

Tenth Quarterly Progress Report

November 1, 2008 to January 31, 2009
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Neurophysiological Studies of Electrical Stimulation for the Vestibular Nerve

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Challenges:

1. Our primary challenge is that we have still not received our specially designed hybrid multisite recording electrodes from Neuronexus – FHC. At this point in our contract, we should have received 240 electrodes. Thus far we have received two electrodes, which were fabricated to roughly meet our specifications, and 2 dummy electrodes that were non-functional and not constructed to our specifications. Furthermore, the two working electrodes had a diameter at a connection point along the shaft of the electrode in excess of our specifications, which precluded placing them in our cannulae and actually using them in vivo. We have been promised 9 additional electrodes, but these have not been forthcoming. The primary issue is that Neuronexus has turned the fabrication of the electrodes over to the Frederick Haer Corporation, and they have not been able or willing to produce electrodes for the contract. They have made electrodes for other research projects, but either our requirements have been difficult to meet, or the other projects have been prioritized ahead of ours.

Our solution to meeting this challenge has been to use single unit tungsten microelectrodes that have worked well, and tetrodes from Thomas Recording. The Thomas Recording tetrodes are useable in our application, but the site spacing is such that in the brainstem it is difficult to isolate more than one unit. We have been reassured by NeuroNexus that they remain fully committed to our project, and that we will receive our electrodes. We are cautiously optimistic that this is the case, and we are fully configured to use the electrodes when we receive them. We have hardware and software in place to isolate and analyze multiple units recorded simultaneously.

2. We had intended to perform two additional implant revision surgeries using vestibular ECAP to guide our electrode placement, but could perform only one such surgery in Quarter 10. Our first surgery was performed successfully and is reported below, but because our surgical nurse informed us that she no longer wishes to work with animals, we could not perform the second surgery in Quarter 10. We used our surgical down time effectively by training additional personnel so that we now have a surgical assistant who is a regular member of the research team trained to assist Dr. Rubinstein in these procedures. In addition, Dr. Shawn Newlands has joined our research team, and can perform revision surgeries with canal plugging as required. Dr. Newlands was highly successful in implanting two monkeys with multiple fine wire electrodes, as described below. These developments should substantially increase our opportunities for surgeries. We now have scheduled two additional implant revision surgeries by Dr. Rubinstein for the second month of Quarter 11.

3. We have not yet received our revised vestibular implants from Cochlear Corporation. This is not so much a current challenge as a cautionary note. We still have one unused vestibular implant of the initial design, and we have several fabricated

fine wire electrode arrays that can be used in the absence of the new implants. We anticipate that the new implants will arrive in the second month of Quarter 11.

Current Successes:

1. We have presented our work at the Society for Neuroscience. Dr. Phillips gave a slide presentation and Dr. Bierer presented a poster.

“A multichannel vestibular prosthesis based on cochlear implant technology”
J. O. Phillips, S. Bierer, A. F. Fuchs, C. R. S. Kaneko, L. Ling, K. Nie, T. Oxford,
J. T. Rubinstein
Society for Neuroscience, 18.10, 2008

“A template-based spike sorting technique to resolve temporally overlapping spike waveforms.”
S. M. Bierer, L. Ling, J. O. Phillips
Society for Neuroscience, 169.19, 2008

In addition, Dr. Phillips has been invited to give a presentation at the Conference on Implantable Auditory Prostheses, July 12 - 17, 2009, Lake Tahoe, California.

Dr Rubinstein had a paper on our surgical strategy accepted for AOS/COSM.

“Prosthetic implantation of the semicircular canals with preservation of rotational sensitivity: A hybrid vestibular implant”
J. T. Rubinstein, L. Ling, K Nie, A F. Fuchs, J. O. Phillips
American Otologic Society, 2009

2. We have reproduced much of the data obtained in our first successful first monkey with an implanted device in a second monkey. The threshold for activation of nystagmus with this implant (Implant 2) was extremely low, with movements evoked at the lowest currents available from the device, which is approximately 20 μ A. The amplitudes of the eye movements and frequency of the fast phases were comparable to those of the previous implant (Implant 1), although at lower stimulus currents. As with Implant 1, increasing stimulus current or frequency produced increasing slow phase velocities of nystagmus. The velocities of nystagmus that could be achieved with Implant 2 were extraordinary, with peak velocities of several hundred degrees per second at the highest stimulus frequencies. The relationship between stimulus current and velocity is shown for two representative stimulus frequencies in Fig. 1. As shown in the figure, there is a consistent increase in both horizontal and vertical velocity with increasing stimulus current. The direction of nystagmus remained relatively constant as the current was increased. Similar behavior was elicited by Implant 1. Because Implant 2 had a very low current threshold, we obtained our best modulation of slow phase velocity with stimulus frequency at low stimulus currents.

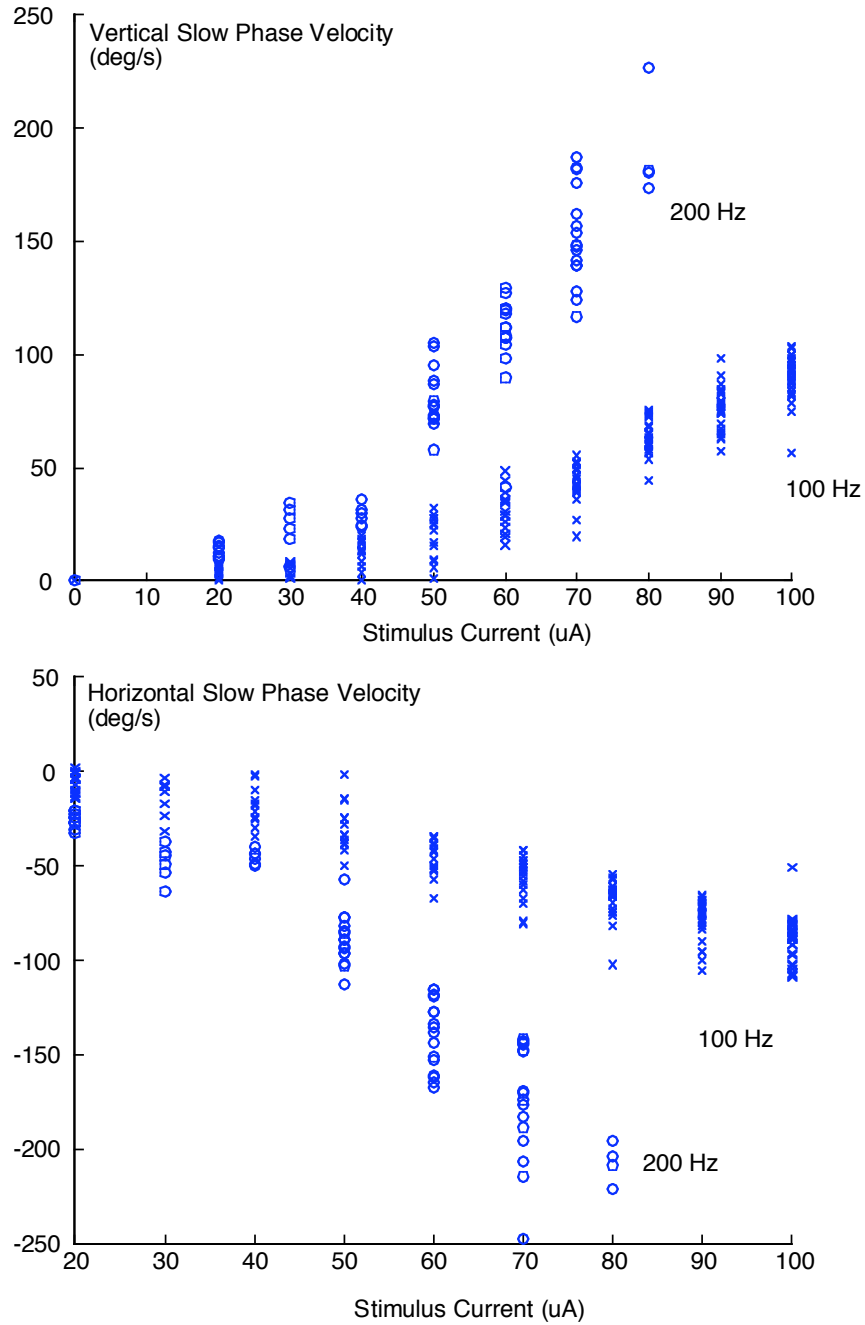


Figure 1. Slow phase velocity versus stimulus current for two different frequencies of stimulation in the left lateral canal. Vertical velocity components are displayed in the upper graph, and horizontal velocity components are displayed in the lower graph. The stimulus parameters were 400 μ s per phase, 8 μ s interphase gap, X = 100 pps or O = 200 pps, monopolar stimulation at the distal site, with a case and implanted ground return.

Fig. 1 also illustrates another very important feature of the behavioral response recorded during stimulation of Implant 2; i.e., there was a significant vertical component evoked during stimulation of the right lateral canal. This vertical component was larger than that evoked by stimulation of Implant 1, and may be related to the very low threshold for activation of nystagmus. The surgery for Implant 2 placed the most proximal electrode much closer to the ampulla of the lateral canal than in Implant 1, and therefore much closer to the afferent fibers of both the lateral and the superior canals. Ideal placement, therefore, may require complete insertion of the electrode with placement intermediate to the sites of Implant 1 and Implant 2. This is the target of the next surgical placement.

3. We successfully utilized intraoperative ECAP (Electrically Evoked Compound Action Potential) recording during surgical implantation of one rhesus monkey with our device. Dr. Paul Abbas assisted us with recording intraoperative and post-operative vestibular ECAPs in a monkey that we reimplemented. We not only optimized the ECAP recordings with his help, but we also demonstrated the value of intraoperative recording and gained insights into the variable nature of the efficacy of stimulation post-operatively in our previous surgical attempts. Fig. 2 shows the results of ECAP recording during surgery and 1 week following surgery. It should be emphasized that the remarkably low thresholds demonstrated in Fig. 1 are a direct result of the use of this technology for operative placement of stimulating electrodes.

The ECAP recording in Fig. 2A shows the robust potential obtained during surgery with the electrode array fully inserted in the end organ. The ECAP recording in Fig. 2B shows the potential recorded during stimulation with the electrode array withdrawn 1 mm from its full insertion position. A comparison of these traces shows that at a stimulus intensity of 100 μ A, there is a robust potential when the lead is fully inserted, and very weak potential when the lead is withdrawn. As stimulus current is increased above 100 μ A, which is displayed in Fig. 2B but not in Fig. 2A, it is still possible to record a reduced ECAP. This ECAP at high currents is very similar to the ECAP recording from an ineffective electrode placement in one of our earlier animals, Fig. 3. In that animal, we were unable to drive either eye movements or recorded vestibular neurons with a working but ineffective implanted device. Note that the scale in Fig. 3 is different from those in Fig. 2, so the amplitudes of the ECAPs in Fig. 3 are actually quite small. In Fig. 2C, we show that the ECAP is maintained one week after surgery. Fig. 2D shows the overall input / output function of the post surgically recorded waveforms. Unlike previous surgeries, Dr. Rubinstein secured the fully implanted position of the electrode array with a suture in a manner comparable to the technique routinely used in the past for securing Nucleus 22 straight arrays. Currently this same fixation technique is used clinically for the placement of Nucleus Hybrid 100mm arrays in the basal cochlea. This surgery produced a robust ECAP, and a fully functional electrode array that drove eye movement at low stimulus current. The device is still functional at the writing of this report.

4. We have now more fully evaluated PDA-based stimulation in our animals. In Quarter 9, we conducted stimulation experiments with the PDA stimulator from Dr. Loizou and we observed stimulus driven nystagmus. However, these data were not

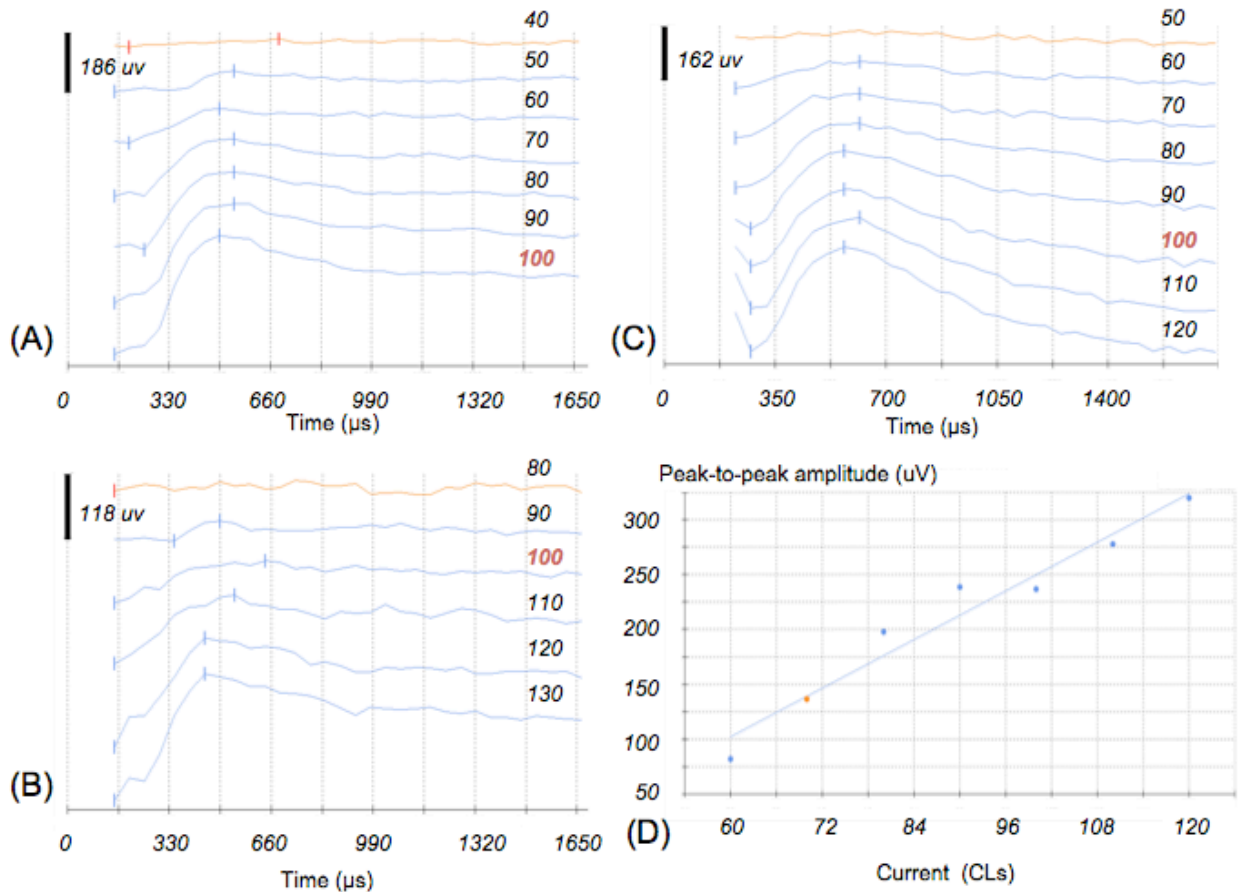


Figure 2. Electrically evoked compound action potentials (ECAPs) measured in a rhesus macaque monkey using neural response telemetry. A. ECAP series recorded during surgery, with a full insertion of the electrode array into the lateral canal. Numbers above each waveform indicate the stimulus current. B. During surgery with a partial array insertion. C. One week after surgery. D. Input-output function of the waveforms shown in C. Potentials were obtained using a forward masking technique to minimize contamination by stimulus artifacts, and the first 150 μs were not sampled to block out the largest component of residual artifacts; a monophasic “artifact reduction” pulse was also used in C. Stimuli were delivered monopolarly between one array electrode and a distant return electrode. Pulse width, 50 $\mu\text{s}/\text{phase}$; pulse rate 50 Hz; masker level, 10 CLs above probe.

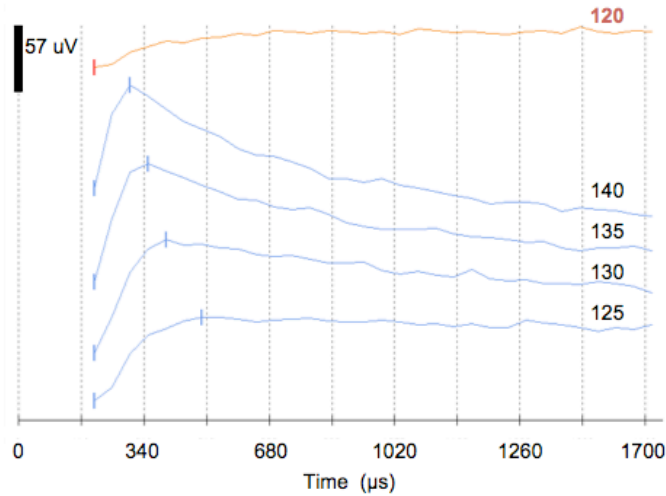


Figure 3. NRT compound action potentials measured in a previously implanted animal several months post-surgery. (Note that the waveforms in this figure are not all sequential with respect to current level.)

collected in complete darkness because the PDA stimulator had to be controlled in the recording booth at that time. Also, the stimulus artifact did not allow us to identify individual stimulus pulses. This quarter, we installed a remote Pocket Controller that allows us to start and stop the electrical pulse stimulation with a laptop outside the recording booth. We also changed the PDA software to generate pulse trains with pulse width at 400 μ s, to be consistent with previous stimulation experiments with the NIC2 stimulator. On the PDA control interface, a button ‘SET’ was added to manually control the start of a pulse train when changing current levels. This function is particularly useful, since the current PDA is unable to take input trigger signals. We need to manually trigger a pulse train, and turn off the fixation target, while watching the eye position of the monkey.

As shown in Fig. 4, the pulse train generated by the PDA stimulator is not sustained at a constant rate. Rather, the device strobesc because it loses pulses due to the buffer limitation of the Rev. 1 PDA SDIO board that we have available. This issue cannot be resolved with the current PDA board. In future quarters, the strobing may be solved if there are dual buffers in the FPGA, one for reading and one for writing, so that both read and write can take place at the same time. On the newer boards being developed at UT

Dallas, there will be double buffering and the communication interface will be parallel CPU-Like with a 16-bit data bus rather than the Serial Peripheral Interface that runs on the current board. Therefore, the PDA will be able to send data to the FPGA 16x faster than with the current board and the time taken is a 16x smaller fraction of the frame duration.

The PDA device is capable of driving nystagmus, but at the higher stimulus rates used in the experiment in Fig. 4, the nystagmus has a choppy character. The eye velocity increases and decreases as the device strobos on and off.

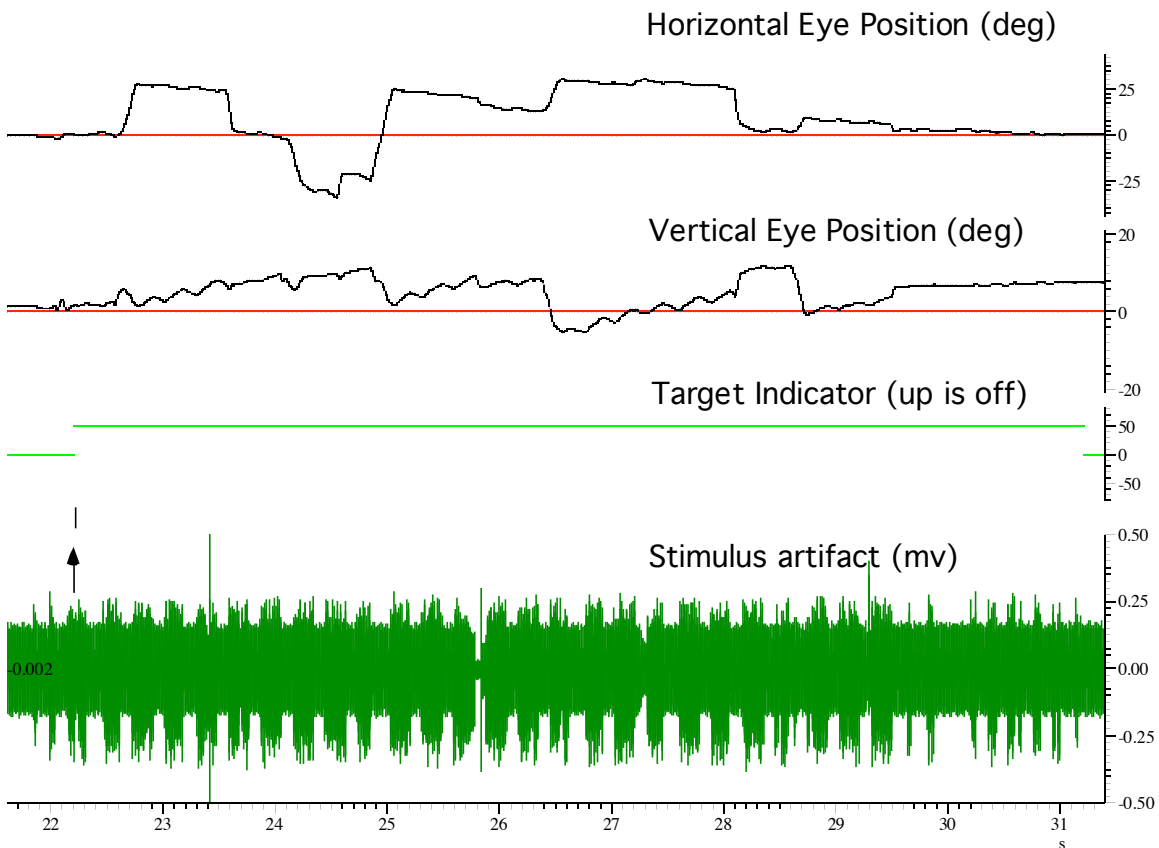


Figure 4. Recorded eye movements in response to a commanded 600 Hz stimulation train at 100 μA delivered to a single distal electrode site in the lateral canal. Stimulation parameters are 400 μs pulse width, 8 μs interphase gap. The vertical arrow indicates the offset of the fixation target.

5. We replaced the stimulation electrodes in two monkeys with fine wire electrodes and canal plugging, and characterized the behavior of the implanted and plugged monkeys. Under general anesthesia, Dr. Shawn Newlands inserted fine wire monopolar electrodes in two of our animals and simultaneously performed canal plugging of all canals ipsilaterally. The monkeys were expected to provide valuable short-term data on the effects of canal plugging and electrical stimulation. The surgeries were highly successful as both animals had viable electrodes, which produced robust nystagmus that could be elicited throughout Quarter 10. Recording in one of the animals continues at this time. With these new animals, we could elicit behavioral responses in a total of four monkeys. Two of the monkeys had lateral canals successfully implanted, and two of the monkeys had two canals successfully implanted, one with working lateral and posterior canal electrodes and one with working lateral and superior canal electrodes. As mentioned above, two of the animals were ipsilaterally canal-plugged, and two were intact, implanted without canal plugging and with the minimally invasive technique.

As we stated in earlier QPRs, we observed no loss of vestibular function in the animals that we had tested following minimally invasive implant surgery. It was possible, however, that the sinusoidal rotation stimuli that we used were not sufficiently sensitive to detect a unilateral loss of function from a canal impaired by surgery. The canal-plugged monkeys gave us an opportunity to determine whether our test of vestibular function was sufficiently sensitive. Fig. 5 shows the results of vestibular testing in a canal plugged monkey and a monkey that was implanted using the minimally invasive technique. The testing involved steps to constant velocity chair rotation in the plane of the implanted right horizontal canal. There are four comparable stimulus steps, 1) from rest to 100 deg/s to the right, 2) from 100 deg/s to the right to rest, 3) from rest to 100 deg/s to the left, and from 100 deg/s to the left to rest. Normally, each step should elicit a nystagmus in the opposite direction, with an initial slow phase eye velocity of approximately 80 deg/s in the direction opposite to that of the step, and a slow decay of eye velocity with a time constant of more than 20 seconds. If a canal is non-functional, rotation toward that canal should elicit a low velocity response and a short time constant. In Fig. 5A, the canal plugged animal shows such a response. The time constant for rotation toward the implanted and plugged canal is reduced, as is the initial gain of the response. In Fig. 5B, the response is symmetrical and robust, with a high gain and long time constants in response to steps in both directions. We conclude from this test, and the sinusoidal testing reported in the previous progress reports, that the vestibulo-ocular reflex is intact following the minimally invasive implantation of the monkey in Fig. 5B.

In other respects, the electrically evoked eye movement responses of the canal plugged and the minimally invasively implanted monkeys were fairly comparable. We were able to drive robust nystagmus in all four animals. The responses were largely in the plane of

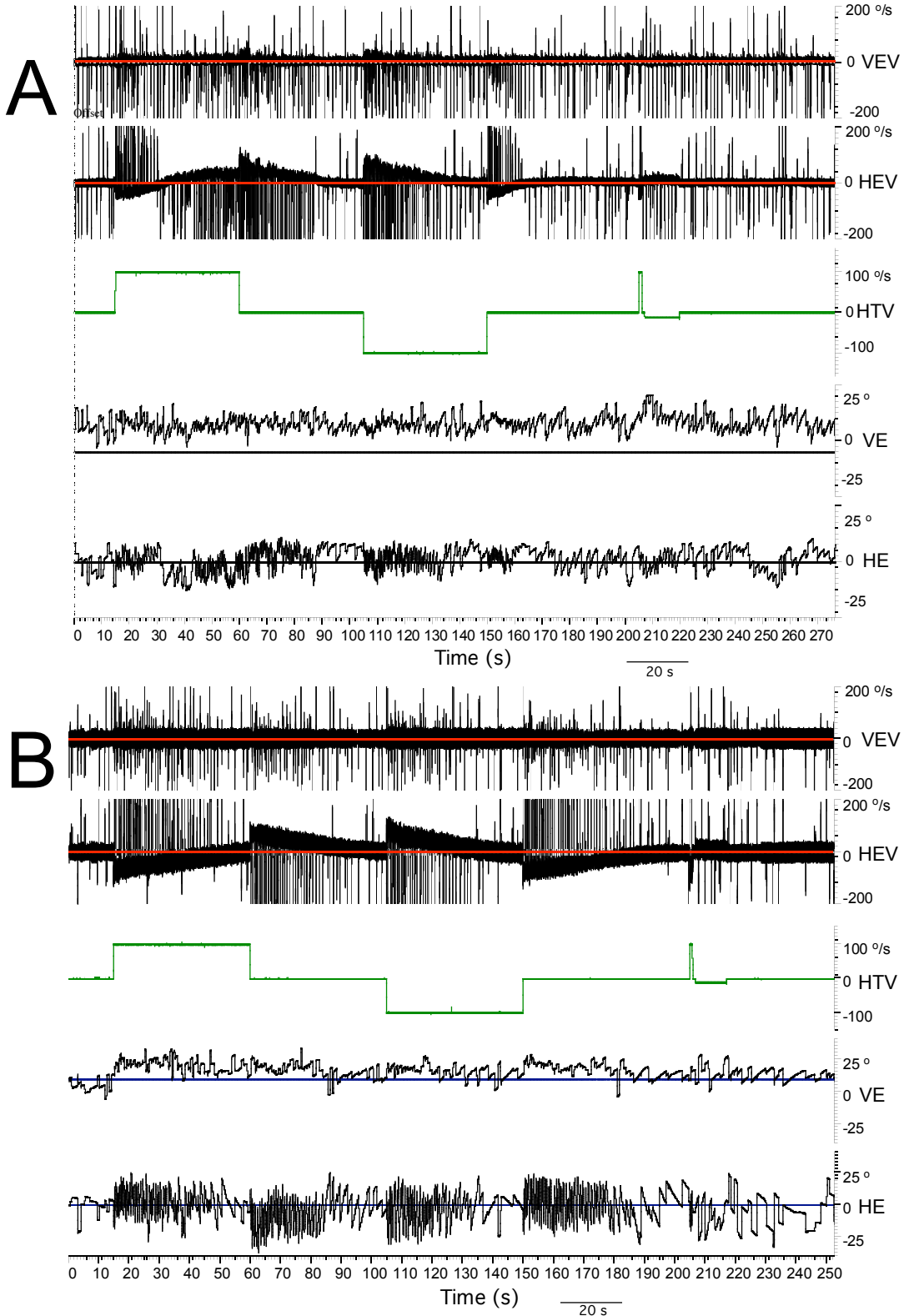


Figure 5. Step vestibular testing in two monkeys in the plane of the implanted left horizontal canal. Traces, from top to bottom in each panel, are vertical eye velocity, horizontal eye velocity, chair velocity, vertical eye position, and horizontal eye position. A) Unilaterally canal plugged monkey, B) Minimally invasively implanted monkey.

the implanted canals. We were able to use very low currents of $\leq 30 \mu\text{A}$ to produce nystagmus in all four animals. Increasing stimulus current, pulse width, or stimulus frequency increased the velocity of the slow phases of the nystagmus in all four monkeys. Stimulation via the fine wire electrodes in the canal-plugged monkeys could be used over a more restricted current range than for the minimally invasively implanted electrodes, because they produced twitching of the facial musculature at much lower current thresholds, suggesting that current had spread to the adjacent facial nerve.

6. We recorded from identified vestibular neurons and then stimulated those neurons electrically using the implanted electrode arrays. Neurons with Type 1 horizontal rotation sensitivity recorded ipsilateral to the stimulating electrode array were driven by the lateral canal electrode array, but were not driven the posterior canal electrode array. Neurons with posterior canal sensitivity were driven by the posterior canal electrode array and not by the lateral canal electrode array. We did not record from any Type 1 neurons that were driven by both canal arrays. An example of a neuron with such response selectivity is shown in Figs. 6, 7 and 8.

Fig. 6 shows the rotational response of a neuron recorded in the right vestibular nucleus during passive en-block rotation in yaw and pitch. The animal is fixating an earth stationary point target in an otherwise darkened room. During horizontal yaw rotation, the unit is not modulated. However, during vertical pitch rotation the unit increases its firing rate in phase with up chair velocity. In Fig. 7, the discharge of the unit is displayed during stimulation of the right lateral canal. In the upper left panel, several consecutive sweeps of recording are superimposed triggered on the onset of the stimulus artifact during canal electrical stimulation that was suprathreshold for eye movement at higher frequencies. There is no time locked action potential, indicating a failure to drive the unit. In the upper right panel, several consecutive sweeps of the action potential of the recorded neuron, triggered on the falling phase of the action potential, are displayed, demonstrating that the neuron was present, but not time locked to the electrical stimulus. The lower panel shows the unit discharges randomly with respect to the stimulus pulses. The blue arrow indicates an occasion when the action potential was randomly superimposed on the stimulus artifact, also shown in the blue trace in the upper left panel. Fig. 8 shows the driven activity of the unit during stimulation of the right posterior canal. In the upper left panel, several consecutive sweeps of recording are superimposed triggered on the onset of the stimulus artifact during suprathreshold (see above) electrical stimulation. There is a clear time locked action potential recorded. In the upper right panel, several consecutive sweeps of the action potential of the recorded neuron, triggered on the falling phase of the action potential, are displayed, demonstrating that the same neuron was present (compare Figs. 7 and 8). The lower panel shows the unit discharging immediately following almost every stimulus artifact during electrical stimulation. This suggests that the same unit was driven by the posterior canal electrode but not the lateral canal electrode, and that this unit responded to rotation in the appropriate direction for natural activation of the effective canal.

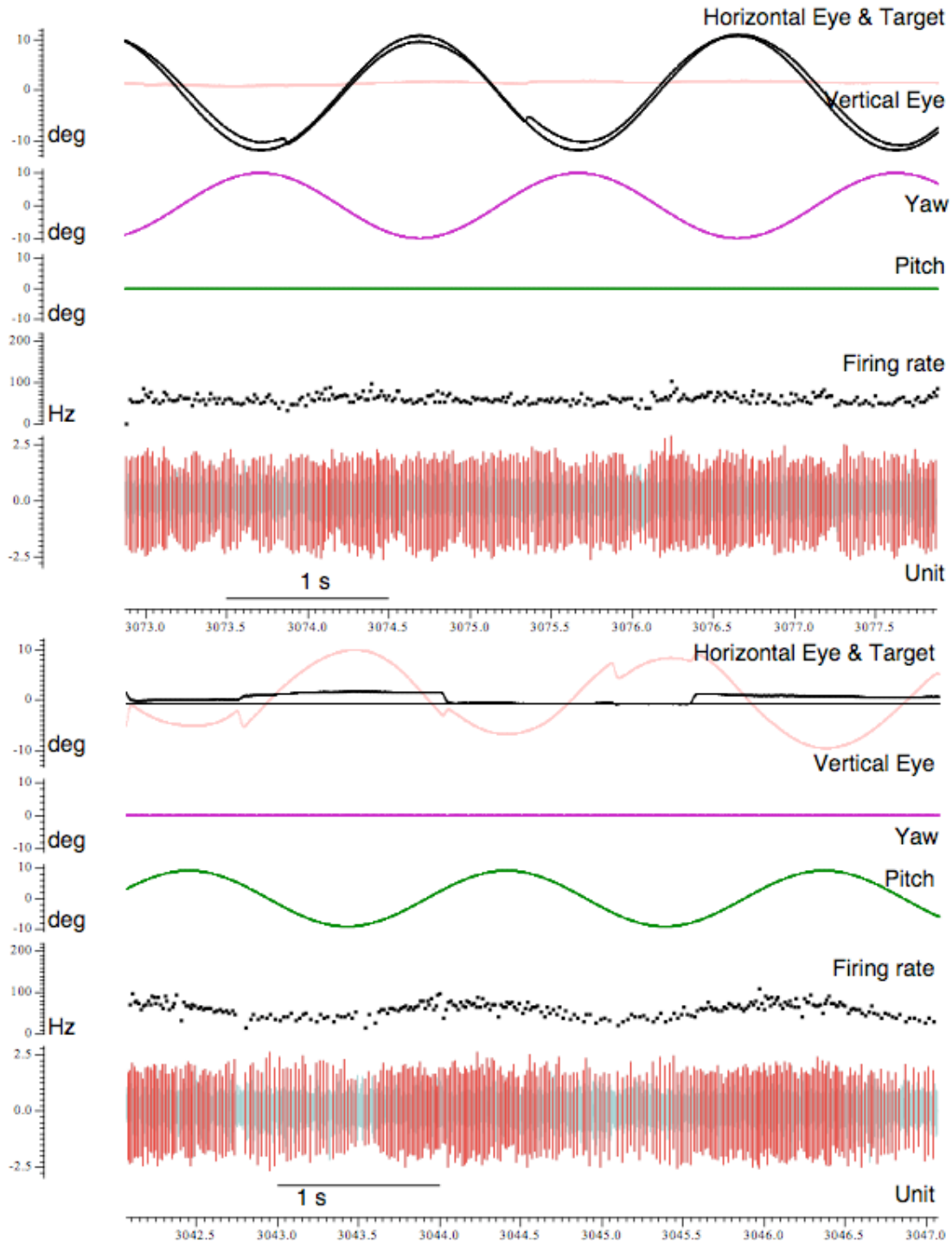


Figure 6: Vestibular responsiveness of a neuron recorded in the right vestibular nucleus. The upper panel displays the response during yaw rotation, and the lower panel displays the response during pitch rotation. In each panel, the traces are horizontal eye and target position, vertical eye position, yaw and pitch chair position, instantaneous unit (neuron) firing rate, and the recorded unit (neuron) discharge.

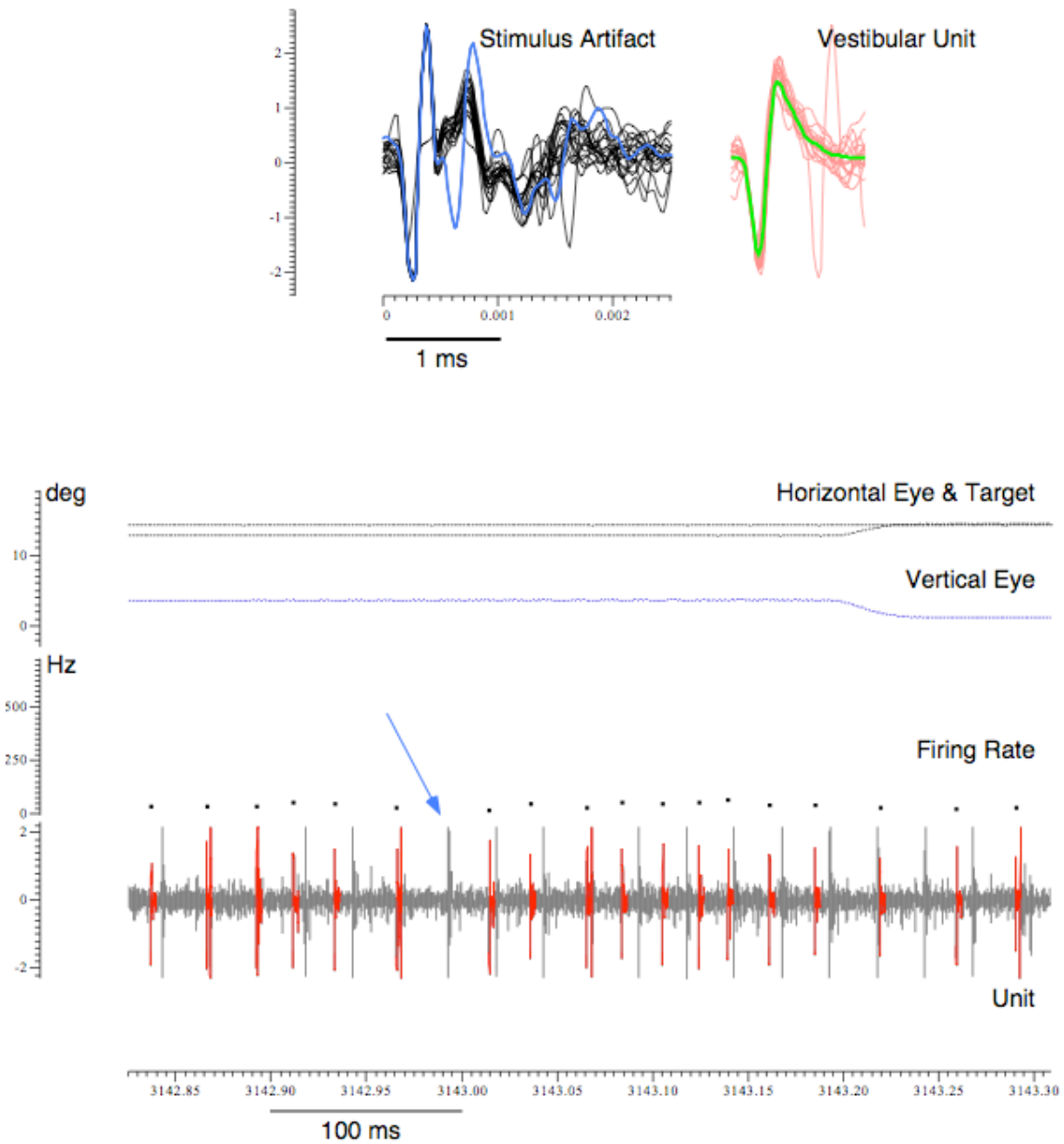


Figure 7. Failure of activation of the vestibular neuron displayed in Fig. 6 by electrical stimulation of the distal electrode site in the right lateral canal. The upper left inset displays the stimulus artifact resulting from stimulation of the canal. There is no associated time locked action potential. The upper right inset displays the action potential of the isolated unit. The lower traces show the horizontal and vertical eye and target position, the isolated neuron in red and the stimulus artifact in grey, during a constant frequency electrical stimulation.

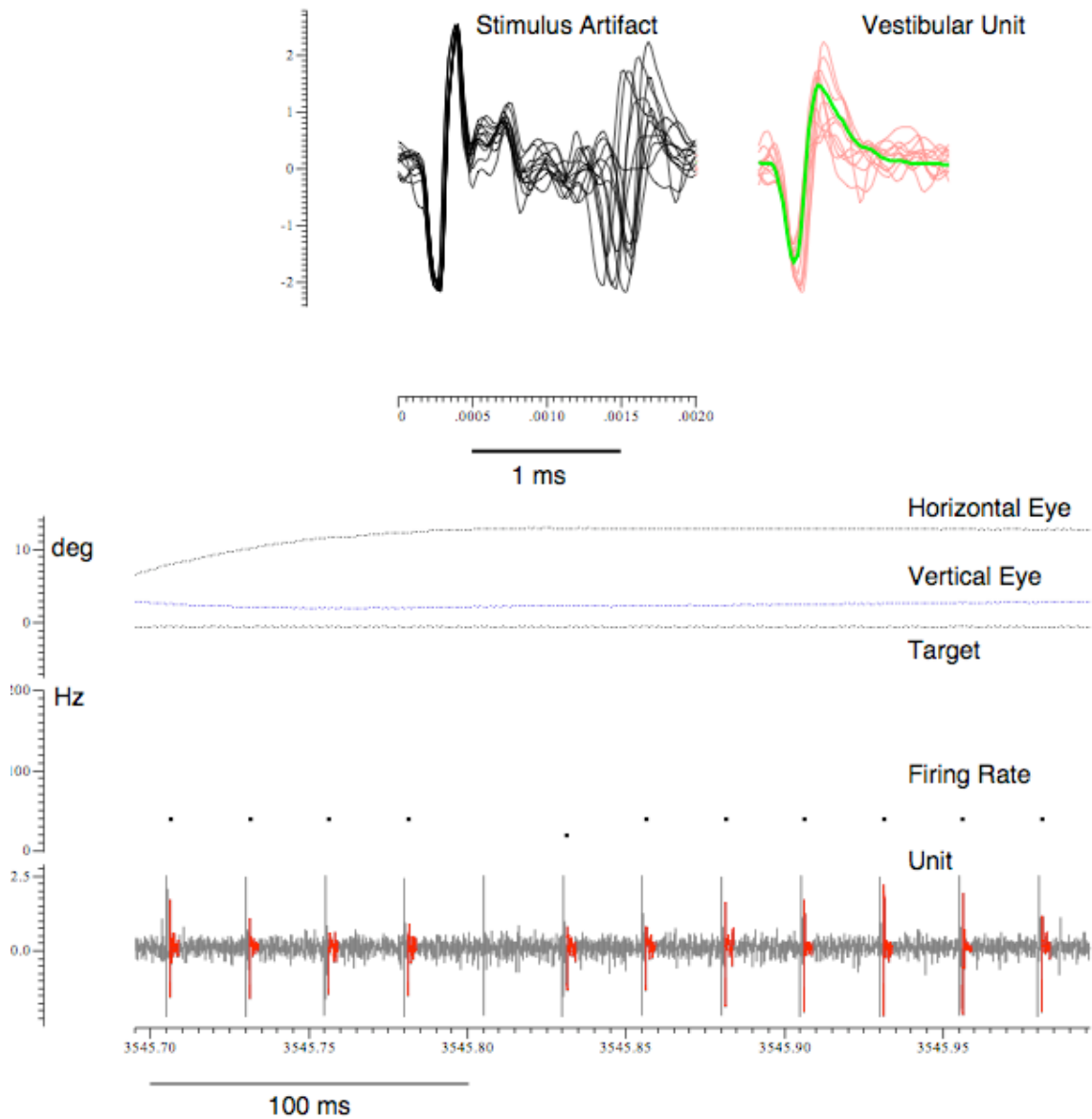


Figure 8. Activation of the vestibular neuron displayed in Fig. 6 by electrical stimulation of the distal electrode site in the right posterior canal. The upper left inset displays the field potential resulting from stimulation of the canal. There is an associated time locked action potential from the isolated neuron. The upper right inset displays the action potential of the isolated unit. The lower traces show the horizontal and vertical eye and target positions, the isolated neuron in red and the stimulus artifact in grey, during a constant frequency electrical stimulation.

In the next quarter:

- 1. We will perform revision implant surgeries in two additional monkeys.** We will use either the current implant design, or the second generation implant design depending on the availability of the devices.
- 2. Dr. Nie will complete a manuscript describing vestibular NRT.** This paper, titled ‘Characterization of the electrically-evoked compound action potential of the vestibular nerve’ is currently in progress and is to be submitted to the journal *Otology and Neurotology*.
- 3. We will continue recording behavior in both the minimally invasively implanted monkeys and in canal plugged and implanted monkeys.** We will compare and contrast the behavioral responses in both implant types in response to natural stimulation with passive rotational stimuli, natural head unrestrained behavior, and behavior elicited by electrical stimulation. We will apply electrical stimulation in various combinations with natural behavior and passive rotation.
- 4. We will continue recording from neurons in the vestibular nucleus in our implanted monkeys.** Our plan is to record from behaviorally identified neurons in minimally invasively implanted monkeys, focusing on neurons that receive input from a single canal. We will: 1) isolate a neuron by recording during a rotational search stimulus, 2) record rotational and eye movement related responses to quantify the directional tuning of the neuron, 3) attempt to drive the neuron with low frequency trains of electrical stimuli from the appropriate canal; i.e., the canal providing the input that produces the rotational responses, 4) determine the threshold of stimulation that drives the neuron, 5) attempt to drive the neuron from a canal not aligned with the rotational activation direction of the neuron, 6) quantitatively explore the frequency following characteristics of the neuron’s response to electrical stimulation. In the canal plugged and implanted monkeys, we will follow the same approach, but we will use electrical stimulation and rotation as our search stimulus. Since the implanted canals should not be responsive to rotational stimuli, we will explore the extent to which commissural input to Type 2 vestibular neurons participates in the creation of electrically elicited nystagmus in the canal plugged monkeys. In both minimally invasively implanted and canal plugged and implanted monkeys, we will be contrasting the current threshold for stimulation and the frequency modulation of the neurons, with the threshold for activation of nystagmus and the frequency versus velocity relationship of the observed behavior in the same animal.
- 5. We will extend our recording studies with multiple single unit recording with either the Thomas Recording tetrodes or the NeuroNexus - FHC axial electrodes.** Our choice of an electrode will be determined entirely by the availability of NeuroNexus – FHC device. We hope to be able to record simultaneously from multiple neurons in the vestibular nucleus during rotational stimulation and during electrical stimulation from the implanted canals.