
Second Quarterly Progress Report

November 1, 2006 to January 31, 2007

Contract No. HHS-N-260-2006-00005-C

Neurophysiological Studies of Electrical Stimulation for the Vestibular Nerve

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Introduction

It is a pressing goal for us to design and fabricate a chronic vestibular implant so that the implant and chamber animal surgeries can be performed as soon as is feasible. Extensive personnel changes at Advanced Bionics due to the retirement and departure of several senior executives have slowed work in this area. Dr Rubinstein was subsequently approached by Dr. Chris Roberts, CEO of Cochlear Corporation, who showed great interest in their involvement in this project. It is our intent to acquire a device with the necessary design features and software capabilities at the earliest possible opportunity from whichever vendor can supply a device with the necessary specifications at the earliest time. Advanced Bionics has an advantage as we already know how to do the necessary software development on that platform. Cochlear Corporation has greater experience with the thin hybrid electrode design that is similar to what we plan for the semicircular canal implantations. We are therefore proceeding as if either vendor will supply the device. This QPR will focus on surgical, hardware, and software developments toward this goal.

Implant anatomical dissections (Surgical Implant Team)

Performing temporal bone dissections on cadaveric rhesus materials in our temporal bone dissection laboratory required approval from the animal care committee, as well as from laboratory safety committees. Drs Rubinstein and Phillips attended biohazard classes in order to obtain this approval. This approval was obtained in this quarter allowing the initiation of the dissections. Drs Rubinstein and Santos have performed temporal bone dissections in the rhesus monkey to develop the proposed surgery, as well as to make normative measures necessary to design appropriate electrode arrays for vestibular implantation.

These dissections revealed the following:

1. The surgical anatomy of the rhesus mastoid and labyrinth are, as expected, quite similar to the human but significantly smaller.
2. As expected, the lateral and posterior semicircular canals are surgically accessible targets for implantation via a transmastoid approach without any intracranial dissection.
3. The superior semicircular canal will be exceedingly difficult to access via a transmastoid approach without violating other labyrinthine structures. If needed, it could be accessed intracranially.
4. A semicircular canal electrode must be smaller than 150 microns in diameter (preferably 120 microns) in order to insert it into the lateral and posterior canals without obviously compressing the membranous labyrinth.
5. The desired stiffness of the intralabyrinthine component of the electrode array was determined to be comparable to 0.12 mm platinum wire, which is commonly used to make “fat-wire” stapes prostheses in temporal bone dissection courses. A

sample of such wire has been provided to the electrode design engineers at both Cochlear and Advanced Bionics to define the desired electrode stiffness.

6. It should be possible to place either a Cochlear or Advanced Bionics receiver/stimulator subcutaneously on the skull between the occipital chamber and the frontal connector for the eye coils. The region will be crowded with hardware, but adequate space should be available for healthy skin flaps to survive.
7. Placement of electrodes within the labyrinth while preserving vestibular sensitivity will require extreme care, similar to hybrid cochlear implant surgery in humans which preserves residual hearing. Dr. Rubinstein has performed three hybrid surgeries during the quarter and preserved acoustic sensitivity in all three, increasing confidence that the desired labyrinthine preservation will be possible given an appropriate electrode array and surgical instruments.

Implant device development (Vestibular Implant Development Team)

Based on the dissections described above, a mockup of a possible prototype vestibular implant was provided by engineers at Cochlear Corporation and is seen in Figure 1.

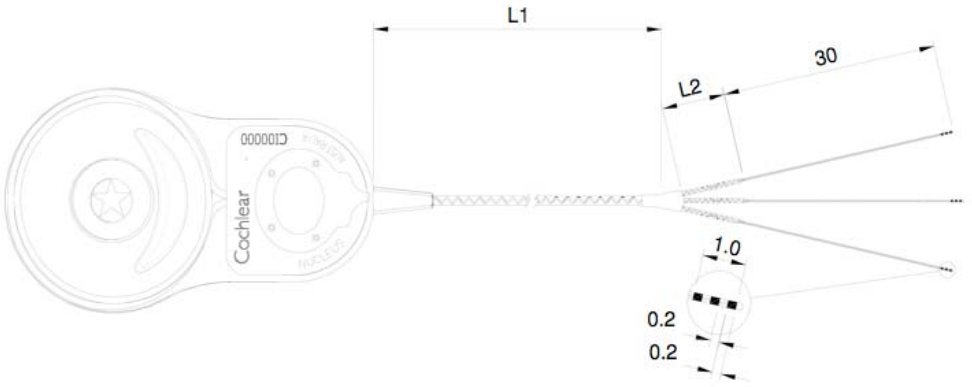


Figure 1: *Prototype vestibular implant from Cochlear Corporation*

Discussions are ongoing about a number of features shown here but the general design scheme appears sound based on our anatomical work and surgical experience with a variety of cochlear implants in humans. It is anticipated that initial surgeries will only use one of the three electrode arrays and will be placed in the lateral semicircular canal 180 degrees opposite from the vestibule, i.e. as far from any neuroepithelium as possible

to minimize risk to vestibular sensitivity. Subsequent surgeries could implant both lateral and posterior canals, leaving a third array available for possible otolithic stimulation via implantation of the vestibule, possibly via a round or oval window approach, or via the common crus. As with hybrid cochlear implant surgery, a small piece of fascia can be placed around the electrode array to seal the "canalostomy" and the array will be inserted 1 mm into the canal with a hub on the device (not pictured) preventing deeper insertion and potential damage to the ampulla. Three electrode contacts will permit monopolar and bipolar stimulation with a third contact available for backup in case of individual electrode failure, a quite likely event given the exceedingly small size and delicate nature of these arrays. We are currently awaiting similar drawings from Advanced Bionics but will proceed with Cochlear Corporation as a vendor if they are able to provide us with the necessary hardware and software tools for signal processor design.

Implant processor software development (Software Development Team)

Advanced Bionics was initially our preferred vendor as the PSP speech processor and software tools in our current possession, BEDCS and BEPS, were adequate for reaching our goals for signal processing. With the vendor now uncertain, we have made significant efforts to acquire and learn tools on the Nucleus platform.

Dr. Nie investigated the possibility of generating the frequency-modulated pulse trains described in the proposal. Animal studies have shown that head velocity and acceleration are coded in the frequency of discharge of vestibular fibers. The proposed optimal stimulus is a frequency modulated pulse train at a base rate of 100 pps (pulses/sec). In this feasibility study, the Nucleus Implant Communicator 2 (NIC 2) research interface was used. The interface and its associated software were just released at the October 2006 workshop organized by the Cochlear Corp in Seattle. After acquiring the NIC 2, we spent most of the time learning how to set up the system, initialize communication links and debug codes that can generate the desired stimuli.

In this feasibility test, a PC was connected to the Freedom BTE speech processor through a USB port. RF signals were picked up by the Nucleus CIC4 receiver board for generating biphasic pulse trains. A digital oscilloscope Bitscope BS442 was used to observe and store pulse train signals. Dr. Nie programmed the NIC 2 interface in Matlab to create a sinusoidally frequency-modulated pulse train varying from 40 pps to 160 pps. The Matlab code generated the necessary stimulus sequence, initialized a streaming connection and sent the sequence to the speech processor.

Figure 2 presents the desired pulse rate as a function of pulses (top) and the real stimulation pulse train recorded on electrode 1 (bottom). As can been seen, frequency modulated pulse trains can be successfully generated with the NIC 2 interface so we are confident in our ability to generate the necessary signal processing software to utilize the Nucleus Freedom device as a possible alternative to the Clarion HiRes 90K. However, the Freedom BTE unit limits the total number of uninterrupted pulses (~250) and the minimal pulse rate (~76 pps) due to its small buffer size and low communication speed. The minimum pulse rate limitation can be obviated by utilizing multiple cycles to

generate a single pulse, as is demonstrated below. The more powerful L34 portable speech processor will be available to us in the next quarter. With it, we should be able to generate more sophisticated and continuous stimuli. This will be necessary in order to provide ongoing stimuli to our implanted animals as described in our proposal. In the next quarter we also hoping to receive drawings for an implant device concept from Advanced Bionics as well as determinations from both vendors as to the feasibility of constructing the devices proposed. The small size of the intralabyrinthine electrode array is a challenge to the fabrication processes of both potential vendors. When this information becomes available, we can settle on a final vendor.

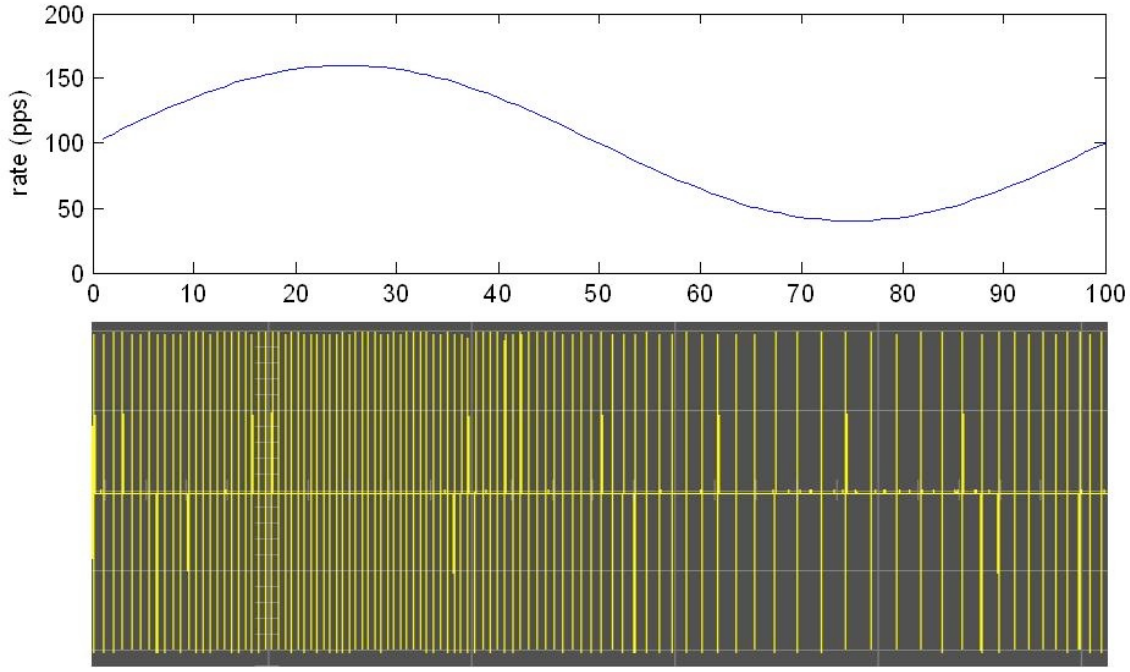


Figure 2*: Pulse rate function (top) and the frequency-modulated pulse train (bottom). As can be seen in this figure, sinusoidal modulation with pulse rates below 76 pps can be achieved with the current processor.*

Additional activities and accomplishments

Multi-channel recording setup (Multiple Single Unit Electrode Development Team)

Although the deep-penetrating NeuroNexus/FHC electrodes were not available in quarter 2, we continued our efforts to develop the hardware and software necessary to record multi-channel neural data. The Multiple Single Unit Electrode Development Team has performed initial testing on the new 16-channel amplifier system from Plexon, Inc. The requirements for this system are very stringent, given the potential for artifacts from the magnetic eye-coil system and the vestibular stimulating electrodes.

The important elements of the Plexon amplifiers are as follows:

1) Unity-gain headstage to prevent front-end saturation by electrical artifacts.
2) DC-coupled inputs to improve common-mode rejection.
3) High-impedance inputs and metal-shielded headstage output cable to minimize noise contamination.
4) 5000-10000 adjustable post-amplifier gain to boost signals prior to computer acquisition.
5) 100 Hz - 8 kHz 6-pole filtering, distributed between the headstage and post-amp. These cutoff values should be sufficient to remove high frequencies produced by the magnetic coil system and low frequencies related to drift and motion artifacts.

We tested the Plexon amplifier system under the same conditions in which recordings will be made in behaving animals, including the presence of a high-frequency magnetic field. We concurrently tested the single-channel amplifier system currently in use in our laboratory. The results are shown in Figure 3.

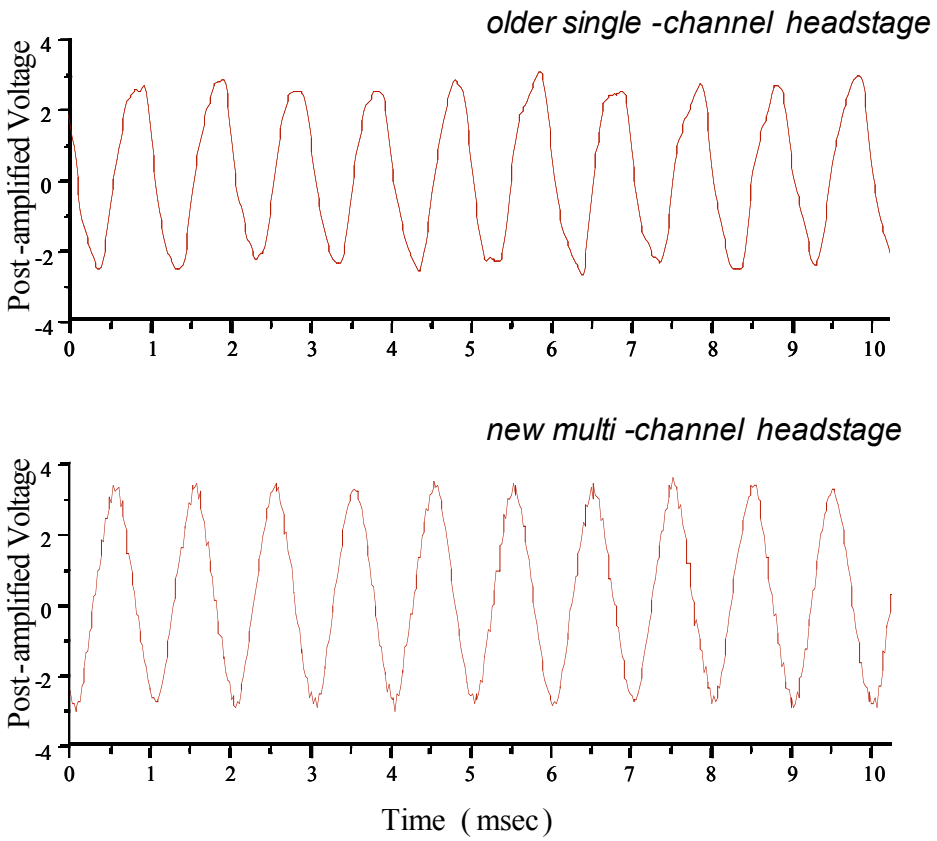


Figure 3. *Comparison of output signals for the single-channel headstage in current use in the laboratory (top panel) and for one channel of the new Plexon multi-channel headstage (bottom panel). Both signals are reasonably uncorrupted by the high-intensity magnetic field utilized in the eye-tracking system.*

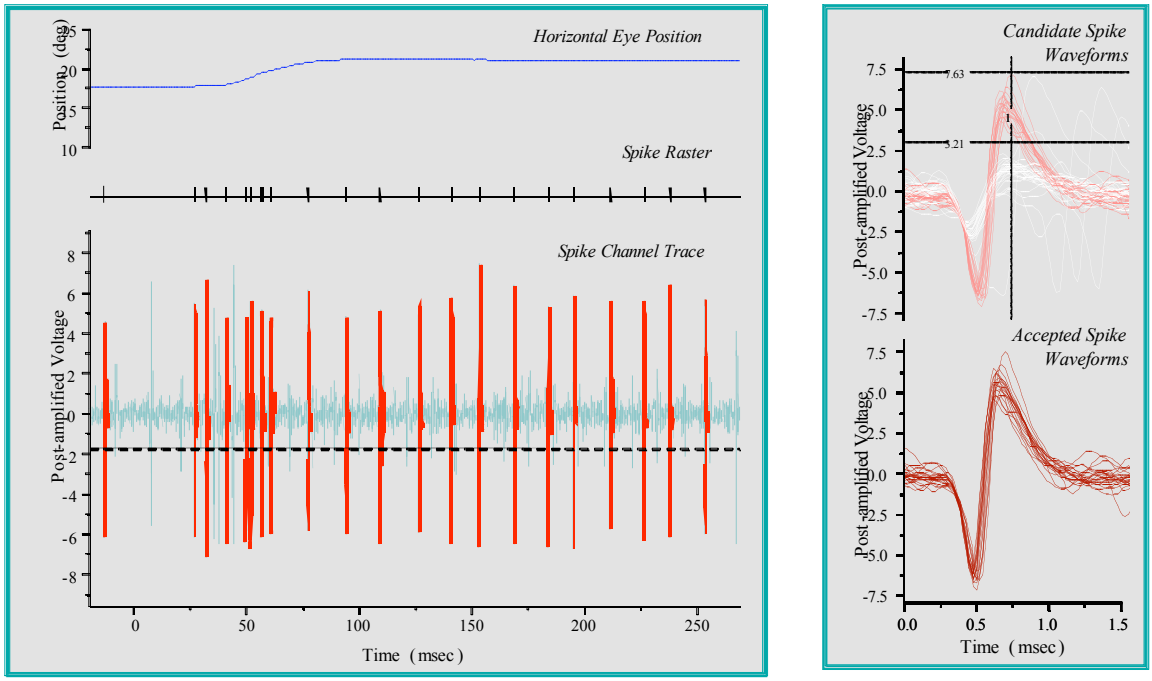
A signal generator producing a low-level 1 kHz sinusoidal signal, of 1 mV peak-to-peak amplitude, was connected to the headstage inputs through a 1 MOhm resistor (taking the place of a high-impedance electrode). After additional amplification and filtering, the resulting 1 kHz component of the signal (*lower trace*) was approximately 6 volts p-p in amplitude for the Plexon system and 5 volts p-p for the older single-channel system (*upper trace*).

Based on the cycle-by-cycle amplitude, the output waveform of the Plexon system was less affected by low frequency noise, which spectral analysis revealed to be harmonics of line noise. On the other hand, the Plexon signal had a somewhat greater noise level at high frequencies. These differences may be attributed to the slightly different cutoff frequencies of the two systems and the fact that the output leads of the Plexon post-amplifier were not shielded. It should be noted that the input signal itself was likely corrupted by noise, inductively and capacitively coupled to the unshielded signal generator leads. This type of noise would be much smaller during actual neural recordings because of the short length of the recording electrode. (By comparison, the single-channel amplifier typically acquires well-isolated action potentials with very little apparent noise.) Overall, the Plexon amplifier system exhibits a suitable signal-to-noise ratio and should perform well in the normal recording environment.

Off-line analysis of neural data (Multiple Single Unit Electrode Development Team and Software Development Team)

Drs. Leo Ling and Steven Bierer have begun to develop off-line software tools to analyze multi-channel spike trains. These tools were created using the Spike2 (Cambridge Electronic Design) programming environment. A critical first-step in the analysis procedure is to extract isolated single-unit spike waveforms (i.e. those belonging to a unique neuron) from every recording channel. Although the timing of spike events is routinely logged during data acquisition, we anticipate several reasons why such on-line spike detection may be unsuitable for multi-channel vestibular nucleus recordings. First, even for single-channel recording, the spike identification process is generally complicated by background noise arising from various sources, especially spikes belonging to neurons other than the target neuron. Second, the position of each recording site on the multi-channel electrode will not be independently adjustable, making the isolation of single-unit spikes difficult and leading to cross-talk between channels. Finally, transient artifacts caused by electrical vestibular stimulation may be "spike-like" in shape, further degrading the ability to detect and discriminate single-unit spikes.

The display portion of an off-line spike detection and sorting program developed by Dr. Ling is shown in Figure 4. The data was acquired from the deep cerebellar nuclei using a single-channel tungsten microelectrode. The figure shows a short time window of the acquired neural signal (*A*) and the waveform shapes of the detected spikes (*B*). Spikes classified as belonging putatively to a single neuron are highlighted (see figure caption for details). This software is currently being expanded to accept more than one spike waveform shape, handle multi-channel signals, discriminate overlapping spike waveforms, and remove transient artifacts caused by electrical stimulation.



(A) (B)

Figure 4. *Demonstration of the off-line software for spike classification. (A) Single trial of neural data recorded from the cerebellum of a rhesus macaque using a tungsten electrode (lower trace), showing a mixture of small and large spike events. The dashed line is the user-chosen threshold for spike detection. Detected spikes subsequently classified as belonging to a single neuron are highlighted in red (and indicated by the vertical markers in the middle trace). For reference, the animal's eye position is also displayed (top trace); the eye signal indicates that a horizontal saccade occurred around the 50 ms time mark, correlating with a burst of spikes. (B). The top panel shows all of the candidate spike events triggered by the threshold in A; the event waveforms which pass through a pair of user-chosen discrimination levels (horizontal dotted lines with number labels) at a specific time point (vertical dotted line) are accepted for further analysis. These spikes are shown in light red, while all of the other "rejected" events are shown in white. The discriminated spikes are also shown by themselves in the lower panel. Note that the rejected events consist mainly of small spike waveforms (likely produced by one or more other neurons) some of which overlap with the larger spike waveforms.*

Data collection software (Software Development Team)

In the second quarter, we made several significant changes to the data acquisition and analysis programs to allow for multiple single unit recording and analysis during natural and electrical stimulation. These changes are summarized as follows:

1) A setup menu that provides the choice of 1-channel or multi-channel neural recording, up to a present total of 16 channels
2) An additional graphic panel that displays the ongoing acquired activity, as a multi-channel oscilloscope
3) A menu button to choose which of the channels should be highlighted for spike detection, spike waveform display, and further on-line analysis
4) An on/off button to toggle the status of saving signals to the hard drive; this option will help reduce the amount of data to process off-line

Primate surgery and training (Behavioral and Neural Recording Team)

In the past quarter, two rhesus macaque monkeys were surgically implanted with eye coils and head restraints. These implants are necessary for maintaining a fixed head position and monitoring eye movement during behavioral and neural recording experiments, and during behavioral training. Both animals were preadapted to the laboratory environment prior to implantation for several weeks During this time, the general health and weights of the animals were closely monitored, and surgical implantation was not performed until the animals successfully transitioned to the lab environment while maintaining a normal growth curve. Both animals have begun preliminary behavioral training under the supervision of Monica Ibarreta. The purpose of the training is to further acclimate the monkeys to the experimental environment and to train them to perform the behavioral tasks reliably over each experimental session.

Overall Progress:

We have made good general progress toward the objectives of this contract. We have implanted two monkeys with the hardware necessary for behavioral training and recording of eye movements, and we have begun training these animals. Much of the hardware required for multiple single unit recording is in place ahead of schedule and testing of that hardware has been initiated. Software development for both stimulation and recording is progressing satisfactorily. Our greatest challenges in the next quarter will be related to the acquisition and testing of the implantable stimulation and recording devices. We have been promised beta test versions of the multiple single unit recording electrodes and should be able to begin evaluation of these in the third quarter.