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

	XCaMP
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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
　（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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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.