REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ORGANIZATION.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
31-05-2007	Final Technical Report	May 20, 2005 - September 1, 2006

4. TITLE AND SUBTITLE

Implantable Neural Interfaces for Sharks

5a. CONTRACT NUMBER

HR0011-05-C-0018

5b. GRANT NUMBER

NA

5c. PROGRAM ELEMENT NUMBER

NA

6. AUTHOR(S)

Prof. Daryl R. Kipke, Project Director, University of Michigan
Prof. Jeffrey Carrier, Albion College
Dr. David J. Anderson, NeuroNexus Technologies, Inc.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

NA

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Michigan Dept of Biomedical Engineering
1101 Beal Avenue / 2212 LBME
Ann Arbor MI 48109-2110

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Defense Advanced Research Projects Agency
Advanced Technology Office
3701 North Fairfax Drive
Arlington VA 22203-1714

10. SPONSOR/MONITOR'S ACRONYM(S)

DARPA / ATO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

NA

12. DISTRIBUTION/AVAILABILITY STATEMENT

Unclassified and unlimited

13. SUPPLEMENTARY NOTES

NA

14. ABSTRACT

Sharks swimming in the open ocean represent impressive examples of biological underwater sensor systems. Their chemical, electrical, and acoustic senses are finely tuned to survival in a complex, dynamic, and dangerous environment. While shark behavior and physiology is gaining increasing attention by marine biologists, the shark nervous system remains largely unexplored. However, it is the nervous system that holds an important key for understanding their sensory and motor acuities, and how this translates to behavior. The primary goal of this project was to develop an innovative implantable neural interface technology that would begin to pave the way for researchers to interface with highly specific targets in the nervous system of swimming sharks to monitor (record) and stimulate (write) neural activity. This project was focused at developing leading-edge neurotechnologies, MEMS technologies, and electronics into novel implantable neural interfaces in freely swimming sharks in order to investigate neural coding associated with sensory processing and natural behavior.

15. SUBJECT TERMS

Implantable Neural Interface, Neural recording, Neural Stimulation, Silicon Microelectrode Arrays, Peripheral Nerve, Local Field Potentials, Extracellular action potentials, electrophysiological recordings

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Dr. Daryl R. Kipke
U	U	U	UU		19b. TELEPHONE NUMBER *(Include area code)* 734-764-3716

Implantable Neural Interfaces for Sharks

Final Technical Report
May 2007

Sponsored By:
Defense Advanced Research Projects Agency
Advanced Technology Office (ATO)
Program: Implantable Neural Interfaces for Sharks
ARPA Order No. T885/00
Issued by DARPA / CMO under
Contract No. HR0011-05-C-0018

Authors:
The University of Michigan, Prof. Daryl R. Kipke, Project Director
Albion College, Prof. Jeffrey Carrier
NeuroNexus Technologies, Inc., Dr. David J. Anderson

Technical Contact:
Dr. Daryl R. Kipke
Biomedical Engineering
1101 Beal Avenue
2212 LBME
Ann Arbor, MI 48109
Phone: 734-764-3716
Fax: 734-936-1905
dkipke@umich.edu

Administrative Contact:
Ms. Kathryn DeWitt
3003 S. State St.
Ann Arbor, MI 48109-1274
Phone: 734-763-6438
FAX: 734-764-8510
dewitt@umich.edu

Project Abstract- **Sharks swimming in the open ocean represent impressive examples of biological underwater sensor systems. Their chemical, electrical, and acoustic senses are finely tuned to survival in a complex, dynamic, and dangerous environment. While shark behavior and physiology is gaining increasing attention by marine biologists, the shark nervous system remains largely unexplored. However, it is the nervous system that holds an important key for understanding their sensory and motor acuities, and how this translates to behavior. The primary goal of this project was to develop an innovative implantable neural interface technology that would begin to pave the way for researchers to interface with highly specific targets in the nervous system of swimming sharks to monitor (record) and stimulate (write) neural activity. This project was focused at developing leading-edge neurotechnologies, MEMS technologies, and electronics into novel implantable neural interfaces in freely swimming sharks in order to investigate neural coding associated with sensory processing and natural behavior. This report summarizes the work of this initial project. While this initial project accomplished its research goals, the sponsor decided to not continue the project beyond the first phase.**

1.1 Introduction

The neuroscience community has developed several methods for recording from and stimulating the nervous system. The major technologies are microwires, the Utah array, the Michigan silicon technology and a variety of polymer based devices. All of these technologies have contributed to progress toward neural interface methods, but only the Michigan probe technology has been successful in all major brain volumes; cortex, peripheral nerve spinal cord and deep brain. The major reason for success is the technology's very large design space. The ability to conform to any two dimensional shape is the most powerful of asset but an integral cable technology and the ability of incorporate onboard electronics are other favorable characteristics. The work presented here demonstrates development of this multielectrode array technology for recording and stimulating from the auditory and olfactory sensory nervous systems of the awake, swimming nurse shark, *G. cirratum* (Figures 1,2).

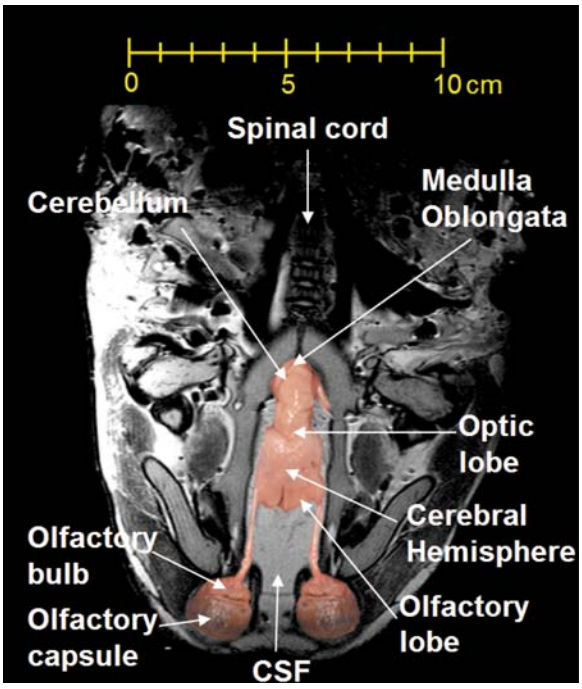


Figure 1: Photographic overlay of the central nervous system of the nurse shark on a horizontal MR image.

The experimental plan consisted of a progressive series of experiments for developing and demonstrating procedures for interfacing with the shark auditory, olfactory, and motor systems (Figures 3,4,5). The initial experiments were conducted in anesthesized, acute preparations in a standard shark tank in the lab of Dr. Carrier at Albion College. These experiments helped to establish proof-of-concept for the surgical techniques, neural recording/stimulation procedures, and device design layout. The primary challenges that were met include the

development of specialized implantable microelectrode arrays that could be implanted into targeted neural structures and then to package these neural probes with the appropriate housing to provide for a fully implantable system in freely swimming animals. Following these demonstrations, specialized devices were implanted in the peripheral and central olfactory structures on a semi-chronic (tethered) preparation. Successful neurophysiological recordings were made from both the peripheral and central olfactory structures as well as auditory-evoked local field potentials (multi-modal sensory responses) from both anesthetized and awake animals. Finally, evidence exists that microstimulation of the olfactory system could lead to patterned behavioral responses in the swimming animal.

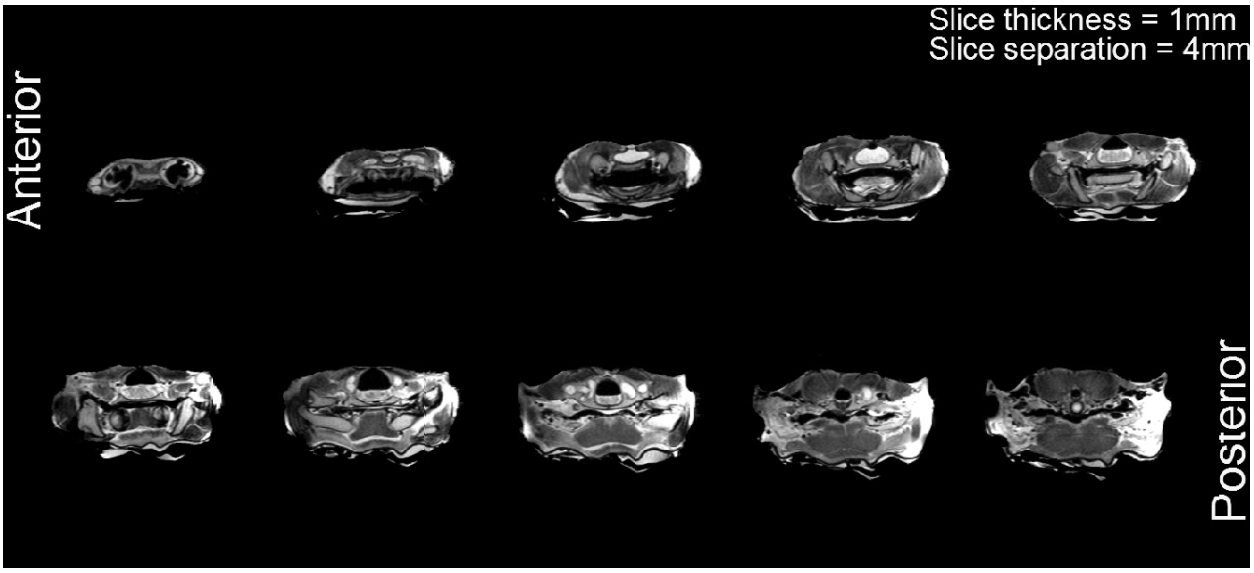


Figure 2: Coronal MR image of a juvenile animal starting most anterior (upper left) and moving posterior (left to right, top to bottom).

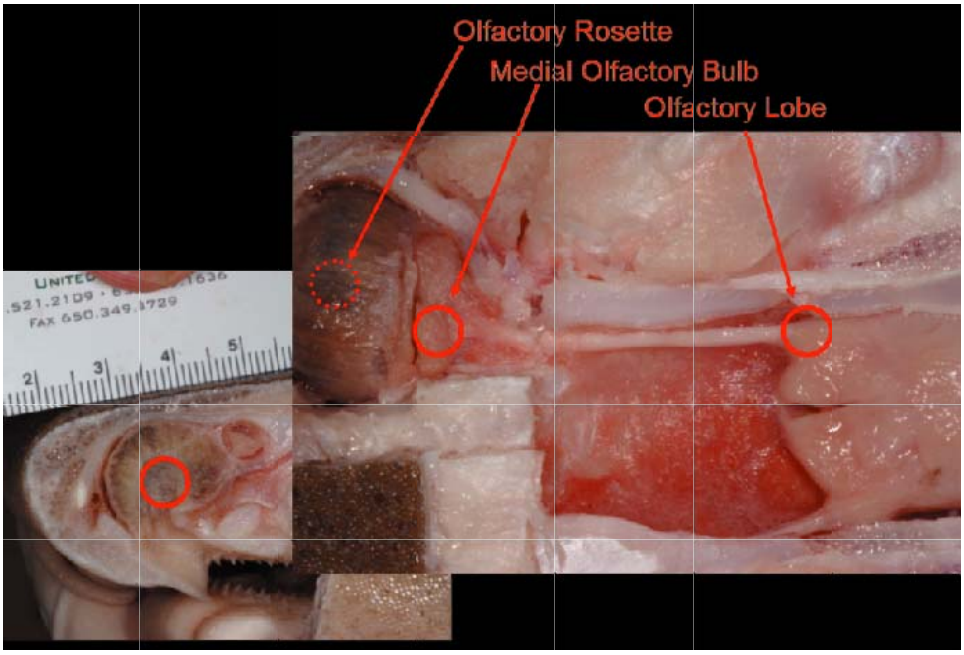


Figure 3: Location of the target structures of the peripheral and central nervous system

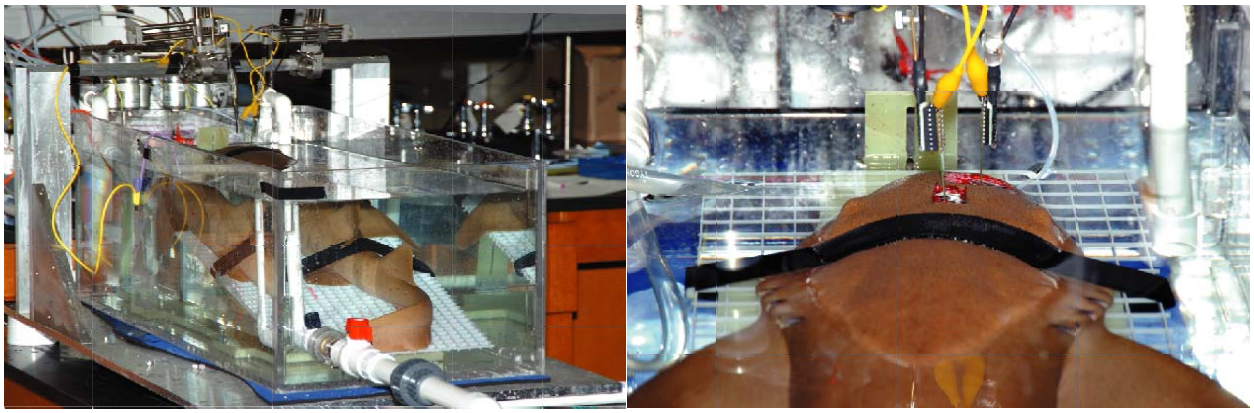


Figure 4: Stereotactic tank / frame (left), and one electrode array containing 16 electrodes each in the olfactory bulb & olfactory lobe (right).

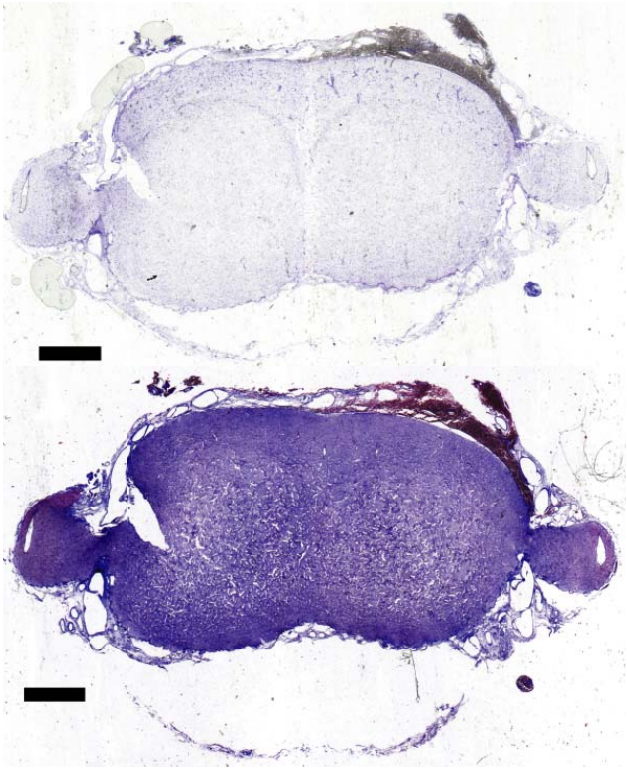


Figure 5: Histological stain with Cresyl Violet (top) and Mason's trichrome (bottom) of coronal sections 1.8 mm posterior from the most anterior edge of the shark brain. Scale bar is 200 mm.

2.1 Milestones

The following tables summarize the project goals with respect to recording (reading) and stimulating (writing) neural codes to the shark nervous system. Each quarterly table summarizes the progress and innovations made in the auditory, olfactory, and interfacing technology development categories. Below the tables are representative data that were collected in support of each of these milestones.

2.2 Milestones – 1st quarter

Auditory system	**Olfactory system**	**Technology**
Record neural codes from peripheral nerve using electrode arrays; Use complex auditory stimuli & multiple locations; *Determined to not be feasible after conducting initial experiments. Developed an alternative, functionally equivalent approach by targeting structures in the central auditory system.*	Record neural codes from peripheral nerve using electrode arrays; Use simple chemical stimuli & multiple locations Completed *Amino acid – evoked responses & electrical stimulation – evoked responses*	Silicon microelectrode arrays, no electronics, wired, acute animal preparation Completed *Successful recordings through all acute probe architectures*

Recording of neural codes in sensory structures:
Simultaneous electrophysiological recordings (Tucker-Davis Technologies) were made from up to 32 electrodes including:

- Extracellular action potentials in the ipsilateral olfactory bulb and lobe
- Local Field Potentials (LFP) in the bulb and lobe
- Electrical stimulation of the seawater space within the ipsilateral rosette
- Odorant perfusion across the olfactory rosette (amino acids: histidine, glutamate, cysteine)

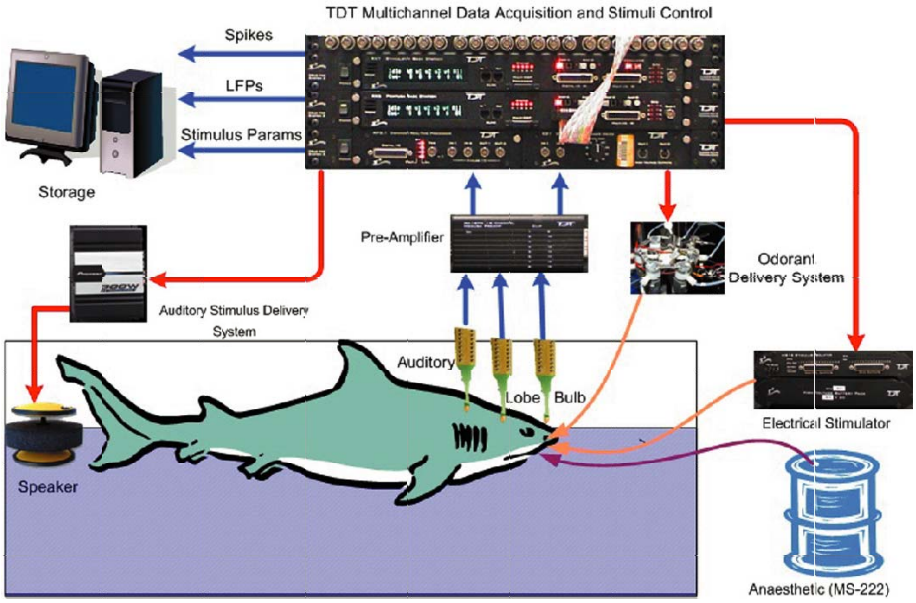


Figure 6: Schematic representation of the experiment.

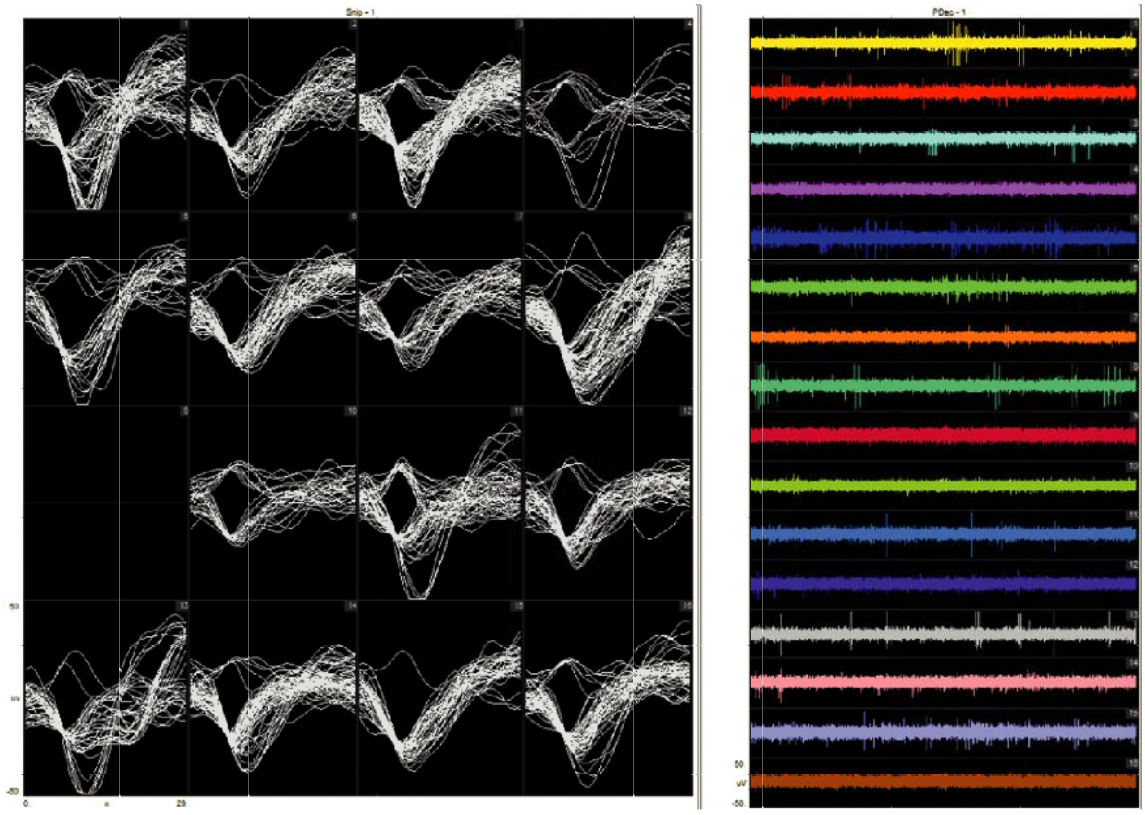


Figure 7: Single- and multi-unit recordings from neurons in the olfactory lobe. Each panel represents individual voltage waveshapes recorded on a single electrode in the array (left panel). Time of each window is 1.2 msec. The panel on the right is a three second history of spikes recorded simultaneously across the olfactory lobe.

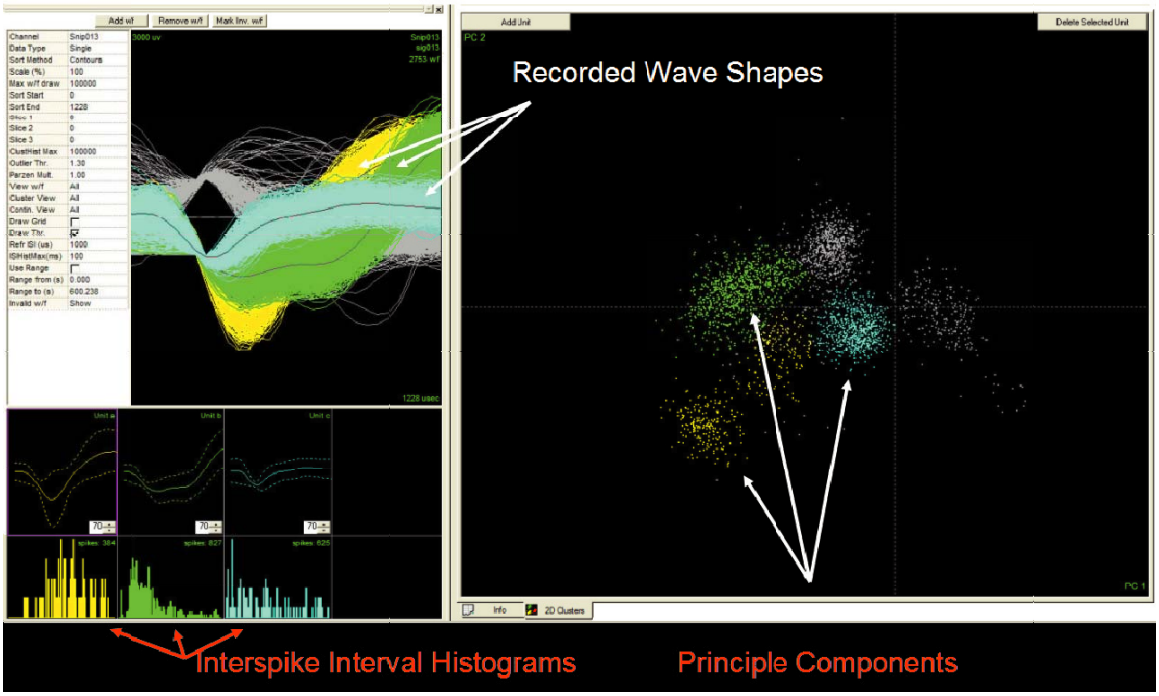


Figure 8: Multiple units recorded on a single electrode in the olfactory lobe (upper left), their respective interspike interval histograms (lower left), and unit clusters (right).

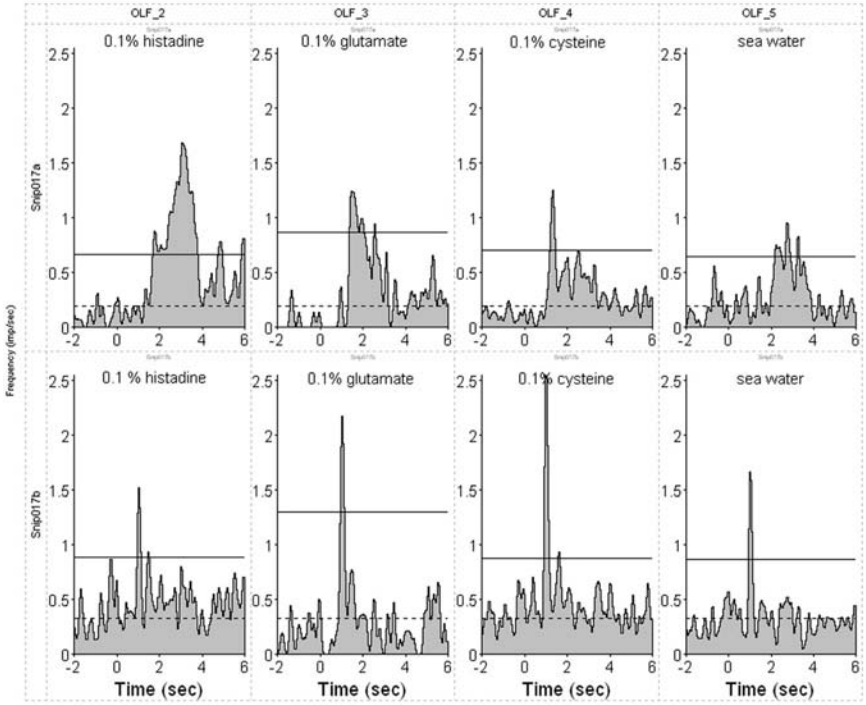


Figure 9: Odorant Detection and Discrimination. Odorants were randomly presented through a six-channel computer-controlled odorant delivery system. Odorants were presented for 1 second every 30 seconds. Each row is one of two neurons recorded in the bulb. Each column is one of four odorants. Data are the mean response of each neuron to the odorant (at time = 0) in 50 msec bins. Dotted line is the mean and solid the 95% confidence around the mean.

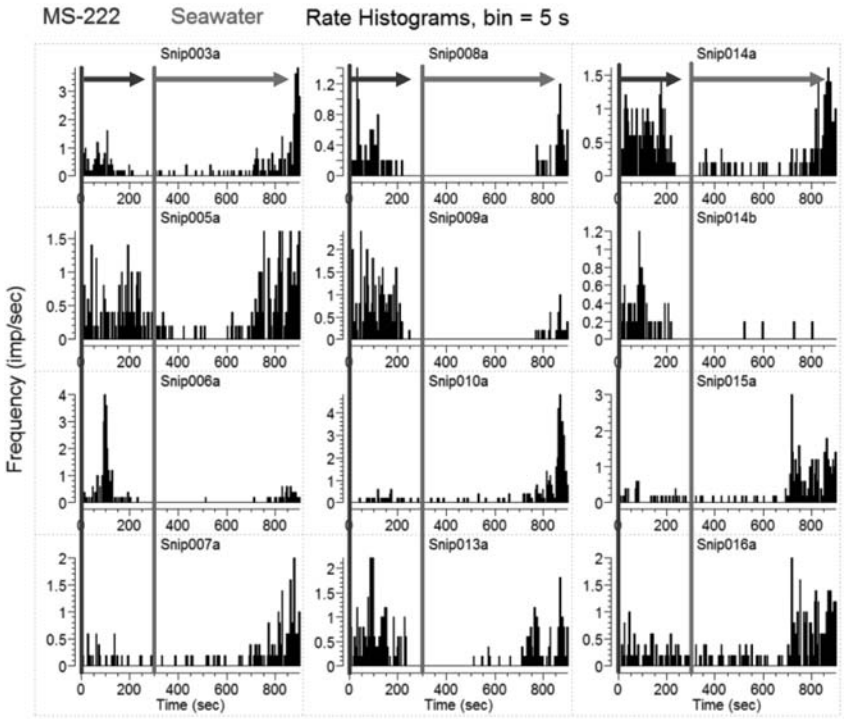


Figure 10: Effects of Anesthesia on Recordings. Data are representative of the effects of MS-222 (tricaine methane sulphonate) at 100 mg/L on spontaneous activity recorded in the olfactory lobe. Rate histograms in 5 sec bins as a function of time. The dark grey bar represents administration of MS-222 (100 mg/kg) anesthesia in artificial seawater. The light grey bar represents administration of seawater only. Note that MS-222 quickly abolished all neural responses.

2.3 Milestones – 2nd quarter

Auditory system	Olfactory system	Technology
Simultaneously record neural codes from peripheral and central auditory structures (tectum); Use complex sound source at multiple locations; Correlate peripheral code with central code	Simultaneously record neural codes from peripheral and central structures; Use several chemical stimuli & multiple locations; Correlate peripheral code with central code	Multiple, silicon and polymer microelectrode arrays; increased site count and refined designs
Write neural codes to elicit predictable direction-oriented movements *Through experiments, determined that peripheral structures were not feasible. Then focused on developing approaches to target central auditory structures, with limited success.*	*Completed. Obtained 32-channels of recordings from olfactory bulb & lobe*	*Completed. Developed custom 32-channels for recording from left & right olfactory lobe*

Simultaneous recording of peripheral and central sensory structures.
Neural activity between the olfactory bulb & olfactory lobe was correlated in time. The timing between odorant-evoked activity in the bulb preceded odorant-evoked activity in the lobe as expected.

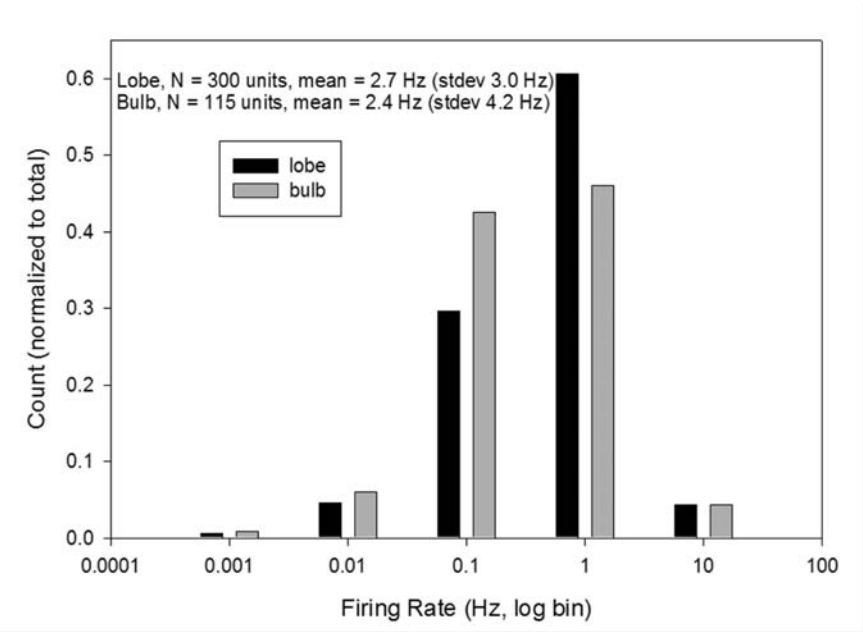


Figure 11: Distribution of firing rates from recorded neurons in the olfactory lobe (black bars) and bulb (grey bars). Data are from four animals.

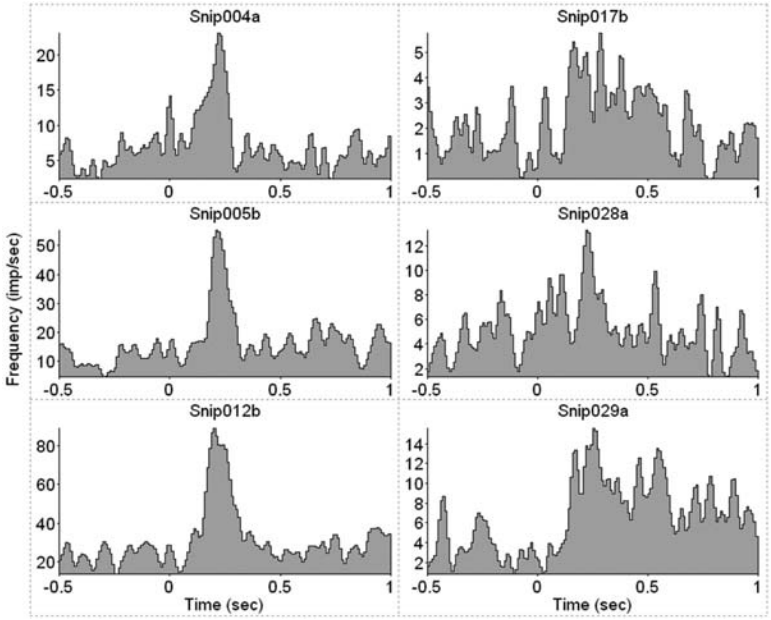


Figure 12: Peri-stimulus time histograms of odorant-evoked activity in the olfactory bulb (left column) and the olfactory lobe (right column) in 10 msec bins. Data are the average response of six units recorded simultaneously to four different odorant stimuli in one animal.

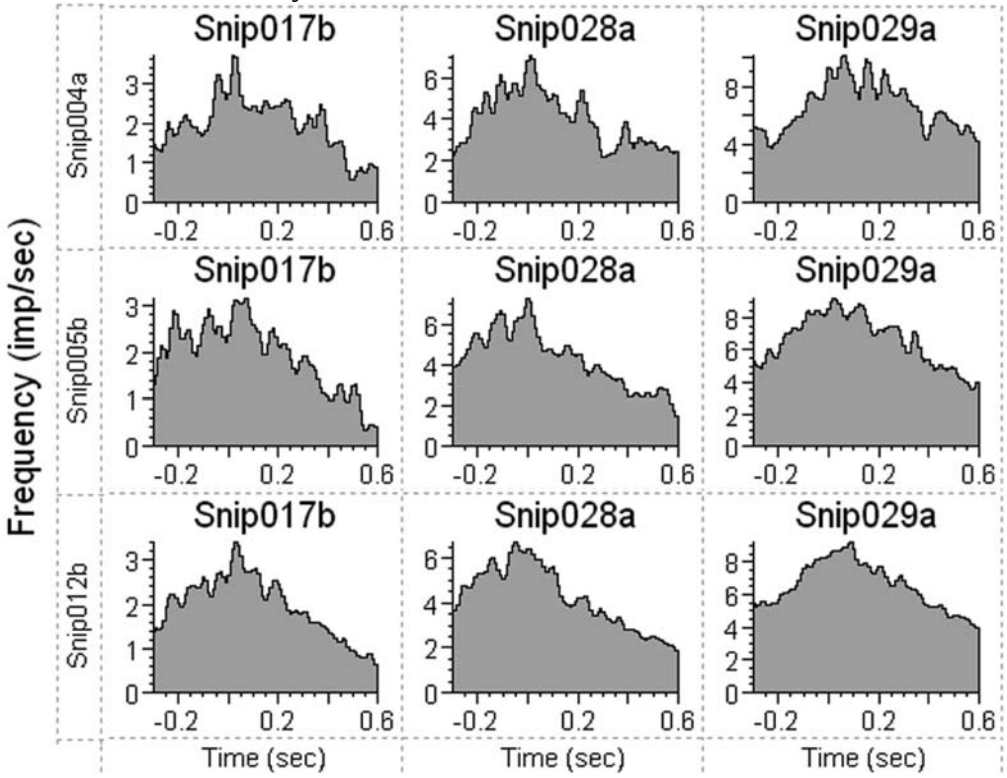


Figure 13: Crosscorrelation of the six units shown in Figure 12. Units recorded in the olfactory bulb are along the rows and units recorded in the olfactory lobe are along the columns. The timing of all spiking events from each unit in the lobe (columns) is plotted as a function of all spikes occurring on each unit in the bulb (rows). Note that neural activity in the bulb precedes activity in the lobe.

2.4 Milestones - 3rd quarter

Auditory system Olfactory system	Technology
Simultaneously record from auditory and olfactory areas in the CNS and periphery. Use multi-modal sensory stimuli at multiple locations. Increase number of recording sites and stimulus complexity *Completed. Auditory-evoked local field potentials in the olfactory lobe through chronic probe architectures*	*SharkCube ß2* device: Customized multiple, silicon and polymer microelectrode arrays, implantable electronics, wired connection
Investigate auditory and olfactory neural codes at multiple levels of the central nervous system and periphery. Investigate a real-time model for movement using minimal, noisy sensory (input) channels and motor command (output) channels *Through experiments, identified technical and biological issues in obtaining long-term neural recordings from tethered, swimming animals*	*Long-term testing of electronics housing attachment to the animal's skull* Use of multiple devices implanted in central and peripheral areas
Stimulate (write) neural codes using patterned microstimulation in CNS to elicit predictable movements associated with multi-modal sensory inputs. Demonstrate more complex targeting movements *Through experiments, determined issues with eliciting reproducible, predictable behavioral responses with microstimuation*	*Used separate, custom 32-channel devices for recording from left & right olfactory lobe* Semi-chronic animal preparation

Electrical stimulation of sensory pathways.

Electrical stimulation of driven activity was observed. An Ag|AgCl wire was inserted through the inlet naris into the seawater space within the olfactory rosette ipsilateral to the recording areas in the olfactory bulb and olfactory lobe. The Ag|AgCl electrode was then stimulated with charge-balanced, biphasic, constant current pulses, 1 - 2 Hz, 250 msec / phase, cathodic first. In the olfactory lobe it was found that electrical stimulation evoked activity occurred maximally ~120 msec after stimulus onset.

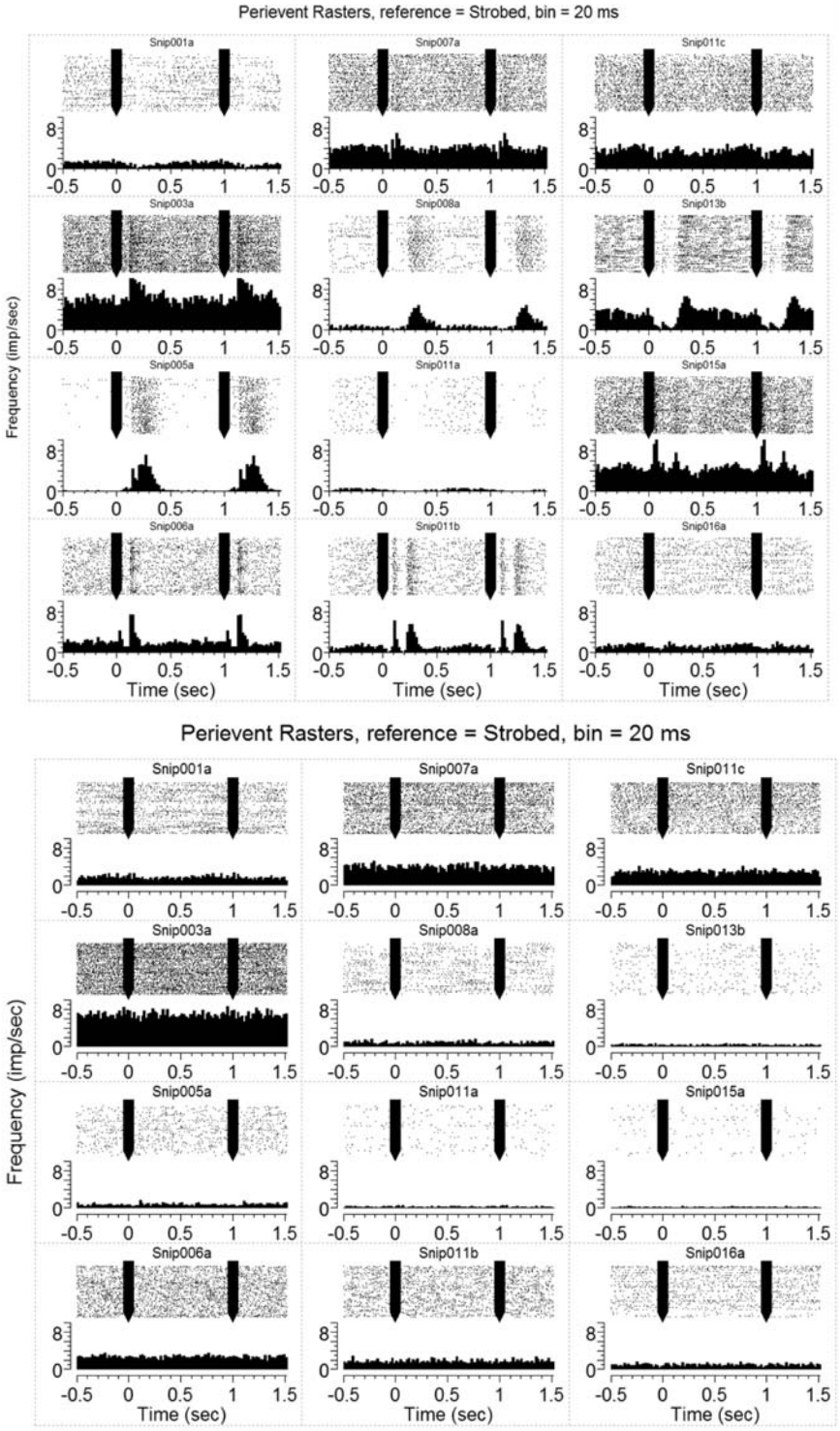


Figure 14: Perievent rasters of units recorded simultaneously in the olfactory lobe. Each tick of the raster represents a spiking event. Each row of the raster represents a trial of an electrical stimulation pulse in the olfactory rosette. Below the rasters are the mean of all trials in 20 msec bins. Inverted black triangles mark the onset of the stimulus pulse (top, 3 mA, 250 µsec, 1 Hz; bottom, no stimulus).

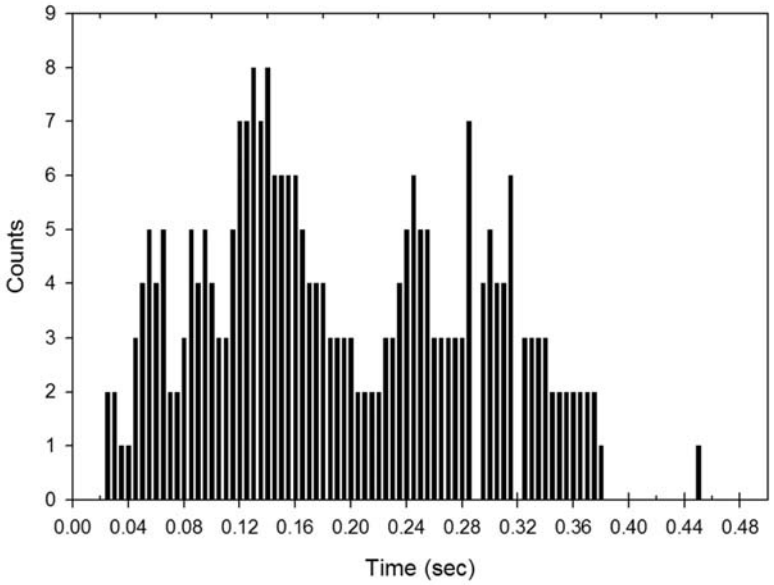


Figure 15: Distribution of significant response times (90% confidence) of lobe neurons following electrical stimulation pulses (time = 0) at 1 Hz in the rosette. Data are from 25 units in one animal. Bin size is 5 msec.

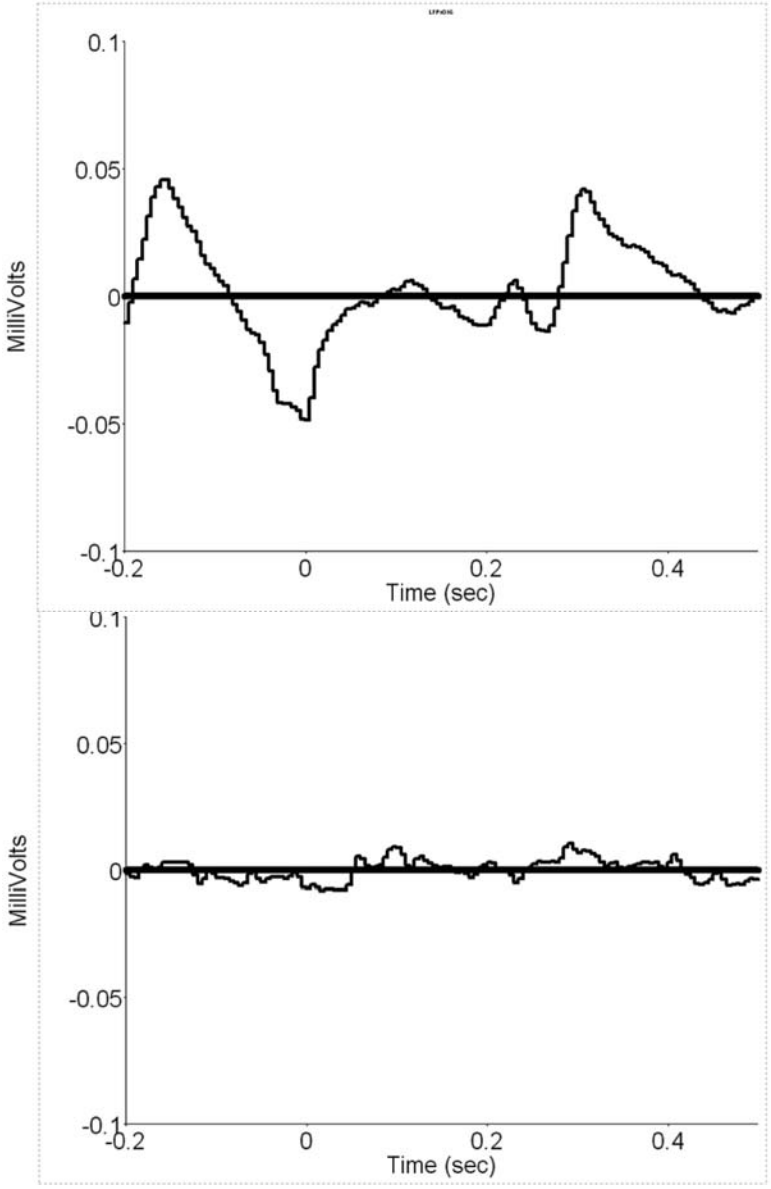


Figure 16 LFP activity recorded in the olfactory lobe aligned to electrical stimulation pulse in the rosette (top, 300 mA, 250 msec, 1.7 Hz) vs. no stimulus (bottom) as a function of time. Data are the mean of ~100 pulses in 5 msec bins.

Long-term electrode array implantation:

An animal was implanted with a sham chronic neural interface and released to its tank to assess the longevity of the implant.

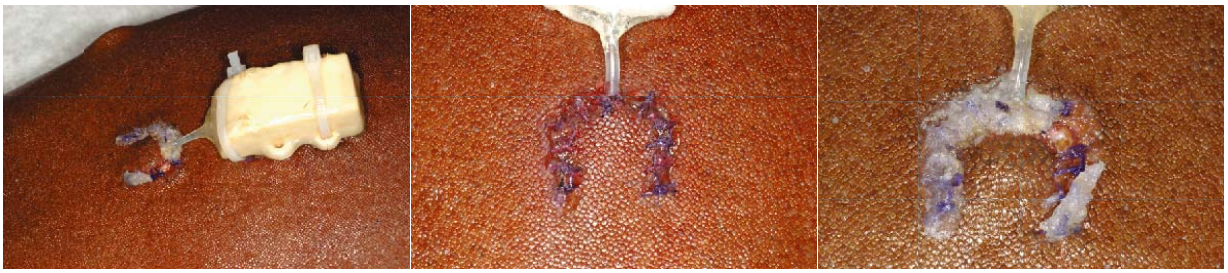


Figure 17: Implantation of a sand-casting of the proposed chronic neural interface including the hardware housing and flexible probe cable (left panel). Denticle flap at the time of implantation (middle panel). Denticle flap three weeks post implantation (right panel). Note that three weeks after implantation, the site of the wound has nearly completely healed around the flexible cable.

2.5 Milestones – 4th quarter

Auditory system Olfactory system	Technology
Simultaneously record from auditory and olfactory areas in the CNS and periphery; Use multi-modal sensory stimuli (sound source eliciting odorant) at multiple locations. Vary the stimulus complexity *Completed. Auditory-evoked local field potentials in the olfactory lobe*	*SharkCube ß1* device: Customized multiple, silicon and polymer microelectrode arrays, no implantable electronics, wired connection
Investigate auditory and olfactory neural codes at multiple levels of the central nervous system and the periphery. Investigate the progressive transformation & integration of neural codes from periphery to central sensorimotor areas *Partially Completed. Electrical stimulation of the olfactory rosette and auditory stimuli, recording from multiple areas*	*Completed. Successful recordings through all acute probe architectures* Semi-chronic animal preparation
Stimulate (write) neural codes using patterned microstimulation in CNS to elicit predictable movements associated with either auditory or olfactory inputs. Demonstrate discrete directional movements *Partially Completed..Microstimulation in the left & right olfactory lobe in semi-chronic animal*	*Completed. Successful recordings through chronic probe architectures*

Multi-Modal Responses Between Spikes and LFPs:

Electrodes were implanted in the olfactory lobe and presented with low-frequency, wide-band auditory noise (20 Hz - 500 Hz) for 10 msec.

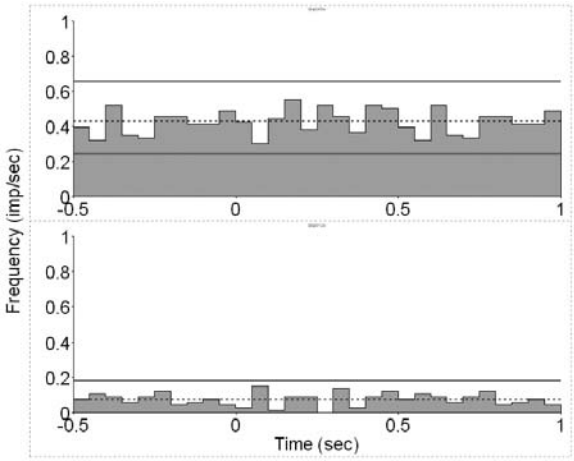


Figure 18: Peri-stimulus time histograms of two units (spikes) in the lobe to auditory stimulus in 50 msec bins. Note there is no significant response to the auditory stimulus.

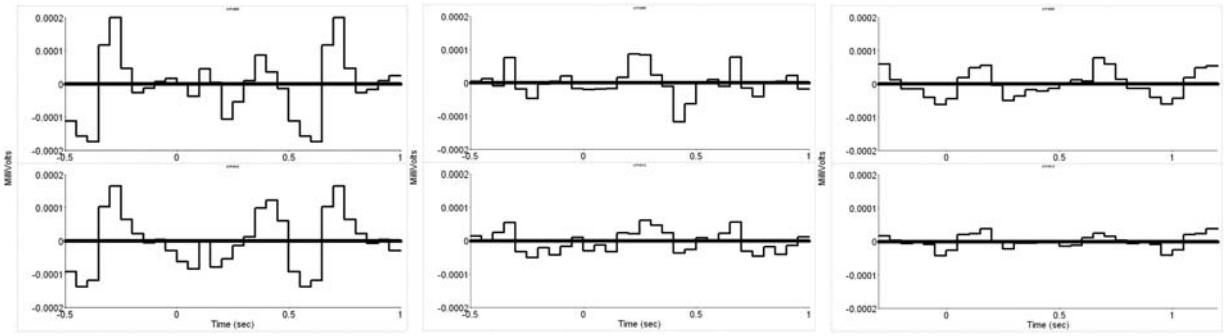


Figure 19: Peri-stimulus time histograms of LFP activity recorded on the same two electrodes that recorded the units in Figure 18 to auditory stimulus (left panel), no auditory stimulus (center panel), and electrical stimulation in the olfactory rosette (right panel). 50 msec bins. Note that whereas the recorded units did not respond to auditory stimuli (figure 18), the local field potential responded with robust deflections following stimulus onset.

Semi-chronic recording capabilities: Electrode arrays were designed to record from both left and right olfactory lobes including electrical stimulation on a semi-chronic basis. All electrodes were sealed in a water tight connector mounted to the animal's skull allowing recording and stimulation sessions to occur over the course of days in the freely swimming (tethered) shark.

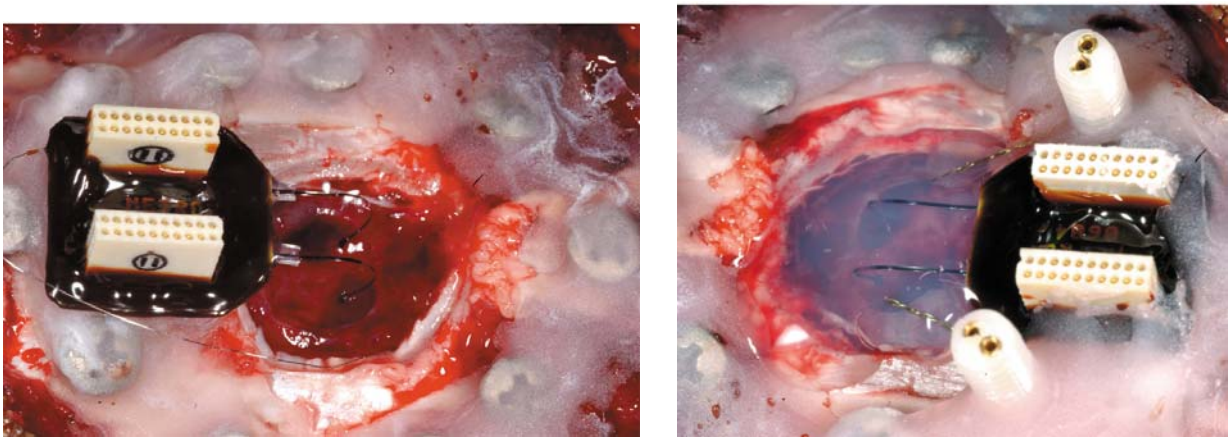
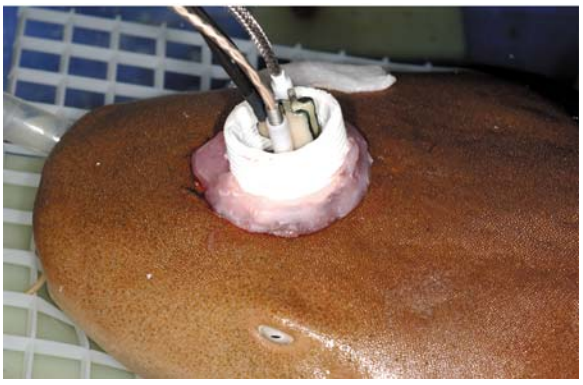


Figure 20: Two sixteen-channel probes are implanted in each olfactory lobe (NeuroNexus, left panel) along with two platinum stimulation electrodes (Plastics1, right panel).

Figure 21: Two sixteen-channel x1 headstages (Tucker-Davis Technologies) allow semi-chronic access to the electrode arrays through a waterproof housing (partially shown).



3. Bibliography

Lehmkuhle, M.J., Vetter, R.J., Parikh, H., Carrier, J.C., and D.R. Kipke. “Implantable Neural Interfaces for Characterizing Population Responses to Odorants and Electrical Stimuli in the Nurse Shark, Ginglymostoma cirratum.” AChemS Abs., 2006.

NeuroNexus Technologies
-MAKING THE CONNECTION-

3985 Research Park Dr. Ste #100
Ann Arbor MI 48108
USA

DAVID J. ANDERSON, PHD
RESEARCH SCIENTIST
NEURONEXUS TECHNOLOGIES

VOICE: 734-786-0061
MOBILE: 734-645-4520
EMAIL: dja@NeuroNexusTech.com

6 July 2007

Memo To: Daryl R. Kipke

From: David J. Anderson, NeuroNexus Technologies project PI

Subject: Final Report for N2T subcontract on DARPA contract entitled Implantable Neural Interfaces for Sharks

Daryl- The following is a final report of NeuroNexus' activities supporting the 'Sensors and Systems' task of your laboratory's University of Michigan's contract with DARPA entitled Implantable Neural Interfaces for Sharks which you are the principle investigator. The original statement of work for this activity is attached as Appendix N2T 1. All in vivo experiments at Albion were supported by at least one N2T employee. It should be noted that the contract was truncated and not all aspects of the work statement were addressed.

The format of our report is an item by item discussion of the eight items listed on the original statement of work from N2T to NEL with documents produced in the process attached as appendices. The exception is Dr. Vetter's report on surgical procedures and the design of the implant structure.

Implantable Neural Interfaces for Sharks

Summary Report

May 2007

Submitted by
NeuroNexus Technologies, Inc., Dr. David J. Anderson
3985 Research Park Dr., Ste. 100
Ann Arbor, MI 48108
734-913-8858

In response to
BAA 04-09
DARPA Bio-inspired Undersea Sensors Program
Other Educational
Identification # 1386006309A1
4 October 2004

Table of Contents

Statements of work with brief statements of accomplishment
(original statement of work in Appendix N2T1)

1. **Working with the NEL group to develop neural probe design specifications which will meet the needs of implants called for in the core project description**

In the course of the several live experiments utilizing nurse sharks at Albion College's shark facility, Rio Vetter, Dave Anderson and Jamie Hetke took an active part in the experiments. Rio Vetter performed all of the surgeries and most of the electrode placement for both acute and chronic experiments in direct support of the NEL and Albion personal who recorded the data and maintained the animals. Dr. Vetter's report on the surgical procedures developed and the experiments performed follow.

2. **Design, create mask sets, specify process flow and fabricate silicon-substrate and polymer-substrate microelectrodes**

For the period of the contract the NEL group used N2T neural probes available from our catalog or stock off catalog probes. These probes were mostly packaged as acute probes in our standard dual inline substrate. Chronic electrodes were utilized in later experiments when we were progressing toward an implant structure.

3. **Using test results and expert evaluation from NEL and Albion, NeuroNexus will specify mechanical and electronic sub-system performance criteria for the various stages of implant development-**

Using experience derived from acute surgeries, progress was made on designing an implant structure and the electronics that would reside therein. The plans for the first version of the implant and photographic documentation of the device are included in Dr. Vetter's report in surgery and implant design. Planning and design work for the electronics was ongoing at the termination of the project. A preliminary set of specifications was derived and components were identified that could meet the requirements of the specifications. In several cases, there were multiple candidates for implementation but further design work will be required to narrow the choice or determine that custom design would be necessary.

Outside the original scope of the project I supervised a University project course project that explored the signal processing necessary to compress the neural data. For a high data rate system such as this, compression is needed to keep the data rates of the implant device within the bandwidth of the transmission technology available to us.

4. **Being responsible for fabrication and/or procurement of all mechanical and electronic sub-system components and for the final integration of the neural probes and sub-systems into functional prototypes that provide for a patent data path from the neural interface to NEL monitoring equipment used for bench testing and *in vivo* testing-**

The project was stopped far too early for significant progress to be made on compact electronic systems that move data from the sensors to a tracking data system but preliminary designs and component selection were accomplished. In addition, a subcontractor was approached who has the appropriate experience in component design and packaging that would be required for a deliverable system. The specifications for such a system are presented in an appendix labeled N2T 2. The cost estimates for the integration of the electronic design into the implant package is given in appendix N2T 3.

5. **Assuring that the data path from the neural interface to NEL's data systems performs to commonly accepted neurophysiological signal processing principles**

The data path used for the laboratory experiments at Albion was through a standard TDT data acquisition system and analyzed by custom software supplied by NEL. Units and slow-wave activity were recorded for each acute experiment and stored in a NEL database. The planning for a mobile electronic system is mentioned in items 3 and 4 above.

6. **Designing an internal temperature sensor and an internal pressure sensor that can be integrated with the *SharkCube* system as auxiliary inputs to internal physiological states of the animal**

This task was not addressed by NEL in the truncated time period of the contract and was therefore not required from N2T.

7. **Conducting bench tests of the probes, sub-systems, and prototype packages, data path and participating in the in vivo testing and analysis.**

All N2T supplied components used in the Albion College experiment were tested at N2T's facility before delivery to NEL or Albion. The most complex systems delivered were mockups of chambers utilizing N2T chronic probes interfaced to the laboratory electronics as were the acute probe systems.

8. **At every stage of specification, design, fabrication integration and testing, NeuroNexus will provide documentation of the effort including drawings, performance results and instructions on use**

At the close of the project there were no delivered items that where not familiar to NEL personal. The chamber designs being fabricated by N2T are documented in the surgical report.

Surgical Procedures and Protocols

In support of this project, engineers and scientists from NeuroNexus worked closely with researchers at the University of Michigan to develop procedures and protocols compatible with technologies being developed at NeuroNexus.

Our microfabricated microelectrode arrays, which originated from the "Michigan" probe technology, are well suited for neural recording and stimulation in the shark nervous system. Our approach was to use our existing family of silicon-substrate microscale neural probes to develop specialized probe systems for interfacing with the shark brain and peripheral nerves. These probes are currently well validated for chronic neural recording and stimulation in the nervous system of fish, insects, birds, and mammals. The various components of this probe system have been developed over the past decade. It has several favorable attributes including batch fabrication, high reproducibility of geometrical and electrical characteristics, easy customization of recording site placement and substrate shape, small size, and the ability to integrate it with a silicon ribbon cable and the ability to include on-chip electronics for signal conditioning. The extensibility of the platform technology enables custom designs for diverse applications. For example, planar probes have been assembled into multi-plane arrays for precise three-dimensional placement of recording sites in the brain, and the probes have been combined with a polymer ribbon cable to form a hybrid assembly to provide additional mechanical flexibility. These silicon probes are currently used by many investigators and are now well validated for recording both spike activity and field potentials in diverse brain structures over acute and semi-chronic time durations up to several months.

The initial experiments were conducted in anesthesized, acute preparations in a standard shark tank at Albion College. These experiments helped to establish proof-of-concept for the surgical techniques, neural recording/stimulation procedures, and device design layout. The primary challenges that were met include the development of specialized implantable microelectrode arrays that could be implanted into targeted neural structures and then to package these neural probes with the appropriate housing to provide for a fully implantable system in freely swimming animals. Following these demonstrations, specialized devices were implanted in the peripheral and central olfactory structures on a semi-chronic (tethered) preparation. Successful neurophysiological recordings were made from both the peripheral and central olfactory structures as well as auditory-evoked local field potentials (multi-modal sensory responses) from both anesthetized and awake animals.

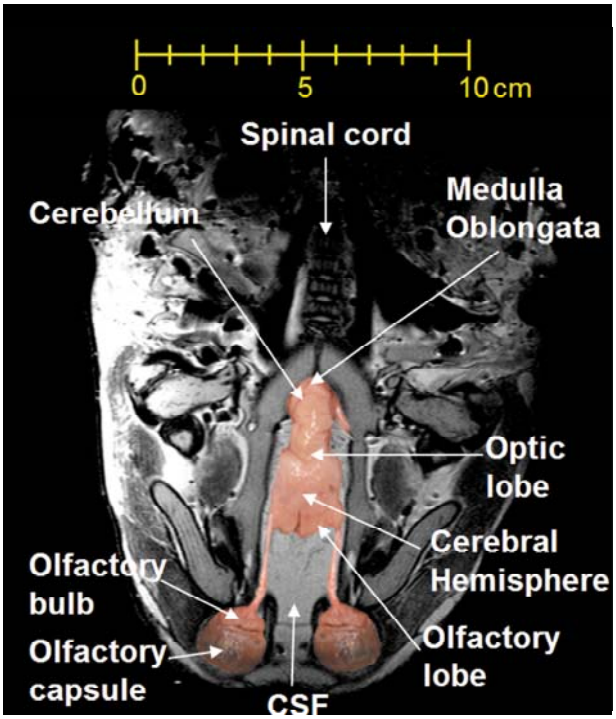


Figure 1: Pre-operative MR image of shark central nervous system, and part of the peripheral nervous system (i.e., olfactory system).

Device design, fabrication and assembly were driven by the anatomy of physiology of the nurse shark and the capabilities and limitations of the technology. As a result, scientists from NeuroNexus were heavily involved and even led much of the planning and execution of the operative procedures. This was important not only because of the experience and expertise they have with handling/implanting the electrodes, but also so that the critical issues of interfacing with the shark's nervous system could be better understood.

The following tasks were lead by NeuroNexus scientists:

- *Pre-operative imaging of shark nervous system:* Prior to conducting surgical procedures, advanced MR imaging techniques were used to identify key neuronal structure of the shark central and peripheral nervous system.
- *Exploratory probing of nervous system to identify/locate auditory and olfactory neural structures:* Prior to conducting any procedures associated with any auditory or olfactory experiments, several exploratory procedures were conducted. These involved dissecting perfused animals so that anatomical structures of the nervous system could be located and identified. Dissected animals were first imaged using MRIs, which helped navigate stereotactically. Approximate stereotactic coordinates were for target areas were identified. These coordinates were subsequently used for exploratory mapping procedures. With the used of 16-channel acute NeuroNexus probes, were recorded neural activity from various places in the nervous system. This allowed us to refine our stereotactic coordinates in order to minimize the invasiveness subsequent experimental procedures.
- Developed/optimize surgical techniques to implant microelectrode arrays for acute sensing of neuronal activity. Based on previous experience of implanting silicon probes, NeuroNexus developed new procedures for implanted the probes in the olfactory bulb, the olfactory nerve, and the olfactory lobe. New methods were developed for various stages throughout the procedures (e.g., handling of the skin, exposing the neural tissue, inserting the electrodes to minimize compression/damage to the tissue, etc.).

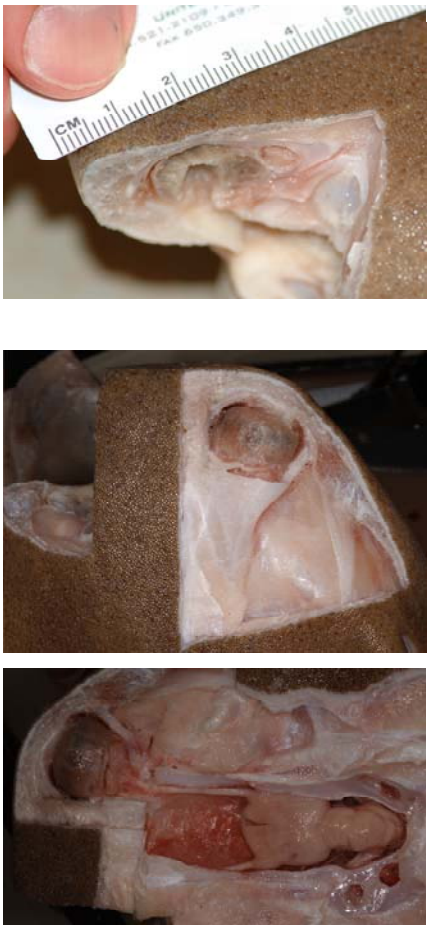


Figure 2: Dissection of the nurse shark olfactory system.

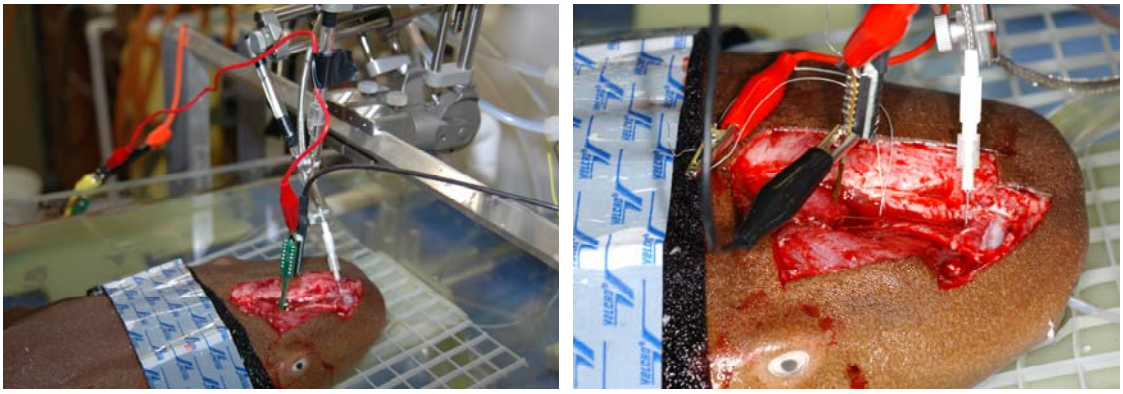


Figure 3: Acute 16-channel probe inserted in olfactory lobe and 2-channel stimulating electrode implanted in olfactory bulb.

- *Implanted single-shank and multi-shank devices*: With the large design space available with the use of this technology, several different probe configurations were fabricated. These ranged from single-shank devices where the sites are oriented in a linear arrangement, to a 8-shank device where each shank contains 4 sites (Fig. 4). Both 16-channel and 32-channel devices were developed and implanted.
- *Recorded olfactory driven spikes & local field potentials:* With the use of electrical stimulation or chemical stimulation of the olfactory bulb, spike and local field potentials could be evoked. Both spikes (representing single- and multi-unit activity) as well as local field potentials could be recorded from each electrode contact implanted in the tissue. A snapshot of recorded spike activity is shown in Fig. 5.
- *Recorded auditory evoked field potentials*: With the use of any underwater speaker, both auditory noise bursts and pure tones were delivered to the animal through the saltwater bath. Although there was difficulty in identifying the central auditory structures, evoked potentials were recorded from various locations surrounding the central nervous tectum.

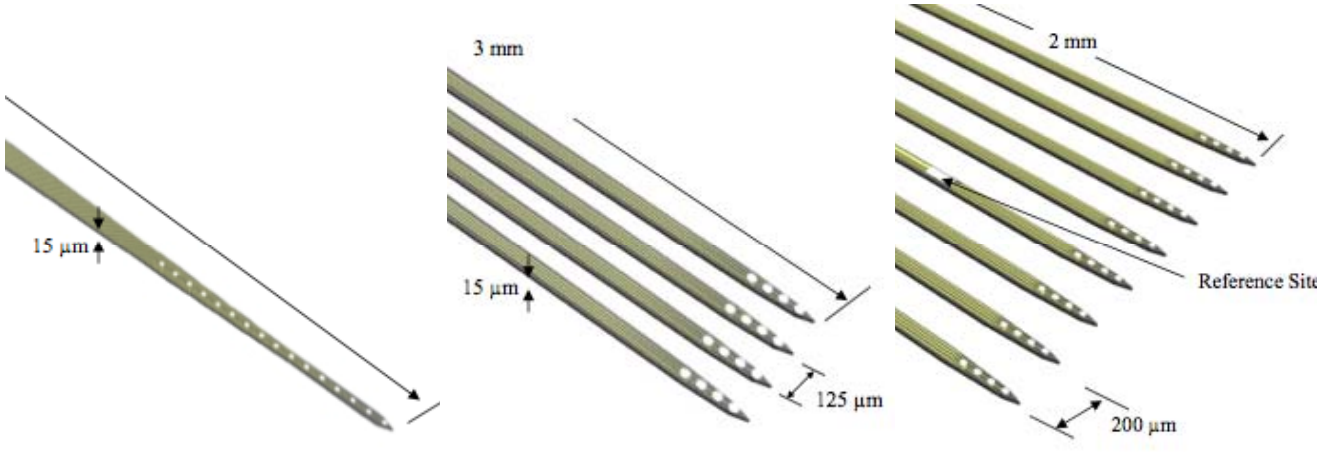


Figure 4: Example electrode configurations: (A) single-shank, 16-channel electrode, (B) four-shank, 16-channel electrode, (C) 8-shank, 32-channel electrode.

- *Developed/optimized surgical techniques to implant microelectrode arrays for chronic sensing of neuronal activity:* Surgical techniques were further modified to account for chronic implantation of the electrode arrays. This was especially challenging as the electrode had to be protected from the outside environment (i.e., salt water) and sufficiently robust to withstand the harsh environment of repeated mechanical disturbances. Additionally, the electrode assemblies and packaging had to be modified to so that they could be permanently implanted. As part of this, a series of "sham" procedure were performed. Non-functional devices were permanently implanted in the animals, which allowed for validation of the chronic surgical procedures (Fig. 6).This required integrating a flexible ribbon cable between the electrode sites and the external circuit board and connector.

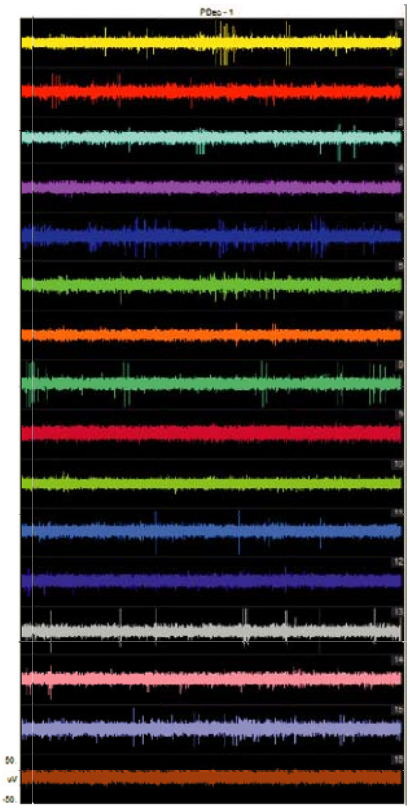


Figure 5: Recorded single- and multi-unit activity from 16 channels implanted in the olfactory lobe.

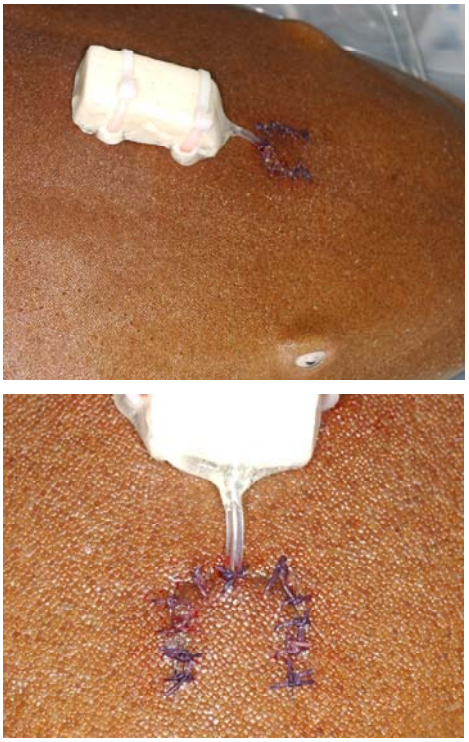


Figure 6: Sham surgery to test surgical techniques and mechanical design of chronic implant.

Chronic implants consisted of a dual 16-channel assembly, like that shown in Fig. 7. A typical procedure is shown in Fig. 7. A 1-cm craniotomy was created through the dorsal cartilage. With the use of several "bone screws", this cartilage was found to be sufficient for anchoring the electrode/headcap assembly. Each electrode assembly had two 16-channel assemblies, which allowed for implantation into both hemispheres of the olfactory lobe. In addition to the 32-channels of recording, two bipolar platinum microwire assemblies were implanted, one in each hemisphere of the olfactory lobe. The connectors (white cylinders) can be seen in the rightmost photo of Fig.7. Also shown in this figure is a headstage preamp plugged in for recording purposes. What is not shown is the outermost chamber that is attached for protect of the headcap/electrodes.

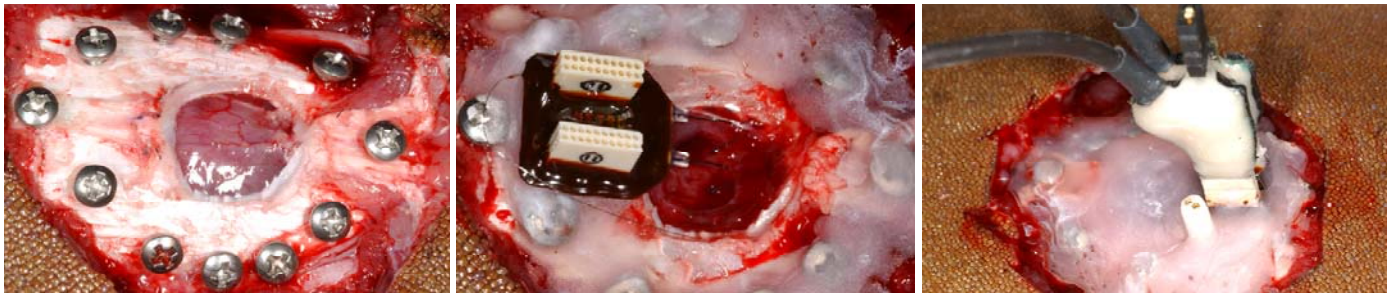


Figure 7: Chronic implant procedure. (A) Craniotomy with screws inserted into cartilage for mechanical support of the head cap, (B) Two 16-channel electrode arrays implanted in olfactory lob, (C) Craniotomy encapsulated with dental cement, head stage amplifiers plugged into electrode connectors. Note the cylindrical connector located adjacent to the rectangular electrode connector. This provides connection to a bipolar stimulating electrode.

Development of Chronic Assembly Housing:

A chamber was designed to house both the electrodes and the electronics so that it could be mounted to the head of the shark while remaining waterproof. In order to start the experiment as soon as possible, in the initial phase we decided to use existing headstage amplifiers and designed the chamber to accommodate two 16-channel Tucker-Davis Technologies headstage amplifiers. The chamber is consisted of two parts – a base chamber which contains the electrodes and a removable auxiliary chamber that contains the amplifiers. The two exit holes in the base chamber allow electrodes to be installed and leaving only the connectors inside the chamber. The holes will then be sealed with waterproof epoxy. In the similar fashion, the headstages will be installed in the auxiliary chamber with cables coming out of the two rear exit holes. Figure 8, shows the CAD designs of the chamber. The minimal feature size and wall thickness are limited by the machining capability. Stainless steel is chosen as the material of the chamber because of its excellent corrosion resistance property.

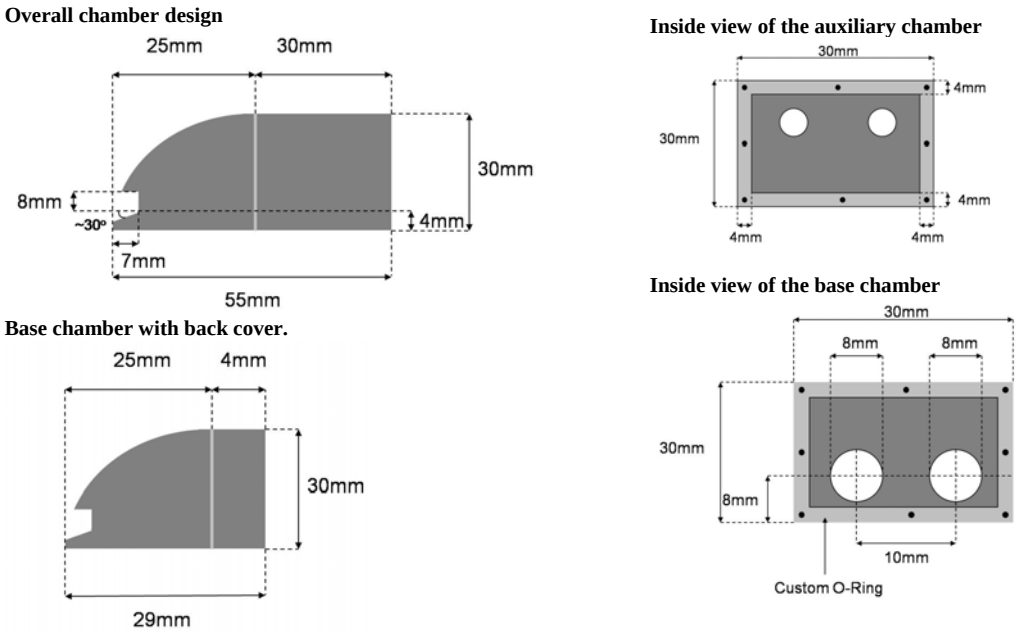


Figure 8. CAD design of the chamber

The base chamber will remain with the shark permanently. During an experiment, the back cover of the base chamber can be removed to allow access of the electrodes. Once the headstages are plugged into the electrodes, the auxiliary chamber can then be installed and sealed with the setscrews. The cable of the headstage can be made as long as 10ft to allow the sharks to feely swim in the tank during the experiment. Figure 9 shows some pictures of the machined chamber. We will be working on packaging and the final assembly of the electrodes and the headstages in the chamber in the next few weeks and getting it ready for the in vivo experiment.

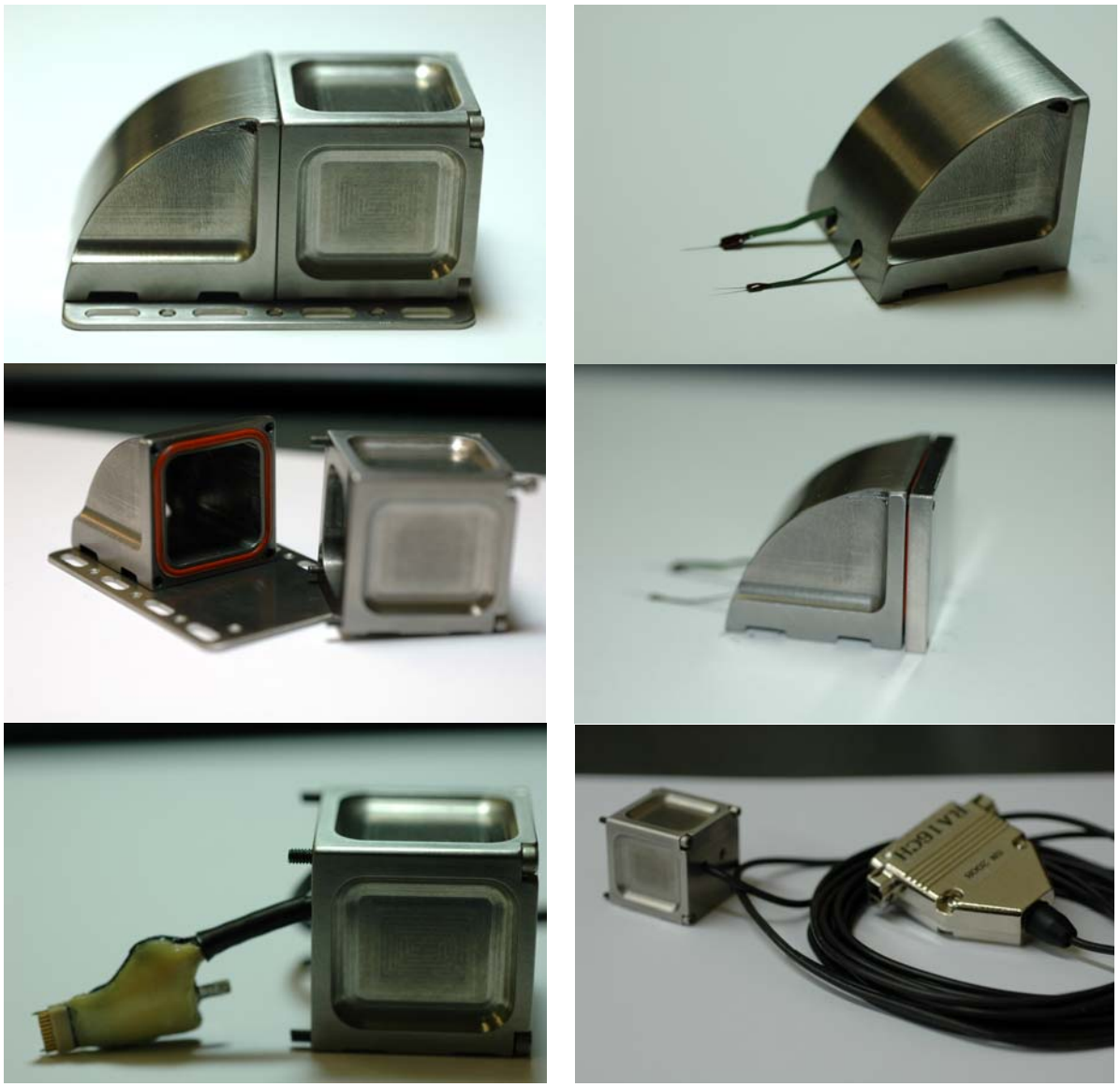


Figure 9. Pictures of the machined chamber

Appendix N2T 1

November 19, 2004

Memo To: Daryl R. Kipke

From: David J. Anderson, NeuroNexus Technologies project PI

Subject: Statement of work for NeuroNexus Subcontract

Daryl- The following details NeuroNexus' proposed commitment in the execution of the University of Michigan's contract with DARPA entitled Implantable Neural Interfaces for Sharks which you are the principle investigator.

Statement of work

NeuroNexus will provide management, technical support and developed hardware to your Neural Engineering Laboratory (NEL) as required to meet the milestones and timeline of the core project. As the PI for the NeuroNexus subcontract, I will be part of the overall management team and will be responsible for coordination of the NeuroNexus effort with the University of Michigan and Albion Collage.

The primary duty of NeuroNexus will be to design, develop and deliver implant and external support hardware to the research teams at NEL and Albion after participating in team exercises to establish specifications. In particular, NeuroNexus will support the 'Sensors and Systems' task by:

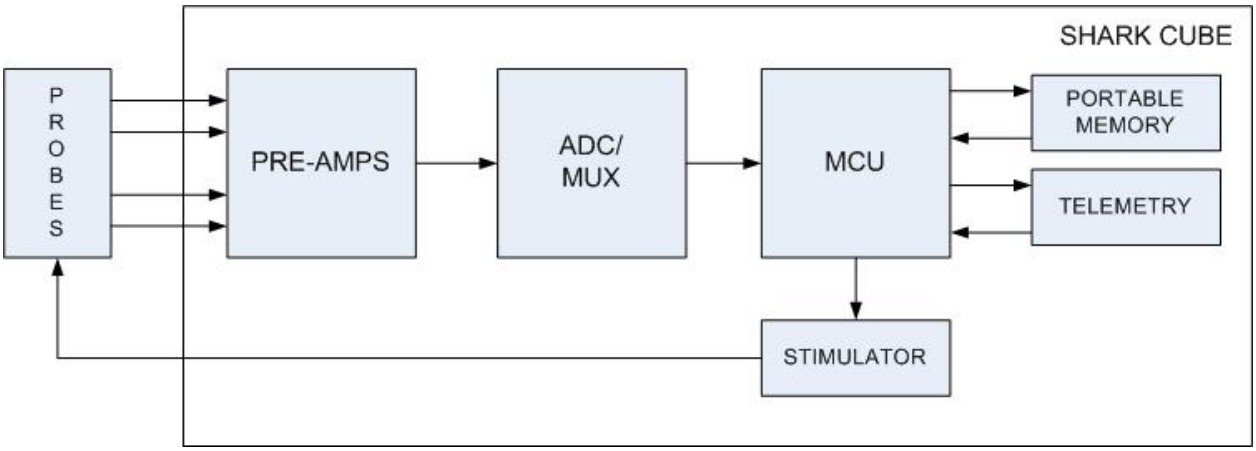
1. Working with the NEL group to develop neural probe design specifications which will meet the needs of implants called for in the core project description
2. Design, create mask sets, specify process flow and fabricate silicon-substrate and polymer-substrate microelectrodes.
3. Using test results and expert evaluation from NEL and Albion, NeuroNexus will specify mechanical and electronic sub-system performance criteria for the various stages of implant development.
4. Being responsible for fabrication and/or procurement of all mechanical and electronic sub-system components and for the final integration of the neural probes and sub-systems into functional prototypes that provide for a patent data path from the neural interface to NEL monitoring equipment used for bench testing and *in vivo* testing.
5. Assuring that the data path from the neural interface to NEL's data systems performs to commonly accepted neurophysiological signal processing principles.
6. Designing an internal temperature sensor and an internal pressure sensor that can be integrated with the *SharkCube* system as auxiliary inputs to internal physiological states of the animal.
7. Conducting bench tests of the probes, sub-systems, and prototype packages, data path and participating in the in vivo testing and analysis.
8. At every stage of specification, design, fabrication integration and testing, NeuroNexus will provide documentation of the effort including drawings, performance results and instructions on use.

NeuroNexus will supply the devices needed to support the 'Neurophysiology and Behavior' task. In addition NeuroNexus will provide training the experiment team members on the use and care of NeuroNexus provided devices. As the experiments are being conducted, NeuroNexus will provide technical support of the devices.

Appendix N2T 2
Electronic specifications for the implant

SHARK CUBE β1 : THE DATA LOGGER

SYSTEM SPECIFICATIONS



Expectations:

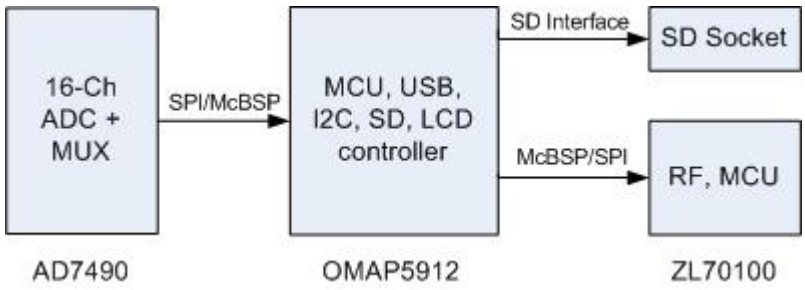
# Channels:	16/32
ADC Sampling Frequency:	> 25 KHz per Channel
Telemetry:	Low bandwidth transmission of firing rates, etc., reception of control signals
Memory:	Removable SD card interface
Wired Connection:	USB
Stimulator:	# Channels – Current range –

SD Card Storage*:

Memory	Recording Time
1 GB	29.83 mins
2 GB	59.65 mins
4 GB	119.30 mins

* #Channels = 16, Fs = 25 KHz, Resolution = 12 bits

SharkCube β1: Component and Interface Details



Working Table:

# Channels	16	
Amplifiers		
Gain		
Cut-off Frequencies		
ADCs	**AD7490**	
Sampling Frequency	1 Msps	
Resolution	12 bit	
Conversion Type	Successive Approximation	
Power Dissipation	12.5 mW @ V_{DD} = 5V	
Interface with Processor	High-Speed SPI	
Throughput	1 Msps @ V_{DD} = 5V	
Processor	**OMAP5912**	
Core	ARM	C55xx
Operating Frequency	0-192MHz	0-192MHz
Power Active Mode Idle Mode		
On-chip Memory	16KB Inst Cache 8KB Data Cache	32Kword DARAM 48Kword SARAM 16Kword ROM 24KB Inst Cache

	250KB Shared Internal SRAM	
Peripherals	32-bit Timers (3) USB1.1 (3) SD/MMC GPIO (upto 16) LCD Controller Keyboard Interface	32-bit Timers (3) DMA (6) McBSP (2) MCSI (2)
	GP Timers (8) SPI UART (3) I^2C SD/MMC McBSP GPIO (upto 64) 32kHz Synchro Counter	
SD Card Storage		
Max Transfer Rate	12.5 MBps	
USB Interface		
Transfer Rate: USB1.1	12 Mbps	
Telemetry	**ZL70100**	
Up-link Frequency	402 – 405 MHz or 433 – 434 MHz	
Down-link Frequency	402 – 405 MHz or 433 – 434 MHz	
Raw Data Rate	800 kbps	
Encoding Scheme	Reed Solomon	
Power Consumption	Continuous TX\RX: 5mA Low power mode: < 1mA	
Interface with Processor	SPI	
Simulator		
# Channels		
Current Amplitude		

Appendix N2T 3
Cost estimates for the electronic integration

Phases

Phase	Description
Requirements	Finalize Functional, Power, Data Collection and Transmission, Data Storage and Download, and Packaging Requirements
Preliminary Design	Major Component Selection, Risk Assessment, Electronic Schematic, Rough Packaging Design
Detailed Design	Circuit Layout, Build of Materials, Firmware Design, Final Power Circuit
Build and Integration	Circuit Board Build, Power Source Build / Procurement, Firmware Development, Integration of All Subsystems, System Level Test
Delivery	Delivery and Demonstration of Prototype to Neuro Nexus for Field Testing

Major Challenges

Currently we foresee the following as the major challenges in the project:

- Power Consumption
  - RF Transmission
  - Size
- Miniaturization of Circuit and Power Source
- Packaging
- Noise Floor

Pricing

Below is a rough estimate of pricing which covers project activities up to and including delivery of a limited number (5-10) of functional prototypes:

Line Item	Estimated Price
Requirements	$ 7,000
Preliminary Design	$ 23,000
Detailed Design	$ 40,000
Development	$ 45,000
Integration	$ 15,000
Delivery and Training	$ 5,000
Documentation	$ 5,000
Project Management	$ 20,000

Documentation includes fundamental items that would be required at a later date to create a Design History File for future FDA filings.

Pricing includes anticipated travel for Senior Electrical Engineer based in Minnesota.