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Masking of Infrared Neural Stimulation (INS) in hearing and deaf guinea pigs

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Abstract

Spatial selective infrared neural stimulation has potential to improve neural prostheses, including cochlear implants. The heating of a confined target volume depolarizes the cell membrane and results in an action potential. Tissue heating may also results in thermal damage or the generation of a stress relaxation wave. Stress relaxation waves may result in a direct mechanical stimulation of remaining hair cells in the cochlea, so called optophony. Data are presented that quantify the effect of an acoustical stimulus (noise masker) on the response obtained with INS in normal hearing, acutely deafened, and chronic deaf animals. While in normal hearing animals an acoustic masker can reduce the response to INS, in acutely deafened animals the masking effect is reduced, and in chronic deaf animals this effect has not been detected. The responses to INS remain stable following the different degrees of cochlear damage.

Keywords

infrared neural stimulation; hearing; cochlea; laser; masking

1. INTRODUCTION

Spatial selective infrared neural stimulation (INS) has demonstrated potential for improved neural prostheses, including cochlear implants (CIs). Contemporary CIs use electrical current to activate neurons that are stimulated in a pristine cochlea by the hair cells of the Organ of Corti. With CIs, it is difficult to stimulate small populations of auditory neurons due to the spread of current in the tissue. In comparison to electrical stimulation, optical radiation can be delivered to a more focused area and INS has demonstrated higher spatial selectivity [1, 2]. Hence, employing INS technologies in cochlear implants could lead to higher fidelity cochlear prostheses.

Limitations of INS include radiation scattering or absorption by the tissue between the optical source and the neuron. Scattering would increase stimulation spread while absorption of the photon before it reaches the target neuron will result in additional tissue heating and eventual thermal damage. An unintended absorption effect of the radiation can be optophony, the direct mechanical stimulation of cochlear hair cells by stress relaxation waves [3–5]. This effect might arise from the rapid heat absorption and expansion of the confined target volume during laser stimulation. It has been demonstrated that INS results in tissue heating that changes the capacitance of a cell's plasma membrane, depolarizes the cell and elicits an action potential [6]. Heating of the target tissue as well as the interstitial fluids by the laser may generate a stress relaxation wave. This pressure wave can mechanically stimulate cochlear hair cells. If stress relaxation waves produce an audible response then optoacoustic stimulation would occur [3, 4] in addition to a direct stimulation of the neuron with the laser by changes of the membrane capacitance.

Here, data are presented that support the view that cochlear responses during INS result from the direct interaction between the laser radiation and the auditory neurons and not from an acoustic event resulting from a stress relaxation wave. The effect of an acoustic stimulus on the response obtained with INS in normal hearing, acutely deafened and chronic deaf animals has been studied.

2. MATERIALS AND METHODS

2.1 Animal procedures

Pigmented guinea pigs (200–1500 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 and were approved by the Animal Care and Use Committee of Northwestern University. As described before, animals were anesthetized with urethane 1.3 g/kg i.p.. Urethane injections, supplemented with ketamine (44 mg/kg) and xylazine (5 mg/kg) at the beginning of surgical procedures. 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 animal was anesthetized, a tracheotomy was made and a plastic tube (1.9 mm outer diameter, 1.1 mm inner diameter, Zeus Inc., Orangeburg, SC) was secured into the trachea. The tube was connected to an anesthesia system (Hallowell EMC, Pittsfield, MA). The animals were ventilated on oxygen throughout the length of the experiment. Next, the animal was placed in a stereotactic head holder (Stoelting, Kiel, WI). Once the head was secured with the ear bars, a custom-made post was attached to the holder to affix a stabilizer post to the skull using 1.5 mm cortex screws (veterinary orthopedic implants, St Augustine, FL) and methyl methacrylate (Teets, Diamond Springs, CA). After the methyl methacrylate cured, the left ear bar was removed to allow for better surgical access and unobstructed pathway for auditory stimulus presentation with the Beyer DT770Pro speaker. A c-shaped skin incision was made behind the left ear lobe and the cervicoauricular muscles were removed. The cartilaginous outer ear canal was exposed and cut for acoustic stimulus presentation. The left bulla was exposed and opened approximately

2×3 mm with a motorized drill (World Precision Instruments, Sarasota, FL). The basal turn of the cochlea was identified and a cochleostomy was created with a 0.5 mm Buckingham footplate hand drill (Richards Manufacturing Co., Memphis, TN) or with the motorized drill and a 1 mm drill bit. The optical fiber was inserted through the opening of the cochlear wall. To measure compound action potentials (CAPs), a silver ball electrode was placed on the round window with a reference in the tissue near the neck.

2.2 Laser stimulation

Cochlear stimulation was achieved with a diode laser (Lockheed Martin Aculight Corp., Bothell, WA). The laser was coupled to the optical fibers (Ocean Optics Inc., Dunedin, FL) with a core diameter of 200 μm . The numerical aperture was 0.22, and the acceptance angle was 25.4° in air (P200-5-VIS-NIR, Ocean Optics, Dunedin, FL). Individual optical fibers were mounted to a micromanipulator (MHW103, Narishige, Tokyo, Japan) to ensure consistent orientation during stimulation. The radiation wavelength was 1862 nm and the pulse duration was 100 μs . The pulse energy of the laser was controlled directly by varying the current to the laser diode and was between 0 and 127 $\mu\text{J/pulse}$ at the tip of the optical fiber in air. Pulses were presented at 10 Hz repetition rate.

2.3 Acoustic masking procedure

Two stimuli of the same type (acoustical, electrical, or optical) will interact when delivered to the cochlea if the level of the stimuli is sufficient. In the present study, the interaction between an optical stimulus and an acoustical masker was tested. The driving voltage for the white noise stimulus was generated with a waveform generator (HP Type 3211). The signal was fed to an audio amplifier to drive a Beyer DT770Pro speaker. The speculum of the speaker was coupled to the outer ear canal. The effect of the acoustical masker on the responses obtained with INS was tested as follows: (1) measure input-output contour for INS (record radiant energy versus compound action potential (CAP) amplitudes for increasing radiant energies); (2) select the radiant energy for a mid level response from the input-output contour; (3) stimulate the cochlea simultaneously with infrared pulses of the selected radiant energy and the acoustical stimulus with increasing noise level. The changes in CAP amplitude to the infrared stimulus provide information on the interaction between the optical and the acoustical stimulus.

2.4 Deaf animals

Acute deaf—During the experiments some of the animals had normal hearing. The masking effect of the noise masker was recorded in the pristine cochlea. Next, the cochlea was acutely damaged by the injection of less than 50 μL of 20 mM neomycin in Ringer's Lactate heated to 38 °C into scala tympani, after some of the perilymph was wicked away. The effect of the neomycin injection was documented with CAP threshold tuning curves. The laser responses after damaging the cochlea were characterized and the changes in the masking effect were determined.

Chronic deaf—The experiments were conducted in hearing acute and chronic deaf animals. To deafen the animals, neomycin (20 mM) dissolved in Ringer's Lactate was heated to 38 °C and was injected into the middle ear. The animals were allowed to survive

for at least four weeks to allow for neural degeneration to occur. Before INS was applied, lack of cochlear function was confirmed by recording acoustically evoked responses.

2.5 Data analysis and statistics

Acoustic responses to optical stimuli in normal hearing, acutely deafened, and chronic deaf animals at varying masking levels were analyzed using Igor Pro software (WaveMetrics). CAPs in normal hearing and neomycin deafened animals were recorded to obtain sound thresholds. For each masker level, a CAP recording at each noise stimulus was recorded and plotted against time. CAP amplitudes after each deafening were normalized by the maximum amplitude and plotted against the noise masker level to observe the effects of masking on the audible responses.

3. RESULTS

For the present study, 5 guinea pigs were used. Neural responses evoked by infrared laser pulses could be masked with an acoustical masker in normal hearing animals. Acute deafening resulted in a rapid decrease in the acoustically evoked CAP amplitude (elevation of the CAP threshold, Figure 1) but had little effect on the change in CAP amplitudes evoked by the laser stimulation (Figure 2). As shown in Figure 3, the response to INS could be masked in normal hearing animals. Elevation of auditory thresholds due to acute or chronic deafening resulted in a loss of masking.

Interaction between acoustic stimuli and INS is shown by a depression in CAP amplitude as the noise stimulus is increased (bottom to top trace) in normal hearing animals. The left column in Figure 2 demonstrates this masking effect. This masking effect is largely reduced or disappears completely in the middle and right columns.

In Figure 3 the effect of masking on an acutely deafened animal is quantified. The difference in the maximum (P1) and minimum (N1) CAP amplitudes were normalized and plotted against noise level. CAP amplitudes in normal hearing animals decrease as the noise level increases, hence indicating that interaction between the acoustic stimulus and INS occurs. After the cochlea is injected with neomycin and auditory thresholds change significantly, the masking effect disappears.

In Figure 4 the effect of masking on a chronic deaf animal is quantified. The normalized CAP amplitude recorded in response to varying noise stimuli remained constant. This indicates that no interaction between laser and acoustic stimulation occurred and as a result, no masking is observed.

4. DISCUSSION

The results from the present study support the hypothesis that INS results as a direct interaction between the optical radiation and the tissue, which is an important finding for the development of prostheses using INS. While previous studies provided proof of principle of INS, our understanding of its mechanism remains incomplete. Progression towards useful clinical applications, such as CIs, depends on this understanding. In principal, laser-tissue interactions can occur via various biophysical mechanisms, including photochemical,

photothermal, photomechanical and electric field effects [7–9]. Wells and coworkers have investigated the various mechanisms that could be responsible for optical stimulation [10, 11]. They suggested that the primary mechanism of INS is a transient temperature increase that results from the absorption of the optical radiation by water in the tissue. Potential mechanistic candidates include thermal activation of a particular ion channel or thermally induced biophysical changes in the membrane. Results from a recent study to determine the mechanism of INS show that the rapid local increase in temperature with each laser pulse transiently alters the electrical capacitance of the plasma membrane, generating depolarizing currents in *Xenopus laevis* oocytes, mammalian cells and artificial lipid bilayers [12]. As a result of the local heating, volumetric tissue expansion, and stress relaxation waves were considered. Pressure waves resulting from the INS-induced volumetric expansion [4] and from the use of lasers with laser parameters that result in stress confinement [3, 5, 13] were investigated in more detail and must be considered during stimulation of the auditory system (see discussions in [4, 14]).

Here, masking experiments were conducted on cochleae of normal hearing, acutely deafened, and chronic deaf animals to determine contribution of pressure waves on INS in the cochlea. For a normal hearing animal, it should be possible to mask the response evoked by INS with a noise masker. In case both INS and the acoustic masker vibrate the basilar membrane and mechanically stimulate the hair cells, both responses should largely diminish after deafening the animal but the masking effect should remain. However, if INS is the result of a direct interaction between the optical radiation and the neuron, the response to INS should change little after deafening of the animal due to the cochlear damage sustained during the deafening, but the masking effect should diminish with increasing elevation of the acoustic threshold.

Figure 3 shows that masking occurs in normal hearing animals as expected. Following acute deafening, this masking effect disappears, possibly indicating the absence of interfering mechanoacoustic stimuli. In animals with impaired cochlear function, this effect is largely reduced but does not disappear completely. Data from chronic deaf animals show no masking at all (e.g. Figure 4).

In conclusion, the experiments provide evidence that stimulation is dominated by direct interaction between neurons and radiation, not by mechanical stimulation.

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References

1. Richter CP, et al. Spread of cochlear excitation during stimulation with pulsed infrared radiation: inferior colliculus measurements. *Journal of neural engineering*. 2011; 8(5):056006. [PubMed: 21828906]
2. Izzo AD, et al. Selectivity of neural stimulation in the auditory system: a comparison of optic and electric stimuli. *Journal of biomedical optics*. 2007; 12(2):021008. [PubMed: 17477715]

3. Schultz M, et al. Nanosecond laser pulse stimulation of the inner ear-a wavelength study. *Biomedical optics express*. 2012; 3(12):3332–45. [PubMed: 23243582]
4. Teudt IU, et al. Acoustic events and “optophonic” cochlear responses induced by pulsed near-infrared laser. *IEEE transactions on bio-medical engineering*. 2011; 58(6):1648–55. [PubMed: 21278011]
5. Wenzel GI, et al. Green laser light activates the inner ear. *Journal of biomedical optics*. 2009; 14(4): 044007. [PubMed: 19725719]
6. Shapiro MG, et al. Infrared light excites cells by changing their electrical capacitance. *Nature communications*. 2012; 3:736.
7. Jacques SL. Laser-tissue interactions. Photochemical, photothermal, and photomechanical. *Surg Clin North Am*. 1992; 72(3):531–58. [PubMed: 1589829]
8. Niemz, MH. *Laser Tissue interactions: fundamentals and application*. 2. New York: Springer; 2004.
9. Welch, AJ.; van Gemert, MJC. *Optical-Thermal Response of Laser-Irradiated Tissue*. New York: Plenum Press; 1995.
10. Wells J, et al. Biophysical mechanisms of transient optical stimulation of peripheral nerve. *Biophys J*. 2007; 93(7):2567–80. [PubMed: 17526565]
11. Wells JD, et al. Biophysical mechanism responsible for pulsed low-level laser excitation of neural tissue. *SPIE*. 2006; 6084:60840X1–7.
12. Shapiro MG, et al. Infrared light excites cells via transient changes in membrane electrical capacitance. *Nature Communications*. 2012; 3:736.
13. Schultz M, et al. Pulsed wavelength-dependent laser stimulation of the inner ear. *Biomedizinische Technik. Biomedical engineering*. 2012; 57(Suppl 1)
14. Richter CP, et al. Spread of cochlear excitation during stimulation with optical radiation: Inferior colliculus measurements. *J Neural Eng*. 2011; 8(5):056006. [PubMed: 21828906]

Figure 1.

Compound action potential threshold curves. To characterize cochlear function, the sound level was determined that is required to evoke a visible compound action potential in the voltage trace recorded at the round window of the cochlea. The dotted line is the gold standard for good hearing animals. The gray thick line is the maximum sound level that can be produced by the speaker. The open circles are recorded as a baseline auditory response, the green circles with + in the middle show auditory response immediately after deafening. The injection of neomycin into the cochlea significantly elevates CAP thresholds.

Figure 2.

Compound action potentials evoked by the laser in the presence of an acoustic masker. The noise level increases from for each trace from bottom (0 dB SPL) to top (75 dB SPL). Sound level is the average sound level. Left column is at the beginning of the experiment, the middle column after the first injection of neomycin into scala tympani of the cochlear basal turn and the right columns after the second injection of neomycin. The radiant energy for stimulation was 43 $\mu\text{J}/\text{pulse}$, pulse length was 100 μs , and pulse repetition rate was 10 Hz.

Figure 3.

The effect of masking can be seen in the three traces. While for a normal hearing animal the CAP response amplitude to INS is reduced by the acoustic masker (filled circles), the masking effect disappears after deafening the animal.

Figure 4.

A Compound action potential threshold curves. To characterize cochlear function, the sound level was determined that is required to evoke a visible compound action potential in the voltage trace recorded at the round window of the cochlea. The dotted line is the gold standard for good hearing animals. The gray thick line is the maximum sound level that can be produced by the speaker. Only at 5 kHz a response could be evoked. No click response could be evoked. **B** No masking can be seen in the trace.