論文の内容の要旨

Competitive synaptic plasticity of dendritic spines in hippocampal CA1 pyramidal neurons

（海馬CA1錐体細胞樹状突起スパインにおける競合的シナプス可塑性の研究）

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Competitive stabilization and elimination of synaptic connections play a key role in the refinement of neuronal networks during development, learning and memory. Cellular mechanisms mediating heterosynaptic competition, however, remain poorly understood.

In the cerebral cortex, excitatory synapses are made on small protrusions of dendrites, dendritic spines, in the pyramidal neurons. Selection of dendritic spines should be achieved by structural plasticity of spines, their enlargement and shrinkage, which are associated with long-term potentiation (LTP) and depression (LTD), respectively. It has been proposed that the modest increases in cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) selectively activate protein phosphatase PP2B, calcineurin, while large increases in $[Ca^{2+}]_i$ activate Ca^{2+}/calmodulin kinase II (CaMKII) to induce LTD and LTP, respectively. It has also been proposed that spine shrinkage is induced by a signaling cascade, involving calcineurin and actin depolymerizing factor, ADF/cofilin. ADF/cofilin is activated upon dephosphorylation by slingshot, and severs and depolymerizes actin fibers. On the other hand, spine enlargement is induced by a signaling cascade, involving activation of CaMKII and Rac1, which causes phosphorylation of PAK, LIMK, and cofilin. Cofilin is deactivated by LIMK-induced phosphorylation. The spatial organizations of the two pathways and their interactions, however, have not been investigated.

Two-photon glutamate uncaging has enabled stimulation of individual spines, and demonstrated the enlargement of dendritic spines underlies LTP at the level of single spines. Although electrical stimulation of presynaptic fibers can induce spine shrinkage and LTD, spine shrinkage has not been induced with stimulation of identified spines, which have hampered the understanding of synaptic competition where spine shrinkage and elimination would play a major role.

In this thesis, I report that spine shrinkage could be readily induced when repetitive glutamate uncaging was applied in the presence of GABAergic inhibition. This could be achieved either by the

spike-timing protocol where glutamate uncaging was paired with a spike in the postsynaptic neuron, provided that each spike was associated with uncaging of caged-GABA compound (1 Hz, 80 times), or with the continuous presence of a selective $GABA_A$ receptor agonist, muscimol. Importantly, I have found that, although spine enlargement was confined to the stimulated spine, spine shrinkage tended to spread into neighboring spines over 10 μm, even when only one spine was stimulated (Fig. 1).

I next examined whether this spreading shrinkage induced heterosynaptic competition between enlargement and shrinkage of spines along a dendrite. For this purpose, two neighboring spines were challenged with repetitive pairing with a spike and glutamate uncaging, one with LTP and the other with LTD timing. I found that spreading spine shrinkage was induced using the LTD protocol except for the spine which was specifically stimulated with the LTP protocol, where spine enlargement was induced. Thus, spine enlargement could outcompete the spreading shrinkage, while shrinkage spread beyond the enlarged spines. Moreover, I found that the bidirectional structural plasticity was mediated by the competition between phosphorylation and dephosphorylation of cofilin (Fig. 2).

Since cofilin regulates both directions of the plasticity depending on its phosphorylation, I hypothesized cofilin is a pivotal molecule for synaptic competition. I therefore investigated the diffusive properties of cofilin using photoactivatable GFP (PAGFP) fused with rat cofilin1 or S3A mutant of PAGFP-cofilin, which mimics dephosphorylated active cofilin. In the resting spine head, the most PAGFP-cofilin or PAGFP-cofilin (S3A) molecules gradually diffused out from the photoactivated spines and readily spread along dendrites over 10 μm within a few minute (Fig. 3). Thus, active cofilin readily diffuses along a dendrite. Moreover, to test whether cofilin could induce spine shrinkage, I applied cofilin protein via whole-cell pipette into the soma of pyramidal neurons. Cofilin perfusion induced shrinkage and reduction of PSD95 of many spines in the dendrite within 30 min (Fig. 4). These experiments indicate that cofilin can diffuse effectively along dendrites, and induces spreading spine shrinkage.

Finally, I examined the diffusive properties of wild type (WT), S3E or 3SA mutants of cofilin during spine enlargement. The S3E mutant of cofilin is known to mimic phosphorylated cofilin. The expression of cofilin and its mutants did not significantly affect the spine enlargement induced by repetitive glutamate uncaging in a Mg^{2+}-free solution. I found that WT and S3E mutant of PAGFP-cofilin were accumulated in the stimulated spines over 30 min during the spine enlargement, similarly as the stable enlargement pool of F-actin. In contrast, S3A mutant of PAGFP-cofilin rapidly diffused out from the enlarged spine, as it did in the resting spines (Fig. 5). These data indicate that LTP protocol induced selective generation and accumulation of phosphorylated cofilin, while dephosphorylated cofilin was excluded from the stable F-actin. Thus, the diffusion of cofilin was dependent on the type of stimulation in the way accounting for the synaptic competition at the level of single spine.

In summary, I have established the conditions to reliably induce shrinkage of identified spines, and found that it tended to spread due to the diffusion of cofilin, and gave rise to local competition of spine synapses. Moreover, the spine shrinkage was greatly promoted by the activation of $GABA_A$ receptors (Fig. 6). My study thus revealed the highly interactive nature of synapses along dendrites.

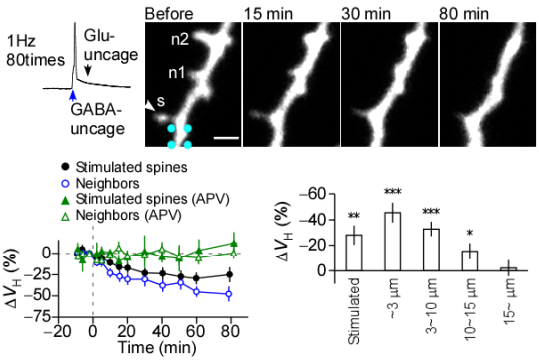

Figure 1. Spine shrinkage induced by two-color uncaging of glutamate and GABA.
Spine shrinkage was reliably induced when GABA uncaging was applied at the onset of spike at the dendritic shaft close to the stimulated spine. Spine shrinkage tended to spread into neighboring spines over 10 μm and was dependent on Ca^{2+} entry through NMDA receptors.

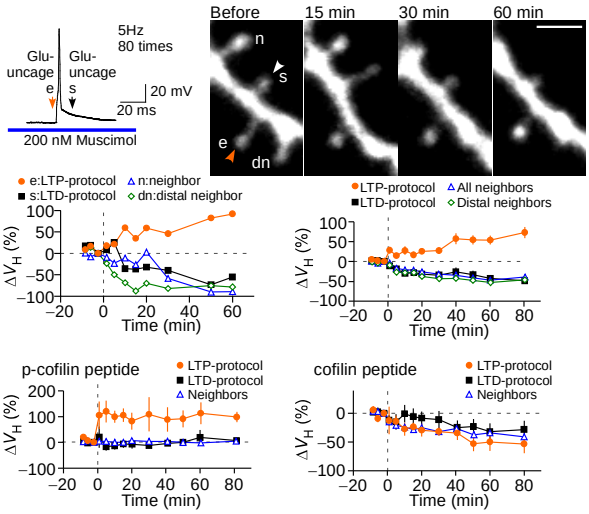

Figure 2. Competition of enlargement and shrinkage of spines with the spike-timing protocol.
Spine enlargement was confined to the stimulated spine even when spine shrinkage spread to the nearby spines. Competitive structural plasticity reflected the competitive phophorylation of cofilin, because the phosphorylated cofilin peptide (p-cofilin peptid) abolished spine shrinkage, and it hastened the enlargement, while the dephosphorylated cofilin peptide blocked spine enlargement. Cofilin peptide and p-cofilin peptide inhibits phosphorylation and dephosphorylation of cofilin, respectively.

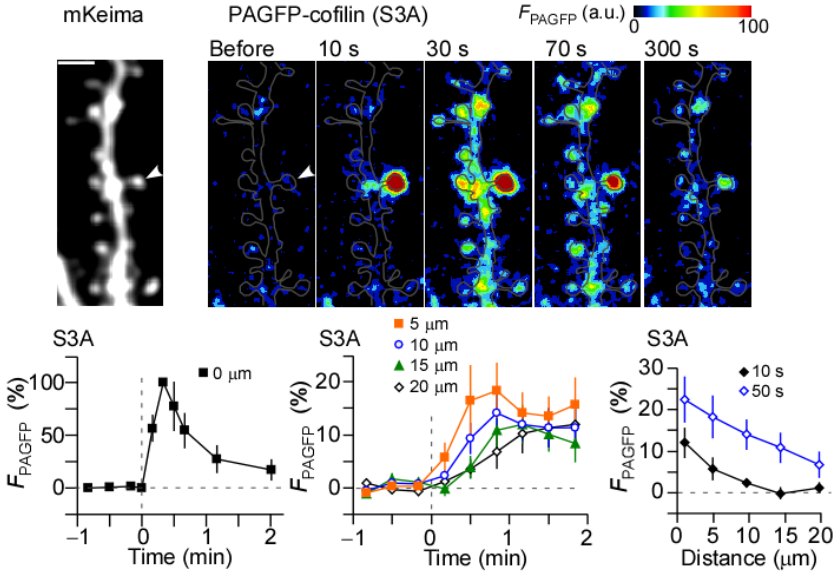

Figure 3. Spread of cofilin along dendrites imaged with photoactivation of PAGFP-cofilin.
In the resting spine, PAGFP-cofilin (S3A) molecules gradually diffused out from the photoactivated spines and readily spread along dendrites over 10 μm within a few minute.

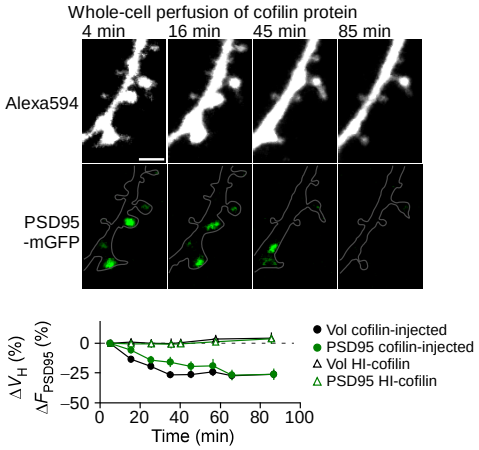

Figure 4. Spine shrinkage induced by whole-cell perfusion of cofilin protein.
Cofilin protein was applied via whole-cell pipette into the soma of pyramidal neurons. Cofilin perfusion induced shrinkage and reduction of PSD95 of many spines in the dendrite, while heat inactivated (HI) cofilin did not.

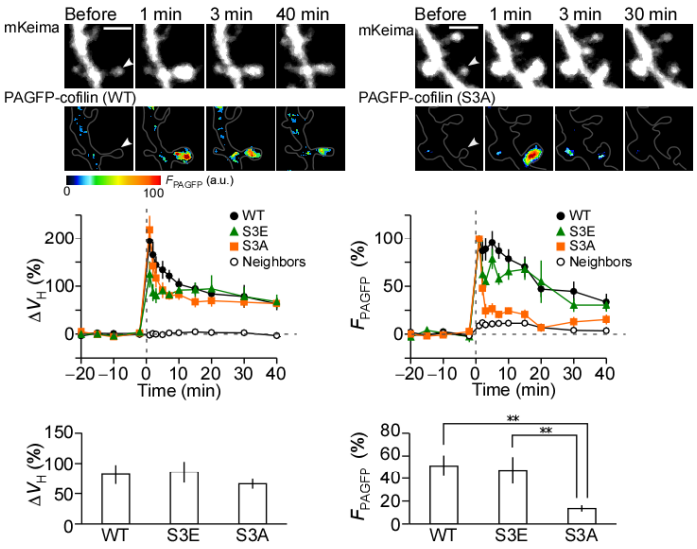

Figure 5. Accumulation of PAGFP-cofilin in the enlarged spines.
WT and S3E mutant of PAGFP-cofilin were accumulated in the stimulated spines over 30 min during the spine enlargement. In contrast, S3A mutant of PAGFP-cofilin rapidly diffused out from the enlarged spine, as it did in the resting spines. S3E and S3A mutant of cofilin is known to mimic phosphorylated and dephosphorylated cofilin, respectively.

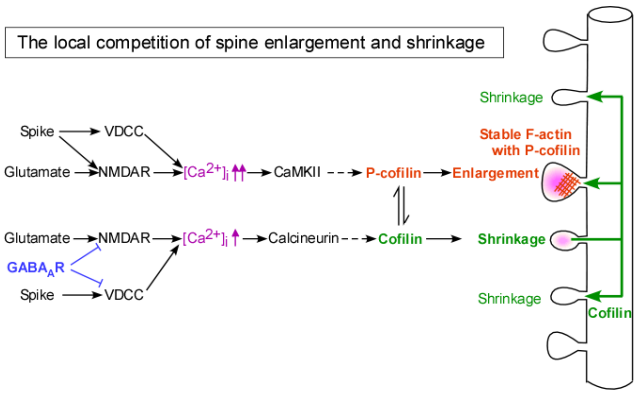

Figure 6. Current hypothesis of the local competition of spine enlargement and shrinkage along a dendrite.