature, which is documented by the change in color (black to gray/colorless) of the ink within the gel. For the measurements, excised guinea pig cochleae were sliced along their mid-modiolar plane to produce a hemicochlea as described previously (Teudt and Richter, 2007). The hemicochlea specimen was then bathed in the ink-agarose solution for 30 minutes and maintained at 39 $^{\circ}\text{C}$. The hemicochlea was removed from the solution and allowed to cool down such that the ink-agarose solution gelled (34–37 $^{\circ}\text{C}$ gelling temperature). The specimen was immersed in Ringer's lactate solution and was placed under a Leitz Medilux microscope (Leitz, Grand Rapids, MI) for imaging. Similar to the *in vivo* experiments, a 200 μm -diameter optical fiber was placed through a cochleostomy in the cochlear wall. To calibrate the effect of the temperature, the hemicochleae were bathed in distilled water at 41 $^{\circ}\text{C}$. The bath was allowed to cool down and images were captured at each 1 $^{\circ}\text{C}$ drop in its temperature. The change in color was recorded via images captured with a digital camera (DCR-TRV, Sony, New York, NY). Following the calibration, the hemicochleae were irradiated to determine the beam path by monitoring the color changes in front of the optical

fiber. The captured images from the calibration and the irradiation experiments were subsequently analyzed using MATLAB (MathWorks, Natick, MA) and ImageJ.

Results

Twelve guinea pig cochleae were used to determine the beam path: three specimens were whole mounts, six cochleae were reconstructed from serial sections, and three cochleae were used for the microCT scans. It was difficult to determine distances along the cochlea in the whole mount preparations. Therefore, the three whole mount specimens were not considered for the quantitative analysis.

Physiology

Neural activity at 16 contacts along the ICC electrode array was recorded (representative example, Figure 1). To examine the neural responses recorded from the ICC, raster plots (1A), PSTHs (1B), average firing rate (1C) and cumulative d' (1D) were calculated for responses recorded at each contact. For the raster plot in Figure 1A, each sub-panel along the x-axis shows the neural activity for increasing radiant energies. The length of the x-axis in each sub-panel is the time after the stimulus presentation (100 ms). The energy was measured in air at the tip of the optical fiber and was varied, in the given example, between 2.2 and 72 $\mu\text{J}/\text{pulse}$. Typically the energy was increased from 2.2 $\mu\text{J}/\text{pulse}$ to up to 127 $\mu\text{J}/\text{pulse}$. Along the y-axis, each panel shows the neural activity following 400 presentations of the IR pulse at each of the contacts along the electrode array. The dots represent the times at which an action potential occurred. If dots are randomly distributed, no stimulation occurred (e.g. at contacts 1 and 2). On other contacts, for example contacts 4–13, a neural response was evoked following the stimulus. In this example, the threshold for INS for the given orientation of the optical fiber was 7.5 $\mu\text{J}/\text{pulse}$ (contact 11).

While most of the responses were excitatory, in this example IR inhibited the neural activity at contact 5. We have compared the INS evoked responses at contact 5 with the acoustically evoked responses prior to damaging the cochlea. Pure tone stimuli did not inhibit neural activity. It is not clear whether the observed inhibition during INS may have resulted from the damaging of the cochlea or by changes in the recording conditions at the electrode contact. A systematic examination of clusters that showed inhibition was not possible because inhibition was only seen on a total of three contacts out of all of our recordings (see also Discussion).

To better quantify the responses to the stimulation, the data from Figure 1A were used to construct PSTHs (Figure 1B) and evoked rate of action potentials (Figure 1C). For each energy level (x-axis) and for each electrode contact (y-axis) the PSTH is shown in Figure 1B. The resolution (bin size) of the PSTH was 0.1 ms. Figure 1C uses the same data and shows the spontaneous and evoked rate of action potentials. The y-axis of Figure 1C shows the electrode contacts of the multi channel electrode array, contact 16 being the most superficial electrode contact and contact 1 the contact that was inserted the deepest into the inferior colliculus (1.5 mm). Following the calibration with an acoustical stimulus, each of the electrode contacts was correlated to a best frequency, as shown. Along the x-axis the energy per pulse has been plotted. The color bar gives the average response rate, which was calculated as an average rate 0.1 s after the stimulus presentation using 50 stimulus repetitions at a given energy level. The resulting spatial tuning curve from these plots is similar to that obtained from the raster plot.

Figure 1D shows the d' values that were calculated using ROC analysis. Similar to Figure 1C, the y-axis of Figure 1D shows the electrode contacts of the multi channel electrode array. From the calibration obtained with an acoustic stimulus, each of the electrode contacts

could be correlated to a best frequency, as shown in Figure 1D. The color bar gives the cumulative d' values, which were calculated as described in Methods. The contour plot obtained here indicates a multiple peaked spatial tuning curve. Based on the results obtained from the rate analysis, the PSTH, and the raster plots for this data, we treat the multi-peaked plots as a broad single peaked spatial tuning curve spanning a range of 4 to 16 kHz (Figure 1D).

Whole mount

After recording neural responses from the ICC, a CO₂ laser was used to ablate the bone and tissue along the trajectory of the stimulation beam path. Tissue carbonization as a result of the CO₂ laser irradiation was evident as dark marks in the cochlea; an example is shown in Figure 2(A and C, black arrowheads). The carbonization marks were then used to estimate the optical beam path through the cochlea. With the whole mount dissection, detailed sketches of the cochlea were made (Figure 2B and D) parallel and perpendicular to the modiolus. The sketches and the carbonization marks were matched to published data of the frequency representation along the guinea pig cochlea (Figure 2D). The cochleostomy, through which the optical fiber was positioned, is marked by blue arrowheads (Figure 2A, and C). A straight line was traced from the site of the cochleostomy to the ablated tissue (arrow in Figure 2D). The structures located along this line that would be irradiated are highlighted in red - roughly the region of cochlea responding to 2 and/or 12 kHz. (Figure 2B and D). Since it is difficult to determine the exact distances from the basal end of the cochlea to the marked sites along the cochlea, the whole mount preparations were only used for qualitative characterization of the beam path in the cochlea.

Reconstruction of the beam path in the cochlea - Histology

Reconstructions of serially sectioned cochleae were used to determine the beam path in the cochleae. The cochleae were sliced perpendicular to the modiolus and coordinates for the inner pillar foot, the center of the spiral ganglion, the center of the ablated area, and the cochleostomy were recorded. Figures 3A–C show three sequential serial sections of an example guinea pig cochlea, that were 50 μ m apart. As described in Methods, three-dimensional reconstructions of the cochleae were made from multiple serial sections that were aligned and stacked with visual histological markers (Figure 3D). The reconstructions of the cochlear sections showed the spatial correlation among the main cochlear structures. Three representative reconstructions are shown in Figures 4–6. In each of the figures, the reconstructions are illustrated in three different orientations (A–C). The green markers denote the position of the inner pillar feet, the black markers show the center of the spiral ganglion, red markers show the ablated sites, and the blue markers denote the cochleostomy. By aligning the red and the blue markers the orientation of the beam path can be estimated and the neural structures in the beam path can be identified. Histology was then correlated with physiology. For this purpose, a frequency-place map obtained from previous publications (Tsuji and Liberman, 1997) was used to convert the best frequencies obtained from the STCs, which were constructed from neural activity in the ICC, into distances from the basal end of the cochlea. The STC were added to the reconstructions, represented as diamonds (Figures 4–6). The largest of the diamonds denotes the best frequency and the smaller diamonds represent the range of the stimulated area. Hence, electrophysiological data derived from ICC recordings in response to IR in each cochlea (panel D in each figure) could be compared to histological data in each reconstructed cochlea. To determine the trajectory of the optical beam, the reconstructed cochlea was oriented such that the cochleostomy, through which the stimulating optical fiber was introduced, and the ablated site were aligned (panel C in Figures 4–6). When oriented in this fashion, the reconstructed cochleae show a correspondence between the cochleostomy, the site of tissue ablation, and the location of stimulation corresponding to STCs. From the analysis of 6 cochleae

reconstructed in this manner and the corresponding STCs, spiral ganglion locations corresponding to frequencies between 2 and 15 kHz were successfully targeted. The neural structures within the beam path include, the spiral ganglion cells, the dendrites and the axons in the center of the modiolus and the organ of Corti. In the example shown in Figure 4, the location of the ablated tissue occurs approximately 3.5 mm from the base. With the assumption that stimulation occurs at the spiral ganglion neurons, aligning the cochleostomy and the area of ablation reveals two possible sites of stimulation along the cochlea, one at about 3–4 mm from the base and the other at about 4.5–6 mm (Figure 4A–C). According to the frequency map used, the corresponding frequencies are 7.9–12.8 kHz and 15–20 kHz. The frequencies obtained from the STC, which was constructed from the response in the ICC to optical stimulation of the cochlea, showed two sites of stimulation, 10.8 and 16.1 kHz. This suggested a correlation between the histology and the physiology. The finding supports the view that the spiral ganglion neurons are the target for stimulation. The site closest to the beam seems not to be completely in the beam path as indicated by the gray line in Figure 4A, which is representing the optical fiber and its beam path. Therefore, no response or only a small response would be expected at the site closer to the optical fiber. Since the beam path crosses parts of the nerve trunk in the center of the modiolus stimulation, stimulation could also occur at this site. This issue is addressed in more detail in Discussion.

For the example in Figure 5, the ablation site was at the same approximate location of Figure 4 (~3.5 mm from the base). The corresponding ICC recordings (Figure 5D) showed stimulation at lower frequencies (4 kHz), which corresponded to 8 mm from the base (Tsuji and Liberman, 1997). Once the cochleostomy and the ablated tissue were aligned in the reconstructed cochlea to replicate the optical path, the stimulated site also aligned with the trajectory of the beam. For this example, spiral ganglion neurons, dendrites, and axons that are close to the neurons, are in the beam path. The reconstruction reveals that the nerve trunk in the center of the modiolus is not included. Rather the beam passes through the thin bony layer of spiral lamina of the more basal turn and includes the spiral ganglion neurons at a more apical site (approximately 8 mm from the base). Since no response could be recorded from neurons located opposite of the tip of the optical fiber, stimulation of the axons in the spiral lamina might not have occurred.

In a different example (Figure 6), the electrophysiology data show a broad area of stimulation, ranging from 11 kHz to 6 kHz, with maxima at 8 kHz (data from the same animal are also shown and described in Figure 1). The stimulation overlapped with the ablation site and a contiguous site giving the appearance of multiple peaks in the frequency place map graph. For this selected example, the beam path was tangential to the spiral ganglion and included spiral ganglion cells, the corresponding dendrites, and the axons of the neurons in spiral lamina. Again, the axons in the nerve trunk in the center of the modiolus were not in the beam path.

Reconstruction of the beam path in the cochlea - microCT

Three guinea pig cochleae, stimulated with IR using a 200 μ m diameter optical fiber, were scanned using the microCT after the optical fiber was cemented in place with dental acrylic. Figures 7A, 7D, and 7G show reconstructed cochlear sections for the three cochleae, which are oriented parallel to the optical fiber. The sections reveal the axis of the optical fiber and the potential target structure for stimulation. The projection of the optical fiber can be extended towards the modiolus and the spiral ganglion. The intersection of the extension of the fiber and the modiolar structure identifies possible sites of stimulation (Figure 7). Moreover, the distance between the tip of the optical fiber and the neural structure was calculated. In the present experiments, the separation of the optical fiber from the target was 481.9 (Figure 7A), 304.5 (Figure 7D), and 481.7 μ m (Figure 7G). The distances of the

stimulation sites from the basal end of the cochlea were estimated from the STCs. The values were 3.5–5.8, 4.9–5.7, and 4.8–7.3 mm, respectively.

Figures 7B, 7E, and 7H show schematics of the sections and identify the relevant physiological structures. The optical fiber, the auditory nerve in the center of the modiolus (N) the spiral ganglion (SG), and the bone have been labeled. The simplified sketches show that the beam path does include the spiral ganglion neurons, dendrites, and the section of the axons in spiral lamina. Only in one case, one can argue that the beam path includes the nerve trunk in the center of the modiolus (Figure 7H).

Figures 7C, 7F, and 7I show 3D segmented volumes from the microCT data made using the Amira Software. The auditory nerve in the center of the modiolus is represented in green, the scala tympani in purple, Rosenthal's canal which houses the spiral ganglion in red, and the optical fiber in yellow. The orientation of the optical fiber and beam path relative to the neural target structures can be examined, which included the spiral ganglion neurons and their dendrites and axons.

Beam path imaging in hemicochlea with thermochromic ink

To obtain a direct visual confirmation of the beam path, the measurements were repeated using the thermochromic ink-agarose gel in a hemicochlea (Figure 8). Panel A shows a 200 μm core-diameter optical fiber inserted into the ink in the basal turn of the hemicochlea. The orientation and placement of the optical fiber mimic the *in vivo* placement. Panel B shows the change in the colorization of the gel in response to a single optical pulse of 2 ms duration and the energy was 2.72 mJ/pulse. It should be noted that the energy per pulse used in this experiment was approximately 200 times the threshold energy per pulse used to stimulate the cochlea. The ink-agarose gels do not show a detectable change in colorization at the lower, stimulation-level energy ranges. For the selected laser parameters, the absorption of the radiation by water in the gel led to a local change in temperature and the resultant decolorization of the thermochromic ink is noticeable. The overall change is shown in Panel C, which was obtained by subtracting the background (panel A) from panel B, to remove any effect of the ambient light. The decolorization from the single pulse spread along the axis of the optical fiber but extended very little to the side (perpendicular to the beam path). The area of the hemicochlea that showed a temperature change was $\sim 580 \mu\text{m}$ (axial) by $\sim 480 \mu\text{m}$ (lateral). A temperature-color calibration curve was obtained from the ink-agarose gels (not shown). Panel D shows a sketch of cochlear structures, which can also be identified in panels A and B. It can be seen that the orientation of the fiber selected and the resulting beam path allow for optimal targeting of the spiral ganglion cells.

Discussion

Effect of optical fiber orientation

The optical beam path determines the selectivity of INS. For the auditory system, depending on the orientation of the optical fiber, different sections along the cochlea can be irradiated. With the assumption that the spiral ganglion neurons are the main target structure for optical stimulation, a longer section along the spiral ganglion is irradiated if the beam is tangential to the structure, and a smaller section is irradiated if the beam is perpendicular to the ganglion. Current experiments showed both situations. In the example of Figure 6, the beam path was tangential, whereas for the other two examples shown (Figures 4 and 5), the beam path was perpendicular to the spiral ganglion. The corresponding spatial tuning curves, obtained from the ICC, tend to be narrow when the beam path was perpendicular to the spiral ganglion. Among the broad stimulation profiles that were recorded when the optical fiber was in the tangential orientation, some had multiple maxima as demonstrated in Figure

6. The profiles suggest that distinct sites along the cochlea were stimulated. According to the beam path, INS can occur at the nerve trunk, at the spiral ganglion neurons, at dendrites or axons of the spiral ganglion neurons. However, direct stimulation of the auditory nerve is less likely because the nerve trunk in the center of the modiolus is not irradiated in the tangential orientations of the beam path.

In the multi peak STCs, although the d' value for the locations between the peaks did not reach threshold values ($d' \approx 1$), the presentation of the data in Figure 1 reveals that neural responses in the ICC were indeed evoked by optical stimuli at those locations. Therefore, the response profiles for such cases are considered as one confluent response area as indicated by the black lines in Figure 6. The profile would be expected to be broad for a beam path that is tangential to the spiral ganglion as shown in the reconstruction (Figure 6). In case the optical fiber is oriented perpendicular to the spiral ganglion, the above explanation does not hold and for multiple peak profiles two distinct sites of stimulation should be considered. An example is shown in Figure 4.

The possibility of stimulation at the nerve trunk cannot be eliminated. However, stimulation of this structure is unlikely since for many examples this structure is not included in the beam path. Another argument that support the view that stimulation unlikely occurs at the nerve trunk but at the spiral ganglion cells is the threshold for stimulation. The stimulation threshold for peripheral and cranial nerves is significantly higher (0.312–1.22 J/cm², Wells et al., 2005; Teudt et al., 2007; Wells et al., 2007) when compared to the stimulation threshold found for the present experiments (~0.016 J/cm²). One can argue that stimulation occurs rather at the cell body than at the axons of the spiral ganglion neurons.

Since the optical fiber was inserted through a cochleostomy in the cochlear base, neural responses to optical stimulation were expected at sites of the multi-channel electrode that responded to acoustic frequencies between 10 and 25 kHz. However, optical stimulation resulted in responses with best frequencies at electrode sites that responded to acoustical frequencies between 2 and 15 kHz. Responses at electrode sites that correspond to 2 kHz can only be explained if neurons in the turn just above the basal turn were irradiated. Note that for this case the beam path does not cross the center of the modiolus. The tip of the optical fiber is in front of the spiral lamina and the structures in the beam path could include the bone and the dendrites of the spiral ganglion cells.

Using the current surgical access, stimulation of cochlear locations at the very base is difficult. Furthermore, those sites correspond to best frequencies above 25 kHz that are not monitored by the multi channel electrode array.

Frequency mapping

Frequency tuning curves that were obtained from single fiber recordings and subsequent staining of the corresponding neurons with HRP have been used to correlate stimulation sites along the cochlea with characteristic frequencies (Robertson, 1984; Tsuji and Liberman, 1997). A frequency-place map of the guinea pig cochlea has been constructed from those *in vivo* measurements. The data have been fitted to an equation that provides the

frequency-place map for the guinea pig cochlea: $f = 10^{-\left(\frac{d-66.4}{38.2}\right)}$, where f denotes the frequency in kHz, and d the distance from the basal end of the cochlea in % (Tsuji and Liberman, 1997). From this equation, the lowest frequency that the guinea pig responds to is 0.132 kHz and the highest frequency is 54.7 kHz (Tsuji and Liberman, 1997). In the present experiments, a frequency range between 1 kHz and 25 kHz could be monitored with the multi-channel electrode array inserted in the ICC, which is similar to the frequency range obtained by other groups in the guinea pig using a similar method (Snyder et al., 2004a;

Snyder et al., 2008). With the total length of the guinea pig basilar membrane of 18–20.8 mm (von Békésy, 1960; Robertson, 1984; Tsuji and Liberman, 1997; Hofman et al., 2009), the sites that can be monitored with the multichannel electrode along the cochlea are between 2.5 and 12.4 mm from the basal cochlear end. In other words, the very base and the apex of the cochlea could not be monitored. This has implications on the orientation of the optical fiber. The cochleostomy is about 2–3 mm from the basal end of the cochlea. When the optical fiber is oriented towards more basal sites along the cochlea, it is likely that neural response will not be recorded with the multi channel electrode because the stimulated locations are outside of the measurable range. Similar, more apical orientations of the optical fiber will likely not result in a measurable response because it is not in the range for the multi channel electrode array. The sites along the cochlea that are in the direct beam path for the radiation are approximately between 3 and 6.5 mm from the basal end of the cochlea. Those locations correspond to frequencies between 6.5 and 20 kHz. If the beam path passes through the spiral ligament to the second turn sites between 10 and 11 mm from the basal of the cochlea are in the beam path. The corresponding frequency range is between 1.5 and 2.3 kHz.

Beam path in the cochlea

An overall analysis of the beam path in the cochleae was performed from the histological reconstructions and the microCT scanned cochlea. For the histological reconstructions, the distances from the basal end of the cochlea to the ablated sections along the cochlea were determined. The center of the ablation mark was determined and was plotted as a black circle in Figure 9. Using the cochlear reconstructions, sites along the spiral ganglion that would be in the beam path were added to the figure. The two possible sites were plotted in the Figure 9. The black diamonds in Figure 9 are the corresponding sites for the best frequencies obtained from neural responses in the ICC. The data reveal a good match between the histology and the physiology.

The beam path of any optical radiation will be determined by the wavelength of the optical source and the optical tissue properties at that wavelength. For INS experiments, the radiation wavelength is in the near infrared, 1862 nm, and the relevant tissues are cochlear fluids, lipids, blood and bone. At this radiation wavelength, the laser tissue interactions are governed, to a first approximation, by water absorption. The absorption coefficient for water, and similarly the cochlear fluids, at this wavelength is 12 cm^{-1} , which corresponds to about 0.8 mm penetration depth (Falk and Ford, 1966; Hale and Querry, 1973; Walsh and Cummings, 1994). In other words, the radiant energy decreases to $1/e$ (1/Euler number) or to 37% of its initial value over this distance. At these wavelengths, scattering plays a minor role in the laser-tissue interaction in water. Consequently, the optical beam does not diverge much over the distance through the fluids (the divergence angle is about 1°). Measurements of the optical properties of bone at shorter wavelengths ($<1.0\text{ }\mu\text{m}$) show significant scattering (Antonio, 1949; Firbank et al., 1993; Ugnell and Oberg, 1997). While it is likely that bone also scatters radiation to some extent at 1862 nm, the absorption and scattering coefficients of bone have not been quantified near this wavelength. However, according to our own measurements, the divergence angle of 1862 nm radiation incident on a thin section of bone is $\sim 26^\circ$ (unpublished data). As a result, the optical spot increases in diameter.

Once the optical radiation has been absorbed by water, most of the energy is dissipated as heat, which causes a local temperature rise. In the present experiments this fact has been used to estimate the beam path in the cochlea by studying the heating in the path of the optical beam (Figure 8). A hemicochlea was filled with the thermochromic ink. Color changes of the ink were correlated with the ambient temperature. The delivery of single pulses resulted in a measurable temperature change if the radiant energy was selected high enough, here about 200 times the radiant energy required to evoke an action potential in the

cochlea. The higher energy required to measure a detectable change in the colorization of the thermochromic ink may either be due to a limitation of our setup or the sensitivity of the ink. The higher energy laser pulses resulted in transient temperature changes of 1.8 °C. Note that the frame rate was at 30 Hz. This rate does not allow measurement of peak temperature changes; rather temperature averages over 33 ms intervals are examined. The peak temperature for a single pulse might therefore be larger than what has been measured with this approach. The heat profiles were 450 μm wide, which is about twice the diameter of the optical fiber. Although the time course is still considered to be short enough for thermal confinement, heat could already be dissipated out of the irradiated target volume, and hence increasing the width of the temperature profile from the irradiation.

In a previous manuscript it has been calculated that the temperature change that can evoke a CAP is 0.15 °C at the tip of the optical fiber and 0.08 °C at the spiral ganglion cells (Izzo et al., 2008), which occurs using radiant energies that are approximately 200 times smaller than the radiant energy used for the thermochromic ink imaging experiments.

Neural stimulation with INS

INS has recently been investigated as an alternative method to electrical stimulation of neurons. While the mechanism is currently unproven, it is believed to involve neural depolarization secondary to a transient temperature increase in the tissue. Recent reports show that an acoustic signal is produced in a hydrated environment from a diode laser similar to the one used for INS here (Teudt et al., 2011). Other experiments using a q-switched, frequency doubled Nd:YAG laser ($\lambda = 532 \text{ nm}$) have demonstrated non-localized activation of the cochlea upon irradiation (Wenzel et al., 2009; Zhang et al., 2009). This Nd:YAG laser produces very short laser pulses, which will generate a significant pressure transient in the irradiated medium. The present findings reveal that only sites along the beam path could be activated and support the hypothesis of a direct interaction between the incident radiation and the neural tissue resulting in the observed neural responses. The data does not suggest a secondary effect caused by mechanical stimulation of hair cells, for example.

Limitations

The CO₂ laser can give information about the trajectory of the optical beam because the waveguide was oriented through the cochleostomy and had the same trajectory as the stimulating optical fiber. However, the absorption and scattering characteristics of tissue differ between wavelengths used for stimulation (1.86 μm) and for ablation (10.6 μm). Therefore, absolute values of penetration depth or spot size for the ablated tissue will not be as applicable.

The monitoring system for the temperature changes had a limited resolution, here 4 steps per °C. Therefore, temperature changes that were expected at stimulation threshold could not be monitored with the current setup. A more detailed study examining the temperature load during optical stimulation will need to be carried out.

Conclusions

This manuscript presents data that support the INS mechanism of direct interaction of IR and spiral ganglion cells. The spatial selectivity of the ICC responses was determined by the optical path in the cochlea and the incident spot on the spiral ganglion. These data will assist in building an optical interface, e.g. size and orientation, for use in future optical cochlear implants. Furthermore, we have demonstrated that microCT allows imaging the optical fiber in the unopened cochlea. Future experiments will need to determine the position of the

optical fiber in the cochlea in chronically implanted animals, perhaps using in-vivo microCT or MRI scanning.

Acknowledgments

This project has been funded with federal funds from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN260-2006-00006-C/NIH No. N01-DC-6-0006. We thank Northwestern University MicroCT facility for use of the microCT.

Abbreviations

CAP	compound action potential
CCD	charge-coupled device
CO₂	Carbon dioxide
EDTA	ethylenediamine tetraacetic acid
HRP	Horse Radish Peroxidase
ICC	central nucleus of the inferior colliculus
INS	infrared neural stimulation
IR	infrared radiation
NIH	National Institute of Health
microCT	micro-computed-tomography
PBS	phosphate buffered saline
PSTH	peri stimulus time histogram
RL	Ringer's Lactate
ROC	receiver-operating characteristics
SPL	sound pressure level, in dB referenced to 20 μ Pa

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Research Highlights

- Optical stimulation of neurons target spiral ganglion cell bodies
- Only neural structures in the beam path are stimulated
- Selective stimulation of the cochlea is possible
- Selectivity is a function of the beam path

Figure 1.

A–D show the responses recorded from the ICC during optical stimulation of the cochlea. A shows the raster plots, B the PSTHs, C the average rate and D the cumulative d' calculations. All plots reveal a spatial tuning curve. While the cumulative d' calculations and the rate plots indicate a multi peaked curve, the raster plots and the PSTHs suggest that the spatial tuning curves should be treated as a broad stimulation area.

Figure 2.

A, C show the whole mount image of a guinea pig cochlea from a lateral view and top view respectively. The black arrow on the basal turn of the cochlea indicates carbonization, a result of the tissue ablation with the CO₂ laser. The blue arrow indicates the cochleostomy. B, D. Schematic drawing of the guinea pig cochlea. The black solid line traces the inner pillar cells. The black dashed line represents the carbonized tissue. The red dots and the red line represent the sites in the cochlea located along the beam path, which is indicated by the arrow. Approximate frequency place map in the cochlea from previously published data is shown for reference.

Figure 3.

A. Histological section indicates the coordinates of important structures used to produce a 3D reconstruction: Spiral ganglion neurons (black triangle), tissue ablation by CO₂ laser (red triangle), inner pillar foot (green triangle), cochleostomy (blue triangle). B. and C. Histological sections showing sequential images used to create 3D reconstruction 50 μm apart from each other. (Scale bar is 500 μm .) D. 3D reconstruction of a guinea pig cochlea. The green markers represent the inner pillar foot, the black markers the spiral ganglion neurons, the blue markers the cochleostomy and the red markers the CO₂ laser ablation site. The gray cylinder represents the approximate orientation of the 200 μm optical fiber inserted through the cochleostomy.

Figure 4.

A, B. 3-D reconstruction of a guinea pig cochlea rotated at different angles. Each structure has a different color of the marker: inner pillar feet are green, the center of the spiral ganglion is black, the center of the sites that were ablated are red the site of the cochleostomy is blue. The orange diamonds show the corresponding electrophysiological recordings. The orientation in panel C is that cochleostomy and ablated sites are aligned. The figure shows that the stimulated site along the cochlea is in this projection. D shows corresponding spatial tuning curve obtained from neural activity recorded from ICC. Measurements show a peak of stimulation at 10.8 and 16.1 kHz.

Figure 5.

A, B. 3-D reconstruction of a guinea pig cochlea rotated at different angles (similar to Figure 4). D shows corresponding spatial tuning curve obtained from neural activity recorded from ICC with a peak of the stimulation at 4 kHz.

Figure 6.

A, B. 3-D reconstruction of a guinea pig cochlea rotated at different angles (similar to Figure 4). D shows corresponding spatial tuning curve obtained from neural activity recorded from ICC. Based on PSTH and rate plots, a broader stimulation area should be considered covering a frequency range of 6–11 kHz. The peak response was observed at 8 kHz.

Figure 7.

A, D, and G show microCT slices of guinea pig cochleae. B, E, and H are illustrative sketches of A, D, and G. A 200 μm optical fiber inserted through the cochleostomy into scala tympani can be seen. The optical fiber points towards the spiral ganglion cells in Rosenthal's canal (gray area). The auditory nerve (N) runs in the center of the modiulus. The basal and second cochlear turns can be seen in the images. The beam path includes the spiral ganglion cells and may also include the eighth nerve in the center of the modiulus. G, F, and I are 3D reconstructions of the cochleae after segmentation. The auditory nerve in the center of the modiulus is green, while the fluid filled spaces such as scala tympani, scala media, and scala vestibuli are purple and transparent. Rosenthal's canal that houses the spiral ganglion neurons is red. The optical fiber is yellow.

Figure 8.

Shown is the basal turn of a guinea pig hemicochlea. After sectioning, the cochlea was filled with a 1% agar solution containing temperature sensitive ink. The tip of a 200 μm diameter optical fiber was oriented towards the spiral ganglion. A shows the optical fiber in position with the laser off. B shows the same preparation during optical stimulation. C: To demonstrate the changes in temperature, A was subtracted from B. D is a sketch of the A and B, in which the key structures are labeled.

Figure 9.

Results from histology or microCT (circles) and physiology (diamonds) were compared. Histology and the microCT scans were used to determine the segment along the cochlea, which could be irradiated by the infrared radiation delivered through the optical fiber inserted into the cochlea. The distances for the stimulation sites along the cochlea are given in mm from the basal end of the cochlea. From the neural responses recorded in the inferior colliculus, corresponding segments along the cochlea that are stimulated could be determined. The multi electrode arrays, which were inserted into the ICC, were calibrated and provided the frequency-range over which neural activity was evoked by pulsed IR. The equation for the frequency place map published by Tsuji and Liberman (1997) was then used to convert the frequency range into distances along the cochlea. The results are shown with the diamonds. Histology and function are in good agreement. In four of the examples the site closest to the optical fiber shown as gray circles was not stimulated but a second site further along the cochlea.