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Fundamental short-term memory of semi-artificial neuronal network

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Abstract— **Spatiotemporal pattern of neuronal network activity is a key component of brain information processing. Cultured rat hippocampal neurons on the multielectrodes array dish are suitable for analyzing and manipulating network dynamics and its developmental changes. We applied paired electrical inputs at various inter-stimulus intervals (ISI) and analyzed the spatio-temporal pattern of evoked responses. We found that the pattern of evoked electrical activity was affected by existence of a prior input in the case that ISI of paired stimuli was within 2 s. These results suggest that a semi-artificial neuronal network on a culture dish has a fundamental component of short-term memory, and the origin of this hysteresis is transition among the internal states of the network, undertaken by synaptic transmissions.**

I. Introduction

Recently biological components are focused on as materials for regenerative medicine or bio-integrated systems. Especially, a living neuronal circuit is recognized as a target for both of biological research and biomimetic engineering. Cultured rat hippocampal neurons form a complex network on a multi-electrodes array (MEA) dish, and spontaneous electrical activity has been observed [1-3]. Fig. 1 shows examples of the MEA dish and cultured neurons on the MEA dish. Neurons keep biological and electrical features even in such semi-artificial condition [4,5]. The spontaneous activity pattern in hippocampal neurons changes depending on culture days, and the network structure is modified by mutual interaction between neurons [6,7]. Especially, extreme High Frequency Bursts (HFBs) of action potentials were transiently observed around 40 Days In Vitro (DIV). Developmental changes of synchronized electrical activity have been confirmed also in the rat cortical culture system [8, 9]. The phenomenon is a common feature of reconstructed network on a culture dish. As for temporal structure of the firing-pattern, the bursting activity was reported to be observed from DIV 14 to DIV 30, though the bursting activity changed to be fragmented over DIV 30 [10, 11]. Neuronal network has various "state" in spontaneous activity, which influences on the spike activity pattern evoked by an electrical stimulation [6,12]. We applied a single electrical stimulation and paired stimuli, expecting to change the spatiotemporal pattern of spontaneous electrical activity, which compose internal states of the network. As a result, we observed hysteresis of living neuronal network, in other words, a kind of fundamental short-term memory in semi-artificial living neuronal network.

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II. Material and Method

A. Cell Culture

Rat hippocampal region of brain was cut off from Wistar rat on embryonic day 18 (E18) [13-15]. Hippocampal neuronal cells were dissociated by 0.175% trypsin (Invitrogen-Gibco, U.S.A.) in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS^-) supplemented with 10 mM glucose for 10 min at 37℃ . Neuronal cells were cultured on a MEA dish (MED probe, alpha MED scientific Inc, Japan)[16,17]. The MED probe has 64 planer microelectrodes on the bottom, and the length of one side of an electrode is 50 μm. The distance between electrodes (center to center) was 450 μm. The density of seeded cells was 7800 cells/mm^2. Rat hippocampal neurons were dissociated and placed in a cloning ring with an inner diameter of 7 mm arranged on the center of a MEA dish. Culture medium consists of 45% Ham's F12, 45% Dulbecco's modified minimum essential medium (Invitrogen-Gibco, U.S.A.), 5% horse serum (Invitrogen-Gibco, U.S.A.), 5% fetal calf serum (Invitrogen-Gibco, U.S.A.), 100 U/ml penicillin, 100 μ g/ml streptomycin (Invitrogen-Gibco, U.S.A.), and 5 μg/ml insulin (Sigma-Aldrich, U.S.A.).

The conduct of all experimental procedures was governed by the "Kwansei Gakuin University Regulations for Animal Experimentation".

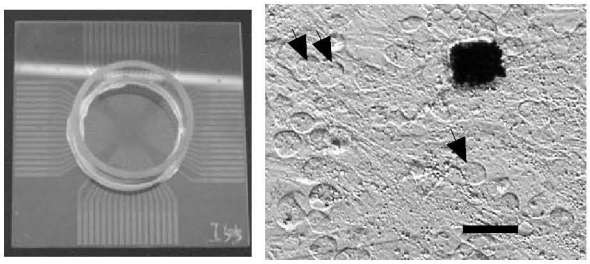

Figure 1. Left picture indicates an example of MED probe . Right picture indicates an example of differential interference contrast micrograph of dissociated cells (black arrowhead, for example) and an electrode (black square). Black line indicates 50 μm.

B. Electrophysiological experiments

Neuronal electrical activity was recorded as extracellular potential by 64 microelectrodes on the bottom of a MED probe. The recording was performed by the MED64 integrated system (Alfa-MED scientific Inc, Japan). A/D

conversion of measured signals was carried out at a sampling rate of 20 kHz and quantization bit rate of 16 bit. The system was controlled by recording software, MED Conductor 3.1 (Alfa-MED scientific, Japan). We applied sequential shot of electrical stimuli to the neuronal network cultured on a MEA dish via a selected electrode. Electrodes with ability to evoke the largest number of spikes was selected as stimutionelectrodes. A biphasic current pulse was used for evoking spikes. The duration and amplitude of stimulation was 0.1 ms/phase and 10 μA, respectively. The interval of the paired electrical stimuli was set to 1 s 2 s, 2.5 s, 5 s and 10 s. Electrical stimuli were applied 3 times at a sweep. First stimulation, stim1 was a single stimulation with an enough inter-stimulus-interval (ISI, more than 60 s) for recovering state of network activity. Then a paired stimuli, stim2 and stim3, with short ISI were applied. This experimental scheme of the sweep was performed repeatedly for 30 times. Electrical spikes were detected and analyzed, using MEDFAUST software, developed in our Lab. MEDFAUST detects synaptic events, according to amplitude and slope of spikes. Peaks with amplitude and slope larger than the thresholds are detected as spike events. Refer details of algorithm to previous paper [18].

C. *Analysis of neuronal activity patterns*

Electrical activity patterns within a certain time window (in our case, 100 ms) constitute a feature vector of 64 spike numbers detected at each electrode. The feature vectors were synthesized repeatedly. To elucidate similarity between the spatiotemporal patterns of evoked activity, we calculated Euclidian distances between feature vectors of electrical spike patterns during a recording time. Assume $\mathbf{p}=(p_1, p_2 \cdots p_{64})$ and $\mathbf{q}=(q_1, q_2 \cdots q_{64})$ are focused two feature vectors, and the Euclidean distance between p and q is given by:

$$D(\mathbf{p},\mathbf{q}) = \sqrt{\sum_{i=1}^{64}(p_i - q_i)^2}$$

III. Results and Discussions

Paired stimulation evoked activity in different spatiotemporal patterns with activity evoked by a single input. Spike pattern after stim1 (a single stimulation) and stim3 (the second stimulation of paired stimuli) were different in frequency and in spatiotemporal pattern. In the case of 1 s and 2 s ISI, the number of spikes after stim3 often decreased, while no obvious differences are observed in the case of 5 s and 10 s ISI (see Fig. 2, 75-125 DIV). Such changes were not observed in the 25-40 DIV. Fig. 3 shows electrical responses at 64 electrodes evoked by a single (stim1, upper panel) and by paired stimuli (stim2 and stim3, ISI = 1 s, lower panel). At interval of 1 s, the inhibition of activity evoked by stim3 was confirmed at several electrodes, while there were no obvious activity attenuation at some electrodes (arrowhead in

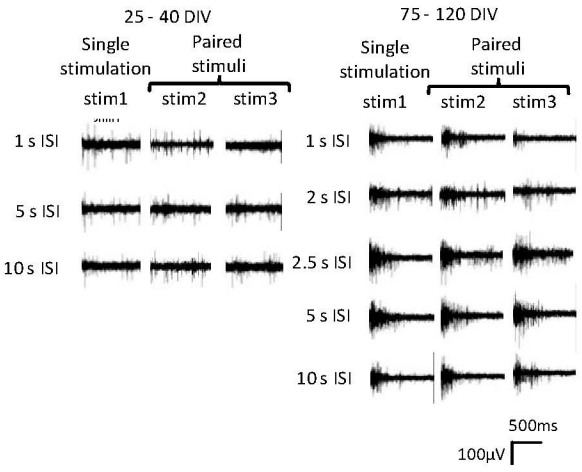

Figure 2. Waveforms obtained from a single electrode. ISI is the time between stim2 and stim3.

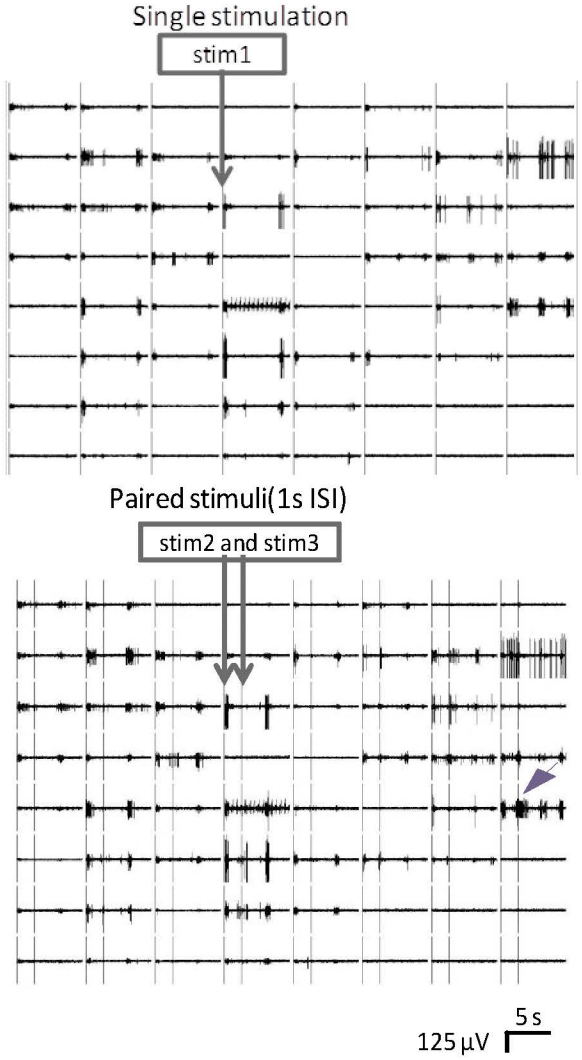

Figure 3. Examples of electrical activity evoked by a single stimulation and paired stimuli. The grey arrows represent stimulation electrodes. Arrow head indicates a site with no obvious activity attenuation.

Fig.3, lower panel). The attenuation of activity was not spatially uniform but heterogeneous at different sites. These results suggest that spatiotemporal pattern of network activity was influenced by the presence of prior stimulation, stim2. In other words, short-term hysteresis is expressed in a cultured network of neurons. The duration of the hysteresis is approximately 1 s to 2 s.

In order to quantify differences between activity pattern evoked by stim1 and stim3, Euclidian distances between the feature vectors at each 100 ms time window were calculated. We performed two types of analysis. First is comparing Euclidian distances with different ISIs among the data obtained from different neuronal network (labeled as "Different cultures", Fig.4). Second is comparing Euclidian distances with different ISIs among the data obtained from the same one neuronal network (labeled as "Single cultures", Fig.5). In both type of experiments, Euclidean distances between activity pattern evoked by stim1 and stim3 is small in the case that culture day is shorter than 30 days (short-term culture). There were no significant differences in the Euclidean distances among ISIs. However, the Euclidean distances were large in the case that culture day was longer than 75 days (long-term culture). The difference of the activity pattern among different ISIs was more obvious in analysis in "Single culture" method. In the case of long-term culture and single culture analysis, the difference between Euclidean distances for 1 s ISI and 10 s ISI was significant (Mann-Whitney U-test, $p<0.01$). In the case of long-term cultures and 1s ISI, the largest Euclidean distances in total recording time appeared at 220 ms after the stimulus. The feature of trend of Euclidean distances was that large values distributed within 300 ms immediately after the stimulation. In the case of ISI 2.5 s, 5 s, and 10 s, Euclidean distances increased at earlier stage after stimulation, though the difference was not obviously significant. Summation of Euclidean distances within 300 ms after stimulation were 529.9 ± 133.2 (ISI = 1 s, mean ± SE), 367.7 ± 113.1 (ISI = 2 s, mean ± SE), 268.2 ± 84.5(ISI = 2.5 s, mean ± SE), 305.4 ± 53.4 (ISI = 5 s, mean ± SE), 188.3 ± 31.6 (ISI = 10 s, mean ±SE) in long-term culture. In short-term cultures, summation of Euclidean distances within 300 ms after stimulation were 267.7 ± 9.8 (ISI = 1s, mean ± SE), 280.3 ± 10.5 (ISI = 5s, mean ± SE), 253.3 ± 5.1 (ISI = 10 s, mean ± SE). Euclidean distances of the electrical pattern between evoked by stim1 and stim3 at more than 5 s ISI were similar to the Euclidian distances between fluctuated patterns of spontaneous spikes.

Short-lasting hysteresis of a neuronal network living in semi-artificial condition has been identified in network level. In the case of 1 s and 2 s ISI, the internal state of the network evoked by a prior input was sustained in a whole network. Internal state of a single neuron evoked the prior stimulation recovers quickly within 1 or 2 s, and the hysteresis is

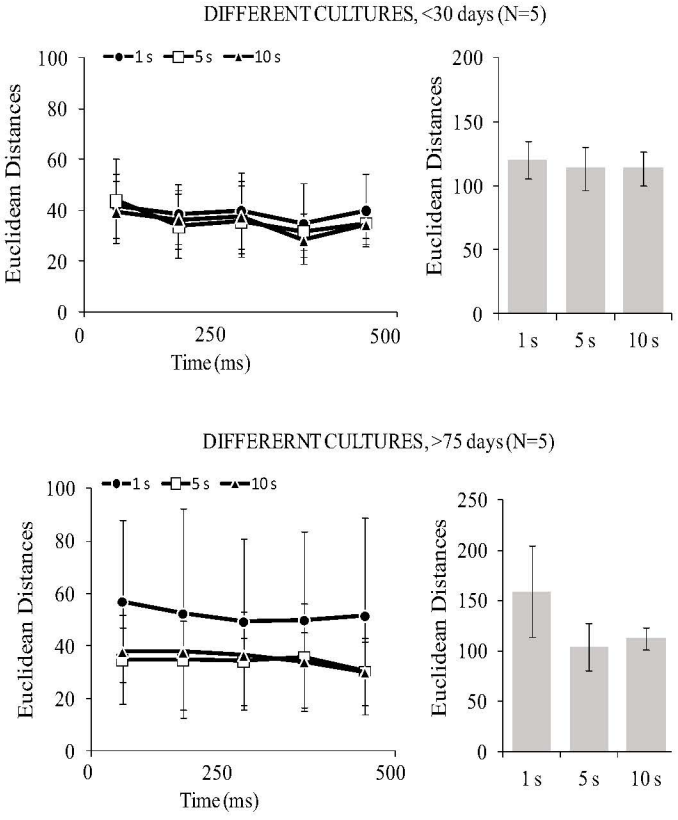

Figure 4.Summary of hysteresis in different culture. Left panel: trends of Euclidean distances of electrical pattern between evoked by stim1 and stim3. Right panel: Ssummation of Euclidean distances within 300 ms after stimulation with various ISIs. Error bar indicates standard deviation (N=5).

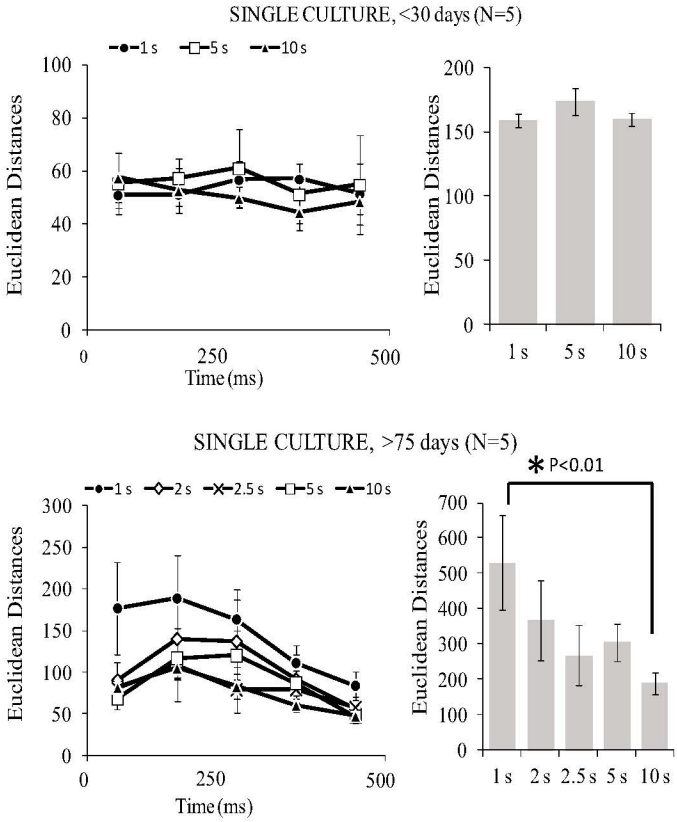

Figure 5. Hysteresis summarized by a single culture. Left panel: trends of Euclidean distances of electrical pattern between evoked by stim1 and stim3. Right panel: Ssummation of Euclidean distances within 300 ms after stimulation with various ISIs. Error bar indicates standard deviation (N=5). Asterisk indicates a significant change (Mann-Whitney U-test).

considered to be undertaken by network mechanisms, which expected to be expressed as a summation of cellular level components, such as refractory period or intracellular Ca^{2+} elevation.

The hysteresis was expressed only in long-term cultures. Evoked activity pattern was significantly different between a long-term culture and a short-term culture, and the frequency of spikes evoked by the stimulation was obviously high in long-term cultures. Accompany with maturation of neuronal network, density of synaptic connections increases and a network structure changes to be more complex. So electrical input is well propagated throughout the network in a long-term culture. This strong connectivity among network is considered to be required for the expression of this hysteresis in cultured neuronal network. This hysteresis of the living neuronal network can be utilized as short-term memory system. Living neuronal network also possesses synaptic plasticity, which is able to modify the functional and physical structures of own network. These features of living neuronal network in semi-artificial environment are useful for composing of biointegrated systems. In addition, a brain system of a creature also considered to possesses such hysteresis, so the elucidating the mechanism of the hysteresis is critical for regenerative medicine for the brain system.

IV. Conclusion

Hysteresis of network activity is observed in a semi-artificial cultured neuronal network, depending on the time between paired stimuli. The hysteresis is expressed only in a long-term culture with enough synaptic connections. This short-term memory lasts for approximately a few seconds. This feature of network dynamics is expected to contribute to information processing in a brain system of an animal.

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