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Research paper

Recovery characteristics of the electrically stimulated auditory nerve in deafened guinea pigs: Relation to neuronal status

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ABSTRACT

Successful cochlear implant performance requires adequate responsiveness of the auditory nerve to prolonged pulsatile electrical stimulation. Degeneration of the auditory nerve as a result of severe hair cell loss could considerably compromise this ability. The main objective of this study was to characterize the recovery of the electrically stimulated auditory nerve, as well as to evaluate possible changes caused by deafness-induced degeneration. To this end we studied temporal responsiveness of the auditory nerve in a guinea pig model of sensorineural hearing loss. Using masker-probe and pulse train paradigms we compared electrically evoked compound action potentials (eCAPs) in normal-hearing animals with those in animals with moderate (two weeks after ototoxic treatment) and severe (six weeks after ototoxic treatment) loss of spiral ganglion cells (SGCs). Masker-probe interval and pulse train inter-pulse interval was varied from 0.3 to 16 ms. Whereas recovery assessed with masker-probe was roughly similar for normal-hearing and both groups of deafened animals, it was considerably faster for six weeks deaf animals ($\tau \approx 1.2$ ms) than for two weeks deaf or normal-hearing animals ($\tau \approx 3$–4 ms) when 100-ms pulse trains were applied. Latency increased with decreasing inter-pulse intervals, and this was more pronounced with pulse trains than with masker-probe stimulation. With high frequency pulse train stimulation eCAP amplitudes were modulated for deafened animals, meaning that amplitudes for odd pulse numbers were larger than for even pulses. The relative refractory period (τ) and the modulation depth of the eCAP amplitude for pulse trains, as well as the latency increase for both paradigms significantly correlated with quantified measures of auditory nerve degeneration (size and packing density of SGCs). In addition to these findings, separate masker-probe recovery functions for the eCAP N_1 and N_2 peaks displayed a robust non-monotonic or shoulder-shaped course in all animals. The time interval between the N_1 and N_2 correlated with neuronal refractoriness, suggesting that the N_2 peak reflects a second firing of part of the SGC population. We conclude that – compared to the commonly used masker-probe recovery functions – recovery functions obtained with pulse train stimulation may provide a means to augment differences and, by doing so, to more potently discriminate between auditory nerve conditions.

1. Introduction

Sensorineural hearing loss (SNHL) is commonly characterized by loss of cochlear hair cells. Severe SNHL can be treated with cochlear implantation. Cochlear implants (CIs) convert sound into electrical pulses that are conveyed to the spiral ganglion cells (SGCs), which constitute the auditory nerve. Animal models consistently show that these SGCs degenerate shortly after SNHL induction (Ylikoski

Abbreviations: ABR, auditory brainstem response; CI, cochlear implant; eCAP, electrically evoked compound action potential; IPI, inter-pulse interval; MPI, masker-probe interval; PP, peripheral process; SNHL, sensorineural hearing loss; SGC, spiral ganglion cell

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et al., 1974; Spoendlin, 1975; Webster and Webster, 1981; Versnel et al., 2007; Ramekers et al., 2014). This secondary SGC degeneration has been shown to occur in human CI users as well (Fayad and Linthicum, 2006), and it has recently been reported that word recognition is positively correlated to SGC count (Seyyedi et al., 2014).

An important tool to study the functional condition of the auditory nerve is the CI's electrically evoked compound action potential (eCAP) recording function, with which the nerve's input to the central auditory system can be recorded. Comparison of eCAP characteristics with psychophysical measures in humans (e.g., Kirby et al., 2012; Carlyon and Deeks, 2013) or measures for speech perception (e.g., Kim et al., 2010) can be used to assess the relation between eCAP characteristics and CI performance.

Animal research is complementary to these clinical techniques; the comparison of eCAP characteristics with SGC histology in animals provides an opportunity to indirectly relate human capability for speech understanding with a CI to detailed histology of the auditory nerve. For instance, introducing an inter-phase gap into a biphasic current pulse leads to a decrease in both animal eCAP thresholds (Prado-Guitierrez et al., 2006; Ramekers et al., 2014) as well as human behavioral thresholds (Carlyon et al., 2005). Since on the eCAP level the effect of inter-phase gap varies with the degree of SGC degeneration (Prado-Guitierrez et al., 2006; Ramekers et al., 2014), an inter-phase gap effect on behavioral thresholds in human CI users might show similar variation and would then provide a link between the condition of the auditory nerve and sound perception.

In the present study we aimed to provide similar objective tools to relate eCAP recovery characteristics to quantified histological measures for SGC degeneration. Since it is crucial that the SGC population is able to follow high pulse rate stimulation for proper CI use, focus was on the temporal response properties of the electrically stimulated auditory nerve, and, in particular, on changes in these properties that are associated with SGC degeneration as a result of hair cell loss. Functional and morphological alterations of the surviving SGCs may lead to changes in temporal responsiveness. For example, the reduction in SGC size that is associated with SGC degeneration after hair cell loss (Ramekers et al., 2014) may lead to reduced membrane capacitance (Limón et al., 2005). Shepherd et al. (2004) additionally reported that the absolute refractory period of single SGCs becomes longer after deafening in rats. In order to assess these alterations, we first recorded eCAPs using masker-probe paradigms to characterize in detail the refractory properties of healthy and degenerating SGCs, which we compared to similar studies in both animals (e.g., Stypulkowski and van den Honert, 1984) and humans (e.g., Botros and Psarros, 2010). Second, to mimic more realistic situations we examined neural recovery using pulse train paradigms, in which firing synchrony and neural fatigue play a prominent role.

2. Materials and methods

2.1. Animals and surgery

The data presented in this report form a subset of data obtained from a series of acute experiments. During these experiments a second set of eCAP data was acquired, which has been published previously (Ramekers et al., 2014). Hence, for details about animal care, surgical procedures and recording techniques we refer to the methods section of that paper. In short, eighteen female albino guinea pigs (Dunkin Hartley; 250–350 g; Harlan Laboratories, Horst, the Netherlands) were divided into three groups. One group of six animals functioned as normal-hearing controls (NH), while the other two were deafened either two (2WD) or six weeks (6WD) before the eCAP recordings, by means of coadministration of kanamycin (Sigma–Aldrich, St. Louis, MO, USA; 400 mg/kg s.c.) and the loop diuretic furosemide (Centrafarm, Etten-Leur, the Netherlands; 100 mg/kg i.v.). Before the deafening procedure, and during the acute experiments, the hearing threshold was determined with recording of click-evoked auditory brainstem responses (ABRs), so that threshold shifts caused by the ototoxic treatment could be established.

During the acute experiments the animals were anesthetized with a gas mixture of O_2 and N_2O and 1–2% isoflurane. The right cochlea was exposed via a retro-auricular approach, and a 0.5 mm cochleostomy was drilled in the basal turn, through which a four-contact electrode array (MED-EL GmbH, Innsbruck, Austria) was inserted into the scala tympani. Transcranial screws were placed on the skull for stimulation and recording reference purposes. All surgical and experimental procedures were approved by the Animal Care and Use Committee of Utrecht University (DEC 2010.I.08.103).

2.2. Electrically evoked compound action potentials

eCAPs were recorded using a MED-EL PULSAR$_{CI}^{100}$ stimulator (MED-EL GmbH, Innsbruck, Austria). The electrode array was inserted up to 4 mm into the cochleostomy, so that all 4 contacts – evenly spaced over the first 3 mm of the array – were in the scala tympani. For all data presented here, the most apical contact was used for stimulation and the most basal one for recording. The implant was controlled by a PC via a Research Interface Box 2 (RIB2; Department of Ion Physics and Applied Physics, University of Innsbruck, Innsbruck, Austria) and a National Instruments data acquisition card (PCI-6533, National Instruments, Austin, TX, USA). Stimulation and recording paradigms were created in MATLAB (version 7.11.0; Mathworks, Natick, MA, USA).

2.3. Recording and stimulation paradigm

Two different stimulus paradigms were used: masker-probe and pulse train. Both pulse train and masker-probe stimulation consisted of monopolar biphasic current pulses with 30 μs phase duration and 30 μs inter-phase gap. Current level was varied from 0 to 800 current units (1 current unit ≈ 1 μA) in ten steps. Alternating polarity stimulation was applied for stimulation artifact reduction, while recordings to a subthreshold stimulus were subtracted to eliminate measurement onset artifacts. Every stimulation step was repeated 50 times, and the N_1–P_2 and P_2–N_2–P_3 peak amplitudes were calculated from these averages (see Fig. 1A for definition of peaks). The N_1 latency was determined by averaging the latencies of the N_1 peaks obtained with the three highest stimulation levels (640, 720 and 800 μA). The masker-probe interval (MPI) was varied from 0.3 to 16 ms in 18 steps, while the inter-pulse interval (IPI) ranged from 0.4 to 16 ms in 10 steps for pulse trains. Pulse train duration was as close to 100 ms as possible, taking into account variable IPI duration. For both stimulation paradigms, MPIs/IPIs were presented in decreasing order, while the order of current levels was permuted.

As the implant did not allow continuous recording, the stimulation paradigm was designed such that the eCAPs in response to both the masker and the probe (masker-probe stimulation) were recorded in separate (consecutive) runs. Similarly, the responses to the last ten pulses in the pulse train were recorded individually (see Fig. 1A). Furthermore, in case of small IPIs/MPIs (≤1.2 ms) the response to the previous pulse was present in the recording, which