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Research paper

Infrared neural stimulation: Beam path in the guinea pig cochlea

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ABSTRACT

It has been demonstrated that 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.

1. 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 et al. (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, more power is required to generate threshold responses (Kral et al., 1998; Bierer and Middlebrooks, 2002; Snyder et al., 2004). 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, 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, 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

Abbreviations: CAP, compound action potential; CCD, charge-coupled device; CO_2, carbon dioxide; EDTA, ethylene diamine tetra acidic 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 μPa.

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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.

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

2.1. 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 min 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.

2.2. 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 (A2 = 30 μV) 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 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.

2.3. 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 multi-channel penetrating electrode array (A1 × 16-5 mm-100-177, NeuroNexus Technologies, Ann Arbor, MI) was advanced through the occipital cortex. Each electrode contact has a surface area of 177 μm^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 multi-channel electrode passed through the ICC approximately orthogonal to its iso-frequency laminae (Merzenich and Reid, 1974; Snyder et al., 1990, 2008; Smith and Delgutte, 2007). 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 and 25 kHz and the most superficial contact could be stimulated with a 1–2 kHz tone burst. In some instances the