Original Article

An Influence of Bit Timing Fluctuation in Neuron Spike Trains on M-sequences Detection

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Abstract: In our recent studies, we consider some M-sequences are generated to communicate data in neuronal networks. Then, there is a possibility the tempo of bit trimming fluctuate (*tempo fluctuation*) because no mechanism of tempo control of data communication as a clock system of computer is existed in brain.

In this study we analyze the correlation of *margin*, the rate of *tempo fluctuation*, with number of M-sequences from t he time course of stimulated action potentials in cultured neuronal networks Moreover, we tried to analyze the correlation of *margin* with number of M-sequences from simulated neuronal networks and compared with the result from cultured neuronal networks to investigate the reason of *tempo fluctuation*. As results, positive correlations were observed in cultured and simulated neuronal network with *tempo fluctuation*. Meanwhile, no correlation was observed in simulated neuronal network without *clock instability (CI)* which is slippage of clock period. We consider this result suggests that o one reason of *tempo fluctuation* was *CI*.

Keywords: Neuron, Spike, Clock Instability, Tempo Fluctuation , M-sequence

1. Introduction

The brain is recognized as a very large-scale network system in which the basic element is a neuron [1-5]. In recent studies of the memory mechanism in the brain, investigating a formation of information communication is more essential than specifying the region of memory in the brain [4].

To investigate what types of memory circuits are assembled and what types of codes are formed to control data communications in brain is essential [6~9].

We analyzed time series patterns of stimulated spike response in cultured neuronal networks [10-13] and detected some pseudo random sequences, especially 0,1 reversed M-sequences [14], above chance in our previous study [15].

In circuit theory, a binary counter with n-bit logical elements (registers) can count up to 2^n-1. With an adequate feedback link, the loop circuit becomes equivalent to a binary counter, the output of which becomes an M-sequence with length 2^n-1 and is called the period; here, "M" stands for maximum length. If this resulting M-sequence is used as an intrinsic code of its own loop, 2^n-1 loops can be discriminated. For example, a 3-stage linear feedback shift register (LFSR) generates a 7-bits period M-sequence like 1101001 [14].

We consider these results suggest that some equivalent LFSR circuits are assembled and some M-sequences are generated to communicate data in neuronal networks. Then the question arises as to how tempo (timing) of data communication is controlled in neuronal networks. In computer, tempo of data communication is controlled by exact clock period as shown in Figure 1(a). However, it is possible that *clock instability (CI)* which is slippage of clock period is caused (see Figure 1 (b)) because it seems that no mechanism of tempo control of data communication is existed in brain.

This phenomenon causes interference of sequences. For reasons of as described above, there is a possibility the bit timing fluctuation (*tempo fluctuation*) in sequence. Actually, some *tempo fluctuation* was observed in our previous study [15], however, the detail of generation mechanism of *tempo fluctuation* and the correlation between the magnitude *tempo fluctuation* and number of detected M-sequences are still unclear.

In this study, to investigate these questions, we analyze the correlation of the magnitude *tempo fluctuation* with number of M-sequences from the time course of stimulated action potentials in cultured neuronal networks. Cultured, small-scale neuronal networks on multi-electrode arrays (MEAs) are feasible for analysis of network assemblies. MEAs can be used to apply stimulation pulse into neurons with sufficient flexibility and have been used to identify functional connections in neuronal networks [10~13].

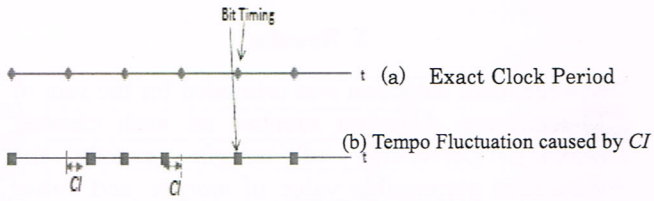


Figure 1 *Clock Instability (CI)* and *Tempo Fluctuation*

Now, we summarize definition of terms in this paper.

- *Clock Instability(CI)* … Slippage of clock Period
- *Tempo Fluctuation* … Bit timing fluctuation in sequence

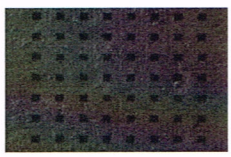
2. Methods

2.1 Cell culture and stimulated spike recording

Cell cultures of hippocampal neurons were dissected from Wistar rats on embryonic day 18 . The procedure was performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of AIST. Hippocampi were dissociated with 0.1% trypsin (Invitrogen , Tokyo, Japan) in Ca^{2+} -free and Mg^{2+} -free phosphate-buffered saline minus at 37°C for 15 min. The dissociated neurons were planted at a density of 3.3×10^5 cells/mm^2 in polyethylentimine-coated MEA dishes (MED-P515A, Alpha MED Scientific, Kadoma, Osaka, Japan) with 8 × 8 planar microelectrodes. The size of each electrode was 50 × 50 μm and the electrode spacing was 150 μm. To locate 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 that contained 5% CO_2 and cultured for 21–40 days in Dulbecco's modified Eagle's medium (Invitrogen) that 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.2 shows a micrograph of cultured neurons in a MEA.

In this study, we prepared 5 cultured cell samples at 22–50 days in vitro (DIV) and named them cultures 1–5



Black rectangles are electrodes. The size of each electrode is 50 × 50 μm and the electrode spacing was 150 μm.

Figure.2 Micrograph of cultured hippocampal neurons in a MEA

2.2. Stimulated spike recording

Stimulated spikes were recorded by an extracellular recording system with 64 channels (MED64, Alpha MED Scientific). The sampling rate of the recording was 20 kHz and the recording time was 3 s. Stimulation was applied at a particular channel (one electrode) 5 ms after the recording started. Stimulation was produced using a current-controlled bipolar pulse (positive, then negative) with a strength of 10 μA and a duration of 100 μs.

2.3 Detection of M- sequence

M-sequences in stimulated spike responses from cultured neuronal network were detected from raster plot of spike timing the same as [15].

Targets of M-sequences for analyze is shown in table 1. The period of all sequences are 7bits which correspond to period of M-sequences generated from 3 stage LFSR (M3, including Rev.M3 which reverse '0' ,'1') because only M-sequences whose period were 7bit were detected in [15]. The state was recognized as "1" if a spike existed; otherwise, it was recognized as "0." Then,
the raster plot was converted to a time course of binary data in order to investigate sequence patterns. All patterns include 3 or 4 numbers of bit '1'. Considering that the data communication must begin at state "1" because the start of communication could not be identified at state "0," the detection of sequence patterns were started from state "1."

Table 1. Targets of M-sequences for analyze

Pattern No.	Sequence Pattern							
3001	1	1	0	1	0	0	0	Rev. M3
3002	1	0	0	0	1	1	0	Rev. M3
3003	1	0	1	1	0	0	0	Rev. M3
3004	1	1	0	0	0	1	0	Rev. M3
3005	1	0	1	1	1	0	0	
3006	1	1	1	0	0	1	0	
3007	1	1	0	0	1	0	1	
3008	1	0	0	1	0	1	1	
3009	1	1	1	0	1	0	0	
3010	1	0	0	1	1	1	0	
3011	1	1	0	1	0	0	1	
3012	1	0	1	0	0	1	1	

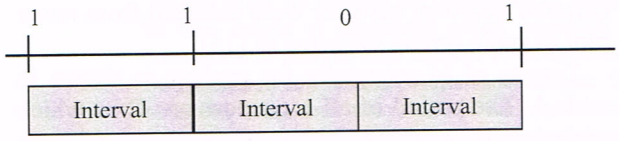
Interval of spikes is dispread variously. So it is crucial to determine the bin size of sequences detection. Some problems of fixed bin always exist [16]. To resolve these problems, the method of

sequences detection was improved as follows.

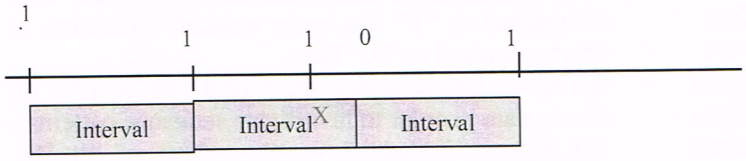
1) All spike intervals were detected before detecting sequence patterns.
2) The start time of sequence detection was '1'.
3) Sequence patterns were scanned with each spike interval with *margin*(see next section).
4) If state of '1' was existed in intervals, the detection was recognized fail.

The outline of sequence detection method was shown in Figure 3.

These analyzing programs were implemented by MATLAB.



(a) Detection was succeeded



(b) Detection was failed

Figure 3. Method of sequence detection

2.4 *Margin*

We called the allowing rate of *tempo fluctuation* when M-sequences were detected was *margin*.

The definition of *margin* was as shown in equation (1) and Figure 4.

$$margin = \frac{sl}{interval \times 6} \times 100\ \% \quad \cdots(1)$$

Where Sl corresponds to the slippage of interval and time stamp ofstate'1' and the denominator of equation (1) equals to the length of detected M-sequence (7bit pattern with 6 intervals).

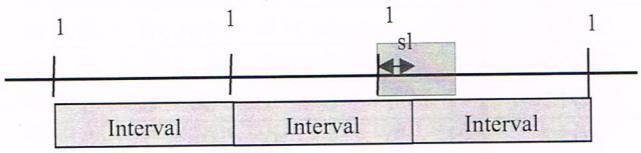
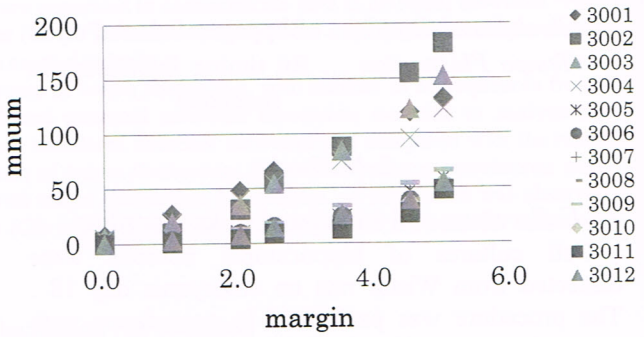


Figure 4. *Margin*

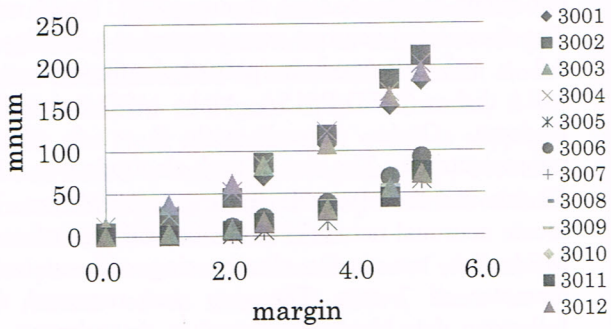
Spike allows to exist in the range indicated sky blue rectangle We detected sequences where the max permissible value of *margin* was 0,1,2,2.5,3.5 and 4.5 %.

3. Results

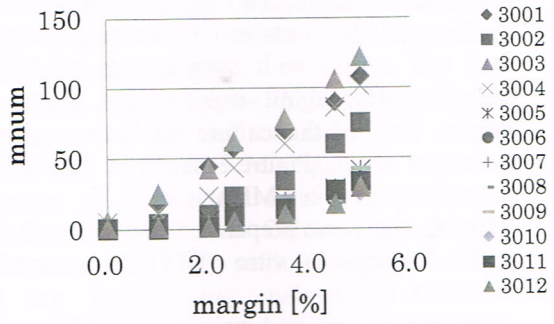
M-sequences detection was estimated for the sum of M-sequences detection number on each channel (*mnum*). Correlation of *margin* ,exactly the maximum permissible value of *margin*, and *mnum* of each sequence type was shown in Figure 5. As shown in these figures, positive correlation was observed in all sequence type.



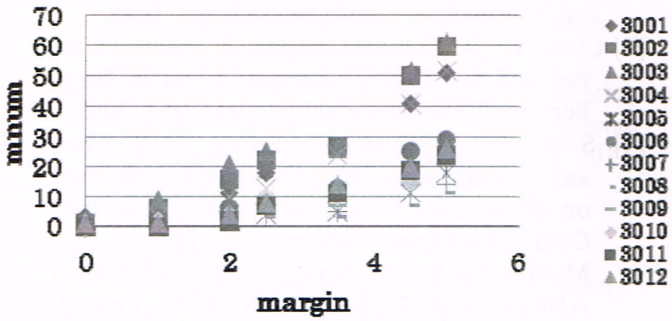
(a) Culture 1



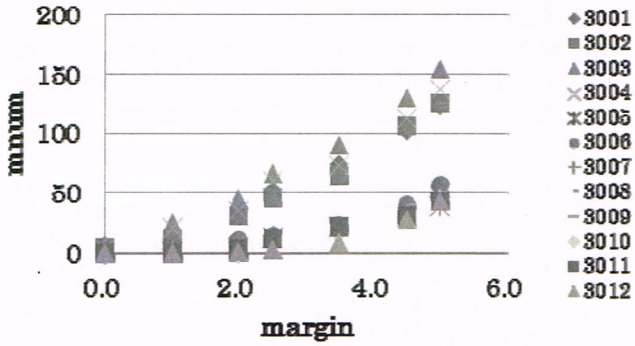
(b) Culture 2



©Culture3



(d) Culture 4



(e) Culture 5

Figure 5 Correlation of *margin* and *mnum*
(Cultured neuronal networks)

4. Discussions

As a analyze result from cultured neuronal networks, the number of detected sequences (mum) is influenced by *margin*. This result shows that *tempo fluctuation* exists in sequences. We assume that one reason of *tempo fluctuation* is *CI*. To prove our assumption, we tried to analyze the correlation of *margin* with number of M-sequences from simulated neuronal networks with different *CI* and compared the result with the result from cultured neuronal networks.

Simulated neuronal network was implemented in MATLAB (Math Works Japan. Tokyo, Japan).

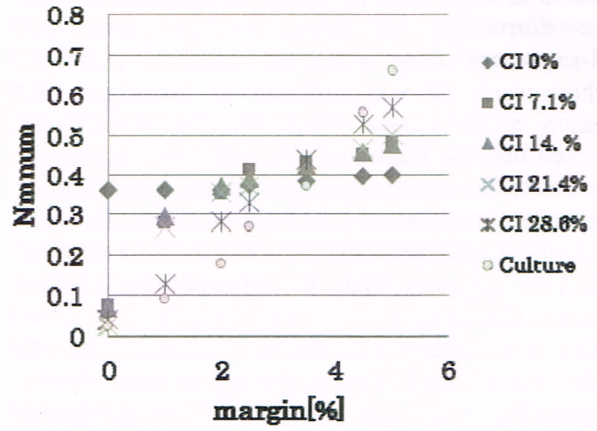
The network consists of n=2000 neurons. Each neuron is connected excitedly or inhibitedly to all other neurons randomly. *Clock period* of neuron connection control was 0.8ms and 5 type of simulated neuronal networks, the rate of *CI*=0%, 6%, 12%, 20%, 25%, were implemented. 64 neurons were selected to analyze spike timings similar to recording spike timing of cultured neuronal network on *MED Prob* [13,15].

Then, we compared the result with the result from cultured neuronal networks as follows. First, mnum was normalized by equation (2) to allow for neuronal networks (cultured and simulated) with different mnum owing to number of spikes, etc.

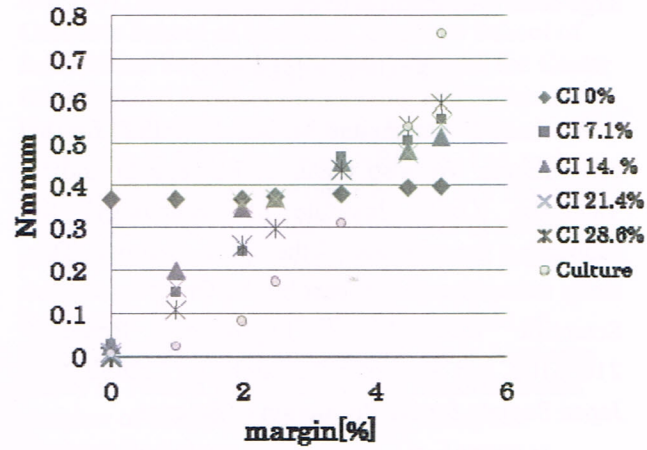
$$Nmnum = \frac{mnum_{ma}}{\sqrt{\sum_{ma}[mnum_{ma}]^2}} \quad \cdots(\ 2\ )$$

Where *Nmnum* is normalized *mnum* and $mnum_{ma}$ is sum of detected number of all Rev.M3 (Pattern No.3001-3004) or Non. Rev.M3 (Pattern No.3005-3012, see Table 1) when *margin* =*ma*. Then maximum value of *mnum* was normalized 1.

Next, the correlation of *margin* and normalized sum of *mnum* of all sequence types *(Nmnum)* was analyzed shown in Figure 6.



(a) Rev.M3



(b) Non Rev.M3

Figure 6 Correlation of *margin* and *Nmnum*
Where *Nmnum* of Culture was calculated from mean

mnum of all cultures

As results, when *CI* was 0% (no t*empo fluctuation*), there was no correlation of *mnum* and *margin*.

While, when t*empo fluctuation* was not 0% (with t*empo fluctuation*), there was correlation of *mnum* and *margin* and few numbers of sequences were detected when *margin* was 0% as cultured neiroral networks.

We consider this result suggests that our assumption that one reason of *tempo fluctuation* was *CI* was proved.

5. Conclusion

In this paper, we analyzed the correlation of *margin* with number of M-sequences from the time course of stimulated action potentials in cultured neuronal networks. As results, positive correlation was observed in each culture and *tempo fluctuation* was caused in sequences. Moreover, we tried to analyze the correlation of *margin* with number of M-sequences from simulated neuronal networks whose each *CI* was different to investigate the reason of the *tempo fluctuation*. As results, we proved that one reason of this phenomenon was *CI*. However, the shape of *margin*-mnum correlation of simulated neuronal networks did not so match up to that of cultured neuronal networks even *CI* was not 0%.This suggests that it is possible that other reasons also exist.

Our future work is to do more detail analyze to solve the reason of *tempo fluctuation* more clearly, especially, why he shape of *margin*-mnum correlation of simulated neuronal networks did not so match up to that of cultured neuronal networks .

We consider results of our study are applicable to large section of medical engineering.

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