BLIND SEPARATION OF NEURONAL ACTIVITIES FROM OPTICAL IMAGING DATA

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ABSTRACT

We applied Independent Component Analysis(ICA) to data of neuronal activities in the brain acquired by optical imaging method. We constructed a model of noise and membrane potential mixed sources, and using suitable parameters detected from its simulation based on ICA, succeeded in estimating activities of neurons embedded in optical imaging data from hippocampal slices. Comparing our results with those obtained by the averaging set of data popular in the application of optical imaging method, it was empirically that our method could extract neuronal activities in the brain by one-shot data without averaging, which may provides a more precise method for the analysis of the complex mechanisms of the brain.

1. INTRODUCTION

It is commonly accepted that the brain is a complicated dynamical information system composed of many neurons. Recently, opitcal imaging method has been developed[8]. It is capable of recording a lot of membrane potentials in cerebral cortex simultaneously. We can measure many neuronal activities simultaneously with this method, and also can investigate the brain as a dynamical information system. When we use the optical imaging method, an important problem is how we can separate neuronal activities and noise from data recorded by optical imaging. Generally, it is difficult to remove noise from data recorded by optical imaging completely, so usually we compute an average from a number of trials to detect them. Taking an average is of practical use to separate neuronal activities and independent noise. Although we can detect neuronal activities that respond in every trial by this method, it is difficult to investigate a characteristic acitivity that appear only in a unique trial. There is some possibility that this unique neuronal activity plays an important role in the brain composed of neurons with synaptic plasticity[1][11][12]. Also, one recorded unit is not always correspondent to an activity of one neuron.

The main purpose of this paper is to extract single neuronal activities from optical imaging data by Independent Component Analysis(ICA)[7] having the ability to separate independent components from their mixtures.

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2. OPTICAL IMAGING

2.1. Preparation

The experiments were performed on transverse hippocampal slices(400μm) taken from guinea-pig (300-350g). The slices was stained for 10 minutes with 0.2mg/ml RH155 in normal medium.

Stimulation was delivered to the Schaffer commissural-collateral(SC) fibers linking CA1 and the area of optical imaging covered CA1 area. The position of the stimulating electrode and the optical imaging area are shown in Fig.1. Signals were recorded from adjacent 4 units. One stimulus(single pulse, 18msec after onset, duration of 0.2ms, intensity of 0.5mA) was applied. The diameter of one unit(14μm) is the same as the diameter of cell body(between 10 and 20μm).

2.2. Optical apparatus

Slices were observed through an optical imaging system, a signal detection system with high spatial reso-

lution by 128 !_ 128 square array of MOS image sensor. The voltage-sensitive dye signals were recorded with an 700±30nm interference filter. Transmitted light was detected by a 128 !_ 128 square array of photodiodes, 1.75 !_ 1.75mm^2 each. The sampling time was set to 0.6ms, which is time resolution of our system (0.6ms/frame). Usually sixteen trials are averaged to improve further the signal to noise ratio of the image in the camera system(HR Deltaron 1700 system; Fujifilm Microdevices), this paper deals with data without averaging.

These conditions indicate that one unit recorded by optical imaging does not always correspond to one neuron. We assume that the recording area can be regarded as Fig.2.

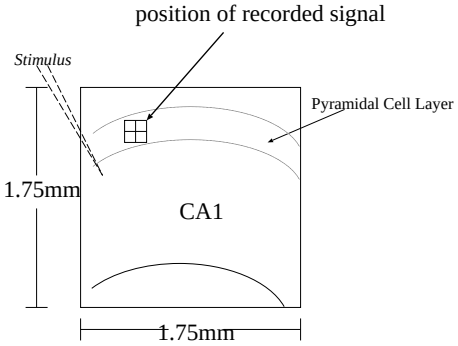


Figure 1: Recording area in hippocampal slice

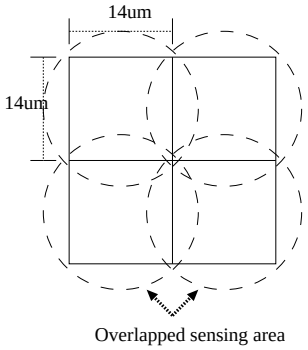


Figure 2: Recording site by photodiode array: one square corresponds to one unit, one circle is an area that is recorded by a photodiode.

3. PROBLEM FORMULATION

In this paper, we consider the standard linear data model.

$$\mathbf{y} = \mathbf{A}\mathbf{s} \tag{1}$$

Here the column vector $\mathbf{s}$ is called the independent component vector. Each component is mutually and statistically independent source signals. Vector $\mathbf{s}$ corresponds to neuronal activities or noise. Vector $\mathbf{y}$ corresponds to data recorded by optical imaging.

It is important to satisfy the following three conditions.

1. Recorded signals are superimposed.
2. The number of recorded signals is equal to that of independent components.
3. Matrix $\mathbf{A}$ is full rank.

Because data recorded by optical imaging are mixtures of independent membrane potentials and noise, the condition 1 is satisfied. One recorded unit does not always correspond to one neuron. Thus, selecting the suitable set of recorded unit, the condition 2 is satisfied. We separate neuronal activities from each adjacent four units selected from optical imaging data. Accordingly we assume that the condition 3 is satisfied.

4. METHOD

In this section, we briefly describe the algorithm used in our work.

4.1. Prewhitening

In consideration of roundoff error, we make use of the following singular value decomposition as prewhitening. Let n be the number of recorded signals, m the number of sampling points and m !_ n matrix $\mathbf{Y}$ describing all of recorded signals.

First, we subtract the mean from recorded signals, and apply singular value decomposition to matrix $\mathbf{Y}$.

$$\mathbf{Y} = \mathbf{U}\mathbf{D}\mathbf{V}^T \tag{2}$$

where $\mathbf{U}$ and $\mathbf{V}$ are upper and lower triangular routines and $\mathbf{D}$ is a diagonal matrices with d_i as the ith element.

Next we calculate decorrelation matrix $\mathbf{C}$.

$$\mathbf{C} = \mathbf{V}[diag(\frac{1}{d_i})] \tag{3}$$

Using this decorrelation matrix $\mathbf{C}$, we whiten data recorded by optical imaging.

$$\mathbf{Y}^* = \mathbf{C}\mathbf{Y} \tag{4}$$

4.2. Independent Component Analysis

To achieve the goal to separate neuronal activities from data recorded by optical imaging, it is necessary that

ICA has the ability to separate them fast and accurately. Thus we introduced natural gradient algorithm[2] for ICA. Moreover, considering nonstationarity of neuronal acitivity, we adopted nonholonomic constraint[3]. The learning parameter η is a small constant. The diagonal matrix Λ has each element of $E[\psi(y^*)y^{*T}]$. The initial value of $\mathbf{W}$ is the unit matrix.

$$\Delta\mathbf{W} = \eta(\mathbf{\Lambda} - \mathbf{E}[\psi(\mathbf{y}^*)\mathbf{y}^{*T}])\mathbf{W} \quad (5)$$

The optimal $\psi(y^*)$ is to choose

$$\psi(\mathbf{y}^*) = -\frac{d\ log\mathbf{r}(\mathbf{y}^*)}{d\mathbf{y}^*} \quad (6)$$

if we can estimate the true source probability distribution $\mathbf{r}(\mathbf{y}^*)$ adaptively[5]. However, we can not estimate the source probability distribution of independent component. Thus, in the following section we determine $\psi(y^*)$ experimentally.

We use a batch gradient for stability reasons. In spite of this, the algorithm may suffer from convergence issues in large-scale problems. So, whitening is applied as a preprocessing step for improving the convergence properties.

$$\mathbf{W} = \mathbf{UV} \quad (7)$$

After learning, we derived independent components $\mathbf{S}$.

$$\mathbf{S} = \mathbf{WY}^* = \mathbf{WCY} \quad (8)$$

The distribution of the intensity of indepedent components takes the form:

$$p_i(t) = \mathbf{W}^{-1}(0, ..0, s_i(t), 0, ...0)^T \quad (9)$$

We estimated each location of independent components $(X_k, Y_k)(1 \leq k \leq n)$ to calculate the center of valance using the result from Eq.9.

$$\begin{aligned} X_k &= \sum_{j=1}^{n} \frac{|max(\mathbf{p}_j) - min(\mathbf{p}_j)|}{\sum_{i=1}^{n} |max(\mathbf{p}_i) - min(\mathbf{p}_i)|} \times x_j \\ Y_k &= \sum_{j=1}^{n} \frac{|max(\mathbf{p}_j) - min(\mathbf{p}_j)|}{\sum_{i=1}^{n} |max(\mathbf{p}_i) - min(\mathbf{p}_i)|} \times y_j \quad (10) \end{aligned}$$

where $max(s_i)$ $(1 \leq i \leq n)$ is each maximum value, $min(s_i)$ $(1 \leq i \leq n)$ is each minimum value. The location of a photodiode(x,y) is x_j,$y_j(1 \leq j \leq n)$.

5. SIMULATION

We adopted the following five sample signals to tune ICA for optical imaging data. These signals have normalized kurtosis that is shown in Table 1. The number of sampling points is 200. The membrane potential was derived from Hodgkin-Huxley Equation[9].

Table 1: Kurtosis of Original Signals

Signal Number	Signal Type	Kurtosis
1	Membrane Potential	26.13
2	Line Noise	-1.49
3	Gaussian Noise	0.22
4	Membrane Potential	26.13
5	Membrane Potential	26.13

5.1. Selection of $\psi(y)$

There are several approaches to separate independent components from super-Gaussian and sub-Gaussian mixed sources [5],[6],[10]. Because each approach has different characteristics, it is necessary that we select a suitable $\psi(y)$ from them.

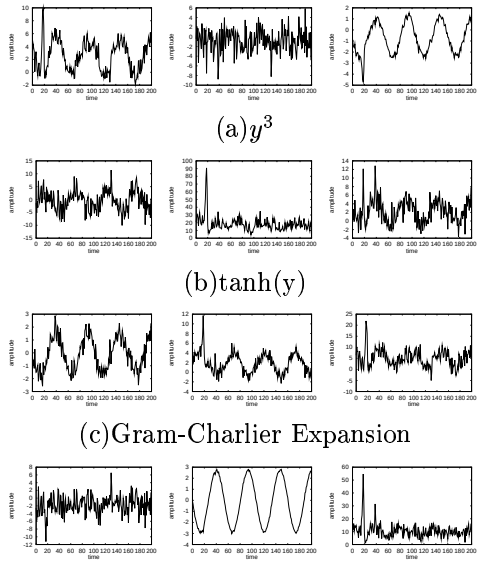
Comparing independent signals extracted by the following four analyses, we selected $\psi(y)$ which is suitable for our problem. Artificial signals are the mixture of signal 1,signal 2 and signal 3. Because the membrane potential of neuron never reverses, matrix $\mathbf{A}$ is set to positive normal random numbers. The number of iterations is 50,000.

1. $\mathbf{y^3}$[6](According to the stability analysis[4], if all signals are sub-Gaussian, this is an efficient function.)
2. **tanh(y)**[6](According to the stability analysis[4], if all signals are super-Gaussian,this is an efficient function.)
3. The function derived from Gram-Charlier expansion [5](Gram-Charlier expansion has an effective property if the probability distribution of independent components nearly corresponds to Gaussian.)
4. Extended Infomax[10](The switching parameter was derived from the stability analysis[4].)

One of the results separated by using these three $\psi(y)$ is shown in Fig.3. We assumed that recovered signals with maximum kurtosis was the membrane potential, and calculated the mean square error of kurtosis of the recovered membrane potential. Table 2 showed that **tanh(y)** had the most precisely approximated kurtosis. Fig.4 illustrates that the accuracy of recoverd signals, and indicated that **tanh(y)** had the most accurate recovering property. We thus adopted **tanh(y)** as the suitable $\psi(y)$ for this problem.

5.2. Combination of original signals

The number of independent components in real data is unknown. Thus we examined results separated from the following three mixtures. The number of iterations is 100,000. Matrix $\mathbf{A}$ is set to positive normal random numbers.



(a)y^3

(b)tanh(y)

(c)Gram-Charlier Expansion

(d)Extended Infomax

Figure 3: Recovered Signals

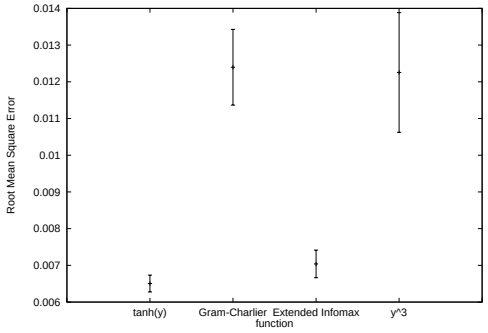


Figure 4: Accuracy of functions in the problem of predicted three signals

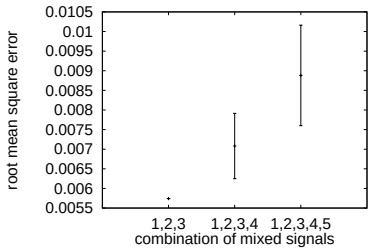


Figure 5: Accuracy of ICA in the problem of combination of predicted five signals

1. The mixture of signal 1, signal 2 and signal 3 (the number of independent components is less than that of recorded signals.)
2. The mixture of signal 1, signal 2, signal 3 and signal 4 (the number of independent components is equal to that of recorded signals.)
3. The mixture of signal 1, signal 2, signal 3, signal 4 and signal 5 (the number of independent components is greater than that of recorded signals.)

From the viewpoint of peak time, we found the membrane potential from independent components. Then we worked out the mean square error of the accuracy of the recovered membrane potential. These results indicated that the fewer the number of independent components was, the better the accuracy of recovered membrane potentials was.

Hence, to extract neuronal activities from data recorded by optical imaging, it is necessary that we arrange recorded units having fewer active neurons. We took sets of adjacent four units for recorded signals.

6. APPLICATION TO OPTICAL IMAGING

We applied the abovementioned method based on independent component analysis to the analysis of data recorded from hippocampal slices taken from guinea-pig by optical imaging.

Fig.6 shows the adjacent four signals selected from optical imaging data. Fig.7 illustrates independent components separated from these signals. The learning parameter η is set to 0.0001. The number of iterations is 100,000. Fig.8 indicates each estimated location of independent components which is derived from Eq.10.

The peak time of the independent component 4, shown in Fig.7, is 20.4 msec. This peak may have been evoked from a stimulus. Besides, the estimated location of independent component 4 is shown in Fig.8. We assumed that the independent component 4 was the membrane potential. Other independent components have random fluctuation. The estimated location of these components is the center of four units. Hence we assumed that the independent components 1, 2 and 3 were Gaussian noise.

We also applied our independent component analysis to other units. We found very similar results. Besides we could identify the results of separation, similar to the simulation. Fig.10 shows that the independent component 4 is nearly equal to 0. This result suggested that the number of independent components is three, similar to the result of the simulation in subsection 6.2.

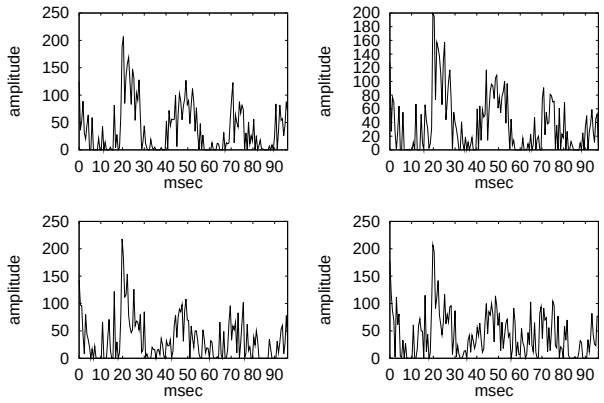
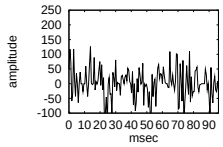
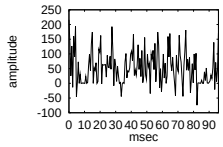


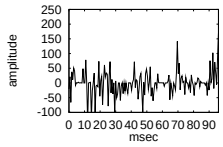
Figure 6: Original signals recorded from hippocampal slice using optical imaging method



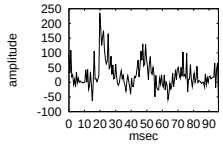
(a)Independent component 1



(b)Independent component 2



(c)Independent component 3



(d)Independent component 4

Figure 7: Independent components separated from original signals by independent component analysis

7. DISCUSSION

7.1. Effectiveness of the proposed method

In this paper, we used a stimulus that evoked one action potential. Hence, we can suppose that the peak time of the averaged membrane potential(20.4msec), which is shown in Fig.9, corresponds to that of action potential. In the previous section, we indicated that the peak time of the independent component 4, which was assumed to be the membrane potential, is 20.4msec. So the peak time of the averaged membrane potential was actually correspondent to that of the independent component 4. Moreover, other adjacent 4 units showed similar results.

The results of simulation suggest that the fewer the number of membrane potentials is, the better membrane potential are recovered accurately. Hence there is a good possibility that the independent component 4, shown in Fig.7, is the membrane potential of one neuron.

We suggest that if the proposed conditions in subsection 2.3 are satisfied, our proposed method is effective to separate neuronal activity from optical imaging data.

7.2. Advantage of the proposed method

In this subsection, we examine the feature of independent components extracted from data recorded by optical imaging method. We assume that the independent component contains a membrane potential and a periodic wave (40Hz). There may be some possibility that this periodic wave is line noise. However, from the independent component analytical point of view, the results of the simulation showed that the line noise was different from the membrane potential. We concluded that this periodic wave was a part of the membrane potential, and suggest that this was a gamma rhythm which is considered to be the cause of long term potentiation. This argument suggests that this proposed method gave us another explanation of optical imaging data.

8. CONCLUSION

The results presented in this paper indicate that we may investigate optical imaging data without averaging, using independent component analysis. Optical imaging method with voltage sensitive dyes had been used as an effective method for recording many neurons simultaneously. We can detect individual neuronal activity and it's location from these data. Thus we suggest that the proposed method is effective to explore the brain function.

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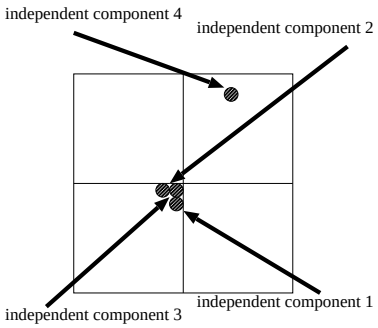


Figure 8: Estimated locations of independent components

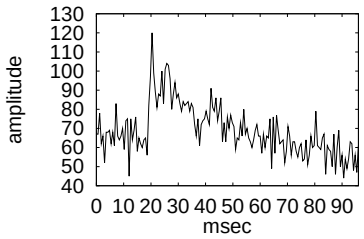
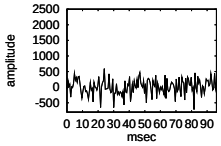
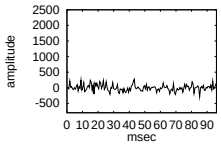


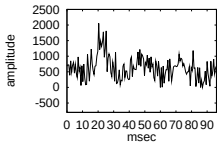
Figure 9: Averaged membrane potential at the same place as original signals(average of 16 trials)



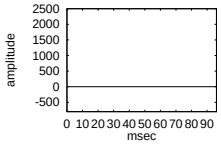
(a)Independent component 1



(b)Independent component 2



(c)Independent component 3



(d)Independent component 4

Figure 10: Independent components separated from original signals by independent component analysis

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