QnAs with Karl Deisseroth

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When Karl Deisseroth, a neuroscientist at Stanford University, recently set out to improve a trusty molecular tool for finessing the activity of brain cells in living animals, he had nary a clue that nature had long-anticipated his fastidious feat of engineering. Deisseroth, whose name has become nearly metonymous with optogenetics, a molecular technique for manipulating neurons using light, has developed exquisitely precise tools to probe brain function. Central to optogenetics are proteins called channelrhodopsins, ion-transporting channels found in microscopic algae. When slipped into neurons, the channels serve as light-activated switches to control brain function. Conceived as basic research tools, the channels have helped limn circuits involved in psychiatric and neurological conditions. In his PNAS Inaugural Article (1), Deisseroth and his collaborators describe how the previously unresolved power of channelrhodopsins can be unleashed to control animal behavior. Using insights from crystallography, his team has engineered improved and versatile channelrhodopsins, which are not only more potent than conventional channels but can dampen neuronal firing, unlike traditional counterparts that trigger it. More than a year after Deisseroth first unveiled the inhibitory channels, researchers reported a naturally occurring channel with a nearly identical design, revealing that nature had taken Deisseroth’s tack, but over the plodding course of evolution. A testament to his unerring instinct, the report proved to be a ringing endorsement of Deisseroth’s structure-guided engineering approach by a force no less than natural selection. Deisseroth, a member of the National Academy of Sciences, tells PNAS that inhibiting selected groups of neurons using the next-generation inhibitory channels may lead to a refined understanding of long-elusive aspects of mood and movement disorders.

PNAS: Your foray into optogenetics began with the publication of your 2005 report in Nature Neuroscience (2). From the vantage of history, can you describe the experiment that led to that article?

Deisseroth: The conceptual history leading to optogenetics is decades old. As early as 1979, Francis Crick posed the challenge of controlling neuron types in his Scientific American article (3). In the late 1990s, he offered a hypothetical solution, suggesting that light might be a way to achieve such fine control. Subsequently, several pioneering teams, including that of Gero Miesenboeck, put forward strategies to achieve this goal. Meanwhile, biochemists and biophysicists had been studying microbial opsins (single-protein light-activated ion conductance regulators) since the 1970s, from the German biochemist Dieter Oesterhelt, who discovered the first microbial opsin in Halobacterium, to my collaborator Peter Hegemann, who led prediction and identification of the light-activated channel subtype in Chlamydomonas.

Though theoretical possibilities were discussed, the neuroscience and microbial opsin worlds converged experimentally in our small team at Stanford. The first experiment came in July 2004, when I introduced a microbial opsin gene into cultured neurons and discovered that the microbial protein, a channelrhodopsin, could be properly synthesized and transported to the surface membrane in these neurons and cause them to be activated by light. (I delivered light through the microscope and then quantified its effects on membrane depolarization-activated cell signaling.) Over the next several years, beginning with creative work led by graduate students Ed Boyden and Feng Zhang and many other scientists, our team developed fast optical strategies for spiking, cell type-targeting viruses, and fiber–optic interfaces compatible with free behavior, as well as neural projection targeting and recording. That first experiment had demonstrated the promise of optogenetics, but challenges relating to application in mammals took years of innovation to solve.

PNAS: You are among the few neuroscientists who are also practicing psychiatrists. Did your experience treating patients shape your interest in optogenetics?
Deisseroth: I started in neuroscience and came back to neuroscience from psychiatry. For my PhD in neuroscience, way back in the 1990s, I did patch-clamp electrophysiology and imaging. Along the way, I had seen challenges in psychiatry and neuroscience, not due to the fault of any investigators, but simply because the fields were lacking the necessary tools. My experience working with patients heightened my sense of inadequacy in doing the kinds of experiments that many neuroscientists, including myself, had long wanted to do.

PNAS: With optogenetics you have continued to pull back the curtain on brain function. As mood disorders go, which insights have been the most compelling?

Deisseroth: For the 10th anniversary of optogenetics, I reviewed the field’s major discoveries across a wide range of categories, such as sensory processing, cognition, memory, and motor behavior (4). Some of the most exciting involved the discovery of novel concepts and pathways. Anxiety, for example, is an adaptive response that can become maladaptive and pathological, and optogenetics has allowed us to observe how anxiety-related pathways are assembled and disassembled across the brain. Among the surprises, as we reported in Nature last year (5), was discovery of a previously unknown top-down pathway—from the brain’s cortex to a deep subcortical region called the basomedial amygdala—in the control of anxiety. This is important because many existing treatments in psychotherapy and brain stimulation are focused on the cortex. Similarly, our Science paper (6), published earlier this year, reported top-down control from the medial prefrontal cortex to subcortical regions in the control of reward and motivation. We found that the prefrontal cortex not only affects downstream structures but also influences how those structures communicate with each other; this is a fundamental second-order insight that we had not expected to find.

PNAS: From the standpoint of uncovering disease mechanisms, which disorders have benefited the most from optogenetic studies?

Deisseroth: We have already made substantial headway in understanding causal circuits underpinning anxiety-related symptoms, individual symptom forms of depression, including anhedonic states and hopelessness like states. On the neurological front, we and others have made advances in understanding circuits involved in Parkinsonism and epilepsy, for example.

PNAS: In what ways are the channelrhodopsins you report in your Inaugural Article (1)? (C++ and SwiChR++) next-generation optogenetic tools?

Deisseroth: Channelrhodopsins have always been intriguing as opsins because they are channels, not pumps; when activated, they actually open a pore in the cell membrane through which ions can pass. To engineer them into inhibitory tools, we needed high-resolution structures of the pores for rationally guided design, but the only opsins for which crystal structures were available were pumps. For many years, we did site-directed mutagenesis and functional studies to unravel the structure of channelrhodopsins, and the breakthrough came when we published the crystal structure of a chimeric channelrhodopsin in Nature in 2012 (7) with our collaborators in Tokyo. We could see that the pore was largely lined with negative amino acid residues, so the surface electrostatic environment in the pore was negative. This suggested how the channel could mostly transport cations, which trigger excitatory impulses in neurons. We then tried to engineer the pore so that it had a positive electrostatic environment and transmitted anions, which would make the channel inhibitory to neurons.

That was a truly tall order because there are so many delicate moving parts in the channel—including the embedded chromophore retinal—that must all work together. We made hundreds of constructs, eventually got it to work, and showed that the engineered channelrhodopsin was functional in cultured neurons in a 2014 Science paper (8), published back-to-back with a paper from Hegemann’s group (9) that arrived at the same result using a different set of mutations. But that was just a proof-of-principle; we hadn’t shown any behavioral modulation with these engineered channels. And it’s not a useful optogenetic tool until it can be used to control behavior.

PNAS: Which is where your Inaugural Article (1) enters the picture.

Deisseroth: Right. So in 2015, we set out to do further rounds of engineering of the channel to create a next-generation channelrhodopsin that would be strong enough to control behavior. We wanted to both increase photocurrents through the channel and enhance chloride selectivity. And that’s just what we demonstrated in the Inaugural Article (1). In two separate behavioral tests—focused on dopamine neurons in the ventral tegmental area (VTA) and the amygdala—the team showed how this improved channelrhodopsin functions as an optogenetic tool in mice.

PNAS: How did you demonstrate the usefulness of the improved channelrhodopsins?

Deisseroth: Our collaborators in Toronto had developed a mouse model of fear memory rooted in the amygdala [a tone paired with an electric shock activates a fearful freezing response in a mouse trained to associate the tone with the shock, even when the tone is unaccompanied by the shock]. They developed a system in which they can influence which neurons in the amygdala are involved in the fear memory by affecting the memory’s engram, which is the population of neurons associated with that memory. And by introducing the engineered channelrhodopsin into selected neurons in the engram, they were able to reduce the freezing response in mice. This suggested that the channel’s inhibitory influence was working. Similarly, Rob Malenka and his team at Stanford showed that this channelrhodopsin can also be used for...
conditioning aversion to specific contexts in mice by controlling dopamine neurons in the VTA.

**PNAS:** Your engineering approach was essentially a recapitulation of nature’s own strategy for pore engineering over the slow march of evolution. Can you describe how your work was externally validated, as it were, by natural selection?

**Deisseroth:** This was a serendipitous finding. While we were developing the next-gen inhibitory channel, John Spudich and his team in Texas reported a naturally occurring chloride channel called GtACR2 in a microbe called *Guillardia theta*. What was amazing was that we could see that the internal electrostatic environment of this channel would be predicted to be almost identical to that of our engineered channelrhodopsins, including the one we had published more than a year earlier. The pore that we had conceived by structure-guided design and engineering was essentially the same as that nature had arrived at after presumably millions of years of evolution. That was truly thrilling because it validated the structural model and our understanding of the channel’s pore.

**PNAS:** What are the immediate next steps in honing these optogenetic tools?

**Deisseroth:** At the moment, we can control cell types, which is sufficient for the vast majority of applications, but for some experiments we would like to be able to control arbitrarily defined ensembles of neurons at the single-cell level. The Toronto experiment is a step in that direction, and we’d like to be able to control single cells beyond merely biasing which cells are likely to be recruited. It requires advanced optics and the ability to manipulate light in complex 3D patterns, which we have been working on. It’s an exciting prospect.

**PNAS:** You are an avid reader of fiction and something of a creative writer yourself. Would you care to discuss these less well-known pursuits?

**Deisseroth:** I am enchanted with the power of the written word and its ability to stir feeling. I just returned from a trip to India, and at the moment I am reading Indian writers writing in English. I find writing therapeutic and am writing some fiction now, but it might be premature to promise too much.

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