S3-2 Simultaneous measurements of synaptic input-induced calcium rise and changes in synaptic efficacy in cortical neurons loaded with a low-affinity Ca^{2+} indicator. Hiroki Yasuda and Tadaharu Tsumoto. Department of Neurophysiology, Osaka University Medical School, Suita, 565, Japan

To test the hypothesis that long-term potentiation (LTP) is induced if an increase in Ca^{2+} concentration at postsynaptic sites during tetanic synaptic inputs is higher than the certain threshold, while long-term depression (LTD) is induced if it is below the threshold, one can use microscopic fluorometry with a fluorescent Ca^{2+} indicator. Since high-affinity Ca^{2+} indicators such as fura-2 may interfere with physiological processes for the induction of LTP or LTD, we used another indicator, rhod-2 which has a much weaker chelating action than fura-2. Initially, rhod-2/AM (membrane-permeable type) was applied to visual cortical slices of young rats. Field responses to test stimulation of layer IV of the cortex were recorded from layer II/III. Fluorescent signal of rhod-2 was detected through a window (50 x 50 μm) placed near the recording electrode in layer II/III. In slices in which LTP of field responses was induced by tetanic stimulation of layer IV, tetanus-induced rise in fluorescent signal was significantly larger than that in LTD-induced slices. Next, we injected rhod-2 directly into pyramidal-cell like neurons of layer II/III through a patch pipette from which excitatory postsynaptic potentials (EPSPs) were recorded. During tetanic stimulation, we often observed a marked increase in fluorescence intensity at apical dendritic areas about 50 μm distal from the soma. In neurons in which LTP of EPSPs was induced, the fluorescent increase at this area was significantly larger than that in neurons in which LTD was observed. These results seem to be consistent with the above-mentioned hypothesis.

S3-3 REAL-TIME IMAGING OF A-KINASE AND CALCINEURIN ACTIVITIES IN NEURAL CELLS AND THEIR ACTIVATION BY GANGLIOSIDES.

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Cyclic AMP-dependent protein kinase (PKA) and protein phosphatase 2B (calcineurin) play important roles in many biological reactions such as LTP and LTD. Real-time observation of spatial image of these enzyme activities should provide significant information for the study of such a complex phenomenon. For the imaging of PKA activity, we constructed a fluorescent-labelled peptide substrate for PKA using a partial sequence of regulatory subunit II of PKA which contains an autophosphorylation site. The resulted substrate was cell permeable and its fluorescence increased by phosphorylation. In NG108-15 cells, the phosphorylation reaction was markedly in cytosol near nuclei. Glutamate induced PKA activation was observed in primary culture of hippocampal neuron. A few kinds of gangliosides activated PKA in the cultured neuron. The same peptide substrate with a phosphorylated serine residue was a specific substrate for calcineurin and its fluorescence decreased by dephosphorylation. Increment of intracellular Ca^{2+} level activated calcineurin markedly near nuclei in NG108-15 cells. Nano molar concentration of gangliosides activated calcineurin in the cells.

S3-4 THE PROPERTIES OF CALCIUM SPIKES AND ASSOCIATED INCREASE OF $[CA^{++}]_i$ IN HIPPOCAMPAL NEURONS. ITO KEN-ICHI, MIURA MASAMI, *MIYAKAWA HIROYOSHI, KATO HIROSHI, Dept. of Physiol., Yamagata Univ. Sch f Med., Yamagata, 990-23, * Laboratory of Life Science, School of Life Science, Tokyo College of Pharmacy, Hachioji 192-03, Japan.

The membrane potential and the concentration of intracellular calcium ion ($[Ca^{++}]_i$) were simultaneously recorded in CA1 pyramidal neurons of guinea pig hippocampal slices with intracellular recording and fluorometry. In the presence of $GABA_A$ antagonists, bicuculline (25 μM) and picrotoxin (10 μM), narrow and broad spikes in all-or-none fashion were elicited by the stimulation of Schaffer/commisural afferents and by the current injection into soma. The broad spikes were blocked by Cd^{++} (200 μM), but were resistant to both TTX (1 μM) and QX-314, while the narrow spikes were blocked by both TTX and QX-314. Based on these findings we concluded that the narrow and broad spikes are Na^+ and Ca^{++} spikes, respectively. The Ca^{++} spike was accompanied with the large increase of $[Ca^{++}]_i$ compared to that induced by Na^+ spike. To evaluate the contribution of voltage gated calcium channels (VGCCs) to the large $[Ca^{++}]_i$ increase with Ca^{++} spikes, the blockers of VGCC were applied. Nifedipine (30 μM), L-type VGCC blocker, suppressed the generation of Ca^{++} spikes, while Ni^{++}(T-/R-type VGCC blocker, 100 μM) and ω-Agatoxin-IVA (P-type VGCC blocker, 60 μM) had little effects on them. Around 35% of $[Ca^{++}]_i$ increase was suppressed by Nifedipine while less than 10% of $[Ca^{++}]_i$ was suppressed by Ni^{++} or by ω-Agatoxin-IVA.