Seventh Quarterly Progress Report

February 1, 2008 to April 30, 2008

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Neurophysiological Studies of Electrical Stimulation for the Vestibular Nerve

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Accomplishments for Quarter 7:

1. **We have now manufactured 7 devices based on existing Cochlear Corp. vestibular implant design.**
2. **We have 6 animals at various stages in the process of outfitting and testing.**
3. **We have produced software to analyze data obtained from multiple unit studies.**

We have continued refining the spike sorting procedures for single-channel and multi-channel recordings. These tools are necessary to properly detect and identify the occurrence of action potentials produced by an ensemble of simultaneously recorded neurons. As reported in QPR 5, our approach is to create a set of templates, each representing the prototypical spike waveform of a single neuron. These templates are created from a subset of threshold-triggered events that have similar waveform shapes (via principal components) and peak-to-peak amplitudes. A set of "overlap" templates is also derived from the time-shifted addition of any two templates. Every candidate spike event is then matched to the best-fitting single-neuron template or overlap template.

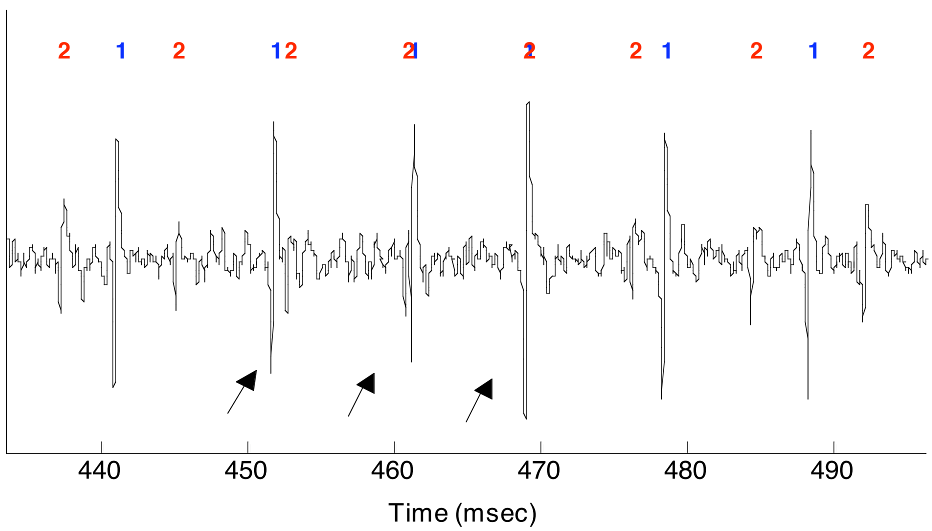


Figure 1. Classification of spikes from two oculomotor neurons using the overlap template method. Arrows indicate overlapping spike waveforms as identified by our overlap template.

Figure 1 demonstrates the results of applying our template-matching technique to spike data recorded from the hindbrain of a rhesus macaque. Shown is a 60 ms section of the raw voltage trace during a period when the animal's eyes were fixated on a visual target. At least two classes of spike waveforms are apparent, judging from the peak-to-peak magnitudes. The regular firing of the small and large spikes, characteristic of the vestibular and oculomotor neurons found in this region of the brain, suggests that the spikes were produced by two neurons. Because the firing rates of the two neurons were different, the spikes occasionally overlapped (arrows), producing distorted waveform shapes. Nevertheless, the overlap-template procedure properly handled the overlaps and accurately classified every spike event.

4. **We have produced software to allow stimulation of multiple electrode arrays using interleaved stimulus pulses.**

The previous pulse train generator only permits single-channel stimulation in one experimental run. In order to program up to 3 channels simultaneously, we significantly changed the data structure, Matlab codes and Python codes of the old software. We created a number of new Matlab and Python programs to do multichannel stimulation. In the new software, the stimulation pulse train on each channel is set separately and then all 3 pulse trains are merged into one interleaved train. Since our vestibular implant is based on a Cochlear Corp. Nucleus implant that only has a single current source, pulses from different channels should be interleaved in time. A search-and-delay algorithm was used to combine conflicting pulses from different channels. All three channels can be programmed with either unmodulated, amplitude-modulated or frequency-modulated trains respectively (Figure 2). The pulse generator also supports bipolar and monopolar mixed stimulation on different channels.

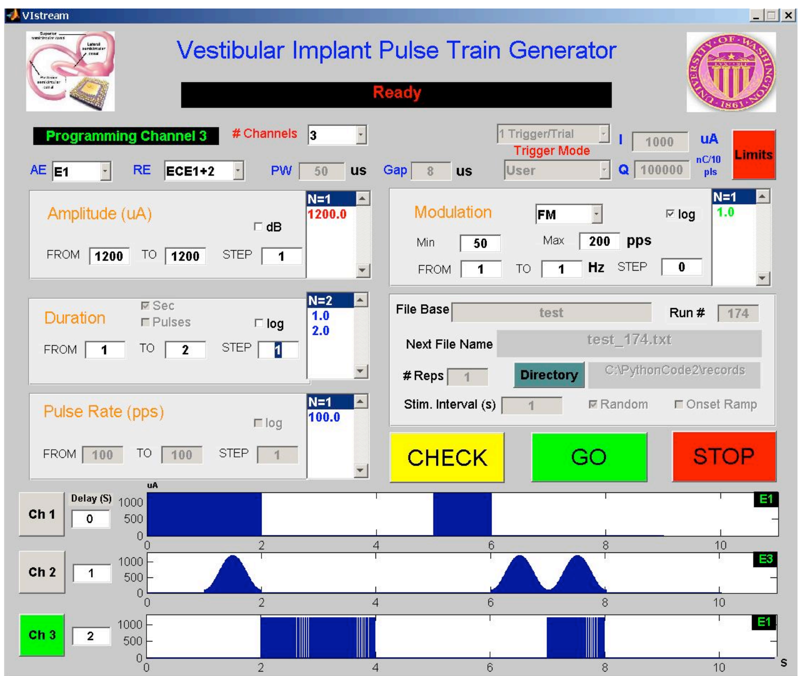


Figure 2. New user interface allowing independent control of three channels of simultaneous stimulation.

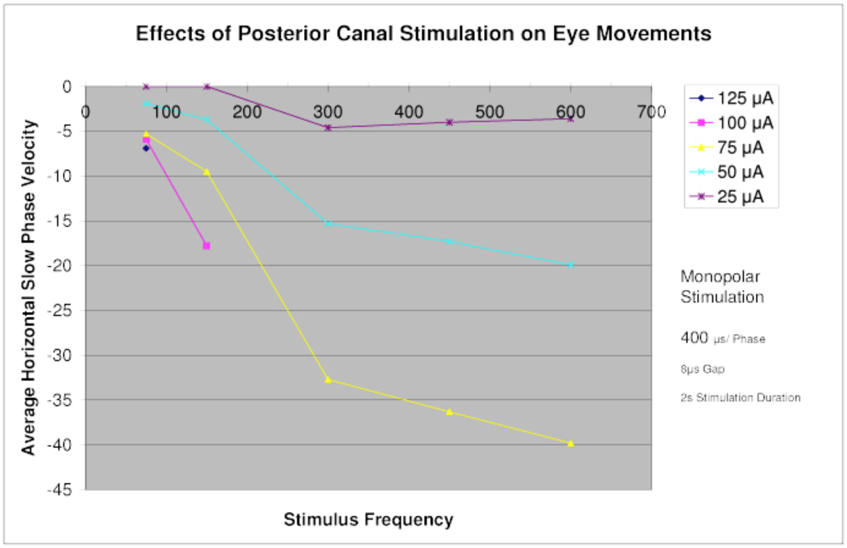
5. **We have produced software to allow prolonged stimulation using programming loops in Python.**

We utilized the loop definition in Python to perform continuous stimulation without having to reload the buffer on the L34 processor. This feature provides up to several hours of uninterrupted stimulation depending on the specified pulse train pattern. The continuous operation mode will allow us to explore the long-term adaptation properties of vestibular implant stimulation.

6. **We have successfully revised a single canal device to a six-channel two-canal device.**

Pertinent surgical findings at the time of revision included new bone formation sealing the site of the prior labyrinthotomy in the lateral semicircular canal around the electrode, which was intralabynthine. A new lateral semicircular canal labyrinthotomy was performed much closer to the ampulla by fenestrating just medial to the incus. The posterior canal was fenestrated just medial to the descending facial nerve and two three-electrode arrays were inserted into the labyrinthotomies. A blood seal was allowed to form prior to soft-tissue closure.

7. **We have demonstrated that the position of the electrode insertion is critical to the performance of the device.**
    a. **Initial implantation away from the ampulla of the lateral canal produced small responses at high current thresholds that were not in the canal plane. These were associated with limited dynamic range (current limits and recruitment of facial nerve were a problem)**
    b. **Revision of the electrode placement closer to the ampulla produced:**
        i. **Thresholds an order of magnitude lower.**
        ii. **No cross talk to the facial nerve.**
        iii. **Large, higher velocity nystagmic eye movements.**
        iv. **Slow phase velocities that scale with the frequency of stimulation (Figure 3).**
        v. **Slow phase velocities that scale with the stimulus current (Figure 3).**
        vi. **Velocities greater than >50°/s and amplitudes >10° (Figure 4).**



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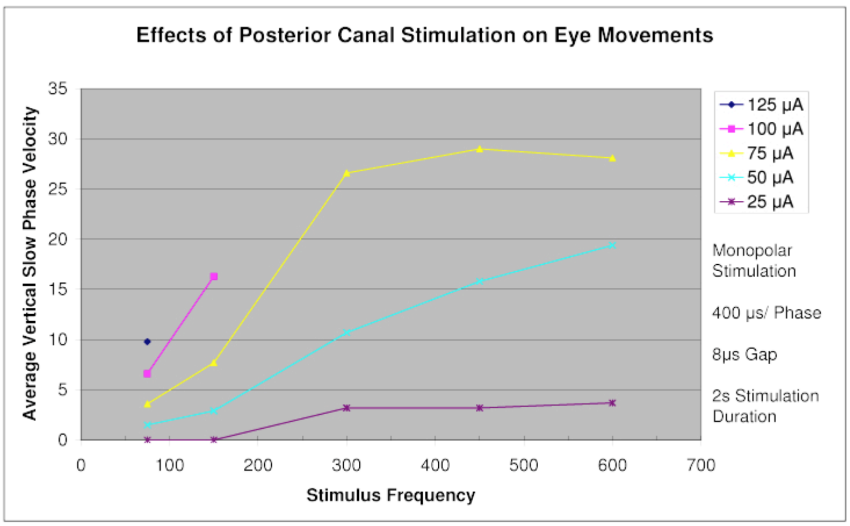

B

Figure 3. Slow phase eye velocity versus stimulus frequency for different stimulus currents during brief (2 s) monopolar stimulus trains with pulses at 400 μs/phase, 8μs gap. A) Horizontal slow phase eye velocity. B) Vertical slow phase eye velocity.

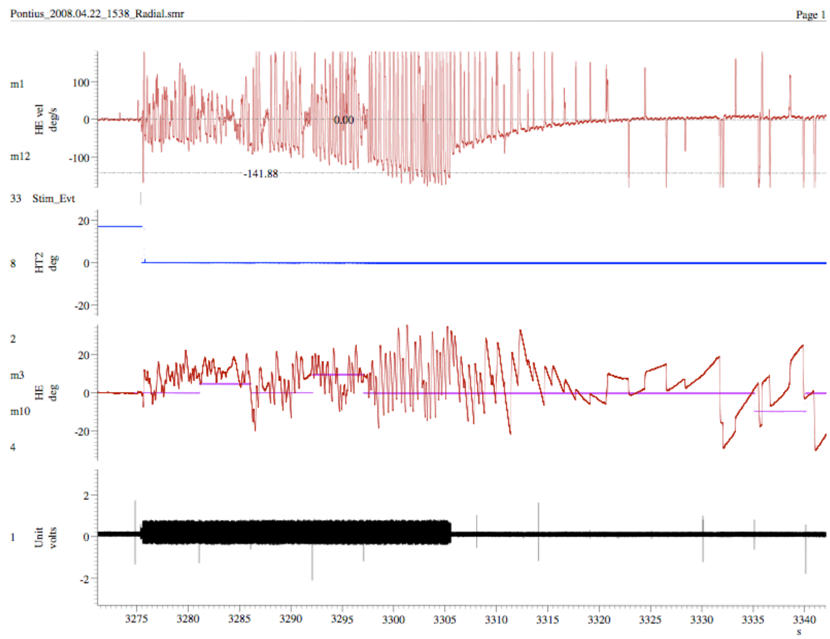

Figure 4. 30s biphasic stimulation (thick bar, bottom trace) at 600 Hz with the target off (upper middle trace) showing high velocity (top) and large amplitude (lower middle) nystagmus, afternystagmus, and after-after nystagmus.

8. **We have successfully implanted a second six-channel two-canal device.**
9. **We have successfully implanted and then used a chamber-based system for stimulation of the vestibular nerve, vestibular nucleus, and extraocular motor nuclei.**
10. **We have confirmed the integrity of the vestibulo-ocular reflex in all implanted animals.**
11. **We have demonstrated that we can record from and stimulate the nerve and brainstem.**
12. **We have demonstrated that we can simultaneously record from and isolate multiple single units in the hindbrain.**
13. **We have implanted multiple canals in individual animals.**
    a. **The revision surgery implanted the posterior and lateral canals, as did a second initial surgery.**
    b. **The animals retained VOR function post surgically.**
14. **We have demonstrated that canal stimulation produces directionally appropriate behavioral effects (i.e., implantation of the lateral and posterior canals produces horizontal and oblique movements from each of the two electrodes, respectively).**
15. **We have demonstrated that changes in frequency and current do not alter the direction of the responses elicited by stimulation of a single canal across a**

significant dynamic range, but thereafter produce eye movements consistent with activation of multiple canals.

16. **We have demonstrated that prolonged bipolar stimulation produces nystagmus, afternystagmus, and after-after nystagmus consistent with functional reflexes (Figure 4). Monopolar stimulation produces high velocity slow phases but little after nystagmus.**
17. **We have demonstrated that the effects of stimulation are context dependent (e.g. related to eye position) but cannot be fully suppressed volitionally.**
18. **We have demonstrated that downstream stimulation produces context dependent behavioral responses, suggesting that agonist – antagonist co-contraction may influence behavioral responses.**
19. **We have demonstrated that stimulation of 5 minutes produces adaptation only at higher frequencies.**
20. **We have examined the time course of adaptation.**
21. **We have demonstrated that repeated trials of the same stimulus parameters produce habituation of the response.**
22. **We have demonstrated that sinusoidal modulation of stimulus frequency (Figure 5) or current (Figure 6) produces a sinusoidally modulated eye velocity. There is a velocity bias consistent with the known physiology of the system (Figures 5 and 6).**

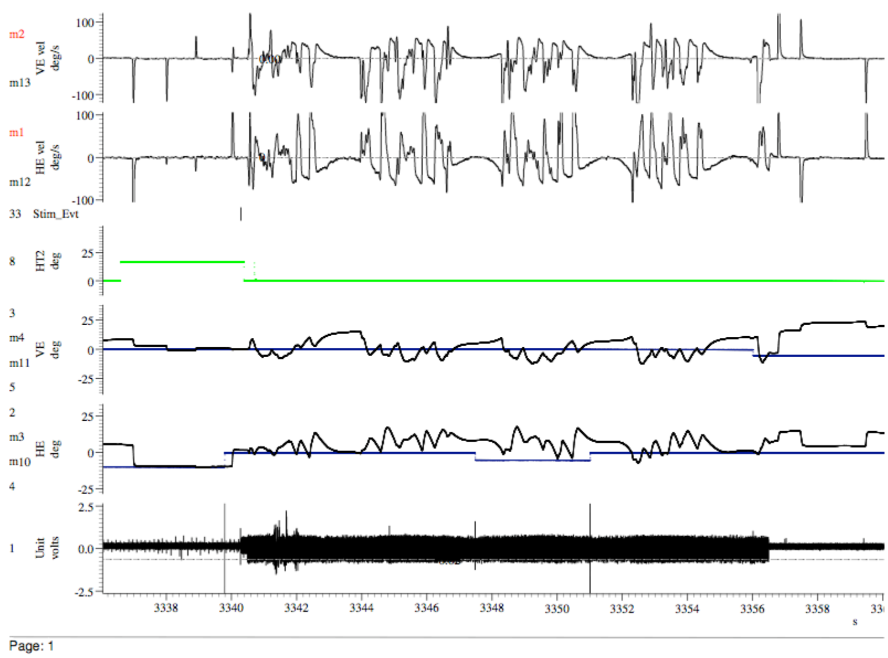

Figure 5. A sinusoidally frequency modulated stimulus train (bottom trace) with the fixation target extinguished (middle trace) evokes sinusoidally modulated slow phase eye velocity (upper traces) and large changes in eye position (lower traces). Note that eye velocity does not cross the zero velocity line (upper traces).

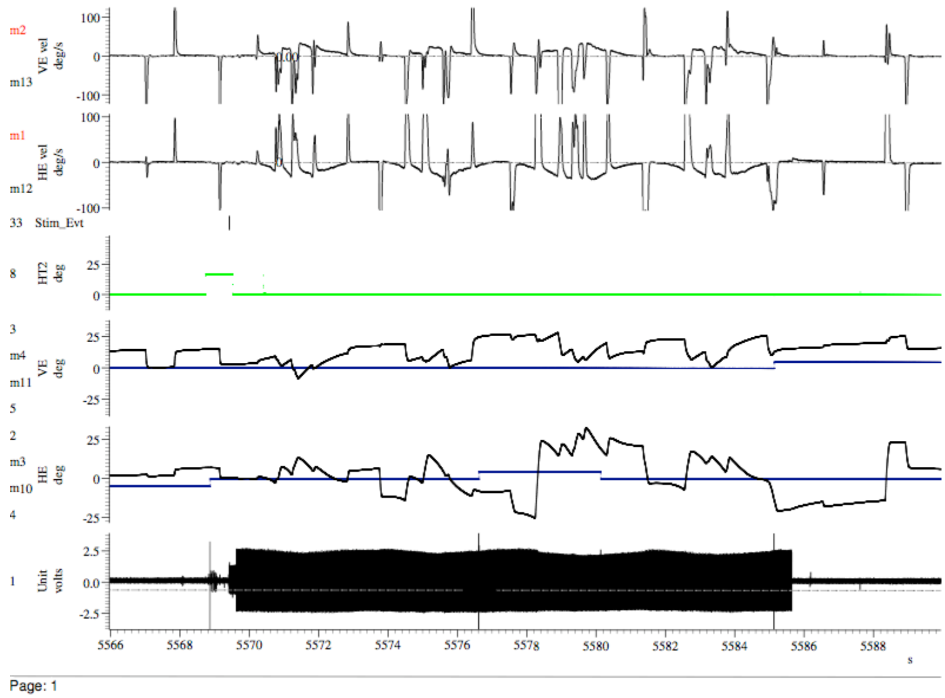

Figure 6. A sinusoidally amplitude modulated stimulus train (bottom trace) with the fixation target extinguished (middle trace) evokes sinusoidally modulated slow phase eye velocity (upper traces) and large changes in eye position (lower traces). Note that eye velocity does not cross the zero velocity line (upper traces).

23. We have created and validated techniques to obtain primate ABR to confirm the unilateral integrity of the auditory system (behavioral techniques can only reveal binaural responses in awake animals).

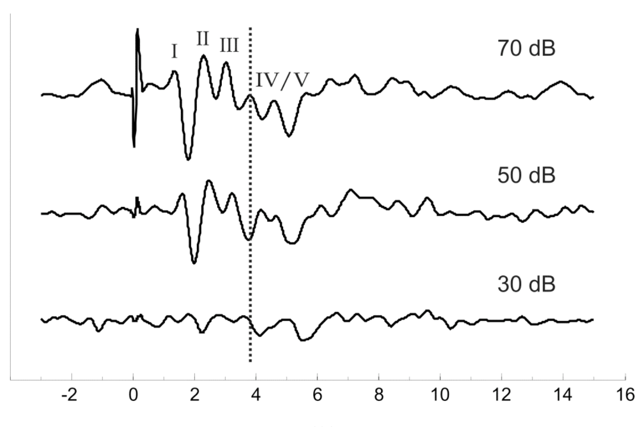

Figure 7. Auditory brainstem evoked potentials recorded in a monkey in response to 1000 click stimuli presented at different sound levels.

We have performed preliminary studies to show the feasibility of obtaining reliable oculomotor and auditory recordings from juvenile monkeys under a chronic monitoring protocol at the Infant Primate Research Laboratory. Figure 7 shows a series of ABR waveforms obtained in a male 44-day old pigtailed Macaca nemestrina. After sedation with Telazol, subdermal needle electrodes were placed on the supraorbital ridge and at the left and right mastoids. A calibrated earphone, driven by an M-audio Delta Series sound card and controlled by custom software, was placed loosely in the left ear canal. The evoked potentials were measured between vertex and the left mastoid and amplified (10,000 gain, 100-3000 Hz bandpass filtering). The stimuli were 100 μs clicks delivered at a rate of 10.7 per second. The resulting waveforms are averaged over 1000 clicks. At 70 dB SPL, at least 5 distinct positive-negative waves are seen following the initial artifact. The most prominent of the positive peaks are labeled according to the conventions of other investigators. At progressively lower click intensities, the amplitudes of all the peaks decrease and the latencies increase, as observed in previous studies.

24. **We have worked with the group of Philipos Loizou (Open Architecture Research Interface for Cochlear Implants - N01-DC-6-0002) to implement a PDA based programming strategy for our animals.**

25. **We have submitted 3 abstracts based on our ongoing work:**

    1. Phillips, J.O., Bierer, S., Fuchs, A.F., Kaneko, C.R.S., Ling, L., Nie, K., Oxford, T., and Rubinstein, J.T. A multichannel vestibular prosthesis based on cochlear implant technology. Society for Neuroscience 2008, submitted

    2. Bierer, S.M., Ling, L., Phillips, J.O. A template-based spike sorting technique to resolve temporally overlapping spike waveforms. Society for Neuroscience 2008, submitted

    3. Phillips, J., Bierer, S., Fuchs, A., Kaneko, C., Ling, L, Nie, K., Rubinstein, J. A minimally invasive prosthesis for electrical stimulation of individual canal channels in the vestibular nerve. CORLAS 2008, submitted

Objectives for Quarter 8 :

1. **We will implant multiple canal arrays in all 6 of our monkeys.**
2. **We will implant recording chambers in all of our monkeys.**
3. **We will reproduce the behavioral recording and stimulation studies in all animals.**
4. **We will extend the behavioral recording experiments to include multichannel stimulation to look for canal interactions.**
5. **We will extend the behavioral recording experiments to look at combined natural and electrical stimulation.**

4. We will begin stimulation and multi-channel neural recording experiments in 2 animals.

5. We will begin ABR recording in all animals after receiving IACUC approval for addition of this procedure to our protocol.

6. We will accept delivery of and begin local development of software for PDA based stimulation paradigms.

7. We will submit two manuscripts for publication in peer-reviewed journals.

Challenges and solutions for Quarter 8.

1. (problem) Currently we are implanting only 2 canals, based on surgical judgment about risk. This limits our recording studies to analysis of cells with horizontal or posterior canal sensitivity, or convergence of these inputs.

(solution) We are working toward 3-canal stimulation, first with a single animal and then with revisions in two additional animals.

(rationale) This strategy allows us to capitalize on our successful surgical approach of implantation, completed studies, and subsequent revision. This worked well for a single animal moving from one to two canals. We now have performed a two canal initial surgery.

2. (problem) We have designed multiple channel single unit electrodes but delivery has been delayed due to fabrication issues.

(solution) We have acquired tetrodes from Thomas recording to complete our studies and we are manufacturing our own single unit electrodes in large numbers. Both of these have been assessed through recording experiments.

(rationale) We will use available technology.