Short-wavelength Near Infrared Stimulation of the Inner Ear Hair Cells

Nan Xia, Fei Peng, Xing Wang, Xiao L. Zheng, Xiao P. Wan, Wei Yuan, Wen S. Hou

Abstract—**To explore whether the short wavelength near infrared laser can stimulate the functional hair cells, pulsed laser with wavelength of 808-nm was used to stimulate guinea pigs cochlea. Compound action potential (CAP) and auditory brainstem responses (ABR) were recorded during the experiments. We successfully recorded photomechanical responses from normal hearing animals and demonstrated the responses were not induced by optical acoustic events. Furthermore, we studied the effect of different stimulation parameters on neural response. The results show that cochlear activation can be modulated with different optical parameters.**

I. Introduction

Pulsed near infrared laser has been widely investigated to stimulate the sciatic nerve [1-3], facial nerve [4], auditory nerves [5] and somatosensory cortex [6] due to the advantages of high spatial precision and no contact or stimulation artifact [7-8]. Among these studies, the infrared neural stimulation (INS) using in cochlea provides a novel approach to improve the stimulus resolution and could be potentially used in the design of a cochlear implant [9].

Although the mechanism for INS is far from clear, it is now generally accepted that photothermal effect plays a decisive role for INS with wavelengths ranging from 1.8 μm to 2.2 μm [9-12]. In another word, infrared energy are directly absorbed by water in spiral ganglion cells, producing a rapid local temperature increase. This heating reversibly alters the electrical capacitance of cell membrane, causing the target neuron depolarization [13]. On the other hand, Anders Fridberger and Tianting Ren demonstrated that infrared light with wavelength of 813-nm can produce mechanical vibration [14], which can be converted into electrical signals by the sensory hair cells, causing the spiral ganglion cells depolarization. Similar mechanical responses were also got from 523-nm pulsed laser stimulation [15]. In fact, there are quite a few hearing impaired individuals have residual hearing which means their hair cells can still be activated. If we can combine these two kinds of effects together for those patients, it will be more effectively repairing their hearing.

Our preliminary work has already demonstrated that 808-nm infrared pulse can evoke auditory nerves response in acute deafened animals [16]. However, we found the amplitude of optical evoked compound action potential (oCAP) in deafened animals was significant smaller than the oCAP amplitude before deafness. In addition to the original conclusion, we speculate that part of inner and outer ear hair cells in normal hearing animals involved in the laser response, that means the laser we use in our experiments may also have photomechanical effect. So the objective of present work is to test whether the short wavelength near infrared laser with wavelength of 808-nm can stimulate the functional hair cells.

II. Materials and Methods

Albino guinea pigs (purchased from the Experimental Animal Center of Chongqing Medical University) of either sex (weight 250-350g) were used in the experiment. Five normal hearing and two acutely deafened guinea pigs of either sex were used in this study. All procedures were performed in accordance with protocols of the Care and Use of Laboratory Animals approved by the Third Military Medical University.

A. Animal Surgery and Optical Fiber Placement

Animals were anesthetized by an intraperitoneal injection of Ethyl carbamate (1.2 g/kg body weight in 20% sterile saline). Depth of anesthesia was assessed by toe pinch every 30 to 60 min, and maintained with 0.16 g Ethyl carbamate/kg body weight to ensure the anesthesia state of animal. A thermostatic bath (HSS-1, Chengdu Instrument Manufactory, Chengdu, China) was used to maintain the body temperature of animals at 38 ℃ throughout the experiment. Surgical procedure were similar to the previously described [16]. Briefly, an incision was performed behind its right ear, and cervicoauricular muscles were removed by blunt tweezers to get the right tympanic bulla. Then the bulla was opened approximately 2×2 mm to visualize the RW. The optical fiber was fixed on a micromanipulator and inserted through the bullaostomy access to the round window (RW) membrane. The distal end of the fiber was positioned 1 mm from the RW membrane oriented toward the basilar membrane (see Fig. 1), and the fiber did not contact any tissue.

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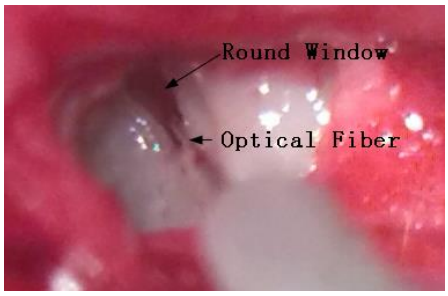

Figure 1. Optical fiber placement.

B. Acoustic and Optical Stimuli

Metronome software was adopted for generating acoustic stimuli with a personal computer, it can generate 0-80 dB pulse sound with repetition rate 4 Hz. The acoustic signal was generated by loudspeaker (D1080-IV, HiVi, SEOAP Trading Co., Ltd. agent in Chongqing, China) which was placed 20 cm away from the animal.

For infrared stimulation, we utilized 808-nm pulsed laser (maximum output power 3.2W) sources as the optical source. The laser output was coupled to a 105-μm diameter optical fiber. The pulse durations operated among 30 and 1000μs.

C. Deafening Procedure

To verify if hair cells involved in optical response, we repeated optical stimulation in deafened guinea pig cochlea. A cochleostomy was created with a 22# syringe needle, and 50μl neomycin (25mM) was injected through the cochleostomy using a 100μl microsyringe. The lack of acoustic CAP at 80dB SPL was defined as the criterion for a successful deafening procedure. Electrical stimulation was recorded to assess if auditory neuron still alive after deafness. A stimulation electrode was inserted into cochlea through the cochleostomy and a ground electrode placed under the neck muscle. Single symmetric biphasic current pulse (250μs per phase) at a rate of 4Hz was used in our experiment. To avoid huge artifact we recorded ABR for deafened animals.

D. Data Recording and Analysis

During the experiments, three acupuncture needles were used as CAP recording electrodes. The acupuncture needle electrodes were insulated with Polydimethylsiloxane (PDMS), and only two ends of the electrodes can conduct electricity. The record electrode was inserted into the bony rim of RW from the outer ear canal, the reference electrode and ground electrode were placed in the ipsilateral auricula and under the skin of nose, respectively. The electrodes were connected with Cerebus multichannel physiological signal acquisition system (Cerebus 6.01, Blackrock Microsystems, Salt Lake City, USA) to record the CAP signals. The sampling rate was set to 30 kHz. The recorded signal was filtered between 100 and 3000 Hz. All data were analyzed offline in Matlab R2008a, the CAPs were averaged 50 times due to the stimulation synchronization signals. The CAP peak to peak amplitudes (see Fig. 2) which measured from the first negative peak (N1) to the following positive peak (P2) were used to measure the intensity of responses.

III. Results

A. Optical Stimulation Before and After Deafness

We can easily record CAPs evoked both by acoustical stimuli and 808-nm laser pulse from normal hearing guinea pigs, Figure 2A shows an example from one guinea pig. The shapes of oCAPs and aCAP were similar with each other. Basically, they both had sequential components of one negative peak (N1), followed by a positive peak (P2). To exclude the possibility of photoacoustic effect, we moved the optical fiber away from the round window to stimulate the bulla bone in the middle ear, and we didn't record any oCAPs (Fig. 2 A), which indicated that the activity is not inducted by the acoustic events created by laser pulse. In figure 2 B we can see that both acoustic and laser responses disappeared even using a high stimulation level after deafness, while electric current can still activate neurons which verified that spiral ganglion cells remained alive, that means optical fiber at the orientation which mentioned above can only stimulate organ of Corti.

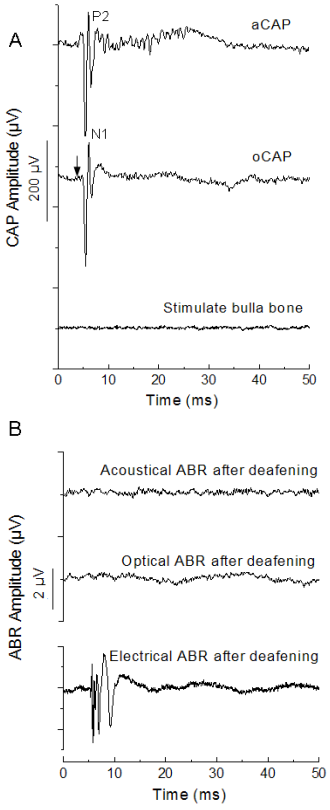

Figure 2. CAPs and ABRs recorded from normal hearing and deafened animals. A: CAPs evoked by acoustical stimuli (80dB SPL), optical pulse (100μs pulse duration, 320μJ/pulse)before and after moving optical fiber to stimulate bulla bone; B: ABRs from acoustical stimuli (80dB SPL), optical pulse (100μs pulse duration, 320μJ/pulse) and electrical current (500μA) after deafness.

B. Optical stimulation with different parameters

To understand the effects of photomechanical stimulation, oCAPs with different pulse energies and different pulse duration were recorded. Figure 3A shows the oCAPs evoked

by different pulse energy levels at the pulse duration of 30μs and the repetition rate of 2 Hz. The CAP amplitudes increased monotonically with the pulse energy increasing (Fig. 3B). This demonstrates that cochlear activation can be modulated with different laser intensity.

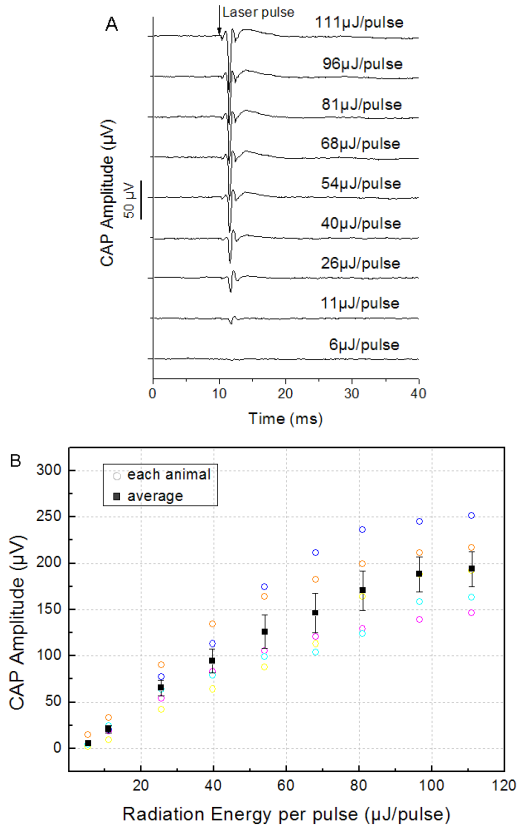

Figure 3. oCAPs evoked by different pulse energy levels. A: level series of oCAPs to laser stimulation; B: input- output functions for laser stimulation. Circle in different colors represent each animal and the mean is shown by solid square with the standard error.

To study the effect of different pulse durations on CAPs , we changed pulse duration from 30μs to 1ms at pulse energy of 96μJ per pulse. Figure 4A shows the CAPs induced by different pulse durations. We can see from those waveforms that the CAP waveforms became more complex at longer pulse duration: the second negative peak (N3) became larger, and the appearance of the second positive peak (P4) shows at the 1000μs pulse duration, which means more neurons responded to the longer optical pulse or the same neuron firing a second time [11]. The CAP amplitude was slightly increased with the pulse duration below 100μs, and was follow by a plateau at the pulse duration longer than 100μs (Fig.4B). The findings indicated that the smaller pulse duration (less than 100μs) is more selective for stimulating organ of Corti.

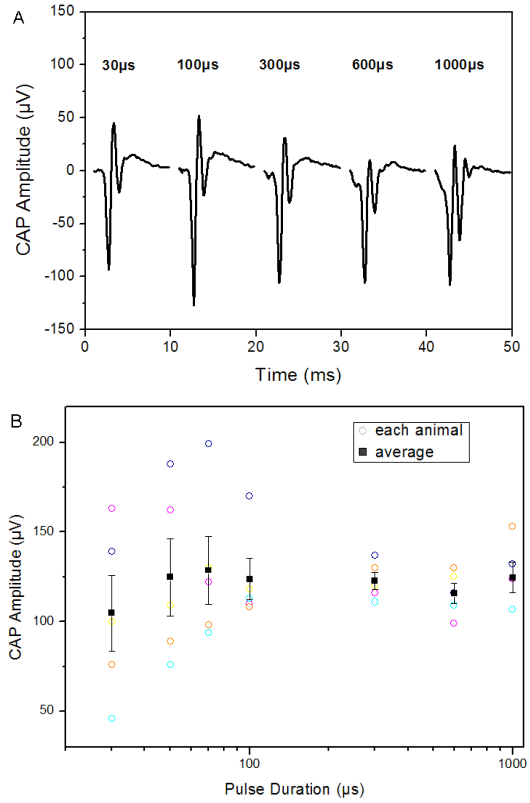

Figure 4. oCAPs evoked by different pulse durations. A: Shape of oCAPs evoked by laser pulse from 30μs to 1ms at pulse energy of 96μJ per pulse. B: input- output functions across pulse durations (pulse energy: 96μJ/pulse).

IV. Conclusion

In this study we demonstrated that 808-nm laser pulse can also stimulate the functional hair cells in normal hearing guinea pigs. And our control experiment (Fig. 2 A) further validated the oCAPs is not induced by audible acoustic events which may appear at INS [17].

Consider that the laser we used in this study can also cause the photothermal responses from the spiral ganglion cells [16]. We placed the optical fiber outside the round window membrane oriented toward the basilar membrane which was different from our previous work to separate the responses from directly stimulation of auditory nerve and mechanical stimulation of hair cells. And we also used neomycin to acutely damage hair cells [18] to further verify the laser at that orientation can only stimulated hair cells. This encouragingly results gives us a new idea of designing a cochlear implant which can be used both in patient with severe to profound sensorineural hearing loss using photothermal stimulation [13,19-20] and hearing impaired individuals with functional hair cells using photomechanical stimulation [14,15].

To further understand the photomechanical effects of 808-nm on organ of Corti different laser stimulation parameters on photomechanical responses were measured. The experimental results shows that the hearing response can be modulated by changing the pulse energy and pulse duration. This study is only a preliminary study to understand the role of short wavelength infrared light in cochlear stimulation.

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