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Protective Effects of Patterned Electrical Stimulation on the Deafened Auditory System

Submitted by:

Julie A. Bierer, Ph.D.

Russell L. Snyder, Ph.D.

***John C. Middlebrooks, Ph.D.**

Patricia A. Leake, Ph.D.

*** Kresge Hearing Research Institute, Neuroscience Program, University of Michigan, Ann Arbor, MI.**

University of California, San Francisco

Department of Otolaryngology – Head and Neck Surgery

Epstein Hearing Research Laboratories

533 Parnassus Avenue, Room-U490

San Francisco, Ca 94143-0526

Abstract

This Quarterly Progress Report presents preliminary results of a study evaluating the temporal response patterns of neurons of the central nucleus of the inferior colliculus (ICC) in response to electrical stimuli presented by a cochlear implant. A preliminary report of findings from this study was presented at the 2002 Mid-winter Meeting of the Association for Research in Otolaryngology at St. Petersburg Beach, Florida.

It is known that the temporal and spatial distribution of neural activation within the central nucleus of the inferior colliculus (ICC) can vary depending upon several aspects of the signals delivered to the cochlea. This study focuses on the temporal features of responses of multi-neuronal clusters elicited by acoustical and electrical stimulation of the cochlea in guinea pigs. We measured responses across the tonotopic gradient of the ICC (i.e., across the range of represented frequencies) using a standardized trajectory orthogonal to the tontopic gradient. Activity was recorded simultaneously at 16 depths along this trajectory using a single-shank, multi-channel recording probe.

The acoustical stimuli employed included pure tones, broadband noises, and clicks. Electrical stimuli consisted of single, biphasic pulses, which varied in phase duration and were delivered using different electrode configurations. Electrical stimuli were presented to the cochlea either through a Nucleus banded array (Cochlear Corp., Englewood, CO), which was similar to the clinical Nucleus-22 electrode but reduced to 6 channels; or, alternatively, pairs of independent platinum/iridium wires, terminating in 250 μm balls, were placed on specific intracochlear landmarks under visual control. Each experimental animal was first stimulated using acoustic tones to characterized the IC location of each recording channel by measuring its characteristic frequency (CF). Typically, CFs ranged from 2-20 kHz across the 16 channels of the probe. Subsequent to this acoustic "calibration" procedure, the recording probe was cemented in place, and the animal was acutely deafened with an intrascalar injection of neomycin sulfate. Then intracochlear electrical stimulation studies were conducted.

For pure tone stimuli, neurons with CFs close to the stimulus frequency exhibited sustained responses, whereas neurons with adjacent CFs usually displayed onset-only responses. For tones and broadband noise stimuli, neural responses were often pauser-like, with a robust onset response followed by a smaller sustained response. For acoustical clicks, an onset burst of activation was observed with a duration of 5 to 10 ms. For all acoustical stimuli, the first-spike latency of responses decreased by as much as 10 ms with increasing stimulus level. For electrical stimuli, regardless of the electrode configuration or the phase-duration, responses were usually sustained and frequently exhibited a clear periodicity. The first-spike latency for responses to electrical signals decreased by a maximum of 5 ms as a function of increasing current level.

Temporal Response Properties of Inferior Colliculus Neurons Responding to Acoustical and Electrical Cochlear Stimulation

Introduction

Cochlear implants (CIs) are one of the most successful neural prostheses in use today. Thousands of severely-to-profoundly deaf adults, and more recently young children, have been implanted with CIs, and most recipients receive significant benefit from the implant compared with conventional hearing aids. Mawman et al., (2000) reported that 83% of CI patients talk on the telephone on a regular basis.

The development of multi-contact electrode arrays and multi-channel speech processing strategies has led to great improvement in patient performance with CIs. Ideally, in a multichannel CI each channel (usually comprised of one or two closely spaced contacts) will effect selective stimulation of a restricted population of auditory nerve cells. However, electrophysiological studies that have recorded neural activation patterns within the central auditory system in response to cochlear implant stimulation have shown that the *spatial distribution* of the responses is much broader than one might predict based on electrical models.

In addition to this issue of selectivity of implant channels, within each cochlear implant channel there is also a specific *temporal pattern* of electrical pulses or sinusoids that are delivered. As technology advances, the presentation rate of pulsatile electrical stimuli are becoming quite high. The latest versions of clinically applied speech processing strategies employ electrical signals delivered at rates up to 4000 pulses per second, and experimental studies have used even higher rates, up to 10,000 pps. To better understand the perception elicited by such stimuli, it is first necessary to have a detailed understanding of the response patterns of central auditory neurons to the simple components of pulsatile processing strategies, which are single pulse stimuli.

Neurons in the central nucleus of the inferior colliculus (ICC) show a wide variety of temporal response patterns in their responses to electrical stimulation of the cochlea. In the present study we examine the temporal response properties of ICC neurons to both acoustical and electrical cochlear stimulation. Acoustical stimuli consisted of tones, broadband noises, and clicks. Electrical stimuli consisted of single biphasic electrical pulses delivered directly to the cochlea either by a cochlear prosthesis or by silver ball-electrodes placed on identifiable cochlear structures under visual guidance through a surgical microscope. Future studies will examine responses to modulated trains of pulses, similar to present speech processing strategies.

Methods

Anesthesia and surgery:

Data were collected from 13 healthy adult pigmented guinea pigs (500–900 g). Twelve of these animals had normal hearing. One animal was deafened with an intracochlear injection of neomycin sulfate 2 weeks prior to the experiment. Animals were initially sedated with a subcutaneous injection of a 3:2 mixture of ketamine hydrochloride (100 mg/ml) and xylazine (100 mg/ml). Additional intramuscular injections of a 4:1 mixture of ketamine/xylazine were given as needed to maintain an areflexive state. Heart rate, respiratory rate and body temperature were monitored continuously. Core body temperature

was maintained at 38°C with a thermostatically controlled heating pad. A tracheal cannula was inserted to insure an unobstructed airway. A midline incision was made in the scalp to expose the dorsal surface of the skull. The head was stabilized by attaching a phenolic rod, which was fixed to the skull anterior to bregma using small self-tapping screws and dental acrylic. The rod was held in place by a clamp connected to a vertical post that was fixed to a large horizontal metal base-plate. The temporalis muscle was reflected, and a 5 mm opening was made in the right parietal bone just dorsal to the parietal/temporal suture and just rostral to the tentorium. The dura was incised and reflected to expose the lateral and posterior occipital cortex. The cortex was aspirated to visualize the dorsolateral surface of the IC.

Once the IC was visualized and all bleeding controlled, a silicon-substrate, thin-film, multichannel-recording probe provided by the Center for Neural Communication Technology, Ann Arbor, MI (Drake et al. 1988; Najafi et al. 1985) was inserted into the IC. The probe was rigidly mounted on a custom-built pre-amplifier that was held by a micromanipulator (Narishige). The probe was inserted into the center of the IC on a dorsolateral to ventromedial trajectory at an angle of 45º off the parasaggital plane in the coronal plane. Using this trajectory, the probe traversed the central nucleus of the IC orthogonal to the iso-frequency laminae (Snyder et al., 1990, 1991, 2001). Each probe had 16 recording sites distributed in a linear array on a single shank at 100-µm intervals (center to center). The shank was 15 µm thick and 100 µm wide at the most proximal site (nearest the prea-amp) and tapered to a 15 µm width at the most distal recording site. The impedances at each site were 1.5–4 MΩ. In the guinea pig, the distance of 1.5 mm encompassed by 16 recording sites allowed simultaneous recording of responses from neurons sensitive to frequencies spanning approximately 4.5 octaves. The probes were inserted by manual advancement of the micromanipulator until the most distal site recorded activity from neurons with a characteristic frequency (CF) of approximately 18 kHz. Once this location had been reached, the cortical deficit was filled with warm 2% agar dissolved in Ringer's solution. When the agar had solidified, the agar and the surrounding parietal and temporal bones were covered with a thick layer of dental acrylic sealing the bony deficit and fixing the probe in place. After the probe had been fixed in place, acoustic responses from all 16 electrodes were recorded (i.e., from the 12 animals with normal hearing). The acoustic responses were evoked by contralateral tones (see below) presented in random order in intensities 0 to 90 dB (in steps of 5 or 10 dB). Tone frequencies typically were separated by 1/6 of an octave and ranged from 1 to 30 kHz. Once the probe was fixed in place and these preliminary acoustic recordings were completed, the probe was detached from its pre-amplifier and the animals were rotated 180 degrees to allow access to the left (contralateral) cochlea after insertion of the probes into the right IC. After rotation, a second series of acoustic responses was recorded to confirm that the recording electrode had not shifted and the frequency tuning at each recording site was unchanged. In all cases, responses recorded after moving the animal were not detectibly different from the initially recorded responses. These acoustic recordings allowed both a relative depth and a CF to be assigned to each probe recording site, effectively calibrating the sites to a series of cochlear locations. Broadband noise and click stimuli were also presented at that time. After this second series of acoustic recordings, the left cochlear bulla was surgically exposed and opened, to visualize the round window. A silver wire was placed in the round window niche and fixed to the overlying temporal bone with tissue cement. This silver wire served as the active electrode to record the click evoked compound action potential (CAP) from the auditory nerve. Two electrodes in the scalp served as the reference and ground electrodes. The CAP threshold was

determined, and the animals then were deafened by injecting a 10% solution of the cochleotoxic drug, neomycin sulfate, into the cochlea through a small slit in the round window membrane. Infusion of neomycin was continued while recording click-evoked responses until the CAP threshold was elevated beyond the maximum output of our system (>105 dB SPL).

After the guinea pig was deafened, a second silver electrode was placed on the oval window and fixed to the surrounding temporal bone with cyanoacrylate glue. A cochleostomy was made in the lateral wall of the cochlea at the junction of the 'hook' and first turn of the cochlea. A modified Nucleus banded electrode, which consisted of the 6 most distal bands of a human cochlear implant electrode, was inserted into the scala tympani. The individual bands were separated from each other by .75 mm (center-to-center). After insertion, the electrode was activated using the intracochlear electrode contacts in different configurations (monopolar, bipolar or tripolar). When the responses to the stimulation had been recorded across the 16 contacts of the recording probe, the Nucleus electrode was removed. The cochleostomy was enlarged and a modified UCSF electrode was inserted. This electrode consisted of two off-radial pairs (an apical and a basal pair) of ball contacts, 250 μm in diameter, in a silastic carrier (Rebscher et al., 2001). The contacts of the off-radial pairs were separated by 1 mm and the pairs were separated by 2 mm. Responses to activation of this UCSF electrode were then recorded using several stimulation configurations (monopolar or bipolar). Finally, the UCSF electrode was removed and the cochleostomy was further enlarged so that two insulated platinum/iridium wires ending in 250 μm balls could be placed visually within the basal scala tympani using a dual micromanipulator (Narishige). These balled contacts were placed at different locations within the basal turn and the responses evoked by their activation were recorded across the 16 channels of the fixed probe. All procedures were conducted in accordance with the policies of the University of Michigan's University Committee on Use and Care of Animals.

Stimulus generation and calibration:

Acoustic signals: Experiments were controlled by an Intel-based personal computer. Acoustic stimuli were synthesized digitally using equipment from Tucker-Davis Technologies (Gainsville, Florida). The sample rate for audio output was 100 kHz with 16-bit resolution. Experiments were conducted in a sound-attenuating chamber. Sound stimuli were presented monaurally to the ear contralateral to the studied inferior colliculus. A headphone enclosed in a small case was connected to a sound-delivery tube inserted into the external auditory meatus near the tympanic membrane and sealed in place using dental acrylic. The headphone was calibrated using a 1/8-in. condenser microphone (Büel & Kjær) and a 0.3-cc coupler. The resulting calibration table was used for online correction of the headphone response. Tone and noise bursts were 40–60 ms in duration. Tones were ramped on and off with 5-ms rise/fall times, and noise bursts had 0.5-ms rise/fall times. Broadband Gaussian noise bursts had a passband of 1–30 kHz with abrupt cutoffs. Sound levels of tones and noise bursts were equated for root-mean-squared power. The sets of center frequencies, bandwidths, and levels varied from animal to animal. Stimulus center frequencies ranged from 1–30 kHz in either 1/3- or 1/6-octave steps. Levels ranged from 0 to as high as 90 dB sound pressure level (SPL) in either 5- or 10-dB steps.

Multi-channel recording and spike sorting:

The multi-channel probe permitted simultaneous recording of spike activity from all sites. Signals from the recording probe were amplified with a custom 16-channel amplifier, digitized at a 25-kHz rate, sharply low-pass filtered below 6 kHz, re-sampled at a 12.5-kHz sample rate, and then stored on the computer hard disk. Unit activity was isolated from the digitized responses off-line using custom spike-sorting software (Furukawa et al. 2000). Spike times were stored at a resolution of 20-μs for further analysis. Well-isolated single units were recorded as well as multiunit clusters consisting of a small number of unresolved units. However, since in all cases the responses were indistinguishable at the levels of analysis used in this study, multi- and single-units were treated identically. Recordings at particular sites were excluded from further analysis if units did not respond to any stimulus with an average of at least 1 spike/trial or if the mean spike count changed by more than a factor of 2 over the recording period.

Data analysis:

For analyzing the spatial distribution of ICC responses, spike counts were normalized at each recording site according to the 95th percentile of the average spike counts computed within one wideband stimulus (a wide band noise of acoustic stimulation and electrical activation with bipolar electrodes placed on the round window and oval window). By normalizing in this manner, we emphasized stimulus-driven changes in activity rather than differences in absolute spike counts across channels. The results include data from individual recording sites as well as the distribution of activity across multiple recording sites. For individual recording sites, the lowest level that elicited a stimulus-locked response was defined as threshold. The *characteristic frequency* (CF) was defined as the frequency that gave the strongest response at 10 dB above threshold. The temporal distribution of activity across all recording sites was referred to as an IC *spatiotemporal image* (STI). The *centroid* of the image was defined as the spike-count-weighted center of mass calculated for the sites at which the firing rate was at least half the maximum normalized spike count of the distribution.

Raster dot plots are representations of spikes recorded at one site in response to a single electrical stimulus that varied in level. Each dot represents one spike recorded for that level. Ten repetitions were presented. Post-stimulus time histograms (PSTHs) were created by adding the number of spikes that occurred in each time bin from the onset of the stimulus to 40 ms after the stimulus. Spikes were sorted with a resolution of 20 μs and the binwidth for the PSTHs was 1 ms. To examine autocorrelation functions, the spike times were first converted into a point process, a vector of zeros and ones representing the occurrence of a spike in 1 ms bins. An autocorrelogram was created from those point processes, averaged across repetitions (Perkel et al., 1967) and smoothed with a three-point filter.

Results

Spatial Response Properties

At the beginning of each experiment, acoustic stimuli were presented to the ear contralateral to the recording sites in the ICC. The tones varied over a wide range of frequencies, from 1 to 30 kHz, and intensities, from 0 to 90 dB. The clicks and noise bands also varied over a wide range of intensities. At each recording site, frequency response areas were created and the site's characteristic frequency (CF) was determined.

Figure 1 illustrates four frequency response areas, each obtained at a different recording site. The CF at each recording site was determined as the frequency at which the greatest number of spikes was measured when the stimulus was 10 dB greater than threshold for that site. For this example, the CFs were 4.3, 7.5, 17.9 and 20 kHz, respectively.

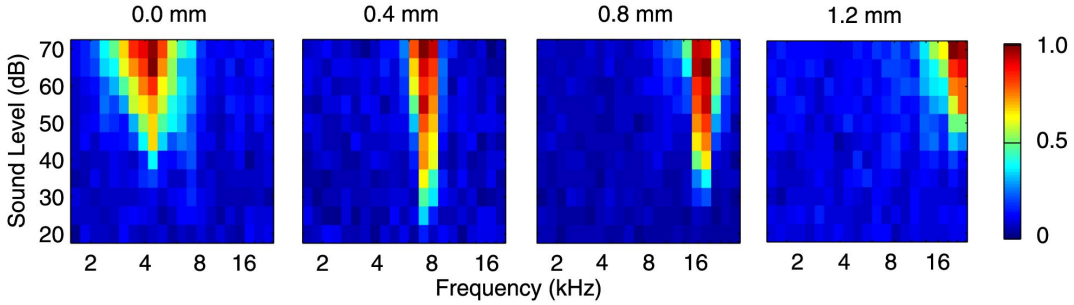

Figure 1: Frequency response areas for four different recording sites, each identified by its location on the recording probe, indicated as distance (in mm) from the most superficial recording site. The abscissa represents stimulus frequency in kHz and the ordinate represents sound level in dB. The normalized spike rate is represented by color (see scale).

Another method of examining these data is to display spike activity for one selected stimulus (i.e., a specific frequency and intensity) as a function of IC recording location along the recording probe versus post-stimulus time. We refer to these analyses as "*IC images*" of neural activation (see Figure 2).

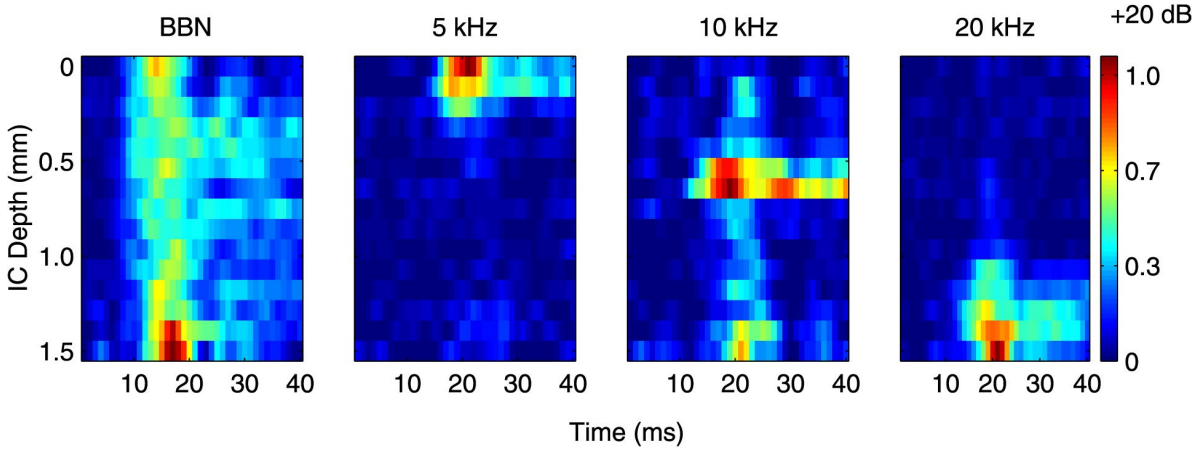

Figure 2: IC images of acoustic tones and noise. Each panel represents the normalized spike activity obtained in response to either a broadband noise (left) or to pure tones of 5, 10 and 20 kHz (right three panels) at 20 dB above the most sensitive site's threshold. The stimulus duration was 40 ms. The abscissa and ordinate represent the time following the stimulus onset and recording depth in the IC, respectively. The magnitude of spike activity is represented by color with red as the greatest activity and blue as the lowest activity. (Data from GP200016)

As described above, following the characterization of the ICC responses to acoustical stimuli, the recording electrode was fixed in place, the guinea pig was deafened, and responses to electrical stimulation were obtained. We hypothesized that with restricted

electrical stimulation of the cochlea, IC response images would be similar in spatial extent to those of pure tones and narrower than those of broadband noise.

The data shown in Figure 3 demonstrate that when a restricted electrical stimulus is delivered to the cochlea via a cochlear prosthesis, a somewhat restricted pattern of activation can indeed be measured in the ICC. That pattern of activation is generally broader than the activation seen with tones, but more restricted than patterns elicited by broadband noise. Moreover, the activation focus shifts systematically from superficial to deep as the stimulus location is shifted from apical to basal in the cochlea, consistent with the cochleotopic organization of the ICC. In comparing Figures 2 and 3, it is clear that the electrical bipolar stimuli at +2 dB relative to threshold are broader in the spatial extent of ICC activation than the acoustical tones at +20 dB relative to threshold. (E.g., compare the IC depth over which a robust response is indicated by the red and yellow pseudocolors.) The extent of ICC activation could be modulated by the configuration and/or placement of the stimulating intra-cochlear electrodes (see 5th Quarterly Progress Report).

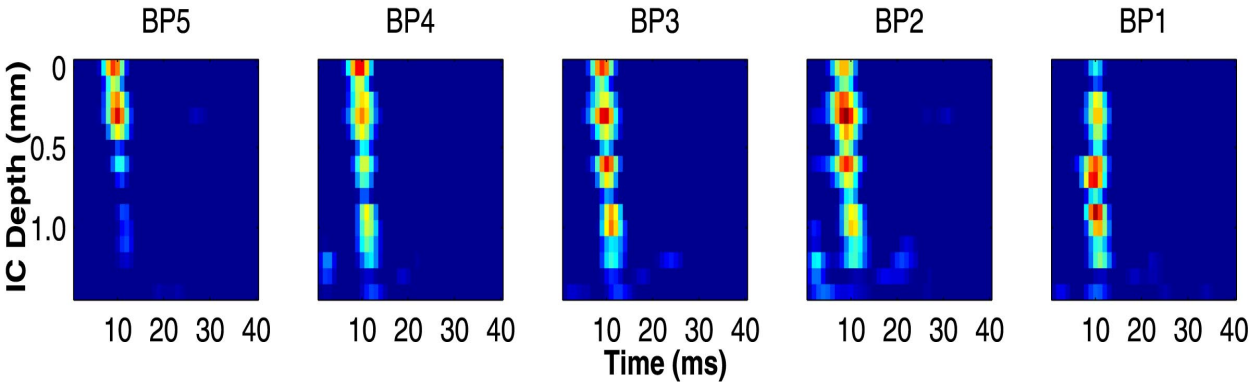

Figure 3: IC images of single electrical pulses delivered by a cochlear prosthesis. Each panel represents the normalized spike activity obtained from a different bipolar (BP) channel of a 6-channel banded electrode array (Nucleus Ltd., Englewood, CO) in response to a biphasic electrical pulse of 160 μs/phase. The electrodes were numbered from 1 to 6, base to apex. The numbers following BP indicate the active electrode and the return electrode was the next apical electrode. Each image represents activity elicited when the pulse was presented at 2 dB above the most sensitive site's threshold. Abscissa, ordinate and color scale conventions are identical to previous figure.

Temporal Response Properties

In all subjects studied, a consistent cochleotopic organization was observed as a function of IC recording depth for both acoustical and electrical stimulation. However, tremendous variability was observed in the timing of responses following the stimulus onset. Our experimental procedures, as described above, allow us to examine a number of different aspects of the responses from a single recording site to various acoustical and electrical stimuli. Figures 4 through 8 present several representative temporal response patterns elicited at one recording site in response to various stimulating conditions.

Figure 4 illustrates the sustained response that was elicited at this recording site when a pure tone (40 ms duration) was delivered at the site's CF. In the raster plot shown in Figure 4A, there is a shift to shorter first spike latencies that occurs with increasing stimulus levels (i.e., toward the top of the plot). There does not appear to be any temporal structure the response. The post-stimulus time histograms presented in Figure 4B clearly show the sustained nature of the response, with a peak at onset with a latency of 18.9 to 15.3 ms. The autocorrelation functions (Fig. 4C) have peak inter-spike-intervals between 1.0 and 1.3 ms for the five highest stimulus intensities. The lowest level shown (45 dB) did not elicit enough spikes for an accurate assessment of the peak interval.

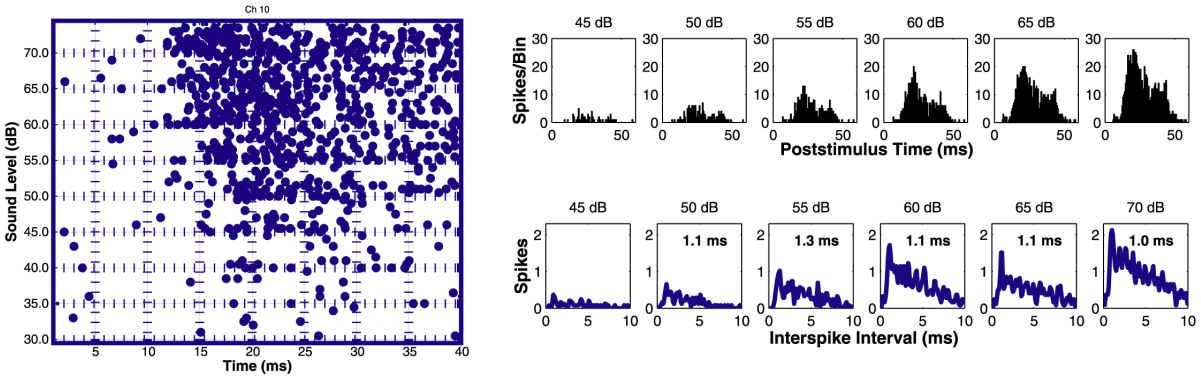

Figure 4: Spike raster display, post-stimulus time histograms, and autocorrelation functions for one recording site. The response is shown for a tone at the CF for this site, which was 15.9 kHz. A) The raster display represents a plot of spikes elicited at this recording site in response to a single electrical pulse presented at various stimulus levels. The ordinate and abscissa represent the stimulus level and post-stimulus time, respectively. Each symbol represents a single spike, and data include spikes elicited by 10 repetitions of the stimulus at each level. Each symbol represents one spike. B) The six panels show post-stimulus time histograms for the 6 highest intensity levels plotted in the raster display (i.e., 45 dB to 70 dB), displayed in 1 ms time bins. C) The six panels show autocorrelation functions, again representing the six highest stimulus levels. The number in each graph indicates the interspike interval at the peak of the function. (GP200016)

The response to a broadband noise (40 ms duration) recorded at this same IC recording site is illustrated in Figure 5. A sustained response is elicited that is similar to the response to the CF tone presented in Fig. 4. Again, no temporal structure is apparent in the raster dot plot. In this case, however, the onset peak is relatively weak compared to the robust sustained response that follows it in the post-stimulus time histogram. As for the pure tone, the autocorrelation functions have peak inter-spike-intervals ranging between 1.0 and 1.3 ms for the highest stimulus levels.

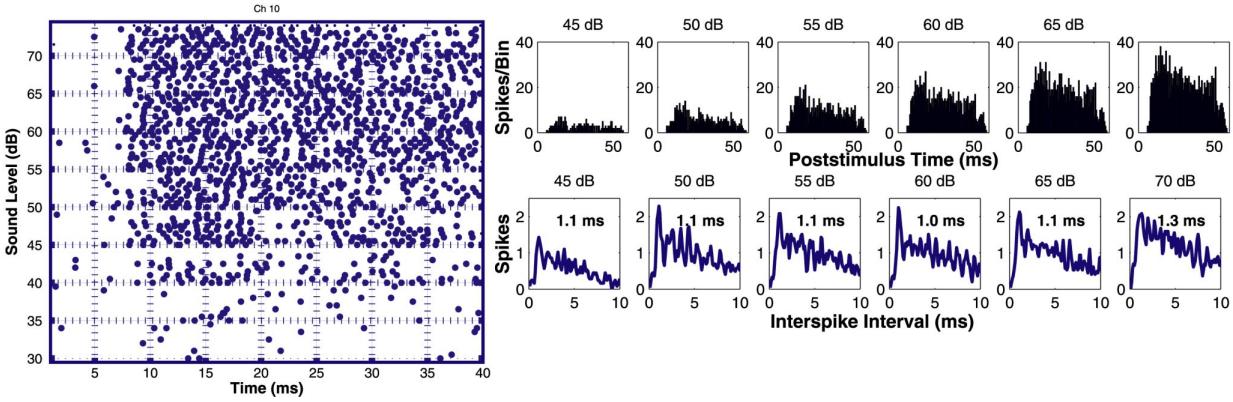

Figure 5: Spike raster, post-stimulus time histograms, and autocorrelation functions for the response to a broadband noise, obtained at the same recording site as in Figure 4. Conventions as in previous figure.

When an acoustic click, which comprises a brief (1ms) but broadband stimulus, was presented, this same recording site in the ICC had responses with durations of 5 to 8 ms as illustrated in Figure 6. The raster display shows that first spike latency again decreased as stimulus intensity increased. No temporal structure is observed in the response. The post-stimulus time histograms are consistent with brief sustained responses. As with tones and broadband noise, peak inter-spike-intervals between 1.0 and 1.3 ms were observed for the highest intensities. In this case, the first three levels did not elicit sufficient spikes to accurately assess the peak interval.

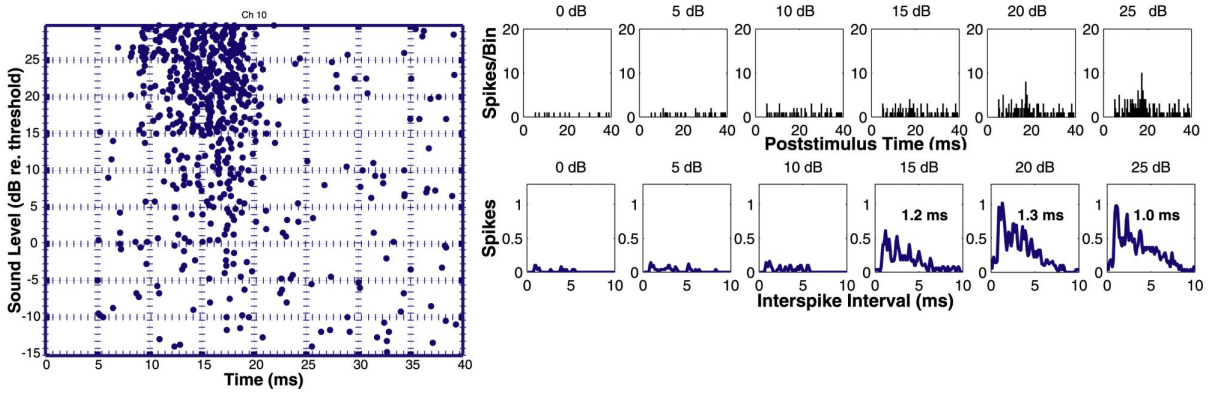

Figure 6: Spike raster, post-stimulus time histograms, and autocorrelation functions for the response elicited by an acoustic click and recorded at the same recording site as the previous 2 figures. Conventions as in previous figures.

An example of one type of neuronal response commonly seen in response to brief biphasic electrical pulse (160 µs/phase) is shown in Figure 7. Recorded at the same ICC recording site as the data in the three previous figures, this response displays some marked differences from responses observed with acoustical stimuli. Note in particular that there appears to be a distinct periodic temporal structure, which is most obvious in the first 5 to 8 ms of the response displayed in the spike raster plot. This periodicity appears to result from a strong tendency for the first spike latency to be highly precise and for subsequent spikes to occur at a precise interval. Such temporal structure is never observed in responses to acoustic signals. In addition, the first spike latency at higher levels is much shorter for electrical stimulation than for acoustic signals, as would be expected with direct electrical stimulation of the auditory nerve, which bypasses the hair cell synapse. Again, a pronounced shift to shorter latencies with higher intensities is apparent. The pattern of the post-stimulus time histograms shows an onset burst, followed by a pause, which in turn is followed by a late response. It is interesting to note that the peak inter-spike intervals measured here were indistinguishable from those measured for responses to acoustical stimuli shown in Figures, 4 through 6 for this recording site. That is, peak inter-spike intervals again ranged from 1.0 to 1.3 ms, in response to the electrical pulses.

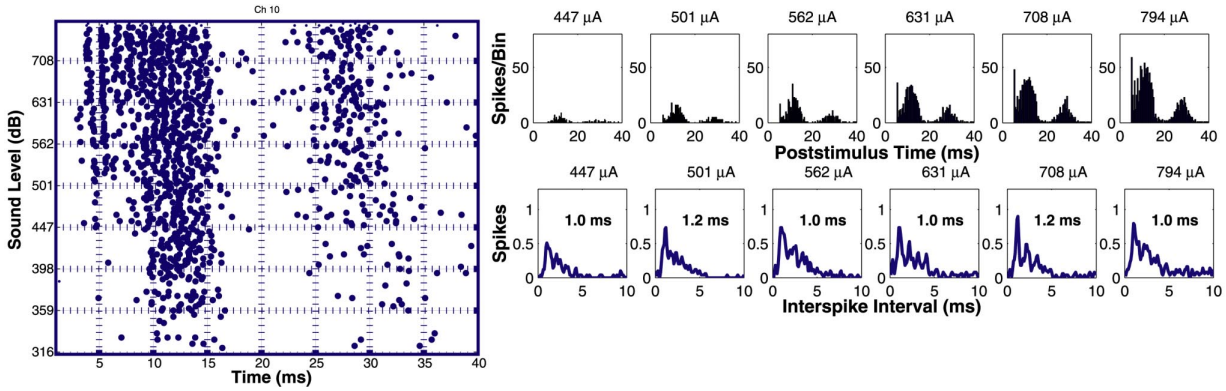

Figure 7: Responses elicited by electrical stimulation and recorded at the same site as the data shown in Figs. 4 through 6. Stimuli here were biphasic electrical pulses (duration, 160 µs/phase) and were delivered through a Nucleus intracochlear electrode, with a BP configuration. Conventions are as in previous figures, except the time window used to compute the autocorrelation was from 5 to 20 ms.

Figure 8 shows an example of an ICC response to electrical stimulation delivered using silver-ball electrodes, which were placed directly in contact with the osseous spiral lamina and activated as a bipolar pair. The temporal response pattern observed is similar to that in the previous example of stimulation with the Nucleus cochlear implant. The response pattern again is comprised of an onset burst, followed by suppression or a pause, and in this case only relatively weak activity representing recovery from suppression is observed following the pause. Some periodicity within the temporal structure again is seen within the onset response in the spike raster display, although it is not as striking as the previous example. This periodicity appears to be the result of a tendency for the first spike to occur with little temporal jitter across stimulus presentations and for latency to 'jump' stepwise from one "preferred" latency to another with increasing level, especially at the lower intensities. That is, the spikes seem to display less temporal dispersion and to occur synchronously at certain poststimulus intervals. The peak inter-spike intervals were again between 0.9 and 1.3 ms.

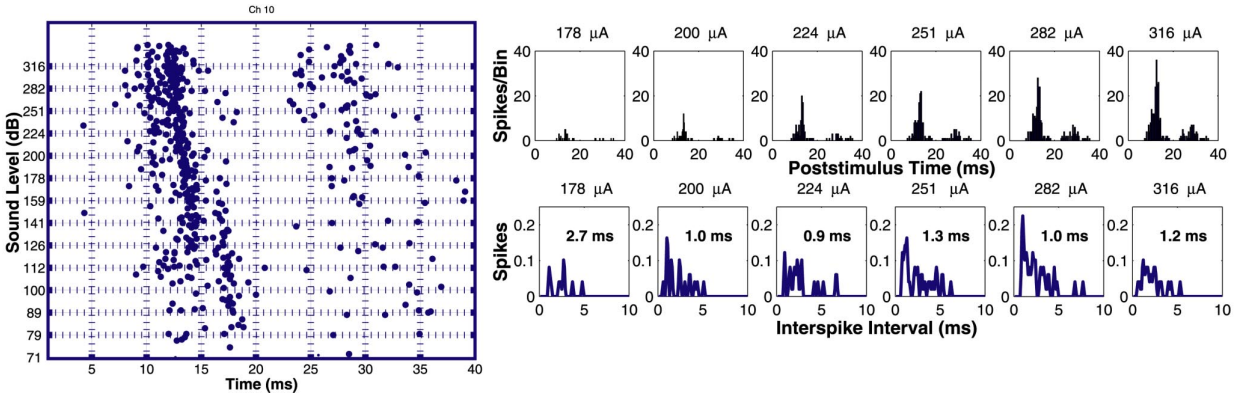

Figure 8: Again, the response shown here was recorded at the same recording site as in the previous Figures 4 through 7. In this case, electrical pulse stimuli (160 µs/phase) were delivered through silver-ball electrodes placed longitudinally in the basal cochlea. Conventions for the spike raster plot, post-stimulus time histograms, and autocorrelation functions are the same as in previous figures.

Figure 9 presents data from 3 different recording sites that illustrate the range of temporal response patterns observed in response to simple electrical pulse stimuli. (Panels C and D show recordings from the same site, using different time windows.) The spike raster display in panel A illustrates a typical example of a recording site that elicits an onset-only response to an electrical biphasic pulse 160 µs/phase in duration. The latency of the response initially becomes shorter with increasing stimulus current level, but above a level of about 1000 µA the latency remains quite stable over a wide range of intensities. The spike raster displayed in panel B shows a second recording site where another common, but more complex response pattern is observed with presentation of the same 160 µs/phase electrical pulse stimulus. This response has an onset burst of 5 to 8 ms duration that is followed by a pause of similar duration, which in turn is followed by a second sustained burst that appears to persist beyond the 40 ms recording window. The other two raster displays shown in C and D illustrate an example of a response pattern that exhibits a periodic response at high

stimulus current levels. In this case, the stimulus was a somewhat longer electrical pulse 640 µs/phase. The first spike latency decreases markedly with higher stimulus intensities, and the latency also exhibits clear stepwise 'jumps' from one latency to another rather than decreasing gradually. Note that neurons are responding for 6 or 7 milliseconds in response to an extremely short stimulus. A second burst of activity is observed at about 26 ms. If one expands the time representation, as shown in Fig. 9D, the highly periodic response pattern can be seen to have a firing rate of approximately 1000 Hz. This periodicity corresponds quite closely to the typical peak inter-spike intervals of ranged 1.0 to 1.3 ms, which were observed for all IC responses as noted above.

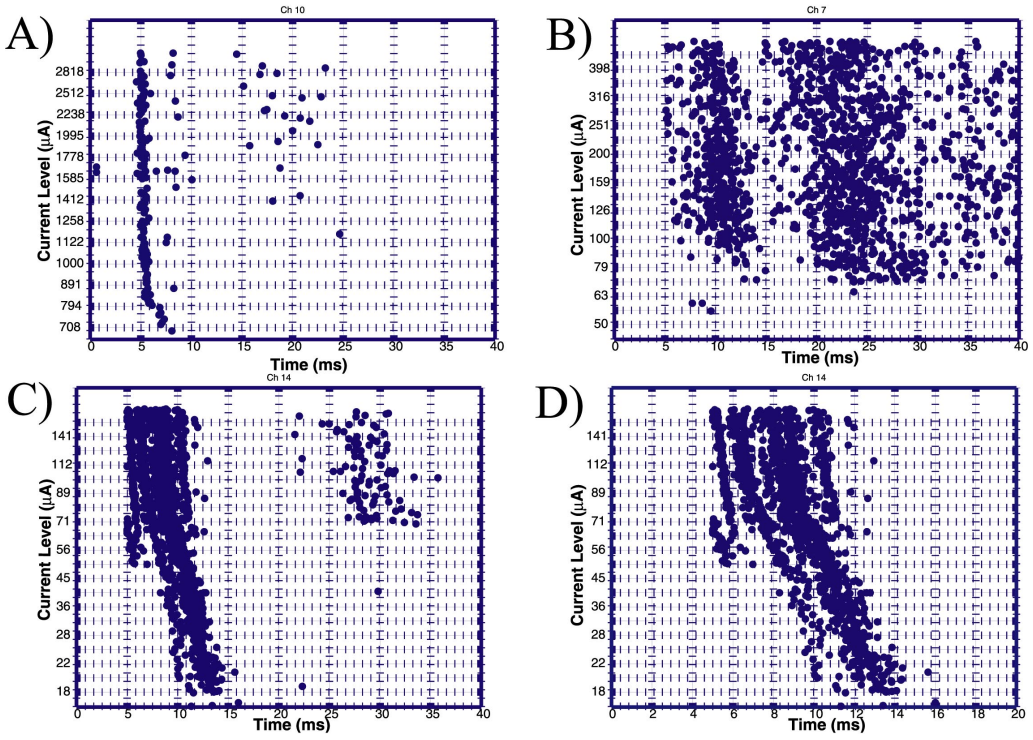

Figure 9: These spike raster displays illustrate the variability in temporal response properties elicited by direct electrical stimulation of the cochlea. Each panel in A,C, and C presents a raster plot of spikes obtained at a different recording site in response to single electrical pulse stimuli presented at various intensities. A. These responses were obtained at channel 10 of the recording array in animal GP20025. Stimuli consisted of 160 µs/phase biphasic pulses, delivered by bipolar stimulation of a Nucleus array (BP4). B. This panel presents the responses elicited at recording channel 7 in animal GP200023. Stimuli were 160 µs/phase pulses, delivered by monopolar stimulation of a Nucleus array (activating between electrode 1 vs. a distant ground). C and D panels display responses obtained at recording channel 14 in animal GP20013. Stimuli consisted of 640µs/phase pulses delivered through two silver-ball electrodes placed longitudinally in the scala tympani under visual guidance. Note that the time scale for the abscissa is 0 to 40 ms in C and 0 to 20ms in D.

When 16 IC locations recorded simultaneously from the multi-channel recording probe are examined, a great deal of variability can be seen in the temporal pattern of the responses elicited by a single biphasic pulse (Figure 10). The temporal response properties vary substantially among the different recording sites. Lower thresholds and stronger responses were obtained at deeper recording sites (those numbered higher). This is consistent with the known cochleotopic organization of the IC (low frequencies represented superficially and higher frequencies deeper in the IC) since the electrical stimulation in this example was delivered at a basal, high frequency region of the cochlea.

Most recording sites show a decreasing first spike latency as a function of increasing stimulus current level, however, channels 5, 9, 11 and 16 all have a constant first spike latency regardless of stimulus level. Also, many — but not all—of the channels exhibit a periodic structure in the first 5 to 8 ms of the response.

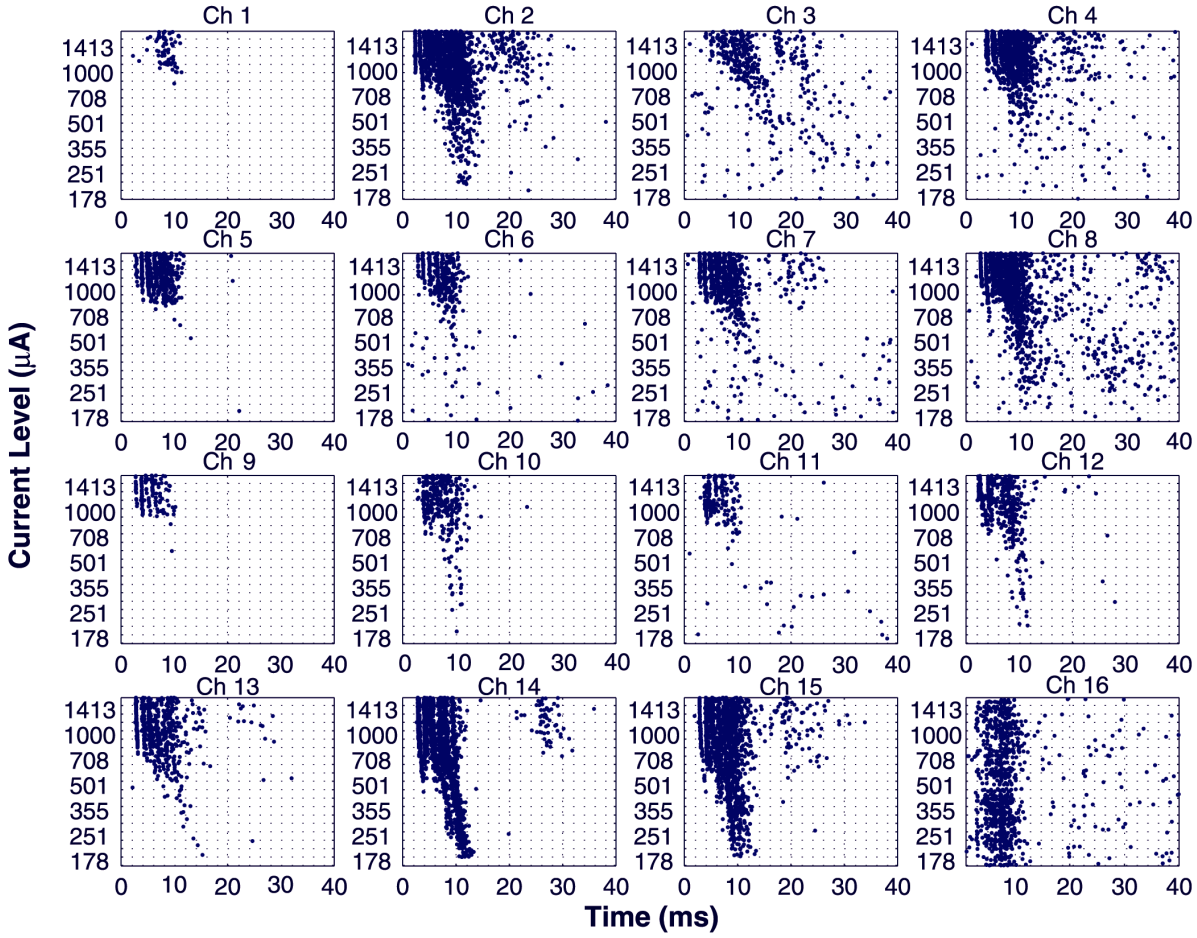

Figure 10: Spike raster plot displays for 16 sites in the IC, with activity recorded simultaneously by a 16-channel probe in response to direct electrical stimulation of the cochlea. Each of the 16 panels represents activity recorded at one site along the array, with adjacent sites separated by 100 µm in the IC. Stimuli were electrical pulses, 640 µs/phase that were varied in intensity. Conventions as in previous figure. Electrical stimuli were presented through silver-wire ball electrodes placed directly on the osseous spiral lamina in a longitudinal orientation in the basal cochlea, ie., under direct visualization through the round window. GP200013.

Future analyses will focus on a couple of important aspects of these data that have not been elucidated in the present report. First, it should be noted that in response to all responses to both acoustical and electrical stimulation, the interspike interval quantified by the peak of the autocorrelation function is approximately 1 ms. This is interesting because this peak ISI is always the same, whether or not a periodicity is observed in the response. The autocorrelation analysis used here might not be sufficient to quantify the periodicity forseveral reasons. First, it should be noted that the peak interval of about 1 ms measured in this analysis is similar to the probable refractory period of the neurons.

Second, the ICC responses obtained may have included some multi-unit clusters of neurons as well as well-isolated single neurons. It should be noted, however, that although we are not able to confirm that recordings were made exclusively from single units, the following specific criteria were used in the spike sorting procedure for each recording site to ensure that recordings were made primarily from isolated single neurons. A cluster of spike shapes was selected based on the first two principle components. The mean of that cluster was used to compute a template waveform. Spikes were accepted that fit the template within a range of 2.5 standard deviations. Further analyses examined the stability of the recording site over the recording period and the range of inter-spike intervals; a peak interval greater than 1 ms further suggested a single unit response allowing for the known refractory period of most ICC neurons. Additional analyses will be required to examine the possible influence of multi-unit responses by analyzing data from the multi- and single-unit responses separately and comparing results.

Finally, it should be noted that methodologies for efficiently acquiring and analyzing data from 16 (and eventually 32) recording sites with the multi-channel recording probes are still under development. Ultimately these analyses will provide a more complete view of single- and multi-unit responses in the ICC to both acoustical and electrical stimulation of the cochlea.

Conclusions and Discussion

Overall, the temporal response patterns for ICC neurons responding to acoustical and electrical stimulation of the cochlea exhibit a number of interesting differences. In response to an acoustic click, which is a broadband transient acoustic stimulus, the response properties of ICC neurons tend to have an onset burst that has no temporal structure. This stimulus is as close to an electrical pulse as can be simulated acoustically.

Biphasic electrical pulses, which were similar in duration to an acoustic click, elicited several response characteristics in the temporal domain that differ from responses to acoustic stimuli. In particular, responses to electrical pulses usually exhibit: 1) a periodic temporal structure; 2) a more marked and/or stepwise shift in latency with level, 3) a pause-type response, and 4) a sustained-type response. One characteristic of these responses was that the onset burst commonly was strongly periodic with a periodicity of approximately 1000 Hz. This finding is particularly interesting because previous studies have demonstrated that ICC neurons respond to a *train* of pulses in a phase-locked manner with a firing rate of up to only about 300 pps and cannot follow pulses up to 1000 pps (Snyder et al., 1995).

A prominent and comparable shift in latency occurred with increasing stimulus level for both acoustical and electrical stimulation, although first spike latencies were markedly shorter for electrical signals. In response to electrical stimulation, a decrease in first spike

latency with increased stimulus level as great as 5 ms was often observed. In contrast, responses of auditory nerve fibers to biphasic electrical pulse show a shift as small as 1 ms when the stimulus level was increased by a few decibels relative to a mA (van den Honert and Stypulkowski, 1987).

For ICC responses, occasionally a step change of first spike latency was observed with increased level (see Figs. 8-10). That shift, however, is not comparable to the 'paradoxical latency shift' that has been observed in the IC of the bat (Galazyuk and Feng, 2001). The paradoxical latency shift represented a step shift but instead of shifting to shorter latency, as was observed in the current study, a shift to longer latency was observed with higher stimulus levels in that study. Also, Galazyuk and Feng discuss the role that paradoxical latency shift might have in echolocation in the bat, a similar pattern in the guinea pig is likely not associated with echolocation.

A pause-like response has been observed in responses of cochlear nucleus and IC neurons in response to acoustical stimulation (Blackburn et al., 1989; Stabler et al., 1996). A similar type of response was observed in the current study, but in response to a brief electrical pulse in contrast to the typical acoustical tone burst of at least 20 ms that elicits this response type.

For acoustical tone bursts, as has been reported previously, a sustained response was elicited. Unexpectedly, however, we also observed a sustained response to transient electrical pulses but not to transient acoustical clicks. The significance of these varied and complex responses and their role in the encoding of specific electrical signal properties in the central auditory system remain to be elucidated.

Literature Cited:

Blackburn CC, and Sachs MB (1989) Classification of unit types in the anteroventral cochlear nucleus: PST histograms and regularity analysis. *J. Neurophysiol.* 62(6): 1303-1329.

Drake KL, Wise KD, Farraye J, Anderson DJ, and BeMent SL. Performance of planar multisite microprobes in recording extracellular single-unit intracortical activity. *IEEE Trans Biomed Eng* BME-35: 719-732, 1988.

Furukawa S, Xu L, and Middlebrooks JC. Coding of sound source location by ensembles of cortical neurons. *J Neurophysiol* 20: 1216-1228, 2000.

Galazyuk AV, and Feng AS (2001) Oscillation may play a role in time domain central auditory processing. *J. Neurosci.* 21: RC147.

van den Honert C, and Stypulkowski PH (1987) Temporal response patterns of single auditory nerve fibers elicited by periodic electrical stimuli. *Hearing Res.* 29: 207-222.

Mawman DJ, Giles EC, Woolford TJ, O'Driscoll M, Hamrouge S, and Ramsden RT (2000) Telephone use by auditory prosthesis users in Manchester. In: Auditory prostheses, S.B. Waltzman and N.L. Cohen, eds., Thieme, New York, pp. 341-342.

Najafi K, Wise KD, and Mochizuki T. A high-yield IC-compatible multi-channel recording array. *IEEE Trans Electron Dev* 32: 1206-1211, 1985.

Perkel DH, Gerstein GL, and Moore GP (1967) Neuronal spike trains and stochastic point processes. I. The single spike train. *Biophys J* 7: 391-418.

Rebscher SJ, Snyder RL, and Leake PA (2001) The effect of electrode orientatin on threshold and selectivity of responses to intracochlear electrical stimulation. *J Acoust Soc Am* 109: 2035-2048.

Stabler SE, Palmer AR, and Winter IM (1996) Temporal and mean rate discharge patterns of single units in the dorsal cochlear nucleus of the anesthetized guinea pig. *J. Neurophysiol.* 76(3): 1667-1688.

Snyder RL Rebscher SJ, Cao K, Leake PA, and Kelly K (1990) Chronic intracochlear electrical stimulation in the neonatally deafened cat. I. Expansion of central representation. *Hear Res* 40: 7-33.

Snyder RL, Rebscher SJ, Leake PA, Kelly K, and Cao K (1991) chronic intracochlear electrical stimulation in the neonatally deafened cat. II. Temporal properties of neurons in the inferior colliculus. *Hear Res* 56: 246-264.

Snyder RL, Leake PA, Rebscher SJ, and Beitel R (1995) Effects of neonatal deafening and chronic intra-cochlear electrical stimulation on temporal resolution of cat IC neurons. *J Neurophysiol* 73: 449-467.

Snyder RL, Vollmer M, Moore C, Rebscher S, Leake PA, and Beitel R (2000) Responses of inferior colliculus neurons to amplitude modulated intracochlear electrical pulses in deaf cats. *J Neurophysiol* 84: 166-184.

Work Planned for the Next Quarter

1) Two manuscripts comprising a two-part series by Dr. Wardrop on temporal bone studies of perimodiolar electrodes will be completed and submitted to the journal Otology and Neurotology for peer review. The second paper, entitled “A Temporal Bone Study of Insertion Trauma and Intracochlear Position of Cochlear Implant Electrodes. II. Comparison of Spiral Clarion™ and HiFocus™ Electrodes” will comprise our next QPR.

2) Histolopathological studies of cochlear specimens from control and chronically stimulated experimental animals will be continued for our new series in which the anti-apoptoti drug desmethyldeprenyl has been administered over the period of several weeks in deafened neonates until the time cochlear implantation.

3) Work will continue on further development of software for multichannel recording experiments examining spatial selectivity of electrical stimulation in guinea pigs and cats using the University of Michigan 16-electrode multichannel recording probes.