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In-Vivo Testing of Vertically Aligned Nanowire Implantable Titanium Electrodes in the Rattus Norvegicus Hippocampus

Lauren Kegley
University of Arkansas, Fayetteville

IN-VIVO TESTING OF VERTICALLY ALIGNED NANOWIRE IMPLANTABLE TITANIUM ELECTRODES IN THE RATTUS NORVEGICUS HIPPOCAMPUS

By Lauren Kegley
Department of Electrical Engineering

Faculty Mentor: Vijay Varadan
Department of Electrical Engineering

Abstract

Miniaturized multielectrode arrays are MEMS devices that have found use as neural prosthetics (Neuro-MEMS). As implants, they can interface with neurons as sensors or actuators and help compensate for loss of sensory input, motor control, or cognitive functions. The microelectrodes studied here were developed in-house. They have a vertically aligned gold nanowire array and are mounted on a sturdy titanium needle with a fine gauge. Hence, the bill of materials and design characteristics encourage their use as a neural probe. For this study, a probe was tested in vivo for signal acquisition in the hippocampus of a Rattus Norvegicus (Sprague Dawley Rat). Using an Institutional Animal Care and Use Committee (IACUC) approved protocol, the neural probe was deployed in the CA1 region of the hippocampus of a sedated rat. The signal was obtained as voltage against time and was filtered for isolated spikes of neural activity, which were sorted in the form of a Spike Train-Raster Plot. The qualitative evaluation of data obtained through the newly developed neural probe was used as groundwork to decide on future research and discuss possible clinical impacts.

Introduction

Neurological disorders and injuries to the central nervous system can have a serious impact on patients and their support group. Neural conditions can cause an individual to lose his or her independence to a spinal cord injury or Parkinson's disease and can be seen in the anguish of a family watching a loved one diminish as a result of Alzheimer's disease. Contemporary medicine uses an electroencephalogram (EEG) as a diagnostic tool for the detection of abnormal neural functions that manifest as the above-mentioned diseases. The EEG technique is based on measurement of the electrical signal that travels through the central nervous system and facilitates communication. In addition, in vivo measurement of neural activity has been widely used among researchers for many years.

Since the experiments conducted by Luigi Galvani (1791) [1] to measure bioelectric forces in living tissue, researchers and practitioners of nerve physiology have been able to acquire and simulate electrical signals that travel through nerve axons. The model proposed by Hodgkin and Huxley in 1952 has often been used [2]. The electrode technology for neural activity measurement has evolved from a glass electrode [3] to a metal electrode [4] and has gone through drastic miniaturization, resulting in microelectrode arrays (MEAs) [5]. The MEA, which can be on a flat substrate or in bundles, allows for multisite recording within the brain tissue on the individual neuron level, which is instrumental for the observation and statistical analysis of region-specific phenomena. In recent years, the MEA system has been reinvented via needle probes with microelectrodes, popularly known as Michigan Electrodes [6] or Utah arrays [7]. Needle probes such as these allow for higher spatial resolution and precision in locating the neural cluster inside the brain.

Currently used implantable neural electrodes were considered a breakthrough in science that allowed doctors to more efficiently treat patients based on the capacity to interface with neurons and provide clinical applications for neural prosthetics. Commercially available neural probes are microwire arrays of flat electrodes mounted on a fine-gauge wire or needle (230-500μm) made of biocompatible metals such as stainless steel and platinum/iridium (Plexton Inc., Dallas, Texas). While these commercially available implantable neural electrodes can effectively monitor brain signals up to individual neuron resolution, further miniaturized nanoengineered neural electrodes can be developed for more accurate sensing of the electrical signals produced by the brain. At the University of Arkansas, such a neural device was created by Yoon and co-workers [8]. Vertically aligned nanowire arrays were grown on the electrodes (<30μm dimension) to enhance performance and functionality, and then the array of electrodes was fabricated on a fine-gauge titanium needle (280x100μm). The uniqueness of this new type of neural probe lies within the materials used to fabricate them; titanium and gold were used to create these flexible and biocompatible electrode array probes. The sturdy fine-gauge titanium probes can provide continuous in vivo monitoring without breaking or having a large impact on the affected organ. This new form of implantable neural probe is also unique because of its implementation of vertically aligned nanowire array technology, which provides a large electrode surface area that improves the sensing capabilities of the whole device despite its smaller size.

The study described here is a preliminary test of this newly developed titanium neural probe in the Rattus Norvegicus (Sprague Dawley laboratory rat) hippocampus. The study was conducted on live (intact) rats to evaluate the efficiency of the probe's signal acquisition and the stereotactic accuracy of the implantation protocol. The hippocampus is the region of the brain that is crucial for the formation of new memory. It acts as a gate for the passage of newly acquired memory – facts, skill sets, or habits – to permanent memory storage. The basic architecture of the hippocampus is a layer of densely packed pyramidal neurons and well-aligned axons originating from them [9]. Since the axons

are the carriers of neural electric pulses, a strong electrical signal of defined polarity shall accompany any neural activity. Therefore, this region provides a very good ground for testing the newly developed titanium neural probe for the efficient measurement of bioelectric signals.

Experimental Setup

The animal experiment was conducted in an Institutional Animal Care and Use Committee (IACUC) approved laboratory situated at the Central Laboratory Animal Facility at the University of Arkansas. The laboratory includes a data collection center, a surgical workspace, and an anesthetic unit. The data collection center consists of a computer that is connected to the data acquisition equipment, with a 32-channel differential amplifier system (Multichannel System, Reutlingen, Germany) that processes the gathered information via MC Rack Software [10]. This software allows the data to be filtered and viewed in many different ways, including analog spikes of neural activity or a raster plot of the spike train, depending on the necessary analysis.

The surgical workstation (Fig. 1) includes the animal experiment and multichannel feed to the amplifier. The animal experiment setup has a thermal mat to help maintain the rat's body temperature and a stereotactic frame (Korp Instruments) that consists of a head holder and brace to help immobilize the subject, calipers to help measure the brain coordinates, and a holder for the neural probes deemed necessary for individual experiments. The entire setup is mounted on an optical bench with a Faraday cage that cancels out electrical and acoustic noise from external sources.

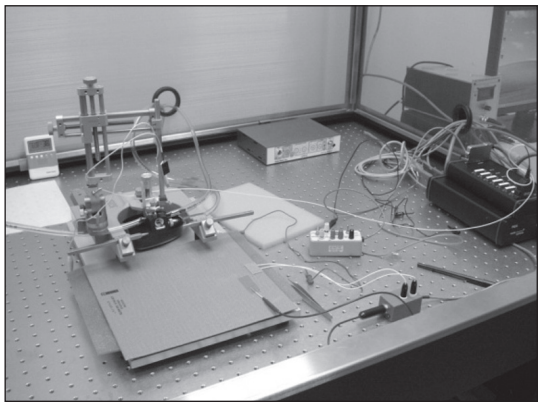

Figure 1. Surgical workstation mounted on an optical bench inside a Faraday cage and equipped with a stereotactic frame.

Once the animal experiments were completed, the collected data were taken to the Brainwave Laboratory at the Engineering Research Center (ENRC) of the University of Arkansas for analysis. This laboratory has a variety of tools to use in data analysis. The collected data were interpreted and conclusions drawn. Since the ENRC also houses the cleanroom (Innovative Nano-Bio Laboratory and HiDEC) facility for fabrication and instrumentation of the titanium neural probes, the lab was also used for experimental preparation and technical changes.

Methodology

The experiment (approved protocol IACUC# 10023) utilized animal subjects of the species Rattus Norvegicus, more commonly known as the Sprague Dawley Rat. The animals were stored in polycarbonate cages with dimensions of 19.5" x 10" x 8" at a population density in compliance with the recommendations listed in the Guide for the Care and Use of Laboratory Animals. Cages were bedded with a 75% aspen chip / 25% cellulose bedding mixture, and rats were fed a standard laboratory rodent diet ad libitum. Tap water was also provided ad libitum. The subjects were split into two separate groups, each containing three Sprague Dawley rats. Group 1 consisted of rats to be tested in the first phase with acute neural recording in the hippocampus, which was done to test the instrumentation of the nanowire probe and to confirm its recordings. Group 2 was earmarked for the second phase, where acute neural recording was done in addition to brain stimulation of mirroring positions in the hippocampus.

Prior to each experiment, the subject was weighed, and an appropriate dose of anesthesia was calculated. The anesthesia method used was intraperitoneal injections of urethane, which was based on a dosage amount of 5.6 ml / kg of body mass. Once calculated, the anesthesia dosage was split into three equal injections that were administered at 2-minute intervals to ensure that the subject would not overdose. Subsequently, the subject was prepared for surgery. Small dosages of anesthesia were set aside and administered subjective to the rat's state of sedation during the course of the procedure. Apart from the anesthesia, no other medication was given to the subject. The neural activity was recorded from the rat in a sedated state, and the animal subject's vital signs were monitored at all times.

The tools and probes were sterilized with a diluted betadine solution to ensure that aseptic techniques were implemented. Prior to sterilization, all the equipment discussed in the experimental setup was checked for appropriate functioning.

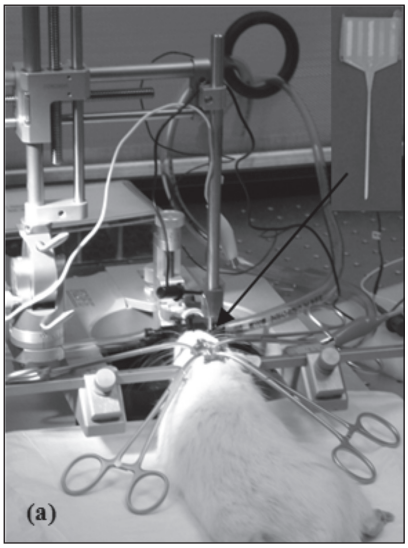

Figure 2. Experimental setup of the laboratory settings: Animal subject on the surgical work station with the neural probe (arrow) mounted on a stereotactic frame.

Once the animal was completely sedated, electric shears were used to remove hair on the rat's scalp between the eyes and ears, carefully avoiding the eyes, whiskers, and ears of the rat. The rat was then placed in a prostate position on the thermal mat, and the head was secured by a head holder and a brace. The eyes were

covered, and the areas surrounding the incision area were blanketed with sterilized towels in order to expose only the incision area. The majority of the body remained visible to allow for the detection of stress responses; however, the area directly surrounding the incision area was covered to maintain sterility. Before an incision was made, the shaved area was cleaned with betadine swabs to further sterilize the area. Next, an incision large enough to access the quadrant of interest was made. Post incision, the subdermal layer of blood vessels and tissue was scraped off with hydrogen peroxide swabs so that the bregma and lambda were visible to serve as medial and baseline references (Fig. 2).

The positions (x, y, and z coordinates) of bregma and lambda were recorded with the help of the calipers of the stereotactic platform. The location of the burr hole was determined by referring to a "Rat Brain Atlas" [11], and the burr hole location was marked with the help of a pointer that was also mounted on the caliper platform. A pre-sterilized drill bit was used to make two burr holes: one for the needle probe and the other for a reference electrode placed away from the probe site. During the operation, the subject's eyes were kept moist with phosphate buffer solution (PBS) swabs, and the subject's vital signs, especially breathing, were monitored via observation. Though sufficiently anesthetized, each rat was also observed for any stress responses (twitching, tremors).

The probe and multichannel acquisition system was prepared by tuning the noise filter to filter the raw signal for DC offset and high-frequency noise. This was done on the MC Rack software console by setting the sampling rate of the band pass filter and allocating the channels to be filtered. The signal acquisition was taken at a specific hippocampus site: interaural 5.76 mm, bregma 3.24 mm, and 2.3 mm deep from the dura. Signal acquisition was commensurate, and the filtered signal was simultaneously sorted for spikes. For immediate feedback of the signal acquisition quality, each spike was heard as a pop on the audio speakers. The acquired data bank was then processed as a pulse train, with -160μV as the threshold, to extract raster plots.

Euthanasia was carried out at the end of the surgical procedure, while the subject was still under anesthesia, by CO2 asphyxiation until clinical death (no perceivable signs of respiration) was determined. Upon completion of the animal experiment, all the surgical materials were disposed of or sanitized according to aseptic technique. The data acquired were filtered, and basic analysis, which was simply the organization of collected data, was completed in the experimentation lab. Extensive analysis was carried out in the brainwave laboratory.

Results

After the testing was complete, the analysis procedure was begun; this phase of the research was essential to determine the accuracy and dependability of the neural probe being tested. The neural spike activity was sorted and stacked to better understand the average peak neural activity in one cornu ammonis (CA1) and the horns of the hippocampus (see the inset of Fig. 3 (a)).

The region in question is densely packed with axons originating from the pyramidal neurons in the outermost layer of the hippocampus. The titanium neural probe was able to detect neural

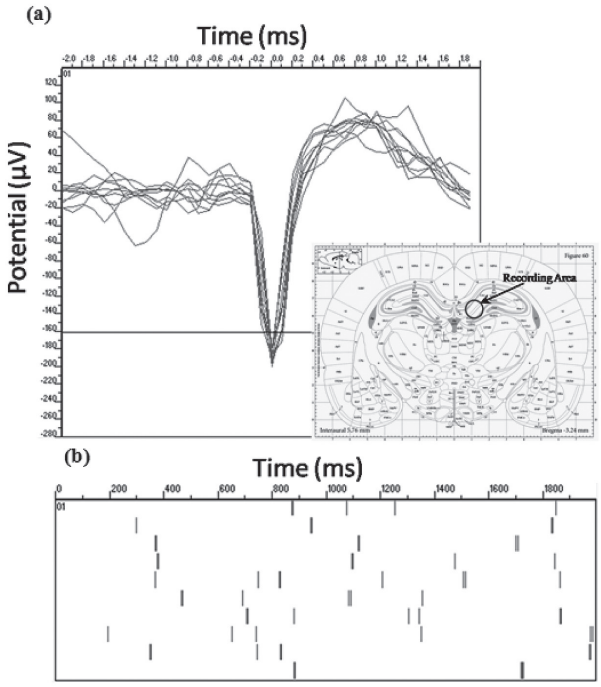

Figure 3. (a) Spikes of neural activity in 4 ms time-amplitude window measured at the CA1 region of the rat hippocampus (b) Spike train Raster Plot with -160 μV threshold.

activity of discernable quality, which can be seen in Fig. 3 (a) and (b).The spikes were sorted based on a pre-specified threshold of -160 μV, and the resultant spike train is represented as a raster plot. The amplitude-time window (Fig. 3 (a)) shows well-isolated potential peaks with a mean amplitude of -180±10 μV. The corresponding spike train (raster plot) along the timeline can be interpreted based on the firing rate of the neuron(s) or the statistical analysis of these spikes/epochs surrounding a documented event (for example, a motor or sensory response to external stimuli) [12].

Discussion

The database acquired from the animal experiment can be filtered and re-plotted for further analysis. Signal processing and analysis facilitates interpretation of data depending on the clinical relevancy of the information. Potential applications of this analysis are provided in the following sections.

Spike train analysis

The spike train from the neuronal ensemble (bundles) can provide control signals for limb movement, represent sensory inputs, or be translated as a highly evolved cognitive signal [12]. The spike train can be represented in the form of spike raster plots (Fig. 3 (b)) that provide information such as spike frequency and spike epoch (width of the raster). This can be treated as a discrete transform of continuous waveforms, which can be characterized on the basis of the functionality of the region of the recording site. For instance, in the hippocampus, spike trains observed in the CA3 and CA1 regions can be respectively categorized as inputs and outputs of the hippocampal memory formulation process [13]. The analysis of such signals can be achieved with covariance/correlation studies between variables or parametric estimation.

Clinical impact

The study detailed in this article focuses on recording neural activity in the pyramidal neuron and axon bundle of the hippocampus. Further experiments shall be able to show that multielectrode needle probes can also be used for measuring (extracellular) field potential (FP) and fiber volley in other neuron bundles; such an ensemble of signals can be analyzed and translated as intended actions or wanted sensory inputs. The evaluation of the titanium needle probe, through animal experiments, will be helpful in accessing the probe's capabilities in various medical applications that employ neuro-MEMS interfaces. These needle probes can be passive or active components that serve as sensors or actuators for technologies such as a neuromotor prosthesis or a prosthesis to replace or restore local neural functions. A fully tested multielectrode probe system has an additional application in neural interfacing for prosthetic limbs [14] to successfully emulate intended movement, controlling other external electronic gadgets for persons with disabilities [12,15], or even for multi-taskers, cochlear implants for the hearing impaired [16], and futuristic applications such as artificial retinas [17] and speech synthesis technology [18]. In the field of neurobiology,a study of neural conditions, such as Parkinson's disease [8,19], Alzheimer's disease [20],and Traumatic Brain Injury [21], involves capturing the spatio-temporal neural activities [13] and then using these multielectrode needle probes, via a sensor or actuator, as an investigative tool and therapeutic aid.

Future research and conclusions

The reported study is a work in progress. Demonstration of quality measurement of neural activity, with the help of vertically aligned nanowire array equipped electrodes on a titanium needle probe, is a precursor to specialized neuro-MEMS interface studies. A recording of neural signals through multiple electrodes should be attempted to analyze the probe's capability to detect activity of a neural ensemble for comprehensive monitoring of functionality. The results of these experiments should be compared with those obtained for commercially available electrodes such as those supplied by Plexon, Inc. (Dallas, Texas). The results of this study shall be instrumental in advancing needle probe applications in neuroprostheses and investigations of pathological neurophysiology.

Evaluation and instrumentation of nano-structured multielectrode array neural probes are important for the development of more advanced technology for the accurate measurement of neural activity. Since the multielectrode arrays are mounted on a sturdy titanium needle with a fine bore, they can be considered implantable devices. Implantable neural probes / electrode arrays that are interfaced with neuron bundles / clusters try to harness the signal strength of the human brain and correct problems internally; such electrodes have been used to treat Parkinson's disease through deep brain stimulation. Extensions of this implantable technology are expected to play important roles in the treatment or management of other neurological disorders. This progressive technology will not only make it possible to improve the lives of many through simple surgeries but will also make it possible to build upon this technology in order to create other life-altering cures.

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Mentor Comments: Professor Varadan describes the commitment of Lauren Kegley to her chosen research domain in the neurosciences.

Lauren is an undergraduate student in the field of Electrical Engineering; she has spent the past two years working on research projects under my supervision and continues to pursue her neuro-scientific research on acquiring pulse trains of neural activity from rat hippocampus. The motivation behind her work is her interest in engineering related medical technologies. It has led her to participate in and carry out research focused on implantable prostheses for monitoring bioelectronic neural activity. This publication showcases her work on a project in which she performed data acquisition, surgical procedure, and final analysis with the supervision and training of two Doctorial candidates, in addition to myself. This translational research shall help in advancement of neural prosthesis technology in the medical field. It will allow neurological disorders, like Parkinson's and Alzheimer's to be treated more effectively. Thus, the project that she has undertaken is a pioneering one. In addition to this publication, Lauren has received a Summer Undergraduate Research Fellowship (SURF) grant and recognition at the NSF Annual Conference for the best poster of an undergraduate researcher. Lauren is a very promising young researcher from whom great things can be expected. As her mentor, I truly believe that she is a very dedicated, hardworking, and inquisitive student.