Recording Place Cells from Multiple Sub-Regions of the Rat Hippocampus with a Customized Micro-Electrode Array

Huijing Xu, *Student Member, IEEE,* Min-Chi Hsiao, *Member, IEEE,* Dong Song, *Member, IEEE,* and Theodore W. Berger, *Fellow, IEEE*

***Abstract*— The hippocampus is a subcortical structure which is involved in memory function. There is a considerable amount of evidence available which indicates that the hippocampal system is necessary for effective spatial learning in rodents and short-term topographical memory in human. Recordings of neural activities from the hippocampus of behaving animals can help us to understand how spatial information is encoded and processed by the hippocampus. In this work, we designed a triple-region microelectrode array (MEA) which took into concern the anatomical structures of the rat hippocampus. The array was composed of 16 stainless steel wires which were arranged into three groups that differed in length. Each group targeted one subregion of the hippocampus. The array was chronically implanted into the rat hippocampus through craniotomy. Neural activities were monitored both during the implantation and after recovery. The triple-region MEA was capable of recording unitary activities from multiple subregions of the rat hippocampus and the spatial distribution of firing rates were analyzed while the animal freely explored in the environment.**

I. INTRODUCTION

The hippocampus is a medial temporal lobe structure which is necessary for declarative memories and the formation of new long-term memories. Damage to the hippocampus can induce severe memory loss. In rodents, impairments in rapid spatial learning and recent memory for places are especially prominent after hippocampus damage [1]. In 1976, O'Keefe et al. first reported that individual hippocampal cells fired rapidly when a freely moving rat was in a specific location. He named these cells "place cells", and the corresponding region where the cell's firing rate increased was called the "place field" [2]. Both pathological evidence and electrophysiological observations supported that the activity of hippocampal place cells was related to the processing of spatial information [3]. Anatomically, the hippocampus is composed of three major cytoarchitectonically distinct subdivisions named the dentate gyrus (DG), the CA3, and the CA1 [4]. Each subregion has its specific contribution to information processing. Although the diversity of functions associated with the DG, the CA3 and the CA1 still haven't been characterized clearly, it is certain that all three subregions are necessary for the transformation of spatial input to output signals [5]. Since the MEA can provide sufficient mechanical rigidity for penetrating the cortex with relatively moderate damages to the tissue, it has been widely used in recording from deep brain structures such as the hippocampus [6]. The impedance of metal electrodes also offers superior signal to noise characteristics in the frequency range of action potentials [7]. These advantages make metal microelectrodes a preferred method for extracellular, unitary recording from behavioral animals. A well designed MEA can record neural activities from multiple subdivisions of the hippocampus simultaneously. This multi-region recording can allow us to examine the encoding of information at different stages of the tri-synaptic circuit and can help us to better understand the function of the hippocampus in spatial information processing.

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H. J. Xu, M. C. Hsiao, D. Song and T. W. Berger are with the Center of Neural Engineering, Department of Biomedical Engineering, University of Southern California, Los Angeles, CA90089 USA (e-mail: huijingx@usc.edu; mhsiao@usc.edu; dsong@usc.edu; berger@bmsr.usc.edu)

II. MATERIALS AND METHODS

A. Electrodes

The layout of the triple-region MEA was first decided based on the anatomical distribution of the hippocampal subregions [8]. The distance between the CA1 and the CA3 cell body layers was also measured by scanning for "complex spikes" which are a series of several single action potentials with interspike intervals shorter than 6msec during the insertion of a single channel electrode to the dorsal hippocampus [9]. The arrangement of the DG group was decided based on its relative position to the CA3 and the CA1 regions. As illustrated in Fig. 1, the triple-region MEA was composed by sixteen stainless steel wires with 25μm diameters. All sixteen recording wires were Polyimide-coated (H-ML) with tips having an exposed length of 25μm. These wires were arranged into three groups. The two longer groups (are shown as set3 and set2 in Fig. 1) with five wires each were designed to target the CA3 and the DG respectively. The two longer groups were in the same sagittal plane and the DG group was 1000μm posterior to the CA3 one. The shorter group which had six wire was aimed at the CA1 region located between the two longer groups on another sagittal plane 250μm lateral to the longer ones. The length difference between the longer group and the shorter group was 1300μm. The separation between each wire was 200μm. Beside the sixteen recording wires, a single wire with a longer (2mm) exposed tip was placed in the CA1 group to serve as reference electrode. The grounding for the MEA was provided by another 5 cm uninsulated wire coming directly off the connector.

B. Apparatus and Behavioral Training

Behavioral training and recording were conducted in a highly simplified round open field. As illustrated in Fig. 2,

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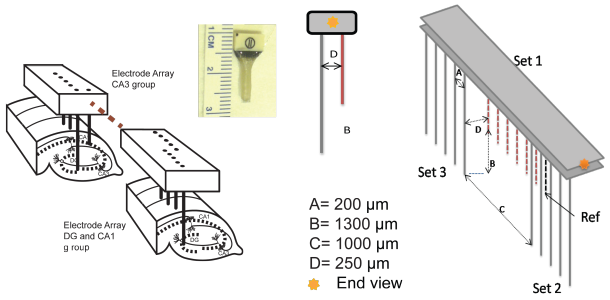

Fig. 1. Schematic diagram of the triple-region MEA. The photo on the top left shows the real size of the triple-region MEA. The lower left figure shows a cartoon of the rat hippocampus and the relative location of the triple-region MEA to the rat hippocampus. The figure on the right side shows the details of the arrangement of the MEA viewed from the rear and the left side.

the recording chamber is solid black with a 76cm diameter and a 50cm height. A movable white cardboard was bent to fit the inner surface of the chamber. This cue card occupied 100 degree of the chamber's wall during all training and recording sessions. In the experiment, the cue card is centered at 12 o'clock, 3 o'clock or 6 o'clock as viewed from the overhead camera. The recording apparatus was surrounded by a black curtain to isolate it from the external environment. A custom-made Faraday cage 82cm in width and height and 164cm in length was used to reduce the power line noise. The chamber was illuminated by a white LED strip which was shaped into a circle and placed on top of the faraday cage. Behavioral activities of the animal were captured by the camera which was located on the Faraday cage over the center of the open field. A commutator was also fixed next to the camera to prevent twining of the recording cables.

All animal preparation and animal care were in accordance with the National Institute of Health and Insitute of Animal Care and Use Committee guidelines and the Department of Animal Resources at the University of Southern California. Animals used in this experiment were housed in 12/12hr light and dark cycle, and all experiments were conducted during

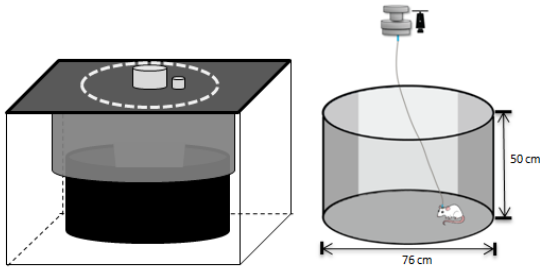

Fig. 2. Schematic diagram of the apparatus and recording set up. The figure on the left side shows the relative location of the Faraday cage, the recording chamber, the black curtain, and the LED strip on top of the frame. The figure on the right side shows a closer view of the recording chamber and the cue card which covers part of the chamber's wall. The location of the commutator and the camera is also shown in the figure.

the light cycle. Rats with *ad libitum* access to water were food deprived to maintain 85% to 90% of their free-feeding body weights. Each day the animal was left in the open field for 30 to 60 min to let it get familiar with the recording environment. The rats were also encouraged to visit all parts of the apparatus by throwing small food pellets into the apparatus randomly at a rate of about 3 pellets per min. With time, most rats would move away from the edge and run after the food pellets. After the animal began spending over 70% of time moving in the field, the triple-region MEA was implanted.

C. Subjects and Surgery

Male Sprague-Dawley (SD) rats greater than three months old and with weights greater than 350g were used in our experiments. Each animal was anesthetized with an intraperitoneal (IP) injection of Ketamine (75mg/100g) and Xylazione (10mg/100g) mixture, and anesthesia was maintained with Isoflurane mixed with oxygen flow (0.5-1.5L/min) during the surgery. The depth of anesthesia was continuously checked by testing the toe-pinch withdrawal reflex during the surgery. The animal's body temperature was maintained with a warm pad. The animal was fixed on a stereotactic frame with its skull flat between Bregma and Lambda. Three small holes were drilled for anchor screws. One of those screws contacted the cerebrospinal fluid to serve as grounding for the electrode. A 2x4mm bone window was made above the dorsal hippocampus and the dura mater was carefully removed. The triple-region MEA was positioned with it's first wire in set3 targeted the coordinates 2.6-2.8mm posterior to Bregma and 2.6-2.7mm lateral to the midline. The array was driven in small steps to a depth of 3.0-4.0mm for the CA3 and DG wires, with the CA1 group automatically located 1.2-1.4mm higher. Neural activities were monitored throughout implantation. After the MEA was placed above or within the cellbody layers of the CA1 and CA3 subfields of the hippocampus, the cranium was sealed with bone wax and dental cement. The scalp wound was sutured and treated with Bacitranci Zinc ointment daily. After the animal recovered from anesthesia, Bupropion (BUP, 0.05mg/kg) was injected to relieve pain and prevent infection.

D. Unit Recording and Data Acquisition

a) During the Implantation: After the MEA was inserted into the cortex, the neural activity was differentially amplified by 1000 and band pass filtered (300-3000kHz) by an AC differential amplifier. The neural signal was visualized with an oscilloscope and monitoted through a speaker. In order to eliminate power line noise, the sound of spikes was also band pass filtered from 300 to 300kHz. Examples of complex spikes recorded from the CA1 and CA3 subregions are shown in Fig. 3.

b) in vivo: After the implanted animal fully recovered from the surgery (7-14 days), unitary activities were recorded while the animal was running freely in the field. Neural signal was amplified by a factor of 20 by the head-stage and then further amplified by the differential amplifier connected

to the data acquisition system (OmniPlex, Plexon, Inc). The signal was digitized at 40kHz and band pass filtered to examine the action potentials and field potentials. Action potentials were sorted both on-line and off-line to get better unit isolation results. Units with signal to noise ratio (SNR) greater than 3 were used for place field analysis.

E. Histological Verification of Tip Locations

After the final recording session, animals were deeply anesthetized with an IP injection of Ketamine and Xylazione mixture. A brief DC current of 300μA was applied to the channel where place cells were recorded to make iron depositions at the tip of the recording electrode. Then the animal was perfused with 10% formalina and 3% KFe. The dissected brain was further fixed with 10% formalina for one day and dehydrated with 18% sucrose solution. 50μm brain sections were sliced with Vibratome and stained with cresyl violet to verify the final tip locations.

III. RESULTS

The triple-region electrode array was capable of recording unitary activities from multiple subregions of the rat hippocampus.

A. Unitary Activities Recorded During the Implantation

Both single action potentials and complex spikes were observed during the implantation of the triple-region electrode. Complex spikes were recorded from both the CA3 and the CA1 when the CA3 recording electrodes were interted at a depth of 3.2-4.2mm. The presence of complex spikes together with the depth of the implantation indicated that the neural signal was from the CA1 and the CA3 subregions of the hippocampus. The number of channels from which unitary activities were recorded were counted during four implantations. Spikels were recorded from 18 recording sites from the CA1 recording group, 14 sites from the CA3 recording group and 11 sites from the DG recording group. Spike sorting was not done at this stage of experiment. Fig. 3 shows an example of complex spikes recorded during the implantation.

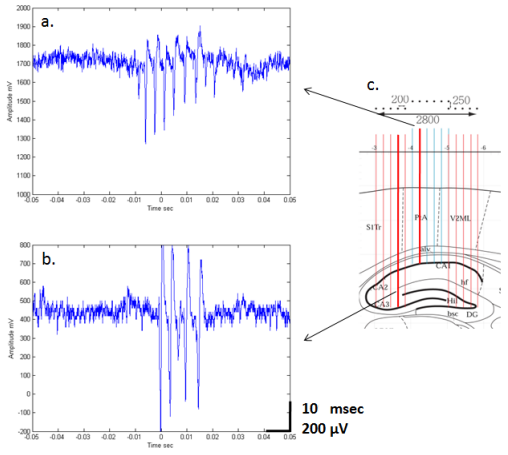

Fig. 3. Complex spikes recorded with the triple-region MEA during implantation. a). A complex spike recorded with an electrode targeted the CA1 cell body layer. b). Shows a complex spike recorded with an electrode which targeted the CA3 region. c). Shows the brain atlas of the sagittal plane corresponding to the implant site. The layout of the electrode array was illustrated with red and blue lines on top of the atlas. Electrode wires from which the complex spikes shown in a) and b) were highlighted with a thick red line.

B. in vivo

The spatial distribution of firing rates was examined in two additional animals which were chronically implanted with the triple-region MEA. After full recovery, unitary activities and the animals' behaviors were recorded simultaneously while the animals were exploring the apparatus described above. The spatial distribution of firing rates was calculated by dividing the open field into 0.2×0.2cm pixels and computing the firing rate for each pixel as the total number of spikes generated in a pixel divided by the total time spent in that pixel. Totally, 25 units (10 units from the CA1

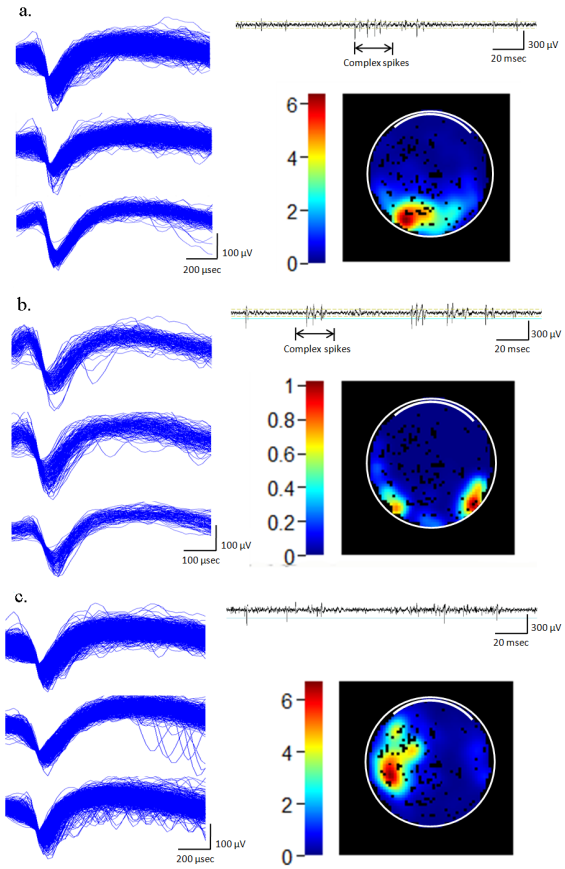

Fig. 4. Neural activities and place fields recorded from all three subregions of the hippocampus. a). Shows a complex spike cell recorded from the CA1 region. b). Shows a complex spike cell recorded from the CA3 region and c). Shows a unit recorded from the DG region. Waveforms plotted on the left panel were spikes recorded for 200sec. From top to bottom the waveforms show spikes recorded from the first day, third day and seventh day of recording respectively. Color maps on the right panel show place fields of those units. Locations where the animal hadn't explored were shown in black. The location of the white cue card is plotted on top of the color maps. Continuous signals on top right shows the complex spikes recorded from the CA1 and the CA3 region and single spikes recorded from the DG region.

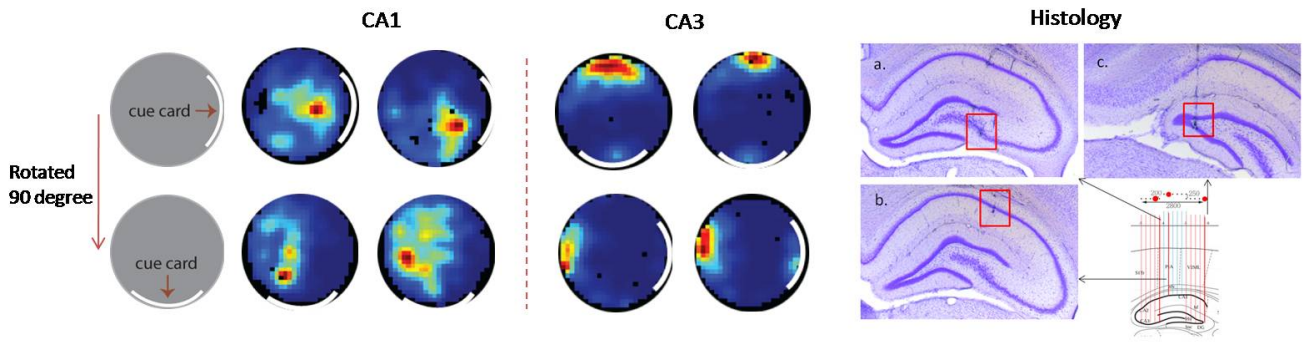

Fig. 5. The first two gray circles on the left show the location of the cue card during the 15min recording sessions. Firing rates of place cells are illustrated in color maps. Bright color represents high firing rate and the highest firing rate (8 Hz) is shown as dark red in the color map. Four color maps in the middle under "CA1" show the place fields of one unit recorded from the CA1 region. Four color maps on the right side under "CA3" show the place fields of one unit recorded from the CA3 region. Color maps on the top row show the place field of the unit for a 15min run with the cue card located at 3 o'clock direction. The bottom row shows the place field of the same units when the cue card was rotated 90 degree clockwise. Histology results are shown on the right side under "Histology". Locations where iron deposition are observed are highlighted with red squares. a). Shows the tip location of one wire in the CA3 group. b). Shows one wire in the CA1 group and c). Shows one wire in the DG group. The brain atlas on the bottom right corner shows the sagittal plane corresponding to the implantation.

region, 7 units from the CA3 region and 8 units from the DG region) with SNR greater than 3 were recorded. 6/10 units recorded from the CA1 region, 5/7 units recorded from the CA3 region and 3/8 units recorded from the DG region exhibited location-specific firing. The ratio of place cells in the CA1 and the CA3 region agrees with other groups' results [10]. The histology results verified that the recording were from the CA1, the CA3 and the DG region respectively. Fig. 4 shows three units recorded from different subregions in one rat.

IV. DISCUSSION AND FUTURE WORK

A. Cue Card Rotation

We also conducted a simple test of how changes in the surrounding environment affect the neural activities in the hippocampus. We let the animal performed a series of two 15min runs in the open field with the cue card rotated 90 degree in the second run. The animal was put back into its home cage for 30 to 60min between each run. We observed that when the cue card was rotated 90 degree, the place fiels rotated accordingly. Many studies demonstrated that the place cells response to the changes in the environment by changing its firing property [11]. This indicates that place cell is involved in the processing of spatial information in the hippocampus. Our next step will focus on the responses of units in different subregions of the hippocampus to the manipulation of the environment and how the spatial information is projected from the input region to the output region.

B. Implantation

The number of units recorded after recovery was relatively low compared to the number of units observed during implantation. It is possible that some units are mechanical stimulated into firing during the implantaion. Also, many projection cells in the hippocampus fire due to behavioral inputs other than spatial location. If the corresponding stimulus does not exist in the experiment, the unit will be silent. However, there is still much to be improved in the MEA design and surgical technique. We will keep working on finding the optimal arrangement of the electrodes and the most accurate sites of implantation to increase the quantity of units recorded from each animal and to enhance the stability of long-term recordings.

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