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Optical stimulation of auditory neurons: Effects of acute and chronic deafening

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Abstract

In developing neural prostheses, particular success has been realized with cochlear implants. These devices bypass damaged hair cells in the auditory system and electrically stimulate the auditory nerve directly. In contemporary cochlear implants, however, the injected electric current spreads widely along the scala tympani and across turns. Consequently, stimulation of spatially discrete spiral ganglion cell populations is difficult. In contrast to electrical stimulation, it has been shown that extremely spatially selective stimulation is possible using infrared radiation (e.g. Izzo et al., 2007a). Here, we explore the correlation between surviving spiral ganglion cells, following acute and chronic deafness induced by neomycin application into the middle ear, and neural stimulation using optical radiation and electrical current.

In vivo experiments were conducted in gerbils. Before the animals were deafened, acoustic thresholds were obtained and neurons were stimulated with optical radiation at various pulse durations, radiation exposures, and pulse repetition rates. In one group of animals, measurements were made immediately after deafening, while the other group was tested at least four weeks after deafening. Deafness was confirmed by measuring acoustically evoked compound action potentials. Optically and electrically evoked compound action potentials and auditory brainstem responses were determined for different radiation exposures and for different electrical current amplitudes, respectively. After completion of the experiments, the animals were euthanized and the cochleae were harvested for histology.

Acoustically evoked compound action potential thresholds were elevated by more than 40 dB after neomycin application in acutely deaf and more than 60 dB in chronically deaf animals. Compound action potential thresholds, which were determined with optical radiation pulses, were not significantly elevated in acutely deaf animals. However, in chronically deaf animals optically evoked CAP thresholds were elevated. Changes correlated with the number of surviving spiral ganglion cells and the optical parameters that were used for stimulation.

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Keywords

cochlea; cochlear implants; deafening; optical stimulation; spatial selectivity

Introduction

Multi-electrode cochlear implants are designed to stimulate discrete spiral ganglion cell populations along the cochlea. Thus, it is possible to restore what is commonly thought of as the tonotopic organization of the normal acoustically stimulated cochlea. High frequency tones activate neurons at the base of the cochlea, while low frequency tones stimulate neurons towards the cochlear apex (Greenwood, 1990; von Békésy, 1960). However, the assumption that discrete neural populations can be electrically activated is not always true. It is widely assumed that stimuli applied between closely spaced bipolar electrodes can locally stimulate spiral ganglion cells, whereas widely spaced electrode pairs will lead to broad electric fields and will result in wide areas of neural activation (Busby et al., 1994; van den Honert et al., 1987).

Nevertheless, for closely spaced electrode pairs at high current levels, a broad region of auditory neurons will be activated (Frijns et al., 1996; van den Honert et al., 1987). Consequently, when two neighboring electrodes are stimulated, a portion of each current field will overlap, resulting in a population of spiral ganglion cells that is stimulated by both electrodes. This overlap in stimulation can have two effects. Firstly, if two electrodes stimulate the same neural population, sound sensation encoded via these two electrode contacts might be confused or even be indistinguishable and this will reduce the number of independent channels of information that can be conveyed to the cochlear implant user. Secondly, the interaction of the current fields can be used to generate additional frequency sensations with frequencies that are between the frequency sensations obtained by stimulating each of the electrode contacts alone. This phenomenon has been examined in the early literature on electrical current field distributions in the cochlea and single fiber recordings in the auditory nerve (Black et al., 1983; Kral et al., 1998) and more recently has been reexamined (Koch et al., 2006). One objective for novel cochlear implant devices is to increase the number of different frequencies that can be encoded with novel speech processing strategies. However, contemporary cochlear implants and coding strategies lack the possibility of simultaneously transferring acoustic information at neighboring electrode contacts because of the current spread in the cochlea. At present, the problem of information transfer at neighboring electrode contacts is largely ameliorated by the use of interleaved pulse trains. In contrast to electrical stimulation, the use of pulsed infrared optical radiation to stimulate spiral ganglion cells could be one step towards a more discrete stimulation of the auditory system, thus stimulating an increased number of independent sub-populations of spiral ganglion cells for parallel speech processing.

Although the concept of using light to stimulate nerves is not novel (Arsonval, 1891; Arvanitaki et al., 1961; Fork, 1971), the use of pulsed mid-infrared lasers to transiently elicit an action potential in a one-to-one manner using low energy radiation is (Wells et al., 2005a; Wells et al., 2005b; Wells et al., 2007). This method of nerve stimulation affords a contact-free, artifact-free stimulation that can improve the spatial selectivity of neural stimulation as compared to electric stimulation. During the last two years, we have demonstrated that the auditory nerve can be stimulated with optical radiation as well (Izzo et al., 2006; Izzo et al., 2007b). The goal of our research is to replace electrodes in a cochlear implant with optical radiation sources, with the intent of stimulating more discrete populations of neurons.

Previous experiments were all conducted on normal hearing animals to establish proof of concept and baseline data for optical stimulation. One may argue that the laser pulse generates a pressure wave, which then stimulates the cochlea by vibrating the basilar membrane. We have conducted a series of control experiments that do not support stimulation of the cochlea secondary to a pressure wave, but through direct interaction between the neural tissue and the optical radiation. The likely mechanism by which optical stimulation occurs is a small, transient increase in tissue temperature upon light absorption by water (Wells et al., 2007), leading to the depolarization of the neurons. Only neurons in the optical path receive suprathreshold levels of infrared radiation and are stimulated selectively (Izzo et al., 2007a). Mid-infrared radiation does not scatter in tissue as electric current spreads (Nevel et al., 2007). A photochemical mechanism is unlikely because there is no single wavelength or narrow wavelength band at which the nerve stimulation is enhanced. In addition, the energy of infrared photons is low ($<0.1\text{eV}$) and is unlikely to result in a rapid photochemical reaction. A photomechanical process has also been ruled out as a possible mechanism. Specifically, no cochlear microphonic (CM) can be detected in the optically evoked CAPs (Izzo et al., 2006). CMs, which are present in acoustic CAPs, indicate deflection of cochlear hair cell cilia in response to a mechanical perturbation of the cochlear fluids. Furthermore, experiments on acute and chronically deafened gerbils indicate that it is possible to stimulate deafened cochleae in which no hair cells exist (present study and Izzo et al., 2006). Moreover, optical stimulation of neurons is wavelength dependent. For radiation wavelengths with shorter penetration depths, the distance between the target structure and the radiation source must be reduced (Izzo et al., 2007c). If the laser pulse would generate a pressure wave, stimulation of the auditory nerve should vary little if the position of the orientation of the optical fiber in the cochlea is changed.

More recently, it has been examined whether optical stimulation of peripheral nerves, including the auditory nerve is safe (Izzo et al., 2006; Teudt et al., 2007; Wells et al., 2005a; Wells et al., 2007). Indeed, a laser can be used to stimulate the auditory system for up to six hours at 13 Hz repetition rate without significantly changing the CAP amplitude (Izzo et al., 2007b). Moreover, auditory neurons could be stimulated for 2 hours at a 400 Hz repetition rate and the CAP peak-to-peak amplitude did not change significantly (Izzo et al., 2007d). Chronic studies are underway but are not completed yet.

Experiments to prove that auditory responses can be evoked with optical radiation pulses were conducted in normal hearing animals. However, a normal hearing ear will likely not reflect the anatomy and electrophysiological responses of cochlear implant users. Laser stimulation parameters may change if the cochlea has been damaged and neural degeneration has occurred. In other words, potential benefits of optical stimulation in the deafened cochlea are unknown and might vary widely depending on the condition of the auditory nerve. Here, we explore the correlation between the number of surviving spiral ganglion cells in the deafened gerbil cochlea and the amplitude of the compound action potential evoked by optical and electrical stimulation. To induce cochlear damage with subsequent neural degeneration, neomycin at different concentrations was injected into the middle ear.

Methods

Adult gerbils (*Meriones unguiculatus*) were used for the experiments. The animals were acutely deafened by a drop ($\sim 10\text{ }\mu\text{l}$) of neomycin, 100 mM in Ringer's Lactate, on the round window, following the surgical procedures described below. Prior to and after deafening the animals, cochlear function was determined by measuring acoustically evoked cochlear compound action potentials (CAPs). To achieve chronic deafness, neomycin at concentrations between 10 and 100 mM in Ringer's Lactate was applied into the middle ear

in anesthetized, but otherwise undisturbed, gerbils. Before animals were deafened, cochlear function was determined by measuring acoustically evoked auditory brainstem responses (ABRs). The gerbils recovered from anesthesia. After 4 weeks, the cochleae of the chronically deaf gerbils were surgically accessed (described below) and deafness was documented by measuring acoustically evoked CAPs.

After determining cochlear function in acutely and chronically deaf animals using tone pips, the cochleae were stimulated optically and electrically and CAPs and ABRs were recorded, respectively.

The care and use of the animals in this study were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and was approved by the Animal Care and Use Committee of Northwestern University.

Animal surgery

Animal surgery was done as described previously (Emadi et al., 2004). Briefly, the gerbils were anesthetized by an initial intraperitoneal injection of sodium pentobarbital (80 mg/kg body weight). Maintenance doses were 17 mg/kg bodyweight and were given throughout an experiment whenever the animal showed signs of increasing arousal, which was assessed every 15 minutes by a paw withdrawal reflex. After the animal was fully anesthetized, breathing was facilitated by performing a tracheotomy and by securing a length of PE90 tubing into the opening in the trachea. Body temperature was maintained at 38°C by placing the animal on a heating pad and the heart rate was monitored with two chest electrodes. For the surgery, the animal was stabilized in a heated head holder. The bulla was exposed and subsequently opened to allow access to the cochlea. A silver electrode was hooked onto the bony rim of the round window of the cochlea, and a ground electrode was placed under the skin at the opposite jaw. For acoustic stimulation, a speculum was cemented with dental acrylic to the outer ear canal. The surgical platform containing the animal was then moved onto a vibration isolation table in a soundproof booth. A series of auditory nerve compound action potential (CAP) threshold curves and auditory brainstem responses were obtained across a range of frequencies in order to determine baseline cochlear function.

Measuring cochlear function with acoustically evoked compound action potentials (CAPs)

Compound action potential (CAP) thresholds were defined as sound levels required for a 30 μ V amplitude (A1) between the first minimum (N1) and the first maximum (P1) of the CAP at a given stimulus frequency (Fig. 1) and were determined using a modified tracking procedure as described previously (Emadi et al., 2004). Acoustical stimuli used to determine the CAP thresholds were tone bursts of 12 ms in duration, including a 1 ms rise and fall time. CAP thresholds were determined by comparing a threshold criterion ($A_2=30 \mu$ V) with the peak-to-peak voltage (A1) 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 with a set of custom programmable filters, the highpass set to 300 Hz and the lowpass set to 3000 Hz. CAP thresholds were determined for a frequency range of five octaves with a resolution of 6 steps per octave. The highest frequency was 50 kHz.

Measuring cochlear function with acoustically, optically, and electrically evoked auditory brainstem responses

After the animals were deeply anesthetized, auditory brainstem responses were obtained by subtracting ipsilateral mastoid potentials from vertex potentials measured relative to a

ground electrode placed in the neck. The electrodes were connected to a differential amplifier (ISO-80, WPI, Sarasota, FL) with a high input-impedance ($>10^{12}\Omega$). The amplifier setting was at 80 dB. Further filtering (300 to 3000 Hz) of the signal was obtained through a Frequency Devices filter (IP90). Sampling rate was 200 kHz, and responses to 100 stimulus presentations were averaged. ABR thresholds were defined as sound levels required for a visible response to acoustic stimuli. The noise floor in an averaged ABR recording is typically 1 μ V. In particular, the appearance of wave II of the ABR was monitored. ABR thresholds were measured for acoustical, optical and electrical stimuli described below.

Acoustical, electrical, and optical stimuli

Voltage commands for acoustical and electrical stimuli were generated using a computer I/O board (KPCI 3110, Keithley) inserted into a PC and were used to drive a Beyer DT 770Pro headphone. For acoustically evoked ABRs and CAPs, tone bursts (12 ms duration, including a 1 ms rise/fall) with different carrier frequencies were presented at a rate of 4 Hz. The sound pressure was measured with a real head coupler (Pearce et al., 2000).

Electrical stimuli consisted of biphasic pulses (250 μ s per phase). The voltage commands controlled a current amplifier (Valhalla, Type 2500), which converted the voltage command into current pulses. The current pulses were delivered to the cochlea via bipolar silver-electrodes (125 μ m outer diameter and 75 μ m core diameter) that were advanced adjacent to the optical fiber and through the round window in the basal turn of the cochlea. Again, pulses were presented at a repetition rate of 4 Hz.

Optical stimulation was achieved with a diode laser (Renoir RINS, Aculight Corp., Bothell WA). The wavelength could be varied between 1.844 – 1.873 μ m and the lengths of pulse durations were 30, 60, 100, 200, 400, 800 and 1600 μ s. The laser was operated at 13 Hz repetition rate, and it was coupled to an optical fiber with a core diameter of 200 μ m. The optical fiber was placed adjacent to the round window membrane, touching but not penetrating it. The orientation of the fiber was such that the optical radiation was directed towards the spiral ganglion cells. The pulse energy of the laser was controlled directly by varying the current supplied to the laser diode. The overall stability of the laser was maintained by internal closed feedback loops that regulate the diode temperature (and thus the emission wavelength) and current while monitoring output power.

Deafening of the animal

Acutely deaf—After auditory responses to acoustic, electric, and optic stimulation were recorded, the animals were acutely deafened. Ten microliters of Ringer's Lactate containing neomycin (100 mM) were applied to the round window. CAP threshold elevations were documented (Fig. 2).

Chronically deaf—Animals were deafened by injecting 100-150 μ l Ringer's Lactate containing neomycin (5, 10, 25, 50, 75 or 100 mM) trans-tympanically into the middle ear cavity. After the injection, the animals were allowed to survive for at least four weeks before hearing was tested. Neural degeneration could occur and the animals were chronically deaf. Cochlear damage was confirmed by either the elevation of CAP thresholds or the absence of acoustically evoked cochlear responses (Fig. 2) and subsequent histology.

Histology

At the conclusion of the electrophysiological experiments, the gerbils were overdosed with sodium pentobarbital (200 mg/kg bodyweight) and decapitated. The bullae were removed and the cochleae exposed. A small opening was made in the cochlear apex. The cochleae were placed in 0.1 M phosphate buffer solution containing 4% paraformaldehyde. The

specimens were fixed for 4 hours and then transferred into 0.1 M phosphate buffer. Embedding in plastic (Araldite Resin) followed a standard protocol: rinse in phosphate buffer three times for 15 minutes, dehydrate in Acetone (15 minutes in 25%, 15 minutes in 50%, 15 minutes in 75%, 15 minutes in 90%, 15 minutes in 100%, 20 minutes in 100%), infiltrate in plastic (7:1 Acetone:Plastic, 1:1 Acetone:Plastic, 1:7 Acetone:Plastic, three times pure plastic). After 12 hours in an oven, the specimens were ready for sectioning on an ultramicrotome (Ultracut, American Ultracut). Slices, 5 μm thick, were cut parallel to the modiolar plane and were placed on glass slides. After staining with toluidine blue, ten pictures of consecutive mid-modiolar slices were captured and the spiral ganglion cells were counted as described below. The number of cells per area provided the spiral ganglion cell density.

Counting of spiral ganglion cells

After slicing the cochleae, ten consecutive mid-modiolar sections were selected to capture images from the spiral ganglion at different locations along the cochlea. Initially a profile count was conducted. Every spiral ganglion cell in a cross section with a nucleus larger than 5 μm and a nucleolus was counted. This approach resulted in a significant overcounting of cells. Therefore, a stereological method was applied. Using serial sections through the mid-modiolar plane of the modiolus, the method “reconstructs” a section of the spiral ganglion and consequently avoids counting a cell in multiple subsequent sections. Because the method is extremely time intensive, only one cochlea at each concentration of neomycin was counted with this method to determine the factor by which a profile count would overcount the number of spiral ganglion cells. It has been determined that the factor is 0.71 by which the raw counts obtained from the profile counts had to be multiplied. The factor was independent from the degree of degeneration that occurred in the cochlea. The basic procedure to count the cells with the stereological method was to locate profiles of the spiral ganglion cells in the first section, which is called the look-up section. The counted spiral ganglion cells had nuclei with a diameter of at least 5 μm and a nucleolus. In the subsequent section (reference section) only spiral ganglion cells were counted that were not present in the look-up section. After counting the cells in the reference section, the reference section then became the look-up section and a subsequent third section became the new reference section. Ten subsequent sections were counted.

In addition to counting cells, the cross sectional area of Rosenthal’s canal and the cross sectional area of randomly selected spiral ganglion cells was determined. Measurements of both structures were taken using ImageJ (Wayne Rasband, NIH, public domain software). The program’s scale was calibrated and the scale setting was changed from pixels to micrometers. This conversion was accomplished by determining the number of pixels between two lines of the image of a standard slide having 10 μm divisions. Area measurements were obtained by tracing the bony opening of Rosenthal’s canal or by tracing spiral ganglion cells at different locations along the cochlea. The total number of pixels within a circumscribed area was calculated and converted into square micrometers or square millimeters.

Statistics

Means and standard deviations were calculated for the electrophysiology thresholds and the spiral ganglion cell counts. An analysis of variance (ANOVA) was performed. If the ANOVA indicated differences among the means, a posteriori test was used for making pairwise comparisons among the means. An honestly significant difference (HSD) test by Tukey was used. The tests are part of a statistical package provided by IGOR[®] (Wavemetrics). Statistical decisions were made for a probability $p=0.05$.

Results

Thirty-one adult gerbils (*Meriones unguiculatus*) of either sex were successfully deafened. Twelve of the animals were examined immediately after deafening and 19 animals were studied four weeks after the deafening: the latter animals were chronically deaf. In contrast to acoustical stimuli, optically evoked CAP thresholds did not change significantly in acutely deaf animals but revealed elevated CAP thresholds in chronically deaf animals. Threshold elevation correlated with the spiral ganglion cell density.

Acutely deaf animals

Application of 10 μ l neomycin (100mM in Ringer's Lactate) on the intact round window membrane elevated the acoustically evoked CAP thresholds by 40 to 60 dB. Changes occurred within 30 minutes. When the acoustic threshold elevations at frequencies ranging from 2 to 10 kHz were more than 40 dB, optically evoked compound action potential thresholds were determined and were compared with responses obtained before deafening the animals. Radiant exposures to evoke a 50 μ V amplitude CAP are summarized in Table 1 and in Figure 3 and 4. Acute deafening of the animals with 10 μ l neomycin (100 mM in Ringer's Lactate) slightly decreased the maximum peak-to-peak optically evoked CAP (Figs. 3 and 4). Differences between the averages were statistically not significant.

Chronically deaf animals

Ears were deafened with neomycin and the animals were allowed to survive at least four weeks before CAP thresholds were determined. The neomycin concentration was 50/100 mM (right/left ear) in 6 animals, 25/75 mM (right/left ear) in 7 animals, and 5/10mM (right/left ear) in 6 animals.

When the ears were deafened with neomycin concentrations of 10 mM, acoustically evoked CAP thresholds were elevated by approximately 10 dB (Fig. 2). No responses to acoustic stimuli could be evoked in any animal after the application of Ringer's Lactate containing a concentration of 25 mM neomycin or higher.

CAP thresholds could be determined using optical radiation pulses. However, CAPs could not be evoked in all animals (Table 2). Since some of the deafened animals had no measurable cochlear responses to optical radiation pulses, we were interested to determine if electrical stimulation would be possible in those animals. In all animals in which optically evoked auditory brainstem responses (oABRs) were present, we were also able to electrically evoke auditory brainstem responses (eABRs; Fig. 5; Table 2). Moreover, in chronically deaf animals in which no oABR could be evoked, no eABR could be evoked either. For animals in which optically evoked CAP thresholds could be determined, the radiant exposures to evoke a 50 μ V CAP at pulse durations of 100 μ s or shorter were similar to those determined in acutely deaf animals. For pulse durations of 200 μ s and longer, radiant exposures for the CAP thresholds were clearly elevated. Threshold radiant exposures for the chronically deaf animals are shown in Table 1. Keep in mind that the averages shown in Table 1 only include the animals for which a CAP could be evoked with an optical radiation pulse.

Radiant exposures up to 127 mJ/cm² or electric currents pulses up to 1 mA failed to evoke a CAP or ABR in animals that were deafened with neomycin concentrations of 50 mM and higher.

Histology

The number of surviving spiral ganglion cells correlated with the neomycin concentration. The radiant exposure required to optically evoke a 50 μ V CAP was correlated with the number of surviving spiral ganglion cells (Fig. 6).

Cell counts—For acutely deaf animals the density of spiral ganglion cells did not change after the application of the neomycin (Fig. 7A). In contrast to acutely deaf animals, chronically deaf animals had lower spiral ganglion cells densities (Fig. 7B, Table 3). When compared with control animals, the spiral ganglion cell counts observed for neomycin applications of 25 mM and greater were statistically significant.

Cell cross sectional areas—For chronically deaf animals, the cells' appearance changed as well. The cells' cross-sectional areas decreased (Fig. 8) with increasing neomycin concentration. Note that the cross-sectional area of the spiral ganglion cells in the control animals is larger in the basal and the apical sections of the cochlea and decreases for the middle section (Fig. 8). The values for the area, in μm^2 , are shown in Table 4. Differences in the cells' average cross sectional area were significant between control and 25 mM, 10 and 50 mM, 25 and 50 mM of neomycin. Differences in the cells' average cross sectional area were not significant between 50 and 75 mM and 50 and 100 mM of neomycin.

Discussion

Deafening of the gerbils by neomycin application (10 μ l of Ringer's Lactate containing 100 mM of neomycin) on the round window elevated acoustically evoked CAP responses by 40 - 60 dB, but had little effect on the optically evoked CAP thresholds. In particular, for short pulse durations (in the present study 100 μ s), minimal changes in thresholds were observed. For longer pulse durations, optically evoked CAP thresholds were elevated. Differences were statistically not significant. Moreover, the maximum CAP amplitude decreased for all pulse durations. Again, changes were statistically not significant.

For acoustic stimulation, it has been established that the CAP amplitude depends on the number of auditory neurons stimulated simultaneously (Davis et al., 1934; Derbyshire et al., 1935). We assume that, similar to acoustical stimulation, the number of spiral ganglion neurons that simultaneously depolarized in response to optical stimuli determine the amplitude of the compound action potential. Consequently, factors that determine the CAP amplitude include the spiral ganglion cell population and the synchronous firing of neurons in response to the optical radiation. Histology did not reveal any decrease in spiral ganglion cell number in the acutely deaf animals. Therefore, factors must be involved that alter the synchronous firing of the neurons and consequently decrease the total number of spiral ganglion cells that can be activated simultaneously. Ongoing experiments, during which neural activities from single auditory nerve fibers are recorded, will be able to determine whether the threshold and the maximum response of the neuron is reduced in the acutely deaf animals.

The number of spiral ganglion cells decreased by about 30% between control animals and animals which received 25 mM neomycin in Ringer's Lactate. For neomycin concentrations above 25 mM, the number of cells did not differ largely between animals, however, the ability to optically or electrically evoke a compound action potential did. Following the application of neomycin at concentrations of 10 mM or below, optically evoked responses were always recorded. After the application of 25 mM neomycin, the optical stimulus evoked an action potential in only approximately 30% of the animals. Moreover, for neomycin concentrations of 50 mM and larger, no cochlear responses could be evoked in response to an acoustical, optical, or electrical stimulus.

For the cases when optical radiation evoked cochlear responses, the compound action potential amplitude was not only correlated with the number of surviving spiral ganglion cells but also with the laser pulse duration. Threshold elevations were minimal for pulse durations less than 200 μ s, but were significant at or above 200 μ s. To discuss this difference in thresholds, it is necessary to consider the underlying mechanism of optical stimulation of the nerve.

At mid-infrared wavelengths, light is primarily absorbed by water in the tissue and converted to heat (Niemz, 2004; Welch et al., 1995). The likely mechanism by which optical stimulation occurs is a spatially and temporally confined small increase in tissue temperature upon light absorption (Wells et al., 2007). The temporal characteristics of this thermal confinement are proportional to a characteristic dimension of the tissue, which is related to the light absorption. There is evidence that this characteristic dimension in optical stimulation of the cochlea may be on the order of 1 – 10 μ m, the diameter of a spiral ganglion cell body or a central projection (Izzo et al., 2007c). If thermal confinement in the spiral ganglion cells governs optical stimulation, then a decrease in the size of the cell due to deafening would affect the temporal characteristics of the stimulation, e.g. the pulse widths at which stimulation thresholds are optimized.

Although the interaction of optical radiation and the tissue will likely result in spatially confined stimulation of neurons, the benefit of optical stimulation for cochlear implant users might still vary widely among patients. A potential factor underlying this variability might be the condition of the auditory nerve and the number of surviving spiral ganglion cells. Several groups have investigated to what degree the condition of the auditory nerve or the number of spiral ganglion cells in the cochlea affect electrically evoked responses from the cochlea (Hall, 1990). The results were equivocal: while some of the studies have not found a correlation between eABRs and surviving spiral ganglion cells (Simmons, 1979; Stypulkowski et al., 1986), others showed that the maximum eABR amplitudes and the slopes of eABR growth function were correlated with the number of spiral ganglion cells (Hall, 1990; Miller et al., 1983; Smith et al., 1983).

The motivation to correlate the eABR with the number of surviving spiral ganglion cells has a sound theoretical basis. Goldstein and Kiang (1958) presented a quantitative model of the round window response including the first negative peak (N_1) of the compound action potential from the cochlea. The N_1 is seen as the convolution of the action potential of a single auditory nerve fiber and the probability density function of action potentials for the entire population of nerve fibers. The model predicts that with the nearly complete synchronization resulting from short current pulses, the maximum compound action potential amplitude is correlated to the number of excitable nerve fibers. Consequently, the eABR should also be directly correlated to the number of spiral ganglion cells in the cochlea.

Although it has been demonstrated that optical radiation can be used to stimulate auditory neurons selectively and after deafening the animals, it remains to be determined if stimulation with optical radiation is safe after a stimulator has been chronically implanted and stimulation occurs over extended periods of time.

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Figure 1.

Shown is a typical example for a recording from the round window of gerbil cochlea before (left) and after (right) deafening with a 10 μ l drop of Ringer's Lactate on the round window containing 100 mM neomycin. The radiant exposure increases from the bottom trace to the top trace. Compound action potential peak-to-peak amplitudes change little before and after deafening.

Figure 2.

Shown are average sound levels to evoke a 30 μ V compound action potential. Cochleae with three different conditions are shown: (1) control, (2) at least four weeks after the application of neomycin at a concentration of 10 mM, and (3) at least four weeks after the application of neomycin at a concentration of 25, 50, 75 or 100 mM. No responses to acoustic stimuli could be evoked for neomycin concentrations above 25 mM. The filled circles, therefore, shows the maximum sound level at which the neurons were tested and not the CAP thresholds obtained during stimulation.

Figure 3.

Optically evoked compound action potential peak-to-peak amplitudes increase with increasing radiant exposure. Different panels show the results for different pulse durations as indicated. The maximum amplitudes are smaller after acutely deafening the cochlea with a 10 µl drop of Ringer's Lactate on the round window containing 100 mM neomycin. Differences are statistically not significant.

Figure 4.

Shown are the optical radiant exposures required to evoke a 50 μ V peak-to-peak compound action potential. Radiant exposures were determined for normal hearing animals, for acutely deafened animals with a 10 μ l drop of Ringer's Lactate on the round window containing 100 mM neomycin, and chronically deaf animals. Deafness was confirmed four weeks after the injection of 150 μ l of Ringer's Lactate containing 10-100 mM neomycin into the middle ear. Acutely deaf animals show small elevations in compound action potential thresholds. Differences are not statistically significant. For chronically deaf animals, small and statistically non-significant changes in radiant exposure exist for radiation pulses 100 μ s and shorter. Statistically significant threshold elevations occur for radiation pulses of 200 μ s and longer.

Figure 5.

Shown are optically (left) and electrically (right) evoked auditory brainstem responses. For chronically deaf animals, the ability to evoke an ABR optically or electrically was tested. In all cases, electrically and optically evoked auditory brainstem responses were either both present or both absent (see also Table 2).

Figure 6.

Spiral ganglion cell densities at different locations along the cochlea (see also figure 6) are compared with radiant exposures required to evoke a 50 μ V peak-to-peak compound action potential. Note that the size of the circles corresponds to the laser pulse duration. Larger diameters represent longer pulses. The number of spiral ganglion cells correlate with radiant exposure. If the number of spiral ganglion cells decreases by a factor of three, the radiant exposure required for the 50 μ V compound action potential amplitude increases by an order of magnitude. Note, the maximum radiant exposure from the laser was limited to approximately 1 J/cm². Also, the correlation appears better for the numbers at the upper base and the apical location.

Figure 7.

Shown is the spiral ganglion cell density, which has been determined at different locations along the cochlea. **A** shows the cell density for the control and for acutely deaf animals after deafening with a 10 μ l drop of Ringer's Lactate containing 100mM of neomycin on the round window. Cell density has not changed significantly. **B** shows the spiral ganglion cell density for chronically deaf animals. Animals were deafened by injecting 150 μ l of Ringer's Lactate containing 10, 25, 50, 75 or 100 mM neomycin in the middle ear. Cell densities were determined four weeks after the injection. Increasing concentrations of neomycin resulted in increasing loss of spiral ganglion cells.

Figure 8.

Cross-sectional areas of spiral ganglion cells at different locations along the cochlea. Data are shown for animals that received no treatment and animals that received 150 μ l Ringer's Lactate in the middle ear containing 10, 25, 50, 75 and 100 mM neomycin. Cross sectional areas of the cells were determined four weeks after the application of the neomycin. Cross sectional areas were larger in the base and apex. With increasing concentrations of neomycin, the cross-sectional area decreased.

Table 1

The average radiant exposure required to optically evoke a 50 μ V CAP. The averages for the chronically deaf animals include animals that were deafened with neomycin concentrations of 10 and 25 mM and only include the animals for which a CAP could be evoked with an optical radiation pulse. For neomycin concentrations of 50 mM and larger, radiant exposures up to 127 mJ/cm² or electric currents pulses up to 1 mA did not evoke a measurable CAP or ABR.

Pulse length [μ s]	control [J/cm ²]	acutely deaf [J/cm ²]	chronically deaf [J/cm ²]
30	0.003 $\pm$ 0.001 (N=10)	0.006 $\pm$ 0.003 (N=9)	0.012 $\pm$ 0.002 (N=3)
60	0.006 $\pm$ 0.003 (N=9)	0.007 $\pm$ 0.002 (N=10)	0.065 $\pm$ 0.091 (N=3)
100	0.007 $\pm$ 0.010 (N=11)	0.009 $\pm$ 0.002 (N=10)	0.164 $\pm$ 0.312 (N=4)
200	0.014 $\pm$ 0.006 (N=10)	0.014 $\pm$ 0.006 (N=10)	0.272 $\pm$ 0.523 (N=6)
400	0.012 $\pm$ 0.010 (N=10)	0.017 $\pm$ 0.001 (N=10)	0.559 $\pm$ 0.104 (N=6)
800	0.020 $\pm$ 0.087 (N=10)	0.020 $\pm$ 0.040 (N=9)	0.719 $\pm$ 1.262 (N=6)
1600	0.020 $\pm$ 0.060 (N=9)	0.037 $\pm$ 0.030 (N=10)	0.887 $\pm$ 0.107 (N=7)

Table 2

Shown are the number of animals which were used for both electrical and optical stimulation and the corresponding qualitative outcome for electrical and optical stimulation.

	base	upper base	middle	apex
corrected raw count				
control	15.8 ± 3.7	12.8 ± 6.0	17.1 ± 6.8	47.6 ± 19.0
10 mM	19.9 ± 5.6	15.5 ± 4.1	13.9 ± 4.0	66.6 ± 30.5
25 mM	8.9 ± 11.8	6.7 ± 10.5	8.2 ± 5.1	42.0 ± 23.1
50 mM	7.8 ± 3.7	6.4 ± 1.9	7.4 ± 2.4	43.7 ± 31.2
75 mM	7.2 ± 0.6	5.0 ± 0.3	5.3 ± 1.8	30.6 ± 7.1
100 mM	7.1 ± 2.1	5.3 ± 1.1	6.0 ± 0.9	11.3 ± 18.7
Rosenthal's canal area [mm ²]				
control	0.0156 ± 0.0011	0.0101 ± 0.0019	0.0123 ± 0.0049	0.0220 ± 0.0088
10 mM	0.0186 ± 0.0029	0.0127 ± 0.0035	0.0094 ± 0.0010	0.0760 ± 0.0206
25 mM	0.0164 ± 0.0048	0.0123 ± 0.0025	0.0127 ± 0.0023	0.0273 ± 0.0148
50 mM	0.0212 ± 0.0062	0.0137 ± 0.0022	0.0151 ± 0.0026	0.0698 ± 0.0326
75 mM	0.0190 ± 0.0017	0.0125 ± 0.0008	0.0123 ± 0.0021	0.0428 ± 0.0086
100 mM	0.0192 ± 0.0026	0.0127 ± 0.0017	0.0137 ± 0.0025	0.0286 ± 0.0240
spiral ganglion cell density [cells/mm ²]				
control	1010 ± 133	1275 ± 262	1397 ± 249	2164 ± 68
10 mM	1070 ± 348	1220 ± 367	1475 ± 564	876 ± 142
25 mM	541 ± 453	545 ± 617	648 ± 641	1535 ± 385
50 mM	369 ± 96	468 ± 169	492 ± 84	627 ± 131
75 mM	377 ± 18	399 ± 58	427 ± 46	715 ± 6
100 mM	368 ± 71	414 ± 134	439 ± 161	397 ± 689

Table 3

The values are the averages $\pm$ their standard deviations from three cochleae (10 slices per cochlea). Numbers are given for the counts, the area, and the density.

	neomycin concentration (mM)	optical stimulation	electrical stimulation
11-June-07 #C11	10	Yes	not done
18-June-07 #c13	10	Yes	Yes
20-June-07 #c15	10	Yes	Yes
21-June-07 #c16	10	Yes	Yes
7-June-07 #c10	25	No	No
14-June-07 #c12	25	No	No
2-July-07 #c19	25	No	No
3-July-07 #c20	25	Yes	Yes

Values of the cross sectional areas of spiral ganglion cells are averages in $\mu\text{m}^2 \pm$ their standard deviations for three cochleae for controls, 10, 25, 50, 75 and 100 mM neomycin, respectively.

Table 4

	Control	10 [mM]	25 [mM]	50 [mM]	75 [mM]	100 [mM]
basal	92.6 $\pm$ 32.6	75.7 $\pm$ 9.2	70.0 $\pm$ 12.4	55.7 $\pm$ 11.3	53.6 $\pm$ 10.6	52.1 $\pm$ 7.9
upper basal	76.6 $\pm$ 18.8	71.7 $\pm$ 9.6	64.7 $\pm$ 12.6	50.7 $\pm$ 10.6	51.3 $\pm$ 7.5	47.8 $\pm$ 9.9
middle	70.0 $\pm$ 16.0	69.1 $\pm$ 9.0	64.4 $\pm$ 15	52.0 $\pm$ 8.2	49.7 $\pm$ 8.6	49.3 $\pm$ 9.2
apical	92.6 $\pm$ 21.8	72.9 $\pm$ 6.8	69.3 $\pm$ 8.2	60.7 $\pm$ 9.6	59.5 $\pm$ 11.5	58.4 $\pm$ 6.8