OPTOGENETICS

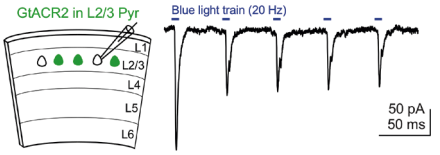
Flipping the switch off

Elife. https://doi.org/10.7554/eLife.38506 (2018).

In order to better understand how neural networks operate and more generally how the brain works, neurobiologists need to be able to turn neurons on or off. To this end, scientists have been developing new tools and one of these toolkits is optogenetics. With optogenetics, proteins are modified so as to be light-activated. Within this collection of proteins, there are pumps, that transmit one ion per photocycle, and channels, that allow multiple ions to flow per absorbed photon. Both pumps and channels can be either stimulatory or inhibitory, depending on the ions transported.

While there are many proteins for activating neurons, there are fewer tools that can successfully turn neurons off. Therefore, inhibitory channels were the focus of a recent *Elife* article by Mingshan Xue, a professor in the Department of Neuroscience at the Baylor College of Medicine, and his lab. The article focused on improving *Guillardia theta* anion channelrhodopsin 2 (GtACR2), a light-activated chloride channel.

While prior work found that GTACR2 was not stimulatory, other light-gated anionic channels were reported to conduct cations and be neuron acitvating, meaning it is possible that GTACR2 might be stimulatory as well. To begin, the Hue lab validated that the GTACR2 channel was an anionic transporter with electrophysiology experiments, validating their belief that it transported negative ions. However, the ensuing experimental data was surprising. First author of the study, Jessica Messier, described how, when neurons expressing this transgenic protein were light-stimulated they released neurotransmitters onto neighboring cells, which is the opposite to what you'd expect if GtACR2 were inhibiting the neurons. This phenomenon occurred in both excitatory and inhibitory neurons in the mouse cortex. In addition, the lab observed this phenomenon in three other light-gated chloride channels, GtACR1, iC++, and iChloC.



Electrophysiology experiment from brain slice. Adapted from *Elife*. https://doi.org/10.7554/eLife.38506 (2018).

Authors speculated that this was the result of differences in the chloride concentration between the soma and distal axon/pre-synaptic terminals, and believed this to be true for a couple of reasons. First, different studies had indirectly shown that other chloride channels (glycine and GABA) also possessed this anomalous pre-synaptic release upon stimulation from brainstem, hippocampus, and cerebellum. Additionally, one other report showed that a high pre-synaptic terminal chloride concentration was responsible for this paradoxical neurotransmitter release, as activation of GtACR2 stimulated pre-synaptic terminals to release neurotransmitter. Messier added that while they did not have a molecular explanation for the concentration differences, it was possibly due to varying amounts of the proteins that determine chloride concentration in the two different regions of the cell.

Because of this contradictory effect of GtACR2 activation in the soma versus pre-synaptic terminals, the utility of GtACR2 as an optogenetic tool is compromised. To try and fix this problem and make the channel purely inhibitory, investigators explored combining the protein with different targeting motifs in the hopes of moving a larger fraction of the GtACR2 protein from the axon towards the soma and dendrites. After trial and error, they found that a novel hybrid fusion targeting motif, Kv2.1C-linker-TlcnC, which combines a motif from the Kv2.1 potassium channel with a motif from the telecephalin protein, was the best at trafficking GtACR2 towards the soma and dendrites. Upon reexamination with electrophysiological assays, the modified protein demonstrated approximately an 80% decrease in the undesired excitatory effect and a 2-3 folds increase in the desired inhibitory effect, commented Xue.

Messier and Xue both thought the most significant part of their work was that it represents the best optogenetic tool to date for neuronal inhibition. Future work for the group includes two different research plans. First, Xue's lab is trying to improve targeting motifs that move the protein more efficiently into the soma and dendrites as opposed to the axons. Additionally, they are trying to introduce a mutation to make GtACR2 a rectifying channel, meaning that the channel would only allow chloride to flow into the cell. This would help reduce the stimulatory effect observed. They are doing this in collaboration with John Spudich's group at the University of Texas Health Science Center at Houston.

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