Spatiotemporal Analysis of Hippocampal Long Term Potentiation Using Independent Component Analysis

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Abstract

Long-term potentiation (LTP) of synaptic transmission is the most widely studied model for learning and memory. However its mechanisms are not clearly elucidated and are a subject for intense investigation. Previous attempts to decipher cellular mechanisms and network properties involved a current-source density analysis (CSDA) of the LTP from small animal hippocampus measured with a limited number of microelectrodes (typically <3), only revealing limited nature of spatiotemporal dynamics. Recent advancement in multi-electrode array (MEA) technology allows continuous and simultaneous recordings of LTP with more than 60 electrodes. However CSDA via the standard Laplacian transform is still limited due to its relatively high sensitivity toward noise, inability of resolving overlapped current sources and sinks, and its requirement for tissue conductivity values. In this study, we propose a new methodology for improved CSDA. Independent component analysis and its joint use (i.e., Joint-ICA) are applied to extract spatiotemporal components of LTP. The results show that ICA and Joint-ICA are capable of extracting independent spatiotemporal components of LTP generators. The ICs of LTP indicate the reversing roles of current sources and sinks which are associated with LTP.

Key words : multi-electrode array (MEA), hippocampus, long term potentiation, independent component analysis (ICA), joint-ICA

I. INTRODUCTION

Long-term potentiation (LTP) refers to a prolonged enhancement of the synaptic transmission after simultaneous activity of the pre/postsynaptic membrane. Due to its synaptic efficacy, LTP has been the most widely studied model for learning and memory [1-3]. The analysis of LTP mechanisms is important since it holds the key for the understanding of memory functions of the human brain. However its mechanisms are not clearly elucidated yet and are a subject for intense investigation. As an attempt to decipher cellular mechanisms and network properties, recent studies applied a current-source density analysis (CSDA) to the measured LTP from a small animal hippocampus with a limited number of microelectrodes (typically <3), but the results only revealed limited nature of spatiotemporal dynamics [1,2].

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To fully monitor the spatiotemporal dynamics of LTP, a multi-electrode array (MEA) system has been recently adopted to record LTP simultaneously and continuously with more than sixty electrodes [3], thus allowing 2-D CSDA via the Laplacian transform [4]. Until now, the Laplacian transformation has been a standard technique to identify locations and magnitudes of current sources and sinks responsible for LTP. However, the Laplacian transform is known to exhibit the following limitations: (1) it is generally sensitive to noise due to its second-order derivatives; (2) the transform produces complex patterns of overlapped or superimposed current sources and sinks; (3) the transform cannot resolve the superposition problem of current generators; (4) the transform requires electrical conductivity values that are not generally available.

In this study, we introduce a new methodology to overcome some of the limitations of the Laplacian transform. Independent component analysis (ICA) is a blind source separation technique that finds many successful applications in the biomedical fields [5-7]. Especially toward biomedical multi-channel data, ICA has proved its capability in identifying statically indep-

endent components in space and time which correspond to the physiologically independent sources. In addition, joint use of ICA (i.e., Joint-ICA) allows simultaneous analysis of multiple data sets differing in their experimental conditions, thus revealing common and uncommon dynamics components within the given data sets.

By adopting this methodology, we can expect the followings: (1) ICA allows a true 3-D CSDA of LTP, revealing spatiotemporal dynamic components;(2) superimposed or overlapped generators of LTP can be separated into individual generator so that we can examine their interacting dynamics;(3) applying Joint ICA, the LTP-free signals (i.e., local field potentials, LFPs) and the LTP-present LFPs can be simultaneously compared to identify unique spatiotemporal components responsible for LTP; (4) no conductivity values are required in the analysis.

In order to test the functionality of ICA toward the analysis of LTP, we have acquired LTP from a hippocampal slice of the rat brain using a MEA system. Then CSDA is done using (1) the Laplacian transform, (2) ICA, and (3) Joint-ICA. Their results have been compared to the spatiotemporal dynamics of raw data. The results presented in this work demonstrate that ICA can reveal the independent dynamics of current sources and sinks for LTP which could not be obtained by the conventional CSDA techniques. The results thus suggest that ICA and Joint-ICA could be an effective tool in studying the dynamic mechanisms of LTP measured on MEA. Some preliminary results have been presented in [8]

II. METHODS

A. Organotypic Culture of Rat Hippocampal Slices

In preparation of hippocamal slices for organotypic culturing, Sprague-Dawley rats (7 days old) were decapitated and their brains were quickly removed under sterile conditions. After isolation of the hippocampi, their dorsal halves were sectioned transversely at 400 μm using a tissue slicer (Mickle Laboratory Engineering Co., Surrey, UK). Slices were placed in chilled HBSS-medium (LB 003-01, Sigma, St. Louis, MO, USA) with 20 mM HEPES (H-4034, Sigma, St. Louis, MO, USA). The slice was placed on a membrane insert (polytetrafluorethylene membranes, 0.4 μm, Millicell-CM, Millipore Co., Bedford, MA, USA), which was set into the 6-well plates filled with 750 μl of culture medium. The medium was replaced every 3 or 4 days. The cultured slices were used in experiments after 14 days in an incubator at 36 °C with 5% CO_2. Fig. 1 (a) shows a cultured slice of hippocampus and four distinctive regions of hippocampus are labeled: namely CA_1, CA_2, CA_3, and Dentate gyrus (DG) respectively.

B. MEA System and LTP Recording

The MEA system used in this study includes electrode array, stimulator (STG1004, Multi Channel Systems MCS GmbH, Germany), amplifier (MEA1060, Multi Channel Systems GmbH, Germany), temperature control units, and data acquisition hardware and software [9]. The array has active 60 electrodes in an 8 x 8 layout grid with electrode diameters of 30 μm and inter-electrode distance of 200 μm with coating of porous titanium nitride (TiN) to minimize the impedance. The amplifier was placed in a Faraday cage with a laboratory-made ground system that is consisted of a four-core copper connecting wire (~7 mm diameter) and a buried three-meter metal bar (1 cm diameter). Fig. 2 (a) shows a typical setup of the MEA system and (b) the layout of multiple electrodes on a hippocampal slice.

The recording of LFPs, evoked by a biphasic pulse of 20~40 μA and 120~160 μs duration at the Schaffer collateral pathway in the stratum radiatum of the CA1 region, was made in

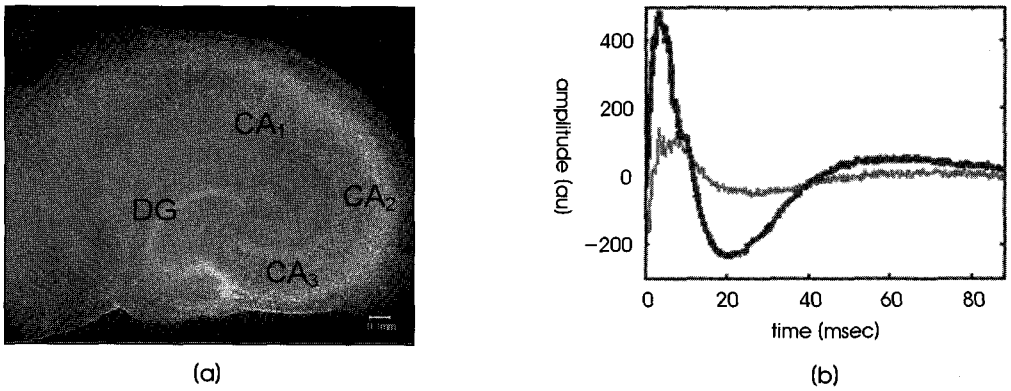
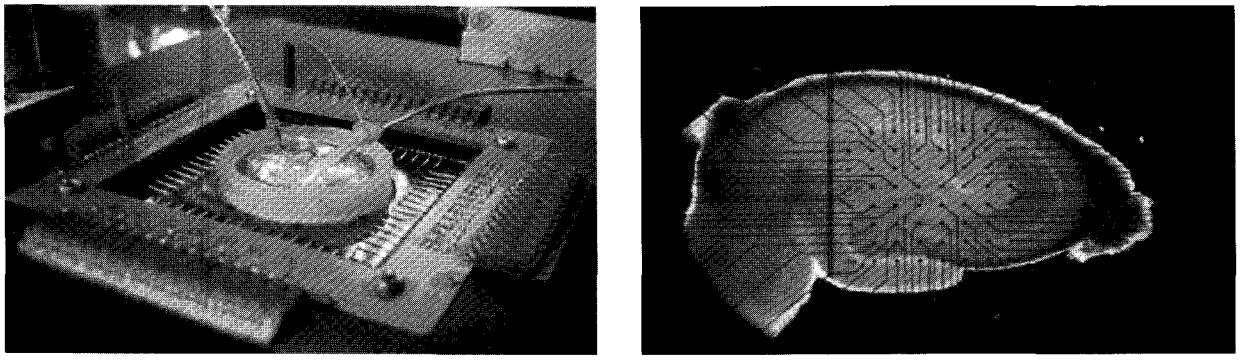


Fig. 1. (a) Organotypic cultured slice of hippocampus. (b) Local potential field from the CA1 region of the hippocampus without LTP in red (lower magnitude; thin line) and with LTP in blue (higher magnitude; thick line). Note the amplitude is shown in an arbitrary unit (au).



(a) (b)

Fig. 2. (a) A view of the MEA system setup. (b) The layout of multi-electrode array on a hippocampal slice.

the CA_1 region. Data were sampled from every channel at 25 kHz and recorded using the Recorder-Rack software (MCS, GmbH). During stimulation, the stimulating channel was disconnected.

In order to induce LTP in the CA1 region, Theta burst stimulation (TBS), consisting of trains of 10 x 100 Hz bursts (4 pulses/burst) with a 250 ms inter-burst interval at the test pulse intensity, was applied to a microelectrode (indicated using a white dot in Fig. 3 (a)) after recording baseline synaptic responses (i.e., baseline LFPs). As a result, a new LFP (i.e., LTP) appeared from hippocampal neurons after TBS. Fig. 1 (b) shows the LFPs measured at one channel before and after the induction of LTP. The LFP in red with lower amplitude are before TBS (i.e., LTP free) and the LFPs in blue with higher amplitude show LFP after TBS (i.e., LTP).

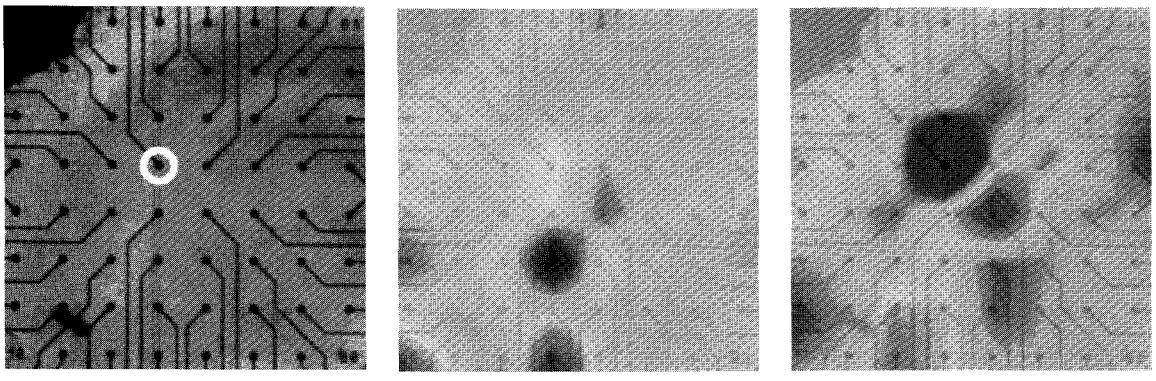
Figs. 3 (b) and (c) show the demonstrative maps of spatial maps of LTP-free and LTP-present LFPs respectively. The maps were created by interpolating the measured potential values at the sixty microelectrodes in an 8 x 8 array. Fig. 3 (a) is used as a background. It is clear that LTP is characterized by higher potential values and broader spatial activities of current generators. Also multiple current sources and sinks are clearly noticeable in the maps of Figs. 3 (b) and (c).

C. Current Source Density Analysis: the Laplacian Transform

The most commonly used technique to identify locations and relative magnitudes of current generators (i.e., sources and sinks) is the Laplacian transform [1-4]. Before MEA became available, 1-D CSDA was employed for the measurements with individual microelectrodes [1,2] and this was expressed as,

$$I_x = -\sigma(\phi_{x-x'} - 2\phi_x - \phi_{x+x'})/4x'^2 \qquad (1)$$

where I_x is the current at x, ϕ is the extracellular potential at each location, and σ is the tissue conductivity (possibly tensor). As described, it involves the approximation of the second spatial derivative of the potentials. The limitation of this approach is



(a) (b) (c)

Fig. 3. (a) Hippocampal slice and overlaid MEA electrodes, (b) A spatial map of LTP-free LFP (1.24 msec after a stimulation onset), and (c) A spatial map of LTP-present LFP (4.44 msec after the stimulation onset). A significantly increased synaptic activities are noticeable in the CA1 regions of the hippocampus. Note the white dot in (a) indicates a microelectrode where stimuli were delivered.

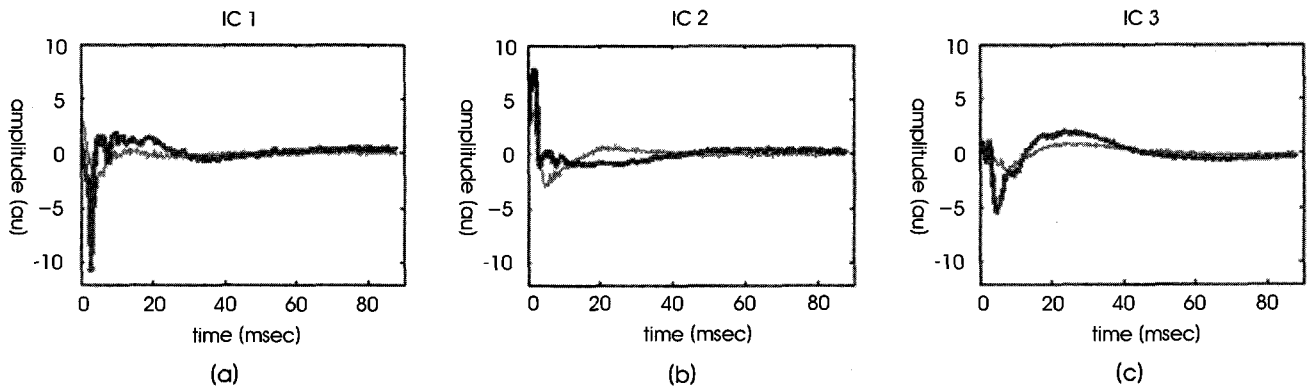


Fig. 4. Temporal independent components of LFPs: without LTP in red (lower amplitude; thin line) vs. with LTP in blue (higher amplitude; thick line)

that the repeated measurements must be made at different locations and multiple sites cannot be examined simultaneously.

However, with the introduction of MEA, for the first time visualization of the evolution of LTP in space and time became possible. Naturally, the Laplacian technique is extended to 2-D, allowing continuous 2-D CSDA as attempted in [4] and can be expressed as,

$$I_m = -(\sigma_x \nabla_x^2 \phi + \sigma_y \nabla_y^2 \phi + \sigma_z \nabla_z^2 \phi) \quad (2)$$

where ϕ indicates the potential values.

One of the major limitations of the Laplacian transform is its need for precise tissue conductivity values. Since electrical properties of tissue are highly anisotropic, the transform is subject to some errors. Other limitations include (1) the transform is sensitive to noise. Because of the second-order spatial derivative, this noise component could be amplified and it possibly obscures true current sources and sinks; (2) our measurements of LFP show that there are multiple current generators are involved in time and space, but the Laplacian cannot separate these current generators. In fact, the generators of LTP were described as alternating current sources and sinks [1,2,12]. What is desirable for more accurate analysis is to separate these current generators into their dynamic temporal and spatial components.

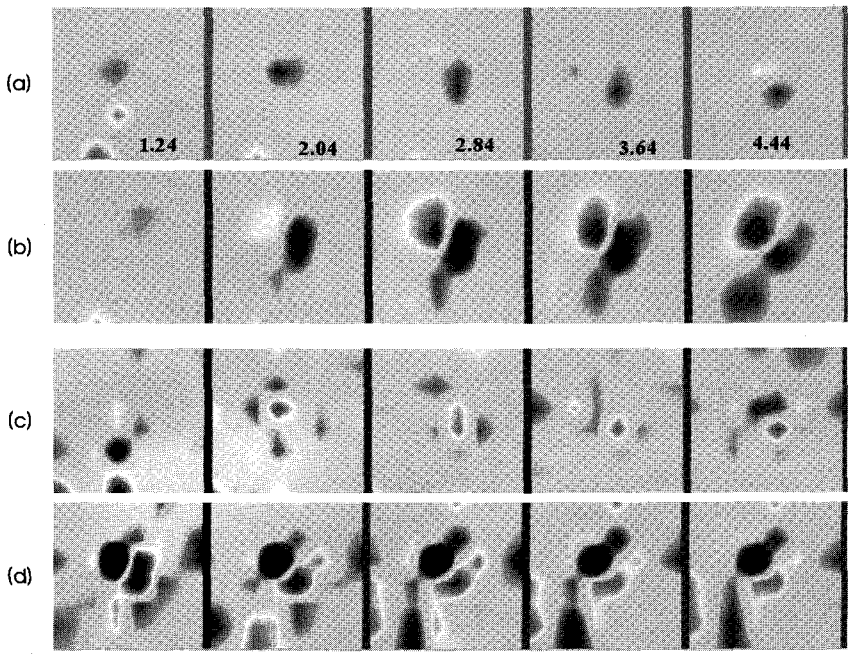


Fig. 5. 2-D CSD maps of LFPs. (a) Raw spatial maps of LTP-free LFP, (b) Raw spatial maps of LTP-present LFP, (c) Laplacian CSD maps of (a), (d) Laplacian CSD maps of (b). Note the numbers indicate time in msec after the stimulation onset.

D. Independent Component Analysis

ICA is such a technique which allows unmixing of overlapped activities of current generators under assumptions that the MEA potential measurements are made by linear mixing of the underlying activities. Therefore the MEA measurements of LFP, X can be expressed as,

$$X = AS \tag{3}$$

where A is a mixing matrix of sources in each row of S. ICA finds the unmixing matrix of A, such that S can be recovered from X (i.e., the technique produces temporally independent component activations and their fixed spatially varying patterns).

Its successful applications to electro-physiological data can be found in various fields [5-7] and its excellent review is available in [7]. The technical details of ICA are referred to the review [7] and our previous paper [5] since it is not the focus of this work.

In this work, we assume that LFPs measured at sixty microelectrodes are a linear mixture of current generators and the number of underlying source activities are less than the number of electrode which is a critical requirement for ICA.

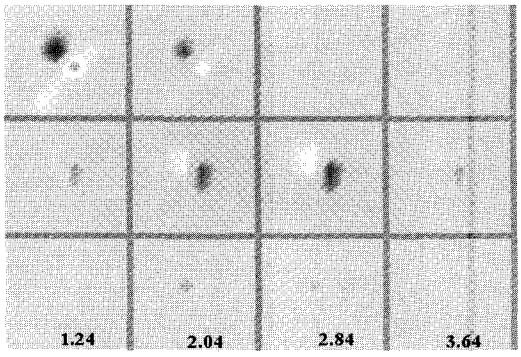
ICA routines are offered through various implementations of the technique and some implementations are readily available from the web [10,11]. In this study, we have utilized our Mixture Density ICA routines [5] for CSDA, although other versions of ICA routines can be utilized as well.

In addition to the capability of ICA to identify the mutually independent components of current generators, ICA can be used to analyze multiple LFPs simultaneously as successfully performed for EEG event-related potentials in [6]. This joint use of ICA (i.e., Joint-ICA) allows the simultaneous comparison of LTP-free and LTP-present LFPs and produces common and unique components of current generators of LTP.

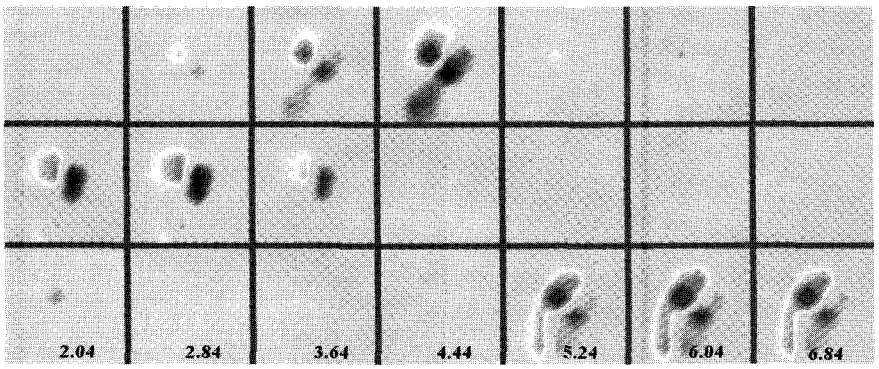
A toolbox has been implemented by incorporating the ICA routines with a functionality of CSDA for a single set of LFP data and a set of LTP-free and LTP-present data [8].

III. RESULTS

In order to compare the efficacy of ICA-based CSDA, we first created the spatial maps of LFPs before and after LTP induction at multiple time locations. The maps were created again by interpolating 8 x 8 measurements into 100 x 100 using the bi-cubic interpolation technique. Secondly, we



(a)



(b)

Fig. 6. 2-D CSD maps of ICs. (a) CSD maps for the LTP-free LFPs. Each row shows the spatial activity changes of the ICs given in Fig. 4 in red. (b) CSD maps for the LTP-present LFPs. Each row shows the spatial activity changes of the ICs given in Fig. 4 in blue.

applied the 2-D Laplacian CSDA and created the spatial maps at the same time instances. Finally, to identify underlying ICs responsible for LFPs, the LFPs of hippocampus before and after the LTP induction were simultaneously processed using Joint-ICA.

Fig. 4 shows three temporal ICs obtained from LFPs without LTP and with LTP. The second IC seems to be common in the early phase, but in the late phase, the source and sink seem to be reversing. The first and third ICs show much faster and intensified source and sink activities for LTP. Three ICs were chosen since the reconstructed signals with those ICs can explain more than 95% of variance of the original signals. This means that the extracted three com- ponents are the major components of the original signals and represent the original signals sufficiently.

The spatial dynamics of current generators are given in Fig. 5 from the raw measurements in Fig. 1 (b). Figs 5 (a) and (b) show the spatial dynamic changes in time for LTP-free and LTP-present LFPs. Much extended and intensified patterns are clearly shown in Fig. 5 (b) 2msec after the stimulation onset.

The spatial CSD dynamics after the Laplacian transform of Figs. 5 (a) and (b) are shown in Figs. 5 (c) and (d) respectively. Although the transform seems to reveal more complex dynamics of current sources and sinks, their understanding might be more difficult. Note the numbers in the maps indicate time in msec and the reversed colors for sources and sinks. The conductivity values in the Laplacian CSDA were assumed to be uniform.

In Fig. 6, the dynamic spatial maps of the three ICs are given. Each row indicates the spatial dynamics of each IC in time. The analysis by Joint-ICA revealed a common temporal IC in the early phase (1.24~3.64 msec) of both LTP-free and LTP-present LFPs (i.e., IC No. 2, second row), showing similar spatial source-sink dynamics, but only differing in their magnitude.

LTP-specific temporal ICs were found in the later phase (>3.64 msec), showing extended spatial activities and dynamic flow of sources and sinks. The sources and sinks responsible for IC No. 1 (first row) lasted only a few msec, but those for IC No. 3 lasted for a prolonged period of time, suggesting that the prolonged LTP are produced by these current generators.

IV. DISCUSSION

Recent CSDA study on spreading depression in hippocampal organotypic culture [12] indicates that depression accompanies reversing activities of current sources and sinks. Our observation of LTP also exhibits much similar changes of current sources and sinks. However, the raw and Laplacian CSDA maps cannot show the dynamic interactions of these reversing sources and sinks: rather they show overlapped current sources and sinks, making it difficult to understand the dynamic behavior of these generators.

In contrast, ICA or Joint-ICA seems to separate these complex dynamics into mutually independent current sources and sinks such that their interactions can be investigated. As shown in Fig. 6, the contributing sources to LTP were divided into three: two of them seem to be transient sources only lasting 2~3 msec (e.g., first and second components), but the last component shows a prolonged activity of the current generators that lasted over a long period (e.g., lasting up to 28 msec). Our speculation on these identified maps is that the first two transient components reflect the short-term memory mechanism. However, the last component reflects the long-term memory that has been facilitated by LTP. In fact, it was shown that LTP in the Schaffer collateral pathway involves two associated mechanisms (i.e., cooperativity and associativity) in learning which leads to Explicit Memory requiring conscious recall. This has been also used a direct evidence for Hebb's rule. [13]. Further investigation will be needed to elucidate these observations and validate our analysis results. On this matter, we are looking for collaboration with neurobiologists.

Nevertheless, our proposed analysis methodology for MEA data clearly demonstrates its own advantages over the conventional analysis methods. One limitation of the ICA approaches can be its validity for CSDA. However its successful applications to EEG or MEG source analysis provides enough evidence for the effectiveness of the technique. In the future work, we plan to investigate more LTP cases. One difficulty remaining is that inter-case studies present some problems since the recordings cannot be made from the exact same regions from each individual hippocampal slice and experimental conditions vary with organotypic culturing of the brain slices. However, we have noticed the stable recordings within the intra-slice measurements.

V. CONCLUSION

The results we presented in this paper indicate that ICA or Joint-ICA is capable of identifying spatiotemporal dynamic components of LTP which are mutually independent in time and space. Also the proposed methodology overcomes some of the limitations exhibited by the conventional CSDA methods. Although the presented results are still preliminary and more cases must be studied, we believe that the methodology should be useful to elucidate the cellular and network mechanisms of LTP by studying the separated components of LTP generators.

REFERENCES

[1] J. S. Taube and P. A. Schwartzkroin, "Mechanisms of Long-Term Potentiation: A Current-Source Density Analysis," *Journal of Neuroscience*, vol. 8, no. 5, pp. 1645-1656, 1988.

[2] I. Vida, J. Czopf, and G. Czeh, "A current-source density analysis of the long-term potentiation in the hippocampus," *Brain Research*, vol. 671, pp.1-11, 1995.

[3] U. Egert, B. Schlosshauer, S. Fennrich, W. Nisch, M. Fejtl, T. Knott, T. Muller, and H. Hammerle, "A novel organotypic long-term culture of the rat hippocampus on substrate-integrated multielectrode arrays," *Brain Research Protocoles*, vol. 2, pp. 229-242, 1998.

[4] K. Shimono, M. Taketani, F. Brucher, D. Kubota, L. Colgin, S. Robertson, R. Granger, and G. Lynch, "Continuous two-dimensional current source density analysis of electrophysiological activity in hippocampal slices," *Neurocomputing*, vol. 38-40, pp. 899-905, 2001.

[5] J. Jeong, T.-S. Kim, S. Kim, and M. Singh, "Application of Independent Component Analysis with Mixture Density Model to Localize the Brain Alpha Activity in fMRI and EEG," *Int. J. Imaging Technology and Systems*, vol. 14, pp. 170-180, 2004.

[6] S. Makeig, T.-P. Jung, A. J. Bell, D. Ghahremani, and T. J. Sejnowsky, "Blind separation of auditory event-related brain responses into independent components," in *Proc. Natl. Acad. Sci.*, vol. 94, 1997, pp. 10979-10984.

[7] J. J. Christopher and W. H. Christian, "Independent component analysis for biomedical signals," *Physio. Meas.*, vol. 26, no. 1, pp. 15-39, 2005.

[8] J. Lee, S. Hwang, D. Lee, S. Lee, J. Park, and T. Kim, "Spatiotemporal analysis of hippocampal long term potentiation using independent component analysis," in *Proc. World Congress on Biomedical Engineering*, Seoul, Korea, 2006, pp. 3405 -3408.

[9] http://www.multichannelsystems.com

[10] http://www.cis.hut.fi/projects/ica/fastica

[11] http://www.cnl.salk.edu/~tewon/ica_cnl.html

[12] P. E. Kunkler, R. E. Hulse, M. W. Schmitt, C. Nicholson, and R. P. Kraig, "Optical current source density analysis in hippocampal organotypic culture shows that spreading depression occurs with uniquely reversing currents," *Journal of Neuroscience*, vol. 25, no. 15, pp. 3952- 3961, 2005.

[13] T. V. P. Bliss and T. Lomo, "Long-lasting potentiation of synaptic transmission in the dentate gyrus of the anesthetized rabbit following stimulation of the perforant path," *J. Physiol (Lond)*, vol. 232, pp. 331-356, 1973.