31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Simultaneous Recording of Brain Activity and Functional Connectivity in the Mouse Brain

Mina Lee, Hee-Sup Shin, and Jee Hyun Choi, *Member, IEEE-EMBS*

Abstract— **Combining of two measurement system of different scale in the mouse brain has been challenging issue due to the small size of the mouse brain. We present a novel approach to record brain activity and connectivity simultaneously by using intracranial electrode and flexible multichannel film electrode. 40 channel ultra-thin nanofabricated microelectrode covering the mouse skull was applied in this study. The null space in the microarray was cut off so that the secondary sensor or stimulator can approach to the brain. Hereby, we performed a simultaneous recording of local field potential and multichannel EEG recording in a freely moving mouse under pharmacologically driven absence seizure. A brain activity in the thalamus was depicted together with cortical EEG map. The connectivity levels between different cortical sites or between thalamus and cortical site were measured during seizure. The time lag map of cortical EEG with respect to the thalamus represents the propagation delay of the seizure.**

I. INTRODUCTION

Electroencephalography (EEG) is one of the widely utilized methods to research the human brain due to its non-invasiveness. However, since EEG reflects the electrical activity of macroscopic neuronal populations, there are too many information to figure out underlying molecular and cellular mechanisms of specific brain functions. Moreover, human scalp EEG system (10-10 and 10-20) has critical limitation in that it detects only cortical activities. On the other hand, if we utilize genetically modified mice, we can find out the molecular and cellular basis of brain states, such as absent seizure [1]. Also, in the mouse EEG we can detect signals from the deeper brain as well, due to smaller size of the mouse brain and thinner skull ranging only 200~600 μm [2]. Therefore, mouse EEG can give us innumerous advantages to explore the fundamental mechanisms of brain functions.

Manuscript received April 7, 2009. This work was supported by a National Honor Scientist Grant from the Ministry of Education, Science and Technology, Korea, by the Centre of Excellence program, and by the Top-Brand program at Korea Institute of Science and Technology.

M. Lee. Author is with the Center for Neural Science, Korea Institute of Science and Technology, Seoul, Korea and Department of Neuroscience, University of Science and Technology, Daejon, Korea (e-mail. sarangmina@kist.re.kr).

H. S. Shin Author is with the Center for Neural Science, Korea Institute of Science and Technology, Seoul, Korea and Department of Neuroscience, University of Science and Technology, Daejon, Korea (e-mail. shin@kist.re.kr).

J.H. Choi. Author is with the Center for Neural Science, Korea Institute of Science and Technology, Seoul, Korea and Department of Neuroscience, University of Science and Technology, Daejon, Korea (corresponding author to provide phone: +82-2-958-6952; fax: +82-2-958-6937; e-mail. jeechoi@kist.re.kr).

Nowadays, in order to improve the low spatial resolution of EEG many researchers utilize the high resolution EEG recordings or are trying to integrate EEG and other modalities, such as EEG-fMRI and EEG-PET. However, it is really challenge to obtain EEG signal with high resolution electrode from the mouse brain mainly due to its small size, and existing multimodal techniques based on the EEG are not adequate for the mouse. To overcome above limitations, we combined cranial recording with high resolution (40 channels), nanofabricated EEG film electrode for the mouse brain [3]. This film electrode is made of polyimide, which has biocompatibility, flexibility, and electrical insulation [4, 5]. Our high resolution mouse EEG system is first incorporated to a conventional local field potential (LFP) to monitor the brain activity and EEG simultaneously, in a purpose of investigation of the relationship between brain activation and the functional connectivity. In this study, thalamic activation was monitored together with cortical activation when the mouse experienced absence seizure, of which the origin is still under investigation, but is known to be a product of interplay between cortex and thalamus. The functional connectivity and time lag between thalamus and cortical regions or different cortical regions during spike-wave discharge (SWD) arrests in millisecond range were successfully assessed to visualize the temporal dynamics of neural networks of absence

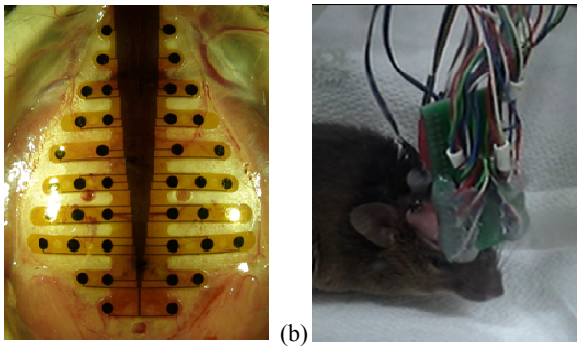
II. METHODS AND MATERIALS

A. Animal preparation

B6-129 hybrid mouse (10 weeks) was generated by mating from C57BL/6J and 129S4/SvJae. Mouse was maintained with free access to food and water under a 12h light/dark cycle with light beginning at 8:00 a.m.

B. Implantation of EEG · LFP & EMG electrodes

The animal was anesthetized by Ketamine/Xylazine (120 mg/kg and 6 mg/kg each). Tail or toe pinching preceded the surgery to inspect the depth of anesthesia. And mouse was fixed by stereotaxic (David Kopf Instruments, Model 902, Calif, USA) to indicate bregma and lambda as landmarks of the skull. This step is indispensable to estimate the position of the brain regions. Middle scalp was incised about 2cm to expose the skull. Any debris on the skull was wiped by saline-soaked cotton tip, which turns out to be an important step in the whole procedure by enhancing the adherence of the electrode to the skull. Using peroxide for removing the residue of the membrane was also effective. A couple of



(a) (b)

Fig 1. (a) A film type EEG microelectrode on the mouse skull. The midline matches the line between the bregma and lambda. The upper two lines of the microelectrodes were located on the frontal area of the mouse. The secondary intrusive electrode or stimulator can access to the brain through the null space. The thickness of the film is approximately 10 μm and the film is transparent except the metal patterns. (b) Mouse with EEG, LFP, and EMG electrodes are implanted. The total weight of the connector did not exceed 3 gram including wire.

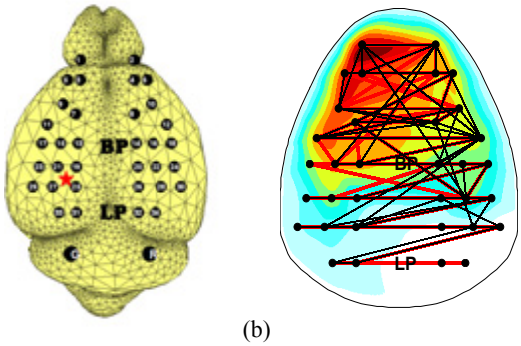
microscrews were used to secure the position of the electrode. Microscrew was used for ground and reference as well. The polyimide based EEG film electrode with 500 μm electrical contacts (the impedance of these contacts were 300kΩ at 30 Hz sine wave on the mouse skull) was positioned to be symmetrical on the mouse skull. And the bregma and lambda were landmarks in placing the electrode. Coordinates of electrode followed by Paxinos and Franklin[6]. These steps take less than an hour.

After placing of EEG film electrode, another two holes were drilled into the skull above the region where the VPM/VPL thalamic nuclei are located (from Bregma, -1.6 mm anteroposterior, ±1.7 mm mediolateral). Teflon coated tungsten electrodes with a diameter of 150 μm were implanted into the holes (3.5 mm dorsoventral) for local field potential (LFP) recording. (Fig 1.(a)) For EMG signal recording, same electrodes were inserted into the nuchal musculature. The location of the electrode was confirmed by brain histology after all the experiments.

C. Recording

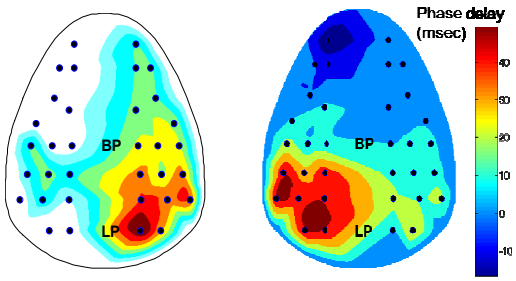
The EEG/LFP/EMG recordings were also captured on video for behavioral arrest analysis. To precipitate the arrests, we put mice in the clean beaker. An 'absent seizure arrest' is defined as a period when the animal suddenly stop its motion and do not blink with high amplitude, 3-6Hz [7] frequency EEG and low EMG.

After recovery from the implantation surgery, both EEG and field potential were measured by Synamps^2. We recorded 1 hr for absent seizure induced by γ–butyrolactone (GBL, 100 mg/kg, i.p.) in freely moving condition. The brain and EMG signals from the mouse were amplified 1,000 times and digitized with a 24bit resolution at 1 kHz sampling rate and filtered with frequency bands between 0.1 to 100 Hz and 30 to 100 Hz, respectively. The spectral and correlation analyses were carried out offline using Matlab.



(a) (b)

Fig 2. (a) Montage of mouse EEG. Due to connection problems of four electrodes, 34 channels were used in this study. The red star notifies the entree position of LFP electrode. (b) The power map of SWD in EEG. Wavelet analysis extracted SWD after normalization. The left frontal area showed high level of SWD activation. Each black dot represents the measurement point. The red and black lines connect two sites with correlation coefficient higher than 0.999 and between 0.99 and 0.999, respectively.



(a) (b)

Fig 3. (a) The coherence map of EEG with respect to the LFP measured from thalamus. (b) The phase delay of EEG with respect to LFP in the thalamus.

D. Data analysis

The petit mal moments of the mouse were detected from the video screening first. We applied wavelet transform both to EEG and LFP to extract spike-and-wave discharge (SWD), which is a typical pattern of absence seizure [8]Figure 2(a) illustrates the channel locations, which were used to draw map contours. The synchronization levels between different channels were evaluated based on correlation methods. The cross-correlation functions were calculated to obtain the phase lag of EEG with respect to LFP. For normalization of EEG, the frequency band between 190-230 Hz was used.

III. RESULTS AND DISCUSSION

The integration of the local field potentials and the skull EEG in the mouse brain successfully monitors the field map and local brain activity at the same time. The color map of Fig. 2(b) illustrates the level of field potential of SWD. The left frontal area experiences bigger response of SWD than other brain sites. The synchronization levels between different brain sites are reported in the same figure. The cross-correlation coefficients between epileptic evens acquired at different location were used to gauge the

synchronization. Most of the connections were between two sites that are located symmetrically with respect to the midline. Considering that the GBL generates a bilaterally synchronous SWD, this result is comprehensible. The most beneficial element of this experimental set up is relating the local brain activity to the cortical activation. Figure 3(a) shows the synchronization levels between LFP and EEG at each site. Compared to Fig. 2(b) which showed high excitation in the left frontal area, right dorsal parietal area shows high synchronization to the thalamic activation. The phase delays between LFP and EEG were calculated, which were shown to be non-zero, that means completely synchronous phenomenon over the whole brain. The maximum phase delays of the cortical field to the LFP were 45 msec and it is interesting to note that the slowest region is the cortical region right above the thalamic area of LFP. The cortical site showing faster epileptic response was also observed, and the region was left frontal which superimpose the highest SWD field region.

IV. Conclusion

An integration of high resolution surface EEG and intracranial recording for mouse brain was successfully performed in freely moving animal. The polyimide based film electrode reduced the surgery time significantly even though much more numbers of electrodes were implanted, as well as presenting high resolution mapping of EEG signal in a less-invasive way. Since the local field potentials are the neurological basis of the surface EEG, the simultaneous measurement of LFP and EEG in the mouse are in favor to study the fundamental origin of EEG in the level of molecular and circuit by using genetic modification. This method is expected to be utilized in the study of the brain activity in regards to the functional connectivity in transgenic mouse brain.

References

[1] D. Kim, I. Song, S. Keum, T. Lee, M. J. Jeong, S. S. Kim, M. W. McEnery, and H. S. Shin, "Lack of the burst firing of thalamocortical relay neurons and resistance to absence seizures in mice lacking alpha(1G) T-type Ca(2+) channels," *Neuron,* vol. 31, pp. 35-45, Jul 19 2001.

[2] M. Weiergraber, M. Henry, J. Hescheler, N. Smyth, and T. Schneider, "Electrocorticographic and deep intracerebral EEG recording in mice using a telemetry system," *Brain Res Brain Res Protoc,* vol. 14, pp. 154-64, Apr 2005.

[3] J. H. Choi, K. P. Koch, W. Poppendieck, M. Lee, T. Doerge, and H. S. Shin, "A flexible, multi-channel electrode for mouse brain and its application to a mouse EEG," in preparation.

[4] K. C. Cheung, P. Renaud, H. Tanila, and K. Djupsund, "Flexible polyimide microelectrode array for in vivo recordings and current source density analysis," *Biosens Bioelectron,* vol. 22, pp. 1783-90, Mar 15 2007.

[5] J. D. Yeager, D. J. Phillips, D. M. Rector, and D. F. Bahr, "Characterization of flexible ECoG electrode arrays for chronic recording in awake rats," *J Neurosci Methods,* vol. 173, pp. 279-85, Aug 30 2008.

[6] G. Paxinos and K. B. J. Franklin, *The mouse brain in stereotaxic coordinates*, 2nd ed. San Diego, Calif. ; London: Elsevier Academic Press, 2004.

[7] M. Aizawa, Y. Ito, and H. Fukuda, "Pharmacological profiles of generalized absence seizures in lethargic, stargazer and gamma-hydroxybutyrate-treated model mice," *Neurosci Res,* vol. 29, pp. 17-25, Sep 1997.

[8] H. Adeli, Z. Zhou, and N. Dadmehr, "Analysis of EEG records in an epileptic patient using wavelet transform," *J Neurosci Methods,* vol. 123, pp. 69-87, Feb 15 2003.