NOVEL CLASSES OF OPTICAL NEURAL CONTROL TOOLS REVEALED VIA SCREENING OF PHYLOGENETIC DIVERSITY

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The heterologous expression of light-activated proteins to enable optical physiological perturbation of genetically specified cells, a field often termed "optogenetics", is a powerful methodology in neural circuit analysis, allowing for spatio-temporal assessment of the necessity and sufficiency of neural activity patterns in brain functions. By screening ecological diversity, we have discovered several stimulation and silencing tools for controlling neural activity over timescales relevant to the probing of neural computation and behavior (Halo/NpHR, Han and Boyden, *PLoS One* 2007; ChR2, Boyden et al. *Nature Neuro* 2005). We here expand upon these tools by exploring entire classes of efficacious neural silencers that build upon our earlier characterization of the novel molecules Arch and Mac (Chow and Han et al. *Nature* 2010), which enable extremely powerful silencing, and blue-light silencing, respectively.

First, we report several new neural silencers from the *Halorubrum* family, related to Arch, that have up to four-fold improved light-sensitivity, enough to enable significant regions of the non-human primate brain to be cleanly, reversibly, and safely silenced. Furthermore, we report on early approaches for enhancing multicolor silencing, including variants of fungal and archaeal opsin that enable well-separated blue vs. red light driving. Finally, we report a fourth class of optogenetic microbial opsins that can mediate both neural activation and silencing in response to two different colors of light, opening up the possibility of precise dialing-in of neural conductance using the full color spectrum range. In summary, boundaries between classes of opsin provide fundamental principles for the identification and design of opsins of desired kinetics, color, and sensitivity, based largely on their phylogenetic origin. We are using these opsins to discover principles of optical neural control that will enable researchers to optimally apply given tools from specific classes toward neuroscientific discovery and the therapeutic treatment of abnormal pathologies.