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The effect of interphase gap on neural response of the electrically-stimulated cochlear nerve in children with cochlear nerve deficiency and children with normal-sized cochlear nerves

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Abstract

Objective: This study aimed to compare effects of increasing the interphase gap (IPG) on the neural response of the electrically-stimulated cochlear nerve (CN) between children with cochlear nerve deficiency (CND) and children with normal-sized CNs.

Design: Study participants included 30 children with CND and 30 children with normal-sized CNs. All subjects were implanted with a Cochlear™ Nucleus® device with the internal electrode array 24RE[CA] in the test ear. The stimulus was a charge-balanced, cathodic leading, biphasic pulse with a pulse-phase duration of 50 μ s. For each subject, the eCAP input/output (I/O) function was measured for six IPGs (i.e., 7, 14, 21, 28, 35 and 42 μ s) at three electrode locations across the electrode array. For each subject and each testing electrode, the highest stimulation used to measure the eCAP I/O function was the maximum comfortable level measured with an IPG of 42 μ s. Dependent variables (DVs) were the maximum eCAP amplitude, the eCAP threshold and the slope of the eCAP I/O function estimated using both linear and sigmoidal regression functions. For each DV, the size of the IPG effect was defined as the proportional change relative to the result measured for the 7 μ s IPG at the basal electrode location. Generalized Linear Mixed effect Models with subject group, electrode location and IPG duration as the fixed effects and subject as the random effect were used to compare these DVs and the size of the IPG effect on these DVs.

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Results: Children with CND showed smaller maximum eCAP amplitudes, higher eCAP thresholds and smaller slopes of eCAP I/O function estimated using either linear or sigmoidal regression function than children with normal-sized CNs. Increasing the IPG duration resulted in larger maximum eCAP amplitudes, lower eCAP thresholds and larger slopes of eCAP I/O function estimated using sigmoidal regression function at all three electrode locations in both study groups. Compared to children with normal-sized CNs, children with CND showed larger IPG effects on both the maximum eCAP amplitude and the slope of the eCAP I/O function estimated using either linear or sigmoidal regression function, and a smaller IPG effect on the eCAP threshold than those measured in children with normal-sized CNs.

Conclusions: Increasing the IPG increases responsiveness of the electrically-stimulated CN in both children with CND and children with normal-sized CNs. The maximum eCAP amplitude and the slope of the eCAP I/O function measured in human listeners with poorer CN survival are more sensitive to changes in the IPG. In contrast, the eCAP threshold in listeners with poorer CN survival is less sensitive to increases in the IPG. Further studies are warranted in order to identify the best parameters of eCAP results for predicting CN survival before this eCAP testing paradigm can be used as a clinical tool for evaluating neural health for individual CI patients.

Keywords

auditory nerve; electrically evoked auditory compound action potentials; interphase gap; cochlear nerve deficiency; neural survival

INTRODUCTION

Electrical stimuli delivered by the cochlear implant (CI) are encoded by the cochlear nerve (CN) before they can be processed by the central auditory system (CAS), and subsequently perceived by CI patients. Theoretically, the functional status (i.e., number and responsiveness of neurons) of the CN affects its ability to faithfully encode and process electrical stimuli, which should be important for CI outcomes. Results of several animal studies appear to align with this theory (e.g., Pfingst et al., 2015b, 2017). Therefore, accurately assessing the functional status of the CN for individual CI patients may provide a better understanding of the substantial inter-patient variations in CI outcomes.

Directly evaluating the functional status of the CN in human listeners is not feasible. Fortunately, useful information can be obtained by assessing basic neural response properties of the CN using electrophysiological measures of the electrically-evoked compound action potential (eCAP). The eCAP is a near-field recorded neural response generated by CN fibers. Its presence depends on the existence of sufficient CN fibers responding synchronously to electrical stimulation. Therefore, it can be used to assess how well CN fibers near each CI electrode respond to electrical stimulation, which is determined by the functional status of these CN fibers.

Animal models have been used to identify eCAP measures that are sensitive to the functional status of the CN (Prado-Guitierrez et al., 2006; Ramekers et al., 2014, 2015). Results of these studies show that sensitivity of the eCAP to changes in the interphase gap (IPG) of a biphasic pulse is associated with neural survival of spiral ganglion cells in guinea pigs

(Prado-Guitierrez et al., 2006; Ramekers et al., 2014, 2015). Specifically, increasing the IPG results in larger slopes of the eCAP input/output (I/O) function (Ramekers et al., 2014), larger eCAP amplitudes at fixed stimulation levels and lower eCAP thresholds (Prado-Guitierrez et al., 2006; Ramekers et al., 2014, 2015). More importantly, smaller changes in the eCAP threshold (Ramekers et al., 2014) and the maximum eCAP amplitude (Ramekers et al., 2015), as well as larger changes in the slope of the eCAP I/O function (Ramekers et al., 2014) have been shown to be associated with higher spiral ganglion cell packing density and perikaryal area. The proposed underlying mechanism for improved eCAPs at longer IPGs is that increasing the IPG makes the action potential generated by the leading cathodic pulse less susceptible to be abolished by the lagging anodic pulse (Shepherd and Javel, 1999; van den Honert and Mortimer, 1979). The reduced susceptibility of action potential abolishment at longer IPGs is caused by a delay in initiating an action potential after the neural membrane reaches a threshold level due to the amount of time required to activate the voltage-sensitive sodium ion channel (Bromm & Frankenhaeuser, 1968). During this “vulnerable period”, the spike initiation induced by one phase can be abolished by the opposite phase. Therefore, including an IPG increases the probability of spike initiation (van den Honert & Mortimer, 1979; Shepherd & Javel, 1999; Rubinstein et al., 2001). As the functional status of CN fibers deteriorates, the permeability of ion channels and the duration of this “vulnerable period” may change, which would affect the effectiveness of increasing the IPG on improving the probability of spike initiation at the CN. Overall, these results in animal models indicate that this new eCAP testing paradigm of systematically changing the IPG may potentially be used to assess the functional status of the CN for individual CI patients. However, due to differences in species, etiology, duration of deafness, and stimulation levels that can be used in anesthetized animals vs awake CI users, results from these animal studies cannot be easily applied to human listeners.

Several studies have investigated the effect of increasing the IPG on the eCAP (Kim et al., 2010; Hughes et al., 2018; Schwartz-Leyzac & Pfingst, 2016, 2018) and its association with speech perception performance in human CI users (Kim et al., 2010; Schwartz-Leyzac & Pfingst, 2016, 2018). Results of these studies show that increasing the IPG results in lower eCAP thresholds (Hughes et al., 2018) and larger eCAP amplitudes (Hughes et al., 2018; Schwartz-Leyzac & Pfingst, 2016, 2018). However, findings regarding the effect of increasing the IPG on the slope of the eCAP I/O function are conflicting. Whereas Schwartz-Leyzac & Pfingst (2016, 2018) reported significantly larger slopes at longer IPGs, Hughes et al. (2018) did not observe any difference in the slope at different IPGs. Similarly, the association between the magnitude of the IPG effect on the eCAP and speech perception performance is also inconsistent. In 10 bilateral CI users, Schwartz-Leyzac & Pfingst (2018) found that the between ear difference in the amount of increase in the slope of the eCAP I/O function at longer IPGs was significantly correlated with between-ear differences in speech perception scores. In contrast, results of Kim et al. (2010) revealed no association between the eCAP sensitivity to changes in the IPG and speech perception scores measured in the same ear in 17 Hybrid-array and 21 standard-array CI users. These inconsistencies may be due to substantial variations in etiology, duration of hearing loss, device type and cognitive function among CI users tested in different studies. Even if these confounding factors can be controlled or matched, confirming the association between these eCAP measures and the

functional status of the CN in typical CI users is still challenging because of the unpredictable CN neural survival pattern in individual patients (Nadol, 1997; Nadol et al., 2012; Long et al., 2014; DeVries et al., 2016; Schwartz-Leyzac & Pfingst, 2016; Sagers et al., 2017). Overall, results of these studies in typical CI users fail to provide conclusive evidence regarding whether evaluating the IPG effect on the eCAP can be used as an objective tool for assessing the functional status of the CN for individual CI patients.

Cochlear nerve deficiency (CND) refers to a small (hypoplastic) or absent (aplastic) CN as revealed by high-resolution magnetic resonance imaging (MRI). CND occurs when inner ear development is arrested during embryogenesis, which results in a partially or completely underdeveloped cochlear nerve (Jackler et al., 1987). As a result, children with CND have fewer CN fibers than typical CI children who have normal-sized CNs. The hypoplastic CN is also less responsive to electrical stimulation than the normal-sized CN (He et al., 2018). More importantly, the likelihood of measuring neural responses generated by CN fibers (i.e., eCAPs) in children with CND is reduced as the stimulating CI electrode site moves from the base to the apex of the cochlea, with many apical CI electrodes showing no measurable CN response. This unique response-deterioration pattern is consistent with the “base-to-apex” sequence of cochlear duct development during embryogenesis (Arnold & Lang, 2001; Jeffery & Spoor, 2004; Yasuda et al., 2007), differs from the previously reported CI-electrode-to-modiolus-distance pattern (Long et al., 2014), and is not observed in children with normal-sized CNs. Therefore, it primarily reflects the unique CN survival pattern in children with CND. As a result, this unique patient population can be considered as a human model for poor neural survival of the CN, which provides an extremely valuable opportunity to verify many important findings reported in animal studies in human listeners. It should be pointed out that due to the lack of histological studies in patients with CND, it remains unknown whether the survival rate of all developed CN fibers in this patient population is 100%. Therefore, the phrase “poor neural survival” used in this study refers to the overall reduced number of CN fibers that can be activated by electrical stimulation.

In summary, accurately assessing the functional status of the CN is important for better understanding the neurophysiological mechanisms underlying substantial inter-patient variations in CI outcomes. Results of animal studies suggest that the size of the IPG effect on the eCAP is associated with neural survival of spiral ganglion cells. However, confirming this important finding in typical CI users is difficult due to the unpredictable neural survival pattern in individual patients, and the heterogeneity in many factors among patients, including, but not limited to, etiology, duration of deafness, and device type. Children with CND can be considered as a human model for poor neural survival of the CN. Therefore, despite the potential difference in etiology, assessing the size of the IPG effect on the eCAP in children with CND and comparing their results with those measured in children with normal-sized CNs could provide conclusive evidence for whether this new eCAP testing paradigm for assessing the IPG effect can be used as a clinical tool for assessing the functional status of the CN in individual CI patients.

This study aimed to compare effects of the IPG on the eCAP threshold, the slope of the eCAP I/O function and the maximum eCAP amplitude between children with CND and children with normal-sized CNs. Duration of deafness, duration of listening experience with

the CI and the internal electrode array of the CI were carefully matched between these two study groups. This study tested the hypothesis that poor CN functional status would affect the effectiveness of increasing the IPG on enhancing responsiveness of the electrically-stimulated CN. Based on this hypothesis, lower eCAP thresholds, larger maximum eCAP amplitudes and steeper eCAP I/O functions at longer IPGs were expected. More importantly, children with CND were expected to show a greater increase in the maximum eCAP amplitude, larger decrease in the eCAP threshold, and less increase in the slope of the eCAP I/O function at longer IPGs than children with normal-sized CNs. In addition, the amount of change in the IPG effect on the eCAP was expected to vary as the stimulating site moved from the base to the apex of the cochlea in children with CND.

MATERIALS AND METHODS

Subjects

This study included two subject groups: the CND group and the control group. Study participants in the CND group were 30 children with CND (CND1-CND30). Study participants in the control group were 30 children with normal-sized CNs (S1-S30). For children with CND, age at implantation and age at testing ranged between 1.0 and 19.9 yrs (mean: 3.8 yrs, SD: 3.6 yrs) and between 1.9 and 23.1 yrs (mean: 6.0 yrs, SD: 4.1 yrs), respectively. For children with normal-sized CNs, age at implantation and age at testing ranged between 0.8 and 10.1 yrs (mean: 3.2 yrs, SD: 2.4 yrs) and between 2.1 and 13.1 yrs (mean: 5.8 yrs, SD: 3.0 yrs), respectively. Results of a 2-tailed Independent Sample *t*-test showed that there was no statistically significant difference in age at implantation ($t=0.74$, $p=0.75$) or age at testing ($t=0.25$, $p=0.65$) between these two study groups.

All subjects had congenital deafness and were implanted with a Cochlear™ Nucleus® device (Cochlear Ltd., Macquarie, NSW, Australia) with an internal electrode array of 24RE[CA] in the test ear using the round window approach. All subjects, including the six subjects in the CND group who had Mondini dysplasia, had a full electrode insertion and at least six months of listening experience with their CIs prior to testing. All 22 electrodes were used in the programming map for all subjects tested in this study. The anatomical status of the CN was determined based on results of MRI scan following the same protocol and criteria as described in He et al. (2018). The anatomical status of the inner ear was determined based on results of high resolution Computed Tomography (CT) temporal bone scans. Incomplete partition type II (IP-II) (Mondini deformity) was diagnosed if the cochlea consisted of 1.5 turns and the middle and apical turns coalesced to form a cystic apex (Sennaroğlu & Saatci, 2002). The internal auditory canal (IAC) was diagnosed to be narrow if its diameter was 3 mm or less (Sennaroğlu & Saatci, 2002). Bony cochlear nerve canal (BCNC) stenosis was diagnosed if the diameter was less than 1.4 mm (Purcell et al., 2015).

Detailed demographic information of these subjects is listed in Table 1. The anatomical status of the CN, the cochlea, the IAC and the BCNC is listed for each child with CND. All subjects included in the control group had no sign of anatomical malformation in the inner ear or the CN. Therefore, their imaging results were not listed. The study was approved by the local Biomedical Institutional Review Board. Written, informed consents were obtained from subjects and/or their legal guardians prior to participation.

Procedures

Testing Electrodes—In implanted children with normal-sized CNs, eCAPs were recorded at one basal, one middle and one apical electrode (i.e., electrodes 3, 12, and 21). In implanted children with CND, three electrodes with measurable eCAPs were tested. These testing sites extended to the most apical electrode location where an eCAP could be recorded and had a relatively equal separation between testing electrodes. For the purpose of this study, these electrodes were considered as the “basal”, the “middle,” and the “apical” electrode based on their relative locations among electrodes with measurable eCAPs. Figure 1 shows a schematic illustration of CI electrode selection for a child with CND (CND30) who had 11 CI electrodes with measurable eCAPs. Electrodes tested in individual subjects are listed in Table 1.

Stimulus—The stimulus was a charge-balanced, cathodic leading, biphasic pulse with a pulse-phase duration (PPD) of 50 μ s. Results of our previous study (He et al., 2018) showed that the eCAP can typically be evoked using a 50 μ s PPD in the majority of children with CND. Therefore, it was used in this study. IPGs tested in this study included 7, 14, 21, 28, 35 and 42 μ s.

eCAP Measures—The Advanced Neural Response Telemetry (NRT) function that is implemented in the Custom Sound EP (v. 5.1) software (Cochlear Ltd, Macquarie, NSW, Australia) was used to measure the eCAP. Responses were recorded using a two-pulse forward-masking-paradigm (Brown & Abbas, 1990). The stimulus was presented to individual CI electrodes in a monopolar-coupled stimulation mode via a Freedom or N5 sound processor interfaced with a programming pod. It has been shown that pulses with longer IPGs are perceived louder than pulses with shorter IPGs when all other stimulation parameters are kept the same (McKay & Henshall, 2003). Therefore, the maximum comfortable level (C level) measured in clinical levels (CLs) for the 42 μ s IPG was used as the highest stimulation level for assessing the IPG effect. The procedure used to measure the C level for each stimulating electrode in each subject was the same as that described in our previous studies (He et al., 2016; He et al., 2018).

The first (masker) pulse was delivered at the C level. The stimulation level of the second (probe) pulse were systematically decreased in steps of 5 CLs until no response could be visually identified, and subsequently increased in steps of 1 CL until at least five eCAPs were measured using this small step size. Other parameters used for eCAP measures included a masker-probe-interval of 400 μ s, an effective sampling rate of 20 kHz, a probe rate of 15 Hz, sampling delays ranged between 98 and 122 μ s, an amplifier gain of 40 dB, and 100 sweeps for the averaged eCAP response. These parameters have been shown to result in a much higher success rate than the manufacture-default parameters for measuring the eCAP in children with CND (He et al., 2019). It took up to six hours to complete the experiment in each subject.

Data Analysis

Custom-designed MATLAB (Mathworks) software was used to conduct statistical modeling of this study. Dependent variables evaluated in this study included the maximum eCAP

amplitude, the eCAP threshold and the slope of the eCAP I/O function. The eCAP amplitude was measured as the amplitude difference between the negative (N1) and positive (P1) peaks of the response (Lai & Dillier, 2000). The eCAP threshold was defined as the lowest stimulation level that elicited an eCAP response with an amplitude of 5 μ V or larger (Patrick et al., 2006). The stimulation level was converted to μ A for data analysis using an equation provided by Cochlear Ltd.

Figure 2 shows eCAP amplitudes plotted as a function of stimulation level in μ A for results measured at three electrode locations in two children with CND (CND4 and CND10) and one child with a normal-sized CN (S16). These data clearly show that there are substantial variations in eCAP amplitude among subjects as well as across electrode locations within each subject. To preserve the relative difference among I/O functions measured at different IPGs, electrode locations and among study subjects, eCAP amplitudes measured in each subject were normalized to the largest eCAP amplitude measured at the basal electrode for the 7 μ s IPG. Normalized eCAP amplitudes plotted as a function of stimulation level in μ A (i.e., eCAP I/O function) for these three subjects are shown in supplemental Figure A1.

Previously published studies investigating the IPG effect on the eCAP used different mathematical functions to characterize the eCAP I/O function (Ramekers et al., 2014; Hughes et al., 2018; Schwartz-Leyzac & Pfingst, 2016, 2018). For example, the linear regression function has been used to estimate slopes of the eCAP I/O function measured in human CI users (Hughes et al., 2018; Schwartz-Leyzac & Pfingst, 2016, 2018). The sigmoidal regression function (Ramekers et al., 2014) and the exponential function (Prado-Guitierrez et al., 2006) have been used to characterize eCAP I/O functions measured in guinea pigs. Results of our previous study showed that eCAP I/O functions recorded in children with CND are better characterized by a sigmoidal than a linear regression and both functions provide a better fit than the exponential function (He et al., 2018). To better compare results of this study with the literature in human CI users, both linear and sigmoidal regression functions were used to characterize eCAP I/O functions measured in this study. More importantly, the IPG effect on the slope of the eCAP I/O function, if it exists, should not be affected or altered by applying different regression functions in the mathematical modeling process. Therefore, using both regression functions to characterize the eCAP I/O function and compare their results also serves as a validation for the true IPG effect. It was expected that results obtained using these two regression functions should yield the same conclusion regarding the overall effect of increasing the IPG on the slope of the eCAP I/O function. Table 2 lists formulas and parameters used in both functions. Parameters of the linear regression function were estimated based on results of linear least squares analysis. Parameters of the sigmoidal regression function were estimated based on results of nonlinear least squares analysis using the trust-region-reflective algorithm.

The size of the IPG effect on each DV was defined as the proportional change relative to the result for the 7 μ s IPG, which is the same as those defined in Schwartz-Leyzac & Pfingst (2016). Specifically, it was defined as

$$d = \frac{\text{DV measured for different IPGs} - \text{DV measured for the 7 } \mu\text{s IPG}}{\text{DV measured for the 7 } \mu\text{s IPG}}$$

Statistical Comparison—All statistical analyses were conducted using SPSS Statistic 25 (IBM Corp.). Dependent variables (DVs) evaluated in this study included the eCAP threshold, the maximum eCAP amplitude, the slope of the eCAP I/O function and the size of the IPG effect on these three variables. The slope of the eCAP I/O function was estimated based on results of statistical modeling. Therefore, how well these two regression functions characterized the eCAP I/O function (i.e., goodness of fit) affected estimated slopes. To minimize the effect of poor function fitting on slope estimation, outliers of the slope were determined based on goodness of fit (i.e., adjusted R^2) using the three scaled median absolute deviations criterion (Leys et al., 2013). Based on this criterion, 98 out of 1080 (9.07%) slopes estimated using the linear regression function and 109 out of 1080 (10.09%) slopes estimated using the sigmoidal regression function were excluded from statistical analysis. The eCAP amplitude and the eCAP threshold were measured based on recorded eCAPs instead of estimations using computational modeling techniques. Therefore, no outliers were determined for these two dependent variables. Generalized Linear Mixed effect Models (GLMMs) with subject group, electrode location and IPG duration as the fixed effects and subject as the random effect were used to compare DVs measured. The GLMM can robustly handle missing data, does not depend on the assumption that the missing data are completely random, and allows comparisons between datasets with different distributions (McCulloch and Neuhaus, 2005). Therefore, it is the most appropriate statistical analysis test for this study. Statistical significance was determined at the 95% confidence level (i.e. $p < .05$).

RESULTS

Figure 3 shows eCAPs with the largest amplitude measured at three electrode locations in CND4, CND10 and S16. It should be pointed out that CND10 and S16 had measurable eCAPs at all CI electrodes. In comparison, CND4 only had measurable eCAPs at electrodes 1–10, which suggests more damage in the CN than the other two subjects (He et al., 2018). It is apparent that eCAPs recorded at the basal electrode in CND4 and CND10 have larger amplitudes and better morphologies than those recorded at the middle and apical electrodes. In contrast, S16 showed more robust eCAPs at the apical electrode than at the other two electrode locations. These observations were confirmed by I/O functions shown in Figure 2.

Table 3 provides a summary of the main statistical findings for raw data of the maximum eCAP amplitude, the eCAP threshold and the slope of the eCAP I/O function measured at different electrode locations and IPG durations in children with CND and children with normal-sized CNs. Table 4 provides a summary of the main statistical findings for the size of the IPG effect on these dependent variables. Details of these statistical findings are reported for each dependent variable listed below. It should be pointed out that the fixed effect or an interaction between or among fixed effects was not evaluated for the size of the IPG effect if its effect on dependent variables did not reach a statistical significance.

The eCAP Amplitude

Figure 4 depicts means and standard deviations of the maximum eCAP amplitude measured at different IPGs at three electrode locations in the two study groups. Despite substantial

variations in these data, these results clearly show that maximum eCAP amplitudes measured at the basal electrode location tend to be larger than those measured at the middle and the apical electrode location in both groups. More importantly, children with normal-sized CNs had larger maximum eCAP amplitudes than children with CND at all three electrode locations. Increasing IPG durations resulted in larger eCAP amplitudes for both study groups at all three electrode locations. Results of the GLMMs (see Table 3) supported these observations. Specifically, there were significant overall effects of subject group ($F_{(1, 862.56)} = 280.20$, $p < 0.001$), electrode location ($F_{(2, 744.71)} = 26.42$, $p < 0.001$), and IPG duration ($F_{(5, 275.03)} = 10.32$, $p < 0.01$) on the maximum eCAP amplitude. There was a statistically significant interaction between effects of subject group and electrode location ($F_{(5, 744.71)} = 4.15$, $p = 0.016$). There was no significant interaction between effects of study group and IPG duration ($F_{(5, 275.03)} = 0.617$, $p = 0.687$), or between effects of electrode location and IPG duration ($F_{(10, 240.58)} = 0.035$, $p = 1.000$). There was no significant interaction among effects of study group, electrode and IPG duration ($F_{(10, 240.58)} = 0.013$, $p = 1.000$).

Results of pairwise comparisons with Bonferroni correction showed that maximum eCAP amplitudes measured at the basal electrode location were significantly larger than those measured at the middle and the apical electrode locations ($p < 0.001$) for both study groups despite an overall significant interaction between effects of study group and electrode location. There was no significant difference in maximum eCAP amplitude measured for the middle and the apical electrode location ($p = 1.00$). The maximum eCAP amplitude measured for the 7 μ s IPG was significantly smaller than those measured for all other IPG durations ($p < 0.001$) except for the IPG duration of 14 μ s ($p = 0.567$). Results measured for the 14 μ s IPG were significantly smaller than those measured for the 35 μ s IPG ($p = 0.021$) and the 42 μ s IPG ($p = 0.002$). There was no significant difference in maximum eCAP measured between any other IPG durations.

Figure 5 depicts the size of the IPG effect on the maximum eCAP amplitude, which was quantified as the percentage of change in normalized maximum eCAP amplitudes relative to the results measured at IPG 7 μ s. Each panel shows results measured at one electrode location. These data clearly show that the size of the IPG effect increases as the IPG duration increases at all three electrode locations. There are fewer variations in the size of the IPG effect in children with normal-sized CNs than children with CND. Results of GLMMs (see Table 4) showed that increasing IPG duration had a significantly larger effect on the maximum eCAP amplitude in children with CND than in children with normal-sized CNs ($F_{(1, 705.050)} = 21.080$, $p < 0.001$). There were significant effects of electrode location ($F_{(2, 464.998)} = 7.888$, $p < 0.001$), IPG duration ($F_{(4, 295.755)} = 70.282$, $p < 0.01$) and a significant interaction between effects of subject group and electrode location ($F_{(2, 464.998)} = 6.300$, $p = 0.002$) on the size of the IPG effect.

Results of pairwise comparisons with Bonferroni correction showed that the size of the IPG effect on the maximum eCAP amplitude was smaller at the basal electrode location than those measured at the middle and the apical electrode locations ($p < 0.001$). There was no significant difference in the size of the IPG effect between the middle and the apical electrode locations ($p = 1.00$). Children with CND showed smaller IPG effects on the

maximum eCAP amplitude at the basal than the middle and apical electrode locations ($p < 0.001$). There was no difference in the size of the IPG effect between the middle and the apical electrode locations ($p = 0.163$). In comparison, children with normal-sized CNs did not show any significant difference in the size of the IPG effect among the three electrode locations tested. The difference in electrode effect between study groups resulted in a significant interaction between effects of electrode location and study group. However, the three way interaction (group $\times$ electrode $\times$ IPG) was not statistically significant ($F_{(8, 196.579)} = 0.354$, $p = 0.943$). Finally, there was a significant increase in the size of the IPG effect for every 7 μ s increase for IPG durations of 14, 21, 28 and 35 μ s ($p < 0.001$) for both study groups. Further increasing IPG duration to 42 μ s did not yield a significant change in the size of the IPG effect on the maximum eCAP amplitude ($p = 0.138$).

In summary, children with CND showed smaller maximum eCAP amplitudes (Figure 4) but a larger IPG effect on the maximum eCAP amplitude than children with normal-sized CNs (Figure 5). Maximum eCAP amplitudes measured at the basal electrode were larger than those measured at the middle and apical electrode locations (Figure 4). For children with CND, increasing the IPG had a larger effect on the maximum eCAP amplitude at the middle and apical electrode locations compared to the basal electrode location (Figure 5). This electrode location effect on the size of the IPG effect was not observed for children with normal-sized CNs. Increasing the IPG generally resulted in larger maximum eCAP amplitudes and greater changes in the maximum eCAP amplitude in both study groups (Figures 4 and 5).

The eCAP Threshold

Figure 6 depicts means and standard deviations of the eCAP threshold measured at different IPGs at three electrode locations in the two study groups. It is apparent that children with CND had higher eCAP thresholds than children with normal-sized CNs at all three electrode locations. They also showed higher eCAP thresholds at more apical electrode locations. In contrast, the middle electrode location showed the highest eCAP threshold in children with normal-sized CNs. Despite the variation in the eCAP threshold measured at different electrode locations, increasing IPG durations resulted in decreased eCAP thresholds in both groups. These observations were supported by results of statistical analysis. Specifically, results of GLMMs (see Table 3) revealed significant overall effects of subject group ($F_{(1, 1016.16)} = 4017.71$, $p < 0.001$), electrode location ($F_{(2, 741.66)} = 171.27$, $p < 0.001$), and IPG duration ($F_{(5, 338.77)} = 8.99$, $p < 0.001$) on the eCAP threshold. There was a statistically significant interaction between effects of subject group and electrode location ($F_{(5, 741.66)} = 93.50$, $p < 0.001$). There was no significant interaction between effects of study group and IPG duration ($F_{(5, 338.77)} = 0.165$, $p = 0.975$), or between effects of electrode location and IPG duration ($F_{(10, 241.07)} = 0.085$, $p = 1.000$). There was no significant interaction among effects of study group, electrode and IPG duration ($F_{(10, 241.07)} = 0.049$, $p = 1.000$).

Results of pairwise comparisons with Bonferroni correction showed that eCAP thresholds measured at the basal electrode location were significantly lower than those measured at the middle electrode location ($p < 0.001$) and the apical electrode location ($p < 0.001$). eCAP thresholds measured at the apical electrode location were also lower than those measured at

the middle electrode location ($p < 0.001$). The significant interaction between effects of study group and electrode location is due to the fact that eCAP thresholds measured in these two study groups demonstrate different patterns. For children with CN, eCAP thresholds measured at the basal electrode location were lower than those measured at the middle electrode location ($p < 0.001$) and the apical electrode location ($p < 0.001$). Thresholds measured at the middle electrode location were also lower than those measured at the apical electrode location ($p < 0.001$). For children with normal-sized CNs, thresholds measured at the basal ($p < 0.001$) and the apical electrode location ($p < 0.001$) were lower than those measured at the middle electrode location. eCAP thresholds measured for the basal electrode location were also lower than those measured at the apical electrode location ($p = 0.011$).

Figure 7 depicts the size of the IPG effect on the eCAP threshold at three electrode locations in both study groups. Inspection of this figure suggests that children with CN had a smaller decrease in the eCAP threshold than children with normal-sized CNs, at least at the most basal electrode, as the IPG increased. Larger IPG effects on the eCAP threshold were observed for longer IPGs. Results of GLMMs (see Table 4) showed that the eCAP threshold was significantly affected by study group ($F_{(1, 283.88)} = 8.28, p = 0.004$), with children with CN having smaller IPG effects than children with normal-sized CNs. Results of GLMMs also revealed significant effects of electrode location ($F_{(2, 354.40)} = 23.27, p < 0.001$), IPG duration ($F_{(4, 182.40)} = 46.25, p < 0.001$), as well as a significant interaction between effects of study group and electrode location ($F_{(2, 354.40)} = 5.02, p = 0.007$).

Results of pairwise comparisons with Bonferroni correction showed that the size of the IPG effect on the eCAP threshold was smaller at the middle electrode location than at the apical electrode location ($p < 0.001$). There was no significant difference in the size of the IPG effect on the eCAP threshold measured between any other two electrode locations ($p > 0.05$). IPG durations of 35 and 42 μ s showed significantly larger effects on the eCAP threshold than IPG durations of 14, 21 and 28 μ s ($p < 0.001$). The effect size measured for the 14 μ s IPG was significantly smaller than that measured for the 21 μ s IPG. Children with CN showed a smaller IPG effect at the middle electrode location than the apical electrode location ($p = 0.002$). Children with normal-sized CNs had a smaller IPG effect at the middle electrode location than the basal and the apical electrode location ($p < 0.001$). For both groups, the size of the IPG effect on the eCAP threshold was not statistically significant between any other two electrode locations.

In summary, children with CN showed higher eCAP threshold and a smaller IPG effect on the eCAP threshold than children with normal-sized CNs (Figures 6 and 7). Increasing IPG durations resulted in lower eCAP thresholds and larger IPG effects in both study groups (Figures 6 and 7). For children with CN, eCAP thresholds increased as the stimulating site moved in a basal-to-apical direction (Figure 6). The size of the IPG effect on the eCAP threshold at the middle electrode location was smaller than at the apical electrode location (Figure 7). For children with normal-sized CNs, the highest eCAP threshold and smallest IPG effect on the eCAP threshold were recorded at the middle electrode location (Figures 6 and 7).

The slope of eCAP I/O Function

For children with CND, the goodness of fit (i.e., adjusted R^2) of the linear and the sigmoidal regression function ranged from -0.14 to 0.99 (mean: 0.89 , SD: 0.15) and -0.33 to 0.99 (mean: 0.90 , SD: 0.15), respectively. For children with normal-sized CNs, the goodness of fit (i.e., adjusted R^2) of the linear and the sigmoidal regression function ranged from 0.43 to 0.99 (mean: 0.95 , SD: 0.06) and 0.42 to 0.99 (mean: 0.96 , SD: 0.059), respectively. Results of an independent-sample t test showed that the slope of the eCAP I/O function was better characterized using both the linear ($F=213.52$, $p<0.001$) and the sigmoidal ($F=228.32$, $p<0.001$) regression functions for children with normal-sized CNs compared to children with CND. Results of a paired-sample t test showed that the sigmoidal regression function provided a better fit for eCAP I/O function than the linear regression fit for both children with CND ($t=2.90$, $p=0.004$) and children with normal-sized CNs ($t=7.88$, $p<0.001$). Overall, these results indicate that the sigmoidal regression function provides a better fit for the eCAP I/O function. The fitting is better for results recorded in children with normal-sized CNs than those measured in children with CND regardless of which regression function is used to characterize the eCAP I/O function.

Linear Regression Function—Figure 8 shows means and standard deviations of the slope of the eCAP I/O function estimated using the linear regression function for both study groups at three electrode locations. These results clearly show that the slopes measured in children with CND were smaller than those measured in children with normal-sized CNs. This observation was confirmed by the significant effect of study group ($F_{(1, 554.04)} = 337.34$, $p<0.001$) revealed by the GLMM analysis (see Table 3). In addition, results of GLMMs showed a significant effect of electrode location ($F_{(2, 450.09)} = 4.08$, $p=0.018$) and the interaction between effects of study group and electrode location ($F_{(2, 450.09)} = 9.84$, $p<0.01$). The effect of the IPG on slope was not statistically significant ($F_{(5, 183.08)} = 0.681$, $p=0.639$). No other interaction reached statistical significance ($p>0.05$).

Results of pairwise comparisons with Bonferroni correction showed that the significant electrode location effect was due to larger slopes measured at the basal electrode location than those measured at the middle electrode location ($p=0.032$). There was no statistical difference in the slope measured between any other electrode locations ($p>0.05$). Children with CND showed smaller slopes than children with normal-sized CNs at all three electrode locations. The significant interaction between effects of study group and electrode location was due to the difference in slope vs electrode pattern observed in these two study groups. Specifically, children with CND showed significantly larger slopes at the basal electrode location than at the middle and apical electrode locations ($p<0.001$). In contrast, children with normal-sized CNs showed significantly smaller slopes at the basal electrode than at the apical electrode location ($p<0.001$). There was no significant difference in slope between any other two electrode locations in these subjects ($p>0.05$).

Figure 9 shows means and standard deviations of the size of the IPG effect (as quantified as percentage of change) on the linear slope for both study groups at three electrode locations. These data show that children with CND had greater variations in the size of the IPG effect than children with normal-sized CN. More importantly, these data demonstrate that

increasing the IPG had a larger effect on increasing the slope of the eCAP I/O function in children with CN than in children with normal-sized CNs. Results of GLMMs (see Table 4) showed a significant effect of group ($F_{(1, 577.02)} = 18.74, p < 0.01$) and a non-significant effect of electrode location ($F_{(2, 455.66)} = 2.43, p = 0.089$).

In summary, children with CN showed shallower linear slopes and a larger IPG effect on the linear slope than children with normal-sized CNs (Figures 8 and 9). Increasing the IPG did not result in significant changes in the slope in either study group (Figure 8). For children with CN, eCAP I/O functions measured at the basal electrode location had the largest linear slopes (Figure 8). For children with normal-sized CNs, linear slopes measured at the basal electrode location were smaller than those measured at the apical electrode location (Figure 8).

Sigmoidal Regression Function—Figure 10 shows means and standard deviations of the slope of the eCAP I/O function estimated using the sigmoidal regression function for both study groups at three electrode locations. Despite variations in sigmoidal slopes, these data clearly show that children with CN had smaller slopes than children with normal-sized CNs. Results of GLMMs (see Table 3) revealed significant effects of study group ($F_{(1, 814.79)} = 789.28, p < 0.01$) and IPG duration ($F_{(5, 287.75)} = 4.17, p = 0.001$). The effect of electrode location or interaction among effects of these factors was not statistically significant ($p > 0.05$). Results of pairwise comparisons with Bonferroni correction showed that slopes measured for the 7 μ s IPG were significantly larger than those measured for the 42 μ s IPG ($p < 0.01$). There was no significant difference in slopes measured between any other two IPG durations ($p > 0.05$).

Figure 11 depicts means and standard deviations of the size of the IPG effect on the sigmoidal slope for both study groups. Similar to patterns shown in Figure 10, these data demonstrate that the size of the IPG effect on the sigmoidal slope differs between children with CN and children with normal-sized CNs. Whereas increasing the IPG resulted in reduced sigmoidal slopes in children with normal-sized CNs, it led to increased sigmoidal slopes at the basal and the middle electrode locations in children with CN. This difference in the data pattern makes the significant effect of study group ($F_{(1, 563.42)} = 34.96, p < 0.01$) less meaningful. Results of GLMM (see Table 4) show that the effect of IPG duration was not statistically significant ($F_{(4, 215.19)} = 1.42, p = 0.228$).

In summary, children with CN had smaller sigmoidal slopes of the eCAP I/O function than children with normal-sized CNs (Figure 10). Increasing the IPG generally decreased the sigmoidal slope in children with normal-sized CNs. This trend was not consistently observed for children with CN (Figure 10).

DISCUSSIONS

This study compared effects of the IPG on the maximum eCAP amplitude, the eCAP threshold and the slope of the eCAP I/O function between children with CN and children with normal-sized CNs. Based on the literature, we hypothesized that poor CN functional

status would affect the size of the IPG effect on increasing neural response of the CN in these listeners.

Group Difference in eCAP Results Measured at Different IPG Durations

Compared to children with normal-sized CNs, children with CND had reduced maximum eCAP amplitudes, elevated eCAP thresholds and smaller slopes of the eCAP I/O function. These results are consistent with those reported in our previous study (He et al., 2018). More importantly, significant reduction in response amplitude and slopes of eCAP I/O functions, and elevation in response threshold have been observed in long-term deafened animal models with reduced spiral ganglion cells (Shepherd & Javel, 1997; Pfingst et al., 2014; Pfingst et al., 2015a, 2015b). Therefore, these results may reflect the greater CN damage in children with CND compared to children with normal-sized CNs. Regardless of this group difference, both study groups showed larger maximum eCAP amplitudes and lower eCAP threshold at longer IPGs, which is consistent with our expectation and the literature (Prado-Guitierrez et al., 2006; Ramekers et al., 2014, 2015; Schwartz-Leyzac & Pfingst, 2016; Hughes et al., 2018). A difference in the IPG effect on the slope of the eCAP I/O function was observed between results estimated using the linear and the sigmoidal regression functions. Specifically, increasing IPG durations did not significantly affect linear slopes measured for both study groups. These results are consistent with those reported in Hughes et al. (2018) but conflict with data reported by Schwartz-Leyzac & Pfingst (2016). It should be pointed out that the linear regression function was used in both of these two previously published studies to estimate the slope of the eCAP I/O function measured in human CI users. Factors accounting for these inconsistent results remain unknown. We speculate that difference in etiology, duration of deafness, internal electrode array, neural survival pattern along the cochlea and electrode placement among CI users tested in these studies may play a role. For slopes estimated using the sigmoidal regression function, increasing IPG duration generally resulted in a decrease in slope, which is not consistent with the results reported in guinea pigs (Ramekers et al., 2014). This inconsistency might reflect differences in species, stimulation level used in anesthetized guinea pigs vs awake human CI users, duration of deafness (6 weeks in guinea pigs vs years in human CI users tested in this study), electrode placement in the cochlea, or how the slope was defined and quantified between these two studies.

The difference in the IPG effect on the slope between results assessed using the linear and the sigmoidal regression function highlight the importance of selecting an appropriate mathematical function for the slope estimation. Unfortunately, there is no universal agreement upon which function can best characterize the eCAP I/O function in human CI users. To date, our previous study (He et al., 2018) is the only study that has compared the goodness of fit of the linear, the sigmoidal and the exponential function for eCAP I/O functions measured in CI patients. Results of that study showed that the sigmoidal regression function yielded the best fit among all three functions tested, which provided evidence supporting the use of the sigmoidal regression function in this study. Consistent with the results of our previous study, the sigmoidal regression function also yielded a better fit than the linear regression function for both children with CND and children with normal-sized CNs in this study. Despite these consistent results, both studies have two limitations. First,

only three regression functions were selected and tested based on the literature. It remains unknown whether the eCAP I/O function can be better characterized by any other mathematical functions. Second, as pointed out in He et al. (2018), the true saturated eCAP amplitude (i.e., the upper limit of the parameter a used in the sigmoidal regression function) could not be measured in human CI users due to subject discomfort, limitation of CI output level, and increased likelihood of stimulus artifact contamination at high stimulation levels, which might reduce the accuracy of estimated slopes. As a result, it still remains unknown which regression function can best characterize the eCAP I/O function measured in human CI users. Further studies are warranted to fill this knowledge gap.

Results of previous studies revealed a greater increase in the IPG effect between shorter IPG durations in both human CI users (McKay & Henshall, 2003; Hughes et al., 2018) and animal models (Ramekers et al., 2014). This trend was also observed in this study for the maximum eCAP amplitude and the linear slope but not for the sigmoidal slope. In addition, children with CNL showed the largest decrease in eCAP threshold as the IPG increased from 7 to 14 μ s. However, this trend was not observed for results measured in children with normal-sized CNs. It should be pointed out that the shortest IPG tested in this study was 7 μ s due to the device limitation, which might partially account for the lack of a similar trend in our results compared to the trend reported in the literature.

Long et al. (2014) reported longer electrode to modiolus, medial-lateral (EMML) distances measured for middle electrode than those assessed for the basal and apical electrodes, which might account, at least partially, for the data pattern of eCAP thresholds measured in children with normal-sized CNs in this study (Figure 6).

Group Difference in the Size of the IPG Effect

Compared to children with normal-sized CNs, children with CNL were expected to show larger IPG effects on maximum eCAP amplitude and the eCAP threshold, and a smaller IPG effect on slope of the eCAP I/O function.

Maximum eCAP Amplitude—Consistent with our hypothesis, children with CNL showed larger IPG effects on the maximum eCAP amplitude compared with children with normal-sized CNs. These results are also consistent with those observed in guinea pigs (Ramekers et al., 2015).

eCAP Threshold—Children with CNL showed less of a decrease in the eCAP threshold as the IPG increased (i.e., a smaller IPG effect) than children with normal-sized CNs. This result conflicts with our hypothesis and the results reported in Ramekers et al. (2014). However, it is consistent with those reported in Prado-Guitierrez et al. (2006). Demyelination of CN fibers in deafened ears has been used to explain the results observed in Prado-Guitierrez et al. (2006). Specifically, demyelination leads to an increased propagation time of action potential along CN fibers (Koles & Rasminsky, 1972). As a result, a long IPG is needed to prevent the action potential generated by one electrical pulse phase from being abolished by the opposite phase. In addition to this possibility, demyelinated auditory nerves can potentially have longer temporal integration windows (Stypulkowski & van den Honert, 1984; Cartee et al., 2006), which can lead to a reduced IPG effect as well. However, both

prolonged propagation time and an increased temporal integration window should theoretically result in a reduced IPG effect on the slope of the eCAP I/O function in addition to the eCAP threshold. Unfortunately, results of this study showed larger IPG effects on the slope of the eCAP I/O function in subjects with poorer CN survival, which does not support this explanation. One more plausible explanation is that better physiological statuses of the CN in children with normal-sized CNs led to higher probabilities of spike initiation near threshold level, which resulted in higher sensitivities to changes in the IPG (i.e., greater IPG effects) in children with normal-sized CNs.

Slope of the eCAP I/O Function—Based on the study hypothesis, children with CND were expected to show less increase in slope of the eCAP I/O function at longer IPGs than children with normal-sized CNs due to poor CN survival. Contrary to these predictions and the previously reported results in guinea pigs (Prado-Guitierrez et al., 2006; Ramekers et al., 2014), our results revealed larger IPG effects on the slope of the eCAP I/O function in children with CND than in children with normal-sized CNs. These results contradicted the proposed study hypothesis.

Result Discrepancy between Current Study and Previous Animal Studies—Several factors may account for the discrepancy between the results of this study and those reported in animal models. One obvious factor is the difference in experimental procedures and study subjects tested in these studies. Anatomical characteristics of the cochlea and neurophysiological responsiveness of the CN in human listeners differ from those of guinea pigs (e.g., Tuncel et al., 2005; Macherey & Cazals, 2016). Other differences include etiology and duration of deafness, rate of neural degeneration, duration of CI use, and CI electrode design and placement. Directly comparing the IPG effect on the eCAP between these two species using the exact same experimental procedure is beyond the scope of this study. Therefore, it remains unknown how much of the discrepancy can be attributed to this factor.

Differences in the method used to quantify the size of the IPG effect are also worth consideration. Whereas the size of the IPG effect in animal studies was defined as the difference in the raw data of the eCAP threshold (Prado-Guitierrez et al., 2006; Ramekers et al., 2014), the maximum eCAP amplitude and the slope of the eCAP I/O function (Ramekers et al., 2014) measured at two IPG durations, it was quantified as the proportional change relative to the results measured at the 7 μ s IPG in this study. It is possible that the difference in how the IPG effect was defined and quantified among studies could account for the result discrepancy. To test this possibility, GLMMs were used to compare the difference between these dependent variables measured at any two adjacent IPG durations in this study. Results of GLMMs showed that children with CND had a significantly smaller IPG effect on the maximum eCAP amplitudes ($F_{(1, 808.28)} = 38.78, p < 0.001$) and the sigmoidal slope ($F_{(1, 175.00)} = 6.31, p = 0.013$), but a significantly larger IPG effect on the eCAP threshold ($F_{(1, 775.11)} = 8.47, p = 0.004$) than children with normal-sized CNs. These results are only partially consistent with those reported in Ramekers et al. (2014). Therefore, the difference in the method used to quantify the IPG effect cannot fully account for the discrepancy between results of this study and those measured in guinea pigs.

Another potential factor is the difference in stimulation level between the experimental group and the control group among studies. In the two animal studies discussed, eCAPs were recorded at either the same fixed ten stimulation levels or the same supra-threshold levels in both healthy and deafened ears of guinea pigs (Prado-Guitierrez et al., 2006; Ramekers et al., 2014). In contrast, higher stimulation levels were used in children with CNND than in children with normal-sized CNs in this study. It has been shown that the probability of discharge increases with stimulation level (Shepherd & Javel, 1997) and the firing rate plays a major role in determining neural output when neural survival is poor (McKay & McDermott, 1998). It is possible that the increase in the speed of the firing rate of each CN fiber was greater in children with CNND than children with normal-sized CNs as the IPG increased. As a result, despite the reduced slope of the eCAP I/O function due to limited CN fibers, children with CNND showed greater IPG effects on this supra-threshold dependent variables than children with normal-sized CNs.

Finally, we speculate that the permeability of ion channels of CN fibers reduces in children with CNND. It is also possible that the vulnerability of the propagating spike caused by neural degeneration (Tasaki, 1955; Cragg & Thomas, 1964; McDonald & Sears, 1970; Rasminsky & Sears, 1972; Pender & Sears, 1984) is higher in children with CNND than in children with normal-sized CN. Both factors lead to a prolonged “vulnerable period”. As a result, increasing the IPG would lead to a greater benefit (i.e., a larger IPG effect) for children with CNND than children with normal-sized CNs. Unfortunately, due to the unknown etiology of the CNND and the inability to directly investigate the pathophysiological damage of the CN in patients with CNND, this speculation cannot be verified.

Electrode Location and the Size of the IPG Effect in Children with CNND

Based on our previous finding (He et al., 2018), children with CNND were also expected to show changes in the IPG effect as the stimulating site moved from the base to the apex of the cochlea. Results of this study demonstrated that children with CNND had a smaller IPG effect on the maximum eCAP amplitude at the basal electrode location compared to the middle and apical electrode locations. A smaller IPG effect on the eCAP threshold at the middle electrode location than at the apical electrode location was also observed. These results conflict with our expectation. The inconsistency might be due to the fact that data recorded at the apical electrode locations were weighted by the results of subjects who showed measurable eCAPs at more electrodes. These subjects might have less damage to their CNs than subjects who only had measurable eCAPs at the most basal electrode sites. The substantial variations in the size of the IPG effect on the slope of the eCAP I/O function (see Figures 9 and 11) may also account for the lack of difference in the slope measured at different electrodes.

Consideration for Clinical Applications

Results of this study have demonstrated that increasing the IPG improves neural responsiveness of the electrically-stimulated cochlear nerve, especially for children with CNND. Therefore, for this unique patient population, using a longer IPG can potentially improve neural encoding and processing of the electrical stimulation at the CN without increasing electrical power consumption. In other words, biphasic pulses with longer IPGs

can be considered more efficient stimuli than those with no or short IPGs for children with CN. The improved stimulation efficiency can lead to longer speech processor battery life due to reduced power consumption and provide an opportunity to develop smaller, more efficient CI devices. More importantly, regardless of the inconsistency in data obtained in human CI users vs animal models, results from this study along with the literature suggest that the size of the IPG effect on the eCAP holds the potential for being a biomarker for neural survival of CN fibers.

Study Limitations

Besides the two limitations of using the sigmoidal regression function to estimate the slope, this study has several additional potential limitations. First, etiology for CN and hearing loss in many children with normal-size CNs remains unknown. Therefore, it is not feasible to control/match this potential confounding factor among study participants. The anatomical size of the CN determined based on results of MRI scans has a relatively low predictive power for the functional status of the CN as assessed using the eCAP (He et al., 2018). Therefore, variations in CN function due to different etiologies among study participants with similar CN anatomies might exist, which could potentially affect results of this study. Second, the pulse phase duration used in this study was 50 μ s, which is longer than what is used in typical CI users. It has been shown that the effect of increasing the IPG on the eCAP is smaller when the pulse phase duration is long (Shepherd & Javel, 1999; Ramekers et al., 2014). Therefore, it is possible that larger IPG effects would have been observed if shorter pulse phase durations were used in this study. Third, the size of the IPG effect was quantified as the percentage of changes in normalized eCAP results. Even though this definition is rational and has been used in one previous study (Schvartz-Leyzac & Pfingst, 2016), it may not be the most optimal way to quantify the IPG effect. Fourth, reliably measuring the C level for each IPG at all three electrode locations was not feasible due to the extensive testing time required and the limited attention span for many children with CN. As a result, the difference between the measured maximum eCAP amplitude and the true saturated eCAP amplitude might have been larger at longer IPGs, which may affect the slope estimated using the sigmoidal regression function. Fifth, only pediatric CI users were tested in this study. Despite the heterogeneity in potential etiology among subjects, all of them had relatively short duration of deafness. Therefore, it remains unknown whether results of this study can be generalizable to adult CI users who are more likely to suffer from longer duration of deafness. Finally, due to the lack of thorough understanding of the pathophysiological damage to the CN in children with CN and the lack of direct access to the CN in human listeners, many of our explanations, even though they are plausible, are based on theories or even speculations. As a result, the findings of this study might have generated more questions than answers, which may facilitate additional research on similar topics.

CONCLUSIONS

Increasing the IPG increases neural responsiveness of the electrically-stimulated CN in both children with CN and children with normal-sized CNs. Larger IPG effects on the maximum eCAP amplitude and the slope of the eCAP I/O function, as well as smaller IPG

effects on the eCAP threshold are associated with poorer neural survival of CN fibers in human CI users. These results do not fully agree with those observed in animal models, which suggests the importance of verifying findings of animal studies in human listeners.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A schematic illustration of how test electrodes were selected for one child with CND who had measurable eCAPs at electrodes 1-11.

Figure 2.

eCAP I/O functions plotted based on raw data measured at six IPG durations and three electrode locations in two children with CND (CND4 and CND10) and one child with a normal-sized CN (S16). Results measured at different IPGs are indicated using different symbols. Each row indicates results recorded from one subject with the subject number indicated in the left panel. Each column shows results recorded at one electrode location (i.e., basal, middle-array or apical electrode). The specific test electrode is indicated in each panel.

Figure 3.

eCAP waveforms with the largest amplitude measured at three electrode locations in CND4, CND10 and S16. eCAPs measured for different IPGs are indicated using different colors. Each row indicates results recorded from one subject with the subject number indicated in the left panel. Each column shows results recorded at one electrode location (i.e., basal, middle or apical electrode). The specific test electrode is indicated in each panel.

Figure 4.

Means and standard deviations of the maximum eCAP amplitude measured at different IPGs and three electrode locations in both study groups. Results measured in children with CND and children with normal-sized CNs (i.e., control) are indicated using filled circles and open squares, respectively. Panels in the top and the bottom rows indicate results recorded in children with CND and children with normal-sized CNs, respectively. Each column shows results recorded at one electrode location (i.e., basal, middle or apical electrode). The ordinate scale is different for these two study groups.

Figure 5.

Means and standard deviations of the size of the IPG effect on the maximum eCAP amplitude, as quantified as the percentage of the change in normalized maximum eCAP amplitude, measured at different IPGs and three electrode locations in both study groups. Results measured in children with CND and children with normal-sized CNs (i.e., control) are indicated in black and grey, respectively. Each panel shows results measured at one electrode location, which is indicated on the top of each panel.

Figure 6.

Means and standard deviations of the eCAP threshold measured at different IPGs and three electrode locations in both study groups. Results measured in children with CND and children with normal-sized CNs (i.e., control) are indicated using filled circles and open squares, respectively. Panels in the top and the bottom rows indicate results recorded in children with CND and children with normal-sized CNs, respectively. Each column shows results recorded at one electrode location (i.e., basal, middle or apical electrode). The ordinate scale is different for these two study groups.

Figure 7.

Means and standard deviations of the size of the IPG effect on the eCAP threshold, as quantified as the percentage of the change in normalized eCAP threshold, measured at different IPGs and three electrode locations in both study groups. Results measured in children with CND and children with normal-sized CNs (i.e., control) are indicated in black and grey, respectively. Each panel shows results measured at one electrode location, which is indicated on the top of each panel.

Figure 8.

Means and standard deviations of the slope of the eCAP I/O function estimated using linear regression (i.e., linear slope) at different IPGs and three electrode locations in both study groups. Results measured in children with CND and children with normal-sized CNs (i.e., control) are indicated using filled circles and open squares, respectively. Panels in the top and the bottom rows indicate results recorded in children with CND and children with normal-sized CNs, respectively. Each column shows results recorded at one electrode location (i.e., basal, middle or apical electrode). The ordinate scale is different for these two study groups.

Figure 9.

Means and standard deviations of the size of the IPG effect on the linear slope, as quantified as the percentage of the change in normalized linear slope, measured at different IPGs and three electrode locations in both study groups. Results measured in children with CND and children with normal-sized CNs (i.e., control) are indicated in black and grey, respectively. Each panel shows results measured at one electrode location, which is indicated on the top of each panel.

Figure 10.

Means and standard deviations of the slope of the eCAP I/O function estimated using sigmoidal regression (i.e., sigmoidal slope) at different IPGs and three electrode locations in both study groups. Results measured in children with CND and children with normal-sized CNs (i.e., control) are indicated using filled circles and open squares, respectively. Panels in the top and the bottom rows indicate results recorded in children with CND and children with normal-sized CNs, respectively. Each column shows results recorded at one electrode location (i.e., basal, middle or apical electrode). The ordinate scale is different for these two study groups.

Figure 11.

Means and standard deviations of the size of the IPG effect on the sigmoidal slope, as quantified as the percentage of the change in normalized sigmoidal slope, measured at different IPGs and three electrode locations in both study groups. Results measured in children with CND and children with normal-sized CNs (i.e., control) are indicated in black and grey, respectively. Each panel shows results measured at one electrode location, which is indicated on the top of each panel.

Table 1.

Demographic information of subjects who participated in this study. M = male, F = female, L = left, R = right, AAI: age at implantation, AAT: age at testing, CN: cochlear nerve, IP-II: incomplete partition type II, IAC: internal auditory canal, BCNC: bony cochlear nerve canal.

Subject number	Gender	Ear tested	AAI (yrs)	AAT (yrs)	Imaging results			Electrode tested	
					CN in the IAC	Cochlea	IAC	BCNC	
CND1	F	L	4.4	8.4	Two small nerves	Normal	Normal	Normal	1,3,7
CND2	F	R	4.0	6.9	Two small nerves	Normal	Narrow	Stenosis	3,12,21
CND3	M	L	1.9	4.1	Two small nerves	IP-II	Narrow	Stenosis	1,3,6
CND4	F	L	1.2	2.5	Two small nerves	IP-II	Narrow	Stenosis	1,5,10
CND5	F	R	1.9	3.4	Two small nerves	IP-II	Normal	Stenosis	3,12,21
CND6	F	L	19.9	23.1	Two small nerves	IP-II	Normal	Stenosis	3,9,16
CND7	M	L	3.9	6.1	Two small nerves	Normal	Normal	Stenosis	1,4,7
CND8	F	R	2.9	8.5	Two small nerves	Normal	Narrow	Stenosis	1,4,8
CND9	F	R	1.2	1.9	One single nerve	Normal	Normal	Stenosis	1,4,8
CND10	M	L	1.1	2.4	Two small nerves	Normal	Narrow	Stenosis	3,12,21
CND11	M	R	2.1	4.1	Two small nerves	Normal	Narrow	Stenosis	1,5,8
CND12	F	R	2.4	6.5	One single nerve	IP-II	Normal	Normal	3,12,21
CND13	F	L	1.3	3.3	Two small nerves	Normal	Narrow	Stenosis	3,12,21
CND14	F	L	3.4	5.4	Two small nerves	Normal	Narrow	Normal	3,10,18
CND15	M	R	1.9	4.1	Two small nerves	IP-II	Normal	Normal	3,12,21
CND16	M	L	1.9	2.4	Two small nerves	Normal	Normal	Stenosis	1,7,14
CND17	M	L	7.9	12.1	Two small nerves	Normal	Normal	Stenosis	3,10,17
CND18	M	L	6.0	7.8	Two small nerves	Normal	Narrow	Stenosis	1,3,7
CND19	F	R	4.6	7.8	One single nerve	Normal	Narrow	Stenosis	3,12,20
CND20	M	L	3.6	9.1	One single nerve	Normal	Narrow	Stenosis	3,12,21
CND21	M	L	1.9	2.5	Two small nerves	Normal	Normal	Stenosis	3,12,21
CND22	F	L	6.0	8.0	Two small nerves	Normal	Normal	Stenosis	3,12,21
CND23	M	R	6.6	8.5	One single nerve	Normal	Normal	Stenosis	3,6,10
CND24	F	L	3.3	5.2	Two small nerves	Normal	Normal	Stenosis	3,12,21
CND25	M	R	1.5	2.4	Two small nerves	Normal	Narrow	Stenosis	1,2,3
CND26	F	L	4.7	6.5	Two small nerves	Normal	Narrow	Stenosis	3,12,21

Subject number	Gender	Ear tested	AAI (yrs)	AAT (yrs)	Imaging results			Electrode tested
					CN in the IAC	Cochlea	IAC	BCNC
CND27	F	L	2.6	4.3	Two small nerves	Normal	Narrow	Stenosis
CND28	M	L	6.0	7.3	Two small nerves	Normal	Narrow	Stenosis
CND29	M	L	2.8	3.3	Two small nerves	Normal	Narrow	Stenosis
CND30	M	R	1.0	3.5	Two small nerves	Normal	Normal	Stenosis
S1	F	R	2.1	4.4				3,12,21
S2	M	R	1.2	2.6				3,12,21
S3	M	L	1.8	3.1				3,12,21
S4	M	R	2.0	3.6				3,12,21
S5	M	R	5.7	7.9				3,12,21
S6	M	R	0.9	2.1				3,12,21
S7	M	R	7.0	8.3				3,12,21
S8	F	R	2.9	4.8				3,12,21
S9	M	R	1.9	5.0				3,12,21
S10	F	R	4.9	6.7				3,12,21
S11	F	R	3.1	7.2				3,12,21
S12	F	R	5.0	7.0				3,12,21
S13	F	L	5.3	9.6				3,12,21
S14	F	L	1.1	4.0				3,12,21
S15	M	L	1.8	4.2				3,12,21
S16	F	R	4.1	9.7				3,12,21
S17	M	R	1.8	5.1				3,12,21
S18	M	R	1.2	2.7				3,12,21
S19	M	R	10.1	13.1				3,12,21
S20	F	R	1.7	2.9				3,12,21
S21	M	L	1.1	2.1				3,12,21
S22	M	L	1.3	3.3				3,12,21
S23	M	R	5.1	10.8				3,12,21
S24	M	L	1.6	3.5				3,12,21
S25	M	L	1.4	4.6				3,12,21
S26	F	R	6.3	9.6				3,12,21

Subject number	Gender	Ear tested	AAI (yrs)	AAT (yrs)	CN in the IAC			Imaging results			Electrode tested
					CN in the IAC			Cochlea	IAC	BCNC	
S27	M	L	7.3	10.3							3,12,21
S28	M	L	0.8	4.0							3,12,21
S29	M	R	1.9	4.9							3,12,21
S30	M	R	4.1	7.4							3,12,21

Table 2.

Linear and sigmoidal regression functions used to characterize eCAP I/O functions measured in this study.
 eCAP_N: normalized amplitude of the electrically evoked compound action potential, L: stimulation level (in
 μA) I/O: input/output

Name	Linear Function	Sigmoidal Function
Formula	$eCAP_N = a + bL$	$eCAP_N = \frac{a}{1 + e^{-b(L - L_0)}}$
Parameters	a : the intercept b : the slope	a : the upper bound of the eCAP _N L_0 : the L-value of the midpoint of the eCAP _N b : the slope

Table 3.

A summary of statistical results for the raw data of the maximum eCAP amplitude, the eCAP threshold and the slope of the eCAP input/output function. eCAP: electrically-evoked Compound Action Potential, IPG: Inter-Phase Gap, B: the basal electrode location; M: the middle electrode location, A: the apical electrode location.

Effects	Amplitude	Threshold	Slope	
			Linear	Sigmoidal
Group	CND<Control	CND>Control	CND<Control	CND<Control
Electrode	B> M and A	B<M and A; M>A	B>M	Not significant
IPG (μ s)	7<21, 28, 35 and 42; 14< 35 and 42	7 >28, 35 and 42; 14>35 and 42	Not significant	7>42
Group*Electrode	An overall significant interaction	For CND group: B<M and A; M<A. For control group: B<M and A; M>A.	For CND group: B>M and A. For control group: B<A	Not significant
Group*IPG	Not significant	Not significant	Not significant	Not significant
Electrode*IPG	Not Significant	Not significant	Not significant	Not significant
Group*Electrode*IPG	Not Significant	Not significant	Not significant	Not significant

Table 4.

A summary of statistical results for the size of the IPG effect on maximum eCAP amplitude, eCAP threshold and slope of the eCAP input/output function. eCAP: electrically-evoked Compound Action Potential, IPG: Inter-Phase Gap, B: the basal electrode location; M: the middle electrode location, A: the apical electrode location; DNT: did not test.

Effects	Amplitude	Threshold	Slope	
			Linear	Sigmoidal
Group	CND>Control	CND<Control	CND>Control	CND>Control
Electrode	B< M and A	M<A	Not significant	DNT
IPG (μs)	14<21, 28, 35 and 42; 21< 28,35 and 42	14 <28, 35 and 42; 21<35 and 42; 28<35 and 42	DNT	Not significant
Group*Electrode	For CND group: B<M, and A For control group: no difference among B, M and A	For CND group: M<A. For control group: B>M; M<A.	Not significant	DNT