35th Annual International Conference of the IEEE EMBS
Osaka, Japan, 3 - 7 July, 2013

Cortical Source Localization of Mouse Extracranial Electroencephalogram using the Fieldtrip Toolbox

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Jee Hyun Choi*

Abstract— **Neuronal source estimation is a general tool for analyzing spatiotemporal dynamics in human EEG. Despite rapidly-evolving interest in human brain, there are few EEG based source estimation tools in rodent brain. Therefore, we implemented source estimation tool in a mouse model, using the FieldTrip open-source software. High resolution EEGs with a known cortical source were recorded with a recently developed 40-channel polyimide-based microelectrode under optical stimulation on optogenetially engineered mice. To obtain realistic mouse head models, the volume conduction model was extracted from in vitro mouse brain MRIs. Segmented compartments (skin and outer/inner skull) were used to form triangular meshes and then applied to the boundary element method. The high-resolution EEGs recorded during various optogenetic stimulation of the mouse brain were inversely source reconstructed using minimum-norm estimate. Estimated source locations and strengths were reconstructed, and their error was calculated to evaluate FieldTrip-based source localization algorithm. In summary, source localization imaging of the mouse brain was successfully achieved, using freely-available open source software. This will be useful to investigate the functional dynamics of mouse brain in noninvasive measure.**

I. INTRODUCTION

The aim of this study is to implement source localization tool in the cortical surface of optogenetically-stimulated mouse brains. Accurate analysis of neuronal current spatiotemporal dynamics during cortical activity is essential for reliable noninvasive and extracranial EEG. Functional brain mapping with human EEG measures discrete electrical potential differences on the scalp, and is a widely-used neuropsychological assessment tool. In comparison with human study, it is difficult to estimate source location in mouse brain due to its tiny volume and the insufficient number of electrode. So far EEG recordings in mice have lacked the spatial resolution, causing difficulty in determining precise cortical origins of rhythms. Limited spatial information is a general hindrance to extended interpretation of EEG data to the level of the neuronal network. Mégevand et al. proposed a spatial mapping technique for modeling large-scale neuronal networks in mouse brains. They used a needle-type electrode cluster to successfully map cortical event-related potentials, but the studies were restricted to using anesthetized mice fixed on a stereotaxic device [1].

Recently, Choi et al. presented a novel technique to obtain spatially-resolved mouse cortical EEG information without invasive brain surgery by fabricating a polyimide-based microelectrode (PBM) array [2]. This PBM-array has 40 electrodes that are uniformly distributed within a limited area of exposed mouse skull. These researchers have also developed topographical- and dynamical-analysis tools to visualize the dynamic changes of spatially-resolved mouse EEG.

To adequately analyze the dynamic neuronal system, however, we require an accurate method for estimating the origin of EEG signals in terms of location and depth of dipole source in mouse brain. An open-source MATLAB toolbox, FieldTrip, was released that included source localization algorithms for invasive/noninvasive human electrophysiological data [3].

The currently released version of FieldTrip, however, does not support source estimation in the rodent brain. In this study, we have implemented a source localization tool based on FieldTrip toolbox to investigate the functional network in the mouse brain. We employed realistic volume conduction model extracted from mouse MRIs. These volumes (inner/outer skull, and skin) of the mouse head were given the electrical conductive properties using the boundary element model (BEM). Traditional inverse method was employed for solving inverse problem such as minimum norm estimate (MNE). We implemented source localization tool and demonstrated the ability of the developed tool using optogenetic stimulation in three

This work was supported by the Global Frontier R&D Program on Human-centered Interaction for Coexistence (2011-0031525) and Brain Research Program (2010-0018944) funded by the National Research Foundation of Korean. The authors gratefully acknowledge the support of the Brain Gain Smart Mix Programme of the Netherlands Ministry of Economic Affairs and the Netherlands Ministry of Education, Cuture and Science.

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different cortical locations (motor, somatosensory, and visual cortex) and at two different depths (layer IV: 0.5 and layer VI: 0.8 mm).

II. METHODS AND MATERIALS

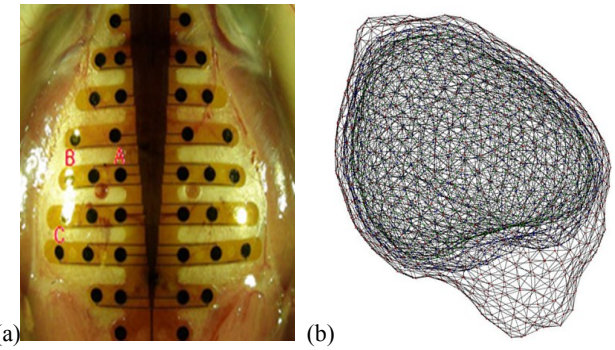
A. PBM Arrays for Mouse Extracranial EEG

The 40-channel PBM arrays were manufactured by nanofabrication process. The electrical contacts, the connection lines, and the interconnection pads were made of 300 nm-thick platinum. The electrical contacts and the interconnection pads were then exposed by selective reactive-ion etching of the polyimide layer (Pyralin 2611; HD Microsystems, Bad Homburg, Germany). A two sided connector with 20 pins on each side (DF16B-40DP-0.5V, Hirose Electric Company, Ltd., Yokohama, Japan) were attached to the interconnection pads using a conductive glue to provide an interface to the EEG recording device.

B. Optogenetic Stimulation and High-Resolution-EEG Recording

We used B6 Thy1-ChR2-EYFP transgenic mice (30-35 g in weight; 12-17 weeks; male). For in vivo recording, animals were anesthetized with intraperitoneal injection of ketamine/xylazine cocktail (120 and 6 mg/kg, respectively) and placed on the stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) with bregma and lambda points on the same horizontal plane. The scalp midline was incised to expose the skull. After removing debris on the skull with tap water soaked cotton balls, the PBM-array was aligned on the line between bregma and lambda. After fixing the PBM-array, holes were made in the skull for the optogenetic stimulation.

For optogenetic stimulation, we used a semiconductor laser (USA & BCL-040-445; 445 nm wavelength and 40 mW/mm^2 maximum output power; CrystaLaser LLC., Reno, NV, USA) that was gated by a pulse generator (575 digital delay, Berkeley Nucleonics Corp., Berkeley, CA, USA). Blue light from the laser was guided to the mouse brain using an optic fiber with clad/core diameters of 125 μm and 3.4 μm, respectively (P1-405A-FC-5; Thorlabs Inc., Newton, NJ, USA). The light intensity measured from the tip of optical fiber was approximately 2 mW/mm^2. A pulse train with a 20 msec pulse width at 1 Hz was delivered in three different cortical regions (primary motor, primary somatosensory, and visual cortex marked as A, B, and C in Figure 1, respectively) at two different depths (0.5 and 0.8 mm ventral from dura mater). After allocating two electrical contacts at the most posterior region as reference and ground electrodes, 38 active channel EEGs were recorded with a SynAmp2 amplifier (Neuroscan Inc., El Paso, TX, USA), digitized with a 1 kHz sampling rate, and band-pass filtered from 0.1 to 100 Hz. Prior to recording, any electrodes in the PBM-array with an impedance greater than 300 kΩ (test frequency of 30 Hz) were excluded.



(a) (b)

Fig 1. (a) PBM array electrode 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. An optical stimulator can access to the brain through the null space (A, B, and C). (b) Volume conduction models are implanted. The total weight of the connector did not exceed 3 gram including wire.

C. Volume Conduction Mode: In Vitro Mouse MRI Image

The volume model was extracted from MRI imaging that was previously used in in vitro studies, and that was downloaded from the open database of the Magnetic Resonance Microimaging Neurological Atlas Group (http://brainatlas.mbi.ufl.edu/). These were MRIs of brain without scalp and skull from four male, 12-week-old mice [4, 5]. A threshold for segmenting the cortex from the MRI image was manually determined. To co-register the extracranial electrodes with the MRI coordinates, we obtained the coordinates of the anatomical landmarks (nasion, preauricular left (PAL), and preauricular right (PAR)) in the MRI data, and then estimated the position of electrodes in MRI coordinates. We translated the respective brain borders in MRI coordinates into the Talairach coordinates (anterior commissure, posterior commissure and upper mid-sagittal, and distances between the anterior commissure and boundaries), with the help of the set parameters of Curry software (Neuroscan Inc., El Paso, TX, USA).

D. Forward and Inverse Problems

To assign electrical properties for the volume compartments, we used the FieldTrip function "ft_headmodel_dipoli"[3]. We assigned isotropic conductive properties for each compartment described by the triangular meshes, i.e., inner/outer skull, and skin surface; as 0.33, 0.0042, and 0.33 S/m, respectively [6]. The tessellation lengths of inner skull, outer skull, and skin were 0.3, 0.35, and 0.4 mm, respectively. To calculate the dipole strength, forward problem has to employ obtaining the lead-field matrix [7, 8]. The lead-field matrix describes the physical relations between the electrode position and the distributed multiple-dipole strength with conductive characteristics of the volume conductor. To calculate this matrix we applied the FieldTrip function "ft_prepare_leadfield", that takes the electrode position, dipole source space, and the previously determined BEM volume conduction model as inputs.

To calculate the multipoles activation by solving an inverse problem, we tested one algorithm - the minimum-

norm estimate (MNE). The MNE is favored for analyzing evoked responses and for tracking wide-spread neuronal activation over time, bringing out the inverse solutions for the strength of a distributed model which discretizes the source space into locations on the cortical surface or in the brain volume using a large number of equivalent current dipoles. The MNE estimates the amplitude of all modeled source locations simultaneously with minimum overall energy [9].

III. RESULTS AND DISCUSSION

Figure 2 shows color maps of MNE results. Focalization ability is presented as the range of red intensity according to the location and the depth of optogenetic stimulation. The simulated potentials on the color maps on the cortical surface were as close to red color as focalized location can provide. By visual inspection, the MNE with the central (A) and the lateral (B, C) cortical source were well focalized as shown in Figure 2. However, these results could not provide their difference with respect to stimulation depth.

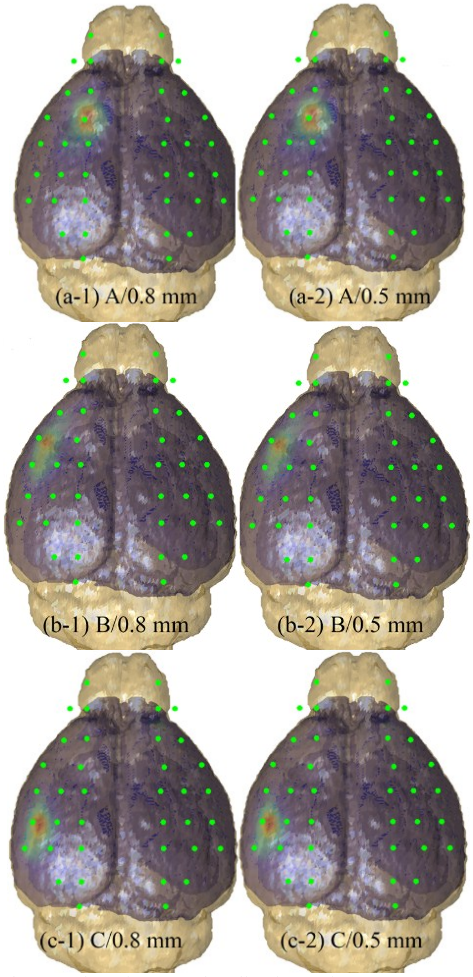


Fig 2. The Results of source localization using MNE. Montages of mouse brain volume. 38 channels were used in this study. The green dots notify the geometry of the PBM array electrode. (a-1) Stimulation A with 0.8 mm depth, (a-2) Stimulation A with 0.5 mm depth (b-1) Stimulation B with 0.8 mm depth, (b-2) Stimulation B with 0.5 mm depth, (c-1) Stimulation C with 0.8 mm depth, (c-2) Stimulation C with 0.5 mm depth

To quantitatively evaluate the performance of the MNE, we calculated the ratio of between the sum of dipole values within boundary and the sum of all dipoles. The approach is visually demonstrated in Figure 3. We set various boundaries (k : 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3, …, and 10 mm) of optical stimulation propagation through cortical tissue from the center points (A, B, and C) because the total transmitted light power is reduced by 90% at 1-mm depth in case of 473nm blue light [10]. These curves in Figure 3 show its focalized ability as both rapidly increasing slop and nearby the upper boundary. According to Figure 3, the shallow depth stimulation (0.5 mm) was more focalized than the deep one (0.8 mm). And the edge of electrode (lateral area, C, green/orange line) could not obtained focalized ability than the central areas.

Mouse source localization has been implemented using open source software, FieldTrip. The minimum norm estimate was used for estimating the source location and distribution on the cortical surface. A quantitative voxel-wise validation of source localization for mouse EEG evoked by optical stimulation was attained in different cortical locations and depths in optogenetic mice. Figure 3 showed that the estimation performance of MNE in constrained source field could achieve the cortical source localization for all cortical source located in primary somatosensory, motor, and visual cortices of mouse brain.

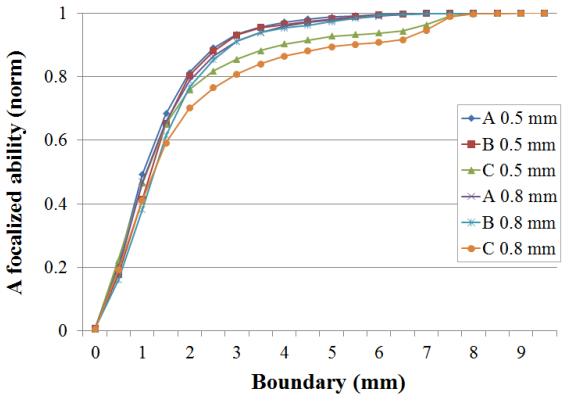


Fig 3. The ratio of within dipoles and all dipoles. The x-axis indicates the boundary from 0 to 10mm. The y-axis indicates the ratio value, sum(within dipoles)/sum(all dipoles). The lateral location (C), orange and green lines, shows inferior result than the central location (A). And the ratio values in deep stimulations (0.8 mm) have relatively lower than shallow ones (0.5 mm).

IV. CONCLUSION

This study shows that the source localization using an open source toolbox FieldTrip successfully localizes the neuronal activation in mouse brain. This toolbox is potentially useful for tracking information flow, identifying functional brain circuit, and localization of evoked potentials or epileptic events or oscillations without

invasive measure in neuroscience using mouse model.

REFERENCES

[1] P. Megevand, C. Quairiaux, A. M. Lascano, J. Z. Kiss, and C. M. Michel, "A mouse model for studying large-scale neuronal networks using EEG mapping techniques," *Neuroimage,* vol. 42, pp. 591-602, Aug 15 2008.

[2] J. H. Choi, K. P. Koch, W. Poppendieck, M. Lee, and H. S. Shin, "High resolution electroencephalography in freely moving mice," *J Neurophysiol,* vol. 104, pp. 1825-34, Sep 2010.

[3] R. Oostenveld, P. Fries, E. Maris, and J. M. Schoffelen, "FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data," *Comput Intell Neurosci,* vol. 2011, p. 156869, 2011.

[4] Y. Ma, P. R. Hof, S. C. Grant, S. J. Blackband, R. Bennett, L. Slatest, M. D. McGuigan, and H. Benveniste, "A three-dimensional digital atlas database of the adult C57BL/6J mouse brain by magnetic resonance microscopy," *Neuroscience,* vol. 135, pp. 1203-1215, 2005.

[5] Y. Ma, D. Smith, P. R. Hof, B. Foerster, S. Hamilton, S. J. Blackband, M. Yu, and H. Benveniste, "In vivo 3D digital atlas database of the adult C57BL/6J mouse brain by magnetic resonance microscopy," *Frontiers in Neuroanatomy,* vol. 2, Apr 17 2008.

[6] M. Fuchs, J. Kastner, M. Wagner, S. Hawes, and J. S. Ebersole, "A standardized boundary element method volume conductor model," *Clin Neurophysiol,* vol. 113, pp. 702-12, May 2002.

[7] R. Grech, T. Cassar, J. Muscat, K. P. Camilleri, S. G. Fabri, M. Zervakis, P. Xanthopoulos, V. Sakkalis, and B. Vanrumste, "Review on solving the inverse problem in EEG source analysis," *Journal of Neuroengineering and Rehabilitation,* vol. 5, Nov 7 2008.

[8] W. Z. Rafik Khemakhem, Ahmed Ben Hamida, Abdelmalik Taleb-Ahmed, and Imed Feki, "EEG source localization using the inverse problem methods," *International Journal of Computer Science and Network Security,* vol. 9, pp. 408-415, 2009.

[9] A. M. Dale, A. K. Liu, B. R. Fischl, R. L. Buckner, J. W. Belliveau, J. D. Lewine, and E. Halgren, "Dynamic statistical parametric mapping: Combining fMRI and MEG for high-resolution imaging of cortical activity," *Neuron,* vol. 26, pp. 55-67, Apr 2000.

[10] A. M. Aravanis, L. P. Wang, F. Zhang, L. A. Meltzer, M. Z. Mogri, M. B. Schneider, and K. Deisseroth, "An optical neural interface: in vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology," *J Neural Eng,* vol. 4, pp. S143-56, Sep 2007.