

Chronicle / *Toolbox*

switch—one that reliably extinguishes neural activity in the same way—seemed only a matter of time. But nearly a decade after channelrhodopsin began turning on neurons, a similar molecule for turning off neurons with light has eluded discovery.

“So,” says Stanford University neuroscientist Karl Deisseroth, “we decided to try to make one.”

Deisseroth, an HHMI investigator and practicing psychiatrist, developed the first channelrhodopsin on-switches in 2005. Channelrhodopsins are essentially ion channels—tubular proteins embedded in neuronal membrane through which ions can flow. In unicellular green algae, the proteins act as photoreceptors to guide the microorganisms’ movements in response to light. Deisseroth and his colleagues demonstrated a technique for genetically inserting the light-sensitive channelrhodopsin into rodent neurons. Shining a pulse of blue light on those neurons triggered the opening of the pore so that positively charged ions flowed into the cell, causing the cell to fire. Thousands of labs around the world now routinely use channelrhodopsins to study the neural basis of a wide range of disorders ranging from Parkinson’s disease to depression and anxiety.

As helpful as they are, channelrhodopsins provide neuroscientists only half the control they need to fully manipulate brain activity. Researchers needed a reliable method to switch neurons off as well. In 2006, Deisseroth’s team found a workable solution in halorhodopsins, light-sensitive ion pump proteins extracted from nature—this time from the microorganism halobacteria, which make opsins, or light-sensitive proteins, selective for the negatively charged ion chloride. By 2010, his lab team had successfully engineered these chloride pumps into tools for inactivating neurons. However, unlike channelrhodopsins, which allow hundreds of ions to pass through per photon of light, all opsin pumps move only a single ion through per photon. Put simply, if a channel is a fire hose, a pump is a dripping faucet. “You still get inhibition, but it’s inefficient inhibition,” says Deisseroth.

After several more fruitless years of searching genomic databases for an inhibitory light-sensitive channel, Deisseroth decided to reengineer channelrhodopsin to make it selective

for negative ions. He knew he’d have to change the polarity of the pore’s lining from negative to positive, so that negative ions, such as chloride, could be shuttled through. One way to do that was to introduce DNA mutations that would swap out negatively charged amino acids in the pore lining for ones that were positively charged. Fortunately, his team had been tinkering with the genetics of channelrhodopsin to make it more light-sensitive and to keep the channel open for longer periods of time. So, they knew such genetic manipulations were possible. They just had to figure out which of the hundreds of amino acids to tweak.

Starting in 2010, Deisseroth’s team, along with biophysicists at the University of Tokyo, committed to solving the structure of a chimera of two channelrhodopsins, called C1C2, using x-ray crystallography. After two years, they published a crystal structure that offered a road map of amino acids to target with mutations. Still, there were hundreds of possibilities, which took another two years to test. Several mutations conferred selectivity for chloride, but the channels failed to conduct current. So, Deisseroth’s team, led by postdoc Andre Berndt, screened more than 400 combinations of mutations, and through a systematic process, ultimately constructed

An Off-Switch for Neurons

Neuroscientists have built a light-sensitive switch for shutting down neural activity.

WHEN FACED WITH a thorny problem, researchers often turn to nature for a solution. One shining example is channelrhodopsin, a protein derived from green algae. The light-sensitive protein can, with the flick of a light switch, instantaneously activate neurons in which it is genetically expressed. Given life’s spectacular diversity, finding a complementary

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a channel with nine amino acid mutations that conducted chloride currents.

The new channel—dubbed inhibitory $C1C2$, or “ $iC1C2$,” and described in the April 25, 2014, issue of *Science*—inhibits neuron firing in two ways: chloride rushing in makes the cell more negatively charged, keeping it from reaching its firing threshold, and, more importantly, all those open channels make the neuron’s membrane leaky. “By making the membrane leaky, you make the neuron harder to fire,” says Deisseroth. “You can’t do that with ion pumps.”

With one final mutation, Deisseroth’s team made the new channel much more sensitive to light overall. The mutation also gave the researchers greater control over the channel. Blue light can open this additional switching tool, called SwiChR, for minutes at a time; red light makes it close quickly. Such control has proven useful in long-term studies where events such as neural development and plasticity play out over minutes to hours. The extended channel-open times also mean less light is needed to inhibit the neurons. Using a weaker light source reduces tissue damage, increases the ability to reach deep brain structures, and opens the possibility of controlling brain functions involving large regions of the brain. According to Deisseroth, these capabilities should facilitate the use of inhibitory channels in animals with large brains, such as primates.

Deisseroth says he is particularly pleased with how his lab was able to weave together years of projects involving crystallography, genetic engineering, and behavioral testing to derive the chloride-conducting channelrhodopsins. “It was certainly a high-risk project,” he says, “and in the end, it was surprising how well it worked and how much we learned about these amazing proteins.”

—Chris Palmer

