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The Ca^{2+} mobility during the induction of Spike-Timing-Dependent Plasticity in the hippocampal CA1 network

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Abstract- In our previous study, spike timing dependent synaptic plasticity (STDP) was investigated in the CA1 area of hippocampal slices using optical imaging and it was revealed that the profiles of STDP were classified into two types, depending upon the layer specific location along the dendrite. The first was characterized by a symmetric time window observed in the proximal region of the stratum radiatum (SR), and the second by an asymmetric time window in the distal region of the SR.

In this study, to investigate how the triggering of STDP is dependant on intracellular calcium concentration. The Ca^{2+} mobility at local areas of CA1 neurons, induced by the protocol of relative timing between pre- and post-synaptic excitation was also spatially investigated by using optical imaging in the hippocampal CA1 area. We found that the magnitude of STDP was closely related to the slope of calcium transient at the local area when applying induction stimuli. Location dependency was analyzed in terms of the Ca^{2+}mobility. Furthermore, it was shown that the relation between the slope and the dynamics of Ca^{2+} transient during the application of STDP-inducing stimuli might be caused of Ca^{2+} release from the internal Ca^{2+} store depending on the stimulus timing.

1.Introduction

It is widely accepted that the precise time of occurrence of individual pre- and post-synaptic action potentials plays an important role in the modification of synaptic efficacy and that the back propagation of action potentials is crucial for the induction of long-term potentiation (LTP) and long-term depression (LTD) (Magee and Johnston[(1)]; Markram et al.[(2)]). Bi and Poo [(3)] later showed that STDP induced in a cultured hippocampal network had an asymmetrical profile, and experimental breakthrough which supported what subsequently was only theoretical. It was further revealed by Nishiyama et al.[(4)] that the profile of STDP induced in the hippocampal CA1 network with inhibitory neurons is symmetrical for the relative timing of pre- and post-synaptic activation.

On the other hand, it is commonly accepted that two separate thresholds exist for the induction of LTP and LTD (Biemenstock et al.[(5)]), and it has been demonstrated with direct elevations of calcium concentration in hippocampal neurons. Ca^{2+} transition for relative timing of pre- and postsynaptic activity (Yuste and Denk[(6)]).

In our previous study (Tsukada et al.[(7)]), the profiles of STDP were classified into two types depending on their layer specific location along the dendrite in the CA1 network. In this study, to investigate how the magnitude of STDP was related to the graduation of calcium intensities when applying induction stimuli, location dependency of STDP profiles was analyzed in terms of Ca^{2+} influx. Furthermore, the dynamics of Ca^{2+} mobility during the application of STDP-inducing stimuli was analyzed. The relation between the graduation and the dynamics of Ca^{2+} mobility was discussed in terms of promotive and demotive factors of STDP induction.

2.Method

2.1 General method

The experiments were performed on hippocampal slices, with a thickness of 400μm, taken from 4 week-old female Wister rats. One bipolar tungsten electrode was placed at a fixed position at a specific region in the stratum radiatum to stimulate the Shaffer commisural coraterals (SC) of the CA3 (Stim.A) in Fig. 1. The other bipolar electrode was placed at a fixed position in the stratum oriens bordering the alvelar (Stim.B). The left border of the optical recording area was fixed at the boundary between CA2 and CA1.

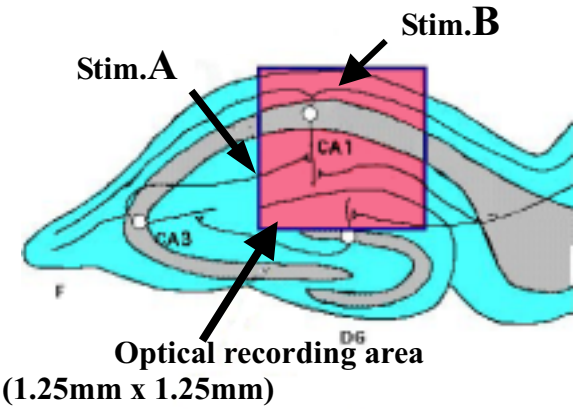

Fig.1 Stimulation and Recording

2.2 Stimulation

The intensity of the electric pulse used to stimulate the Stim.A and Stim.B was fixed at a constant value. It was exactly half the intensity necessary to produce a maximum population in the CA1 region (0.1 – 0.5 mA). The duration of the stimulus pulse, the total number of pulses and the inter-stimulus interval (ISI) were fixed at 0.2 ms, 200 pulses and 2 s, respectively. The stimulus condition, ISI = 2 s, was known not to induce LTP or LTD (Aihara et al.[8][9]). A pair of pulse stimuli were used to stimulate the Stim.A and Stim.B with various sets of relative timing ($\tau = t_B - t_A$), where Stim.A (t_A) was the timing reference for Stim.B (t_B). These sets with τ = 0, ±10, ±20, ±50 ms were used as stimuli to induce STDP.

2.3 Optical imaging

The method of optical imaging using voltage sensitive dye is the technique of Aihara et al. [10] . For optical imaging using Ca^{2+} sensitive dye, slices were stained for 30 min with 10 μM fura-2/AM in normal medium and were then washed and recovered for an additional 10 min. A naive slice was used for each stimulus sequence. Slices were viewed with a 10× objective. The Ca^{2+} measurement were made by ratio imaging of fura-2 with the use of 340/380 nm excitation (Argus50, Hamamatu Photonics Co.) The transmitted light was detected by a 128×128 square pixels, each with a receptive area of 1.25×1.25 mm^2. This had an adequate resolution in space (40×40 μm^2 using 4×4 binning with 10×10 μm^2 / single photopixel) and in time (78 msec sampling, 19.4 ms / single frame) to analyze the spatio-temporal activities of the CA1 neural network. In this experiment, the representative two point of CA1 area, distal area and proximal area from cell body, were used for comparing the characteristics (location dependency) of Ca^{2+} transients or inducing STDP

2.4 Measurement of LTP/LTD

The "test stimulus," (TS, at Stim.A site, single pulse) was applied once every 20 s (0.05 Hz) before and after one of the paired stimuli with τ as a "conditioning stimulus (CS)." The magnitude of LTP/LTD was estimated using mean percentage changes in the EPSP peak in hippocampal CA1 slices (conditioned TS–response / unconditioned TS–response). The detail is shown in a paper (Tsukada et al.[7]). A new slice was used for each stimulus sequence of TS/CS/TS and seven slices were used for each paired stimulus.

2.4 Measurement of Ca^{2+} signals

The Ca^{2+} transient was measured during TS and CS, spike timing dependent synaptic plasticity STDP inducing stimulus. The Ca^{2+} increase was measured as the difference from the resting Ca^{2+} level during TS before CS.

All values were expressed as the mean(%) ± standard error of the mean (SE) and the results were evaluated statistically ($p<0.05$) by analysis of variance (ANOVA) .

3. Results

To investigate how Ca^{2+} transient during the induction of STDP affects the magnitude depending on the location in the CA1 network, the inclination of Ca^{2+} transient during induction of STDP for relative timings was measured at the proximal dendrite (PD) and the distal dendrite (DD) in Fig. 2A. The results at PD and DD are shown in fig. 2C,E, respectively. It was found that the profiles of the inclination of Ca^{2+} transient showed symmetry at PD and asymmetry at DD corresponding to the magnitudes of STDP in Fig. 2B,D. Furthermore, under bicuculline application shown by open squares in Fig.2C,E, the profile at PD changed from symmetry to asymmetry and the low inclination of Ca^{2+} transient at τ =20 was changed to a medium level similar to τ =-50, 50, -10, 10 which showed little

LTP and LTD. The result also corresponded to the change of profiles of STDP shown by open circles under 25 μM bicuculline application in Fig. 2B,D. The inclination of Ca^{2+} transient was closely related to the magnitude of STDP.

To investigate the incident for differences in the inclinations for relative timing, the dynamics of the decay time constant and peak height of the time course of Ca^{2+} transient during STDP induction were measured as the average of five pairings in three periods ; P_1: from the first pairing to the fifth, P_2: at the maximum Ca^{2+} inclinations, P_3: at a stable state of Ca^{2+} transient. The peak height of DD was larger than that of PD for any relative stimulus timing and there was no significant difference among the three periods. While, the time constant of PD was a little larger than that of DD but there was no difference between the three periods at -20 msec of relative timing. However, both time constants of PD and DD were strongly increased in P_2 at 0 msec of relative timing. The level of time constant of PD was gradually decreased and that of DD was further increased. The time constant of PD was smaller than that of DD at 20 msec contrary to the case at -20 msec, though there was also no difference between the three periods.

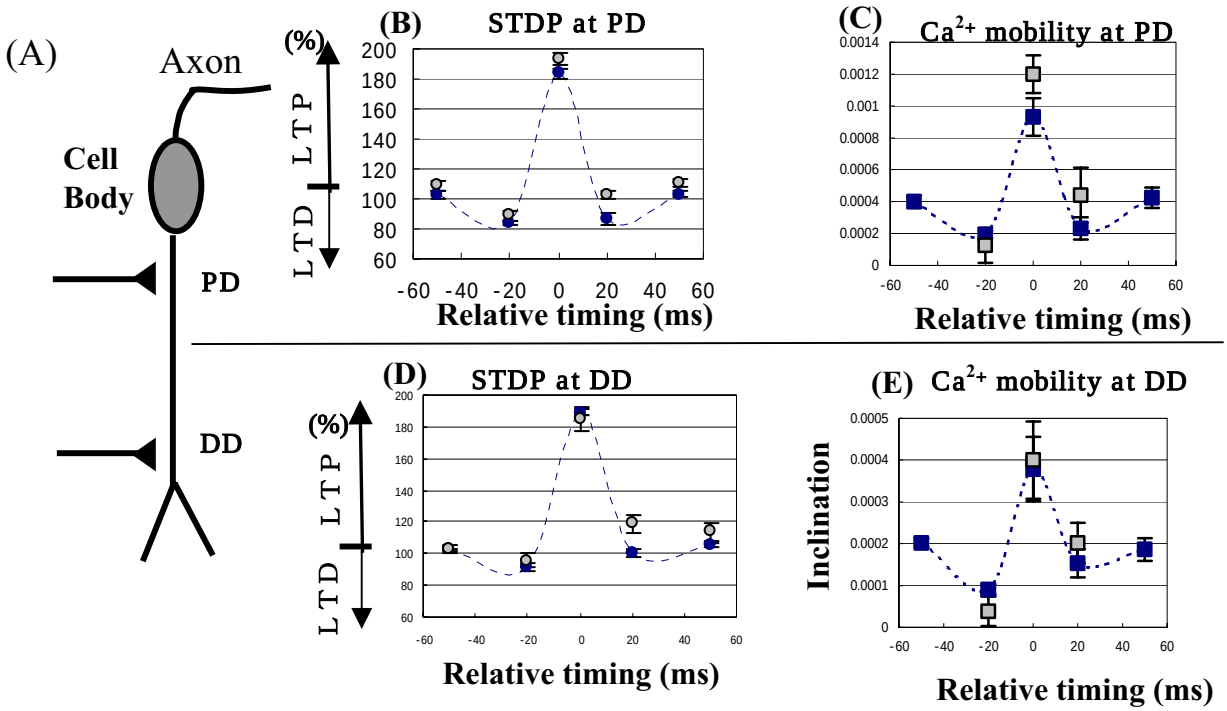

Fig.2 The magnitudes of STDP and Ca^{2+} mobility

4. Discussion

Measurements of the inclination of Ca^{2+} transient showed that the profiles correspond closely to those of the magnitude of STDP depending on the relative timing at both the PD and DD. The application of bicuculline resulted in a reduction of LTD (Fig. 2 B) and an increase in Ca^{2+} inclination (Fig. 2 C) at τ = 20 msec at PD, it was considered that the feed-forward projection of GABAergic inter-neurons was blocked and that the level of Ca^{2+} inclination recovered to a neutral level and, thus, did not induce LTP/LTD. While the projection of GABAergic inter-neurons to the DD area was not dense, there was also no significant change in Fig 2 D,E. As these results show, the high inclination of Ca^{2+} during stimulation induced a significant amount of LTP, while the low inclination of Ca^{2+} induced LTD. Interestingly, between Ca^{2+} inclination for LTP and that for LTD, there was a neutral level which did not induce LTP/LTD. These results suggest that the estimation for the relation between STDP and Ca^{2+} mobility by our measurement of Ca^{2+} transient, inclination, was similar to the extended *BCM rule* (Biemenstock et al.[5]).

Secondly, to investigate the incidence for differences of the relative timing in inclinations of the Ca^{2+} cumulative effect, the dynamics of peak height for each pairing of Ca^{2+} mobility during STDP induction was measured. The results of the decay time constants suggest that the large induction of LTP was dependent on the rapid increase of Ca^{2+} in period P_2. Since the inter-pair interval of stimulus was 2 sec in this experiment and the peak height was kept during the stimulus, the cumulative increase of Ca^{2+} through NMDA channels and voltage dependent Ca^{2+} channels may be very small. This suggests that large amounts of LTP would not be induced unless another factor was not at work, which might be Inositol 1,4,5-trisphosphate (IP_3) induced Ca^{2+} release (IICR) and Ca^{2+} induced Ca^{2+} release (CICR) based on ryanodine receptors. The effect of regulation by IICR and CICR might be apparent in results showing that the time constant of P_3 which has a higher Ca^{2+} concentration, was smaller than that of P_2 at 5msec in DD.

Our optical method was able to address the analysis of spatio-temporal coding in CA1 neural networks by removing the spatial limitation of data acquisition using an electro-physiological technique. Our results showed that the location dependence of STDP is caused by Ca^{2+} mobility modulated by the behavior of IPSP and AHP for the relative timing of input in the network. As suggested by the synaptic modification related to Ca^{2+} mobility in the network observed here, the timing of inputs (spatio-temporal information) is might be an algorithm which manipulates factors (inhibitory connection so on) in the network for inducing LTP/LTD.

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