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

Multi-neuron Action Potentials Recorded with Tetrode are not Instantaneous Mixtures of Single Neuronal Action Potentials

Yasushi Shiraishi, Norihiro Katayama, Tetsuya Takahashi, Akihiro Karashima and Mitsuyuki Nakao, Member, IEEE

Abstract— **Multiunit recording with multi-site electrodes in the brain has been widely used in neuroscience studies. After the data recording, neuronal spikes should be sorted according to the pattern of spike waveforms. For the spike sorting, independent component analysis (ICA) has recently been used because ICA has potential for resolving the problem to separate the overlapped multiple neuronal spikes. However the performance of spike sorting by using ICA has not been examined in detail. In this study, we quantitatively evaluate the performance of ICA-based spike sorting method by using simulated multiunit signals. The simulated multiunit signal is constructed by compositing real extracellular action potentials recorded from guinea-pig brain. It is found that the spike sorting by using ICA hardly avoids significant false positive and negative errors due to the cross-talk noise contamination on the separated signals. The cross-talk occurs when the multiunit signal of each recording channel have significant time difference; this situation does not satisfy the assumption of instantaneous source mixture for the major ICA algorithms. Since the channel delay problem is hardly resolved, an ICA algorithm which does not require the instantaneous source mixing assumption would be appropriate for use of spike sorting.**

I. Introduction

In the brain, it is assumed that information is represented by a spatio-temporal pattern of ensemble neuronal activity (e.g. cell assembly). Therefore, simultaneous recordings of multiple neuronal activities are essential for the study of information processing in the brain. For this purpose, recordings of extracellular action potentials generated from multiple neurons (multiunit) with multi-site electrode such as tetrode have been widely used (Fig. 1) [1]. Since the multiunit signal contains multiple neuronal activities near the electrode, signal processing including detection of neuronal spikes and sorting of action potentials to the spike-generating neurons is required. Recently, many spike sorting methods based on pattern classification technique have been proposed. The method sorts the multi-channel action potential signals according to the pattern of spike waveforms. Thus the pattern classification-based method hardly separates overlapped neuronal spikes due to distortion of spike waveform, which are recorded when neighboring neurons excite synchronously. Since the synchronized neuronal activity is believed to play an important role in the brain information processing, solving the spike overlapping problem would be essential.

One of the methods to solve the spike overlapping problem is inverse filter method [2]. However the construction of the filter requires template spike waveforms, other spike sorting process should be performed before the application of this method.

Another method of resolving the overlapping problem is independent component analysis (ICA) [3]. Unlike inverse filter method, ICA can separate overlapped signals without prior information about the multiunit signals. However, there are some assumptions to be satisfied, e.g. statistical independence of the source signals, and the mixing process of the source signals. Thus, violation of the assumption could degrade the performance of spike sorting by using

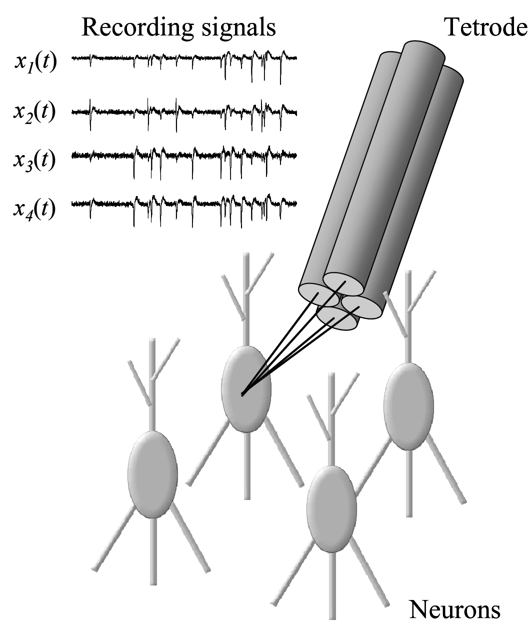

Fig 1. A schematic diagram of extracellular action potential recording from multiple neurons with a tetrode (a bundle of 4 wire electrodes). Each recorded signal is a mixture of extracellular action potentials generated from several neurons near the tetrode.

Manuscript received April 23, 2009. This research was partially supported by the Grant-in-Aid for Scientific Research (C) (No. 19500256), and the Grant-in-Aid for Scientific Research on Priority Areas-Elucidation of neural network function in the brain- (No. 20021001) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Y. Shiraishi, N. Katayama, T. Takahashi, A. Karashima, and M. Nakao are with Biomodeling Laboratory, Department of Applied Information Sciences, Graduate School of Information Sciences, Tohoku University, Japan (corresponding author (YS) to provide Phone/fax; +81-22-795-7159; e-mail: yasushi@biomdl.ecei.tohoku.ac.jp).

978-1-4244-3296-7/09/$25.00 ©2009 IEEE

ICA; this point has not been examined in detail so far.

In this study, we investigate the performance of spike sorting of multiunit signals by using ICA. It is demonstrated that the most of neurons cannot be precisely sorted just by applying ICA algorithm to the multiunit data. It will be suggested that the deficit of the separation is mainly caused by the violation of assumption for ICA regarding the mixing process of single neuronal activities.

II. Methods

A. Independent Component Analysis

Assume that independent n-source signals (neuronal spikes, $\mathbf{s}(t)=[s_1(t),\ldots,s_n(t)]^T$) are recorded with an m-channel electrode ($\mathbf{x}(t)=[x_1(t),\ldots,x_m(t)]^T$), where T indicates transpose and t indicates time. If the transformation from $\boldsymbol{s}$ to $\boldsymbol{x}$ is an instantaneous linear mixture, the following relationship holds:

$$\mathbf{x}(t) = \mathbf{A}\mathbf{s}(t), \qquad (1)$$

where $\mathbf{A}=(a_{ij})$ is a m-by-n matrix whose elements are constant real numbers. If $m = n$, and $\mathbf{A}$ is a regular matrix. the system is regarded to satisfy complete condition. In this paper, we investigate the case of complete condition. ICA is the methods to estimate unmixing matrix $\mathbf{W}$ based on the independence of the source signals:

$$\mathbf{y}(t) = \mathbf{W}\mathbf{x}(t) \quad , \qquad (2)$$

where $\mathbf{y}(t) = [y_1(t)\ ,\ \ldots\ ,\ y_n(t)]^T$ is unmixed signal, each component of which represents single neuronal activity. If $\mathbf{W}=\mathbf{A}^{-1}$, the source signals are completely reconstructed. However, since ICA cannot resolve ambiguity of amplitude and permutation of source signals, this relation generally does not hold. In this study, fastICA [4] and InfoMax ICA with natural gradient algorithm [5] were used to estimate unmixing matrix.

B. Generation of Simulated Multiunit Signals

To evaluate the performance of spike sorting, it is preferable to control the number of source signals because the number of sources are critical for ICA. In addition, the data of correct answer of spike separation would be useful for evaluating the accuracy of spike sorting. Thus, simulated multiunit data was used. The multiunit data were constructed as follows. First, extracellular action potentials of multiple neurons were recorded with a tetrode (a bundle of four stainless steel wire electrodes) in the CA3 field of guinea pig hippocampus [6]. Action potentials (spike) of neurons were detected and extracted from the signal by the threshold method [7]. Extracted spike waveforms were sorted by the conventional pattern classification-based method [1]. To improve the signal-to-noise ratio, spike waveforms were averaged at every sorted neuron. Finally, the averaged spike waveforms were linearly superposed and added independent white-noise as same as actual extracellular signals to obtain a simulated multiunit signal. Each set of simulated multiunit signals were composed of four single neuron's activities, i.e., the number of source signals equals to that of recording channels (=4). Each neuron was assumed to fire randomly and independently at a firing rate of 50 Hz.

C. Crosstalk attenuation ratio

To evaluate the performance of spike separation, cross-talk attenuation ratio (CTAR) [8] was calculated. CTAR for the i-th component is defined as follows:

$$\mathrm{CTAR}_i = 10 \log \frac{\frac{1}{T}\sum_{t=0}^{T}\left|y_i(t)\right|^2}{\sum_{j\neq i}\left(\frac{1}{T}\sum_{t=0}^{T}\left|y_j(t)\right|^2\right)}, \qquad (3)$$

where T indicates recording period. In this study, a single neuronal activity is assumed to be sorted correctly from other neuronal activities if and only if CTAR is nonnegative.

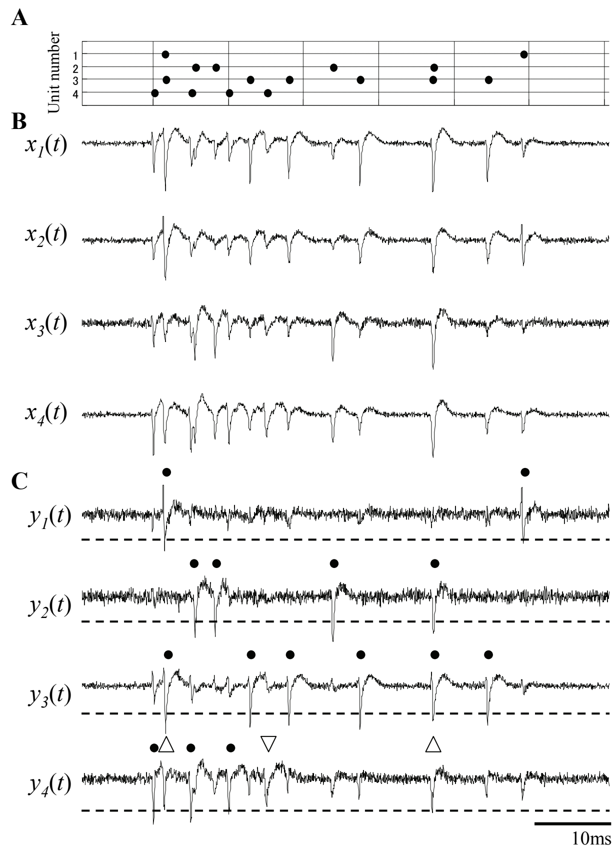

Fig. 2: Example of simulated multiunit data and single neuronal signals obtained by using ICA. (A) Spike timing of four neurons included in the simulated multiunit data. (B) Test signals (**x**) constructed by the superposition of single neuronal action potentials recorded with a four-channel electrode (tetrode) in guinea-pig hippocampus. (C) Separated neuronal signals (**y**) obtained just by using fastICA. Dashed lines indicate the signal level for spike detection by the threshold method. Symbols near the spikes indicate correct answer (black dot), false positive (upward triangle), and false negative (downward triangle) spikes, respectively.

D. Evaluation of time difference between recording channels

If an *m*-channel signal is an instantaneous mixture of multiple sources, the signal emitted from a single source of each channel would be identical except for the amplitude. This implies that distribution of the data (signal) in the *m*-dimensional space has a linear-shaped structure. Contrary, if there was delay between the channels, the distribution should have a circular or complex shape. Therefore, the linearity of the distribution of the multi-channel signal represents how well the instantaneous mixing assumption is satisfied.

The shape of data distribution can be characterized by principle component analysis. In this paper, we used contribution ratio of the first principal component (PC1) to quantify the linearity of the shape of data distribution, because PC1 increases as the shape of trajectory becomes more linear. The contribution ratio of PC1 is defined as follows:

$$\text{(Contribution ratio of PC1)} = d_1 / \sum_{i=1}^{m} d_i \ . \qquad (4)$$

where d_i is the *i*-th eigenvalue of the covariance matrix of the data.

III. Result and Discussion

Figure 2 shows an example of simulated multiunit signals ($\mathbf{x}(t)$) and the separated components ($\mathbf{y}(t)$) obtained by using fastICA. Each component of $\mathbf{y}(t)$ is expected to represent a single neuronal activity, however, the components have significant cross-talk noise from other components except for $y_2(t)$. Due to the cross-talk noise; there are serious spike detection errors (open triangles). The similar result was obtained by using natural gradient ICA algorithm. Therefore, it is suggested that the simple application of ICA to multiunit signal could provide poor spike separation. To evaluate the performance of ICA for spike sorting quantitatively, we investigated the number of single neuron separated from 10 sets of simulated multiunit data by using natural gradient ICA algorithm and fastICA. It was found the average numbers of neuron separated by using fastICA and natural gradient algorithm were 2.8 and 2.5, respectively.

As mentioned in Sec. II-B, the simulated multiunit signals satisfy the independence of source signals and complete condition, i.e. the number of sources equals that of recording channels. Therefore, the error of the spike sorting by ICA is attributed to the violation of another assumption; the immediacy of mixing of source signals. We examined the satisfaction of the instantaneous mixing assumption. Figure 3 and 4 show typical spike waveforms of two neurons. As shown in the Fig. 3, the normalized spike waveforms of all the recording channels are almost identical. The trajectory of the extracellular action potential in signal phase space seems almost linear-shaped (Fig. 3C). The linearity of the trajectory evaluated by the contribution ratio of PC1 was

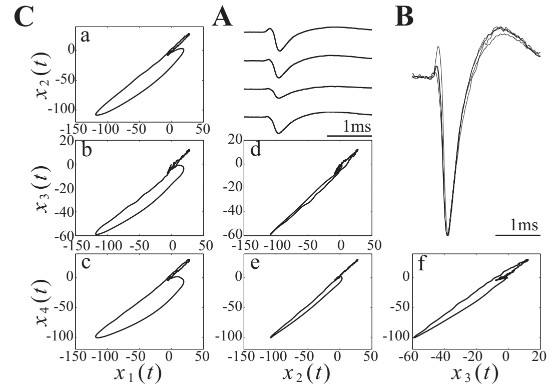

Fig 3. Typical extracellular action potential waveforms satisfying instantaneous mixing assumption. A: Averaged spike waveform. B: Superimposed waveforms of which amplitude is normalized. C: Trajectories of the extracellular potentials in the signal phase space. The contribution ratio of PC1 is 98.8%.

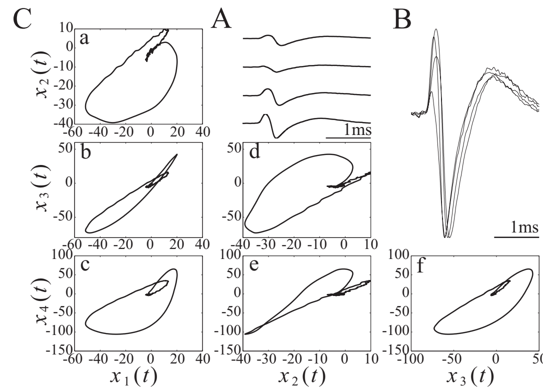

Fig 4. Typical extracellular action potential waveforms not satisfying instantaneous mixing assumption. A: Averaged spike waveform. B: Superimposed waveforms of which amplitude is normalized. C: Trajectories of the extracellular potentials in the signal phase space. The contribution ratio of PC1 is 91.6%.

98.8%. These data suggest that the delay between the recording channels for recording the extracellular action potential generated from this neuron is negligible. On the other hand, in the case of the neuron shown in Fig. 4, the peak times of the action potential are different between the channels. The trajectory of the recorded signal in the signal phase space has a circular-shape. The linearity of the shape of trajectory is relatively low (91.6%). These data suggest that the delay between the recording channels is not negligible for this neuron, i.e. the assumption of instantaneous mixture is violated in this neuron.

The effect of violation of the assumption on the performance of ICA-based spike sorting method was examined. Figure 5A shows multi-site recording data of four neurons recorded simultaneously. Except for neuron U3, linearity of the shape of trajectory is not high (<95%). Simulated multiunit signals were generated by compositing the action potentials and the data were sorted by using fastICA. Figure 5B shows the result of spike sorting. It was

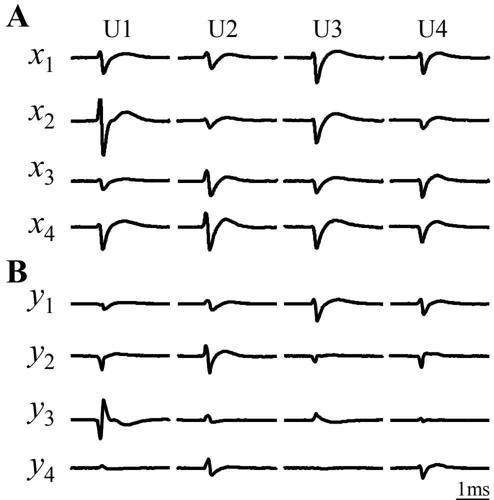

Fig. 5: (A) Example waveforms of extracellular action potentials simultaneously recorded from four hippocampal neurons with a tetrode. The neurons U2 and U3 are identical to the ones shown in Figs. 4 and 3, respectively. The contribution ratios of PC1 of U1-U4 are 93.4%, 91.6%, 98.8% and 92.5%, respectively. (B) A result of spike sorting of the simulated multiunit signals by using fastICA. The simulated multiunit signals was composed of single neuronal action potentials shown in A. There are significant cross-talk contaminations except for U3.

shown that only the signal originated from neuron U3 was concentrated on y_1 component. However, signals from other neurons were not separated well; in other words, significant cross-talk noise invaded to the other neuronal signals. The cross-talk would disrupt the spike detection and deficit the performance of spike sorting.

Figure 6 shows the histogram of the contribution ratio of PC1. The score of 34 out of 114 neurons was below 95 %, suggesting that the action potential of significant fraction of neuron are recorded at multi-site electrode with significant delay; they cannot be regarded as an instantaneous mixture of single neuronal action potentials. The results suggest that spike sorting by using ICA would have a serious problem regarding the accuracy due to the violation of assumption of instantaneous mixture of sources for ICA.

IV. Conclusion

In this study, we have demonstrated that the multiunit signals recorded by tetrode cannot be separated into single unit activity with considerable accuracy just by using ICA. It was found that the most of the single neuronal signals separated by ICA contain significant cross-talk noise from the other neurons, even if the number of neurons included in the recorded signals equals to the number of recording channels (i.e. complete condition is satisfied). It was revealed that the violation of instantaneous mixture of sources assumed in ICA algorithm caused significant cross-talk noise contamination.

According to the neurophysiological studies, dendrites of central nervous neuron exhibit complex spatio-temporal dynamics such as passive and active propagation of action

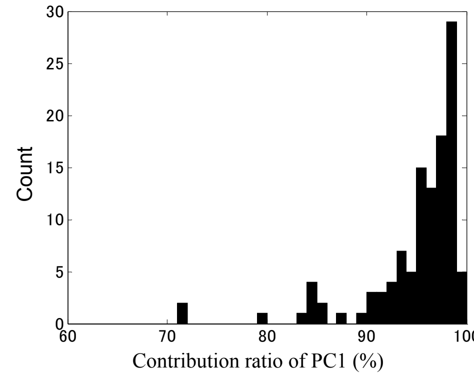

Fig 6. Histogram of the contribution ratio of PC1 (n=114).Mode of contribution ratio is 98-99 %. 29.8 percent (34/114) of neurons are below 95 %. It indicates more than half of neurons dissatisfy instantaneous mixing assumption.

potential making use of their linear and nonlinear electrical properties [9, 10]. Consequently, extracellular potential near the dendrite would also change in a complex manner. This implies that it is very hard to solve the problem regarding the time delay between the recording channels of multi-site electrode. Therefore, to improve the performance of spike sorting by using ICA, it is necessary to adopt an ICA algorithm which does not require the instantaneous mixing assumption.

References

[1] C. M. Gray, P. E. Maldonado, M. Wilson, and B. McNaughton, "Tetrodes markedly improve the reliability and yield of multiple single-unit isolation from multi-unit recordings in cat striate cortex," *J Neurosci Methods*, vol. 63, 1995, pp.43-54.

[2] R. Vollgraf, and K. Obermayer, "Improved optimal linear filters for the discrimination of multichannel waveform templates for spike-sorting applications", *IEEE Signal Proc Lett*, vol. 13, 2006, pp.121-124.

[3] S. Takahashi, and Y. Sakurai, "Real-time and automatic sorting of multineuronal activity for sub-millisecond interactions in vivo," *Neurosci*, vol.134, 2005, pp.301-315.

[4] A. Hyvärinen, J. Karhunen, and E. Oja, *Independent component analysis*, John Wiley & Sons, Inc, 2001.

[5] S. Amari, A. Cichocki, and H. H. Yang, "A new learning algorithm for blind signal separation," In: *Advances in Neural Information Processing Systems 8*, Ed., Touretzky, D., Mozer, M., and Hasselmo, M., MIT Press, Cambridge MA, 1996, pp. 757-763.

[6] O. Kikuchi, N. Katayama, A. Karashima, and M. Nakao, "Temporally correlated neuronal discharges induced by local glutamate application in the recurrent neural network of hippocampus in vitro," *Proc Intl Workshop Biosignal Interpretation (BSI)*, 2005, pp. 179-182.

[7] M. S. Lewicki, "A review of methods for spike sorting: the detection and classification of neural action potentials," *Network: Computation in Neural Systems*, vol. 9, 1998, pp. R53-R78.

[8] J. E. Flood, *Telecommunication Network*, IET, 1997.

[9] W. Rall, "Branching dendritic trees and motorneuron membrane resistivity," *Experimental Neurology*, vol. 1, 1959, pp. 491-527.

[10] G. J. Stuart, B. Sakmann, "Active propagation of somatic action potentials into neocortical pyramidal cell dendrites." *Nature*, vol. 367, 1994, pp. 69-72.