Eighth Quarterly Progress Report

May 1, 2008 to July 31, 2008
Contract No. HHS-N-260-2006-00005-C
Neurophysiological Studies of Electrical Stimulation for the Vestibular Nerve
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Accomplishments for Quarter 8:

1. We have implanted multiple canal arrays in all 6 of our monkeys. We surgically implanted 3 animals with two electrode arrays, one in the lateral canal and one in the posterior canal. A fourth animal had three electrode arrays inserted in two canals; two in the lateral canal and one in the posterior canal. The surgical procedure was grossly identical to the highly successful procedure described in the previous QPR. To stabilize the electrode arrays in the semicircular canals, and to prevent migration of the electrodes post surgically, we attempted to use a surgical sealant, "Duraseal" to fix the electrodes in two animals. In these animals, there was very little behavioral response to electrical stimulation with the prosthesis. In one animal, this resulted in a post-operative infection and loss of the implant. While other surgical cements are available for this purpose, until we can prove that electrode extrusion is in fact an issue, it does not make sense to use them as they are potential toxic to the vestibular neuroepithelium, or are exothermic when setting. We do know that response to rotational stimuli is unaffected by implantation, suggesting that the implanted ear still contains a functional vestibular apparatus.

2. We have implanted recording chambers in four of our monkeys. In the remaining two monkeys, we are planning implant revision surgeries followed by chamber surgeries. In the three animals with chamber implants, stimulation and recording studies were initiated, and are described below. The chambers are aimed at the vestibular nuclei and/or the vestibular nerve.

3. We have performed behavioral recording and stimulation studies in all of our animals. Only one animal has shown robust behavioral responses to stimulation. The new surgeries failed to produce eye movements of significant amplitude or velocity in response to electrical stimulation despite an exhaustive parameter search of the electrode montage, pulse duration, stimulus current, and stimulation rate. We presume that either the precise electrode position is a critical parameter to electrical excitation of the vestibular nerve, or the electrodes are migrating out of the labyrinth either during surgical closure or postoperatively. The latter explanation seems plausible as our first animal's initial surgery placed the electrodes distant from the ampulla, yet did evoke small amplitude eye movements. In addition, the intralabyrinthine component of the electrode array is short and not well stabilized in the semicircular canal.

4. We have extended the behavioral recording experiments to include multichannel stimulation to look for canal interactions. We have observed clear interactions between stimulated canals, producing movements that represent the directional sum of the movements elicited by single canal stimulation. The interaction is non-linear with respect to the velocity of the movements but relatively consistent. Summation of low current stimulation in one canal, using stimulus parameters that would fail to produce a movement during stimulation of that canal alone, alters eye movements produced by stimulation of another canal. Overlap of ongoing stimulation in one canal,

with brief stimulation in a second canal, produces an immediate summation of movement direction and velocity that lasts for the duration of the overlapping stimulation.

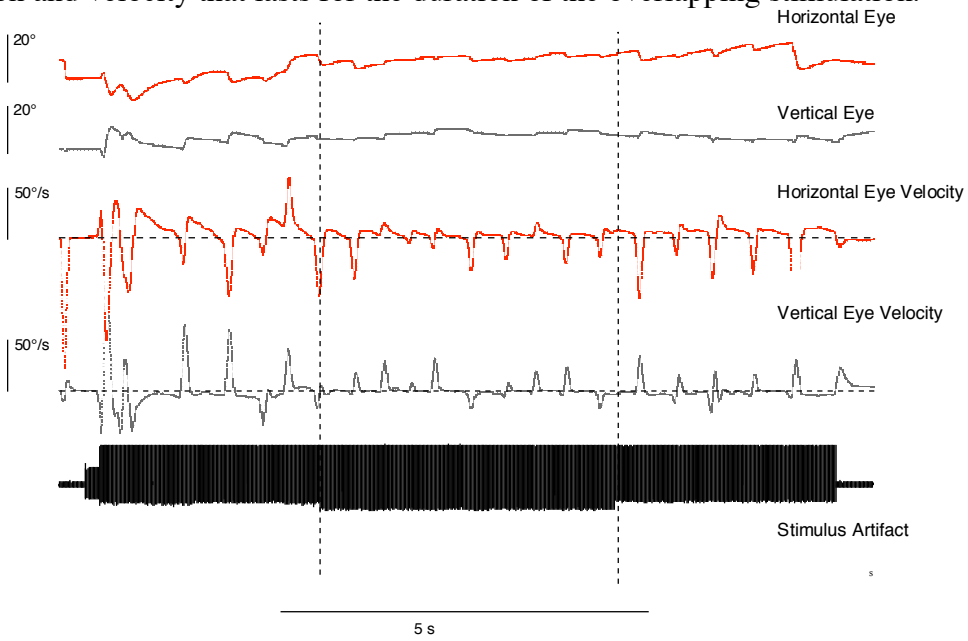

Figure 1: Summation of stimulation in the posterior canal with stimulation in the lateral canal (marked by the vertical hashed lines and increase in the stimulus artifact). Note that the velocity of the horizontal component of the nystagmus is temporarily reduced. Stimulus parameters: (right posterior canal) monopolar, 140 µA, 600 Hz, 100 µs/phase, 8 µs interphase gap; (right lateral canal) monopolar, 40 µA, 600 Hz, 100 µs/phase, 8µs interphase gap

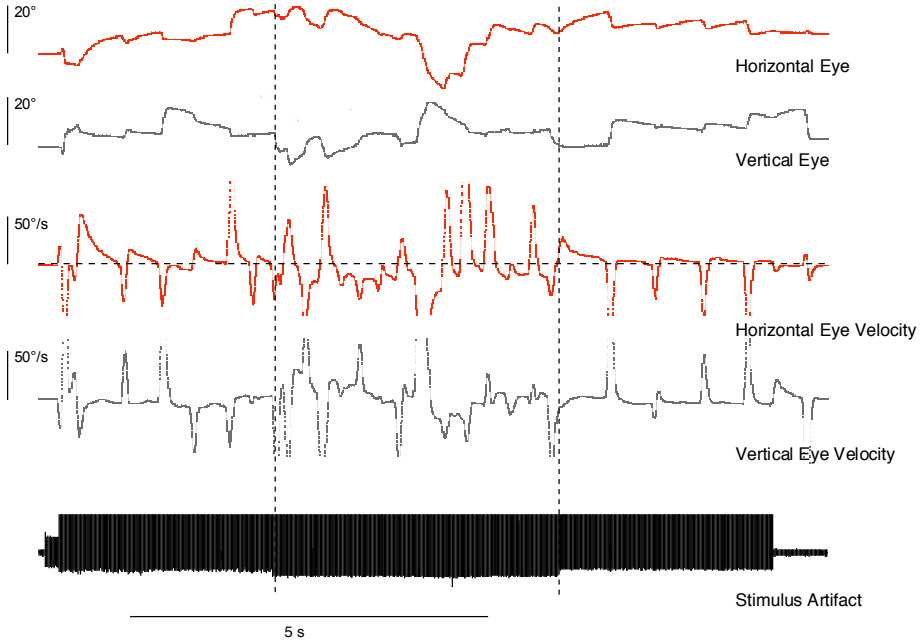

Figure 2: Summation of stimulation in the posterior canal with stimulation in the lateral canal (marked by the vertical hashed lines and increase in the stimulus artifact). Note that the velocity of the horizontal component of the nystagmus is temporarily reversed. Stimulus parameters: (right posterior canal) monopolar, 140 µA, 600 Hz, 100 µs/phase, 8 µs interphase gap; (right lateral canal) monopolar, 120 µA, 600 Hz, 100 µs/phase, 8µs interphase gap

Figure 1 shows the result of single canal stimulation followed by the brief addition of a second stimulus train. In this figure, the initial stimulation of the posterior semicircular canal is followed by stimulation of the lateral semicircular canal. This produces a reduction in the horizontal component of the observed nystagmus during the brief superimposed stimulation. Figure 2 shows the result of an increase in the stimulus current of the superimposed lateral canal stimulation. In this case, the direction of the horizontal component of the nystagmus reverses during the superimposed stimulation. In Figure 3, the progression of horizontal stimulus velocity during superimposed two-canal stimulation is displayed as a function of current level of the second, superimposed stimulus train. This figure clearly shows that as the stimulation current in the lateral canal is increased, the velocity of the slow phase reduces, reverses and then increases in the opposite direction, which is consistent with the summation of canal inputs. Stimulation of both canals produces eye velocities that are intermediate to stimulation of either canal alone.

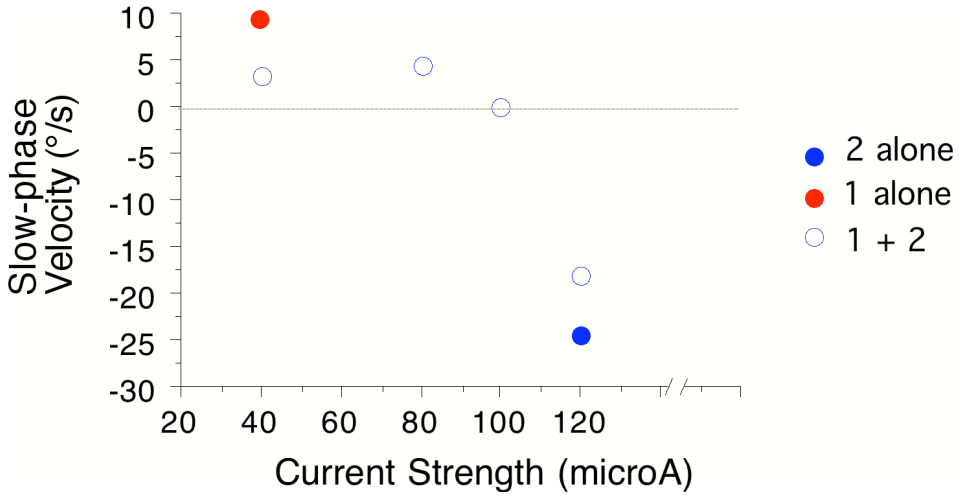

Figure 3: Summation of stimulation in the posterior canal (stimulus 1) with stimulation in the lateral canal (stimulus 2). Horizontal slow phase velocity is plotted against the current strength of stimulus 2 (open circles). Stimulation of the posterior canal alone is plotted as a solid red circle, and stimulation of the lateral canal alone is plotted as a solid blue circle. Stimulus parameters: (right posterior canal) monopolar, 140 µA, 600 Hz, 100 µs/phase, 8 µs interphase gap; (right lateral canal) monopolar, 600 Hz, 100 µs/phase, 8 µs interphase gap

5. We have extended the behavioral recording experiments to look at combined natural and electrical stimulation. These experiments utilize superimposition of stimulus trains and ongoing rotational stimuli. We have observed that response to ongoing rotation is influenced in a predictable manner by addition of brief electrical stimulation. Surprisingly, the gain of the response to rotation is unaffected by the addition of an electrical stimulus. However, there is an offset in the velocity of the response. This shown clearly in Figure 4, which displays the result of horizontal canal stimulation during en-block yaw rotation of the monkey in the dark. Under these conditions, the eye movement velocity resulting from yaw rotation shifts in the direction of the movement elicited by the electrical stimulation of the right lateral canal; i.e., a leftward velocity bias is introduced. The modulation of the eye movement velocity remains unchanged, as does the amplitude of the modulation. This result illustrates that the summation of natural and

electrical stimulation results in the summation of the eye velocity resulting from natural stimulation with the constant eye velocity elicited by a constant frequency stimulus train.

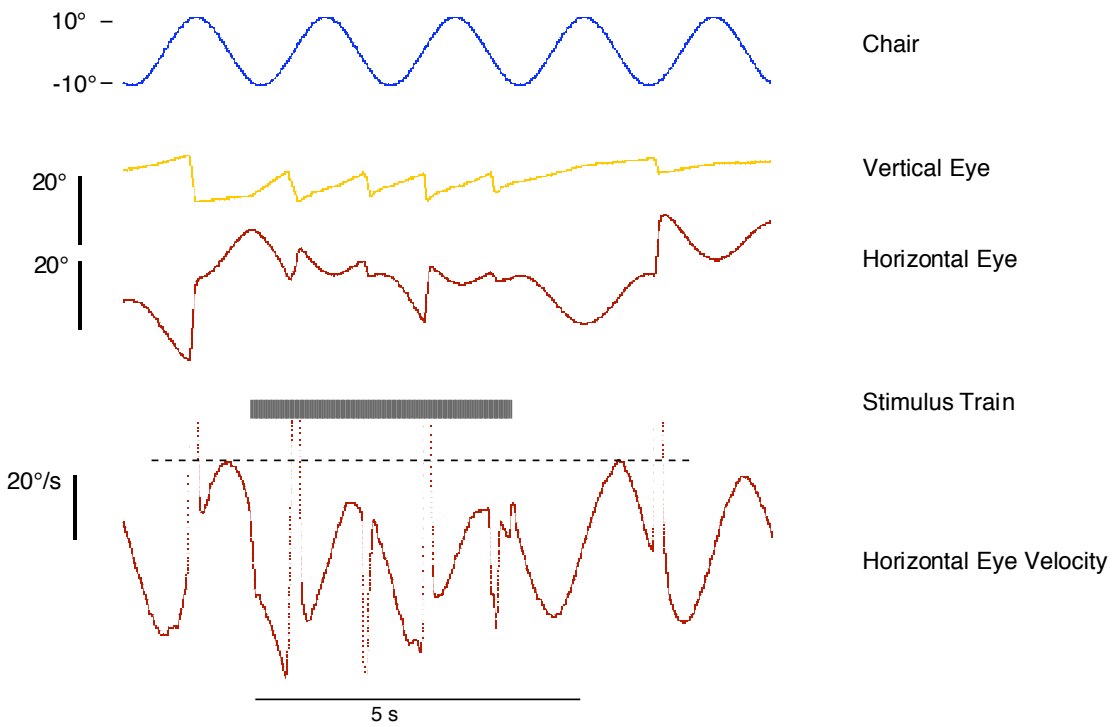

Figure 4. Addition of electrical stimulation of the lateral canal to ongoing natural vestibular-ocular reflex in response to yaw rotation. Traces are horizontal chair, vertical eye and horizontal eye position, electrical stimulus train, and horizontal eye velocity. The dashed horizontal line indicates the average peak rightward eye velocity during rotation in the dark without electrical stimulation. Electrical stimulation parameters are: right lateral canal, monopolar, 50 µA, 600 Hz, 100 µs/phase, 8µs interphase gap

6. We have begun begin stimulation and recording experiments in four of our monkeys using recording in the end organ of evoked neural responses with NRT (neural response telemetry). Our prosthesis has the capability of stimulating through a single electrode in an implanted array, and then recording an evoked neural potential through an adjacent electrode. This technique is analogous to neural response telemetry that is widely used in cochlear implant research. This gives us a tool to evaluate whether our electrode is driving a neural response. In two of four animals tested, an ECAP (evoked compound action potential) response was observed consequent to stimulation from our prosthesis. The stimulation parameters for NRT stimulation are shown in Figure 5, and the resulting ECAPs are shown in Figure 6. In these trials, we stimulated the right lateral canal using monopolar stimulation of the most proximal electrode of the three-electrode array, and recorded from the most distal electrode. The response, which has never to our knowledge been previously recorded in the vestibular end organ displays many of the characteristics of the compound action potential recorded in the cochlea using a similar paradigm in cochlear implants. For example, the biphasic waveform has similar temporal characteristics and shows a similar progression in size with increasing stimulus current amplitude. Interestingly, one of the two animals in which ECAPs were recorded showed a very robust behavioral response to electrical stimulation, while the other animal showed

a very weak behavioral response. This opens the possibility that activation of the vestibular nerve can produce different behavioral responses depending on the specific neural elements that are activated by the stimulation. Of course, the specific source of the evoked neural response to stimulation of the canal is currently unknown. However, given their short latency, morphology, and correlation with behavioral responses they are likely to arise from terminals of vestibular afferents.

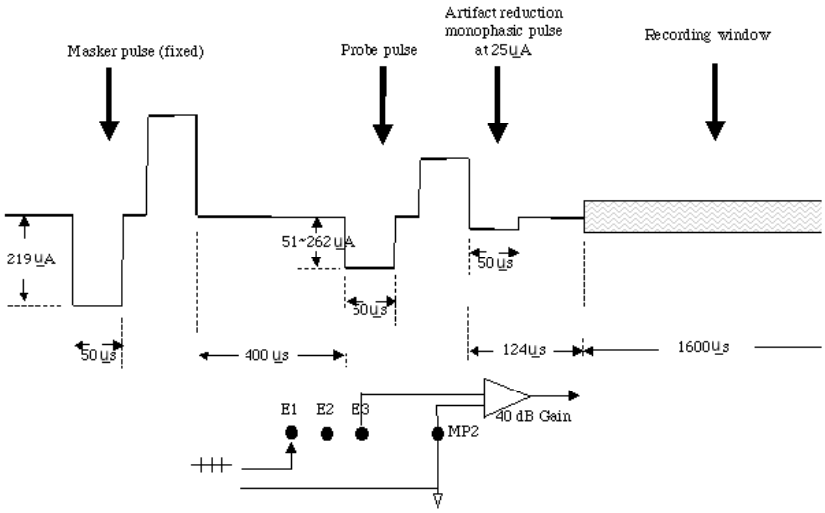

Figure 5. Stimulation parameters for NRT. A biphasic probe pulse of varying current intensity is presented followed by a brief artifact reduction pulse. 124 µs after the probe pulse, the recording window is opened for 1600 µs to record the neural activity produced by the probe pulse. This stimulus is interleaved with a more complex masking stimulus trial where a masker pulse is presented immediately before the probe pulse. The masker pulse eliminates the response to the probe pulse because the neural elements are refractory to further stimulation following presentation of the masker pulse. In this situation, the recording window records only the artifact resulting from the presentation of the probe pulse. By subtracting the masking trials and adding the probe trials, one creates an averaged representation of the neural response to stimulation while greatly reducing the stimulus artifact.

7. We have begun stimulation and multi-channel neural recording experiments in 2 animals. To accomplish this, we needed revise our multichannel stimulation software so that it could provide a better representation of the stimuli being delivered and the trigger pulses associated with the stimulation. A number of functions were added to the existing software and a new version was used in this quarter. A user can preview the pulse train pattern prior to any stimulation. This function allows us to see the actual pulses and triggers generated by the software and we can also zoom in to each individual pulse to check whether it is desirable. The new software can generate trigger signal in two modes, 1 trigger per pulse train or 1 trigger per pulse. Both are useful in the artifact removal after stimulation. We also developed a sophisticated data logging function for the multichannel stimulation. All related parameters are saved to a file and that file can be fully retrieved for future data analysis. This software has been successfully used in our animal stimulation. It allows us to fully explore up to three canals with electrical stimulation and multichannel single unit recording in the brainstem or vestibular nerve. Figure 7 displays the two trigger modes that can be obtained from the new software. In Figure 7A, the original trigger mode defines the start of a stimulus train. In Figure 7B, the trigger defines each stimulus pulse.

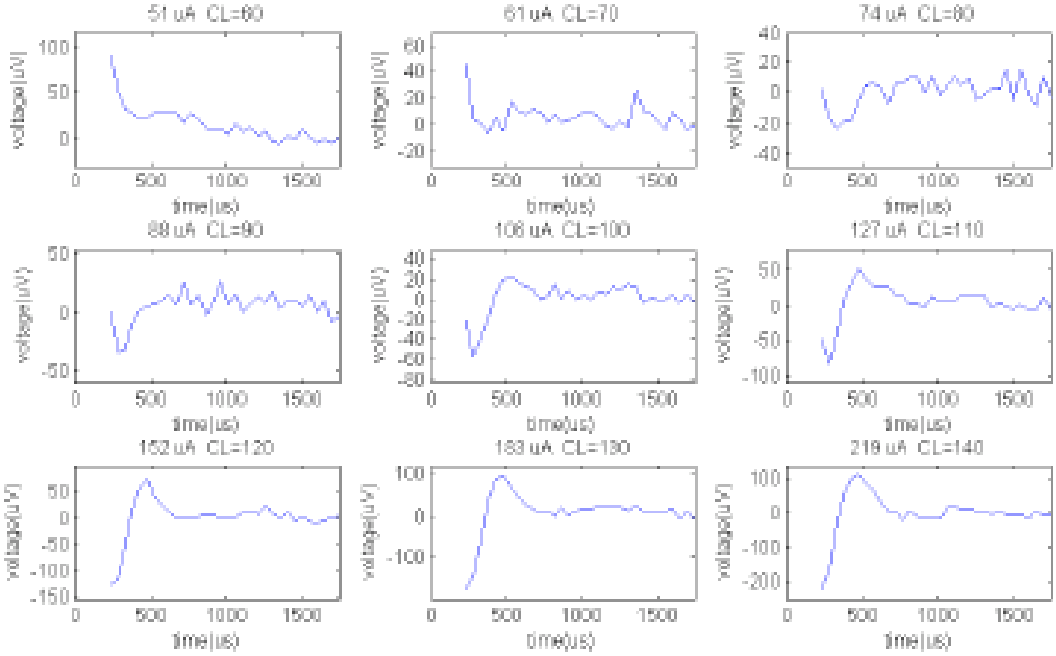

Figure 6. Evoked potentials resulting from NRT stimulation and recording in the lateral canal.

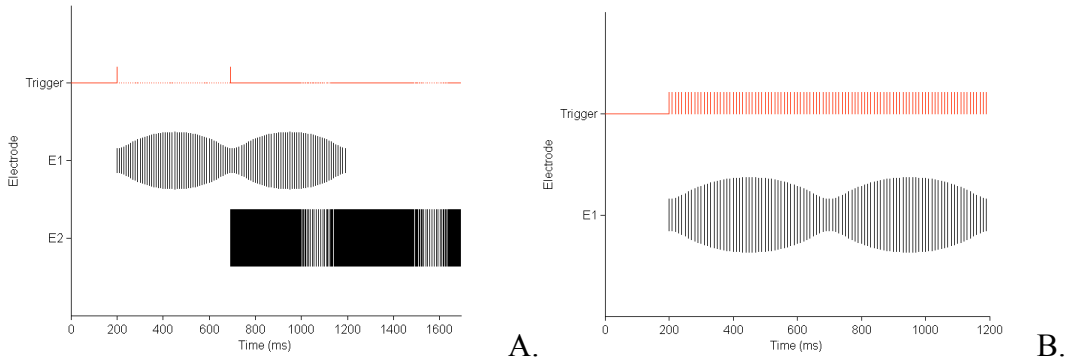

Figure 7. Examples of two trigger modes in the new stimulation software. In panel A, the trigger defines the timing of the start of two trains of stimuli (one amplitude modulated and one frequency modulated) delivered to separate channels. In panel B, the trigger defines the timing each stimulus pulse in a single amplitude modulated train.

Our stimulation and recording experiments have produced very interesting preliminary results. We have recorded from neurons in the brainstem of two monkeys during stimulation of the end organ with our prosthesis. We have demonstrated that the stimulus artifact is not driving the unit amplifiers into saturation beyond the period occupied by the stimulation waveform. However, the high frequency stimulation that elicits the most robust eye movements is not suitable for unit recording. High frequency of stimulation and wide pulse widths reduce the window between artifacts in which unit activity can be recorded. Fortunately, we can still induce reasonable nystagmus with lower frequencies (150 to 200Hz) and narrower pulse widths (50 to 100 μs). Another challenge is presented by the high resting firing rate of units in the vestibular and

oculomotor systems. To examine the pattern of neural activity during stimulation we require resolution of spikes by an overlap template technique. We tested the procedure with units that exhibited vestibular or eye movement sensitivity during activation of the prosthesis. The method could be evaluated on the basis of the signature firing patterns of units in the oculomotor system.

Figure 8 illustrates our recording approach. This figure displays the result of recording from a position vestibular pause (PVP) neuron in the vestibular nucleus during natural and electrical stimulation. This neuron has a horizontal eye position sensitivity, a horizontal head velocity (vestibular) sensitivity, and pauses for saccades. Electrical stimuli, delivered at a frequency of 5 Hz to an electrode in a lateral canal array, fail to elicit a time locked response in this neuron, suggesting that the neuron is not following our electrical stimulation despite the fact that the neuron is a secondary vestibular neuron.

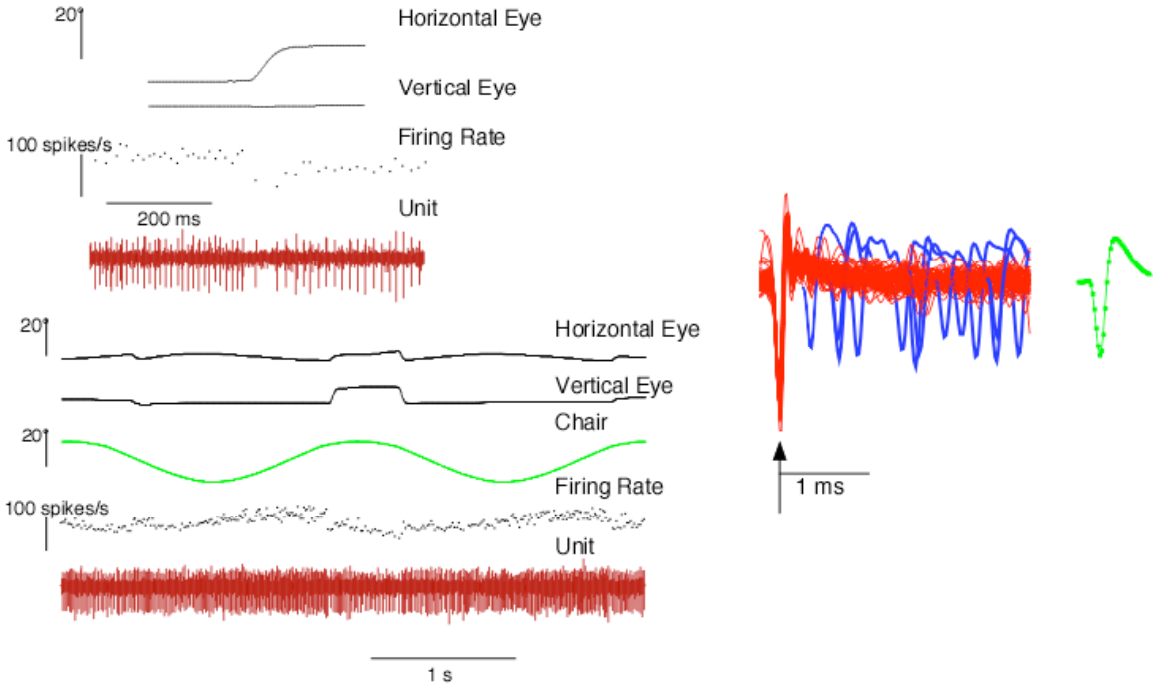

Figure 8. Recording from a Position Vestibular Pause (PVP) neuron during natural stimulation and electrical stimulation. In the top panel, recording from the neuron during a saccade elicited by a target step (not shown) produces a pause in activity followed by a change in the tonic rate of the neuron associated with a new eye position. In the lower left panel, modulation of horizontal chair and head position during en bloc yaw rotation with visual suppression of vestibulo-ocular reflex produces a modulation in the discharge rate of the neuron with head velocity. In the lower right panel, repeated stimulation of the lateral canal fails to elicit a time locked response in the neuron. However, the traces demonstrate that it is possible to disambiguate the stimulus artifact and the resulting neural response.

Figure 9 displays the response of a PVP neuron during a low frequency train of stimuli delivered from a prosthesis implanted in the lateral canal. In Figure 9A, horizontal and vertical eye position traces are shown above simultaneously recorded single unit activity. Prior to stimulation, the spikes had a good signal-to-noise ratio and fired regularly due to the stable eye position. Beginning at ~1200 ms, transient artifacts caused by the stimulus pulses (150 μA biphasic pulses, 50 μs per phase, 100 pulses per second) almost completely obscure the neural spikes when viewed at this time scale. However,

Figure 9A inset displays the portion of the trace defined by the symbol I—I at the onset of stimulation. The expanded time scale in the inset reveals that most spike waveforms are resolvable between the individual artifact pulses. However, occasional spikes temporally overlap with the artifact, such as the spike-artifact pair highlighted by the arrow. Such overlaps can produce errors when analyzing the number and timing of spikes, especially when comparing the rate of spike firing prior to and during electrical stimulation. Figure 9A inset demonstrates the success of classifying both the spikes and artifacts, which have been labeled "1" and "2" respectively. In particular, the spike-artifact complex denoted by the arrow was correctly resolved.

To detect spikes in the presence of electrical artifact, we applied the overlap template technique described in previous quarterly reports. This technique was originally designed to classify spike waveforms generated by two different neurons, but it is equally applicable to the removal of recurring transient artifacts. After templates were constructed of the artifact and neural spike from isolated instances of each type of waveform (as determined by principal component clustering), all detected events were matched to the templates. Those rejected in this first pass were subsequently matched to a series of overlap templates, created by summing the separate templates at different relative time lags.

Figure 9B shows the instantaneous event rates of the classified spikes and artifact pulses. The overlap template matching process resulted in 100% identification of the artifact pulses (red trace), as evidenced by the straight line occurring throughout the duration of the 100 pps stimulus train. Validation of the spike classification is not strictly possible in this example. However, the type of neuron recorded (PVP) is known to fire regularly during eye fixation. As seen in panel A, the horizontal and vertical eye positions were fairly constant just prior to stimulation and during the first 300 ms of the pulse train. During this period, the estimated spike rate (blue trace in Figure 9B) was also relatively constant: a missing spike would have been evident as a sudden rate decrease, but the rate remains fairly level around 80 spikes/second.

Two important features of the response are also seen in the estimated spike rate trace. First, there are two brief dips in spike rate (arrows in Figure 9B) that correspond to small horizontal saccades. Pause during saccades is a defining characteristic of a PVP neuron. Second, and even more interesting is the change in firing rate associated with the stimulus train. In this example, the firing rate increases as stimulation produces a relatively slow leftward horizontal eye movement, appropriate for stimulation of the right lateral canal at low frequency. The unit shows an elevation of discharge rate in association with the movement. This suggests that the unit recording has the potential to reveal functionally significant changes in spike rate in vestibular neurons during electrical stimulation of the vestibular nerve.

8. We have applied for approval from our IACUC for sedation and ABR recording in our implanted animals. As of the end of the quarter, we have not yet received approval but anticipate approval in Quarter 9.

9. We have accepted delivery of a PDA based stimulation solution from the laboratory of Dr. Philip Loizou of UT Dallas and have programmed the device to provide frequency-modulated stimuli to the vestibular end organ in rhesus monkeys. We have

started the local development of software generating electrical stimuli using the PDA cochlear implant platform. Although this version of the PDA platform is not capable of processing rotational analog input signals, it is ideal to conduct stimulation experiments with continuous long-term pulse trains. Much of our effort in quarter 8 was directed toward digesting the existing codes provided by Dr. Loizou's group and then modifying them according to our specific needs. For example, we can change the pulse width, pulse rate or pulse amplitude on a specific electrode. We are able to generate a predefined pulse train on a single or multiple electrodes. This device can provide some functionality not provided by the NIC 2 platform despite its present limitations.

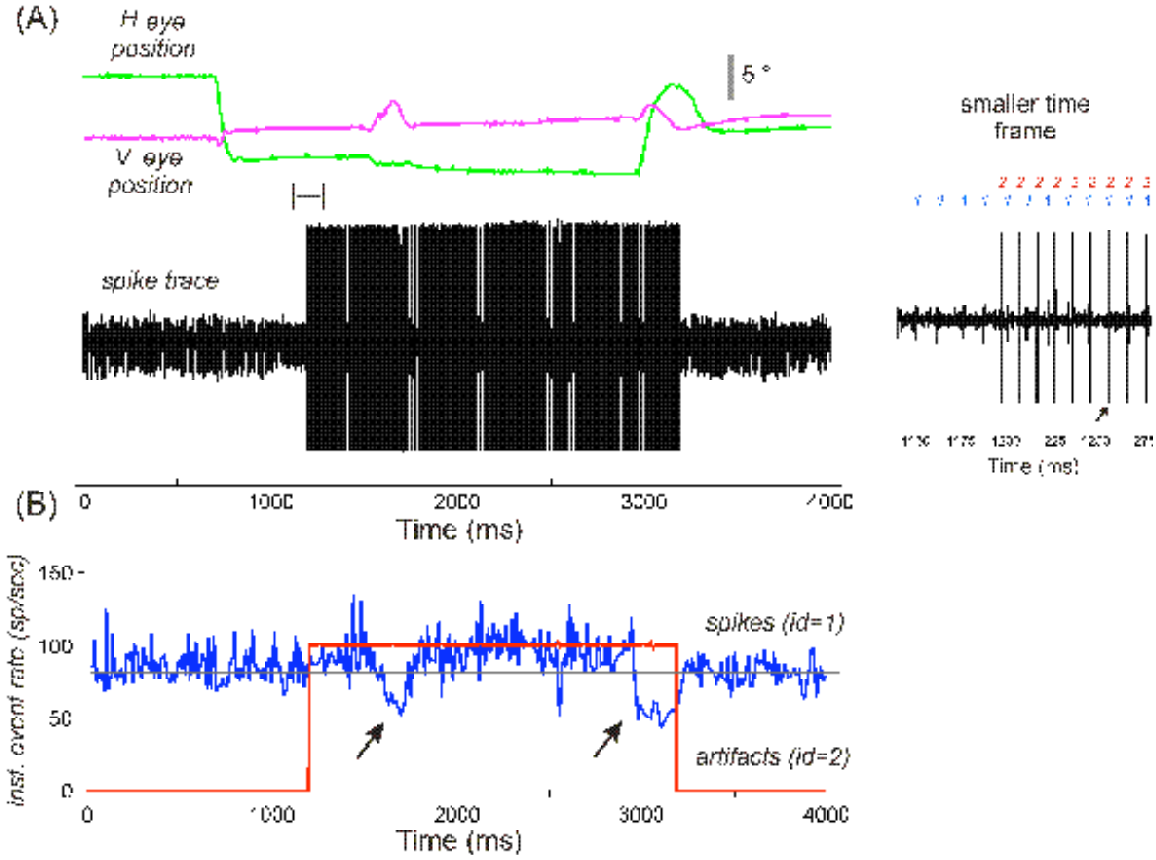

Figure 9. Identification of unit activity during constant frequency stimulation of the lateral canal with a vestibular prosthesis. Panel A shows eye position traces and a spike train before and during electrical stimulation of the lateral canal. Panel A inset displays the spike train and stimulus artifact at the start of stimulation (marked with I—I) in an expanded scale. The arrow identifies an overlapping spike and stimulus artifact. Panel B shows the instantaneous spike rate extracted from a template analysis during the stimulation in panel A. The horizontal black line displays the average pre and post-stimulation spike rate. The arrows indicate decreases in the spike rate associated with saccadic eye movements.

10. We have completed one manuscript, to be submitted to Acta Oto-Laryngologica in association with its presentation at the Collegium Oto Rhino Laryngologicum Amicitiae Sacrum in Berlin, Germany by Dr. Phillips. This paper, titled "A minimally invasive prosthesis for electrical stimulation of individual canal channels in the vestibular nerve" describes our approach to the design of a prosthesis, the

implantation of the device, and some preliminary observations on the relationship between stimulation frequency and amplitude, and the speed and direction of the resulting eye movements. In addition, the paper describes the results of combined rotational and electrical stimulation.

Challenges in Quarter 8:

Our most significant challenge is the continuing issue of the variability in the behavioral efficacy of the implanted electrodes. While we have been able to drive robust nystagmus with our device, three of the animals show very little behavioral response. One of these animals shows neural responses using NRT suggesting that neural activation of the end organ is taking place. The mystery, and most important scientific question is, are we activating the end organ and central neural elements, yet failing to produce overt behavior. There are several possibilities. 1) The electrode arrays are not placed optimally to produce behavior or drive neural responses. We are getting a variable result because of variation in surgical placement at the outset. 2) The electrode arrays are placed properly but become dislodged after placement. The variation in result is related to the design of the electrode array and our inability to fix the electrode in place. 3) The electrode arrays are properly placed to drive neural responses, but the presence of intact vestibular input in either the implanted ear or the contralateral ear reduces the behavioral efficacy of the stimulation. 4) The electrode arrays are placed properly and the stimulation drives both centrally recorded and peripherally recorded neural responses, but the placement is not optimal to drive behavioral responses because a) we are not activating the appropriate combination of afferent types, b) we are not activating a sufficient number of afferent fibers or c) we are not providing a providing a physiologically relevant stimulus. Finally, 5) it is possible that we have damaged the ear in some of our animals, but that damage is not apparent with testing using rotational stimuli.

Answers to those challenges.

Our approach to resolving these issues in Quarter 9 will be to continue to systematically vary the surgical procedure while using tools developed in the last two quarters.

1). First, when we have received approval from our IACUC we will test each animal to confirm that the implanted and contralateral ear have an intact auditory brainstem response (ABR). This will confirm that at least the auditory portion of the inner ear is functioning properly.

2) We will continue to utilize NRT as a measure of neural activation at the end organ. In addition, we plan to have Dr Paul Abbas review our now fairly copious NRT data to assist in optimizing NRT recording and then assist Dr Rubinstein in performing a revision surgery on one of our animals without electrically evoked eye-movements. We will determine intraoperatively how the position of the electrode array affects the ECAP amplitude and morphology and then during closure and in the postoperative period periodically monitor the stability of the ECAP. We are cautiously optimistic that this

approach will greatly improve the robustness of our surgical procedure. If it is successful, we will then implant the second monkey without a chamber to see if we can duplicate our success.

3) We will continue central neural recording to see if we can reveal consistent neural responses in animals that show robust behavioral responses to stimulation and those that show minimal behavioral responses to stimulation. If both groups of animals show neurons that respond to stimulation, but the response differs in the percentage of driven neurons, or the relative distribution of driven neuron types, this will provide a very important clue to understanding the current result. Similarly, absence of neural responses in a large sample of vestibular neurons in animals without behavioral responses would argue strongly that the surgical placement or approach is the problem.

4). We are redesigning our next batch of vestibular prostheses to have longer terminal ends to each tripolar array, which can be inserted between the bony labyrinth and the membranous labyrinth. The new design will allow for a more secure placement of the electrode array in the canal.