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Testing the Efficiency of Vertically Aligned Gold Nanowires on a Titanium Needle Implantable Neural Electrodes in the Rattus Norvegicus Hippocampus

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Testing the Efficiency of Vertically Aligned Gold Nanowires on a Titanium
Needle Implantable Neural Electrodes in the Rattus Norvegicus Hippocampus

An Undergraduate Honors College Thesis
in the

Department of Electrical Engineering
College of Engineering
University of Arkansas
Fayetteville, AR

by
Lauren Elizabeth Kegley

April 23, 2012

ABSTRACT

Miniaturized multi-electrode 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 function. The micro-electrodes, studied here, were developed in-house. They have a vertically aligned gold nanowire array and are mounted on a sturdy fine gauge titanium needle. Hence, the bill of materials and design characteristics encourage its use as 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 it was filtered for isolated spikes of neural activity, which were sorted in form of a Spike Train-Raster Plot. The qualitative evaluation of data, obtained through the newly developed neural probe, was used as ground work to decide on future research and discuss possible clinical impacts.

APPROVAL

This thesis is approved.

Thesis Advisor:



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INTRODUCTION

SIGNIFICANCE OF RESEARCH

If you have ever seen the anguish on a family's face as they watch a loved one diminish as a result of Alzheimer's disease or the pain of an individual losing their independence to Parkinson's disease or a spinal cord injury, you understand the underlying foundation of this research. Neurological disorders and injuries affecting the central nervous system can have a serious impact on the patients and their support group. Not only will it be possible to improve the lives of many with simple surgeries, but it will be possible to build upon this project in order to create other life-altering cures. In an attempt to better understand the human brain for the benefit of modern science, neural probes have been created to try to harness the power of the human brain and correct its problems internally with the aid of an implantable neural electrode.

APPROACH

This study was a preliminary test of newly, in-house developed neural probes, which have a vertically aligned gold nanowire array on them and are mounted on a sturdy titanium fine gauge needle, in the *Rattus Norvegicus*' (Sprague Dawley laboratory rat) hippocampus. The study was conducted on live (intact) rats to evaluate the probe's efficiency of signal acquisition and the stereotactic accuracy of the implantation protocol. The hippocampus is the region of the brain that is crucial for formation of new memory. It acts like a gate for the passage of newly acquired memory – facts, skills, or habits – to permanent memory storage. The basic architecture

of the Hippocampus comprises of a layer of densely packed pyramidal neurons and well aligned axons originating from them [9]. Since the axons are the carriers of neural electric pulse, a strong electrical signal of defined polarity shall accompany any neural activity.

THESIS STATEMENT

The hippocampal region should provide a very good testing ground for the newly developed neural probe's measurement ability of bio-electric signals. Since the neural probe being tested in this experiment contains vertically aligned gold nanowires, there should be an increased ability to sense neural signals due to an increase in the amount of sensors and sensing surface area. In addition to the signal acquisition, the surgical procedure in this experiment should be able to place the probe in a desired region of the brain, which will be verified using Magnetic Resonance Imaging and Histopathology.

BACKGROUND

Contemporary medicine uses an Electroencephalogram (EEG) as a diagnostic tool for detection of abnormal neural functions that manifest as the aforementioned diseases. The EEG technique is based on the measurement of the electrical signal that travels through the central nervous system and facilitates communication. Apart from this, in vivo measurement of neural activity has been widely used among researchers for many years.

Since the experiments conducted by Luigi Galvani (1791) [1] for measuring bioelectric forces in living tissue, researchers of nerve physiology have been able to acquire and simulate (using the model proposed by Hodgkin and Huxley in 1952 [2]) electrical signals that travel through nerve axons. 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 (MEA) [5]. The MEA, which can be on a flat substrate or in bundles, allow for multi-site recording within the brain tissue at the 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 once considered to be a breakthrough in science that allowed doctors to more efficiently treat patients based on their capacity to interface with neurons and provide clinical applications for neural prosthetics. Commercially available neural probes are micro-wire arrays of flat electrodes mounted on a fine gauge wire/needle (230 μ m-500 μ m) 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 nano-engineered neural electrodes can be developed with 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]. In order to do this, vertically aligned nanowire arrays were grown on the electrodes ($<30\mu\text{m}$ dimension), to enhance the performance and functionality, and then the array of electrodes was fabricated on a titanium needle with a fine gauge ($280\times 100\mu\text{m}$). 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. These 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.

FABRICATION PROCESS

The titanium probes used in this study are designed with five separate electrodes down the face of the needle, as shown in Figure 1. The entire design of the probe, required significant research into biologically acceptable materials that would be suitable for fabrication and dimensions that would be non-intrusive or damaging to neural networks. The sizing of the probe was constructed to be 12 mm long to facilitate the probe reaching the hippocampus and olfactory nerves that would provide optimal sensing environments, while the needle is a mere 280 μm wide. [8]

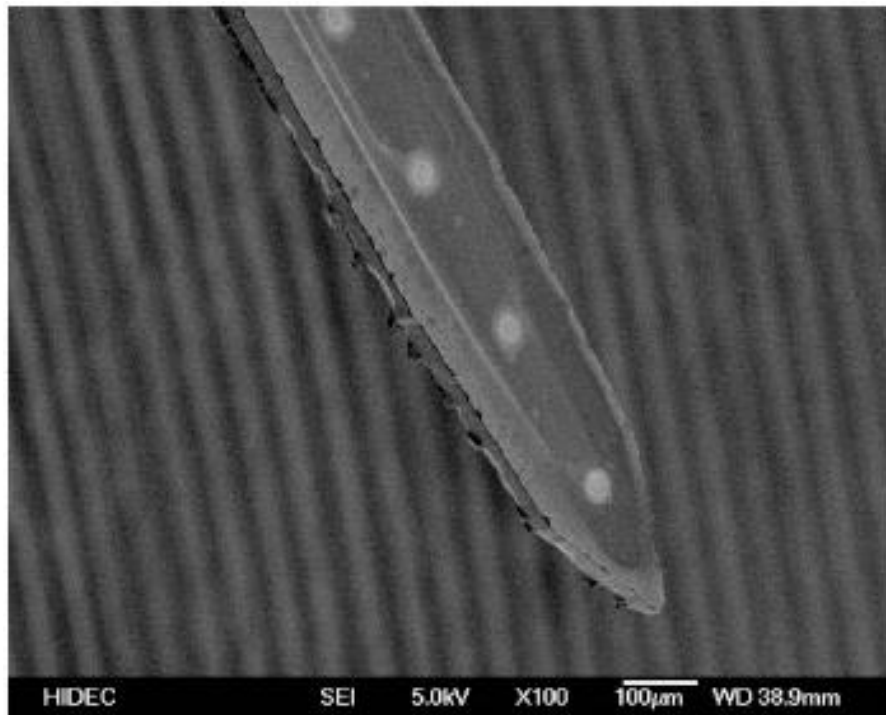


FIGURE 1: SCANNING ELECTRON MICROSCOPE IMAGE OF THE TITANIUM PROBE WITH THE GOLD ELECTRODES POST FABRICATION, SHOWING THE SCALE OF THE NEEDLE.

Fabrication processes combined traditional microfabrication processes with titanium specific processes, such as thin foil lamination and deep reactive ion etching (DRIE). The thin foil lamination process was necessary to provide support and flexibility to the titanium foils during fabrication. DRIE was employed to “release the needle structures from the titanium foil” [8]. The process required a series of different etchings including the use of CHF_3 and Cl_2/Ar gas etchings. In order to provide maximum electrode efficiency, a layer of iridium oxide was used to coat the gold surface via electrochemical deposition. Initial proof of concept testing, such as grass defect analysis of the DRIE process, MRI artifact measurement, and physical property characterization, were all completed on this neural probe by the developers of this unique probe. [8] Therefore, the project detailed in this manuscript intends to serve as a more

thorough proof of concept and the potential clinical viability of this neural probe. The final product is shown in Figure 2.

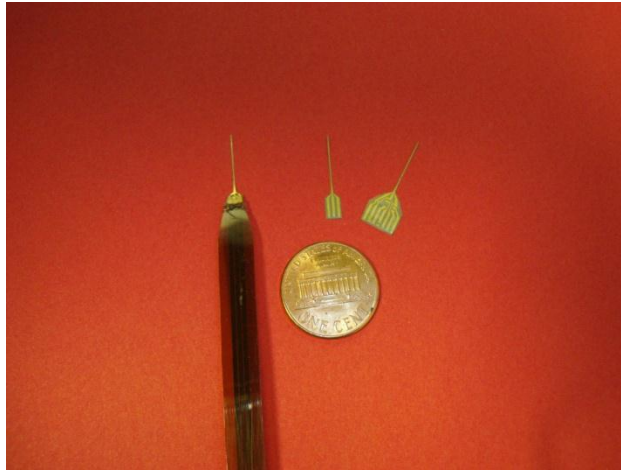


FIGURE 2: SIZE COMPARISON FOR NEURAL PROBE TO BE USED IN THIS EXPERIMENT.

SURGICAL SETUP

The animal experiment was conducted in an Institutional Animal Care and Use Committee (IACUC) approved laboratory, which is situated at 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, such as analog spikes of neural activity or a raster plot of the spike train, depending on the necessary analysis.

The surgical workstation, shown in Figure 3, includes the animal experiment and multichannel feed to the amplifier. The animal experiment setup has a thermal mat to help maintain the subject's body temperature and a stereotactic frame (Korp Instruments) that consists of a head holder / brace to help immobilize the subject, calipers to help measure the brain coordinates, and a holder for the neural probes as deemed necessary by the 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.

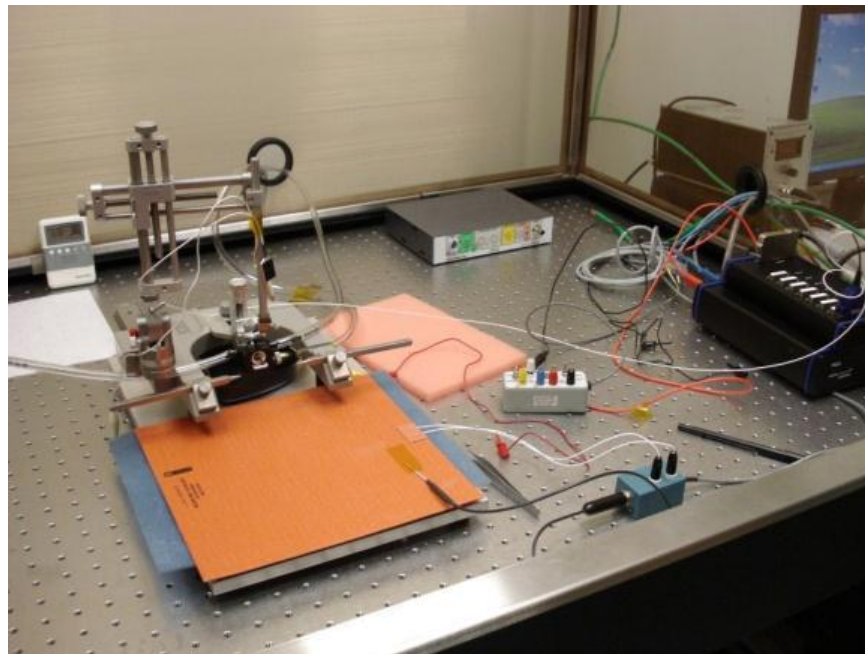


FIGURE 3: SURGICAL WORKSTATION MOUNTED ON AN OPTICAL BENCH, INSIDE A FARADAY CAGE AND EQUIPPED WITH A STEREOTACTIC FRAME.

Once the animal experiments were completed, the collected data was taken to the Brainwave Laboratory, at the Engineering Research Center (ENRC) of the University of Arkansas, for analysis. This laboratory has a variety of different tools to use in data analysis, which shall be discussed in the later section. Through the use of these tools, the collected data

can be interpreted and conclusions can be 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.

SURGICAL METHODOLOGY

The experiment with IACUC approved protocol #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 the 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 used to test the acute neural recording in the hippocampus and to test the instrumentation of the nanowire probe.

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 the 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 administering the anesthesia, no other medication was given to the subject. The neural activity was recorded from the rat in sedated state and at all times the animal subject's vital signs were monitored.



FIGURE 4: (A) ANIMAL SUBJECT ON THE SURGICAL WORK STATION WITH THE NEURAL PROBE, INDICATED BY AN ARROW, MOUNTED ON A STEROTACTIC FRAME. (B) / (C) LAUREN KEGLEY AND PHILLIP HANKINS CALIBRATING THE DATA ACQUISITION SYSTEM TO PREPARE FOR A LIVE ANIMAL EXPERIMENT.

The tools and probes were sterilized with a diluted betadine solution to ensure aseptic techniques were implemented; all the antiseptic agents used in this experiment were approved.

Prior to sterilization, all the equipment discussed in the experimental set-up was checked for appropriate functioning. Once the animal was completely sedated, electric shears were used to remove hair on rat's scalp between the eyes and ears, carefully avoiding the eyes, whiskers, and ears of the rat. The rat was then placed in prone position on the thermal mat and the head was secured with the help of the head holder and a brace. Eyes were covered and the body was blanketed with sterilized towels, in order to expose only the incision area. Before making an incision the shaved area was cleaned with betadine swabs to further sterilize the area. Next, an incision, big enough to access the quadrant of interest, was made. Post incision the sub-dermal layer of blood vessels and tissue was scrapped off with hydrogen peroxide swabs, so that the bregma and lambda on the surface of the skull were visible to serve as medial and base line references.

The position (x, y, and z coordinates) of bregma and lambda was recorded with the help of the calipers on 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, subject's eyes were kept moist with help of phosphate buffer solution (PBS) swabs and the subject's vital signs, especially breathing, were monitored

via observation. Though sufficiently anesthetized, the rat was also observed for any stress responses (twitching, tremors, squealing).

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; in one successful experiment, the coordinates were 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 speakers. The acquired data bank was then processed as a pulse train, with $-160\mu\text{V}$ as threshold, to extract raster plots.

Euthanasia was carried out at the end of surgical procedure, while the subject was still under anesthesia, by CO_2 asphyxiation until clinical death was determined (no perceivable signs of respiration). Upon completion of the animal experiment, all the surgical materials were disposed off or sanitized according to aseptic technique. The data acquired was filtered and early analysis was completed in the experimentation lab; however, extensive analysis was carried out in the brainwave laboratory.

RESULTS

IMPEDANCE TESTING

In order to select the most viable probe to use for successful measurement in the live animal experiments, an impedance test setup was constructed in the Engineering Research Center Innovative Nano- and Bio- Devices and Systems Laboratory Class 100 Clean Room. The setup used a Zahner IM6ex Electrochemical Workstation connected to an individual probe pins on the neural probe being tested, which was submerged in PBS solution. The Zahner IM6ex uses Thales software to run an impedance scan, which can be analyzed in many different formats.



FIGURE 5: IMPEDANCE TESTING SETUP IN THE INNOVATIVE NANO- AND BIO- DEVICES LABORATORY CLASS 100 CLEAN ROOM.

For the purposes of this study, the impedance data was analyzed through the use of impedance scan plots. The plots show both the impedance and phase of the selected pin throughout a frequency sweep from 0 Hz to 100 KHz. From these plots, the probe with the most efficient pins can be determined. Analysis of the response to electric potentials of each pin at different magnitudes and frequency became the way to determine the most viable probe to use during animal experiments. Figure 6 demonstrates both an example of a good and bad impedance scan plot, (A) and (B) respectively. A good plot maintain is very close to linear and will allow for successful frequency measurements due to its smooth characteristic curve, whereas a bad plot is highly nonlinear with erratic data points, resulting in poor measurements.

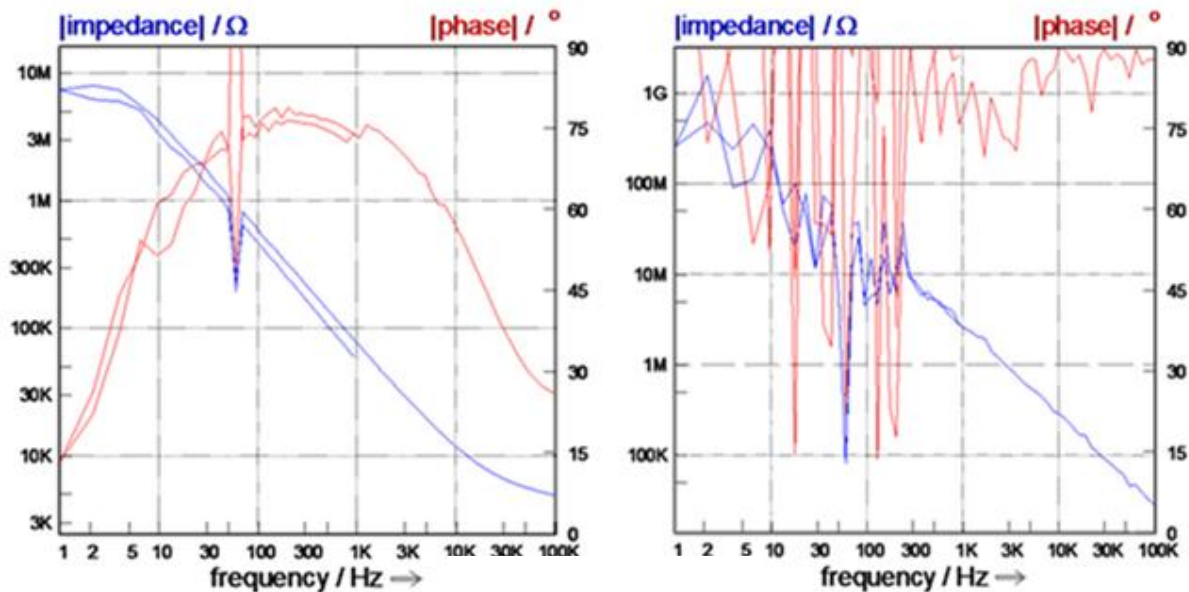


FIGURE 6: (A) EXAMPLE OF IMPEDANCE SCAN FOR A GOOD PIN (B) EXAMPLE OF IMPEDANCE SCAN FOR A BAD PIN

Since each pin on the probe is tested individually, the researchers can determine the best channels to record and analyze data from. The innovative fabrication of the probes previously

discussed is a very low yield process. Thus, it is necessary to run this type of testing to determine the most efficient and successful probes and pins to use to increase the sensing capabilities during the experiment.

LIVE ANIMAL EXPERIMENTS

The database acquired from the animal experiment was filtered and re-plotted for further analysis. Signal processing and analysis facilitates the form of data interpretation, which depends on the clinical relevancy of the information. The spikes were sorted and stacked together to better understand the average peak neural activity in one of the cornu ammonis (CA1) regions and the horns of the hippocampus, see Figure 7 (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 activity of discernible quality, which can be seen in Figure 7 (a) and (b); the spikes were sorted based on a pre-specified threshold of $-160 \mu\text{V}$ and the resultant spike train is represented as a raster plot. The amplitude-time window, Figure 7 (a), shows well isolated potential peaks with a mean amplitude of $-180 \pm 10 \mu\text{V}$. The corresponding spike train (raster plot), along the timeline, can be interpreted based on the firing rate of the neurons or the statistical analysis of these spikes/epochs surrounding a documented event (for example a motor or sensory response to external stimuli). [12]

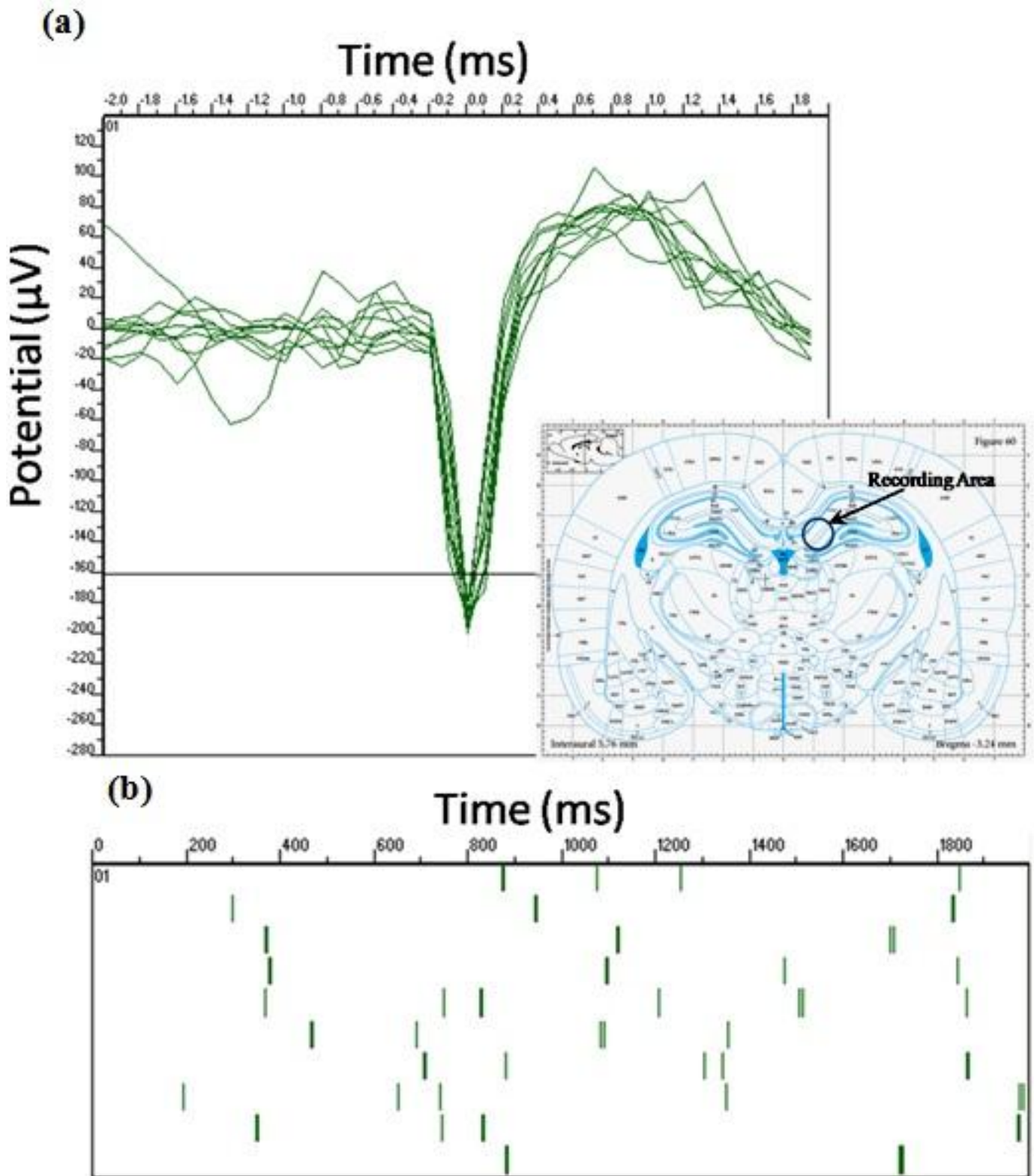


FIGURE 7: DEPECTION OF NEURAL ACTIVITY FOR THE GIVEN RECORDING AREA, PRESENTED IN DIFFERENT FORMS

The spike train from the neuronal ensemble (bundles) can provide control signals for limb movement, represent sensory inputs, or can be translated as a highly evolved cognitive signal. [12] The spike train can be represented in the form of spike raster plots, as in Figure 7 (b), that provide information like 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 variable or parametric estimation.

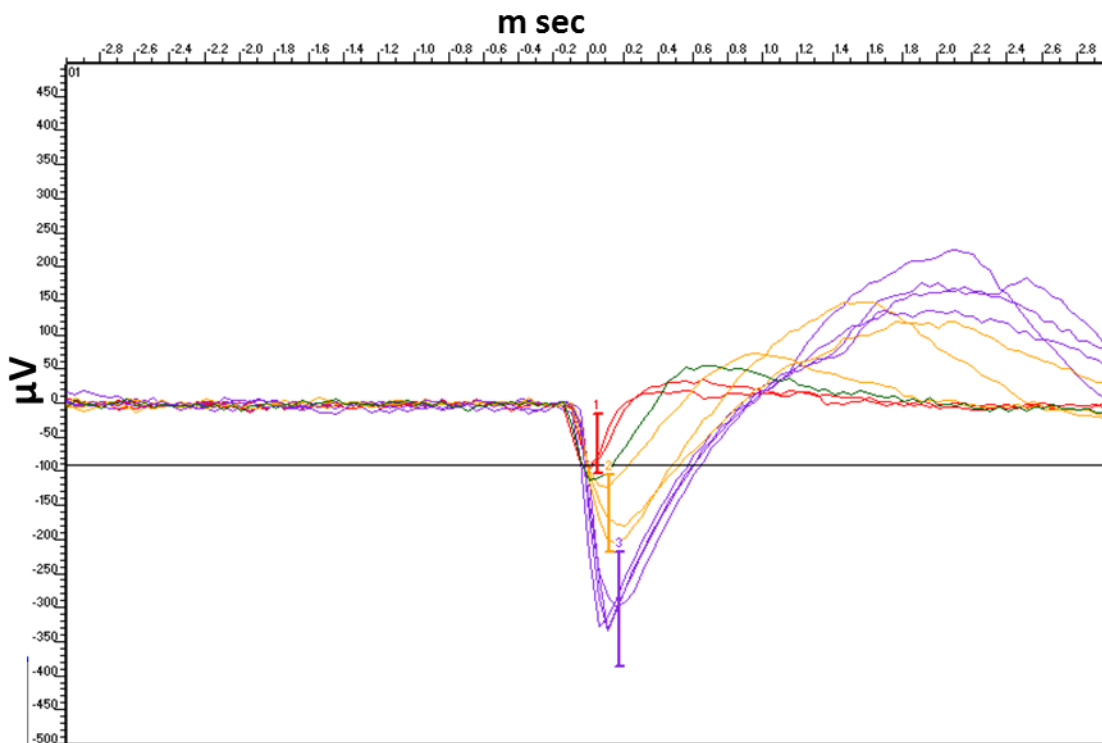


FIGURE: 8 NEURON SPIKES RECORDED ON CHANNEL 1 OF NEURAL PROBE.

Figure 8 shows spikes observed on channel one of the neural probe, these spikes were recorded above a threshold of 100 mv negative polarity. This particular recording occurred at 2.4 mm depth from the top of the brain, Bregma -3.8, and Z of 2.4, which places the needle in the desired CA1 region of the hippocampus; for reference, refer to Figure 9 for the brain atlas figure corresponding to the given coordinates.

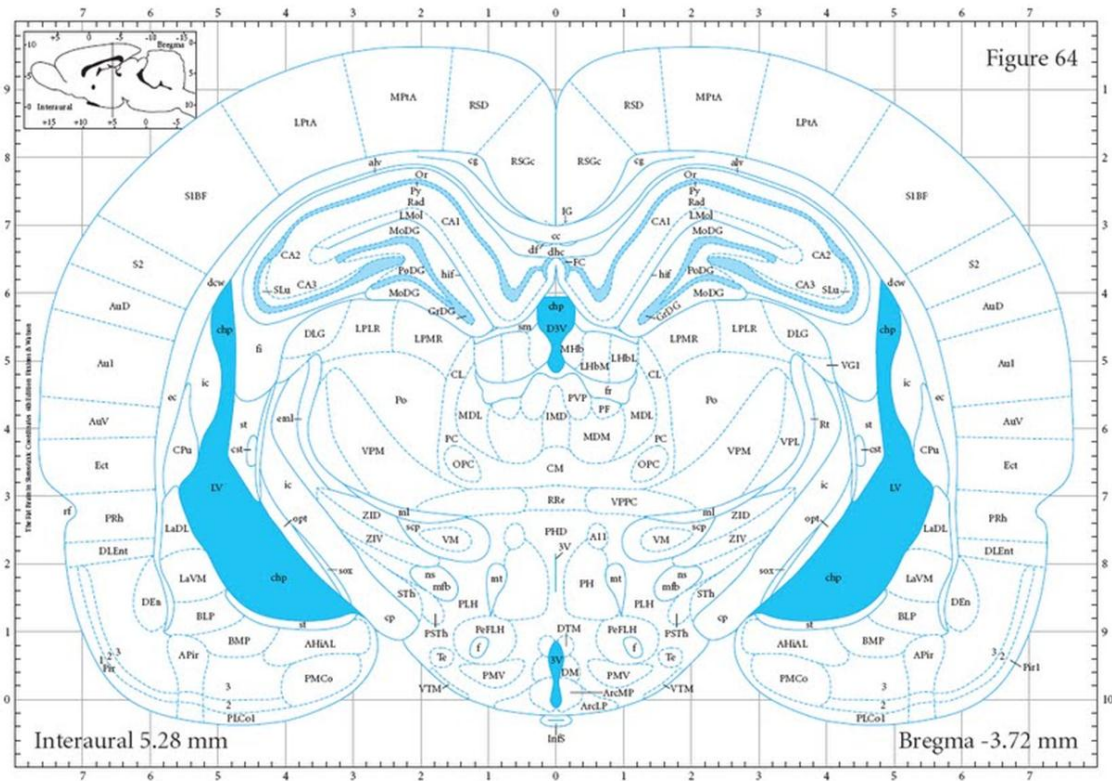


FIGURE 9: RAT BRAIN ATLAS IMAGE SHOWING THE RECORDING DEPTH FOR THE DATA IN FIGURE 7.

RELIABILITY TESTING

MAGNETIC RESONANCE IMAGING

In conjunction with the University of Arkansas for Medical Sciences (UAMS) Department of Radiology's Professor and Director of Research, Dr. Michael J. Borrelli, Ph.D., and his Imaging Specialist, Ms. Terri Alpe, a series of Magnetic Resonance Imaging

experiments were conducted on subjects that underwent live animal experiments. These tests were used to determine the accuracy of the stereo-tactical placement of the neural probe during surgical experimentation. As previously noted, the placement of needle within the brain will largely determine the sensing capabilities and amount of signals that the probe is exposed to during the procedure.

The animal subjects were transported per IACUC approved protocols to the UAMS Radiology Department office in Little Rock, AR. To ensure accurate detection in the imaging, the neural probe was left intact for the duration of the travel and the imaging process. Once the live animal experiment was concluded and the subject was euthanized, the neural probe needle was severed from the rest of the needle directly above the top of the exposed skull. In order to protect the head of the subject, a specially constructed protective box was placed around the head to ensure that no damage occurred during transport and the subject was placed in a chilled box to prevent decay prior to further testing. The magnet being used for the imaging is a Bruker PharmScan 7T, which is designed to image small laboratory animals with high accuracy.

The first attempt at the probe imaging was unsuccessful. No matter the type of scan that as run, the magnet left a blur around the layer with the probe insertion. This yielded no acceptable results as to the probe placement from the first imaging attempt. To remedy this problem, Dr. Borelli and Ms. Alpe worked to create phantoms for use in the imaging system that

would utilize the neural probe and be able to yield useful images. Although this initial imaging did not provide the desired images, the initial imaging procedure and the procedures using the phantoms gave insight into the settings and type of scans to be used during the next attempt.

The second attempt at the probe imaging was very successful. The sagittal image shown in Figure 10 is done using a turboT2 image setup with TE = 36.2 ms, TR = 5319.4 ms, FA = 180, FOV = 3x3 cm, slice thickness = 0.5 mm, with averages = 5.



FIGURE 10: SAGITAL IMAGE INDICATING THE PROBE TRACT.

The first coronal image shown in Figure 11 is also a turboT2 scan with slightly different settings: TE=35.3 ms, TR=3561 ms, while the other settings remained constant from the previous T2 scan.

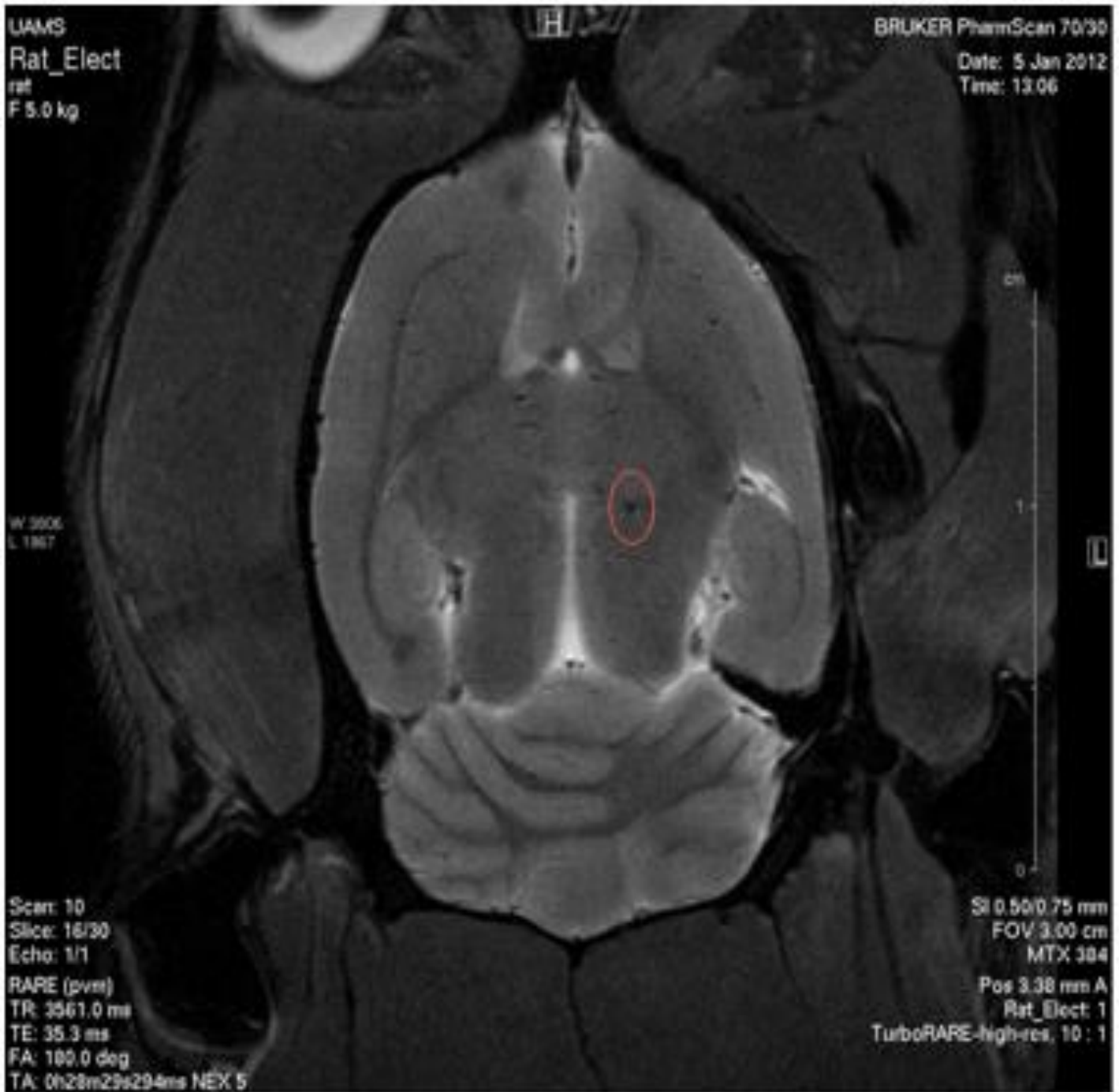


FIGURE 11: CORONAL IMAGE AT SLICE THICKNESS, USING 3 CM X 3 CM SLICE THICKNESS, SHOWING THE PROBE TRACT

The second coronal image shown in Figure 12 is another turboRARE T2 scan. However, TE = 33 ms, TR = 2500 ms, FOV = 4x4 cm, slice thickness = 1 mm, and averages =1; only FA remained the same at 180 in this scan.

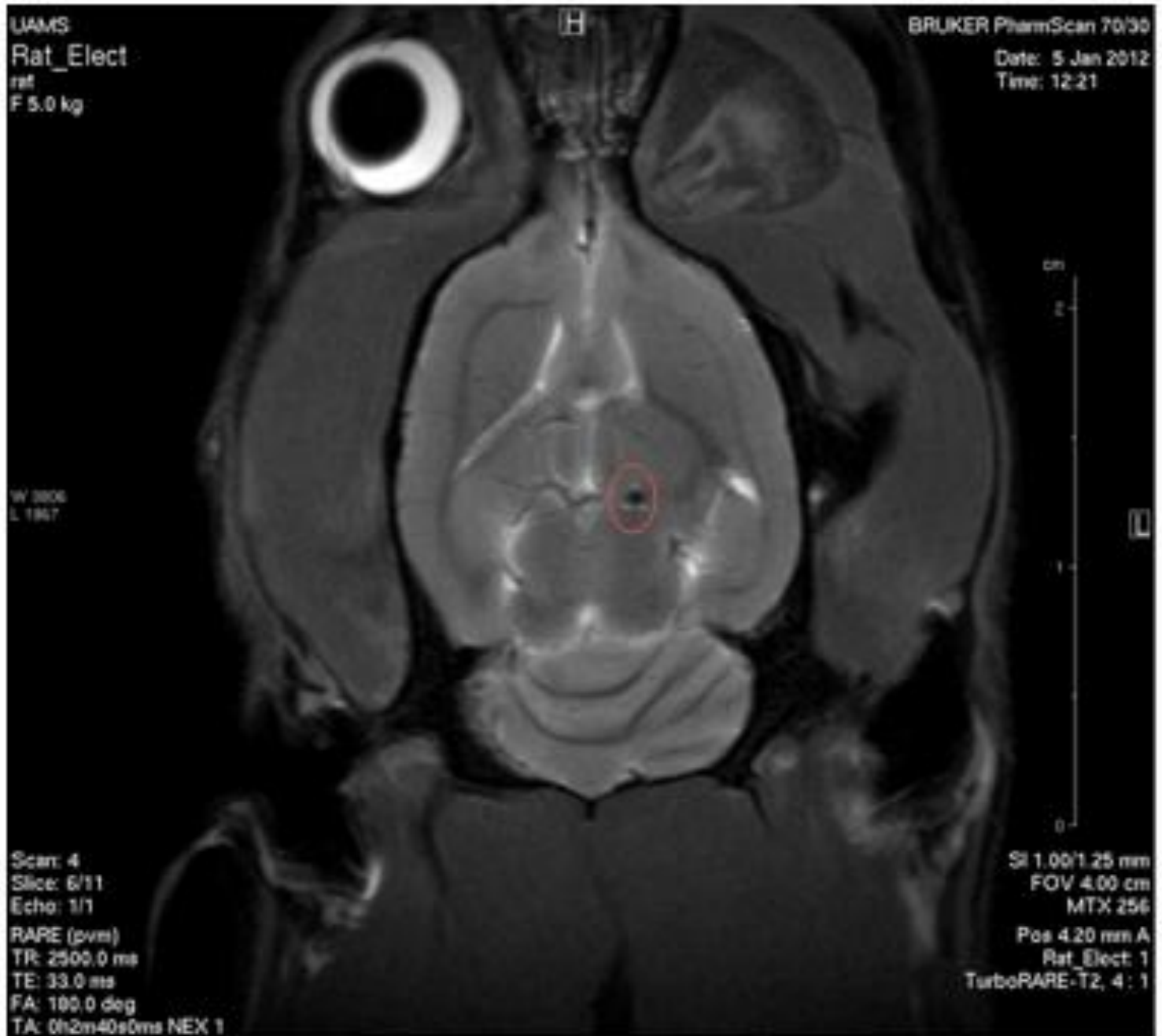


FIGURE 12: CORONAL IMAGE AT SLICE THICKNESS, USING 4 CM X 4 CM SLICE THICKNESS, SHOWING THE PROBE TRACT

In these images, it was confirmed that the desired hippocampal region was obtained during the live experiment. Therefore, this proves that careful extrapolation from the rat brain atlas and diligent experimental procedure can lead to neural probes reaching the desired brain location. This helps to validate the surgical procedure used in this experiment for future experimentation.

HISTOPATHOLOGY

Immediately following the Magnetic Resonance Imaging, the subject was taken to Dr. Leah Hennings, Assistant Professor and Director of the Experimental Pathology Core Laboratory at UAMS. Histopathology was used to determine how intrusive the neural probe needle and nanowires were during the animal experiment. It was also another way to verify the placement of the neural probe. To be considered for clinical use, it is necessary to ensure that minimal damage occurs during the experiment on account of the probe.

Histopathology supported that the hippocampal region was obtained during the experimentation. Figure 13 shows the entry point of the probe and its tract in the hippocampus; it also shows the entry point in more detail. Comparing the MRI location images with the histopathology entry point allows the opportunity to view the entry in two different contexts. This is important based on the specific clinical application in which the probe will be used.

Assessing the relationship between the entry point and the surrounding areas of the brain can help to determine what applications this probe can be used in. Also, it is feasible to calculate the dimensions of a probe to fit applications that the current experimental probe is unsuitable for, i.e. deeper brain recording or stimulation. This could also be used to help determine potential advantages or disadvantages of redesigning the current experimental probe.

This image is crucial because it not only proves that the probe was in the peripheral of the hippocampus, but that it does not leave a large footprint in its wake. In spatial terms, this shows that the probe does not intrude largely into the brain.

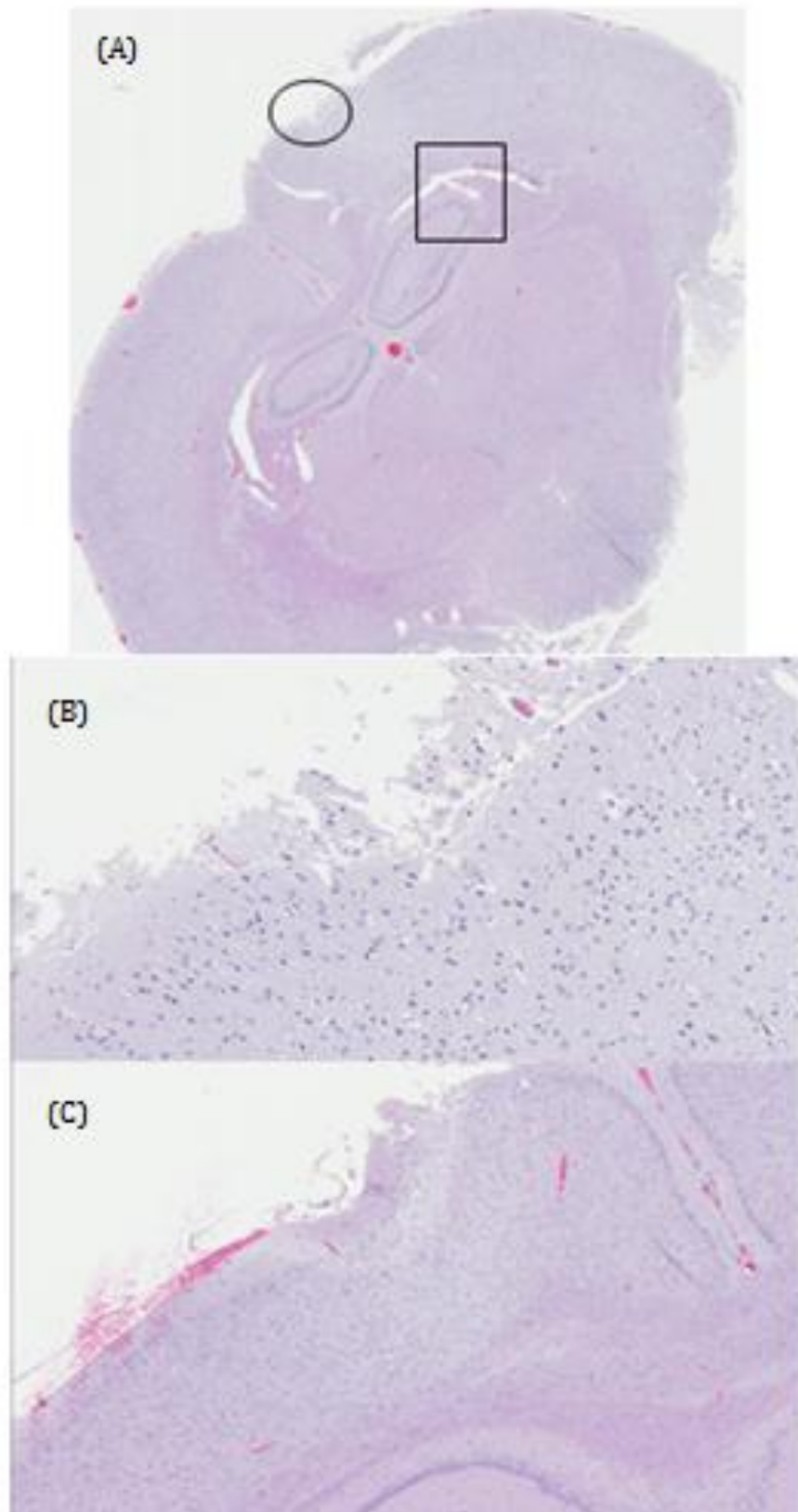


FIGURE 13: (A) BRAIN ENTRY SHOWN IN THE CIRCLE AND THE PROBE'S PATH THROUGH THE HIPPOCAMPUS SHOWNN IN THE RECTANGLE. (B) / (C) SHOW THE PROBE ENTRY AT 100 AND 30 TIME MAGNIFIACION, RESPECTIVELY.

To determine the probes effect on the physiology of the brain, inspection of the ventricles and surrounding cells is key. Figure 14 shows the probe tract with hemorrhage and the opposing, contralateral, brain for comparison in part B. The figure shows slight hemorrhage, visible in red, but the hippocampal neurons were determined to be minimally damaged.

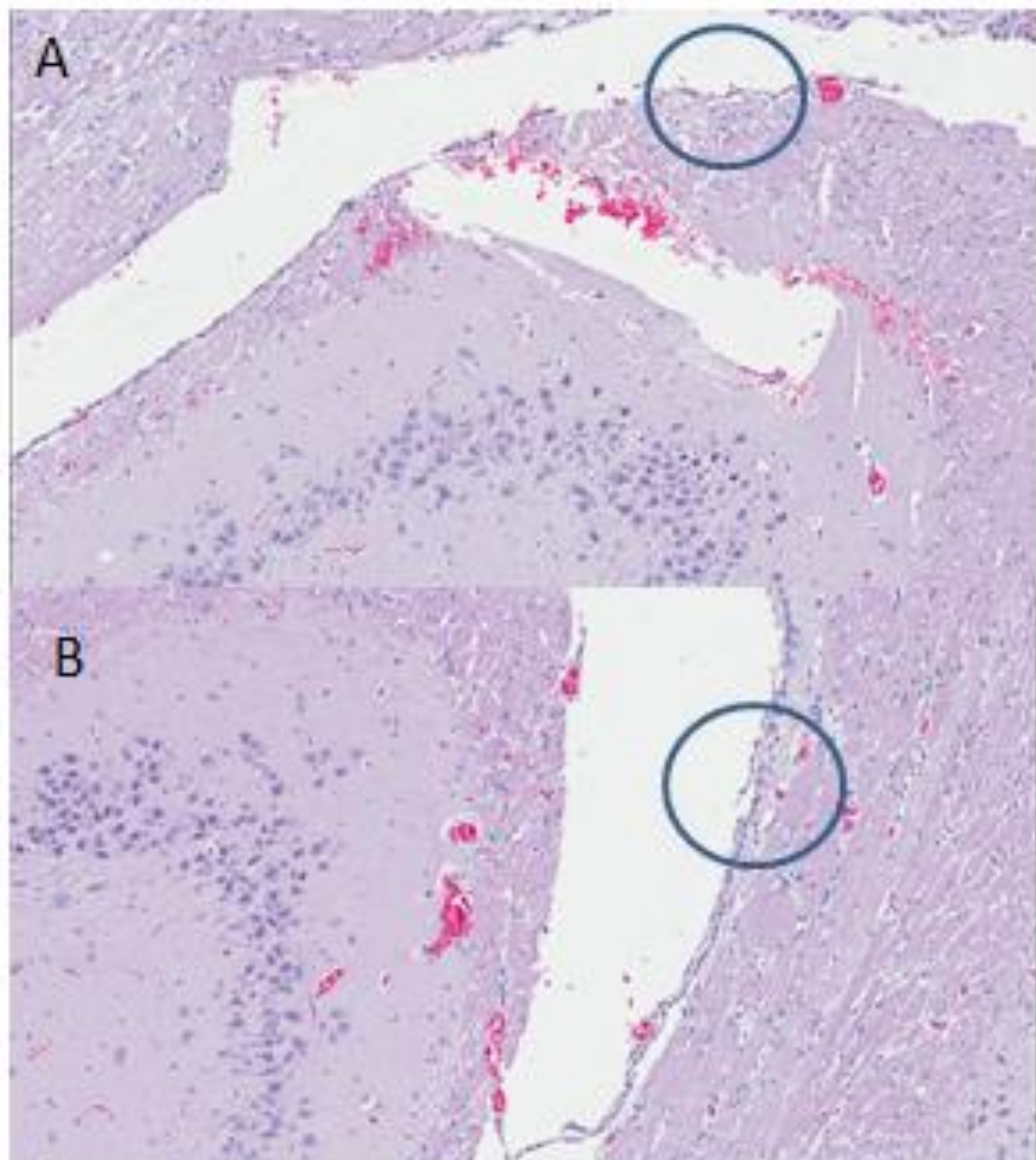


FIGURE 14: HEMORRHAGE ALONG THE PROBE TRACT IN (A) AND THE CONTRALATERAL BRAIN (B) WITH RESPECT TO THE VENTRICLES, INDICATED BY THE CIRCLES.

The area surrounding the probe tract was determined to show perivascular edema with scattered cell necrosis, as shown in part A of Figure 15. The perivascular edema is indicated by the widening of the Virchow-Ribbons spaces; since the spaces did not widen dramatically, the perivascular edema is deemed mild. The pyknotic nuclei of the scattered cells show the cell necrosis surrounding the probe tract. The contralateral brain is shown for comparison in part B of Figure 15.

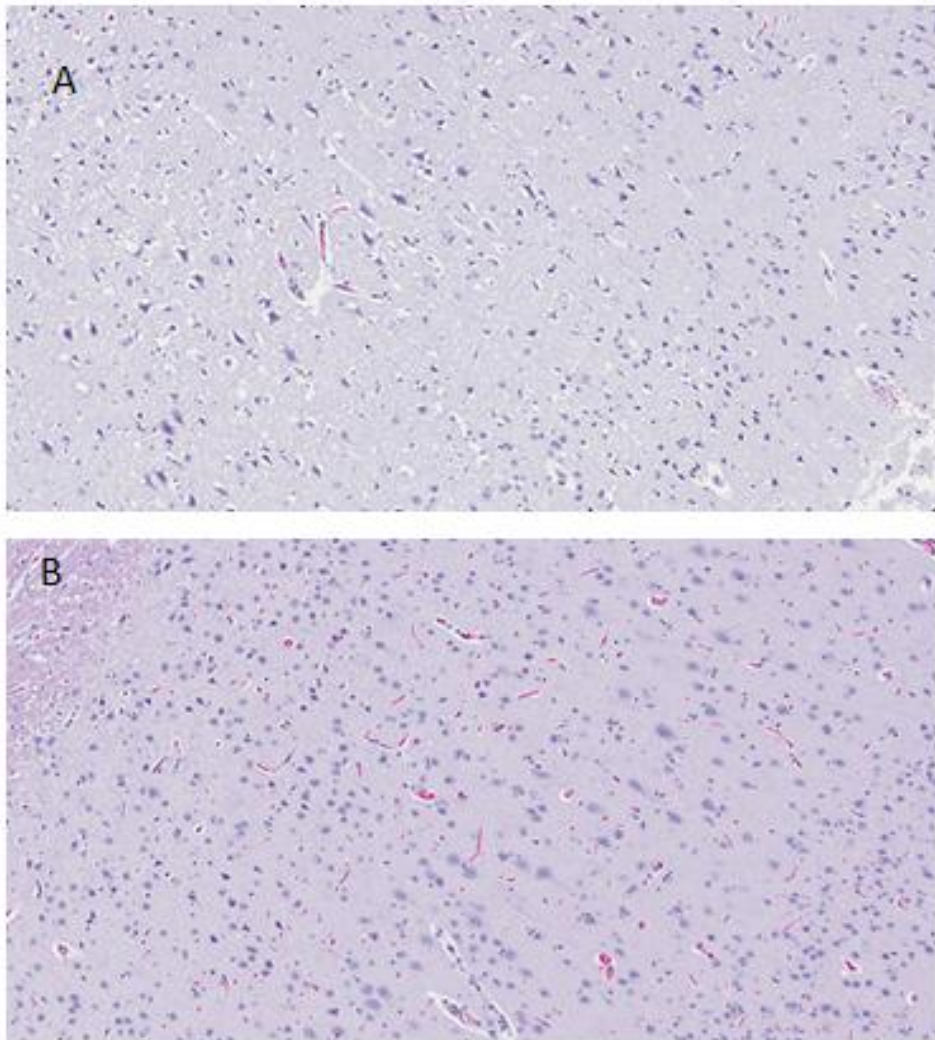


FIGURE 15: AREA SURROUNDING THE PROBE (A) AND THE CONTRALATERAL BRAIN (B), RESPECTIVELY.

Overall, the damage caused by the probe entry caused mild hemorrhage, which is expected mechanical damage for the experimental procedure. The other results of significance are the mild perivascular edema and scattered cell necrosis. However, none of these items were found to be widespread.

CONCLUSIONS

The reported study is one part of a larger 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 the activity of a neural ensemble for comprehensive functionality monitoring. The results from these experiments should be compared with commercially available electrodes, such as those supplied by Plexon Inc. (Dallas, Texas). The results from this study shall be instrumental in advancing needle probe applications in neuro-prosthesis and investigation of pathological neurophysiology.

The impedance testing of the neural probes, prove that though the current fabrication process is inconsistent in yield, there are indeed viable probes created that will allow for efficient in vivo experiments. The live animal experiments, magnetic resonance imaging, and histopathology findings all yield positive results for the predictability of placement with careful

experimental procedure and minimal intrusion of the experimental neural probe. When compared to findings from previous studies, which utilized commercially available probes, the experimental titanium neural probe can be concluded to be comparable in neural signal recording and minimal footprint.

Evaluation and instrumentation of nano-structured multi-electrode array neural probes is important for development of more advanced technology for the accurate measurement of neural activity. Since the multi-electrode arrays are mounted on 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 human brain and correct the problems internally; such electrodes have been shown 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 or handicaps. This progressive technology will not only make it possible to improve the lives of many with simple surgeries, but will also make it possible to build upon this technology in order to create other life-altering cures.

FUTURE WORK

The study detailed in this manuscript focuses on recording neural activity in the pyramidal neuron and axon bundle of the hippocampus. Further experiments shall be able to show that multi-electrode 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 like neuromotor prosthesis or prosthesis to replace or restore local neural functions. A fully tested multi-electrode probe system has additional application in neural interfacing for prosthetic limbs [14] for successful emulation of intended movement, controlling other external electronic gadgets for disabled [12,15], cochlear implants for the hearing impaired [16], and futuristic applications like artificial retinas [17] and speech synthesis [18]. In the field of neuro-biology, a study on 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 multi-electrode needle probes, via sensor or actuator, as an investigative tool and therapeutic aid.

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