博士論文（要約）

In vivo imaging of synaptic and dendritic activity in awake-mouse brain with novel genetically encoded calcium indicator XCaMP

（新規カルシウム指示遺伝子 XCaMP を用いた、覚醒下マウス脳におけるシナプスおよび樹状突起活動のイメージング）

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論文の内容の要旨

論文題目

In vivo imaging of synaptic and dendritic activity in awake-mouse brain with novel genetically encoded calcium indicator XCaMP
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A series of random, targeted or 3D structure–based mutagenesis, performed in several laboratories, have made genetically encoded calcium indicators (GECIs) a powerful tool to investigate the function of complex neural circuits in living animals. In spite of these advances, there still remain several important problems that need to be overcome, such as sensitivity, linearity and slow kinetics of indicators. Here, we developed a new class of GECIs, R-CaMP2 and XCaMPs. R-CaMP2 is the first single red fluorophore GECI to have Ca^{2+}/CaM-sensing domain of CaMKK-α/β (ckkap sequence) instead of an M13 sequence. Characterization of biophysical properties and performance of R-CaMP2 *in vitro* and *in vivo* revealed higher signal-to-noise ratio, better linearity, and faster kinetics of Ca^{2+} transients than conventional CaM/M13-based red GECIs. By using R-CaMP2 and GCaMP6, I demonstrated simultaneous dual-color somatic Ca^{2+} imaging *in vivo* between two populations of neurons: excitatory pyramidal neurons and inhibitory interneurons. I found that SST-positive interneurons in close proximity showed highly correlated responses compared to excitatory neurons. Based on the successful development of R-CaMP2, we further expanded the color repertory of CaM/ckkap-based GECIs and optimized their properties to create XCaMPs.

By using XCaMP-G, I analyzed individual synaptic inputs to layer 2/3 neurons in the somatosensory barrel cortex of awake mice. I found that whisker stimulation induced sparse synaptic inputs distributed throughout entire dendritic trees, in which sensory information is

encoded by reliable activation of a small subset of spines. These results suggest that CaM/ckkap-based GECIs are invaluable research tools to advance our understanding of the cellular and synaptic functions in the living brain.