Examining the Auditory Nerve Fiber Response to High Rate Cochlear Implant Stimulation: Chronic Sensorineural Hearing Loss and Facilitation

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Heffer LF, Sly DJ, Fallon JB, White MW, Shepherd RK, O’Leary SJ. Examining the auditory nerve fiber response to high rate cochlear implant stimulation: chronic sensorineural hearing loss and facilitation. J Neurophysiol 104: 3124–3135, 2010. First published October 6, 2010; doi:10.1152/jn.00500.2010. Neural prostheses, such as cochlear and retinal implants, induce perceptual responses by electrically stimulating sensory nerves. These devices restore sensory system function by using patterned electrical stimuli to evoke neural responses. An understanding of their function requires knowledge of the nerves responses to relevant electrical stimuli as well as the likely effects of pathology on nerve function. We describe how sensorineural hearing loss (SNHL) affects the response properties of single auditory nerve fibers (ANFs) to electrical stimuli relevant to cochlear implants. The response of 188 individual ANFs were recorded in response to trains of stimuli presented at 200, 1,000, 2,000, and 5,000 pulses/s in acutely and chronically deafened guinea pigs. The effects of stimulation rate and SNHL on ANF responses during the 0–2 ms period following stimulus onset were examined to minimize the influence of ANF adaptation. As stimulation rate increased to 5,000 pulses/s, threshold decreased, dynamic range increased and first spike latency decreased. Similar effects of stimulation rate were observed following chronic SNHL, although onset threshold and first spike latency were reduced and onset dynamic range increased compared with acutely deafened animals. Facilitation, defined as an increased nerve excitability caused by subthreshold stimulation, was observed in both acute and chronic SNHL groups, although the magnitude of its effect was diminished in the latter. These results indicate that facilitation, demonstrated here using stimuli similar to those used in cochlear implants, influences the ANF response to pulsatile electrical stimulation and may have important implications for cochlear implant signal processing strategies.

INTRODUCTION

The cochlear implant restores auditory function by electrically stimulating the cochlea to evoke a response in the auditory nerve. Most cochlear prostheses present biphasic, charge-balanced current pulses to electrodes distributed along the base-middle region of the cochlea. Stimuli are presented at per-electrode rates of ~250 to >1,000 pulses/s with electrodes stimulated in a nonsimultaneous manner to avoid interactions between the current distributions generated by closely spaced electrodes. However, even when presented on a single electrode, interactions between stimuli occurring at the level of the auditory nerve fiber (ANF) membrane can lead to changes in nerve excitability (Cartee et al. 2000, 2006; Dynes 1996). While the response of ANFs to stimulation rates of ~1,000 pulses/s have been well characterized (e.g., Miller et al. 2006; Shepherd and Javel 1997; Sly et al. 2007), relatively few studies have directly investigated their response to higher stimulation rates where these interactions are likely to be greater (Litvak et al. 2001, 2003a–c; Miller et al. 2008; Zhang et al. 2007). These studies reveal that ANF responses to higher stimulation rates have lower thresholds, an increased dynamic range, and reduced mean first spike latency. It is likely that these changes are caused, at least in part, by interactions between stimulus pulses on the nerve membrane.

Facilitation, or summation, is defined here as an increase in nerve excitability caused by subthreshold stimulation. Facilitation is greatest immediately following a subthreshold stimulus pulse, decaying approximately exponentially over time (Katz 1937). ANF facilitation has previously been demonstrated using a two-pulse, conditioner-probe stimulus paradigm (Cartee et al. 2000, 2006; Dynes 1996). These studies have shown that a single subthreshold conditioner pulse reduces probe threshold for interpulse intervals <1 ms. However, these observations need to be extended for several reasons. First, the previous studies investigated facilitation using pairs of monophasic or pseudo-monophasic stimulus pulses while cochlear implants use biphasic stimulus current pulses. As stimulus waveform is known to affect ANF response properties (Miller et al. 2000; Shepherd and Javel 1999), it is necessary to examine facilitation using biphasic stimulus current pulses. Second, these studies have focused on the effects of single subthreshold conditioner pulses. As cochlear implants present trains of stimuli, often across a range of stimulation rates, the effects of multiple conditioner stimuli on the ANF response needs to be investigated. Therefore the ANF response at the beginning of trains of biphasic current pulses presented at rates of 200–5000 pulses/s was analyzed to examine facilitation as well as changes to ANF response properties that may be influenced by facilitation.

Prolonged periods of sensorineural hearing loss (SNHL) prior to implantation adversely affect speech perception with a cochlear implant (Blamey et al. 1996). While this is thought to be strongly influenced by cortical sensory deprivation, degenerative changes in the auditory periphery are also known to influence the response of the auditory nerve to electrical stimulation. In particular, the loss of cochlear sensory hair cells associated with SNHL is accompanied by a withdrawal of trophic support to the auditory nerve, which begins to degenerate (for review of SNHL effects, see Shepherd and Hardie 2001). Functionally, chronic SNHL has been shown to prolong the ANF refractory period (Shepherd et al. 2004) and reduce
first spike latency (Shepherd and Javel 1997; Shepherd et al. 2004; Sly et al. 2007), while changes to facilitation have not previously been reported.

Here we describe how chronic SNHL affects the response properties of single ANFs to electrical stimuli relevant to cochlear implants. Importantly, using stimulation rates of between 200 and 5,000 pulse/s, where the potential for interactions between pulses is increased, provided an opportunity to investigate the effects of facilitation on these ANF responses.

**METHODS**

A total of 25 adult Duncan-Hartley guinea pigs, weighing between 300 and 816 g (mean: 488 g, s: 118 g) at the time of the single-unit recording experiments, were used. Normal hearing animals were allocated to one of two experimental groups: the acute SNHL group (n = 15) and the chronic SNHL group (n = 10). Animals allocated to the acute SNHL group were deafened on the side ipsilateral to the deafening procedure. ABRs were recorded using previously described normal acoustic sensitivity and to confirm the success of the chronic deafening procedure was deafened 5 wk prior to the acute ANF recording experiments by a monohydrate (400 mg/kg, Sigma Aldrich), Castle Hill, NSW, Australia) in sterile saline (Hardie and Shepherd 1999). Animals allocated to the chronic SNHL group were bilaterally deafened 5 wk prior to the acute ANF recording experiments by a single co-administration of the otoxic aminoglycoside kanamycin monohydrate (400 mg/kg, Sigma Aldrich) and the loop diuretic furosemide (100 mg/kg, Troy Laboratories Pty) (Hellier et al. 2002).

The auditory brain stem response (ABR) was recorded to ensure normal acoustic sensitivity and to confirm the success of the chronic deafening procedure. ABRs were recorded using previously described techniques (Hardie and Shepherd 1999; Shepherd and Clark 1985; Sly et al. 2007) in response to 100 µs rarefaction clicks, delivered at 33 repetition/s in free-field via a loudspeaker. ABR threshold was defined as the minimum stimulus intensity required to evoke a wave III response with an amplitude that exceeded 200 µV. Wave III typically occurred 3–4 ms following the stimulus onset Normal hearing was defined as an ABR threshold ≤45 dB peak equivalent sound pressure level (pe SPL) in both ears while the chronic deafening procedure was deemed successful when the postdeafening ABR threshold was ≥95 dB pe SPL. All electrophysiological recordings were conducted within a sound attenuated, electrically shielded room.

**Animal care**

All described experimental procedures were developed according to National Health and Medical Research Council of Australia guidelines and were approved by the Royal Victorian Eye and Ear Hospital Animal Research and Ethics Committee (05/118A). All experimental procedures were conducted under general anesthesia using intramuscular injections of ketamine (60 mg/kg; Troy Laboratories) and xyazine (2 mg/kg; Troy Laboratories). The local anesthetic lignocaine hydrochloride (20 mg/ml; Troy Laboratories) was injected subcutaneously prior to all skin incisions as required. Core body temperature was maintained between 37 and 39°C using a custom-made, thermostatically controlled heating pad and ocular ointment applied to prevent drying of the cornea.

Prior to ANF recording experiments, a subcutaneous injection of Anamav (0.1 ml/kg; Movab, Slacks Creek, Qld., Australia), containing acepromazine maleate (2 mg/ml) and atropine sulfate (1 mg/ml), was administered to aid anesthetic induction and minimize bronchial secretions. An intramuscular injection of dexamethasone (0.1 mg/kg; Troy Laboratories) was also given to minimize cerebral edema. During ANF recording experiments, depth of anesthesia was continually assessed by monitoring respiration rate and end-tidal CO₂ (Normocap 200; Datex Instrumentarium, Helsinki, Finland). Additional ketamine (40 mg/kg) and xyazine (4 mg/kg) was administered via intramuscular injection as required, maintaining respiration rate between ~40 and 60 breath/min.

**Surgical procedures**

Anesthetized guinea pigs were placed into a stereotaxic frame (Trent Wells) and the left bulla exposed via a dorsal approach. A hole was drilled through the dorsal aspect of the bulla, revealing the round window of the cochlea. Animals allocated to the acute SNHL group were deafened at this stage. A custom-built cochlear electrode array (Xu et al. 1997), modified to fit the guinea pig, was inserted with the apical electrode band positioned ~3–4 mm inside the round window. The round window incision was sealed using crushed muscle to prevent leakage of perilymph from the cochlea. The cerebellum was exposed by an occipito-parietal craniotomy and the overlying dura removed. The lateral aspects of the cerebellum were aspirated and the doral cochlear nucleus visualized. The location of the underlying nerve was identified using bony landmarks (Wysocki 2004) and by brief medial retraction of the cochlear nucleus.

**Stimuli**

Stimulus pulses were charge-balanced, biphasic current pulses – 25 µs/phase, 8 µs interphase gap, cathodic phase first at the intracochlear electrode. All stimuli were presented using a monopolar electrode configuration between the most apical intracochlear electrode and a stainless steel electrode inserted through the ipsilateral pinna or the skin adjacent to the pinna. Stimuli were generated using a custom-made, battery powered, optically isolated current source stimulator (R. E. Millard, Dept. of Otolaryngology, University of Melbourne) with electrodes shorted between pulses to minimize DC production (Huang et al. 1999). The stimulator was controlled using a PC running custom-written routines in Igor Pro (Wavemetrics, Portland, OR).

Stimulus pulses were presented at a rate of 20 pulse/s for recording the electrically evoked auditory brain stem response (EABR) and for the ANF search stimulus. Experimental stimuli for ANF recordings consisted of 100 ms trains of stimulus pulses presented at 200, 1,000, 2,000, and 5,000 pulse/s, presented at a repetition rate of 4 train/s. Approximately 10–15 current levels were presented over a 200–500 µA range at each of the four stimulation rates, evoking average spike rates between 0 and 250 spike/s. A stimulus trial consisted of a single repetition of all specified stimulation rate-current level combinations, presented in a randomized order. Between 10 and 70 trials were obtained for each ANF.

The maximum stimulus current level was limited to <2 mA to ensure that the maximum charge delivered was below the safety limit for stimulation with platinum electrodes (Brunner and Turner 1977; Huang et al. 1999). Maximum current was also constrained to ensure the absence of stimulus evoked myogenic activity, which causes instability in ANF recordings. In the described experiments, the maximum current typically used was 1.0–1.5 mA for low rate (200 pulse/s) stimulation and 0.8–1.2 mA for high rate (5,000 pulse/s) stimulation.

**Experimental recordings**

EABRs were recorded following implantation of the cochlear electrode array and prior to aspiration of the cerebellum to assess the electrical excitability of the auditory system. Two EABR responses, averaged over 100 stimulus presentations, were obtained at each stimulus current level between 0 µA and maximum current level. EABR threshold was defined as the lowest current level required to evoke a wave III response, which occurred between 1.4 and 2.4 ms following the stimulus pulse onset, with an amplitude that exceed 200 µV. EABRs were recorded with a differential amplifier (Gain: ×100, wide band filter; DAM-5A, WPI, Sarasota, FL) using stainless steel electrodes inserted through skin folds at the vertex of the skull.
(positive), the nape of the neck (negative), and the thorax (ground). The amplified signal was digitized using a 16-bit ADC (National Instruments, Austin, TX) sampling at 100 kHz for 20 ms following the onset of the stimulus pulse. Stimulus artifact was removed from the averaged waveforms using software blanking over the first 800 µs following the stimulus pulse onset. Response waveforms were digitally filtered using a first-order band-pass filter (HP: 150 Hz; LP: 3000 Hz).

The response of individual ANFs to electrical stimulus pulses were recorded extracellularly using quartz glass microelectrodes (1.0 mm OD, 0.7 mm ID, Sutter Instruments, Novato, CA), pulled to an OD, 0.7 mm ID, Sutter Instruments, Novato, CA), pulled to an estimated tip diameter (P-2000; Sutter Instruments). Electrodes were filled with 3 M KCl and connected to the recording system using an Ag-AgCl wire; electrodes typically had an initial impedance of ~50 MΩ at 1,000 Hz. An Ag-AgCl disc electrode was inserted subcutaneously at the nape of the neck to serve as the recording return electrode. Microelectrodes were advanced through the cochlear nucleus and into the underlying auditory nerve in 1–2 µm steps using a motorized micromanipulator (Narashige Scientific Instrument Lab, Tokyo, Japan). Auditory nerve fiber responses were identified on the basis of their latency. Spikes with a latency of <1 ms (Sly et al. 2007) were classified as ANFs while longer latency responses were presumed to arise from the cochlear nucleus and were not recorded.

Electrode potentials were amplified and low-pass filtered (30 kHz, 3 dB/decade) using an Axon Instruments headstage (HS-2A; gain: 0.1) and amplifier (Axoclamp-2B; gain: 10; Molecular Devices, Sunnyvale, CA). These potentials were digitized using a 16-bit ADC sampling at 100 kHz and recorded onto either a Macintosh computer running Chart (v.3.6.9, AD Instruments, Bella Vista, NSW, Australia) or onto a PC running Igor Pro (Wavemetrics). All recorded waveforms were stored for off-line analysis. Stimulus artifact was removed using a sample-and-interpolate technique (Heffer and Fallon 2008) and spike timing obtained using a threshold crossing algorithm.

Spiral ganglion neuron density

At the conclusion of the acute ANF recording experiments, guinea pigs were killed with an intraperitoneal overdose of pentobarbitone (1,000 mg/kg). Cochleae were extracted, locally perfused with neutral buffered formalin (NBF; 10% vol/vol), and submersed in NBF for an hour. Cochleae were decalcified in ethylenediamine tetra-acetic acid (Applichem, Darmstadt, Germany) in 0.2 M phosphate buffered saline for ~14 days. Decalcification was confirmed via radiography. Decalcified cochleae were embedded in optimal cutting temperature compound (ProSciTech, Thuringowa, Queensland, Australia) and snap frozen (Coleman et al. 2009). Cochleae were stored at −80°C until cryosectioned. Cochleae were cryosectioned at a thickness of 12 µm in the modiolar plane and mid-modiolar sections were mounted onto Superfrost slides (Menzel-Glaser, Braunschweig, Germany). Sections were dehydrated in ethanol, stained with hematoxylin and eozin and coverslipped with DPX (ProSciTech).

Cochlea histology was assessed in four acutely deafened and five chronically deafened animals. Animals were selected on the basis of successful cochlear perfusion and processing as well as the obtained ANF recordings. Both the left and right cochleae of the acutely deafened animals were assessed while only the left cochlea of the chronically deafened animals were used. Four randomly selected, nonserial sections from each cochlea were examined to assess the density of SGN cell bodies within Rosenthal’s canal. The number of SGN cell bodies containing a visible nucleus and regular-shaped cytoplasm within Rosenthal’s canal were counted under bright-field microscopy. The area of Rosenthal’s canal was measured digitally (National Institutes of Health Scion Image) and the density of SGNs calculated. Density measurements were made at all available cochlear turns. SGN density for each animal was calculated at each cochlear turn by averaging the results from all histological sections examined.

Statistical analysis

Unless otherwise stated, data were normally distributed and had equal variance and are presented as means ± SE. Data that were nonnormally distributed, or that had unequal variance, are presented using the median and interquartile range. All data pooled across fibers was distributed about a single mode. The effects of stimulation rate and SNHL group were tested using a general linear model ANOVA (normal) or general linear model ANOVA by ranks (nonnormal). Data were ranked according to their position within the entire dataset (i.e., no subsets for SNHL group, etc.). Post hoc multiple comparisons were adjusted using the Bonferroni correction method. All statistical testing was performed in Minitab 15 (Minitab, Sydney, NSW, Australia).

RESULTS

Spiral ganglion neuron density

To assess the effect of SNHL on cochlear pathology, the density of SGNs within Rosenthal’s canal was examined for a subset of animals in the chronic SNHL group (n = 5) and compared with the acute SNHL group (n = 4). Representative mid-modiolar photomicrographs, taken from turn 1 (upper basal) of the left cochleae from acute and chronic SNHL groups, are shown (Fig. 1). The mean number of SGNs within Rosenthal’s canal was reduced from ~1,400 SGNs/mm2 in the acute SNHL group to ~800 SGNs/mm2 in the chronic SNHL group, and this reduction was statistically significant [F(1,134) = 210.17, P < 0.001]. No effect of cochlear turn on SGN density was observed [F(3,134) = 1.95, P = 0.125].
To examine the effect of the acute deafening technique, SGN density of the deafened left cochlea was compared with the untreated right cochlea of the same animal. Across all cochlear turns, mean SGN density was lower in the deafened left cochlea (1,374 ± 69 cell/mm²) compared with the untreated right cochlea (1,504 ± 84 cells/mm²). Statistical analysis confirmed that this reduction in SGN density was significant (t = -3.14, P = 0.007; paired t-test).

EABR

EABRs were recorded for all animals in acute (n = 15) and chronic (n = 10) SNHL groups to examine the physiological effects of SNHL. Representative waveforms (Fig. 2) demonstrate that waveform morphology was similar in both SNHL groups. No evidence of electrophonic activity, as evidenced by a low threshold, long latency response (see Shepherd and Javel 1997), was observed within either group. Quantitative analyses reveal that EABR threshold was higher in the acute SNHL group (median: 700 µA, range: 500–1,000 µA) than the chronic SNHL group (median: 400 µA, range: 400–700 µA). Statistical analyses confirmed that this effect of SNHL group was significant (H₁ = 12.01, P = 0.001; Kruskal-Wallis ANOVA by ranks).

Auditory nerve fiber recordings

Analyses of the ANF response to electrical stimulation are based on fiber recordings from the 15 acutely deafened (n = 114) and 10 chronically deafened (n = 74) guinea pigs. All ANFs included in this study were stimulated at 200, 1,000, and 2,000 pulse/s while a subset of acutely (n = 55) and chronically (n = 42) deafened fibers were also stimulated at 5,000 pulse/s. Representative ANF response waveforms evoked by cochlear electrical stimulation, following stimulus artifact removal, are shown (Fig. 3). Average spike rate typically increased with stimulus current level for all stimulation rates examined. Average spike rate also varied with stimulation rate and decreased (i.e., adapted) over the stimulus pulse train duration. Spike waveform was largely unaffected by stimulation rate (Fig. 3B), current level or SNHL group. To examine whether long-term adaptation effects were present in the data, changes to the average spike rate across successive trials were examined for fibers that were stimulated and recorded for >5 min (n = 104). The median spike rate reduction of these fibers was 5.9 spike/s (Q₁: -13.4, Q₃: -0.01 spike/s), indicating that longer-term adaptation effects were not unduly influencing the observed effects in the majority of fibers.

Spontaneous activity was also measured for each ANF by calculating the average spike rate in the 100 ms, nonstimulus period prior to the stimulus pulse train onset. All fibers in both acute and chronic SNHL groups had an average spontaneous spike rate of <1 spike/s with the majority of those (~85%) having average spontaneous spike rates <0.1 spike/s.

Threshold and dynamic range

As noted previously, the average spike rate decreased over the stimulus duration (Fig. 4). This spike rate reduction is often referred to as ANF adaptation and is consistent with previous studies (e.g., Sly et al. 2007; Zhang et al. 2007). To examine the effects of stimulation rate and SNHL group on the ANF response to electrical stimulation independently of adaptation, the ANF response immediately following the stimulus train onset was examined. The onset response was defined as the ANF response during the 0–2 ms period following the onset of the stimulus pulse train. This response is evoked by a single stimulus pulse at 200 pulse/s, ≤2 stimulus pulses at 1,000 pulse/s, ≤4 stimulus pulses at 2,000 pulse/s and ≤10 stimulus pulses at 5,000 pulse/s. Importantly, across all ANFs, stimulation rates and current levels examined, no more than one evoked spike was recorded during the onset period. Consequently, the ANF onset response was not influenced by either refraction or adaptation.

To examine the effects of stimulation rate and chronic SNHL on the excitability of individual ANFs, the stimulus current required to evoke specified spike probabilities over the onset response period were calculated. Threshold was defined as the stimulus current that evoked an average spike probability of 0.5 over the 0–2 ms “onset” period following the stimulus train onset. The stimulus current levels that evoked an average spike probability of 0.1 and 0.9 were also determined and were used to calculate the onset dynamic range. These threshold and dynamic range levels were chosen to correspond with the 0.1, 0.5, and 0.9 spike probability per pulse used in previous studies in response to pulse train stimuli presented at 200 pulse/s (e.g., Shepherd and Javel 1997; Sly et al. 2007). As onset spike probability was not a controlled variable, the stimulus current required to evoke threshold was generally not directly measured. Rather threshold was estimated using linear interpolation between measured average spike probability versus stimulus current data points. As extrapolation was not used, threshold values were not calculated for all stimulation rates in every ANF.

### FIG. 2. Electrically evoked auditory brain stem response. Example electrically evoked auditory brain stem response waveforms recorded from an acutely and chronically deafened guinea pig. The wave III response occurs ~2–3 ms following the stimulus pulse, and the response amplitude increased with stimulus current level in all guinea pigs studied. At each stimulus current level, 2 averaged response recordings are shown. The first 800 µs (2) of the recording has been “blanked out,” masking the large-amplitude stimulus artifact and the wave I response.
ANF threshold typically decreased with increasing stimulation rate for both acute and chronic SNHL groups over the onset period (Fig. 5A). Quantitative analyses reveal that onset threshold decreased significantly with stimulation rate \([F(3,582) = 41.7, P < 0.001]\) for both groups, although, compared with the acute SNHL group, onset threshold was significantly lower in the chronically deafened group \([F(1,582) = 63.9, P < 0.001]\). Statistical analyses of the effects of stimulation rate and SNHL group at onset spike probabilities of 0.1 and 0.9 produced similar results.

To examine the effects of stimulation rate and SNHL group on the ability of ANFs to encode changes in stimulus current level, the ANFs onset dynamic range was calculated (Fig. 5B). Dynamic range increased with stimulation rate \([F(3,523) = 5.86, P < 0.001]\) and was greater in the chronically deafened group \([F(1,523) = 4.19, P = 0.041]\); no interactions between these factors was observed \([F(3,523) = 0.99, P = 0.4; \text{ANOVA by ranks}]\). Post hoc pairwise comparisons revealed that the effect of stimulation rate was due to dynamic range being significantly greater at 5,000 pulse/s compared with 200 pulse/s \((t = 3.64, P = 0.002)\) and 1,000 pulse/s \((t = 3.8, P < 0.001)\).

These results demonstrate that the encoding of stimulus intensity in the ANF response is influenced by stimulation rate and SNHL and suggest an effect of interactions between stimulus pulses at high stimulation rates. To investigate these interactions and changes with SNHL, onset spike probability was examined as a function of stimulation rate.

**Facilitation**

Onset spike probability was calculated for each stimulation rate and current level for all recorded fibers. For a given current level, the probability of obtaining a spike within the onset period typically increased with stimulation rate. This increase in spike probability with stimulation rate may be due to the increased number of stimulus pulses within the 0–2 ms onset period providing an increased opportunity for spiking. Alternatively, it may also be caused by interactions between these pulses (i.e., facilitation). A simple approach was developed to examine the relative contribution of facilitation to the increased spike probability.

A fiber’s response to a single, independent stimulus pulse was defined as the cumulative spike probability function, calculated over the onset period in 100 μs intervals, in response to 200 pulse/s stimulation. The predicted ANF response to multiple stimulus pulses presented during the onset period, with no facilitation, was calculated as the sum of multiple, independent response functions. Importantly, as only a single spike was ever recorded during the onset period, the predicted spike probability was constrained to a maximum of 1. Facilitation was then estimated by measuring the difference between the measured and predicted onset spike probability for each stimulation rate. For the example shown (Fig. 6), the single pulse response probability was ~0.04, the predicted onset probability at 5,000 pulse/s was ~0.28, and the measured onset probability at 5,000 pulse/s was 1. Thus for this representative example...
fiber, facilitation was estimated to increase onset spike probability at 5,000 pulse/s by 0.72.

Facilitation was examined at three levels across the fibers dynamic range. These levels were defined as those that evoked a mean onset spike probability of 0.1 (range: 0.02–0.18), 0.5 (0.3–0.7), and 0.9 (0.75–0.98) in response to a single stimulus pulse (i.e., 200 pulse/s). Where multiple current levels elicited an ANF response within this probability range, recorded measures were pooled to minimize fiber selection bias. Results were calculated as a function of stimulation rate and SNHL group for each spike probability level (Fig. 7). At low spike probability levels (Fig. 7, E and F), the probability of obtaining a spike increased from 0.1 at 200 pulse/s to 1.0 at 5,000 pulse/s. This increased spike probability was partially attributable to facilitation at 1,000, 2,000, and 5,000 pulse/s. Statistical analyses revealed that facilitation generally increased with stimulation rate \([F(3,374) = 57.0, P < 0.001]\) and was lower in chronically deafened animals compared with the acute sensorineural hearing loss (SNHL) group, thresholds were lower and dynamic range was wider in the chronically deafened cohort.

FIG. 4. Peri-stimulus time histograms. A: peristimulus time histograms (PSTHs), calculated for the auditory nerve fiber shown in Fig. 3, calculated over the entire stimulus duration. The average spike rate varied with stimulus current level and stimulation rate and decreased (i.e., adapted) over the stimulus duration. B: an expanded view of the first 2 ms following the stimulus onset at 600 µA as a function of stimulation rate showing increased activity during the onset period as stimulation rate increased.

FIG. 5. Auditory nerve fiber threshold and dynamic range. A: threshold (means ± SE), calculated at 3 onset spike probabilities (0.1, 0.5, 0.9). B: dynamic range (shown as the median and interquartile range) calculated for the onset period, measured as a function of stimulation rate. As stimulation rate increased, onset threshold decreased and dynamic range increased. Compared with the acute sensorineural hearing loss (SNHL) group, thresholds were lower and dynamic range was wider in the chronically deafened cohort.
First spike latency and jitter were examined. ANF temporal responses, the timing (i.e., latency) and variance to facilitation. To investigate whether facilitation also affects probability with stimulation rate is at least partially attributable (3,287)

\[ F(3,287) = 15.8, P < 0.001; \text{ANOVA by ranks} \]. Interestingly, while onset spike probability was greater at 5,000 compared with 2,000 pulse/s, the increase due to facilitation was not different between 2,000 and 5,000 pulse/s (\( P > 0.8 \)). In addition to causing a smaller increase in spike probability, facilitation was more variable in the chronic SNHL group.

At moderate (Fig. 7, C and D) and high (Fig. 7, A and B) spike probability levels, the probability of obtaining a spike increased with stimulation rate. Facilitation could not be detected at 2,000 or 5,000 pulse/s at these spike probabilities as both the measured and predicted response typically reached an onset probability of one. At 1,000 pulse/s, where the median measured and predicted response onset probabilities were typically \(<1\), the measured response was smaller than that predicted in the absence of facilitation, resulting in “negative facilitation.” Consequently, there was a significant effect of stimulation rate on facilitation at moderate \([F(3,287) = 40.1, P < 0.001]\) and high \([F(3,256) = 35.1, P < 0.001]\) spike probability levels. A significant effect of SNHL group on facilitation was also observed at moderate \([F(1,287) = 9.35, P = 0.002; \text{ANOVA by ranks}]\), but not high \([F(1,256) = 0.11, P = 0.7; \text{ANOVA by ranks}]\), spike probability levels.

These results demonstrate that the increase in onset spike probability with stimulation rate is at least partially attributable to facilitation. To investigate whether facilitation also affects ANF temporal responses, the timing (i.e., latency) and variance (i.e., jitter) of the first spike following the stimulus train onset were examined.

First spike latency and jitter

First spike latency and jitter were calculated for each stimulation rate and current level for all recorded fibers. To reduce the effects of refraction and adaptation on these measures and to ensure consistency with the threshold and dynamic range analyses, only first spikes within the 0–2 ms onset period were included. It is important to note that, at 200 pulse/s, latency and jitter were determined solely by the ANF response to a single pulse. In contrast, at higher stimulation rates, there were multiple stimulus pulses within the onset period, as well as the potential for interactions between these pulses, that may influence latency and jitter measures. Consequently, these analyses will reflect the influence of facilitation on ANF temporal response properties.

The distribution of first spike times at each stimulation rate and current level across multiple stimulus trials, measured over the onset period, were generally skewed toward short (i.e., \(<1\) ms) values. Therefore first spike latency and first spike jitter were defined as the median and interquartile range of all first spike times within the onset period, respectively. To minimize the effects of stimulation rate on ANF threshold, first spike latency and jitter were examined as a function of spike probability at three levels across the fibers’ dynamic range. These levels were centered around current levels that evoked a mean spike probability of 0.1 (range: 0.02–0.18), 0.5 (0.3–0.7), and 0.9 (0.75–0.98) within the onset period for each stimulation rate. For any fiber where more than one current level evoked an average spike probability within a specified range, latency and jitter measures were pooled to minimize fiber sample bias.

The effect of stimulation rate on first spike latency and jitter were greatest at low onset spike probabilities and decreased with increasing level (Fig. 8). At low onset spike probabilities (Fig. 8, E and F), statistical analyses revealed that first spike latency varied significantly with stimulation rate \([F(3,367) = 60.6, P < 0.001]\) and SNHL groups \([F(1,367) = 6.9, P < 0.001; \text{ANOVA by ranks}]\). First spike jitter also varied significantly with stimulation rate \([F(3,367) = 55.3, P < 0.001]\) but not SNHL group \([F(1,367) = 3.0, P = 0.084; \text{ANOVA by ranks}]\), Compared with 200 pulse/s, latency and jitter increased at 1,000 and 2,000 pulse/s, reflecting the fact that spikes were sometimes initiated by the second or subsequent pulse rather than the first pulse. Interestingly, despite a further increase in the number of stimulus pulses, first spike latency and jitter at 5,000 pulse/s were lower than 1,000 and 2,000 pulse/s \((P < 0.001)\) and were not different to 200 pulse/s \((P > 0.36)\).

At moderate onset spike probabilities (Fig. 8, C and D), first spike latency was shorter and jitter reduced when compared with low spike probabilities. However, latency varied with stimulation rate \([F(3,432) = 48.3, P < 0.001]\) and SNHL groups \([F(1,432) = 17.6, P < 0.001; \text{ANOVA by ranks}]\). First spike jitter also varied with stimulation rate \([F(3,432) = 80.2, P < 0.001]\) but not SNHL group \([F(1,432) = 0.07, P = 0.79; \text{ANOVA by ranks}]\). Similar to the low probability results, latency and jitter were greater at 1,000 and 2,000 pulse/s compared with 200 and 5,000 pulse/s \((P < 0.001)\). Again, latency and jitter were not different at 200 and 5,000 pulse/s \((P > 0.8)\).

At high onset spike probabilities (Fig. 8, A and B), first spike latency was shorter and jitter reduced when compared with low and moderate spike probability levels. First spike latency did not vary with either stimulation rate \([F(3,265) = 1.54, P = 0.2]\) or SNHL group \([F(1,265) = 3.16, P = 0.08; \text{ANOVA by ranks}]\). However, while median first spike jitter was \(<0.2\) ms for all stimulation rates, it varied with both stimulation rate
F(3, 265) = 6.97, P < 0.001] and SNHL group [F(1, 265) = 4.20, P = 0.04; ANOVA by ranks].

**Discussion**

**Spiral ganglion neuron survival**

SGN density in the chronically deafened group was ~60% of the acutely deafened group. This is consistent with previous guinea pig data where, using the same chronic deafening technique and duration, SGN density decreased by ≤50% compared with normal hearing controls (Shepherd et al. 2005; Sly et al. 2007; Wise et al. 2005). Using this experimental model, SGN density has been shown to decrease further with increasing durations of deafness (Sly et al. 2007). Similarly, human SGN survival is negatively correlated with increasing duration of SNHL (Nadol et al. 1989). As direct auditory nerve recordings are too invasive to be conducted in humans, our experimental model provides a useful way to investigate the neurophysiological effects of chronic SNHL.

An acutely deafened cohort was used as the control for the chronically deafened animals. Similar acute deafening techniques have previously been used in both guinea pigs (Dodson 1997; Dodson and Mohuiddin 2000) and cats (Cartee et al. 2000; Hardie and Shepherd 1999; Zhang et al. 2007). This control group was selected primarily to ensure the absence of hair-cell mediated (i.e., electrophonic) activity that is often
observed in response to cochlear electrical stimulation in normal hearing animals (Miller et al. 2006; Shepherd and Javel 1997; Sly et al. 2007). Use of an acute SNHL control group also facilitates comparisons to the results of previous research investigating ANF responses in acutely deafened animals (e.g., Zhang et al. 2007).

Our results demonstrate that intracochlear infusion of neomycin caused a reduction in SGN density in the left (deafened) cochlea compared with the right (untreated) cochlea. This is consistent with previous studies that have shown intracochlear infusion of the aminoglycoside antibiotic gentamicin causes “severe disruptions” to SGN ultrastructure throughout the cochlea at time points as short as 1–24 h following deafening (Dodson 1997). These changes may be attributed to the ototoxic effects of the aminoglycoside neomycin as well as the mechanical trauma initiated by the intracochlear deafening technique itself. Consequently, differences between the acute and chronic SNHL groups are likely to reflect both the ongoing degenerative changes in the chronic SNHL group as well as the short-term (i.e., mechanical and ototoxic) changes initiated by the acute deafening technique itself.

**FIG. 8.** First spike latency and jitter. Median first spike latency (A, C, and E) and jitter (B, D, and F) measured as a function of stimulation rate at 3 levels across the fibers dynamic range. Levels, defined by the spike probability calculated over the onset period, are high (0.75–0.98, A and B), moderate (0.3–0.7, C and D), and low (0.02–0.18, E and F). Latency and jitter decreased with increasing level and latency was shorter in the chronically deafened group. At low and moderate levels, latency and jitter were greater at 1,000 and 2,000 pulse/s compared with 200 and 5,000 pulse/s; no significant differences between 200 and 5,000 pulse/s were observed. At high levels, no effect of stimulation rate on latency was observed. Data are displayed using median and interquartile range values.
EABR threshold was significantly lower in the chronic SNHL group when compared with the acute SNHL group. This appears to contrast with previous studies showing that EABR threshold increases with the duration of chronic SNHL (Hardie and Shepherd 1999; Prado-Guitierrez et al. 2006; Shepherd and Javel 1997; Sly et al. 2007) and also increases over time in deafened animals (Shepherd et al. 2005). However, this apparent discrepancy may be understood by comparing the deafening techniques used, the duration of SNHL, and resultant SGN survival.

Analysis of previously published data shows no change in EABR threshold with SNHL after 5 wk, although EABR threshold increased following a 6-mo SNHL duration (Sly et al. 2007). The chronic deafening procedure and duration, and the EABR threshold determination, used by Sly et al. (2007) was the same as in the present study. However, Sly et al. (2007) used a normal hearing control while the preset study used an acutely deafened control. This suggests that the difference in EABR threshold between acute and chronic SNHL groups may be attributable to the acute deafening procedure. That is, the changes to SGN morphology initiated by the acute deafening technique may have caused the increase in EABR thresholds observed in the present study.

Hardie and Shepherd (1999) used almost identical acute and chronic deafening protocols to those described here in cats. Those authors reported a significant increase in EABR threshold in the chronic SNHL group compared with acutely deafened animals which appears to contradict the present study. However, they utilized a significantly longer duration of deafness compared with the present study—≤28 months compared with 5 weeks. Also, a direct result of the longer SNHL duration, Hardie and Shepherd (1999) reported a significantly lower SGN density than the present study when compared with the respective control groups (<20 vs. ~60%). This finding may help to reconcile the discrepancy between the EABR thresholds in the two studies because thresholds are inversely related to SGN survival (Black et al. 1983; Hall 1990; Shepherd and Javel 1997). We speculate that the elevation in EABR threshold resulting from the dramatic reduction in SGN density in the 28-mo deafened cats may have outweighed any short-term effect of the acute deafening procedure, while this was not the case for the 5-wk deafened guinea pigs of the present study where SGN density reductions were more modest.

Nerve facilitation

Onset threshold decreased with increasing stimulation rate and was partially explained by the greater opportunity for spiking due to an increased number of stimulus pulses presented at higher stimulation rates. However, the reduction in onset threshold was also partially attributable to facilitation. The most direct evidence for this was found at low spike probability levels where the measured onset response was greater than predicted and where the difference between the measured and predicted responses increased with stimulation rate. While the predicted response accounted for the increased opportunity for spiking at higher stimulation rates, it assumed independence between pulses. Therefore we attribute the difference between the measured and predicted responses to interactions between pulses (i.e., facilitation).

Facilitation could not be observed in response to 2,000 and 5,000 pulse/s stimulation at moderate and high stimulus levels as the measured and predicted responses reached an onset probability of one. Interestingly, at these levels the measured response was lower than predicted at 1,000 pulse/s, indicating negative facilitation. However, this may be due to the predicted onset response not accounting for refraction. To examine the possible effects of refraction, we considered the response to 1,000 pulse/s at moderate onset spike probabilities, where the chance of obtaining a spike following the first pulse was ~50%. Where a spike was evoked by the first pulse, refraction would likely prevent the ANF from responding to the second pulse. In contrast, where a spike was not evoked by the first pulse, and in the absence of facilitation, the probability of obtaining a spike would be ~50%. This would produce an overall firing probability of ~0.75 and is very close to the observed result.

These results provide evidence that facilitation can increase ANF excitability in response to high rate stimulation at low spike probability levels. These results may have significant implications for models attempting to simulate rate-dependent changes in ANF measures such as threshold and dynamic range (e.g., Bruce et al. 1999). Furthermore, it demonstrates the necessity for modeling interpulse interactions if such models are to emulate auditory nerve behavior at high stimulation rates.

Facilitation, observed here in response to biphasic current pulses, is consistent with previous physiological results in ANFs responding to monophasic or pseudo-monophasic current pulses (Cartee et al. 2006; Cartee et al. 2000; Dynes 1996). Facilitation has also been observed in human (Bostock and Rothwell 1997) and amphibian (Verveen 1961) myelinated peripheral nerve fibers and its effects demonstrated using a biophysical model (Butikofer and Lawrence 1979). Similar to the results reported here, these studies have shown that facilitation decreases nerve threshold (Butikofer and Lawrence 1979) and increased nerve relative spread (a measure of a nerve’s dynamic range) (Hales et al. 2004; Verveen 1961). It has been suggested that facilitation is an active process and may be mediated via nerve membrane sodium channel activation (Bostock and Rothwell 1997). While the present study did not explore the mechanisms underlying facilitation, our results are consistent with this proposition.

First spike latency and jitter

Changes to first spike latency and jitter with increasing stimulation rate may also be explained by the greater opportunity for spiking and/or facilitation. As noted previously, these measures will be influenced by both the number of stimulus pulses presented as well as interactions between these pulses. At 200 pulse/s, where a single pulse is presented during the onset period, spikes occur at a short latency (i.e., <1 ms) with a degree of jitter that is determined by the interactions between the nerve membrane properties and the charge present on the membrane. As a result of these membrane-charge interactions, latency and jitter decrease as stimulus current is increased.

Compared with ANF responses at 200 pulse/s, first spike latency and jitter increased in response to stimuli presented at 1,000 and 2,000 pulse/s at low and moderate spike probability levels. These changes reflect the fact that spikes could be
initiated by the second or subsequent stimulus pulse over the onset period at these stimulation rates compared with a single pulse at 200 pulse/s. Despite a further increase in the number of stimuli, first spike latency and jitter were lower at 5,000 pulse/s when compared with responses at 1,000 and 2,000 pulse/s at low and moderate spike probability levels. No effect of stimulation rate on first spike latency was observed at high spike probability levels. These results suggest that the reduction in first spike latency and jitter at 5,000 pulse/s can be attributed, at least in part, to facilitation.

The reduction in threshold and first spike latency and the increase in dynamic range with increasing stimulation rate are consistent with previous research (Zhang et al. 2007). The reduction in latency and jitter with increasing spike rate is also consistent with previously published results (Miller et al. 2008; Shepherd and Javel 1997; Shepherd et al. 2004; Sly et al. 2007).

**Effects of sensorineural hearing loss on auditory nerve fiber responses**

Facilitation appears to be somewhat diminished after chronic SNHL with the difference between predicted and measured onset spike probability reduced in the chronic SNHL group at low spike probabilities. Consistent with this, the extent to which onset spike probability increased with stimulation rate was also lower in the chronic SNHL group. The reduction in facilitation with chronic SNHL indicates an alteration of nerve membrane properties at the site of spike initiation.

First spike latency was lower in the chronic SNHL group at 200 pulse/s at low and moderate onset spike probabilities and is consistent with previous reports (Shepherd and Javel 1997; Shepherd et al. 2004; Sly et al. 2007). A reduction in latency following chronic SNHL has previously been attributed to a central migration of the site of excitation due to peripheral processes retraction following deafferentation. Changes in first spike latency and jitter with stimulation rate in the chronic SNHL group were otherwise consistent with those seen in the acute SNHL group. The reduction in ANF threshold with increasing stimulation rate in the chronic SNHL group was also similar to that observed in the acute SNHL group.

Changes in membrane properties with chronic SNHL may be caused by a shift in the site of excitation due to peripheral process retraction as has been previously suggested to explain the reduced latency (e.g., Sly et al. 2007). However, other pathophysiological changes, such as cell body shrinkage, may contribute to this effect by directly affecting SGN membrane properties (for review, see Shepherd and Hardie 2001). Another significant effect of chronic SNHL is the progressive reduction in peripheral myelin expression (Hurley et al. 2007). Demyelination is known to increase axonal capacitance (Koles and Rasminsky 1972), increasing the charge required to depolarize the neural membrane. In this way, demyelination may effectively decrease the efficiency of a stimulus to generate and propagate an action potential. However, the time course of facilitation has been shown to increase as myelin thickness is reduced (Cartee 2006). Thus one suggestion is that while a potential for facilitation is maintained following chronic SNHL, increased capacitance due to demyelination reduces the increased membrane excitability observed following sub-threshold stimulation, thereby limiting its effect.

**Conclusions**

This study investigated the effect of stimulation rate and chronic SNHL on the auditory nerve fiber response immediately following stimulus pulse train onset to cochlear implant stimulation. In the acute SNHL group, first spike latency, jitter, and threshold decreased while dynamic range became wider as stimulation rate increased. Similar effects of stimulation rate were observed in the chronic SNHL group, although onset threshold and first spike latency were lower and onset dynamic range greater when compared with the acute SNHL group. Facilitation was observed in both acute and chronic SNHL groups but was diminished in the latter. These results demonstrate that interactions between pulses can affect ANF responses to pulsatile electrical stimulation in a level-dependent manner. This may have implications for cochlear implant signal processing strategies where the intervals between stimulus pulses on the same, or closely spaced electrodes, are relatively short and the potential for interactions are increased.

**REFERENCES**


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