Trends in
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Spotlight

Photoactivatable CaMKII: Rewiring the Brain, One Synapse at a Time

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A recent article by Shibata *et al.* introduces the engineered photoactivatable enzyme paCaMKII. Activation of this new tool is sufficient to induce long-term potentiation (LTP) of hippocampal synapses in slice culture and in intact animals, thereby expanding the existing toolkit for light-induced modification of brain connectivity at the synaptic level.

Recording brain activity has been a mainstay of neuroscience research for many decades, and a wide variety of different techniques have been developed. However, truly understanding the workings of a complex machine usually requires tinkering with the individual elements, in addition to observation. The relatively crude tools of stimulation electrodes and lesions historically used to manipulate brain circuits have been surpassed by more refined methodologies, including optogenetic channels and ion pumps that allow driving or inhibiting the activity of specific neuronal subpopulations with excellent temporal control [1]. The promise of this first optogenetic revolution, to unravel the function of defined brain circuits by controlling the activity of identified neurons, for most researchers seemed to be in the realm of speculative fiction only 20 years ago.

Given that optical control of neuronal activity has rapidly become a standard technique, the attention of some toolmakers has shifted to the very fabric of cellular function: manipulating the activity of enzymes using light. While this is good news for all cell biologists, it is particularly exciting for brain research. Recent breakthroughs in artificial intelligence have underscored the idea that the computational ability of neural networks relies on smart ways to adjust the weight of thousands of synaptic connections simultaneously and in the right amount/direction [2]. This same general principle is relevant in the context of biological neural networks as well. In the domain of artificial neural networks, back-propagation of information about output quality is often used to adjust synaptic weights. It remains unclear whether and how a similar 'magic' is achieved at biological synapses, which seem to have little information about current performance of the whole organism. Arguably, the key to this mystery must lie in the multitude of signaling pathways that modulate and adjust pre- and postsynaptic function at every synapse. Armed with only electrophysiology, pharmacology, and global, organism-wide genetic manipulation, investigating these processes has been difficult at best. To understand the highly compartmentalized intracellular signaling networks in neurons, a new generation of tools is becoming available, allowing control over specific signaling pathways in individual synapses.

Writing in *Nature Communications*, Hideji Murakoshi and coworkers rendered photoactivatable one of the key players in LTP of synapses, CaMKIIα (paCaMKII) [3]. Endogenous CaMKII gets activated by Ca^{2+}/calmodulin-dependent opening of a hinge region. The authors replaced this flexible hinge region with a light-sensitive LOV2 domain and its binding partner Jα, resulting in blue-light-induced, calcium-independent activation of the kinase domain. When expressed in neurons, photoactivation of paCaMKII resulted in long-lasting spine enlargement and increased AMPA receptor-mediated currents, hallmarks of LTP. Localized two-photon excitation of paCaMKII caused potentiation of the illuminated synapse, but not of its neighbors. This specificity is remarkable although not unexpected, as very localized CaMKIIα activation has been reported after two-photon glutamate uncaging onto individual dendritic spines [4].

While it was known that calcium-triggered CaMKIIα activation is vital for LTP induction, the observation that artificial activation of this one enzyme in a single spine is sufficient to potentiate the resident synapse is impressive. After all, induction of LTP via NMDA receptor-mediated calcium influx activates numerous Ca^{2+}/calmodulin dependent pathways, and it was by no means clear whether additional factors are required for synaptic potentiation. As paCaMKII activation does not elevate spine $[Ca^{2+}]_i$, parallel pathways can be excluded. With respect to downstream effectors, the authors demonstrated that the small G-protein Cdc42 is necessary to convey actin-driven enlargement of the spine after paCaMKII activation. The coupling mechanism between CaMKII and Cdc42 activation remains unknown. Thus, seemingly independent biochemical pathways at the cellular level, may interact in the sub-femtoliter volume of a single spine. In volumes this small, average concentrations of reactants are not a good description of reality. Reactions catalyzed by single molecules will rather behave like switches, creating discrete synaptic states [5]. In this context, it would be fascinating to determine whether transient activation of a single CaMKII holoenzyme is sufficient to induce LTP at a given synapse.

On a technical note, it is worth mentioning that the light sensor of paCaMKII, a LOV2 domain, is completely insensitive to wavelengths above 520 nm. Consequently, when paCaMKII is expressed together with a green/yellow light excitable marker, for example, tdTomato, transfected neurons can be visualized without

simultaneously activating CaMKII. As an additional refinement, paCaMKII contains an integrated Förster resonance energy transfer (FRET) sensor with a dark acceptor. Successful photoactivation with blue light can be optically monitored online as an increase in green fluorescence lifetime. This level of sophistication is lacking in most optogenetic tools.

Photoactivatable CaMKII is the latest addition to the growing toolkit of light-activated signaling molecules. Available tools include cyclases to produce cAMP and cGMP [6], calcium channels [7], and small G proteins that are activated by light [8]. It is even possible to strengthen synapses by directly recruiting AMPA receptors to the postsynaptic density using light-induced dimerization, circumventing all endogenous mechanisms [9]. Unfortunately, all of these tools are sensitive to blue light, so multiplexed optical control of several pathways is not yet feasible. Parallel to the development of novel optogenetic tools, holographic light-sculpting devices that allow simultaneous 3D photostimulation of multiple selected regions in intact brain tissue are becoming available [10]. Thus, following the first wave of 'membrane potential optogenetics', noninvasive control of biochemical pathways with subcellular precision is emerging. As the elegant work of Shibata *et al.* shows, the area of synaptic physiology has advanced from mostly observational tools in its early days, to methodologies that allow fine-scale manipulations and enable studying how each individual synapse is regulated by the activity of its various molecular components.

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Declaration of Interests

The authors declare no competing interests in relation to this work.

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