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Design of a Flexible Parylene-based Multi-electrode Array for Multi-region Recording from the Rat Hippocampus

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Abstract — The hippocampus is a critical deep brain structure in several aspects. It is directly related to the formation of new long-term declarative memory. The malfunction of the hippocampus closely relates to various disease and pathological conditions. It is also a model structure for the study of cortical function and synaptic plasticity in general because of its special neuro-anatomical structure and intrinsic connections within the hippocampus formation. Both the understanding of roles that the hippocampus plays in recognition memory and the study of neural plasticity require simultaneously recording of neural activities from multiple sub-regions of the hippocampus from behavioral animals. However the distribution of cells in the hippocampus make the recording from multiple sub-regions a big challenge with the traditional uni-length micro-wire arrays. Well-designed electrode arrays are required to reach multiple regions simultaneously because of the distinctive double C shape of the hippocampus cell body layers. In this work, we designed a multi-shanks electrode which uses Parylene C, a highly biocompatible and flexible polymer, as a base and has multiple recording sites specially positioned along the longitudinal axis to fit the curvy shape of the rat hippocampus.

I. Introduction

The hippocampus and the surrounding anatomically associated cortical areas together compose the hippocampus formation which have been widely studied for the past few decades. Through study of its anatomical characteristics, researchers noticed the unique distribution of cells in the hippocampus. It was quite different from that found anywhere else in the cortex [1]. The majority of cells in the hippocampus are packed densely into a thin layer which curve into a double C structure. Based on the cytoarchitectural distinction of principle cells in the hippocampus, it was divided into three subdivisions as the dentate gyrus (DG), the CA3 and the CA1. Intrinsic connections between hippocampal sub-regions and the entorhinal cortex (EC) form a closed feedback loop [2]. It is widely believed that sensory inputs from cortical regions are propagated through the excitatory connections within the hippocampus and are processed by hippocampal sub-regions into output signals for long-term storage in the cortex [3].
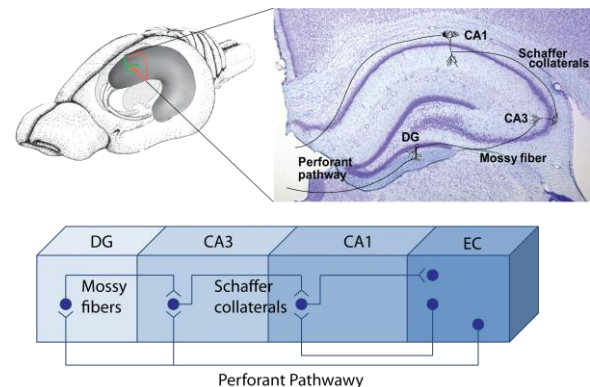

Figure 1. Diagrammatic representation of the rat brain and the relative location of the hippocampal formation on the left side of the brain. Pictured on the top right is a transverse slice of the hippocampus stained with crystal violet to show the cell body layers and hippocampal sub-regions. Intrinsic connections are illustrated with lines superimposed on the histology and are shown in the bottom diagram.

Lesion studies in the rat have indicated that damage that occurs along the longitudinal axis of the hippocampus affects the process of spatial and non-spatial information differently [4]. Neurotoxic lesions made in different sub-regions of the dorsal hippocampus of rats resulted in different impairment in detecting spatial novelty [5]. Based on neural activities recorded from ensembles of CA3 and CA1 neurons from rats, researchers were able to build a mathematical model which can simulate the function of the hippocampus [6] [7]. These studies indicate that simultaneous recordings of neural activities from multiple hippocampal sub-regions not only help the understanding of the mechanisms underlying cognitive function related to the hippocampus but also the development of computational models to mimic the function of the hippocampus. This also benefits the study of how information is processed in the hippocampus. Although micro-wire arrays have been widely used for *in vivo* recordings from the hippocampus, micro-wires with complicated arrangements are required in order to reach multiple sub-regions [8]. Parylene C is a micro-machinable and USP class VI biocompatible polymer commonly used in FDA-approved implants. Compared with other commonly used materials such as metal wires and silicon, the high flexibility and mechanical strength of the Parylene C make it

an ideal structural material for cortical recording electrodes. Parylene devices can be easily fabricated and the placement of recording sites can be easily manipulated using conventional micromachining techniques [9]. These advantages make the use of Parylene C probes for chronic recordings from behavioral animals have great potential.

II. MATERIALS AND METHODS

A rat brain atlas, histology results together with *in vivo* recordings were used to decide the layout of Parylene shanks and the placement of recording sites.

A. Brain Atlas and Brain Slices

The parameter for electrodes implantation was initially decided based on the brain atlas [10]. The most distal edges of the hippocampus and depth differences between the CA1 and the CA3, the CA1 and the DG cell body layers at locations between 2.45 - 4.00 mm posterior to the Bregma were measured. One rat was deeply anesthetized with an injection of ketamine and xylazine mixture and perfused with 10% formalin (PFA). Its dissected brain was fixed with 10% PFA overnight and dehydrated with 18% sucrose solution for one day. 50 µm coronal sections were sliced and stained with cresyl violet to identify the cell body layers (Figure 2).
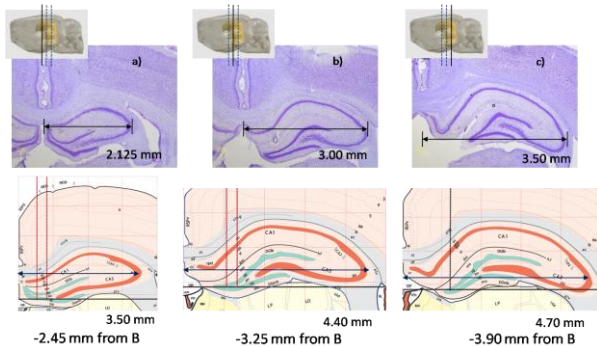

Figure 2. Comparison of histology results (top) with values obtained from the brain atlas (bottom). Histology results were coronal sections obtained from a) 2.45, b) 3.25 mm and c) 3.90 mm from the Bregma.

B. Surgery

With the information from the brain atlas reference and histology results, we further tested and modified the parameter of recording site placement with neural activities observed during surgery and *in vivo* recording from rats chronically implanted with micro-wire electrodes. Both uni-length micro-wire arrays and specially designed multi-length electrode arrays were used. All animal preparation and animal care were in accordance with the National Institute of Health and Institute of Animal Care and Use Committee guidelines and the Department of Animal Resources at the University of Southern California.

Male Sprague-Dawley (SD) rats greater than three months and with weights between 350 to 450g were housed in 12/12hr light and dark cycle, all surgery were conducted during the light cycle. All animals were given *ad libitum* access to food and water. Rats were pre-anesthetized with an intraperitoneal (IP) injection of a ketamine and xylazine mixture. Animals were anesthetized intra-operatively with a maintenance dose of a mixture of isoflurane and oxygen. Toe-pinch withdrawal reflexes were evaluated frequently to ensure proper dosing. Animal body temperatures were maintained with a warm pad. Surgeries were performed on a stereotactic frame. Each animal was fixed on the frame by ear bars holding its skull in place. A 2x4 mm bone window was drilled out of the cranium above the dorsal hippocampus and the dura was carefully removed. Three small holes were also drilled for anchor screws. One screw contacted the cerebrospinal fluid to serve as a ground for the electrode. The cranium was sealed with bone wax and dental cement after the electrode reached desired locations.

The detection of complex spikes (CS), a group of action potentials fired with less than 5 msec inter-spike intervals (shown in Figure 4), was used as the indicator that the micro-wire was approaching the CA1 and the CA3 cell body layers. The observation of CS in the depth between 2.20 - 3.20 mm from the skull was considered as the recording from the CA1 cell body layer and the detection of CS in the deeper region (3.60 - 4.40 mm from the skull) was considered as the recording from the CA3 cell body layer.

Two types of surgery were performed on three animals. One animal was sacrificed immediately after the implantation of a 3x5 uni-length micro-wire array to verify that the CS we observed during implantation were generated by cells located in the CA1 and CA3 cell body layers, respectively. For this non-survival experiment, a brief DC current of 300 µA was applied to the micro-wires right after implantation to make electrical lesions at the tip. The animal was then perfused with 10% PFA and 3% Kfe (Potassium Ferrocyanide into 10% PFA). Brain slices were evaluated to verify tip locations. Micro-wire arrays were also implanted chronically into two animals. One animal was chronically implanted with a 3x5 uni-length micro-wire array and another animal was chronically implanted with a special designed multi-length electrode array (as shown in Figure 5a) to measure the depth difference between the CA1 and the CA3 regions. For chronically implanted animals, scalp wounds were sutured and bacitracin zinc ointment was applied to the cut to prevent infection. Bupropion (BUP) was injected to relief pain. *In vivo* recording were taken after the animal fully recovered. After long-term recording completed animals were sacrificed as described above.

C. Data Acquisition

Neural activities were monitored during the operation of both the non-survival surgery and chronically implantations. During the surgery, signals were differentially amplified 1000 times and band pass filtered from 300 to 3000 Hz to check for action potentials. Filtered neural activities were displayed with an oscilloscope and played through a speaker. *In vivo* data was recorded with an on-line data acquisition system (OmniPlex, Plexon, Inc) and digitized at 40 kHz for storage.

Spike sorting was done by performing a principle component analysis (PCA) to wave forms with an off-line sorting system (Offline Sorter, Plexon, Inc).

D. Parylene Probe Fabrication

The fabrication steps of a single-metal-layer Parylene C based electrode array is illustrated in Figure 3. A layer of 10 μm Parylene C was vapor-deposited on the silicon wafer in a PDS2010 system. Platinum electrode recording sites, traces and contact pads were lithographically patterned on top of the base layer using e-beam deposition at a thickness around 2000 Å. The metal layer was then encapsulated by another layer of 10 μm thick Parylene C. Electrodes and contact pad were subsequently exposed by deep reactive iron etching (DRIE) with an oxygen plasma and the probes was cut out from the substrate. The device was released from the silicon carrier wafer in a water bath.
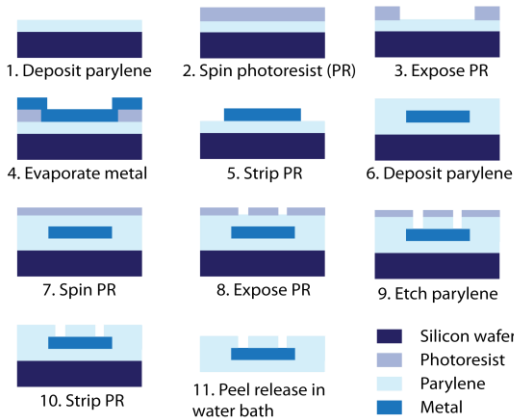

Figure 3. Fabrication processes of the Parylene C based micro-electrode array with a single layer of metal.

III. RESULTS

A. Depth Difference Measurement

The measurement of distances between the CA1 and the CA3 cell body layers in different regions along the anterior-posterior (AP) axis from both the brain atlas and brain slices are listed in Table I. It is obvious that the depth differences are not uniform along the AP axis. In addition, the differences measured from the brain atlas matches that from the histology results. On average, the CA3 cell body layer is about 1.00 to 1.30 mm more ventral than the cell body layer of the CA1 region.

TABLE I. HIPPOCAMPUS MEASURE FROM BRAIN ATLAS AND BRAIN SLICES

AP (mm)	Brain Atlas (mm)		Brain Slices (mm)	
	ML	CA1-CA3	ML	CA1-CA3
-2.45	3.50	1.10	2.25	1.00
-3.25	4.40	1.30	3.00	1.25
-3.90	4.70	1.20	3.50	1.20

AP: anterior to posterior, ML: medial to lateral.

Neural activities together with histology results obtained from the non-survival experiment (Figure 4) verified that using the detection of CS as the criteria for cell body layer localization is accurate. The depth from which CS were recorded from both the non-survival experiment and the chronically implantation of a uni-length micro-wire array are listed in Table II.
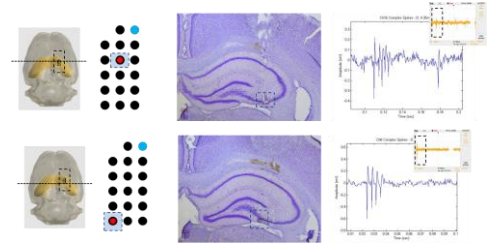

Figure 4. Complex spikes recorded during implantation (left) and the corresponding histology results (middle). Diagram on the right illustrated the channel from which CS were recorded and its relative location along the AP axis.

TABLE II. HIPPOCAMPUS MEASURE FROM BRAIN ATLAS AND BRAIN SLICES

AP (mm)	Rat1		Rat2	
	CA1	CA3	CA1	CA3
-2.45	3.55	3.95	3.65	4.25
-2.70	3.35	4.20	3.65	4.20
-2.95	3.30	4.35	3.60	3.85
-3.20	3.05	4.35	3.60	4.35
-3.45	3.05	4.40	3.35	4.35
-3.70	2.80	4.40	3.10	4.35

Rat1: the animal used in non-survival experiment.
Rat2: the animal used in chronically implantation.

The special designed multi-length electrode array has a length difference of 1.3 mm between the CA1 group with the CA3 and DG group respectively. With this difference, certain channels in each set was capable of recording unitary activities from the cell body layer. The histology results also verified that the recording was from desired region. However, with a uniformed length difference, only a small portion of the wires in each group were able to reach the cell body layer accurately.
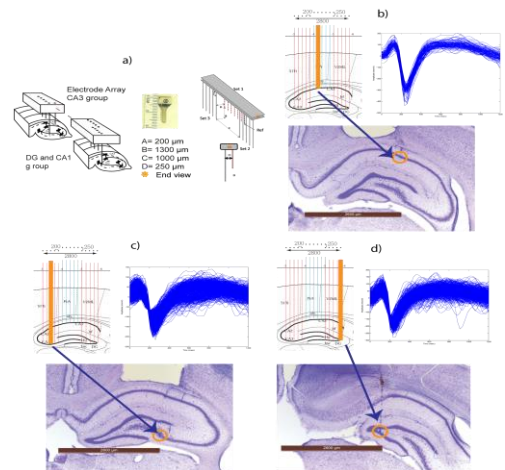

Figure 5. a) Diagrammatic representation of the multi-length electrode array. b), c), d) action potential recorded from the CA1, the CA3 and DG region, respectively, and its corresponding histology result.
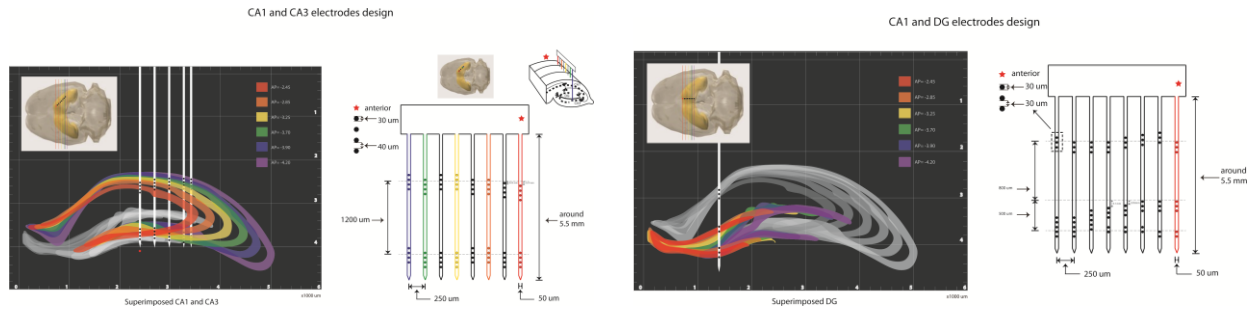
Figure 6. Diagrammatic representation of the layout of the CA1-CA3 Parylene probe (left) and the CA1-DG Parylene probe (right). The arrangement of recording sites is designed base on measurements obtained from histology results and the brain atlas. Each recording site has a diameter of 30 μm and the spacing between recording sites within each recording group is 40 μm.

B. Design of the Parylene Probe

By combining the *in vivo* recording results, histology results and the information attracted from the brain atlas, two 64 channels Parylene probes are designed for the recording from the CA1 and the CA3, the CA1 and the DG sub-region of the rat hippocampus respectively. For the CA1-CA3 probe, two groups of four recording sites are placed on each shank. The spacing between each recording site in one group is 40 μm which make the recording group span a wide enough depth (240 μm) to cover the CA1 and the CA3 cell body layers. The distance between two recording group is specially arranged to match the measurement we obtained above. The CA1-DG Parylene probe has a similar geometry as the CA1-CA3 probe. Since the DG cell body layer has two blades which spans a much larger region than the CA3 ones, five recording sites are placed in the DG group. These five recording sites are further divided into two sub-sites which target at both blades separately. The spacing between recording sites in the CA1group is also 40 μm.

C. Insertion Tool

Since Parylene C is a flexible material, we also designed a insertion tool to guide the Parylene probe during surgical placement into the brain. The insertion tool is composed of eight micro-wires 75 μm in diameter. The Parylene probe will be attached to the insertion tool with polyethylene glycol, a water-soluble biodegradable adhesive. After the Parylene probe is inserted into desired depth, the insertion tool will be retrieved.
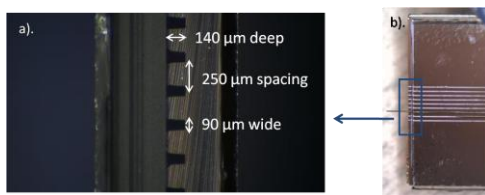

Figure 7. A close look at the geometry of the insertion tool. Eight notches are etched out on the silicon wafer. The spacing between each notch is 250 μm and the diameter of each notch is 90 μm. Pyrex is used to cover the notches and also will hold the micro-wires in place.

IV. FUTURE WORK

The Parylene probe will be implanted chronically into the rat hippocampus. The placement of recording sites will be further optimized to best fit the anatomical distribution of principle cells in the hippocampus. The stability of neural activities recorded with the Parylene probe and the performance of the Parylene probe in long-term implantation will also be evaluated.

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