Fourteenth Quarterly Progress Report

November 1, 2009 to January 31, 2010
Contract No. HHS-N-260-2006-00005-C
Neurophysiological Studies of Electrical Stimulation for the Vestibular Nerve

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Reporting Period: November1, 2009 to January 31, 2010

Challenges: Our main challenge this quarter has been in software development.

1. Our development of software for the lower level SDK programming environment is progressing slowly. Our initial attempts to test the programs that have been developed are described below. Our greatest challenge is that there is a significant difference between the performance of the software on the bench top driving the Implant-in-a-box, and the performance of the same software when driving the implanted devices in-vivo. Specifically, the software produces reliable current output measured across a resistor on the bench top. This can then be tailored to match the output of the device when driven by the NIC 2 programming interface. However, in-vivo the device fails to produce the same amplitude stimulus artifact, or precisely the same behavior, i.e., the same nystagmus velocity. This is perhaps due to the different return path modes and power settings that are available through the SDK programming environment, and the complex regional impedances in the inner ear. This difference between bench top testing and in-vivo testing is currently a significant challenge.

Our response to this challenge has been to ask Dr. Nie to devote his full contract effort of 20% FTE to this subproject in Quarter 15. We have also asked Dr. Rubinstein to commit effort to this activity.

2. Our development of software for the analysis of multiple single units, or the disambiguation and isolation of neural activity and artifact, has also been very challenging. The current software has been optimized to do a reasonable job of separating neural discharge from artifact and identifying the spike when it is embedded in the vestibular field potential, and indeed it is used almost exclusively for this purpose in the project currently. The software can also be used to enhance unit isolation with the use of multiple site recording electrodes. Finally, the software can be used to isolate multiple single units from synchronously recorded channels. This application is demonstrated below. This procedure attempts to discriminate nearly synchronous spike events and often succeeds. However, a limitation to all of these applications is that the user interface for inspecting and editing the results is cumbersome, which limits its application to the large numbers of units that we are currently recording in the laboratory.

Our response to this challenge has been to develop a two step analysis process, where software using more traditional window discrimination techniques is applied first. If this approach fails, the data is subjected to a second analysis using some of the routines developed by Dr. Bierer. This strategy ensures good unit isolation and a more reasonable throughput. Dr. Bierer is responsible for and continues to devote the majority of his effort to this subproject.

3. We have not yet received our multiple single unit recording axial electrode arrays from Lawrence Livermore Laboratories. Dr. Satinderpal Pannu has indicated via email that they have fabricated a “simple model” of our proposed array and that it

"looks promising". We plan to discuss the further development of these devices with Dr. Pannu via email in Quarter 15.

Our response to this continuing challenge is to use Thomas recording tetrodes for all multiple unit recording sessions until we receive other electrode arrays.

Current Successes:

1. In Quarter 14, Drs. Rubinstein and Phillips met in person with the FDA and provided further information in support of the submission of an IDE for a clinical trial of the vestibular prosthesis developed under this contract. The interaction was positive overall and defined the criteria for an appropriate IDE submission. Following this meeting and in response to the written comments and questions received earlier, we have completed a revised IDE. We are currently waiting for a revised package insert from Cochlear Corporation. When we have received this, we will submit the full IDE application.

2. We have presented our results in one international meeting this quarter.

Suppression of nystagmus with an implanted vestibular neurostimulator. Jay Rubinstein, James Phillips, Leo Ling, Kaibao Nie. 7th Asia Pacific Symposium on Cochlear Implants and Related Sciences, Singapore, 2009

3. We have had two abstracts accepted for platform presentations at national / international meetings. Our abstract for the 2010 meeting of the Association for Research in Vision and Ophthalmology in Ft. Lauderdale, Fl was accepted for a 20 minute oral presentation and our abstract for the 20th Annual Conference of the Society for the Neural Control of Movement was accepted for an individual oral presentation.

4. We have submitted two new abstracts for presentations at national meetings.

Behavioral results from an implantable vestibular prosthesis based on commercial cochlear implant technology. Steven M. Bierer, Leo Ling, Kaibao Nie, Albert Fuchs, Trey Oxford, Chris Kaneko, Jay T. Rubinstein, and James O. Phillips, Neural Interfaces Conference, Long Beach, CA, 2010

Neural recording results from an implantable vestibular prosthesis based on commercial cochlear implant technology. James O. Phillips, Leo Ling, Albert Fuchs, Trey Oxford, Steven M. Bierer, Chris Kaneko, Kaibao Nie, and Jay T. Rubinstein Neural Interfaces Conference, Long Beach, CA, 2010

5. We have sacrificed two additional animals and are processing tissue for brain and inner ear reconstruction, as well as temporal bone CT. We are performing temporal bone spiral CT to identify electrode placement in necropsy material at Seattle Children's Hospital. The brainstem tract reconstruction is performed in tissue perfused

with 10% formalin, sectioned, mounted and stained with cresyl violet. The temporal bone reconstruction is performed in decalcified sectioned tissue.

6. We were able to "treat" pathologic spontaneous nystagmus with electrical stimulation in a single monkey. To test the hypothesis that we could block pathological vestibular nystagmus in a monkey model, we perfomed an aggressive revision implantation in the right posterior canal of a single monkey. The revision procedure compromised the ear of this monkey, producing low gain vestibular responses during low frequency rotation. In addition, the animal developed a down and left beating spontaneous nystagmus in the dark post surgically. The implanted electrode array was fully functional despite the spontaneous nystagmus and the change in rotational vestibular function. We then used constant frequency electrical stimulation to "treat" the observed nystagmus. The velocity traces in Figure 1 show that during electrical stimulation, the spontaneous nystagmus is dramatically reduced. The inset in Figure 1 shows horizontal and vertical eye position during the last six seconds of stimulation. The animal shows no residual nystagmus during this period.

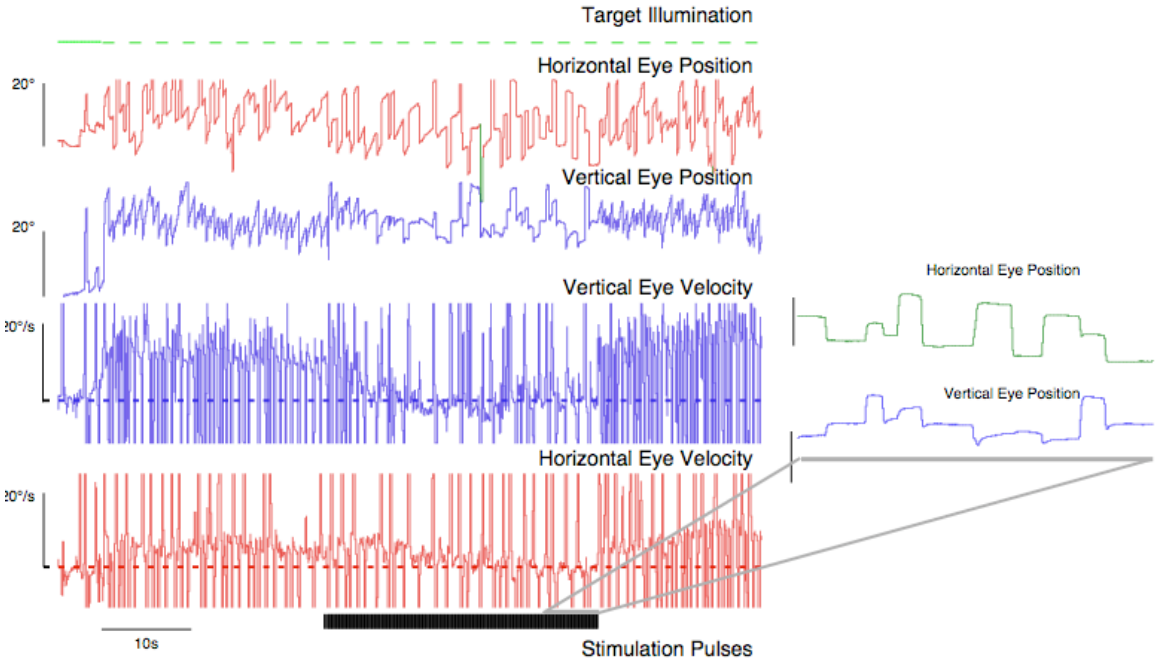

Figure 1. Treatment of spontaneous nystagmus with electrical stimulation in a rhesus monkey. The stimulation parameters for monopolar stimulation through the distal site in the right posterior canal are 400 µs pulse width, 8 µs *gap, 300 pps, 95 µA, for 30s.*

7. We have been able to use real time modulated stimulation to control the gain of the vestibulo-ocular reflex (VOR) in the rhesus monkey. In these experiments, the vestibulo-ocular reflex is generated by rotation of the monkey in the dark. A head velocity signal is used to modulate the amplitude of a 1 KHz carrier, which is used to drive a clinical processor. The result is an amplitude modulated constant frequency electrical stimulus either in phase (gain increase) or out of phase (gain decrease) with head velocity in the direction of the stimulated canal. Using this technique we can drive

the VOR gain up or down. At each gain setting, we performed rotations at different frequencies and amplitudes to see if there was an amplitude or frequency dependence to the observed gain change.

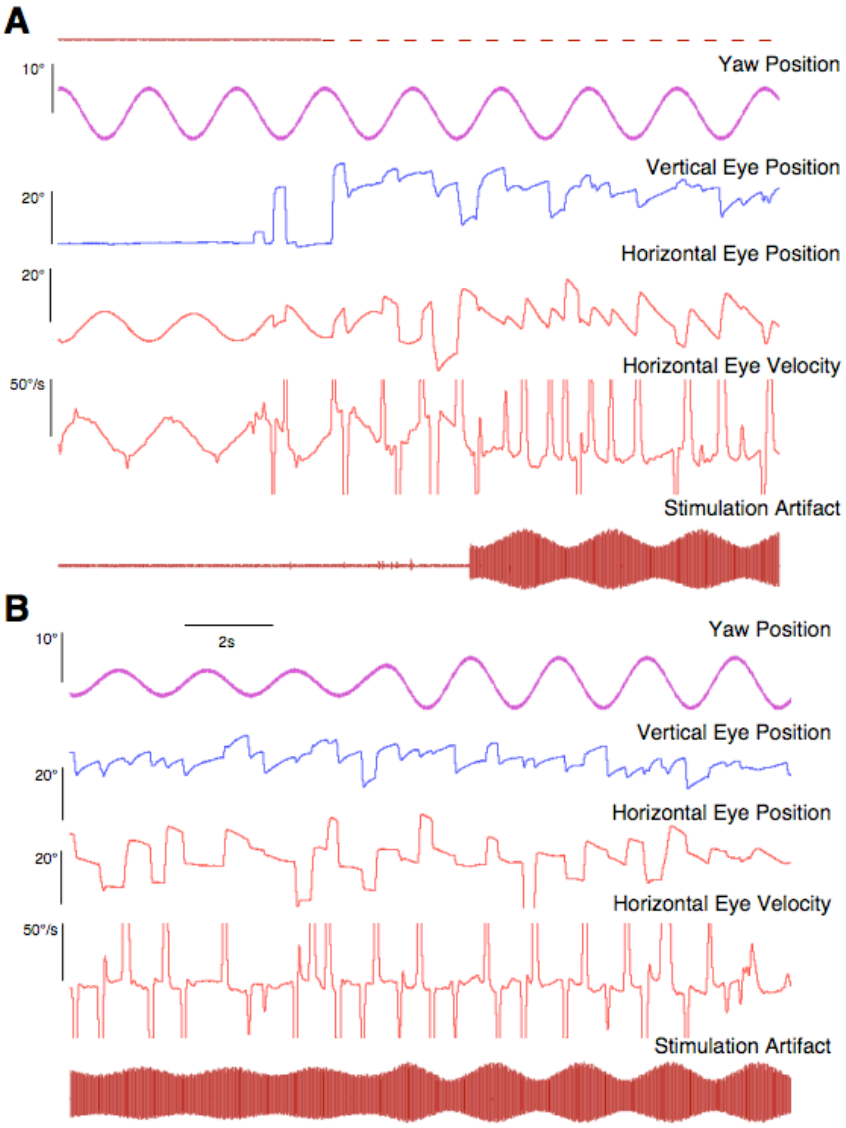

Figure 2. VOR gain modulation experiment. A) VOR gain is reduced to ≈ 0.2 by real time amplitude modulated electrical stimulation. B) VOR gain remains at ≈ 0.2 despite an increase in the amplitude and velocity of rotation.

Figure 2 displays data obtained from a single session of one such experiment. In Figure 2A, the animal initially tracks an earth fixed point target during 0.5 Hz horizontal rotation. After 3 cycles, the point target is extinguished and the animal is rotated in complete darkness for two additional cycles. During this time the VOR gain remains high, as can be seen in the horizontal eye velocity trace. At this point, a real time head velocity controlled amplitude modulated electrical stimulus is applied to reduce the gain of the VOR to approximately 0.2. This electrical stimulus configuration is continued in

Figure 2B, but the amplitude of the rotation is changed. The gain remains at 0.2. In our experiments we have observed that across a range of velocities and frequencies we can maintain a relatively constant VOR gain ranging from 0.0 to 2.0 using this technique.

8. We have been able to create frequency modulated electrical stimuli using the SDK interface. In addition we have used this stimulus to drive behavior in vivo. Specifically, we continued software development by generating frequency modulated (FM) pulse trains on the Nucleus SDK (Software Development Kit) platform. The research interface has the capability of processing up-to-three rotational signals in real time and converting them into amplitude modulated (AM) or frequency modulated (FM) pulse trains. We have implemented the generation of AM stimuli using the Nucleus Freedom speech processor and have conducted a series of animal experiments. However, the clinical interface is not capable of generating FM trains.

For the purpose of creating FM trains, we significantly modified the Nucleus SDK software package, including all Python programming modules and prosperity DSP assembly codes, provided by the Cochlear Ltd. As a first step, we demonstrated that pre-defined FM trains can be produced with the SDK interface. Figure 3 shows 2 s long FM trains in an ongoing stimulus recorded on a digital oscilloscope (exported to Matlab). The pulse train was modulated from 10 pps to 50 pps at a rate of 1 Hz. The pulse width was set to 100 μs to be consistent with our previous settings. The FM pulse train can run forever as long as the processor power is on.

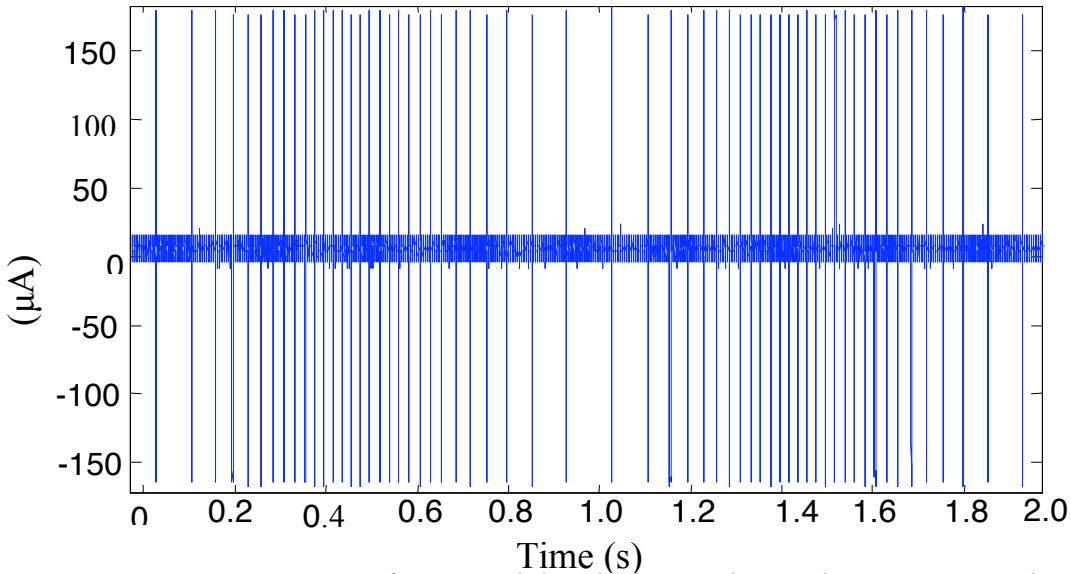

Figure 3. A 2s segment of FM modulated electrical stimulation generated with the Nucleus SDK research platform. The pulse train was modulated between 10 pps and 50 pps at 1.0 Hz.

We developed several programs to overcome the rate limit in regular stimulation settings of the SDK. For example, the default SDK settings can produce pulses only at rates higher than 76 pps. In our program, we added extra null pulses if the desired pulse rate

was lower than 76 Hz, to produce pulse trains as low as 10 pps. We also made changes to the VIstream pulse train generator to produce all of the parameters required by the SDK research interface. The new VIstream has the capability of generating a file that contains the design parameters of the pulse train sequence suitable for the SDK interface. With this functionality, we are able to create the same FM pulse trains on the bench top with the validated NIC2 and the newly-developed SDK. We have verified that the SDK interface produces identical amplitude and frequency modulated pulses on several bench tests, as illustrated in Figure 4.

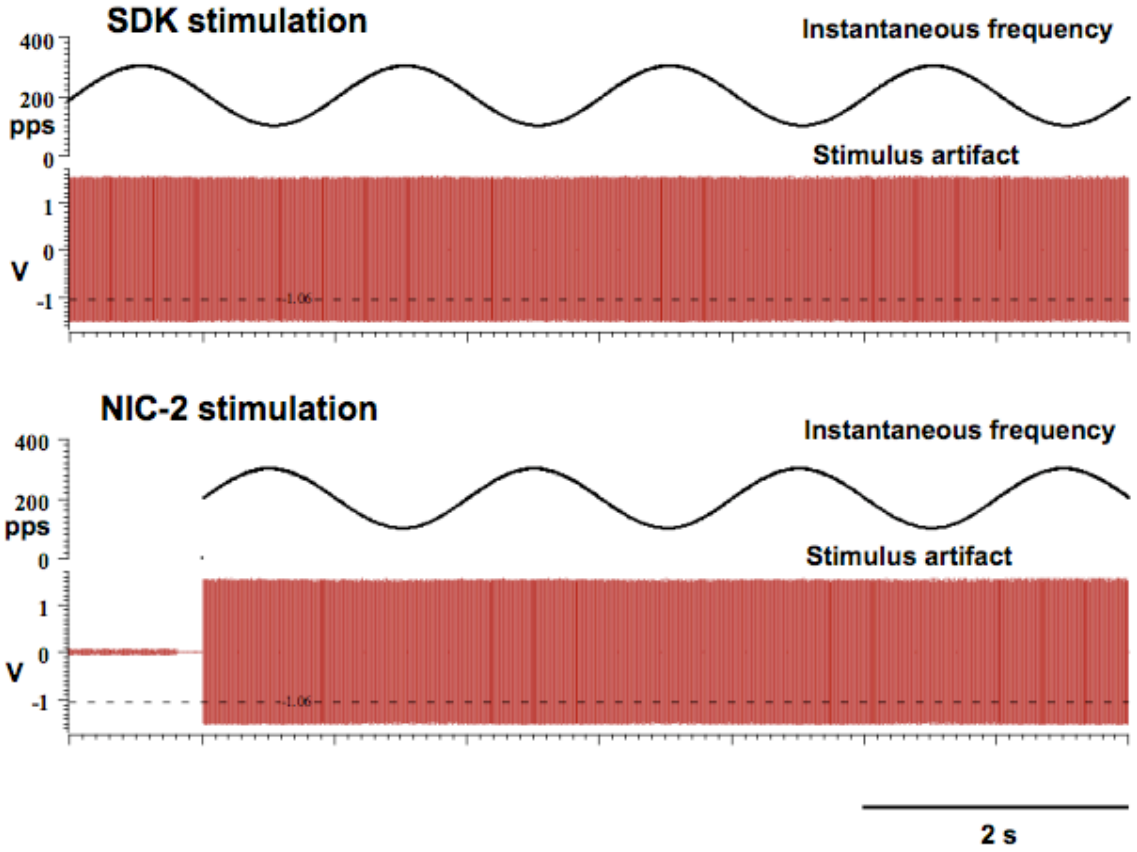

Figure 4. Bench test of the SDK and NIC-2 platforms. Both systems were programmed to deliver an FM train, 100-300pps, at .5 Hz, with a current of 140 µA, pulse width of 100 µs, and 8 µs gap. Upper trace shows the instantaneous frequency and stimulus voltage waveforem measured across a resistor during electrical stimulation with the SDK platform. Lower trace shows the same programmed stimulation using the NIC-2 platform. The stimulation starts late to show the background noise in the recording. The dotted horizontal line shows the trigger level for the instantaneous frequency traces.

As a preliminary test, we also conducted animal stimulation experiments on one implanted monkey with the SDK interface. As shown in Figure 5, we observed a smaller stimulus artifact with the SDK interface and a nystagmus response similar, but not identical to, that produced by the NIC2 interface with the same pulse train settings. This demonstrated that the SDK interface can be potentially used in future studies to

investigate long-term stimulation effects and real-time stimulation. This should give us considerable functionality beyond that available with the current NIC2 interface.

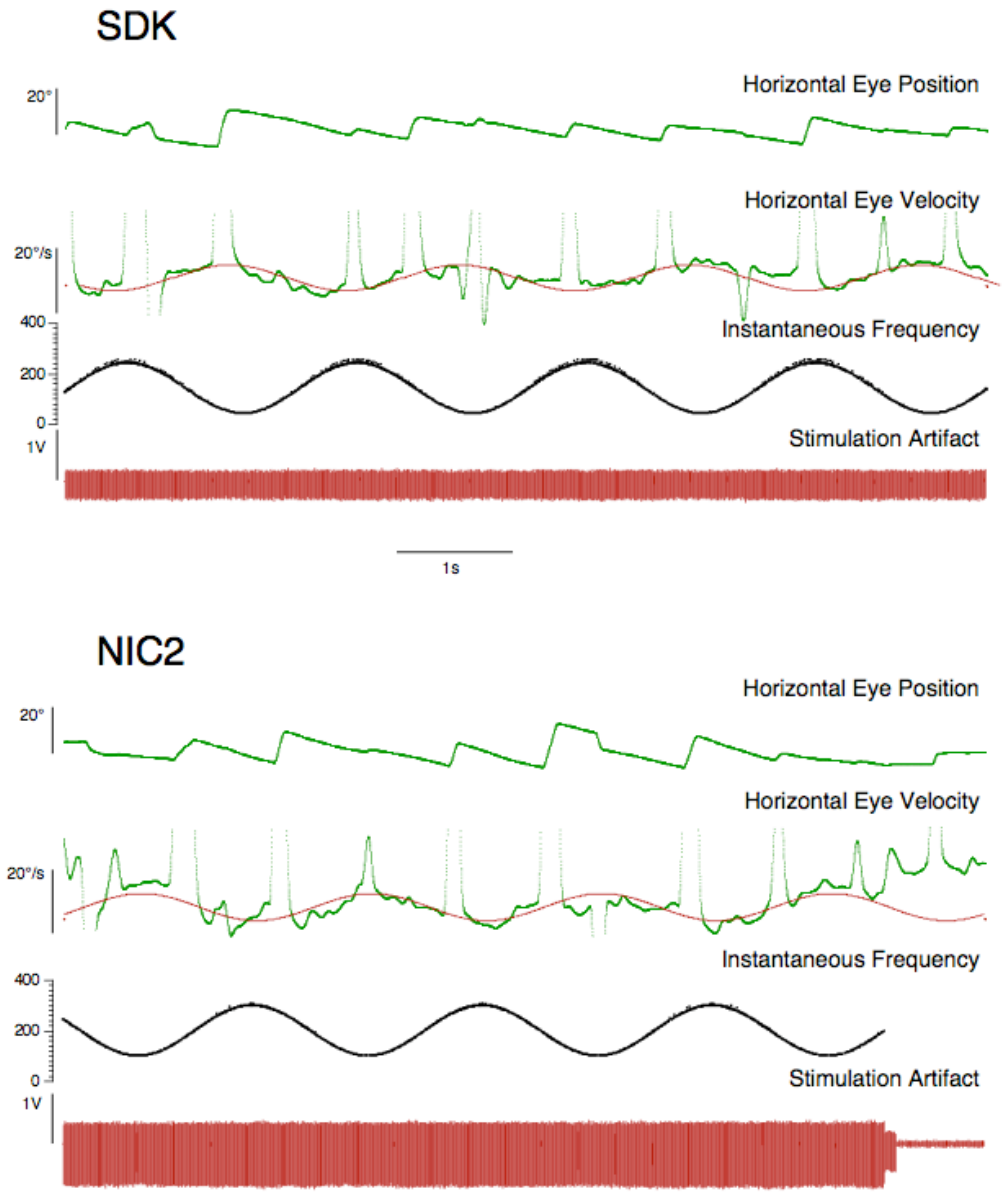

Figure 5. A comparison of comparable SDK and NIC-2 platform stimulation in-vivo. While the instantaneous frequencies of stimulation are the same, and the behavior is similar, the stimulus artifact is larger when using the NIC-2 platform. This suggests that the return path settings are not identical in the programs written using each platform.

9. We have studied short term adaptation conditioning of sine wave modulated FM stimulation. Ordinarily, FM modulated electrical stimulation with our unilateral implant in the intact animal produces modulation in slow phase eye velocity with an offset in

slow phase eye velocity proportional to the mean stimulation frequency. We have examined the effect of using short, constant, high frequency and high current preadaptation stimulation trains to reduce the velocity offset so that the modulation of velocity during FM stimulation occurs around 0 velocity. Initially, the animal is exposed to constant frequency stimulation from 5 to 30 minutes. This stimulation produces a relatively stable slow phase eye velocity for the duration of the stimulation. At the end of the constant frequency stimulation, we use a sinusoidally modulated FM probe stimulus to evaluate the effect of the adaptation. The result of one such experiment is shown in Figure 6.

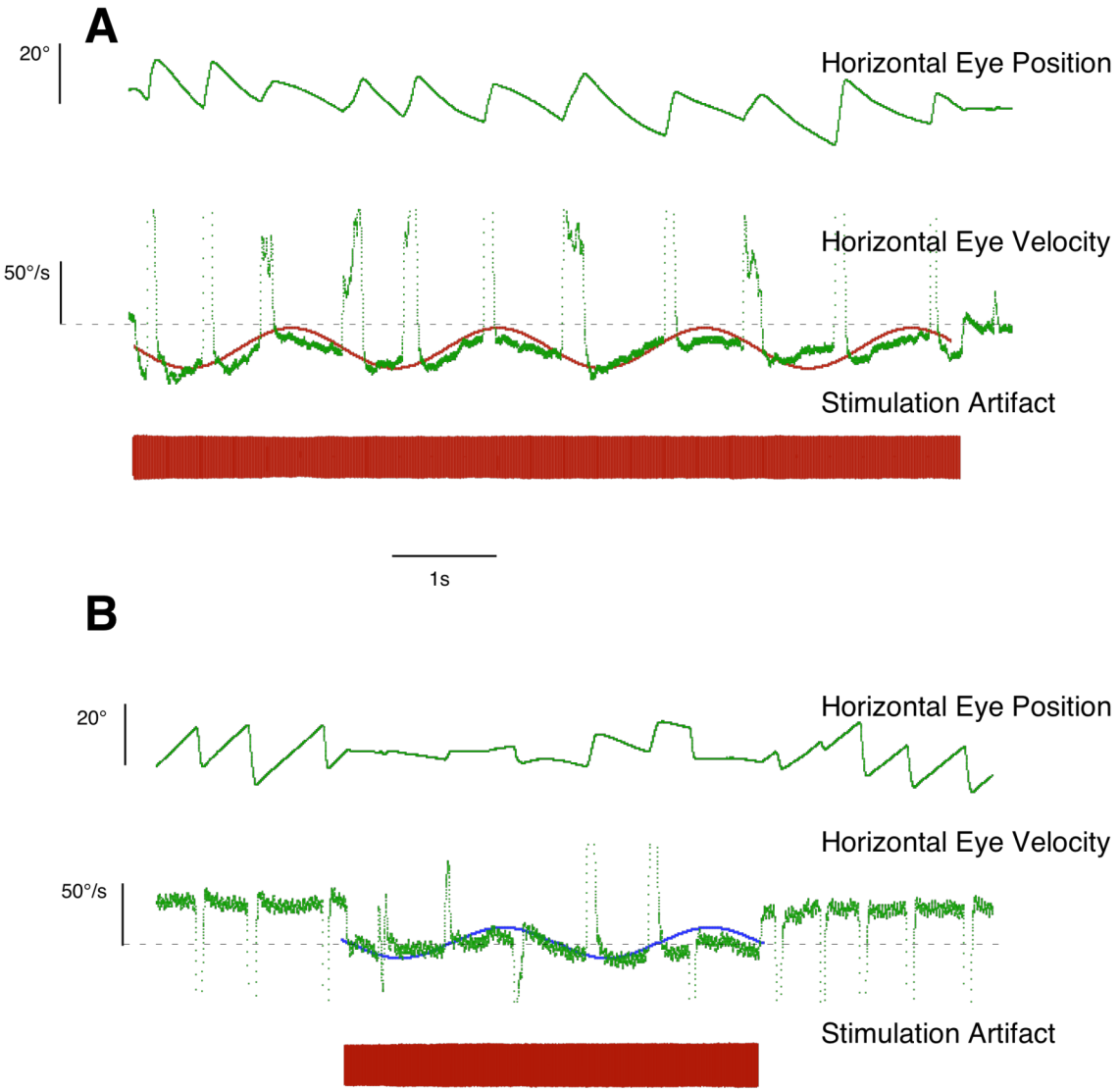

Figure 6. Preadaptation conditioning of FM electrical stimulation. A. Response to FM modulated electrical stimulation before 5 minutes of high frequency stimulation. B. The response to FM modulated electrical stimulation after adaptation. The FM stimulation parameters are 100-300pps, 160 µA, 0.5Hz modulation, 100 µs pulse width, 8 µs gap

Figure 6A shows the response to FM modulated electrical stimulation before adaptation. The FM modulation produces a modulation in slow phase eye velocity with an eye velocity offset as well. In this situation, eye velocity is modulated but always directed away from the implanted ear in the plane of the stimulated canal. The animal then received high constant frequency stimulation for 5 minutes. The slow phase eye velocity elicited by the constant frequency stimulus was maintained throughout the adaptation period (not shown). Figure 6B shows that following adaptation the FM probe stimulus now produces eye velocity both toward and away from the implanted ear, and the mean eye velocity is 0.0 deg/s. The eye velocity is once again modulated in phase with the FM electrical stimulation but without a velocity offset. This result suggests that even brief preconditioning in the intact animal can affect the response to FM stimulation without affecting the response to the constant frequency train.

10. We have continued to process single- and multi-channel neural records to achieve a high reliability of spike classification.

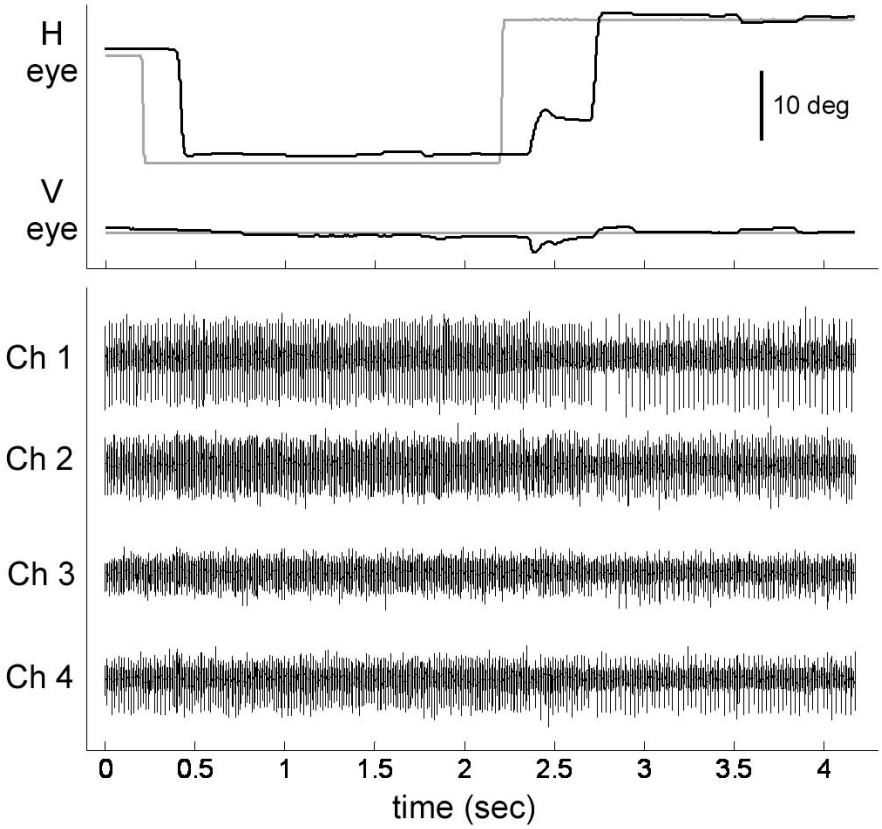

Figure 7. Simultaneous recordings from three brainstem neurons exhibiting eye position sensitivity. Recordings were made with a tetrode device (Thomas Recording) and spatially filtered using the array processing method. Corresponding spike rate traces are shown in Figure 8, and examples of the spike data on a finer time scale is shown in Figure 9.

As described in previous QPRs, the overlap-template matching technique has proven effective at removing electrical artifacts and improving the isolation of neural units when a second unit is recorded on the same electrode. We have now extended these methods to handle multi-channel recordings made with tetrodes. Tetrodes offer an advantage for template matching because two detected neurons (or a neuron and electrical artifact) will generally produce signal energy on each of the four channels with different distributions across the channels. The close spacing of tetrodes also allows common sources of noise to be partially subtracted out via array processing, which we have found to improve both spike detection and the quality of template matching.

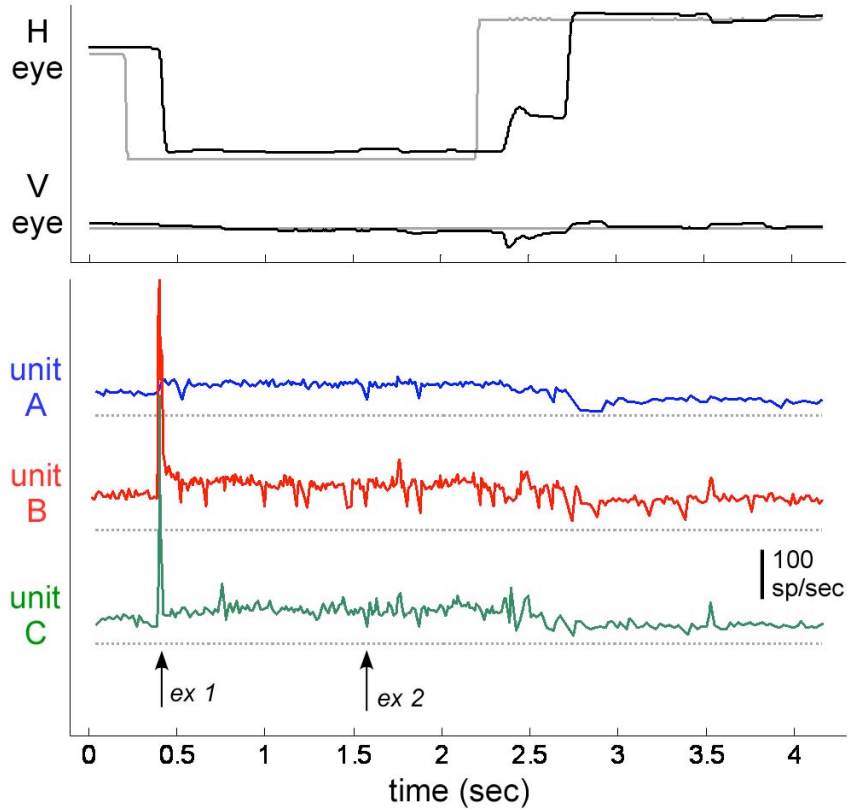

Figure 8. Instantaneous spike rate of the three neurons of Figure 7. The tonic firing rate of each unit increased when the eye made a leftward horizontal saccade at 0.5 s. Units B and C also exhibited a burst of spikes just prior to the saccade ("ex 1" arrow); the multi-channel recording traces during this burst is shown at a higher time resolution in the left panel of Figure 9. The occasional rapid increase or decrease in rate may be the result of a spike detection or classification error; the second arrow highlights a common example ("ex 2"), in which all three units occurred within ~1 ms of each other, creating an overlap of their spike waveforms that the spike sorting algorithm didn't correctly interpret.

The result of the template matching technique applied to tetrode data is illustrated in Figures 7-9. Activity from three neurons is represented across the four tetrode channels, labeled Ch1 – Ch4 in the bottom panel of Figure 7, with each unit appearing primarily on one of the channels. The largest unit (unit A) appears primarily on Ch1, while the spikes from units B and C are most evident on Ch 2 and Ch 4, respectively. All three neurons

were eye position related, having higher tonic spike rates for leftward eye positions (horizontal and vertical eye position traces are shown in the top panel of Figure 7). This behavior is most easily seen in the bottom panel of Figure 8, which plots instantaneous spike rate for each unit, following the spike sorting procedure. For units B and C, the increase in tonic firing for the leftward saccade is preceded by a transient burst of spikes, characteristic of burst-tonic units (arrow "ex 1"). The individual spikes making up each burst can be better seen in the left panel of Figure 9.

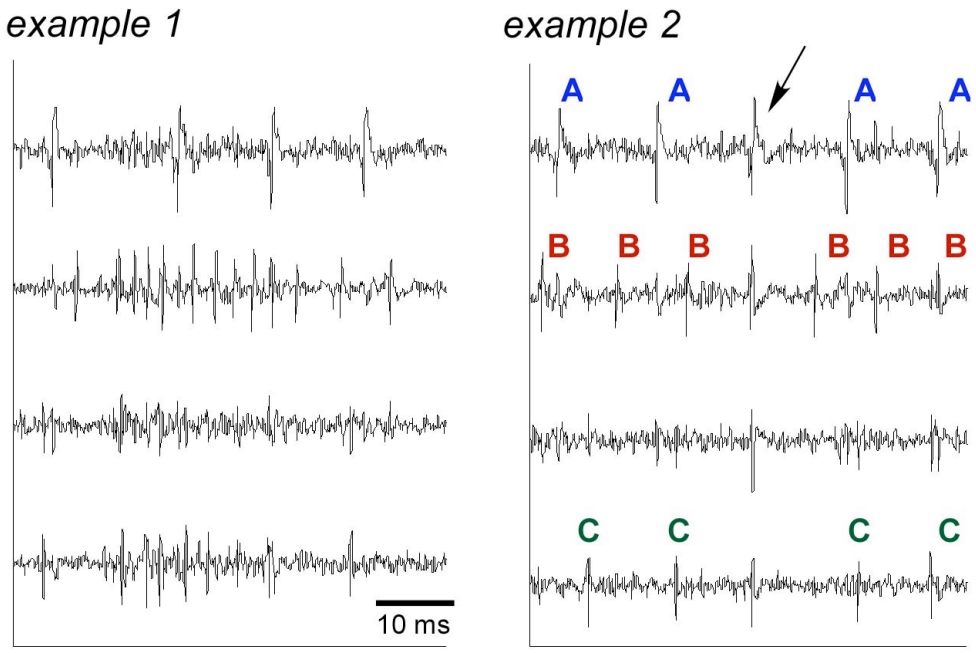

Figure 9. The unique spike waveforms of the three units shown in the previous figures are more apparent on a finer time scale. The left panel corresponds to the initial spike bursts (left arrow in Figure 8), and the right panel is an example of a misclassification during the template matching process. The letters in the right panel indicate spikes that were properly assigned to a spike class (i.e. to a single-unit template), and the arrow points to an overlap of spike waveforms from all three units.

The spike rate traces in Figure 8 reflect blind application of the overlap-template matching procedure (not including the generation of the single-unit templates, which were defined via manual clustering). Thus, many of the fast increases and decreases in rate, such as those apparent in the unit B trace, likely reflect errors in spike sorting. The risk of misclassification is a limitation of the spike sorting process, but the most obvious errors can be corrected manually. Nevertheless, analysis of rate traces like these can provide useful feedback about the quality of spike sorting. Based on the fairly regular firing rates of these types of neurons when eye position is steady, it appears that false-negatives (omitted spikes) were much more common than false-positives (added spikes)

in this recording sample. Closer inspection reveals that missed spikes were very infrequent for the largest unit (A), and for unit B occurred only a small fraction of the time. An example of a missed spike classification is shown in the right panel of Figure 9 (arrow "ex 2" in Figure 8). In this particular case, the detected spike waveform was actually the simultaneous occurrence, or overlap, of all three units. Indeed, the template matching algorithm is currently configured to handle overlaps of only two units at a time.

11. We have continued our single unit recording and electrical stimulation experiments. We have concentrated on neurons stereotaxically located in the medial portion of the medial vestibular nucleus (1 mm posterior and 1 mm lateral to abducens). In this region, there are neurons with vestibular and also eye position sensitivity.

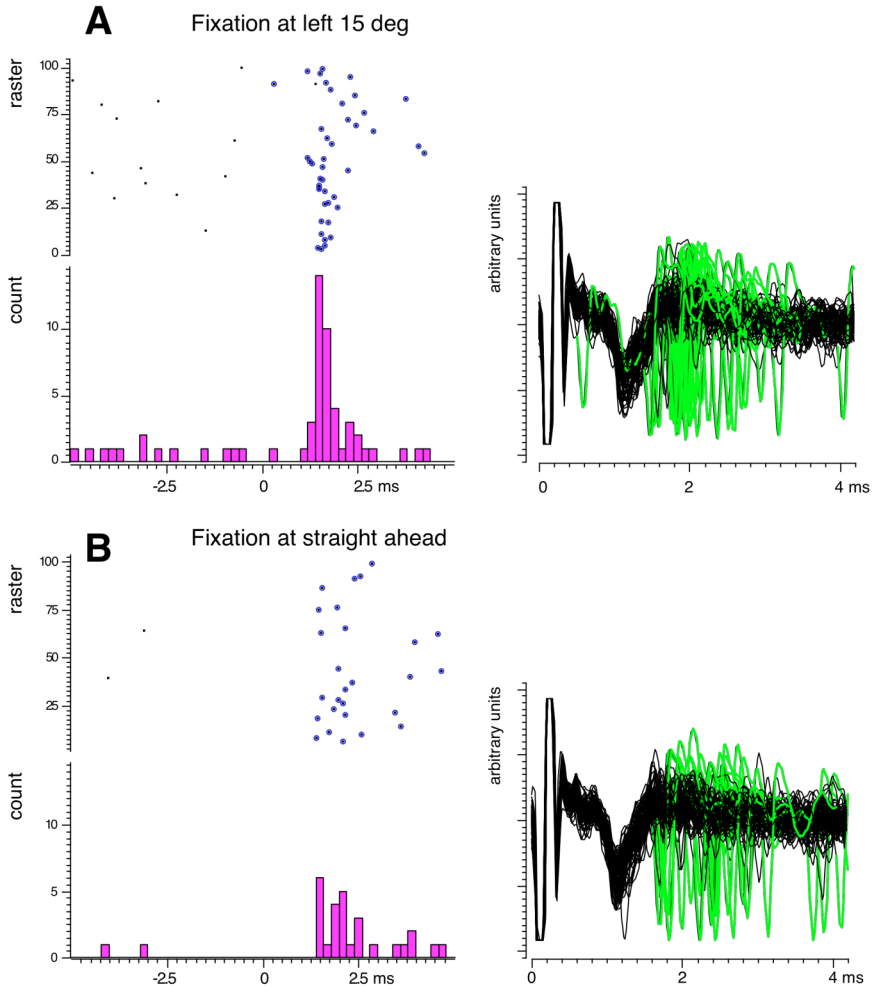

Figure 10. Unit discharge aligned on electrical stimulus artifact. A. Unit discharge raster, spike histogram, and time display during fixation at 15 deg to the left. B. Unit discharge raster, spike histogram, and time display during fixation at 0 deg eccentricity. In the rasters, the first spikes following each stimulus artifact are labeled in blue. The major ticks on the histogram and rasters represent 2.5 ms. In the time display, accepted unit spikes are labeled in green.

During electrical stimulation, we typically record a field potential and then if he unit is driven a spike follows at a short and consistent latency. Unlike other neurons we have encountered at relatively short latency, many of these recently recorded short latency neurons have higher thresholds for excitation with electrical stimulation. Furthermore, some of these neurons show a facilitation of unit discharge for electrical stimulation with horizontal eye position in the on direction for the neuron. This is illustrated in Figure 10. In this example neuron, recorded in the region of the right medial vestibular nucleus, eye position to the left increases the reliability of discharge following a 10 Hz electrical stimulus. This neuron typically has a very ragged increase in its tonic discharge rate for eye positions to the left. In Figure 10A, the unit raster shows the first unit spike following each electrical stimulus in blue and spontaneous discharge in black, and the spike histogram shows the distribution of all spikes. The actual spikes are shown in the inset immediately to the right of the unit raster. The vestibular field potential is also clearly visible in the inset. The neuron in Figure 10A often discharges in a time locked manner with the electrical stimulation while the animal is looking 15 deg to the left.

In Figure 10B, the unit discharge is again aligned on the electrical stimulus. Here however, there is only a ragged alignment of unit discharge on stimulus onset, with very few spikes actually occurring in the time window of the raster; i.e., most stimulation trials do not elicit a driven spike. Taken together, Figure 10 illustrates that this neuron accurately relays the electrical stimulation primarily in leftward eye position, and not in primary position. This serendipitous finding is interesting, because it suggests that the population coding of the electrical stimulus may change with tonic eye position in this portion of the vestibular nucleus.

Objectives for Quarter 15.

1. We will submit our full IDE for our device to the FDA.

2. We will continue our single unit recording experiments during electrical stimulation in rhesus monkey. We plan to record in animals during natural stimulation, during electrical stimulation, and following adaptation with high frequency stimulation trains. We also plan to further evaluate the eye position sensitivity of the response to electrical stimulation.

3. We will record with tetrodes, or our new arrays from Lawrence Livermore Laboratories, with the objective of recording multiple single units during natural and electrical stimulation.

4. We will evaluate the affects of adaptation parametrically in multiple animals to see the time course of the adaptation and post-adaptation effects, and the sensitivity of the effect to adaptation train, and probe, frequency and current.

5. Based on the work this quarter, we will develop the software to process rotational analog input signals fed through the audio input ports of the SDK platform, and then transform them into FM pulse trains similar to those shown above. This function will

enable us to conduct a variety of FM stimulation experiments on monkeys by using real rotational signals to drive modulated electrical stimulation over long durations.

6. We will implant an additional animal with stimulating electrodes in three canals, and evaluate our ability to steer eye movements in multiple directions in response to preprogrammed electrical stimuli, and combined electrical stimulation and rotational stimuli.