
Fourth Quarterly Progress Report

May 1, 2007 to July 31, 2007

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

Neurophysiological Studies of Electrical Stimulation for the Vestibular Nerve

Submitted by:

James O. Phillips, Ph.D.[1,3,4]
Steven Bierer, Ph.D. [1,3,4]
Albert F. Fuchs, Ph.D. [2,3,4]
Chris R.S. Kaneko, Ph.D. [2,3]
Leo Ling, Ph.D. [2,3]
Kaibao Nie, Ph.D. [1,4]
Jay T. Rubinstein, M.D., Ph.D. [1,4,5]

[1] Department of Otolaryngology-HNS, University of Washington, Seattle, Washington
[2] Department of Physiology and Biophysics, University of Washington, Seattle, Washington
[3] Washington National Primate Research Center, University of Washington, Seattle, Washington
[4] Virginia Merrill Bloedel Hearing Research Center, University of Washington, Seattle, Washington
[5] Department of Bioengineering, University of Washington, Seattle, Washington

---

Reporting Period: May 1, 2007 – July 31, 2007

(1) Stimulation Device Development

In the past quarter we received a prototype of the UW/Cochlear primate vestibular implant (see Addendum). This was surgically placed in the posterior semicircular canal of a macaque temporal bone and several design modifications were defined. These modifications were communicated to Cochlear Corporation who provided us with modified device drawings (see Addendum). These appear to be appropriate based on our temporal bone dissections and implantation of the prototype device. Cochlear Corporation believes they can provide us with the first sterile devices toward the end of September, 2007. Negotiations over the contract for providing these devices with the University of Washington are ongoing. We are tooling the Primate Center operating room for the first surgery anticipated in early October, 2007. The Anspach Corporation has agreed to provide the project free of charge, an appropriate high-speed surgical drill for the procedures.

(2) Software Interface Development

We have continued development of the software interfaces for both stimulation and recording. We have revised the SacTrack software interface (written for Spike 2) for recording and stimulus control through the CED Power 1401 hardware interface so that it allows viewing of multiunit data during recording. The revised version of SacTrack also now includes a provision to pause data collection to disk, allowing for more flexible recording of multiunit data.

(3) On-line Control of Stimulation

We have continued working on the software programming to generate patterned stimuli. This software will allow us to intensively explore the electrical stimulation parameter space appropriate for exciting vestibular nerves. The software is now fully functional. It is able to generate biphasic pulse train bursts with varying amplitudes, rates and durations. It is also capable of producing either amplitude-modulated (AM) or frequency-modulated (FM) pulse trains at various modulation rates and modulation depths.

We have evaluated the functionality of the software with the Spike 2 neural recording system and SacTrack interface software described above. The pulse train output from the Nucleus implant-in-a-box and the trigger output from the Nucleus programming pod were fed into the analog input channels of the CED Power 1401. We recorded the desired pulse train signals specified by the programming interface. In addition, a synchronized trigger signal was generated at the first biphasic pulse of each trial, with fixed duration and amplitude. These trigger signals will be used as stimulation events to retrieve actual pulse sequence, analyze neural spikes, and remove stimulation artifacts. The software will be fine tuned early in the next quarter, to prepare for the forthcoming neural stimulation and recording experiments in implanted monkeys.

(4) Multi-channel Recording Device Development

This quarter we submitted a mask design to NeuroNexus Technologies to initiate production of electrodes suitable for deep-brain recording in primates. The fabrication and packaging of these custom Axial electrodes is expected to be completed in the next quarter. The mask layout at the tip of the electrode (shown in Figure 1) meets all of our requirements for recording in the vestibular nuclei. The 12 recording sites span approximately 850 μm, long enough to permit simultaneous recordings from neurons in different prescribed regions of the nuclei. In addition, the sites are clustered in threes to increase the chance of recording from each area. These "triodes" should also facilitate the assignment, or sorting, of spike waveforms to different single neurons, as proposed for four-channel tetrode recordings. The overall length of the Axial electrode, 140 mm, is sufficiently long to reach the deep brainstem structures and fit securely into an electrode holder designed for tungsten microelectrodes.

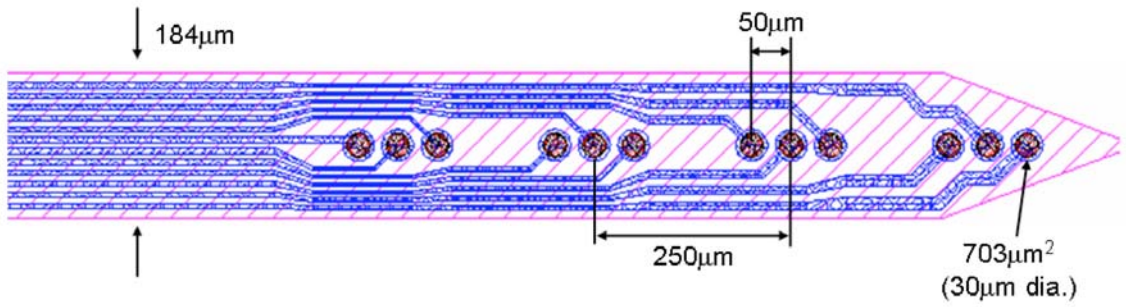

Figure 1. *Schematic layout of a NeuroNexus Axial electrode, designed for recording in the primate brainstem. 12 recording sites are arranged in four groups of three, each "triode" separated by 250* μm. *Total length of the device, from tip to connector, is 140 mm. The tip itself is a functional tungsten microelectrode, providing a 13th site.*

Certain aspects of the Axial electrode design reflect resolution limitations of the microfabrication technique. These include the width of the individual conducting leads and the diameter of the electrode contacts. Nevertheless, even with 12 leads, the device is only 184 μm wide and should fit easily into the 500 μm I.D. metal cannulas used to protect the electrode during brain penetration.

As a second option for multi-channel recording, we have received and tested tetrodes from the Thomas Recording company (Giessen, Germany). These 4-channel electrodes meet many of the same criteria as the Axial electrode. In particular, we have specified their length to 130 μm, suitable for recording from the vestibular nuclei. Because the insulating quartz shaft is thin and very flexible, we found it difficult to handle without the optional stainless sheathing to support the electrode shaft. The sheath is narrow enough

(~250 µm) to fit inside our cannula system, but it does not cover the portion of the electrode that passes from the end of the cannula to penetrate brain tissue.

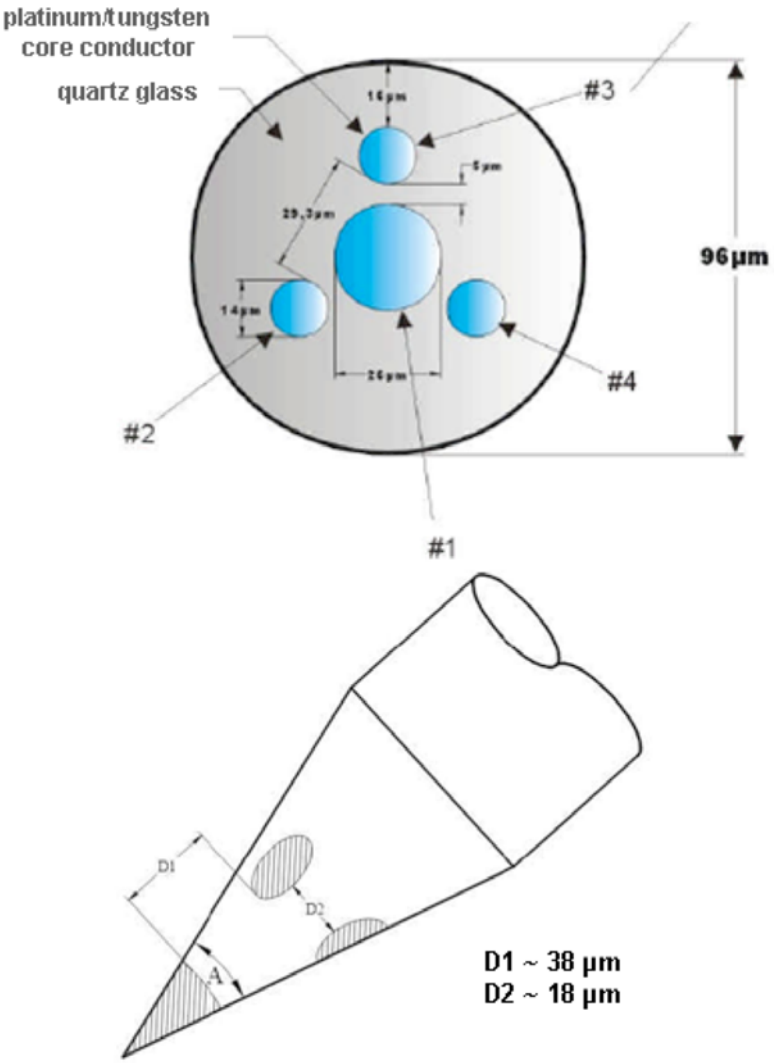

Figure 2. *Schematic drawings showing the cross-section and profile dimensions of the Thomas Recording tetrode device. Total length of the quartz shank is 130 mm.*

As shown in Figure 2, the four sites are grouped at the end of the electrode, one at the tip and the others embedded around the diameter of the shaft just above the tip. The sites are spaced closely together, making it likely that the same unit can be recorded on multiple channels. As described in the next section, we have successfully used the Thomas Recording tetrodes to record from multiple units in the cerebellum, including units responsive to head rotations.

(5) Multichannel Unit Recording

In this section we describe our initial experiments with the Thomas Recording tetrodes. The recordings were obtained in a rhesus macaque monkey trained to fixate its gaze on a visual spot target (see QPR 2). The target region was the cortex of the cerebellum, in which specific regions contain neurons that are responsive to eye4 and or head movement. Initial impedance measurements, which ranged from 1.5 to 2.5 MOhm, were high compared to the single-channel tungsten electrodes commonly used in our laboratory. The first attempt at recording with these electrodes was unsuccessful, yielding only background neural activity and some artifact related to the animal's movements. For subsequent experiments, we first electrolytically cleaned each electrode site in saline by passing enough anodic and cathodic DC current to evolve gas. We next plated with iron, using a ferric sulfate solution (SIFCO, Cleveland, OH) and applying 1.5 volts across each site. The impedance of each site typically dropped an order of magnitude, ranging from about 80 kOhm to 300 kOhm. This plating procedure is now repeated prior to each recording session.

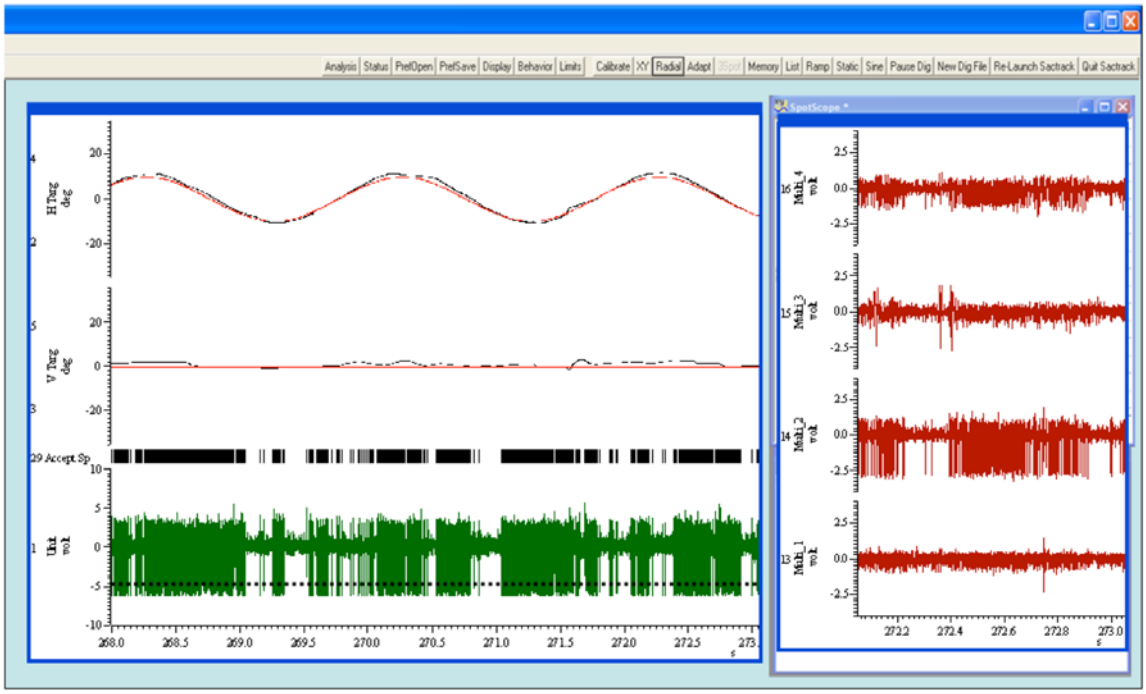

Figure 3. A screen shot of the SacTrack user interface used for data collection in the laboratory.

Figure 3 depicts the revised user interface, "SacTrack", described in section 2 above and designed to collect multi-channel neural data with the Spike2 data acquisition system (Cambridge Electronic Design, Cambridge, UK). The data shown were collected in the cerebellar cortex while the animal's chair and visual target were counter-rotated, eliciting a visually enhanced VOR response. A 1 s interval of recording from all four recording channels is displayed in the right panel. The channels are numbered 1 to 4 from bottom

to top. In this recording session, the site at the tip of the tetrode is represented in the third trace from the top (channel 2). This channel has been selected for real-time spike triggering, with a threshold defined by the dotted line, and is redisplayed, at a broader time scale (5 s interval), in the bottom trace of the left panel. The visual target and eye movements are also shown in this panel (horizontal in the top set of traces, vertical in the second set of traces). Also shown is a series of acceptance pulses indicating the triggering of selected spikes. In this particular example, there is no clear correlation between eye movements and spike firing.

Note in the right panel of Figure 3 that the four electrode channels record spikes that are common to two or more channels (e.g. channels 2 and 4), as well as spikes that appear primarily on only one channel (e.g. isolated spikes on channels 1 and 3). Thus, the tetrode is capable of increasing the yield of isolated single-units compared to a single-channel electrode, while providing an opportunity to improve spike sorting by the comparison of spike waveform shapes across channels.

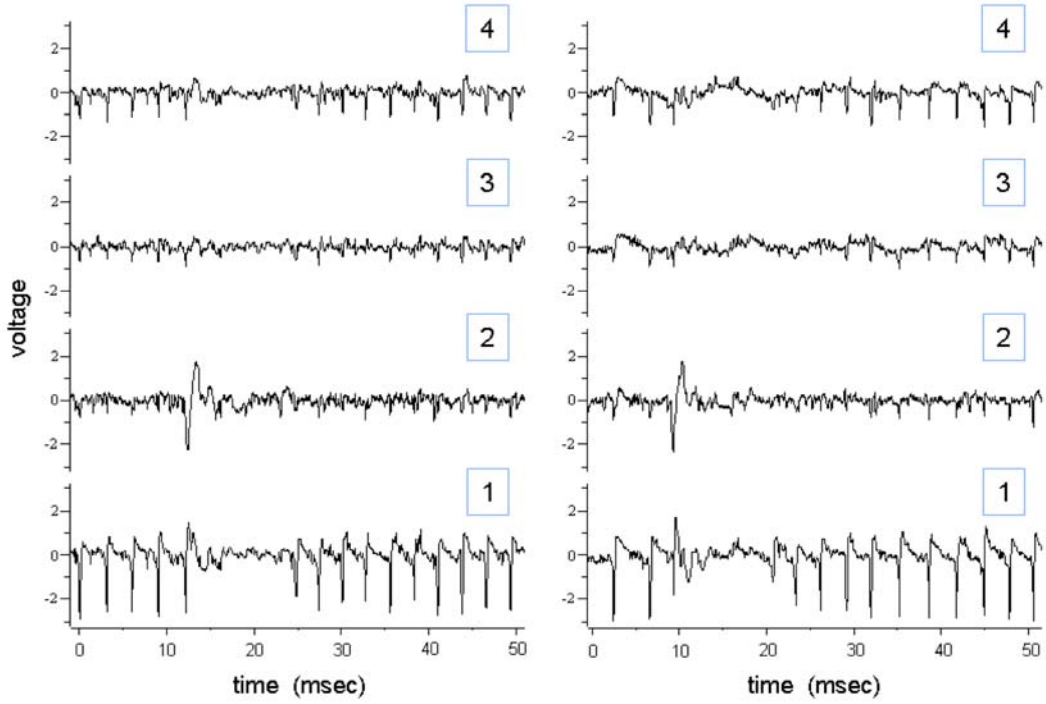

Figure 4. *Fine temporal resolution multichannel tetrode recording in our laboratory. Channels are indicated by the boxed numbers 1-4.*

Another example of tetrode recording is shown in Figure 4. As in the previous figure, the recordings were obtained from a region of the cerebellar cortex. The left and right panels display two 50 ms time windows recorded 2 s apart. In this case, the tip channel appears in the bottom trace (channel 1). Two primary waveform shapes are apparent. In channel 1, the spikes are biphasic in shape, negative phase first, and occur at fairly regular intervals. Smaller versions of these “simple” spikes appear on channel 4 as well,

suggesting that the corresponding site on the tetrode is physically close to the same neuron. On channel 2 a single large spike is evident in each time window. These spikes are multi-phasic in shape, and appear at a smaller magnitude on channel 1. Each of these "complex" spikes is followed by a 10 ms cessation of firing by the simple spike. Our observation is that the simple and complex spikes appear with different spatial patterns across the tetrode channels, which is consistent with their different biophysical origins.

(6) Modeling and 3D histologic reconstruction:

We have been working on developing the tools necessary for 3D histologic reconstruction and visualization of the vestibular end organ. This will be necessary for confirmation of the integrity of the vestibular end organs following prolonged stimulation, and following administration of gentamicin to eliminate vestibular hair cells, in addition to more precise traditional micro-histological assessment of the sliced tissue. Also, such reconstructions allow for the possibility of modeling current distribution using data driven modeling assumptions.

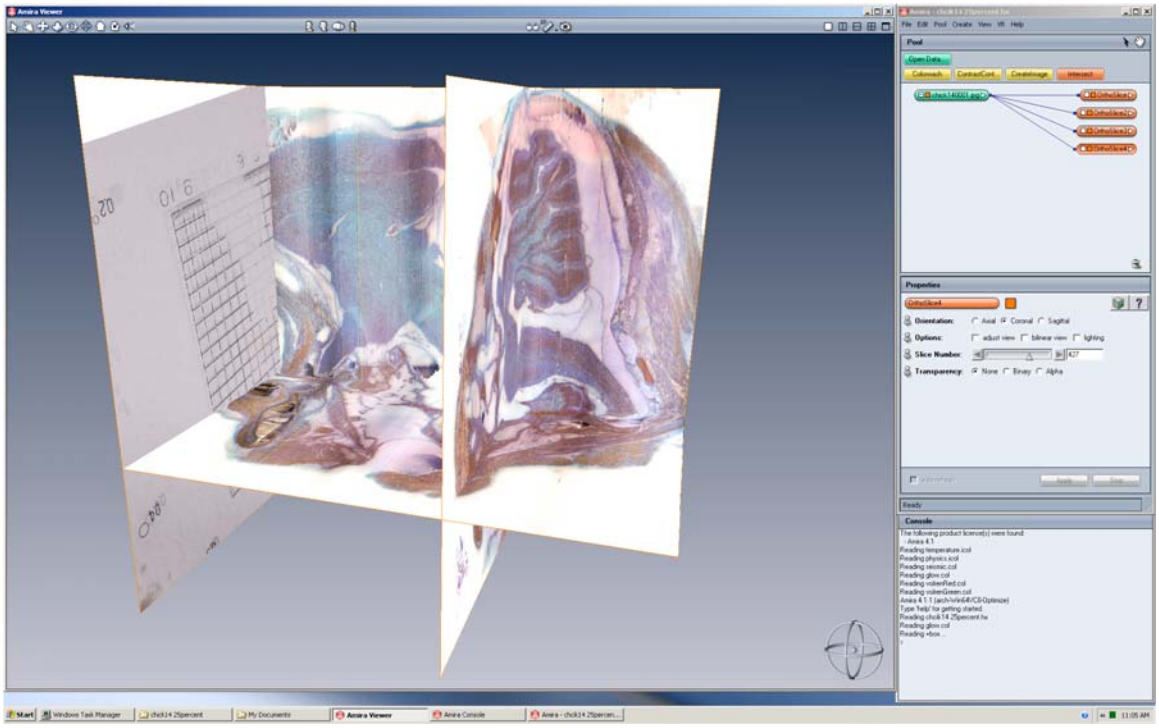

Figure 5. *A screen shot from the Amira user interface showing 3 dimensional reconstruction of guinea pig temporal bone.*

Our current approach to 3D reconstruction is to utilize guinea pig temporal bone sections obtained from direct imaging of the blocked tissue on a microtome. This way the sections are available for further histologic assessment and the 3D reconstruction can proceed rapidly. For reconstruction, we have utilized Amira software and photomicrographs obtained with a Nikon high-resolution imaging system. The resulting 3D image reconstruction is displayed in Figure 5.

(6) Dissemination of Information:

An important continuing objective of the contract is the dissemination of information to clinical and research groups locally and nationally. During the fourth quarter, we have continued our local presentations with a comprehensive update to the Clinical Vestibular Disorders Group, which meets monthly. In addition, we have had two related presentations to the research meeting for affiliates of the Neuroscience Division of the Washington National Primate Research Center. We have implemented I-Visit, which is now available for virtual conferences. We have continued to supplement our contract Web site with downloadable information. The site now lists 28 separate computer programs, each developed through the contract, that are available for unrestricted download. We have submitted two abstracts on topics related to the project.

Steven M. Bierer, Leo Ling, Kaibao Nie, Jay T. Rubinstein, and James O. Phillips Design and Validation of a vestibular prosthesis. NWAVM, 2007

David M Harris, Steve Bierer, Jonathon Wells, James Philips Photonic stimulation for a vestibular prosthesis. SPIE, 2008

The first of these abstracts describes the approach that we have utilized and our current progress. The second abstract compares and contrasts our approach and the challenges we face with that of optical stimulation, and the challenges that entails.

We continue to maintain an active dialog with other researchers in the neural prosthesis program. We have had visits from John Middlebrooks Ph.D. and Claus-Peter Richter, M.D., Ph.D. Both have provided valuable input on unit recording and alternative approaches to stimulation. In addition we have had productive discussions with Philipos Loizou, Ph.D. about the use of PDA based processing to address challenges in the implementation of real time processing strategies. After the end of the fourth quarter, but prior to the completion of this report, we had a site visit from Roger Miller. Ph.D. who provided us with a clear definition of future priorities for Quarter 5.

(7) Overall Progress

We have now implemented multiple single unit recording in the laboratory utilizing tetrode technology. The rhesus monkey subjects of these experiments are fully trained. All of the recording and analysis tools are in place. We have finalized the design of our stimulation implant, and expect receipt and implantation of the device in Quarter 5.

We have made plans for an alternative stimulation technology if the preferred approach is unavailable in Quarter 5. We continue to pursue our negotiations with NeuroNexus/FHC and expect to receive multiple single unit recording Axial electrodes in Quarter 5.

(8) Future Emphasis

We have a clear direction for the future and plan for accomplishing our objectives.

a. In recognition of the importance of proceeding with electrical stimulation we have committed to a "drop dead" date for receipt of the stimulation implant from our commercial suppliers. That date is September 30, 2007. After that date we will proceed with implantation of locally manufactured electrodes with the use of a connector head plug embedded adjacent to the recording chamber. The stimulation paradigms will be implemented with the use of the research interface provided by the implant manufacturer, so in all other regards the stimulation experiments will proceed as planned.

b. We will continue to develop and optimize the full stimulation prosthesis with the intention of implanting that device when it becomes available. We currently have a commitment from the manufacturer to provide devices prior to September 30, 2007, but if that date slips we will proceed with the stimulation experiments.

c. We will continue to develop the multiple unit deep recording technologies. However, in the next quarter our primary objective is to evaluate electrical stimulation. To accomplish this objective in the most direct manner available, we will focus first on behavioral recording in stimulated animals and second on single unit recording from traditional tungsten microelectrodes. Our objective will be to integrate multiple single unit recording into our experiments at later date, after our stimulation experiments are well under way.

d. We will continue to provide software for download and updated schematics of technologies developed on this contract through our Web site. We will modify the site so that the interface tracks the use of the information and provides a description of the information, increasing its utility to the community.

Addendum A (CONFIDENTIAL: This addendum is for the exclusive use of the Project Officer and should not be published.)

Revised engineering design of the Cochlear Corporation Final Vestibular Prosthesis (scheduled to ship prior to September 30, 2007).