Twelfth Quarterly Progress Report

May 1, 2009 to July 31, 2009
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Neurophysiological Studies of Electrical Stimulation for the Vestibular Nerve

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Challenges:

1. We have not received an adequate supply of axial multiple single unit recording electrodes. As of the end of Quarter 12, we still have not received the bulk of our multiple single unit axial recording electrodes from NeuroNexus – FHC. We have modified our recording system to allow recording from the two oversized prototype electrodes that we have received. We have dedicated one monkey to this preliminary recording project. This monkey had previously lost its percutaneous stimulation leads for canal plugged stimulation, and so any damage done by the larger shaft of the prototype axial electrode array will not cause us to lose valuable recording data from an animal with an intact vestibular prosthesis.

Our preliminary data from recording experiments, presented below, suggest that the shaft of the axial array may not provide optimal recording sites for recording simultaneous multiple units from the brainstem of an awake monkey. However, we will continue working with this animal and the two axial array electrodes that we have until the axial electrodes are no longer viable (i.e., until we have either bent the tip of the tungsten shaft running tracks or damaged the axial array through repeated insertion in the recording cannula). This approach should provide us with preliminary information about the utility and durability of these electrodes in everyday use. We will then refocus our efforts away from the use of the axial arrays until such time as NeuroNexus-FHC moves forward with production of our devices.

2. It is taking us a much longer time than we had anticipated to map the chambers of the implanted monkeys and identify the optimal recording sites within the brainstem. We suspect that the stereotaxic placement of the chamber after implantation of the vestibular prosthesis is compromised by new bone growth in the temporal bone adjacent to the external ear canal. While this has not prevented continued success with our recording studies, it has added considerably to the effort required to locate physiologically identified landmarks within the recording chambers. As a result, we are revising our protocol to implant the chambers first in our monkeys, along with the eye coils. Then we will map the chambers, identifying all of the important landmarks but running a minimum number of penetrations through the vestibular nuclei. After the chambers are fully mapped, we will implant the vestibular prosthesis and begin stimulation experiments. All of these changes have been incorporated into our 3-year renewal of our IACUC protocol. This has delayed implantation of the new monkeys with the prosthesis, but should greatly simplify our ongoing recording experiments. It should be noted that this change is not without risks, because maintaining the recording chamber and running electrode penetrations does expose the animal to increased risk of infection or damage to tissue adjacent to the electrode tracks. We will continue to use silicone elastomer to seal the chambers, and we will clean and treat the chambers on a regular basis and before any penetrations are run to reduce these risks.

Current Successes:

1. **In Quarter 12 we examined nulling of optokinetically induced eye movement with electrical stimulation.** We presented full field visual motion stimuli to monkeys to produce optokinetic nystagmus of a specific velocity. We then used constant rate stimulus trains with a frequency and current that were previously determined to produce nystagmus in darkness with slow phase eye velocity matching, or exceeding, the velocity of the optokinetic nystagmus. This stimulus paradigm is an analog of the one that we propose using to treat nystagmus and vertigo in human subjects with Meniere's disease. Short trains of stimuli produced an elimination of optokinetic nystagmus when the slow phase velocity elicited by the train in the dark matched the velocity of the optokinetic nystagmus. When the stimulation elicited higher slow phase velocities in the dark, it produced a reversal of nystagmus when paired with ongoing optokinetic nystagmus. The resulting slow phase velocity of the paired stimulus could be predicted by the linear summation of the optokinetic nystagmus velocity without stimulation, and the velocity of the electrically elicited nystagmus in the dark. This is shown in Figure 1, where a series of three 2s stimulus trains are presented during 40 °/s optokinetic nystagmus. The first stimulus train was constructed to match the slow phase velocity of the nystagmus; i.e., produces a slow velocity of 40 °/s in the opposite direction in the dark The second train doubled the current and roughly doubled the slow phase velocity elicited by stimulation in the dark. The third stimulus train tripled the current and further increased the slow phase velocity of the electrically elicited nystagmus in the dark. The first stimulus train eliminated the nystagmus, but the second and third trains produced reversal of nystagmus with higher and higher slow phase velocities. This suggests that a constant rate stimulus train, pre-calibrated by the velocity of nystagmus that it elicits in darkness, can be used to stop ongoing nystagmus of a predicted velocity.

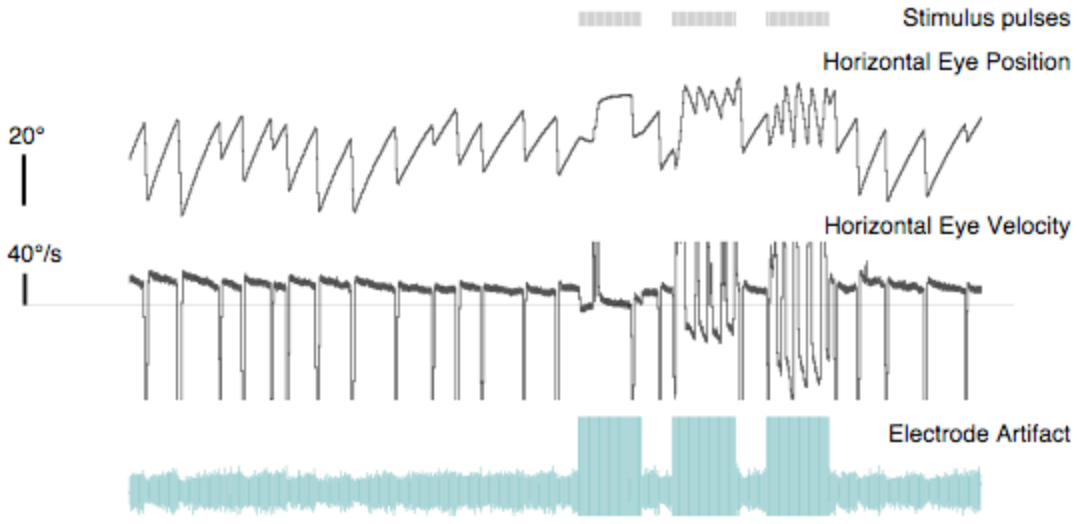

Figure 1. The effect of short duration electrical stimulation of ongoing optokinetic nystagmus. Two-second constant rate stimulus trains of 300 pps at 40, 80 and 120 μA are presented to the right lateral canal during 40 °/s optokinetic nystagmus.

Although not shown in Figure 1, there is a limitation to this paradigm to study nulling of nystagmus. Longer trains reliably stopped nystagmus in all of the monkeys tested, but then the continuing visual stimulation produced a resumption of nystagmus. Given the closed-loop nature of visually elicited optokinetic nystagmus, this is not surprising. However, it means that we were unable to examine the effects of long duration stimulation with this paradigm.

2. We began to evaluate head unrestrained behavior during electrical stimulation with the vestibular prosthesis. We observed that electrical stimulation of the vestibular end organ during stable gaze holding reliably produces both head and eye movement in the same direction, as would be expected from natural stimulation of the vestibular end organ. An experiment examining head and eye movement during head restrained and head unrestrained electrical stimulation is shown in Figure 2. Electrical stimulation of the right lateral canal in a monkey with the head restrained elicits primarily a right beating horizontal nystagmus. Electrical stimulation, with the same stimulus parameters, with the head unrestrained again elicits a right beating horizontal nystagmus, but also elicits a leftward head movement, which also beats to the right at a lower frequency. The beats of head nystagmus are damped by the large inertial load of the head. The resulting slow phase horizontal gaze velocity (sum of head velocity plus eye in head velocity) in the head unrestrained stimulation is roughly equal to the slow phase eye velocity in the head restrained stimulation. Figure 2 suggests that the electrical stimulation is producing a natural activation of the vestibular system, which is distributed to the eye and head motor plants appropriately.

3. We examined the characteristics of eye movements elicited with a revised version of the PDA controlled vestibular prosthesis. We purchased a new PDA with a higher processing speed and paired that device with a modified PDA SDIO card from Dr. Loizou at UT Dallas. The codes associated with the new PDA card were then significantly modified to create pulse trains similar to those created with the NIC 2 processor. We then demonstrated on the bench top that the new card and PDA were capable of generating constant rate pulse trains of different frequencies without the dropout of pulses (strobing) that we observed with the previous PDA card.

Figure 3 shows the result of our bench top testing. The PDA processor controller was connected to an implant simulator (implant in a box) to measure the electrode outputs. Stimulation currents were converted to voltage outputs as measured across a 5kΩ resistor. Inset 3A shows the control interface that was used, with onscreen buttons to start and stop stimulation and a slider to set the current level during stimulation. Panels 3B and 3C show 40 ms duration pulse trains recorded with a high-resolution and high-bandwidth Tektronix DPO 4034 oscilloscope. In Figure 3B, the pulse rate was set to 380 pulses/s and the pulse width was fixed at 100 μs. In Figure 3C, the pulse rate was increased to 600 pulses/s and the pulse width was set to 61 μs. Because of the limitations of the FPGA program on the SDIO card, the pulse width and pulse rate are correlated with each other and we are currently unable to set the pulse width and pulse rate independently. This is a significant limitation of the current PDA controller. Figures 3B and 3C demonstrate that

the PDA controller can produce consistent and reliable pulse trains. In addition, it can produce pulse trains of essentially unlimited duration. We have discussed with Dr. Loizou the trade-off between pulse rate and pulse width and we will need new FPGA codes to address this issue in future experiments.

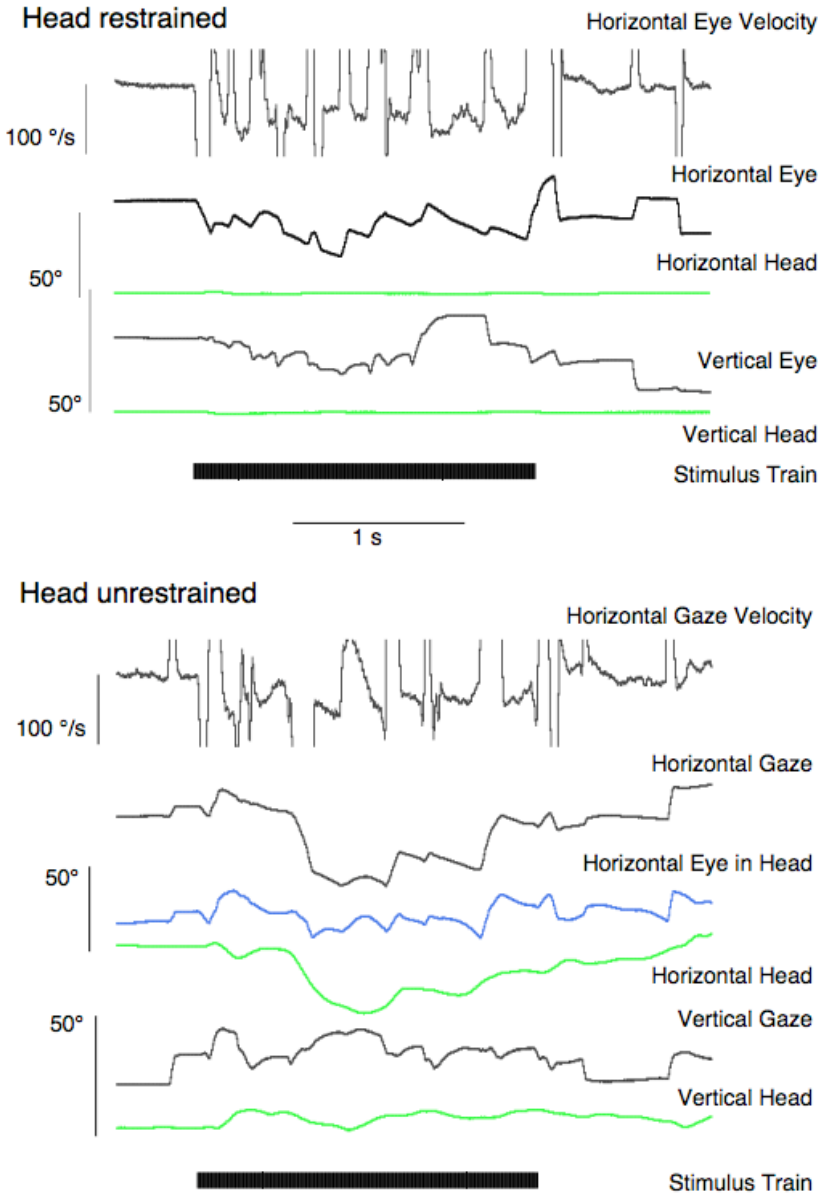

Figure 2. Electrical stimulation of the right lateral canal elicits equivalent slow phase eye and gaze velocity in the head restrained and head unrestrained monkey, respectively. The upper panel shows eye nystagmus resulting from stimulation of the right lateral canal in the head restrained monkey. The lower panel shows combined eye and head nystagmus, or gaze nystagmus (eye in head plus head = gaze, or eye in space) resulting from the same stimulus applied after the head was free to move.

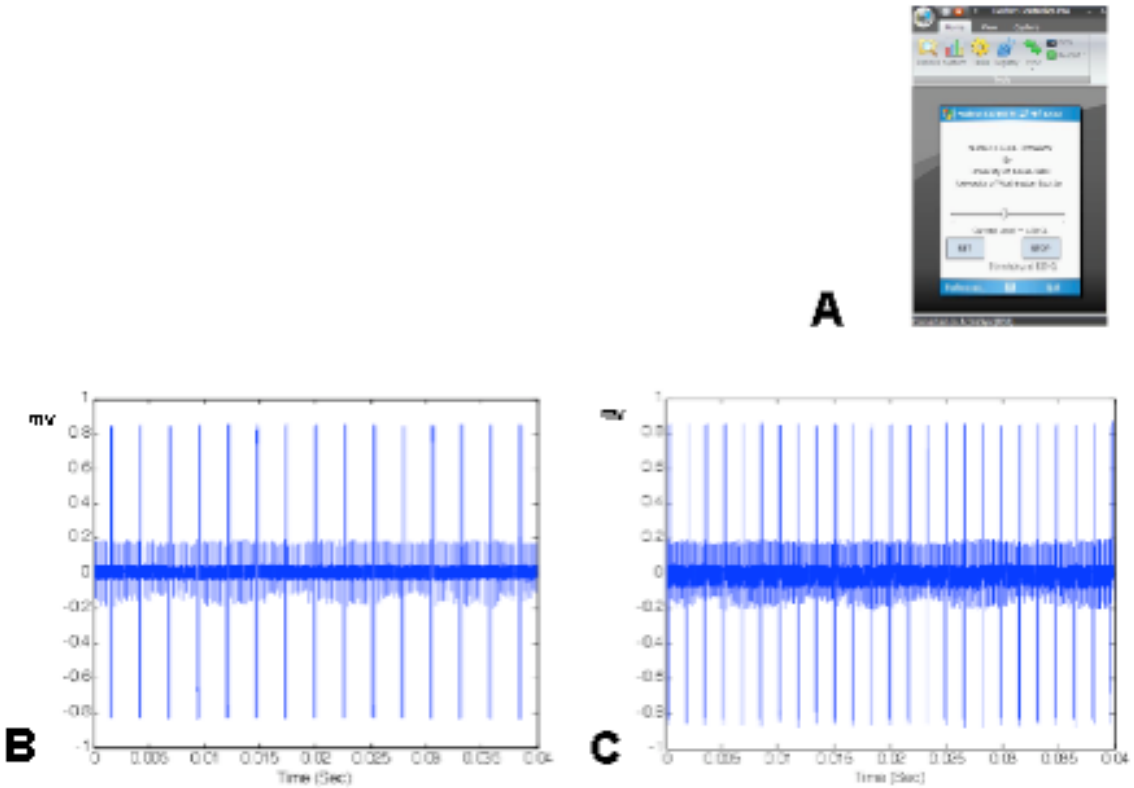

Figure 3. Bench top stimulation with a PDA controller and an implant in a box. A. Inset showing the PDA GUI interface for controlling stimulation parameters. B. Stimulation at 380 pps with a pulse width of 100 μs. C. Stimulation at 600 pps with a pulse width of 61 μs.

The stimulation parameters used for the bench top test of the PDA controller were also used for experiments driving an implanted vestibular prosthesis in a monkey. Figure 4 compares the result of electrical stimulation using the NIC 2 interface and VIstream with the result of stimulation using the PDA interface through the same distal lateral canal electrode in the same session. In both cases, a constant frequency pulse train is used. In the upper panel, stimulation with the NIC 2 interface at 300 pps, 100 us pulse width, and 150 μA current amplitude produces a clear right beating nystagmus with little vertical component. In the lower panel, stimulation with the PDA controller at 380 PPS, 100 us pulse width, and 130 CL current amplitude (≈183 μA) produces a similar horizontal nystagmus. These experiments suggest that the revised PDA controller now produces a very similar stimulus output to the NIC 2 interface on the bench, and elicits similar behavioral responses when paired with an implantable vestibular prosthesis.

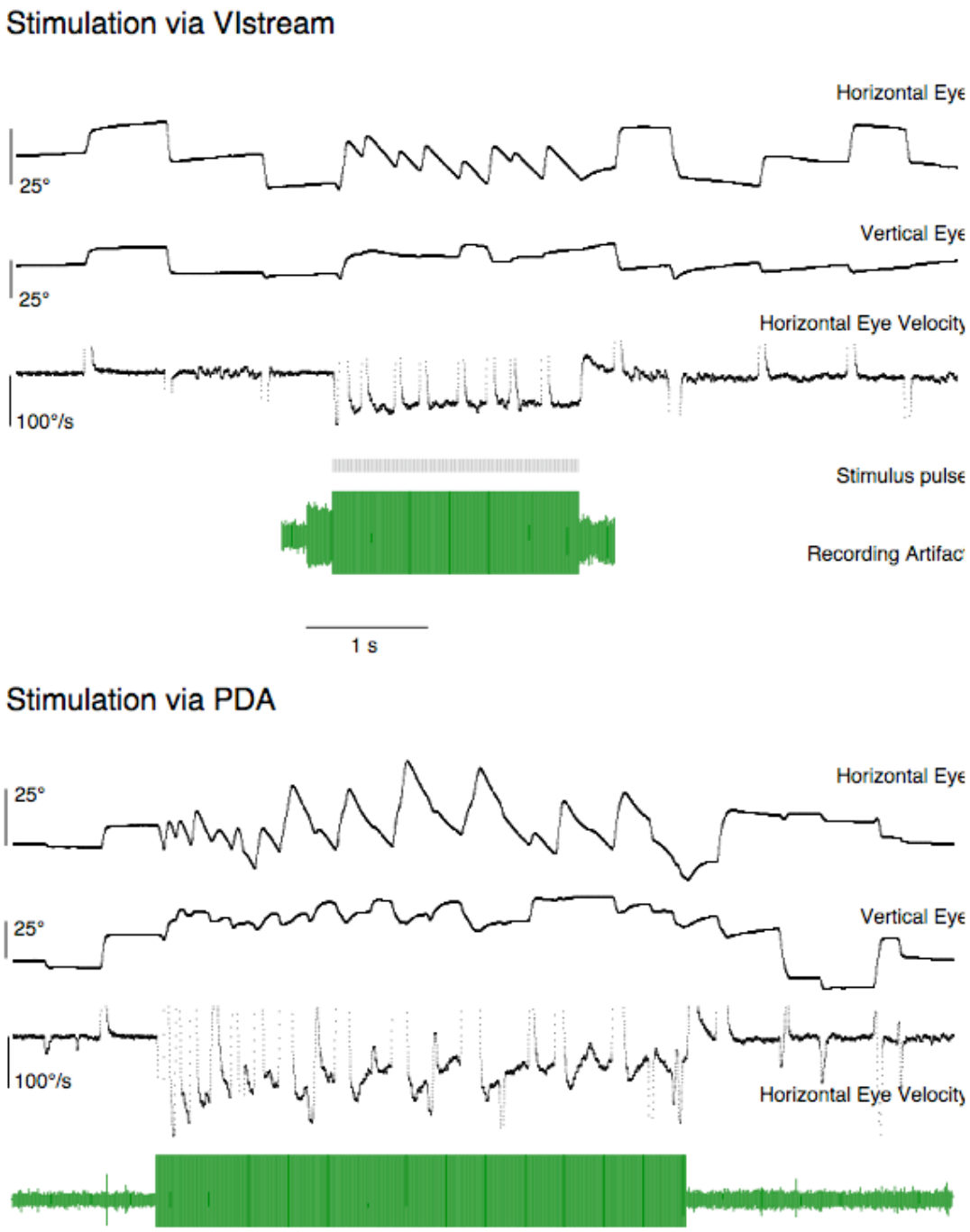

Figure 4. Comparison of nystagmus from stimulation using the NIC 2 interface and VIstream controller with nystagmus produced using the PDA controller. Upper panel: Nystagmus elicited by stimulation with the NIC 2 and VI stream for 2 s duration, 300 pps, 100 us pulse width, 150 µA. Lower panel: Nystagmus elicited by stimulation with the PDA controller for 4 s duration, 380 pps, 100 us pulse width, 130 CL ≈ 183 µA.

4. We sacrificed and perfused three animals for histology and high resolution micro CT. Three of our monkeys with non-working prostheses were sacrificed and perfused with formalin for histological reconstruction of recording tracks. Brains in two of these animals have been blocked, sectioned and stained with cresyl violet, and are undergoing microscopic examination. In addition, the temporal bones of two animals were preserved to allow for histological examination of the anatomy of the end organ following prolonged stimulation with a vestibular prosthesis. In one animal, the non-working vestibular implant was maintained intact in the implanted ear to confirm the location of the stimulating electrodes with very high resolution micro-computed tomography. This device will allow very thin section (up to 5 μm resolution) reconstruction of the semicircular canals and should show the precise placement of the electrode arrays within the end organ. These experiments are ongoing. In addition, we are preparing a Bloedel Traveling Scholarship to allow Dr. Stephen O'Leary to come to the laboratory to work with us, and to prepare the temporal bones for histological analysis in the laboratory of Dr. Rob Shepherd. This would allow us to confirm that the end organ is histologically intact following electrical stimulation.

5. We have obtained recordings using the Neuronexus - FHS axial array electrode in one animal. In Figure 5, we first recorded in the brainstem with tungsten microelectrodes to determine the characteristics of the neurons in the region, and then with the axial array electrode in the same location so that a direct comparison could be made between the quality of the recordings and behavior of the animal in both situations. Recording with the tungsten single unit microelectrode showed numerous burst tonic neurons at various depths throughout the recording tracks, suggesting that the recording site was in the immediate vicinity of the abducens nucleus. We chose this site because it contains many neurons with similar, but distinct, discharge properties (i.e., burst tonic neurons with different eye position thresholds, rate versus eye position relationships, and rate versus eye velocity relationships).

Recordings obtained with the multiaxial array in this and other experiments failed to record multiple single units simultaneously in the brainstem. As can be seen in Figure 5, the multiaxial array recorded a single unit in this region from one of the recording sites along the shaft on the array. The other sites, including the tip of the tungsten core electrode, failed to record this unit as the electrode was advanced, despite the fact that they presumably passed by it. In addition, the multiaxial electrode array produced significant changes in the behavior of the animal as the electrode was advanced into the region of interest. The animal began to display a gaze holding nystagmus, suggesting that the size of the array was displacing tissue and disrupting normal function of the brainstem gaze holding integrator. Many of the neurons in this region are thought to perform this function. These initial recordings suggest that the size of the prototype array, which is far larger than our original specifications, may be a significant issue for prolonged brainstem neural recording in awake behaving primates. Furthermore, it may be that the current design of the prototype device, with a relatively flat and wide surface, causes it to become covered during penetration into the brainstem. Finally, the low electrode impedances suggest that 1) the sites may be too large (~700 μm^2) for recording in the macaque brainstem; 2) the electrode leads may be shorted to one another; or 3) there may

be leaks in the insulation layers, effectively shorting the small voltage signals to ground. We are currently considering strategies to improve the recording quality of the axial array, including iron plating.

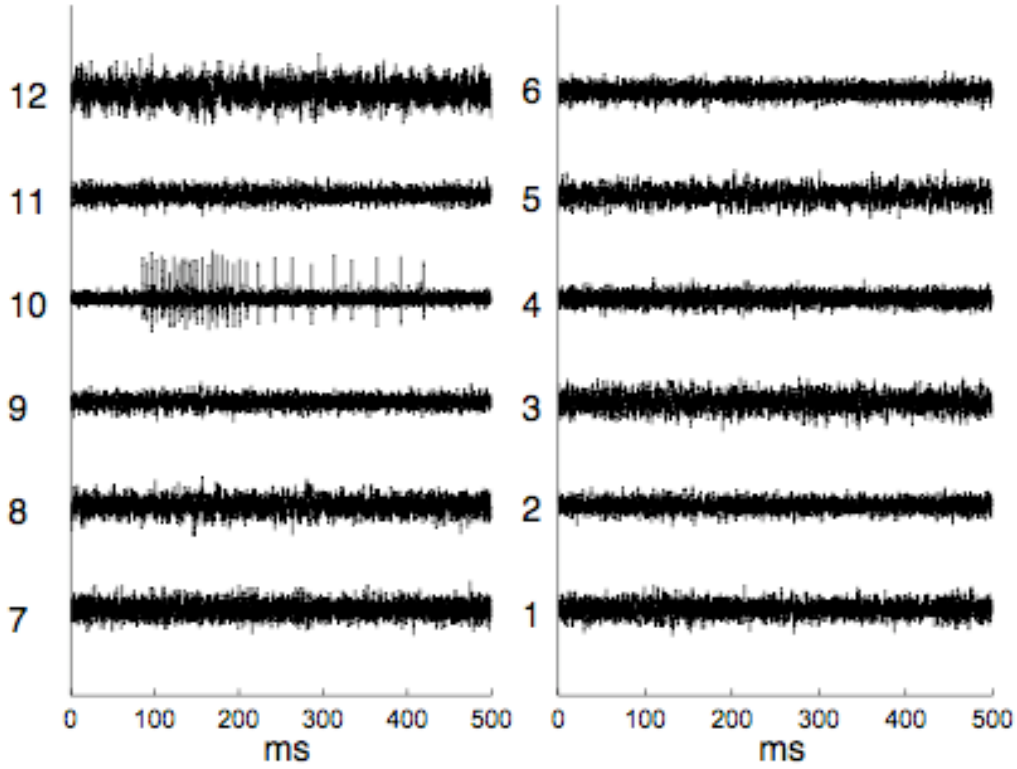

Figure 5. Recording in the vicinity of the abducens nucleus with a prototype axial array electrode. The voltage traces are ordered from the back of the array (channel 12) to the tip (channel 1).

6. We continued our recording experiments in the vestibular nucleus and adjacent nuclei, comparing higher order neurons driven by electrical stimulation at multisynaptic latencies with secondary vestibular neurons driven at monosynaptic latencies. We have revealed several interesting properties of the higher order neurons. Figure 6 illustrates the properties of a secondary vestibular neuron receiving monosynaptic input from the vestibular end organ. At high stimulation currents, Figures 6A and 6B, the neuron responds reliably (filled blue circles) and at short latency to the electrical stimulus pulses at 10 Hz (indicated by the dotted vertical line). As the stimulus current is decreased, the unit begins to fire more sporadically in response to the electrical stimuli (Figure 6C). Finally, at the lowest current, Figure 6D, the unit discharges randomly with respect to the electrical stimuli. This progression is common to the neurons driven at monosynaptic latency. Such neurons, as discussed in the previous QPR, are typically driven from the canal that is activated by natural stimuli in the plane of the eye movement evoked during electrical stimulation; e.g., horizontally sensitive neurons are driven by vertical axis rotation toward the stimulated canal.

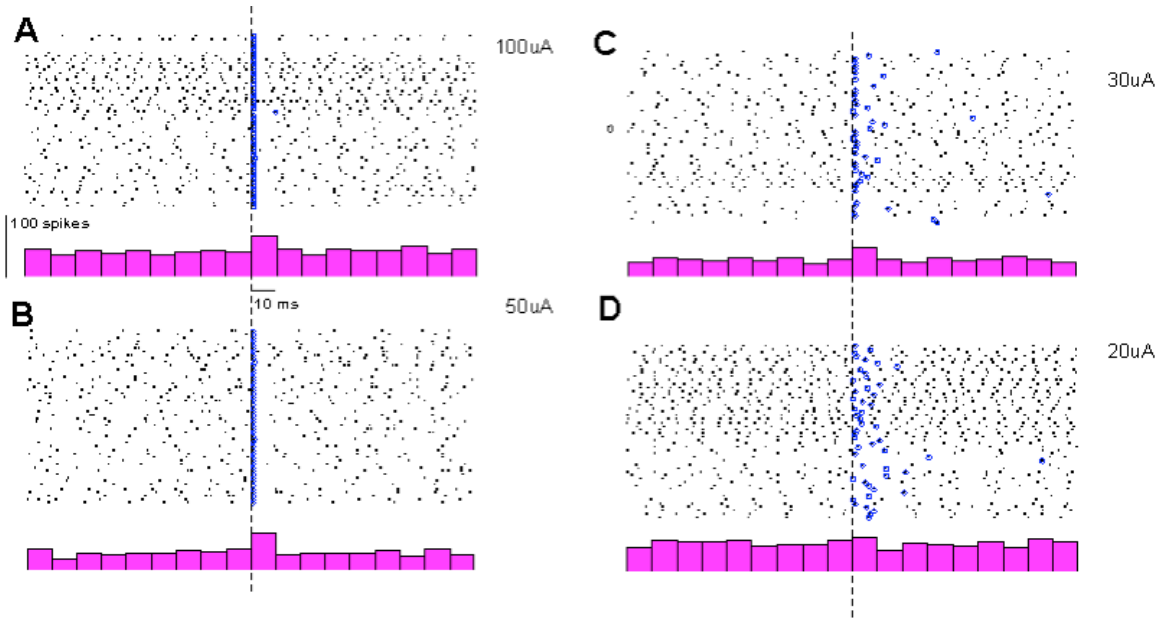

Figure 6. Rasters of spike discharge of a single neuron during 10 Hz electrical stimulation of the right lateral semicircular canal. Rasters are aligned on electrical stimulation onset (dashed vertical line). Filled circles indicate the first spike following each electrical stimulus pulse. A. Stimulation with a current amplitude of 100 μA. B. Stimulation with a current amplitude of 50 μA, just above threshold. C. Stimulation with a current amplitude of 30 μA, just approaching threshold. D. Stimulation with a current amplitude of 20 μA, below threshold. The spike histograms indicate the number of spikes in 10 ms bins preceding and following the onset of stimulation.

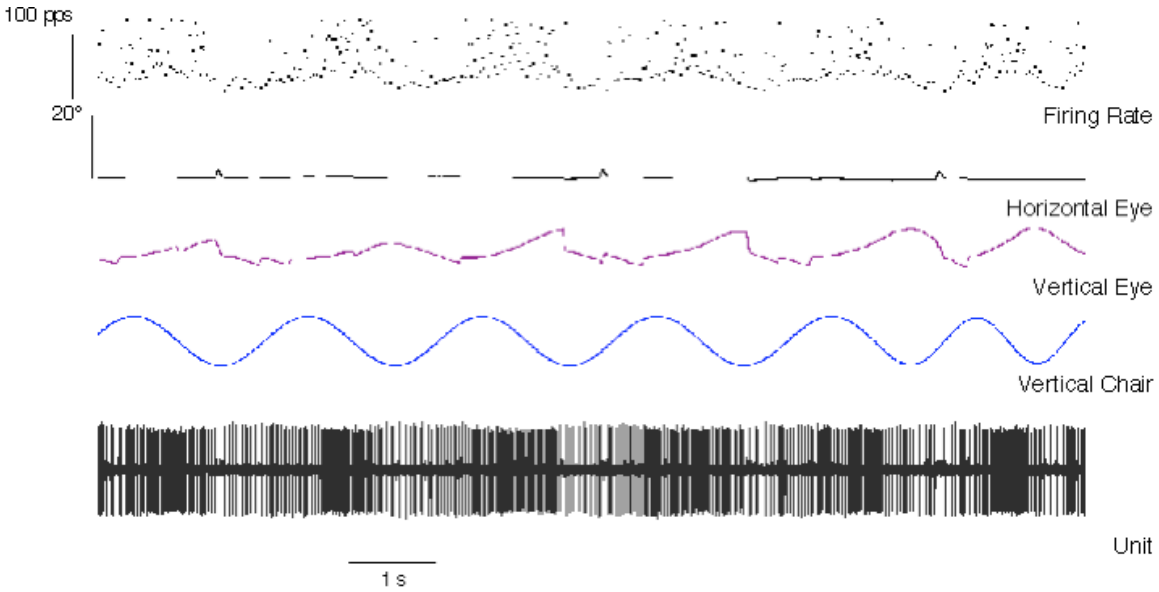

Figure 7: Modulation of an irregularly discharging neuron in the vestibular nucleus during pitch rotation.

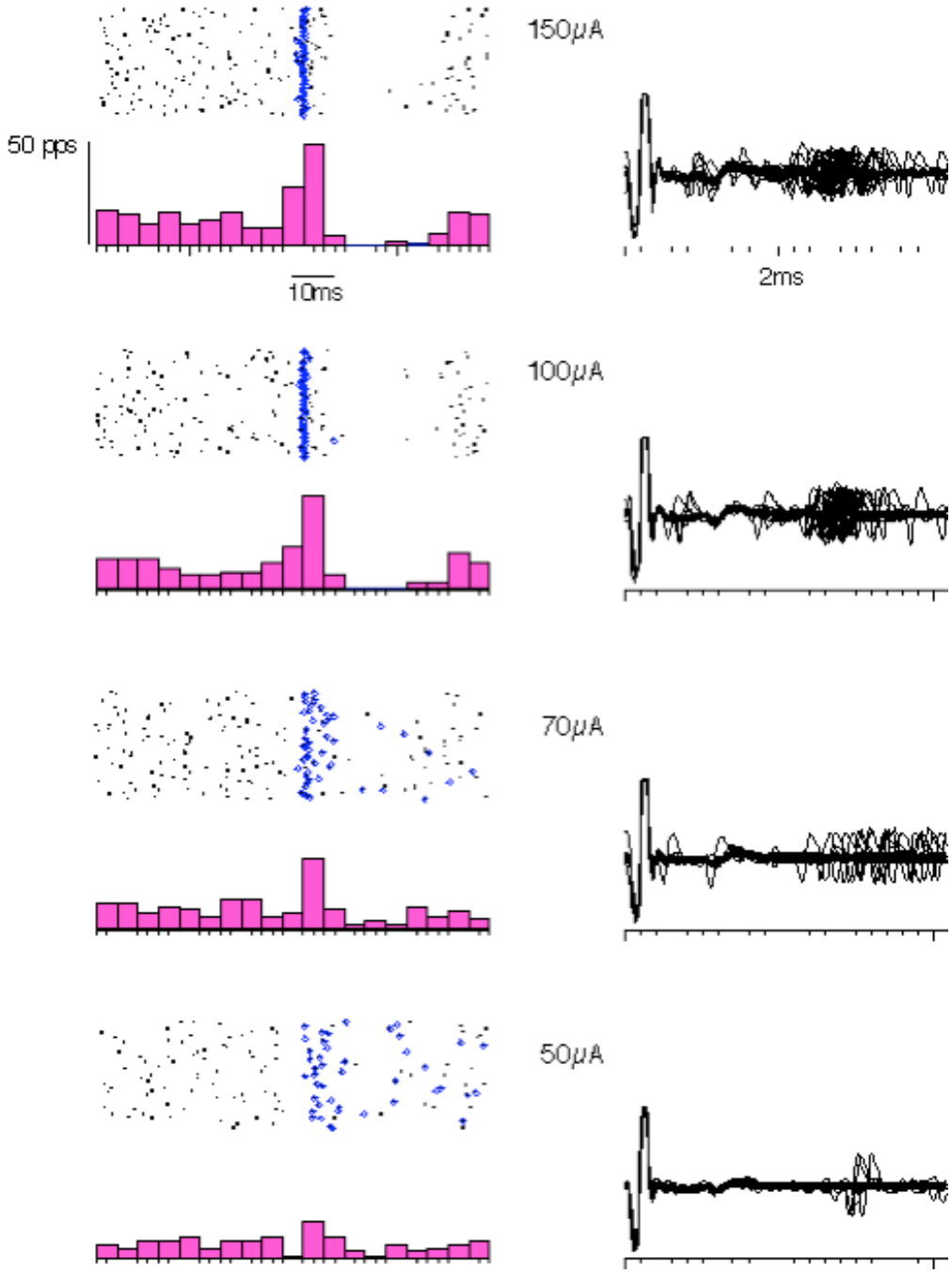

Figure 8. *Spike discharge of the neuron shown in Figure 7 during 10 Hz electrical stimulation of the right lateral semicircular canal at different currents. Rasters are aligned on electrical stimulation onset. Filled blue circles indicate the first spike following each electrical stimulus pulse. The left column shows rasters of spike discharge and spike histograms indicating the number of spikes in 5 ms bins preceding and following the onset of stimulation. The right column displays the spike waveforms triggered on the electrical stimulus artifact (stimulus onset).*

Neurons driven at longer latency show more complex characteristics. Such a neuron is illustrated in Figure 7. This irregularly discharging neuron increased its discharge in

association with down chair velocity during pitch rotation. It was not modulated during horizontal rotation. Based on this observation alone, if this neuron was a secondary vestibular neuron monosynaptically activated by a single canal, one might expect that the cell would be driven by electrical stimulation of the superior canal. However, as shown in Figure 8, this neuron was excited by stimulation of the right (ipsilateral) lateral canal at long latency (approx. 3 ms). There is considerable temporal jitter in the stimulus elicited discharge of such neurons, especially near threshold. However, as the stimulus current is increased, the latency of the stimulus evoked discharge becomes more reliable, until the first spike following the each stimulus pulse, blue filled circles, is relatively time locked to the stimulus. This characteristic is also revealed by the spike histogram, which shows the emergence of a clear peak in the discharge of the neuron at higher stimulus currents.

Another interesting feature of the electrically elicited discharge is also clearly present in the rasters and spike histograms of Figure 8. As the stimulus current is increased, there is the emergence of an even later component to the response, i.e., a pause in the resting activity of the neuron. This pause is clearly present at 100 μA and 150 μA current intensities, and is not clear in the discharge at 50 μA. Such a pause may represent the arrival of a longer latency inhibition, or a disfacilitation of the neuron. It is likely that it reflects the activity of intrinsic connections within the brainstem, and not the activity of the vestibular nerve or secondary vestibular neurons, simply because it is not seen in neurons activated at monosynaptic latencies. The complex discharge characteristics of the neurons driven at longer latencies may provide an important clue to the processing of vestibular signals in the brainstem during electrical stimulation, and help to explain the relationships that we have observed between overt eye movement behavior and the parameters of electrical stimulation with the vestibular prosthesis

7. We have written two manuscripts describing the results of our NRT experiments and our behavioral stimulation experiments. Both manuscripts will be submitted in the first half of Quarter 13. We have also presented our data at the Conference on Implantable Auditory Prostheses 2009 at Lake Tahoe, California as an invited presentation and in a poster presentation. We have presented our data at the combined Otological Spring Meetings in Phoenix Arizona, and at the ENT World Congress IFOS Brazil 2009, in Sao Paulo, Brazil. We have published an abstract for the Neuroscience 2009 Annual Meeting in Chicago, Illinois.

8. We have submitted a pre - I.D.E. application to the Food and Drug Administration (F.D.A.) in preparation for a Phase One clinical trial of our vestibular prosthesis. In addition, we have submitted an R01 application to the NIDCD, which proposes an expanded Phase One clinical trial of the vestibular prosthesis in 10 human subjects.

Objectives for Quarter 13:

1. We will continue to record multiple single units with the axial array. We will complete this activity when the two prototype electrodes fail, unless we receive additional electrodes from NeuroNexus-FHC.

2. We will continue to record from single neurons in intact monkeys in response to natural and electrical stimulation. We will also perform recording experiments in new monkeys with premapped chambers. One of these monkeys will be canal plugged and the other monkey will receive transtympanic gentamicin injection. We will continue to explore the apparent dichotomy between secondary vestibular neurons and those driven at longer latency, presumably higher order vestibular neurons. In addition, we will begin to characterize neurons using more complex head unrestrained behavioral tasks.

3. We will continue to evaluate optokinetic nystagmus and vestibular interactions with electrical stimulation. We will combine saccadic tracking of point targets and smooth pursuit tracking tasks with the optokinetic and vestibular stimuli, to see if these are facilitated by stimulation nulling of ongoing nystagmus. If the stimulation improves performance on tasks that are compromised by rotational stimuli, either en-block rotation or rotation of the visual world, we will have additional evidence that the stimulation is effectively nulling the vestibular input.

4. We will work toward implementation of a real time, head velocity input controlled frequency modulated stimulus using a clinical processor. We now have access to lower level programming tools that should allow us to implement this new stimulation strategy. Currently, we are limited to an amplitude modulation strategy in real time. Although the amplitude modulation strategy appears successful, frequency modulation capability would add considerable functionality to the device and is an important objective in continuing development of the vestibular prosthesis.

5. We will meet with the F.D.A. to discuss our pre-I.D.E. submission, and implement modifications to allow a full submission to the F.D.A.

6. We will work with Dr. Stephen O'Leary to finalize a traveling scholars application and research plan so that he can begin working in our laboratory in Seattle.

7. We will present our work at Neuroscience 2009. We will also submit abstracts for presentation at the Asia Pacific Symposium on Cochlear Implants and Related Sciences 2009 meeting and the 33rd Association for Research in Otolaryngology Midwinter Meeting.