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Insertion trauma and recovery of function after cochlear implantation: Evidence from objective functional measures

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ABSTRACT

Partial loss and subsequent recovery of cochlear implant function in the first few weeks following cochlear implant surgery has been observed in previous studies using psychophysical detection thresholds. In the current study, we explored this putative manifestation of insertion trauma using objective functional measures: electrically-evoked compound action potential (ECAP) amplitude-growth functions (ECAP amplitude as a function of stimulus level). In guinea pigs implanted in a hearing ear with good post-implant hearing and good spiral ganglion neuron (SGN) survival, consistent patterns of ECAP functions were observed. The slopes of ECAP growth functions were moderately steep on the day of implant insertion, decreased to low levels over the first few days after implantation and then increased slowly over several weeks to reach a relatively stable level. In parallel, ECAP thresholds increased over time after implantation and then recovered, although more quickly, to a relatively stable low level as did thresholds for eliciting a facial twitch. Similar results were obtained in animals deafened but treated with an adenovirus with a neurotrophin gene insert that resulted in good SGN preservation. In contrast, in animals implanted in deaf ears that had relatively poor SGN survival, ECAP slopes reached low levels within a few days after implantation and remained low. These results are consistent with the idea that steep ECAP growth functions require a healthy population of auditory nerve fibers and that cochlear implant insertion trauma can temporarily impair the function of a healthy SGN population.

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1. Introduction

While cochlear implants already perform remarkably well at restoring or improving hearing for deaf or hearing-impaired individuals, there is still room for improvement (Wilson and Dorman, 2008). Recently there has been increased attention to preserving the biology of the implanted cochlea, including improved implant designs, “soft surgery” procedures, and tissue engineering (e.g., Cohen, 1997; von Ilberg et al., 1999; Wise et al., 2005; Van De Water et al., 2010; Budenz et al., 2012; Ramekers et al., 2012; Havenith et al., 2013; Usami et al., 2014). In previous studies we have observed significant loss of cochlear implant function following cochlear implant insertion and subsequent recovery of function, suggesting a temporary negative reaction to cochlear implant insertion surgery (Pfingst, 1990; Miller et al., 2000; Su et al., 2008). We use the term “insertion trauma” to refer to the reaction to the entire surgical procedure in which an implant is inserted into the scala tympani of the cochlea. In our animal models, cochlear implant insertion was often done shortly after the ear was deafened by local perfusion of neomycin into the scala tympani so it is possible that the deafening procedure contributed to the temporary loss of neural function in those cases. However, we have seen similar changes over time in implant function when the implant was inserted into a hearing ear, suggesting the surgical insertion procedure alone can produce significant temporary functional impairment (Su et al., 2008). These observations were made in nonhuman primates and guinea pigs using psychophysical detection thresholds as the measure of cochlear implant function. In these studies, psychophysical detection thresholds, when they

Abbreviations: AAV, adeno-associated virus; DPI, days post implantation; ECAP, electrically-evoked compound action potential; IHC, inner hair cell; MSL, maximum stimulus level; μA, microampere; N1, first negative potential; *Ntf3*, gene for neurotrophic factor 3; P2, second positive potential; SGN, spiral ganglion neuron

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could be recorded within a day or two after surgery, started out at a low level, increased (showed a loss of sensitivity) over time during the first several days after implantation, and then slowly recovered to near or below the original levels. More commonly, thresholds were high when the first reliable thresholds were obtained and they decreased over a period of several weeks. These initial reactions to implant insertion are of interest for several reasons. First they probably reflect at least a temporary traumatic reaction to the surgical procedure and implant insertion, which could potentially have long-term consequences. Second, the unstable functional response to electrical stimulation during the early days following implant insertion is a concern for experimental studies that take place during this period. Finally the phenomenon of apparent loss and recovery during this relatively restricted time period defines a potentially interesting model for study of features of the implanted cochlea that influence cochlear implant function.

A plausible explanation of the systematic fluctuation in electrical-stimulus detection in the days following implantation is a temporary disruption of neural function, perhaps due to an inflammatory reaction to the implant insertion (Van De Water et al., 2010; Seyyedi and Nadol, 2014). However, one can not completely rule out changes in behavioral performance, due perhaps to the animal not feeling well and not delivering its best performance. Indeed, many animals did not perform the behavioral task at all for several days after the surgery and we were only able to observe a decrease in thresholds over time after the animal began performing the psychophysical task reliably. To address these issues we have used an objective measure of cochlear implant function: electrically evoked compound action potentials (ECAPs). These potentials can be recorded from awake animals daily starting on the day of surgery and thus can provide a more frequent, direct, and objective measure of the response of the auditory nerve to electrical stimulation. Furthermore, using ECAP amplitude-growth functions (ECAP amplitude as a function of electrical-stimulus level) we can monitor both threshold and suprathreshold responses. Here we report on ECAP amplitude-growth functions following implant insertion in three guinea pig treatment groups.

2. Methods

2.1. Overview

Nine adult male specific pathogen free pigmented guinea pigs were used for these experiments. The guinea pigs were bred and maintained by the Unit for Laboratory Animal Medicine at the University of Michigan. The animal-use protocol was reviewed and approved by the University of Michigan Committee on the Use and Care of Animals. The guinea pigs used to follow ECAP amplitude-growth functions over time after implantation were also used for a variety of other experiments; thus there were several different treatment groups and we were able to follow changes over time after implantation in the context of several pre-implant treatments. These groups are described below and summarized in Table 1.

In one group ($n = 3$), guinea pigs received a cochlear implant in a previously-normal ear. Thus the effects of the surgery and implant-insertion could be studied in the absence of any other cochlear trauma. Implanting in a hearing ear typically results in fair to good hearing preservation and presence of surviving hair cells in the region of the cochlear implant (Kang et al., 2010; Pfingst et al., 2011).

The remaining animals were deafened prior to implantation by injection of neomycin into the scala tympani. 10 μl of 5% (w/v) neomycin sulfate solution in sterile water were injected into the scala tympani through the cochleostomy at 5 μl/min. Hair cells are typically destroyed within a few days by this procedure (Kim and Raphael, 2007). Deafening the ear introduced an additional cochlear trauma but allowed us to assess the responses to electrical stimulation in the absence of functioning hair cells.

Three of the deafened animals received inoculation of the ear with an adeno-associated viral vector containing a neurotrophin gene insert (AAV.*Ntf3*). The adeno-associated virus used was AAV2 and the concentration was 1×10^{12} pfu/ml. 5 μl were infused into the scala tympani through the cochleostomy at a rate of 1 μl/min. Treatment of the cochlea with neurotrophin following deafening can have long-lasting protective effects on the auditory nerve (Budenz et al., 2012), but results are variable across animals. The effects of the neurotrophins on the early changes in sensitivity to electrical stimulation following implant insertion have not been reported previously to our knowledge.

Finally, three animals were deafened and then inoculated with an empty adeno-associated virus (AAV.Empty) as a control for the effects of the AAV alone. Neomycin deafening alone (i.e. without neurotrophin support) typically results in ears with no inner hair cells (IHCs) and very low spiral ganglion neuron (SGN) densities (Kang et al., 2010; Pfingst et al., 2011).

2.2. Cochlear implants

The cochlear implant electrode arrays (supplied by Cochlear Ltd., Lane Cove, Australia) consisted of 8 full-band electrodes encircling a straight silicone rubber carrier and spaced at 0.75 mm center to center. Electrodes were labeled A through H with A being the most apical. The implant was gently inserted into the scala tympani through a cochleostomy which was made at approximately 0.7 mm apical to the round window. In the guinea pig, the cochleostomy allows a straighter and deeper insertion than a round-window approach. The diameter of the implant near its apical end was 0.4 mm. Because the scala tympani in the guinea pig narrows dramatically past the first half turn, the implant could be advanced only about 4.5 mm from the cochleostomy without doing physical damage. The primary electrode used for stimulation in the current experiments was the second most apical electrode (Electrode B) which was located an average of 2.83 mm apical to the cochleostomy and typically sat close to the modiolar wall. ECAP potentials were recorded from Electrode A except in one case where the connection to Electrode A was not reliable and Electrode C was used (see Table 1).

2.3. Electrically-evoked compound acton potential (ECAP) amplitude-growth functions

ECAP amplitude-growth functions (ECAP amplitude as a function of stimulus level) were recorded in awake guinea pigs while the animals were standing in a test cage. A MED-EL "Pulsar" CI100 receiver/stimulator, connected to the implant through a percutaneous electrical connector, was used for stimulation and recording. The output of the receiver/stimulator was connected to a Research Interface Box (RIB II; University of Innsbruck). Custom software controlled the stimulus delivery and ECAP recording.

Monopolar electrode configurations were used for stimulation and recording with the stimulating electrode referenced to a skull screw at bregma and the recording electrode referenced to a vertex skull screw. The stimulus was a biphasic pulse, with 45 μs phase duration and 2.1 μs inter-phase interval. Pulses were delivered with alternating leading-phase polarity at 50 pps for 20 iterations. The recording amplifier was blanked for 165 μs following electrical pulse onset to avoid saturation artifact.

The maximum stimulus level (MSL) that could be used for ECAP recording was determined each day prior to the recording session. The MSL was typically set approximately 2 μA below the current

Table 1
Characteristics of subjects with ECAP amplitude-growth functions measured over time after implantation. Animals received a cochlear implant in a hearing ear (implant only) or following deafening and inoculation. Inoculations were with an adeno-associated virus (AAV) containing an *Ntf3* gene insert (AAV.*Ntf3*) or containing no gene insert (AAV.Empty). The fourth column shows the hearing status after hearing had stabilized after implantation. For the three animals with residual hearing, the pure-tone thresholds at 8, 16 and 24 kHz are shown. Animals with no measurable hearing are labeled "deaf". The fifth column shows the percentage of inner hair cells surviving in the basal three half turns of the cochlea left to right, with the most basal half turn (the area occupied by the implant) to the left. The last column shows the SGN densities in these three half turns. NA = not available.

Subject, implant, stimulation site – recording site	Treatment	Survival time in days post implantation (DPI)	Residual hearing at 8, 16 & 24 kHz (dB SPL)	IHC status near implant (% survival)	SGN density near implant (cells/mm^2)
453L1, B-A	Implant only	341 DPI	39, 6, 23 dB	80%, 80%, 100%	1015, 950, 913
456L1, B-A	Implant only	246 DPI	87, >88, >91 dB	0%, 80%, 100%	479, 980, 946
580L1, B-A	Implant only	On-going	69, 33, 52 dB	NA	NA
451L1, B-A	Neomycin AAV.*Ntf3*	377 DPI	Deaf	0%, 0%, 0%	484, 172, 154
455R1, B-A	Neomycin AAV.*Ntf3*	426 DPI	Deaf	0%, 0%, 0%	138, 258, 39
448L1, B-A	Neomycin AAV.*Ntf3*	309 DPI	Deaf	0%, 0%, 0%	73, 34, 41
449L1, B-A	Neomycin AAV.Empty	299 DPI	Deaf	0%, 0%, 0%	30, 82, 30
454L1, B–C	Neomycin AAV.Empty	206 DPI	Deaf	0%, 0%, 0%	62, 79, 73
457L1, B-A	Neomycin AAV.Empty	176 DPI	Deaf	0%, 0%, 0%	74, 71, 36

level eliciting a facial twitch. This level was invariably below the compliance limit of the stimulator. To obtain an ECAP amplitude-growth function, the program selected stimulus levels at 15 amplitudes evenly spaced from zero to the MSL and presented in a permuted order.

For every amplitude step, responses to an anodic leading and a cathodic leading pulse were averaged to reduce stimulation artifact and the response to zero-amplitude stimulation was subtracted from this average to reduce recording artifact. The resulting waveforms obtained for each of the 15 amplitude steps were plotted and analyzed using custom software. The software program picked all negative peaks (Ns) and all positive peaks (Ps), which were then verified by visual inspection of the waveforms. Given that P1 could occur during the blanking time, the N1 to P2 response amplitudes (μV) were used. The N1 to P2 amplitudes were plotted against stimulus current (μA) to obtain input–output amplitude-growth functions. To fit the data and to avoid for measurement error at low stimulus levels, only ECAP amplitude response values ≥100 μV were used to calculate slopes of the amplitude-growth functions. Linear regression was applied to fit all data points between 100 μV and the MSL, providing the ECAP amplitudes continued to increase as a function of level. If ECAP amplitudes started to decrease with increases in stimulus level as levels approached the MSL, which rarely happened, these points were excluded from the fit. The threshold ECAP response was defined as the current required to achieve a 100 μV response in the linear regression fit to the input–output function.

In the initial studies ECAP amplitude-growth functions were recorded every 2–5 days starting from the implantation day (Day 0) until Day 30 and then every 30 days until the study was complete. The duration of the study in days post implantation depended on the other experiments in which the animals were used. The times of sacrifice in days post implantation are reported in Table 1. After all tests were completed, histological analysis was performed on the ears of these animals. A more frequent ECAP recording schedule was used in one animal. In this case, ECAP measurements were performed daily for fourteen days starting on Day 0, then every other day up to Day 30, then every ten days until Day 120. Studies using this animal were still in progress at the time of this report so histology had not been obtained.

The other experiments in which all of the animals participated included psychophysical measures and other electrophysiological measures. The animals received intermittent stimulation during those experiments for about 2 h per day, 4–5 days per week.

2.4. Assessment of acoustic hearing

Acoustic hearing was assessed using positive-reinforcement psychophysical procedures. Prior to implantation, the animals were trained using the method of successive approximations to press a button on the floor of the test cage to initiate a trial, wait through a variable foreperiod before the onset of the acoustic stimulus and then release the button to signal detection of an acoustic stimulus. Further details of the training and testing procedures are available in previous publications (Kang et al., 2010; Pfingst et al., 2011). In order to assess acoustic hearing in the implanted ear free from influence of the contralateral ear, the contralateral ear had been previously deafened by neomycin injection. To characterize acoustic hearing in the cochlear region near the implant following implantation, we assessed detection thresholds for pure tone stimuli at 8 kHz, 16 kHz and 24 kHz starting 4–5 weeks after implantation and up to the time of sacrifice in animals that retained any acoustic hearing. The amount of residual hearing is reported in Table 1. Animals that retained no acoustic hearing are labeled "deaf" in Table 1. A number of variables can affect acoustic hearing following cochlear implantation including disruption of middle ear and cochlear mechanics and loss of hair cells and auditory neurons. Thus, while the presence of acoustic hearing in the implanted ear is an indication that there are some functioning inner hair cells, it might not be a reliable indicator of the percentage of IHCs present.

2.5. Histological procedures

After collection of all functional data was complete, animals were anesthetized and perfused intravascularly with 4% paraformaldehyde. Temporal bones were extracted with the implant remaining in place in the implanted ear. The tissues were decalcified in 3% EDTA for 3–6 months until the bone was soft. When decalcification was complete and the cochlear-implant electrodes were visible through the bone, the cochlea was marked in the lateral wall at the location of the primary electrode that was used for stimulation in this study (Electrode B). The implant was then gently removed. Tissues were embedded in JB-4 (Electron

Microscopy Sciences, Hatfield, PA, USA) and sectioned with glass knives to obtain 3 μm thick sections in a near-midmodiolar plane centered at the location of the previously made mark designating the location of Electrode B. Approximately 45 sections were collected per cochlea.

The first section chosen for analysis was usually the section closest to the mark that indicated the location of the primary stimulating electrode. If that section was damaged, the next closest section was used. Four other sections were then selected at intervals separated by a minimum of 6 sections (~18 μm) in order to prevent counting a cell more than one time. The peri-midmodiolar sections used for SGN counts were stained with toluidine blue. The specimens were observed with a Leica DMRB epi-fluorescence microscope (Leica, Eaton, PA, USA) and photographed with a CCD Cooled SPOT-RT digital camera (Diagnostic Instruments). The cross-sectional area within Rosenthal's canal containing SGN cell bodies was determined using Image J software and the cell bodies in each of the 5 selected sections were counted. Density of SGNs was calculated by dividing the number of cells counted by this cross-sectional area. Only cells with diameters of 12–25 μm and nuclei of 5–9 μm or more in diameter were considered. Of these cells, only healthy-appearing cells were counted; cells that had a poorly defined cell membrane or that appeared to be shrunken or atrophying were not counted. Inner hair cells (IHCs) in each profile, if present, were also counted. To avoid counting the same hair cell in two sections, IHCs were only counted if both the nucleus and stereocilia were present.

The percentage of IHCs remaining in cross-sectional profiles of the organ of Corti and the SGN densities in cross-sectional profiles of Rosenthal's canal for the basal three half-turns of the cochlea (the areas occupied by the implant and just apical to the implant) are reported in Table 1.

3. Results

ECAP growth functions at multiple time points after implantation for Subject 580, which was implanted in a hearing ear, are shown in the top panel of Fig. 1. The time points are coded by color as detailed in the legend. On the day of implantation (Day 0), approximately 4 h after the implant was inserted, the ECAP amplitudes increased as a function of stimulus level at a moderate rate (red trace in the top panel of Fig. 1), giving a slope of 6.5 μV/μA (bottom panel of Fig. 1). Over the following days, the slopes of the ECAP growth functions decreased, reaching a minimum on Day 5. After Day 5, they began to increase over time, reaching a plateau after about Day 41.

Changes over time in ECAP thresholds for this animal followed a similar but not identical time course (Fig. 2, top panel). ECAP thresholds decreased slightly over time and then rose to a peak around Day 9, then decreased, reaching a relatively stable level by Day 30. Note that the ECAP thresholds decreased very slowly after Day 30, while slopes of ECAP growth functions were still increasing rapidly over time.

The maximum stimulus levels (MSLs), which were based on the threshold for eliciting a facial twitch, showed patterns over time that were very similar to those for the ECAP thresholds. The changes in the MSL over time are reflected in variation in the current range over which ECAPs were measured, as is evident in top panel of Fig. 1.

The patterns of changes in the ECAP growth functions as well as the ECAP thresholds and the MSLs over time after implantation seen in the other two animals that were implanted in a hearing ear were similar to those for Subject 580. Fig. 3 shows the growth-function data for an animal that was tested over a longer time period, but less frequently within the time period (Subject 453). In

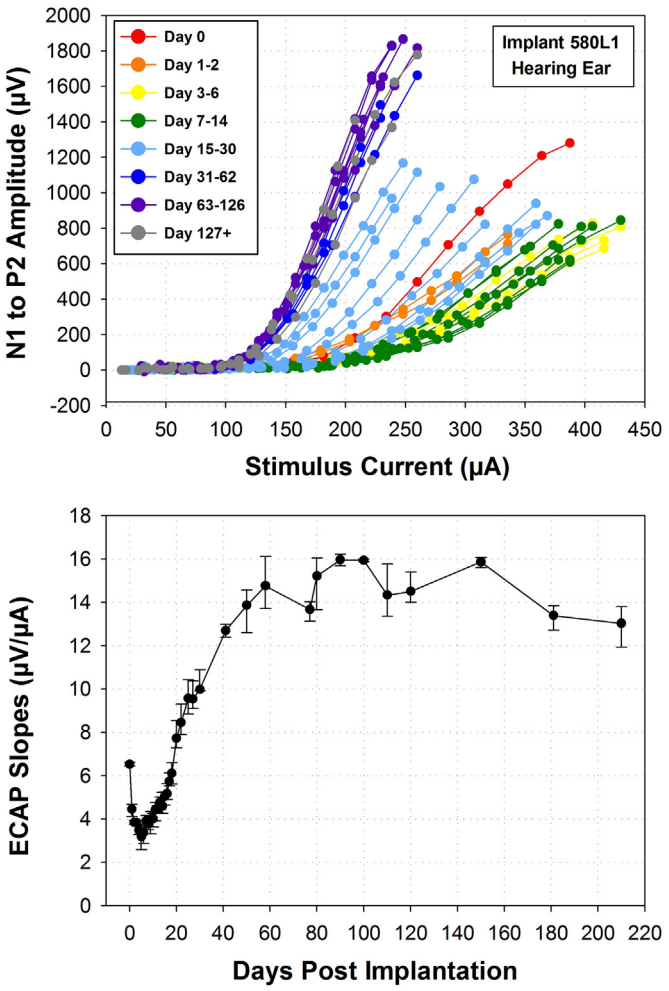

Fig. 1. Changes over time after implantation in ECAP amplitude-growth functions for Subject 580. This animal received a cochlear implant in the left ear, which had normal hearing prior to implant insertion. After implant insertion some hearing was preserved (Table 1). The top panel shows amplitude-growth functions color coded to indicate the time post implantation. The spectral colors (red, orange, yellow, green, blue, indigo, violet) represent successive time periods after implantation starting with red on the day of implantation (Day 0). The number of days included in each time period starts with one day on Day 0 and doubles on each successive period. This animal was still undergoing testing and had been tested up to Day 210 at the time of this report. Each of the amplitude-growth functions shown for this subject for a given day is based on the average values from three repeated measures. The slopes of the amplitude-growth functions are shown as a function of days post implantation in the bottom panel. Data points represent the mean slopes for three functions obtained on a given day and the error bars indicate the range of those three slopes (highest and lowest values).

this case the ECAP slopes decreased after implant insertion, reaching a minimum around Day 11, then increased to a relatively stable level by Day 120 and remained stable up to the time of sacrifice 341 days after implantation. ECAP thresholds and MSLs for this animal also followed patterns similar to those for Subject 580. For Subject 453, the ECAP thresholds became relatively stable by Day 40 while the ECAP growth function slopes continued to increase up to about Day 120.

All of the animals implanted in a hearing ear maintained some degree of residual acoustic hearing in the implanted ear. The two which have undergone histological processing showed large populations of remaining inner hair cells and moderate to good spiral ganglion cell survival. Details are reported in Table 1.

Two of the three animals that were treated with neomycin followed by AAV.*Ntf3* inoculation (Subjects 451 and 455) showed

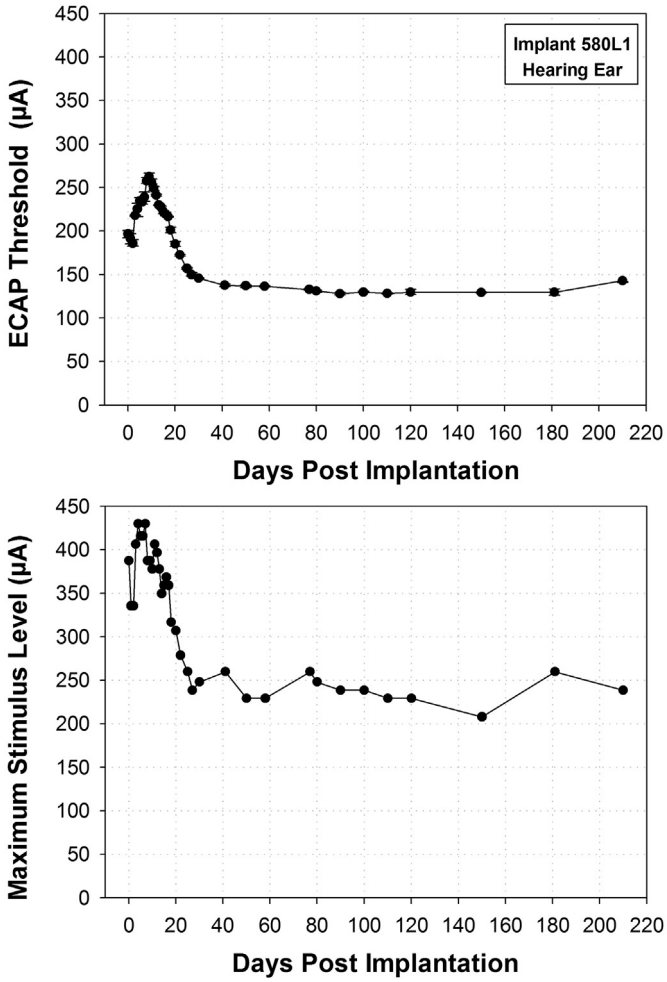

Fig. 2. Changes over time in ECAP thresholds (top panel) and maximum stimulus levels (MSLs; bottom panel) plotted as a function of days post implantation for the guinea pig represented in Fig. 1. Thresholds for a 100 μV ECAP response were derived from the ECAP amplitude-growth functions shown in Fig. 1. Each data point represents the mean of the thresholds for the three functions obtained on a given day and the error bars indicate the range of those three thresholds. The MSLs shown in the bottom panel were based on the lowest current level that elicited a visually observable facial twitch. This level was assessed prior to ECAP data collection each day and determined the upper limit for electrical stimulation on that day.

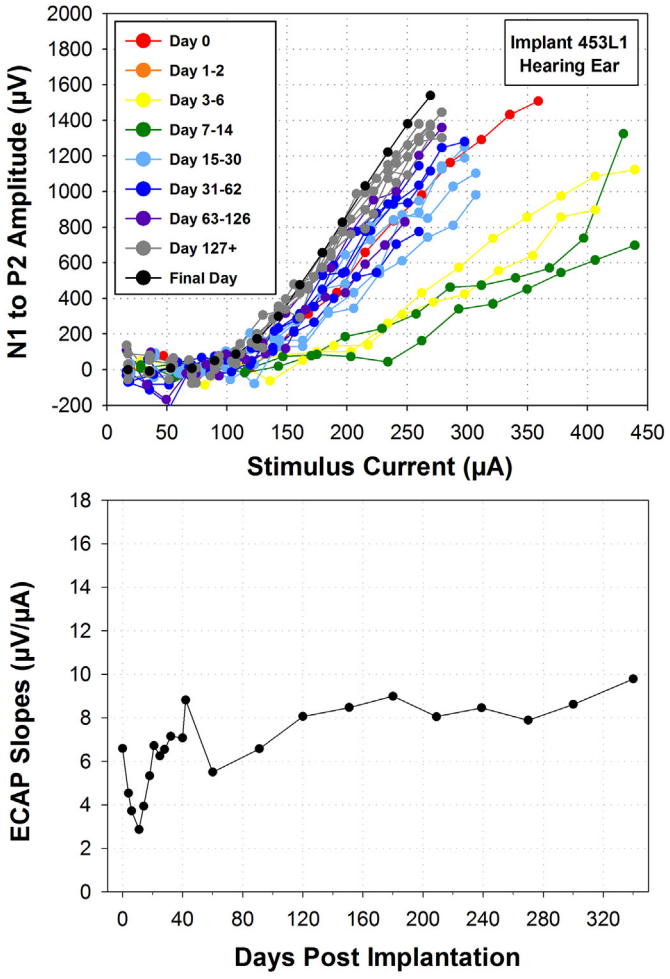

Fig. 3. Changes over time after implantation in ECAP amplitude-growth functions for Subject 453. This animal received a cochlear implant in the left ear, which had normal hearing prior to implant insertion. After implant insertion some hearing was preserved (Table 1). This animal was tested for 341 days post implantation and then euthanized for histological examination. Dissection of the left cochlea revealed good hair cell survival in all cochlear turns. SGN density in this ear was high (Table 1). The top panel shows amplitude-growth functions color coded to indicate the time post implantation as described in the caption for Fig. 1. Days after Day 126 are represented by gray symbols except for the final test day which is represented in black. The bottom panel shows the slopes of these functions. Only one function per day was obtained in this case.

patterns of ECAP growth function slopes, ECAP thresholds and MSLs that were similar in most respects to those seen in the animals implanted in a hearing ear. Data for Subject 451 are shown in Figs. 4 and 5. This animal had no residual hearing and no hair cells or supporting cells were found throughout the implanted cochlea. However, SGN densities in the region close to the implant were moderately high in the half turn occupied by the implant (Table 1). The ECAP growth functions slopes followed the familiar pattern described above except for a sudden drop in slope of the growth function on the last test day. ECAP thresholds remained stable on this day, but there was a slight increase in the MSL. Note that, as in the cases described above, the ECAP thresholds and the MSLs reached a stable level long before the ECAP growth-function slopes became stable.

The third AAV.*Ntf3* inoculated animal (Subject 448) showed a decrease in ECAP growth function slopes over the first 8 days after implantation but then the slopes remained low. This animal was deaf with no IHCs, and SGN densities were very low (Table 1).

The three animals that were deafened with neomycin and inoculated with AAV.Empty also showed little or no signs of recovery in the ECAP growth-function slopes. In two of these cases, ECAP slopes started out moderately high and decreased over time after implantation but they never recovered to high levels. An example is shown in Fig. 6 (Subject 454). In the other case, the slopes started out low and fluctuated over time but remained relatively low for the duration of the experiment. These animals all had no residual acoustic hearing and had no IHCs and low SGN densities (Table 1). Interestingly, the MSLs in the AAV.Empty-inoculated subjects decreased over time after implantation, showing a pattern similar to those of the hearing animals. ECAP thresholds in the AAV.Empty animals were variable over time and across subjects.

4. Discussion

It is evident from this and previous studies in animals that functional responses to electrical stimulation of the cochlea can be

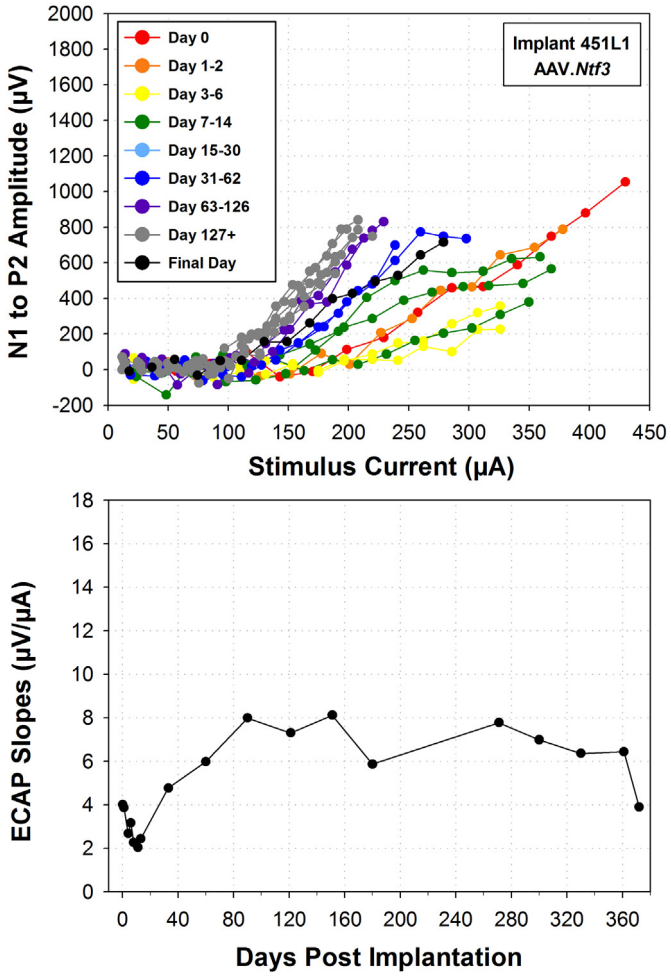

Fig. 4. Changes over time after implantation in ECAP amplitude-growth functions for Subject 451. This animal received a cochlear implant in the left ear, which had been deafened with neomycin and inoculated with AAV.*Ntf3* prior to implant insertion. Histological examination showed no surviving IHCs, but moderately good SGN densities (Table 1). The top panel shows amplitude-growth functions color coded to indicate the time post implantation as described in the captions for Figs. 1 and 3. The bottom panel shows the slopes of these functions. Only one function per day was obtained in this case.

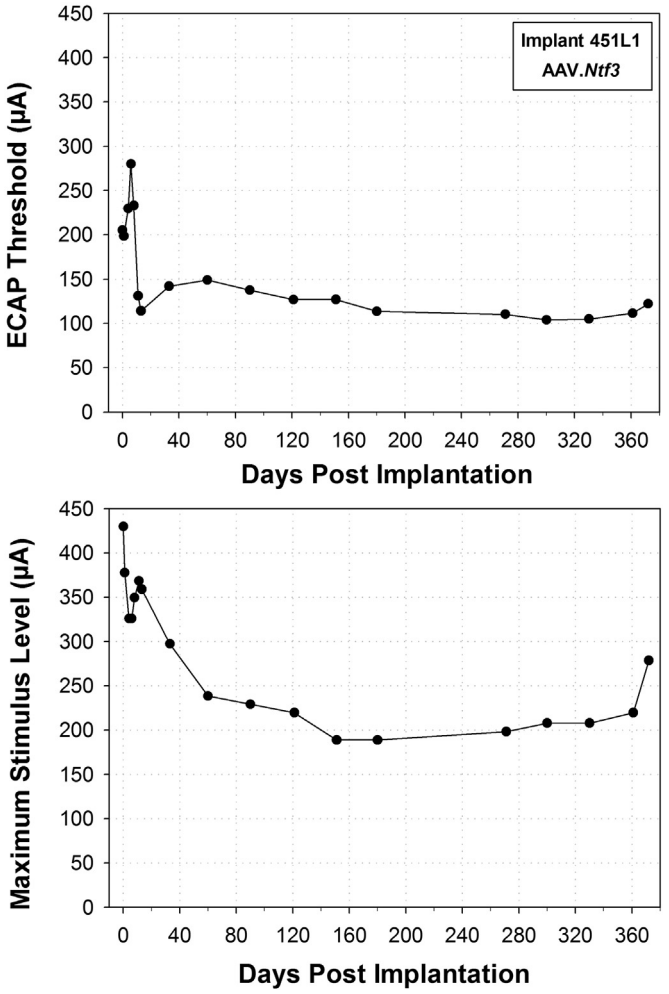

Fig. 5. Changes over time in ECAP thresholds (top panel) and maximum stimulus levels (MSLs; bottom panel) plotted as a function of days post implantation for the guinea pig represented in Fig. 4. ECAP thresholds were derived from the ECAP amplitude-growth functions shown in Fig. 4. The maximum stimulus levels shown in the bottom panel were determined prior to each daily run based on the lowest current level that elicited a visually observable facial twitch. This level was determined prior to ECAP data collection each day and defined the upper limit for electrical stimulation on that day.

temporarily impaired following surgical insertion of a cochlear implant and that they can recover, in many cases, over a period of several weeks to reach a relatively stable level of function (Pfingst, 1990; Miller et al., 2000; Su et al., 2008). The use of ECAP amplitude-growth functions to assess these changes in this study has allowed a more detailed assessment of these functional changes free of dependence on behavioral responses from the subjects. These measures show that the temporary loss of function occurs at both threshold and suprathreshold levels.

It is important to note that the functional changes reported in this manuscript were made from an electrode in the cochlear-implant electrode array. Thus, the primary contributors to the recorded potentials were nerve fibers near the implant. Insertion trauma that might have occurred in regions apical to the implant, due for example to increased fluid pressure during insertion, could potentially be different from that which occurred in the region where the implant was physically present.

In all of the cases that we observed, the recovery of the ECAP-growth-function slopes was much slower than the recovery of ECAP thresholds. This suggests that thresholds and growth-function slopes might reflect different underlying mechanisms, or that the growth-function slopes are just more sensitive to the underlying conditions. In any case, it seems that ECAP thresholds alone are not sufficient to get an accurate assessment of the functional changes following implant insertion.

4.1. Potential mechanisms

A plausible explanation for the loss of sensitivity and the changes in growth function slopes over time after implantation is that the auditory nerve function has been temporarily impaired by the tissue response to the implant insertion surgery. The impairment peaked around 5–9 days after implant insertion, consistent with a model in which an inflammatory reaction grows over time and then dissipates. Several previous studies have shown that the slopes of amplitude-growth functions of electrophysiological potentials in response to electrical stimulation are correlated with the number of surviving auditory neurons (Smith and Simmons, 1983; Hall, 1990; Pfingst et al., 2015). It is unlikely that the number of SGNs decreased and then increased following implant insertion.

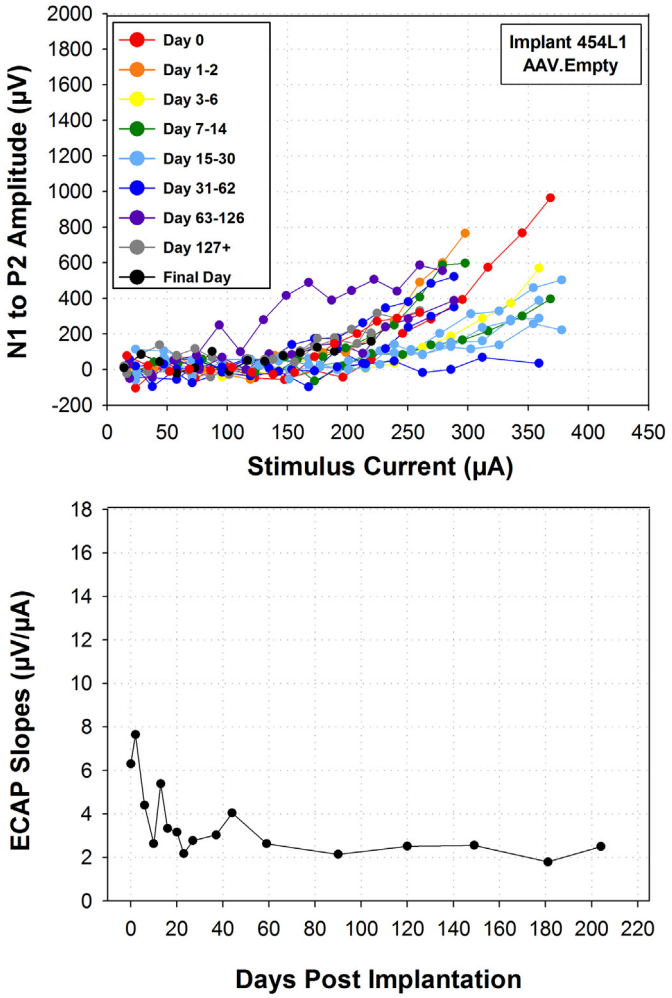

Fig. 6. Changes over time after implantation in ECAP amplitude-growth functions for Subject 454. This animal received a cochlear implant in the left ear, which had been deafened with neomycin and inoculated with AAV.Empty. Histology showed no IHCs and poor SGN survival (Table 1). The top panel shows amplitude-growth functions color coded to indicate the time post implantation as described in the captions for Figs. 1 and 3. Only one function per day was obtained in this case. The bottom panel shows the slopes of these functions.

There is no evidence that SGN cell bodies regenerate in a mature ear once they are lost. However, it is quite possible that many SGNs became temporarily disabled or weakened as a result of the insertion trauma and then recovered. Possible mechanisms underlying this temporary disability include changes in membrane properties, changes in myelination of the neurons, or die-back and regeneration of the SGN peripheral processes. All of the ears with good SGN survival potentially had neurotrophic support, either from surviving hair cells and supporting cells in ears with preserved hearing, or from transfected cells in deaf ears successfully inoculated with AAV.*Ntf3* (Budenz et al., 2012). These neurotrophins probably helped support the survival of the SGN cell bodies and could potentially have supported the survival or regeneration of other neural elements including SGN peripheral processes. Immunological reaction to the implant surgery could also contribute to the observed post-surgical changes (Anderson et al., 2008). Further research is needed to examine possible biochemical and anatomical changes associated with the observed functional changes.

A potential contributor to the recovery of implant function following implantation is the delivery of electrical stimulation to the cochlea. Some previous studies have shown that chronic electrical stimulation of the implant can enhance but not replace the effects of neurotrophins on SGN survival (e.g., Shepherd et al., 2005) while others have shown protective effects of electrical stimulation alone (e.g., Miller and Altschuler, 1995). It is important to note that the amount of electrical stimulation received by animals in the current study was far less than that received in studies using chronic stimulation. Psychophysical testing lasted for a maximum of two hours per day 4 days per week and involved mostly short bursts of pulsatile stimuli at levels near the detection threshold. Electrophysiological testing lasted only about 20 min per day. Furthermore, it is clear from the AAV.Empty cases that electrical stimulation alone, at the levels used for psychophysical and electrophysiological testing in animals used in the current experiment, was not sufficient to support an increase over time in the slopes of the ECAP growth functions.

In other studies we found that psychophysical acoustic detection thresholds increased and then recovered following cochlear implantation in cases where electrical stimulation was not initiated until after the acoustic thresholds had stabilized (Colesa and Pfingst, 2015). This also suggests that electrical stimulation is not a necessary contributor to recovery from insertion trauma.

The fact that the MSL (i.e., threshold for eliciting a facial twitch) showed a similar pattern to the ECAP thresholds suggests that the facial nerve might also have been affected by the implant surgery. Consistent with this idea, we note that the facial twitch was always on the side of the implant; no bilateral twitch response was observed. There were signs of facial weakness (impaired blinking) for a few days after surgery in some animals. The threshold for eliciting a facial twitch with cochlear-implant stimulation might be a more sensitive means of assessing facial nerve involvement quantitatively over a longer time period.

The data for most of the animals tested in this study followed one of two distinct patterns over time. In one pattern, ECAP slopes decreased during the first week after implantation and then increased over the next several weeks reaching a relatively stable condition (e.g., Figs. 1, 3 and 4) and in the other pattern, thresholds decreased over time, or started out low, and remained low for the duration of the experiments (e.g., Fig. 6). The first pattern was associated with moderate to high SGN densities and the second with relatively low SGN densities. While presence of IHCs is associated with, and probably supports, high levels of SGN survival, the first pattern of data was also observed in cases where hair cells were absent as long as SGN counts were relatively high, associated with the AAV.*Ntf3* inoculations (e.g., Fig. 4). If the AAV.Empty inoculations were used or if the AAV.*Ntf3* inoculations failed to preserve at least a moderate SGN density, the second pattern of ECAP slopes was observed. We suggest that when the SGN density was moderate to high, the fluctuation in growth-function slope after implantation was due to a temporary impairment in the existing fiber population whereas if the fiber population was low after the initial impairment, there were not enough fibers to achieve the steeper slopes.

We can also consider that the loss of sensitivity and growth-function slope after implant insertion might be due to a temporary disruption of the current paths from the cochlear implant to the neurons. However, we note that similar loss and recovery patterns over time after implantation have been observed for acoustic detection thresholds (Colesa and Pfingst, 2015). Obviously, the patterns over time in these cases were not due to disruption of electrical current paths. Further research is needed to determine how closely the patterns for acoustic and electrical stimulation match in the same subject. If these patterns are very similar, that would support a neural rather than a biophysical

(current path) explanation of the post-implant changes in function.

4.2. Implications

The observed changes in implant function in the first weeks after implant insertion have multiple implications. The most immediate concern is for acute or short-term studies of implant function. It is clear, at least in our animal models, that conditions in the first weeks after implantation are not representative of the long-term state of the implanted cochlea. It takes several days after implant insertion for the peak decrement in responses to occur, so data collected in acute studies might be less affected by the insertion trauma. However, we note that in most cases the ECAP growth function slopes measured a few hours after implant insertion were shallower than those measured several weeks after insertion. We urge caution in interpreting data collected during this early post-surgical period. On the other hand, a careful study of the physiological and anatomical conditions that parallel the functional changes over time during this early period after implantation could yield a better understanding of some of the mechanisms underlying variation in implant function, which in turn could lead to improved therapeutic procedures.

Currently it is unknown if the short-term temporary impairments in implant function have long-term implications. We do sometimes see long-term functional changes following the initial stable period in guinea pigs, but the mechanisms underlying these changes are unknown. More detailed examinations of the anatomy and physiology of the cochlea, before and after temporary manifestations of cochlear implant insertion trauma, are needed to determine if the effects of the insertion trauma are actually temporary or if they have long-term implications.

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