Two-Color, Bi-Directional Optical Voltage Control of Genetically-Targeted Neurons

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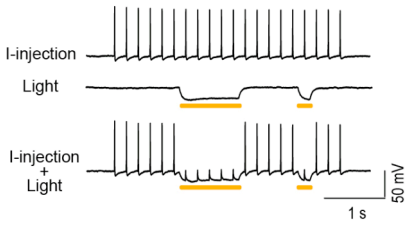
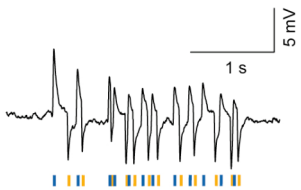
Much effort in neuroscience is devoted to determining the contribution of neural activity in specific brain regions or neuron classes towards specific behaviors, neural computations, and pathological states. This quest would be greatly aided by a technology that enables rapidly inducible and reversible neural activation and inactivation at the millisecond timescale, while having no side effects on cell physiology or survival, and requiring no exogenous chemicals to be delivered. Having found a powerful method for activating neurons with blue light in the protein Channelrhodopsin-2 (ChR2) [1], we sought to augment the toolbox by finding a single-component system capable of mediating light-elicited neuronal inhibition. We identified a powerful tool, the mammalian codon-optimized version of the light-driven chloride pump halorhodopsin, from the archaebacterium *Natronobacterium pharaonis* (here abbreviated Halo) [2].

We report that cultured hippocampal pyramidal neurons expressing Halo-GFP under the CaMKII promoter experienced strong hyperpolarizations (> -20 mV) upon exposure to brief pulses of moderate-intensity yellow light (~565 nm). In the absence of light, Halo-expressing neurons were physiologically indistinguishable from wild-type neurons. Halo could mediate 100% optical blockade of neuronal spiking induced by somatically injected intracellular current pulses (~300 pA), with millisecond-timescale onset and offset of the blockade (***right***). In addition, Halo could mediate naturalistic trains of inhibitory voltage deflections at physiologically relevant frequencies, with almost no attenuation of voltage amplitude from pulse to pulse. We also demonstrated that in individual neurons expressing both yellow-light driven Halo and the blue-light driven cation channel ChR2, neural inhibition and excitation could be efficiently and independently controlled at the millisecond timescale, by interleaving brief pulses of yellow and blue light (***left***). Thus, Halo powerfully extends our ability to analyze and engineer neural circuits, and will facilitate determination of the time-resolved causal roles of specific neurons and neural activity patterns in behavior, computation, and disease.





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References
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