VARIANCE OF SPATIOTEMPORAL SPIKING PATTERNS BY DIFFERENT STIMULATED NEURONS IN CULTURED NEURONAL NETWORKS

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ABSTRACT

How a neuronal network of ambiguously behaving neurons establishes a highly reliable information processing system, distinct data communication, and organized communication links remains unclear. To solve this mystery, we must spatiotemporally analyze the spike trains in neuronal networks. In our previous study, we observed spike propagation as a cluster of excitation waves in simulated neuronal networks. We call this phenomenon spike wave propagation. In this paper, we attempted to observe spike wave propagation in cultured neuronal networks. In addition, we tried to calculate the dynamic time warping (DTW) distance of the temporal spiking forms spread from several different stimulated neurons in the cultured neuronal network. Using this distance, called the Inter DTW distance, we investigate whether stimulated neurons can be similarly identified in physiological neuronal networks. To this end, we subjected the same neurons to 5 stimulation events and calculated the DTW distances within the trials. The resulting distances, called the Local DTW distances, were significantly smaller than the Inter DTW distances, particularly for neurons far from the stimulated neurons. Moreover, the spatial patterns of the electrodes in this scenario were significantly different for different stimulated neurons. These results suggest that stimulated neurons can identify distant neurons by the spatiotemporal patterns in the network and that distinct data communications occur via multiple communication links in the brain.

Keywords: Cultured neuronal networks, Spike wave, Spatialtemporal patterns,

INTRODUCTION

The brain is recognized as an intellectual information processing system [1-5]. The neurons in a neuronal network exhibit ambiguous behaviors; yet, they establish highly reliable information processing system, form and store memories, communicate different data through organized communication links, and perform other high-level tasks. The mechanism by which the neuronal network accomplishes these feats remains unknown.

To resolve this question, we must analyze the spatiotemporal patterns of the spike trains in the neuronal network. The spatiotemporal form of spike activity is considered as the fundamental generator of natural intelligence in the brain [6-10]. Olshausen proposed a minimum network component for information processing in the brain [7]. Bell et al. attempted to extract the receptive field characteristics in a neuronal network simulated by ICA [8]. However, these methods analyze the static or spatial states of the network but not the temporal behaviors. Spatiotemporal analysis has been attempted in several other studies [9-10]. For example, in the Synfire Chain model proposed by Abel, neuron groups

synchronously fire with temporal patterns [11]. We have frequently observed this phenomenon in our own study. Takahashi et al. observed that some of the motor cortical neurons in non-human primates spatially coordinate their spiking activity, such that the activity resembles wave propagation in the beta oscillatory band of the local field potential [12]. However, the volley firing of neuron groups was not clarified in these studies. Therefore, the above research question has yet to be resolved.

In our recent study, we focused on distinct and different data communications in the brain. We cultured a neuronal network on an 8 × 8 array of multi-electrodes, applied one-shot electrical stimulations, and extracted brief sequence codes, which were greater than expected, from the spike trains [13]. Moreover, we showed the simultaneity of these code sequences in stimulations of pairs of electrodes [14]. From these results, we concluded that the flow of the code sequences reflects data communication in the brain. However, we did not clarify the generation of these sequence flows.

To investigate this question, we simulated neuronal networks on small two-dimensional (2D) meshes (9 × 9 and 25 × 25) and observed spike propagation as a cluster of excitation waves [15]. This phenomenon, which we call spike wave propagation, exhibited individual spatiotemporal patterns corresponding to the differences among the stimulated neurons. These results confirmed that the abovementioned code sequence flows were fragments of spike waves but not whether spike wave propagation is a physiological phenomenon.

Therefore, in this study, we analyzed the spatiotemporal patterns of stimulated spikes in several cultured neuronal networks. The observed spike wave propagation matched that of our previous simulation. Moreover, we compared the spatiotemporal spiking forms generated by different stimulated neurons in the cultured network, and found that stimulated neurons can identify distant neurons by the spatiotemporal pattern of the spike train. As these phenomena have not been discussed in previous studies, we here report the abovementioned results in detail and relate them to the distinction of different data communications in the brain..

METHODS

Cell cultures

Cell cultures of hippocampal neurons were dissected from Wistar rats on embryonic day 18. The procedure conformed to the protocols approved by the Institutional Animal Care and Use Committee of AIST. Hippocampi were dissociated with 0.1% trypsin (Invitrogen, Tokyo, Japan) in Ca2+- and Mg2+-free phosphate-buffered saline minus at 37°C for 15 min. The dissociated neurons were planted at a density of 3.3 × 105 cells/mm2 in polyethylentimine-coated MEA dishes (MED-P515A, Alpha MED Scientific, Kadoma, Osaka, Japan) with 8 × 8 planar microelectrodes. The size and spacing of the electrodes was (50 × 50) μm2 and 150 or 450 μm, respectively. To position the neuronal networks in the central area of each MEA dish, we used a cloning ring with an inner diameter of 7 mm. The ring was removed the following day. Neurons adhered to the substrate of the MEAs, covering all electrodes.

Neurons were maintained at 37°C in a humidified atmosphere of 5% CO2 and cultured for 21–40 days in Dulbecco's modified Eagle's medium (Invitrogen), which contained 5% horse serum and 5% fetal calf serum with supplements of 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 μg/ml insulin. Half of the culture medium was renewed twice per week. Figure 1 shows a micrograph of the cultured neurons in an MEA.

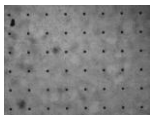


Figure 1 Micrograph of cultured neurons in an MEA (×20)

In this study, we prepared 3 cultured cell samples at 22–50 days in vitro (DIV) and named them Cultures 1–3.

Stimulated spike recording

Stimulated spikes were recorded by an extracellular recording system with 64 channels (MED64, Alpha MED Scientific). The recording was performed for 3 s at a sampling rate of 20 kHz. A selected channel (one electrode) was stimulated 5 ms after the start of recording. The stimulation signal was a current-controlled bipolar pulse (positive, then negative) with a strength of 10 uA and a duration of 100 us.

Two electrodes in each culture were selected as the stimulation electrodes, and both were subjected to 5 recordings. In this paper, the stimulated electrodes (stimulated neurons) are referred to as StimA and StimB.

This study investigates whether the original stimulated neuron can be identified in particular areas (including multi-neurons), rather than by single neurons. Therefore, spike sorting was not performed [27].

Coding spike trains

The recorded spike trains were coded as follows.
First, peaks above a pre-specified threshold on each channel [16] were detected in the recorded spike responses at a sampling frequency of 10 kHz, and raster plots were generated. The threshold was determined by trial and error. Experimentally, the most suitable threshold was determined as 5 times the RMS of the noise (~0.016–0.024 mV), which almost completely eliminated the noise while preserving the action potential. The suitability of this threshold was also noted in [16]. The spike interval trains were then calculated from the raster plot data.

Estimating the spatiotemporal differences in the spike forms
Temporal pattern of spike trains in each electrode

Classically, simultaneously generated different spike trains are analyzed by cross-correlation [17]. However, the lengths of the spike trains (number of spikes) propagated from StimA and StimB may differ (see Section 2.2). Moreover, the two electrodes may generate the same spike interval train with a time lag. These variations cannot be handled by classical methods such as cross correlation. Therefore, in this study, we estimated the differences between the temporal spike patterns propagated from StimA and StimB by dynamic time warping (DTW) [18], which is outlined below.
Given two sequence data SeqA and SeqB with

SeqA = (a1, a2, …, an); SeqB = (b1, b2, …, bn), the
DTW distance *D(SeqA,SeqB)* is calculated by Dynamic Programming as follows

$$D(SeqA, SeqB) = f(n, m)$$

$$f(t,i) = |a_i - b_i| + min\begin{cases} f(t,i-1) & \text{insertion} \\ f(t-1,i) & \text{deletion} \\ f(t-1,i-1) & \text{match} \end{cases}$$
$$f(0,0) = 0 \quad f(0,t) = f(t,0) = \infty$$

where SeqA and SeqB correspond to the spike interval trains propagated from StimA and StimB.

If SeqA and SeqB are equal, the DTW distance is zero. The larger the DTW distance, the greater the difference between SeqA and SeqB.
However, the characteristics of the spike interval train are influenced by factors such as the synaptic weight and refractory period. To resolve this problem, we conducted the following estimation experiment.

First, we made 5 recordings of the stimulated spikes at both stimulation channels (StimA and StimB) in each culture and calculated the DTW distances for combinations of spike temporal patterns in the trials of the same stimulated channel (Trial A1 vs Trail A2, Trial A1 vs Trail A3 …; see Figure 2). We call this distance the Local DTW distance. Next, we computed the DTW distances for combinations of spike temporal patterns in the trials of different stimulated channels (Trial A1 vs Trail B1, Trial A1 vs Trail B2 …). This distance was called the Inter DTW distance. Next, the Local DTW distances and Inter DTW distances were averaged, and the significance of their difference was assessed by a hypothesis test (t-test). The method is illustrated in Figure 2.

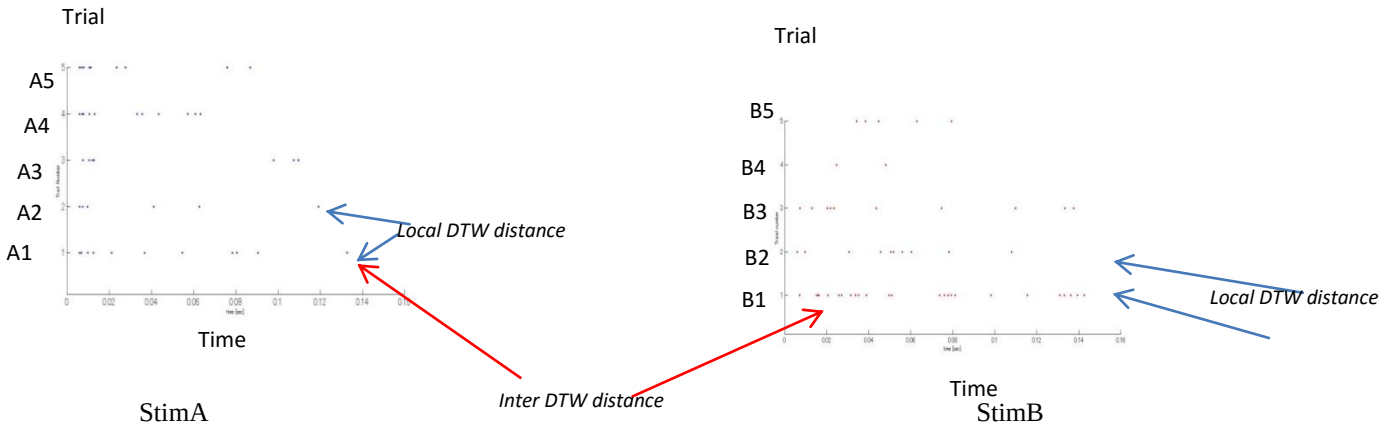


Figure 2 Illustration of Local DTW distance and Inter DTW distance

Figure 2 shows the raster plots (temporal spike patterns) at the stimulated channels StimA and StimB in each of 5 trials.

The DTW distances between pairs of spike interval trains in the same and different stimulation channel(s) are called Local DTW distance and Inter DTW distances, respectively. For 5 trials, the DTW distance list comprises 20 Local DTW distances and 25 Inter DTW distances.

Spatial pattern of hypothesis test results of neuron DTW distances

The hypothesis test results of the DTW distances in each electrode (neuron) were mapped onto an 8 × 8 2D array (see Figures 4 and 5 in the Results). The differences between the spatial patterns of this map were estimated for different stimulated neurons.

RESULTS

Figure 3 presents the raster plots (temporal spike patterns) of the 5 trials at ch(channel)19 under stimulation at ch8 (stim A) and ch57 (stim B) in Culture 1. The spike interval trains are seen to vary among the trials. Moreover, the Local DTW distance (2.82) and Inter DTW distance (11.0) were significantly different ($p < 0.05$; t-test). Presuming that such stimulated neurons might be distinguishable among all neurons in the network, we call these neurons distinguishable neurons.

Figure 4 shows the result of the hypothesis test between the averaged Local DTW distance and Inter DTW distance at every channel in each culture (10 trials). Although distinguishable neurons were localized in particular areas, these significant differences may have been accidental occurrences. To assess this possibility, we averaged 2 Local DTW distances (obtained in 2 sets of 5 trials at the same stimulated channel in the same culture). Figure 5 shows the hypothesis test result of this experiment in Culture 2. Distinguishable neurons are absent, implying that the significant differences in Figure 4 are actually caused by the various stimulated channels.

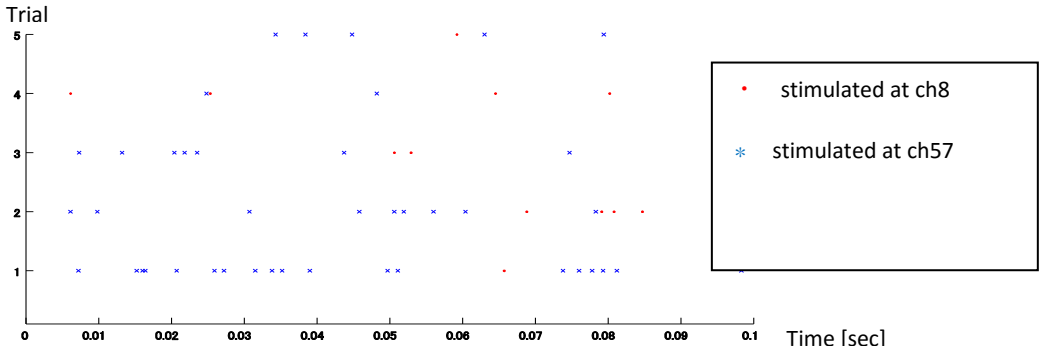
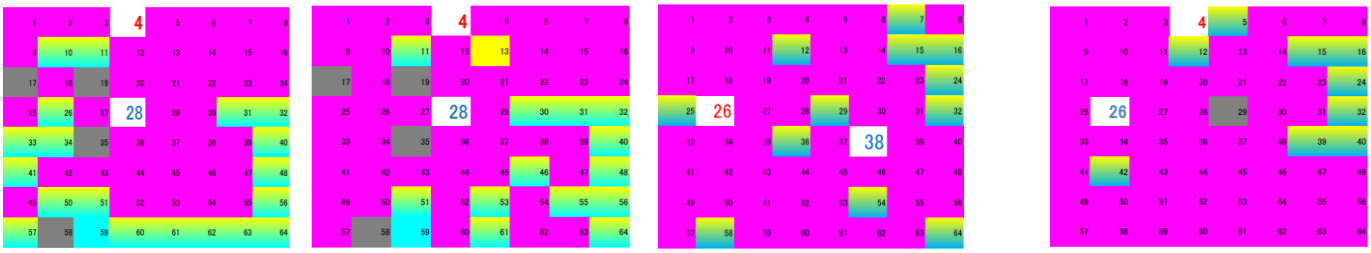


Figure 3 Raster plots (spike interval trains) of 5 trials at ch19 in Culture 1

Incidentally, these results are independent of electrode spacing, which differed between Culture 1 and Cultures 2 and 3.



Culture 1 (b) Culture 2 (c) Culture 3 (stim ch26 vs ch38) (d) Culture 3 (stim ch4 vs ch26)

Figure 4 Results of hypothesis test between averaged Local DTW distances and Inter DTW distances

Local DTW distance and Inter DTW distance are significantly different ($p < 0.05$) (*Distinguishable neurons*)

Spikes observed only from StimA.

Spikes observed only from StimB.

No significant difference between Local DTW distance and Inter DTW distance

Stim A Ch number is displayed in red font.
Stim B Ch number is displayed in blue font
Electrode spacing: 450 μm (Culture 1); 150 μm (Cultures 2 and 3)

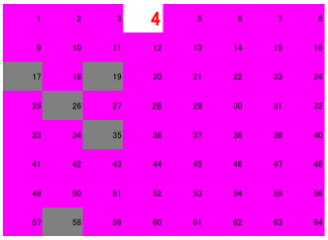


Figure 5 Results of hypothesis test between 2 averaged Local DTW distances (in Culture 2)

DISCUSSION

As shown in Figure 4, distinguishable neurons were observed in particular areas.
This result likely reflects the synaptic weight distribution in the network. In physiology, the neurons in a neuronal network are known to differ by their synaptic weights. Consequently, neurons respond differently to a stimulus and generate a variety of spike wave propagation routes. The spike wave propagation route (which links to the DTW distance) influences the temporal spiking patterns at the neurons.

Because the Local DTW distances are also non-zero, the synaptic weight distribution in a network clearly fluctuates in time. However, because the Inter DTW distance is significantly larger than the Local DTW distance, we consider that the DTW distance is chiefly influenced by differences between the stimulated neurons. This suggests that the variety of the synaptic weight distribution in each spike wave propagation route exceeds the time fluctuations of the synaptic weight distribution. Therefore, the sources of spike wave propagation (stimulated neurons) might be distinguished by several neurons, as observed in the simulation study (see Section 1).

Moreover, the distribution of distinguishable neurons alters when different neurons are stimulated in the same neuronal network (Figure 4(c) and (d)). This suggests that the origin of spike wave propagation can also be identified from the spatial pattern of the DTW distance.
We now link this phenomenon to the data communication mechanism in the brain, which is the primary focus of our study.

To clarify this mechanism, we generated the sound space shown in Figure 6(a). When several sounds are generated in different areas, their origins are correctly identified, even for sounds of identical pitch (frequency), timbre (waveform), and intensity (amplitude). These sound elements are influenced by the process of sound propagation (for instance, diffraction by obstacles, wind direction, and other conditions of the sound propagation route).

In neuronal networks, a stimulated neuron corresponds to a sound source, the form of the spike wave propagation corresponds to the sound propagation process, the neurons calculating the DTW distance correspond to human ears, and the synaptic weight distribution corresponds to the condition of the sound propagation route (see Figure 6(b)).

If the speakers in Figure 6(a) are now regarded as data sources, the field shown in this figure is equivalent to a data communication link established between the speaker and listener.

Comparing this field with the neuronal network in Figure 6(b), we note that spike wave clusters are equivalent to data communication links. Moreover, several neurons in the

network share multiple communication links, each distinguished by its spatiotemporal pattern of spike wave propagation at the received part of the neuronal network.

We regard this phenomenon as a multiplexed spatiotemporal communication. Previously, we constructed such a model in artificial neural networks [19]. Our current results suggest that multiplexed spatiotemporal communication is indeed a physiological phenomenon.

Importantly, as data communication underpins memory in the brain, our results show how a seemingly ambiguous neuronal network can establish a highly reliable information processing system.

(a)Identification of sound origins by a listener (b) Identification of stimulated neurons in a neuronal network

Figure 6 Analogy between identification of sound origins and identification of stimulated neurons

(a) Even when speakers A and B emit the same sounds, humans can identify their origins because sound propagation is affected by distance, diffraction by obstacles, direction of speakers and wind, and other factors
(b) indicates neurons, indicates stimulated neurons and indicates neurons in areas observing the spike propagation from stimulated neurons. Each neuron is connected to several other neurons with different synaptic weights. In the area marked , the stimulated neurons identify the spatiotemporal pattern by the synaptic weight distribution in the neuronal network, which alters the incoming pattern.

CONCLUSIONS

In this study, we compared the spatiotemporal patterns of spikes generated by two stimulated neurons in a cultured neuronal network. The variety of the spike interval trains was quantified by the DTW distance. The Inter DTW distance and Local DTW distance were significantly different ($p < 0.05$) at several neurons (called distinguishable neurons) in each of the cultured networks. Similar results were obtained in our previous simulation study.

Moreover, the spatial pattern of the distinguishable neurons in the neuronal network depended on which neurons were stimulated. This suggests that multiplexed spatiotemporal communication occurs in real neuronal networks.

The results of this study might solve one of the deepest mysteries of neuronal networks, namely how seemingly ambiguous behavior among neurons leads to a reliable information processing system.

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