The Feasibility of an Intraneural Auditory Prosthesis Stimulating Electrode Array

Quarterly Progress Report #10

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By:

Richard Normann, Ph.D., Principal Investigator

Clough Shelton, M.D., Co-Investigator

Todd Hillman, M.D., Co-Investigator

Arun N. Badi, M.D., Ph.D., Co-Investigator

Center for Neural Interfaces

Department of Bioengineering

University of Utah

Salt Lake City, UT 84112

ABSTRACT:

Over this quarter we have accomplished the following tasks:

1. We have established a collaboration with the Scientific Computing and Imaging Institute at the University of Utah that has allowed us to provide augmented visualization of the ultra high resolution small animal CT scans used in identifying the site of the UEA implants in chronically implanted cats.
2. We have been invited by Dr. Russell Snyder at UCSF to observe his acoustic and electrical mapping of inferior colliculus using Michigan 16 site electrode arrays. We will be visiting his laboratory in late November to participate in inferior colliculus experiments conducted there.
3. We have continued mapping of neuronal activation patterns in AI resulting from acoustic stimulation of the ipsilateral ear, and electrical stimulation via USEA's implanted acutely in the contralateral auditory nerve. Electrical stimulation of a subset of implanted electrodes evoked unique excitation patterns in auditory cortex that can be mapped to unique frequency percepts.
4. We have begun a collaboration with Dr. Patrick Tresco at the University of Utah to more closely examine the immuno-histological consequences of USEA implantation into the feline auditory nerve. We have performed immunohistochemistry on two feline auditory cortexes that were chronically implanted with 10 x 10 UEA's and that underwent 60 hour electrical stimulation with our portable back-pack stimulators. Nerve cell body densities were only minimally affected by the implants, but we observed a significant fibrotic response, more localized to the pial surface. There was only a modest fibrotic response near the tips of the electrodes.
5. We have begun anatomical counting of auditory nerve fibers in implanted auditory nerves and in unimplanted auditory nerves from the same cat. We have used the NIH 'Image' software package to compare automated fiber counting with manual counting but were not able to perform reliable counts of auditory nerve fibers with this software package.

1. WORK PERFORMED DURING THIS REPORTING PERIOD.

1***. Ultra high resolution, small animal CT scans used in identifying the site of the UEA implants in chronically implanted cats.***

The demonstration of the site of array implantation in the auditory nerve has proven to be a difficult problem that was resolved with the use of a GE EVS-RS9 computed tomography, small-animal scanner recently installed at the University of Utah. This scanner produces a 131 MB 16-bit image of 425x420x385 samples, with resolution of 27x27x27 microns. The resolution of the scanner can be appreciated in the raw CT images of the implanted cochlear nerve shown in Figure 1. The figure shows a sequence of sections from the apex of the cochlea down to its base, with the bright regions indicating wires that connect to the array. The last image in the series is a tangential section through the array, showing the array substrate (base), the shanks of the

electrodes and the tips of these electrodes.

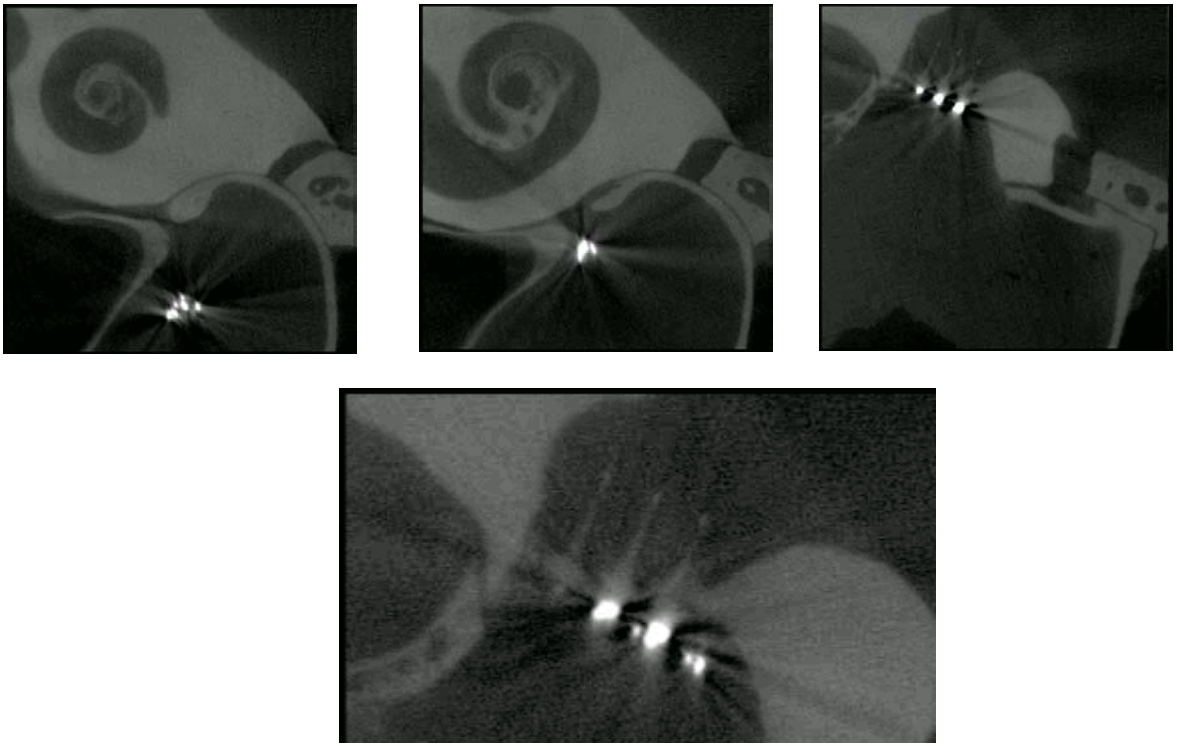

Figure 1. Raw CT images at various planes of section through the cochlea showing the spiraling cochlea, lead wires (bright objects in each section), and the cross section of the implanted array.

3-D visualization of the cochlea, modiolus and implanted electrode array.

Over this past quarter, we have entered into a collaboration with Dr. Chris Johnson, Director of the Scientific Computing and Imaging Institute (SCI) at the University of Utah to expand our visualizations of the implantation site of the array in the auditory nerve. The SCI has developed software that allows virtually real-time manipulations of complex data structures and visualizations of the data. Visualizations of the data were created with a parallel ray-tracing volume renderer developed in the SCI. Ray-tracing is a method commonly used in computer graphics that supports highly efficient implementations on multiple processors for interactive visualization. Volume rendering permits direct inspection of internal structures, without a pre-processing segmentation or surface extraction step, through the use of multi-dimensional transfer functions. There are distinct X-ray attenuation factors for air, soft tissue, bone, and the electrode array, enabling the use of a combination of ray-tracing and volume rendering to visualize the array in the context of the surrounding structures, specifically the bone surface. Figure 2, shows volume renderings of the cochlea, the modiolus (on the right), the implanted electrode array, and the lead wires (in purple) that connect the array to a head mounted connector.

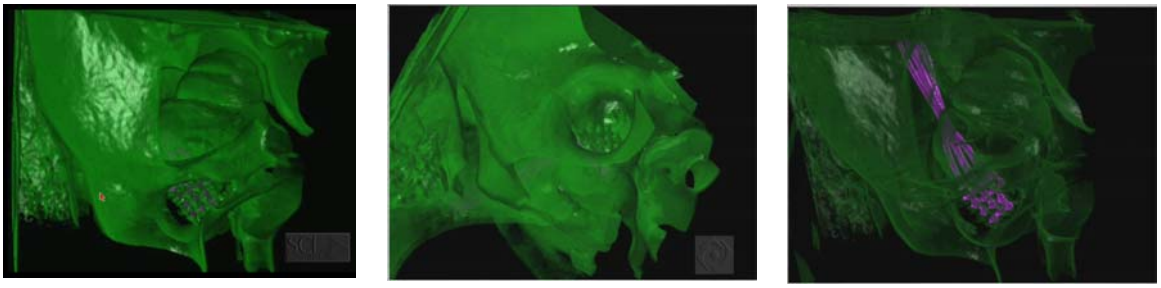

Figure 2. Volume rendered images of the cochlea, a view down the 'barrel' of the modiolus, and the temporal bones with more transparent bone structure allowing the implanted array and lead wires to be easily visualized.

Volume rendering also allows the bone to be rendered as translucent, as on the right panel of this figure, enabling the electrode to be clearly viewed. The combination of high-resolution scanning, image processing, and interactive visualization tools such as ray-tracing, allows non-invasive verification of the implantation site in the anatomical context of the auditory structures in the region that are completely encased in the thick temporal bone. The visualization techniques used to create these images will be presented at the Biomedical Information Science and Technology Initiative (BISTI) Symposium to be held at the NIH on Nov. 6 and 7, 2003.

2. Mapping of auditory nerve electrically evoked neural activity patterns in inferior colliculus.

The complexity of the tonotopic organization of the auditory cortex, and the difficulty we have experienced in making single- and multi-unit recordings from all 100 UEA electrodes implanted in this structure have hampered the exploration of stimulation selectivity described in the following section of this progress report. It has been suggested that the organization of the inferior colliculus (IC) could be used to facilitate this aspect of our research.

At the recent Asilomar conference on cochlear prosthetics (August 17-22, 2003), we had the opportunity to discuss the recordings of electrically evoked activity maps in inferior colliculus with Drs. Russell Snyder and John Middlebrooks. These individuals have had extensive experience in recording from IC and they described at this meeting outstanding electrically evoked maps made from this structure. Dr. Snyder has agreed to allow members of the Utah team to participate in upcoming feline experiments they will be performing using Michigan electrode arrays so that this technology can be exported back to Utah. Dr. Snyder has also agreed to join the Utah team when they perform their first IC experiments, presumably at the end of this next quarter or the beginning of 2004. In order to facilitate this collaboration, we have received 16 site linear Michigan electrode arrays from the Michigan team, and will be modifying our electrode amplifiers and head stages to allow them to work with the Michigan arrays.

3. Selectivity of Electrical Stimulation

We have hypothesized that one of the potential advantages of direct electrical stimulation of the auditory nerve to evoke auditory percepts is augmented selectivity. Specifically, as the tips of the electrodes, implanted in the auditory nerve abut the auditory nerve fibers, electrical excitation of these fibers is expected to excite only a small population of fibers. Thresholds for electrical stimulation are expected to be low, and the number of fibers excited at threshold is expected to be very low.

We are investigating this hypothesis by recording the activation pattern of a subset of neurons in auditory cortex (AI) in response to direct electrical stimulation of the auditory nerve. The selective activation evoked by stimulation via each implanted electrode should be manifest as a localized activation pattern at threshold that is different for each electrode in the array. We will assay selectivity by comparing the electrically evoked AI activation patterns with acoustically evoked AI patterns and ascribing a 'best frequency' to the stimulus pattern evoked by each electrode implanted in the auditory nerve. Selectivity will be indicated as differences in the 'best frequency' for each electrode implanted in AI. We have yet to complete this aspect of our research, but we are progressing towards the realization of this goal. In our previous progress report, we have demonstrated that the cortical neurons in A1 were responsive to electrical stimuli via the UEA implanted in the auditory nerve. However, due to the complexity of these experiments, we were not able to show differences in cortical activity pattern, evoked by selective activation via the stimulating electrodes. We describe below the results of our latest experiment on AI acoustic and electrical mapping. Although we have not yet achieved complete AI mapping with both acoustic and electrical stimuli (not all AI electrodes recorded single- and multi-unit responses), we provide these results to indicate the direction of these experiments and the progress we have made to date.

We used both acoustic and electrical stimulation in these experiments. Electrical stimuli were delivered via a 4x3 USEA that had been implanted in the auditory nerve. Electrical and acoustic stimuli were produced with a custom built Labview program that allowed computer controlled selection of stimulated electrodes, and the stimulation level on each electrode. Electrical stimulation was monopolar with respect to a distant return. The nerve was stimulated cathodically first using a charge balanced 100μs per phase biphasic waveform of amplitudes ranging from 10μA to 100μA. The stimulated electrode and current level were randomly selected within fixed ranges. After the auditory nerve implantation, the eABRs evoked with the UEA were studied in order to check if the tip of each electrode had been properly imbedded in the nerve. Those electrodes that did not evoke eABRs with reasonable thresholds were excluded from the pool of activating electrodes. In this experiment, six electrodes (numbered 1, 6, 7, 9, 10, and 11) were shown to evoke eABRs. The thresholds for these electrodes were about 30uA.

The acoustic stimuli consisted of pure tones (50 ms long) with ascending and descending linear ramps (5 ms duration). 256 such tones of varying intensity (30 ~ 90 dB) and frequency (0.5 kHz ~ 40 kHz) with a 1 sec interstimulus interval were randomly interleaved.

Neural recordings from A1 were made with acute implants of 10x10 UEA's. Single unit responses were seen on a subset of electrodes (20) immediately after implantation into the cortex. After 1 hour, responses were found to stabilize. Spike amplitude thresholds were individually set for each electrode and events crossing these thresholds were recorded. Multi-unit activity was recorded using a multichannel data acquisition system (NSAS, Cyberkinetics, Inc., formerly Bionic

Technologies, Inc.). Subsequent off-line spike sorting algorithms were used for single-unit analysis. An example of the units recorded from AI in this experiment is shown in Figure 3.

Mapping of Best Frequencies (BF)

The acoustically evoked responses were used to generate a set of tuning curves for both the single unit responses and the multi-unit responses recorded on electrodes (multi-unit responses are not shown in Figure 3). Figure 4 illustrates stimulus frequency – sound intensity tuning curves estimated from ipsilateral acoustic stimulation (the auditory nerve implant was performed on the side contralateral to the AI implant). Although not all channels were active enough to show sharp tuning curves (sharp tuning curves were found on only 20 of the 100 electrodes), the tuning curves were used to estimate the BF map shown in Figure 5. The map shows the tendency of the distribution of BFs to form a spatial tonotopic gradient.

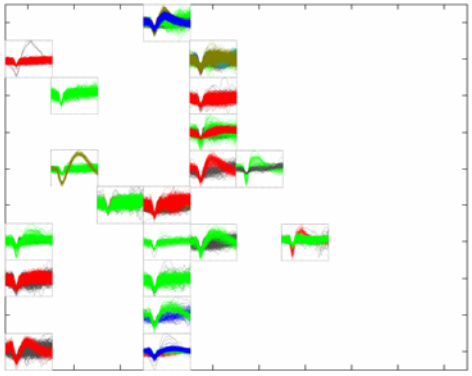

Figure 3. Specimen recordings of units recorded in this experiment.

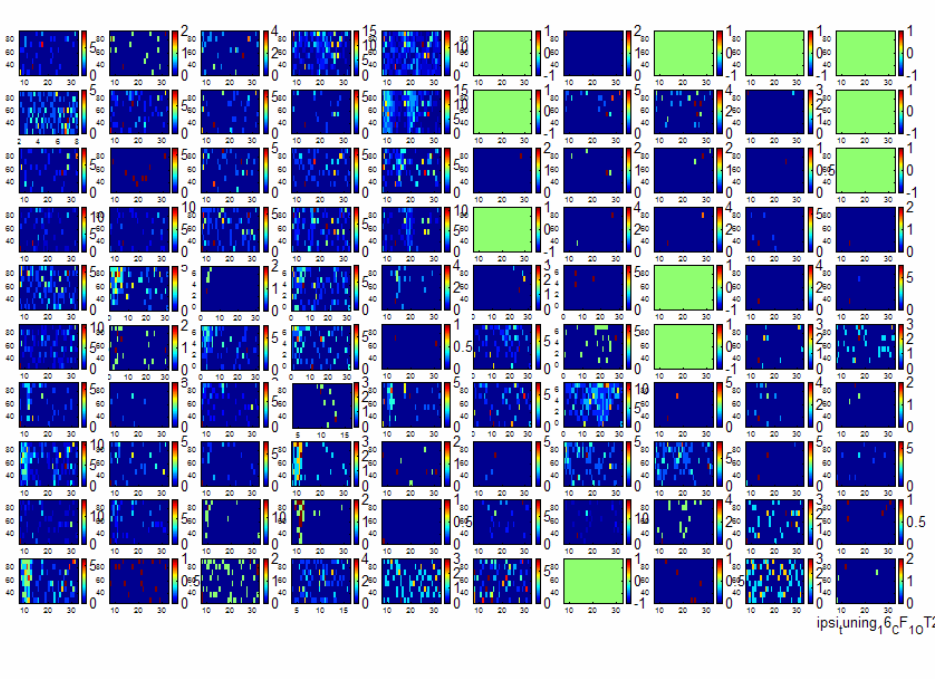

Figure 4. Tuning curves generated from the single- and multi-unit responses recorded in our latest experiment.

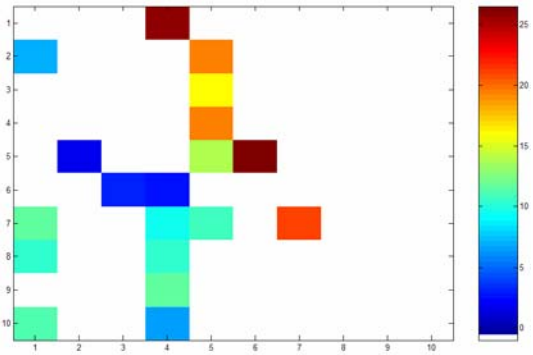

Figure 5. BF map estimated from the tuning curves of Figure 4.

When the period of acoustic stimulation had been completed, we began electrical stimulation via the USEA implanted in the contralateral auditory nerve. We only stimulated those electrodes that had eABR thresholds determined to be acceptable from earlier measurements (electrodes 1, 6, 7, 9,10 and 11). Using responses in AI to electrical stimulation from these electrodes, we performed the following analyses.

Latency of AI responses to auditory nerve electrical stimulation.

Figure 6 shows the 10 x 10 patterns of AI neural activation for four different post stimulus time epochs: 0 to 10 ms post stimulus, 10 to 20 ms post stimulus, 20 to 30 ms post stimulus, and 30 to 40 ms post stimulus. Each pattern was evoked by stimulation via one of the electrodes implanted in the auditory nerve. From these activation patterns, we conclude that the latencies of the AI responses are in the 20 to 30 msec range (1-7). There is little activity prior to or after this period.

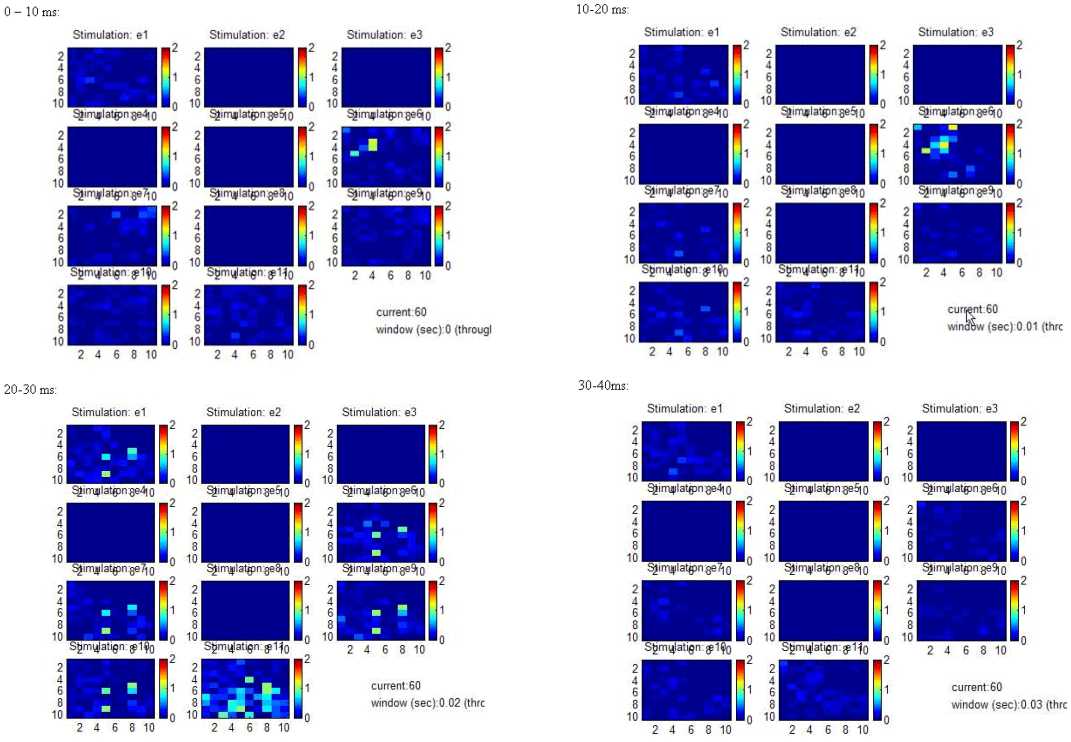

Figure 6. AI neural activation patterns evoked by electrical stimulation of auditory nerve.

BF's estimated from the AI neural activation patterns.

The patterns of AI activation due to electrical stimulation of the auditory nerve via the USEA varied with which electrodes were used to pass the current, and with the amplitude of the currents used. In this experiment, not all USEA electrodes evoked obvious AI activation patterns, so we have illustrated the effects of current injections via two of the USEA electrodes in Figure 7. In this figure, the 20 uamp stimulus levels were below threshold, so the firing patterns reflect background firing (and the waxing and waning of this activity that occured during the course of the experiment). The 50 uamp current injections were about at the threshold level, and the 70 and 100 uamp maps reflect current injections that were superthreshold. The growth of the responses with stimulus intensity is apparent, as is the differential excitation patterns that were evoked by the currents passed through the two illustrated electrodes.

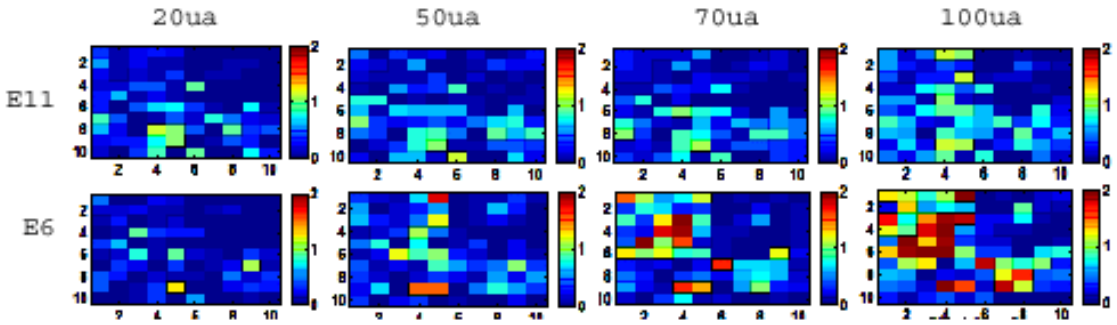

Figure 7. AI neural activation patterns produced by electrical stimulation of auditory nerve via USEA electrodes 6 and 11.

We next superposed these electrical activation patterns onto the tonotopic map (Figure 5) to estimate the BF's associated with stimulation via electrode 6 and electrode 11. In doing this, we restricted our assignment of BF's only to AI electrodes with the largest evoked responses to stimulation via each auditory nerve electrode. The BF evoked by electrode 6 was 16.6 kHz and that by electrode 11 was 8.16 kHz (using a students 't' test, this difference in BF was determined to be significant at the 0.03 level).

We stress once again that this analysis has been done on incomplete AI activation data, and that we have included it in this progress report only to illustrate the direction we are following. Problems we will be attacking in the next quarter are: increasing the yield of single units recorded in AI, obtaining lower eABR stimulation thresholds, and providing a more quantitative measure of independence of the AI activation patterns evoked by auditory nerve stimulation. The experimental techniques and data analysis tools we have developed in the AI mapping studies will be directly applicable to studies we will be performing in the inferior colliculus when our collaboration with Dr. Russell Snyder begins later in the next quarter.

4. Immuno-histological consequences of USEA implantation into the feline auditory cortex.

An important component of our contract has been the demonstration of the safety of the implantation of the USEA in the auditory nerve, and the consequences of electrical stimulation of the auditory nerve via this array of penetrating electrodes. As a prelude to this part of our contract,

we needed to develop histological tools that could be used to determine these anatomical consequences. Thus, we have begun a series of experiments in auditory cortex using our backpack electrical stimulators for stimulation in freely moving cats. We have focused initially on Auditory cortex because of the ease of implantation at this site, and because of other work performed on CNS tissues (8). Cortical implantation has allowed to manage the lead wires that connect the backpack stimulators to the implanted electrode array, to develop histological tools for assessing the impact of the implanted arrays. Our original efforts at performing histological assessment were performed by Dr. Eduardo Fernandez at the Miguel Hernandez University in Alicante, Spain, and this collaboration is continuing. In an effort to get more immediate feedback from these studies, we have approached Dr. Patrick Tresco of the Keck Center for Tissue Engineering at the University of Utah to help in this phase of our work. Dr. Tresco is an expert in cortical wound healing and in the cortical response to penetrating devices. He has agreed to assist in this phase of our work. The results described below were obtained under the direction of Dr. Tresco.

The experiments were conducted on the auditory cortex of a cat that had been implanted for a period of ten months with a 100 electrode UEA. While some current injections had been attempted in this animal, the lead wire management system had yet to be fully developed and the stimulation was not well controlled. As a result, this animal was used to develop the histological tools for the feline model (an important step as Dr. Tresco's antibodies were generally derived for rat histology). Figure 8 shows a cortical section, stained with NeuN, a marker for neuron cell bodies. The micrograph shows a region of about 2.5 x 3.5 mm of the region of the cortex where the array had been implanted, and contains about 48 electrode tracks (not easily visualized in this image). The density of the NeuN stained neuronal cell bodies appears relatively uniform except for the regions of the image where the electrode tracks are located.

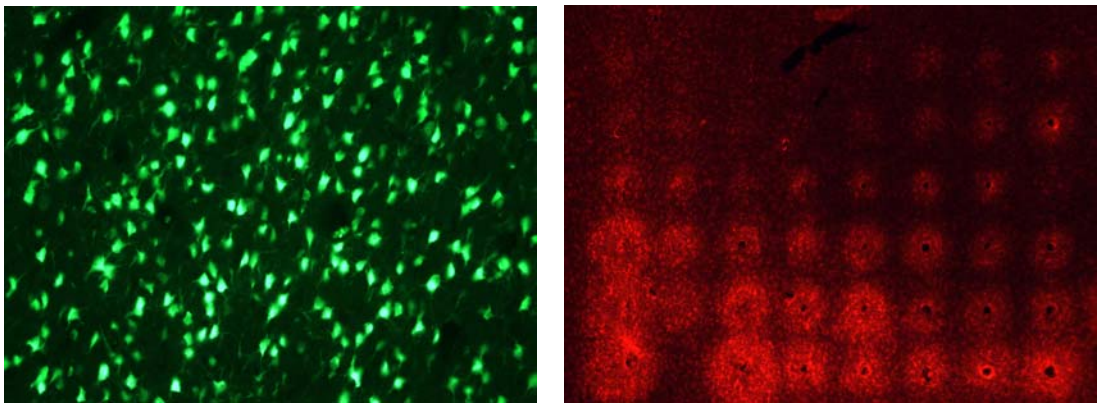

Figure 8. Left panel shows NeuN stained cell bodies in an approximately 2.5 x 3.5 mm section of feline auditory cortex implanted with a 10 x 10 UEA. Right panel shows same region of cortex stained for GFAP.

The same region of tissue was also stained for GFAP, and this section is illustrated in the right panel of Figure 8. The electrode tracks are much more apparent in this GFAP stained section. The tissue illustrated in this figure was sectioned obliquely to the surface, with the plane of the section more superficial in the lower part of the figure. Thus, the heavier GFAP reactivity seen in the lower parts of this figure reflect fibrotic responses closer to the pial surface, while the more modest responses on the top part of this figure reflect fibrotic responses nearer to the tips of the implanted electrodes. Figure 9 shows higher power light micrographs of this same tissue, stained for GFAP and NeuN (left panel) and GFAP and Neurofilament 160 (right panel).

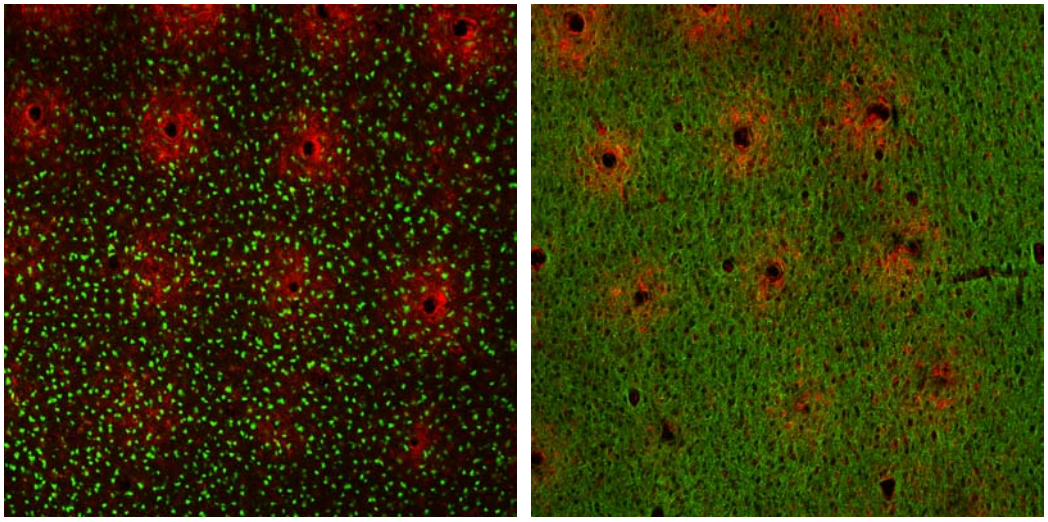

Figure 9 shows higher power superimposed light micrographs of this same region of cortex but with staining for GFAP and NeuN (left panel) and GFAP and Neurofilament 160 (right panel).

One goal of these experiments was to learn if the immunohistochemical antibodies used by Dr. Tresco to study tissue responses in rats could be exported to the feline model. It is clear from Figures 8 and 9 that this is the case. The techniques used to generate these figures will be refined over the next quarter and used to evaluate the consequences of array implantations and current injections in cat auditory cortex. Specifically, we will try to discover if these immunohistochemical techniques are sensitive enough to reveal pathological consequences of electrical stimulation of cortex delivered via our backpack stimulators.

5. We have begun anatomical experiments focused on counting auditory nerve fibers in implanted auditory nerves and in unimplanted auditory nerves from the same cat. We are using image analysis software to do fiber counts and have demonstrated that we are able to reliably count auditory nerve fibers.

We have proposed that the long-term anatomical consequences of auditory nerve implantation can be determined by monitoring auditory nerve fiber survival. To this end, we are proposing to count auditory nerve fibers on implanted and unimplanted sides of chronically implanted cats. We have attempted to use 'NIH Image' software to obtain reliable counts of nerve fibers, by performing 'manual counting' of laryngeal nerves and comparing these counts to automated counting with Image software. We have used laryngeal nerve sections in these experiments because the relatively small number of fibers makes manual counting accurate and reliable. Further, the laryngeal nerve fibers are relatively well separated from each other in the nerve, making automated counting less problematic. In spite of this, hand and automated counts differed significantly.

We have also developed auditory nerve harvesting and processing techniques that should also us to recover auditory nerve sections that will allow us to hand count the fibers in harvested nerves. Over the next quarter, we will attempt hand counting of implanted and unimplanted nerves in cats that have been implanted in auditory nerve for over six months.

2. PLANS FOR THE NEXT REPORTING PERIOD.

Over the next reporting period we will work in the following areas:

1. We will travel to UCSF and participate in Dr. Russell Snyder's mapping experiments performed in cat inferior colliculus.

2. We will continue to map electrical excitation patterns in AI and begin to start work in IC.

3. We will implant four more cats and continue stimulation of auditory cortex using our backpack stimulators.

4. We will analyze the cortical response to implantation and electrical stimulation using immunohistochemical techniques developed during this reporting period, performed with Dr. Patrick Tresco.

5. We will continue our counts of auditory nerve fibers in implanted and unimplanted auditory nerves to document the consequences of array implantation on fiber survival.

3. PUBLICATIONS AND PRESENTATIONS

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Imaging of Utah Electrode Array, Implanted in Cochlear Nerve. Kindlmann, Gordon; Normann, Richard A.; Badi, Arun; Bigler, James; Keller, Charles; Coffey, Richard; Jones, Greg M.; and Johnson, Chris R.. Presented at the Biomedical Information Science and Technology Initiative (BISTI) Symposium, NIH, 2003.

Experimental Studies Of Direct Auditory Nerve Stimulation Via A Penetrating Electrode Array. Richard A. Normann, Arun N. Badi, Todd A. Hillman, Clough Shelton. Presented at the 2003 Cochlear Prosthesis Workshop, Asilomar, CA, 2003.

Feasibility Of An Intraneural Auditory Prosthesis Stimulating Electrode Array. Richard A. Normann, Todd A. Hillman, Arun N. Badi, Clough Shelton. Presented at the Neuroprosthesis Workshop, NIH. 2003

Towards a VIII Nerve Auditory Neuroprosthesis. A.N.Badi, T. Hillman, C. Shelton, R.A. Normann R.A. American Academy of Otolaryngology Head & Neck Surgery Annual Meeting, Orlando, FLA. 2003.

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Differential Stimulation Of The Recurrent Laryngeal Nerve In The Cat Model Using An Intra-Neural Electrode Array. K. Hadley, M.E. Smith, A.N. Badi, R.A. Normann R.A. American Academy of Otolaryngology Head & Neck Surgery Annual Meeting, Orlando, FLA. 2003.

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