Q&A

Optogenetics: 10 years after ChR2 in neurons—views from the community

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On the anniversary of the Boyden *et al.* (2005) paper that introduced the use of channelrhodopsin in neurons, *Nature Neuroscience* asks selected members of the community to comment on the utility, impact and future of this important technique.

Neuroscientists have long dreamed of the ability to control neuronal activity with exquisite spatiotemporal precision. In this issue, we celebrate the tenth anniversary of a paper published in the September 2005 issue of *Nature Neuroscience* by a team led by Karl Deisseroth (*Nat. Neurosci.* **8**, 1263–1268 (2005)). In this study, the authors expressed a light-sensitive microbial protein, Channelrhodopsin-2 (ChR2), in neurons and showed that exposing these neurons to pulses of light could activate them in a temporally precise and reliable manner. In the decade since this paper, 'optogenetic' approaches have been widely and enthusiastically adopted by the field and applied to a vast array of questions both in neuroscience and beyond.

In the intervening years, improvements to early techniques have provided the community with an optogenetics tool box that has opened the door to experiments we could have once only dreamed of. Controlling neuronal activity in real time, we now have the ability to determine causality between activity patterns in specific neuronal circuits and brain function and behavior, enabling researchers to definitively test long-held views and advance our understanding of brain function in both health and disease.

Anniversaries are often a time to reflect and, in light of the seminal influence this technique has had on neuroscience, we were curious to know how researchers in the field feel the advances in optogenetic approaches have influenced their work, what they think the future holds in terms of the application of these techniques and what they see as the obstacles we need to overcome to get there. Toward this end, we've asked a number of scientists to share their thoughts with us in this Q&A. Although we weren't able to ask more than a small fraction of the field, their answers give an exciting view of the power and potential of optogenetic approaches for understanding, and even potentially repairing, the nervous system.

How do you define optogenetics?

John Huguenard: Sensitizing neurons to light, then manipulating neural activity in precise spatiotemporal patterns to answer questions regarding neural circuits and behavior.

Michael Häusser: There's a broad definition and a narrow definition. The broad definition is rooted in etymology: any approach that combines optical interrogation with genetic targeting qualifies as 'optogenetic', and that includes the use of genetically encoded activity sensors. However, most people generally use the term optogenetics to mean the use of probes to manipulate activity, and (as is usual in English) usage normally wins.

Ernst Bamberg: Optogenetics is the use of genetically encoded light-activated proteins for manipulation of cells in an almost non-invasive way by light. The most prominent tool is ChR2, which allows in a cell-specific way the activation of electrical excitable cells via the light-dependent depolarization. The combination of ChR2 with hyperpolarizing light-driven ion pumps such as the Cl^- pump halorhodopsin (NphR) allows, with high temporal and spatial precision, the activation or inactivation of neural cells in culture, tissue and living animals.

Richard Tsien: This 10-year celebration, well-deserved by the authors and journal alike, implicitly points to a narrower definition: use of genetically encoded molecules to excite and inhibit neurons. I would prefer it to mean any genetically encoded tool to record or perturb electrical activity of neurons and other excitable cells. But using a broad name to identify a fairly specific subset of tasks was a brilliant stroke, nonetheless.

Dan Johnston: I suppose that the accurate definition would be genetically encoded optical sensors, but it is most commonly thought of as genetically encoded light-activated channels. It's worth noting, however, and I wasn't aware of it at the time I reviewed the Boyden paper, but there were two papers that predated this one that reported a genetically encoded light-activated channel: Zemelman, B.V. *et al. Neuron* **33**,

15–22 (2002) and Zemelman, B.V. *et al. Proc. Natl. Acad. Sci. USA* **100**, 1352–1357 (2003).


Mark Schnitzer

Mark Schnitzer: The term 'optogenetics' first appeared in 2006 in a short review article published in *Journal of Neuroscience* to accompany a Society for Neuroscience mini-symposium that Karl Deisseroth and I had organized. (Deisseroth, K. *et al. J. Neurosci.* **26**, 10380–10386 (2006)). We considered different options, such as 'photogenetics', but eventually settled on optogenetics as the best term to describe techniques that combined optical and genetic facets. Notably, our original intent was to cover both genetically targeted optical control and imaging under a single umbrella term. Nevertheless, I have subsequently always preferred a narrower interpretation of optogenetics that covers only the control approaches and the wonderful field that grew out of Karl's seminal 2005 paper in *Nature Neuroscience*; the broader interpretation of optogenetics that includes imaging is so general that, in some respects, it can be vague. My impression is that a substantial majority of the usages of the term optogenetics in the neuroscience literature follows the narrower interpretation.

Rachel Wilson: I think of optogenetic tools as a set of wrenches in a larger toolkit of genetically encoded effectors. This includes effectors activated by heat, as well as effectors activated by designer drugs, etc. Optogenetic tools are often the most useful because light can be modulated so rapidly and precisely. However, we should just reach for the tool that suits the job. Sometimes the old tools are best!


Rachel Wilson

What was your first reaction when optogenetics came onto the scene 10 years ago? Did you think it would have such a transformative impact on neuroscience?

Peter Hegemann: We were involved from the beginning, as we discovered the main player, Channelrhodopsin, but we never expected such an enormous impact.

Rob Malenka: I was excited about the possibilities, but was skeptical that optogenetics would really work in the manners that were promised. I thought it might have some finite uses, but did not imagine it would be as "revolutionary" as it turned out to be.


Krishna Shenoy

Krishna Shenoy: I had the great pleasure of being right here at Stanford and knowing Ed and Karl for years, so yes, when their results came in and the paper came out it was clear it would be an enormous advance. Could I have predicted how revolutionary it would be? No, there I'm afraid I would have underestimated the full extent to which it has been a neuroscience-wide seismic shift!

Sheena Josselyn: I thought the data were interesting, but likely not replicable and definitely not generalizable. I thought optogenetics would not work reliably and, even if it did, the technique would be so complicated as to be out of reach for most neuroscience labs. My initial impression was that optogenetics would be highly parameter-sensitive and would take lots of fiddling to get any kind of effect. I was definitely in the camp that didn't think it would have an impact on my kind of neuroscience.

Scott Sternson: I thought that if it worked as advertised, then it would be exactly the tool that I'd been looking for since I started in neuroscience.

Gina Turrigiano: Intense excitement. I thought the work leading up to this study was a beautiful example of basic curiosity-driven research (trying to understand the basis of bacterial phototaxis) leading to an unanticipated transformative outcome.


Kay Tye

Kay Tye: My first reaction was one of wonder and amazement. Seriously? Is this really possible? I was a junior graduate student at the time and it was quite the buzz. Many were skeptical and predicted it would be a fad, and I of course was both curious and skeptical—but really too naive to let my skepticism deter my curiosity. Pretty soon, we just tried working with ChR2, trying to replicate the effects seen in the Boyden *et al.* 2005 paper. It was remarkable how well it worked and it was exciting to get spiking from patching onto cells, and it quickly became clear that the technique was very robust and replicable. Shortly thereafter I decided to do a postdoc focusing on this approach, and that's how I ended up at Stanford with Karl Deisseroth.


Antoine Adamantidis

Antoine Adamantidis: At that time, I was finishing my PhD at the University of Liege, Belgium, and we were investigating the role of a unique population of neurons in the lateral hypothalamus (that is, melanin-concentrating hormone) in controlling 'dream sleep'. In mammals, this deep sleep stage lasts classically few minutes, which made it difficult to study with conventional 'low temporal' approaches (pharmacology, KO, KI, etc.) without altering other sleep stages. Thus, when the Boyden/Deisseroth publication came out, I thought, "This is it! That's what we need!" Since I was joining the laboratory of Professor Luis de Lecea at Stanford University a few months later, I emailed Karl about this idea, who replied, "OK, let's meet when you get here!" We did meet, and, together with Dr. Feng Zhang, brought it to brain slices and freely moving mice to publish the first *in vivo* optogenetic paper establishing a causal role between hypocretin/orexin cells and arousal (Adamantidis, A. *et al.*, *Nature* **450**, 420–424 (2007)). Thus, yes, I believe this was a transformative technology since early on!


Thomas Insel

Thomas Insel: While everyone assumes optogenetics is a great technology, reviewers on NIH study sections in 2005 did not embrace this idea. Fortunately, a very smart program officer at NIMH recognized the promise of this proposal. And soon after this first NIMH K award, the advent of the Pioneer Awards, designed for high risk–high reward research, gave Karl the kind of support needed to take this from concept to tool. Optogenetics was a great object lesson for NIH, revealing the need for mechanisms like the Pioneer Award that could overcome the conservatism of traditional peer review.

Gyuri Buzsáki: The impact of optogenetics was big and instantaneous. I think all 'engineer types' immediately recognized that this was the method we were all waiting for. My lab was doing closed-loop experiments in the hippocampus at that time using electrical stimulation and only speculated about the possibility

of affecting specific neuron types and possibly single neurons in neuronal circuits. As soon as we were able to affect specific neurons optogenetically in my lab, I gave away my two-photon microscope setup, as identifying and manipulating cell types combined with large-scale recording of neurons became possible in the freely moving animal. What more can one wish for?

Richard Tsien: I was very excited about the experimental possibilities it opened up and proud of the joint accomplishment of two former graduate students who had worked at different times in my lab at Stanford.

Ivan Soltesz: I immediately knew that optogenetics was going to have an unprecedented impact on neuroscience because it was a technology that virtually all neuroscientists had been waiting for, one way or another, consciously or not. I think that many people had been keenly aware of the fundamental veracity of Francis Crick's major challenge to neuroscience that he formulated in 1979, that is, that the path forward to understanding neuronal circuits and the associated behaviors was through the development of a technology that allows the selective *in vivo* control of one type of neuron without affecting the activity of all others. Historically, there is no doubt that several scientists, myself included, for at least a decade before the publication of the Boyden *et al.* paper in 2005, had entertained the possibility of making specific types of neurons light sensitive and using light to switch them on and off. Some tried to make it happen and did not really succeed, while others did not even try; for example, when I, as a fresh assistant professor, had raised the possibility of optogenetics to an accomplished senior scientist at my institution in 1995, he rolled his eyes and told me that it would be impossible to express enough of the light-sensitive proteins to generate measurable photocurrents, and I had believed him (entirely my fault, not his). But the point is that optogenetics was 'in the air', something that was expected to arrive one day, and when it finally did, many of us instantly recognized its importance. However, what I, for one, did not expect was how incredibly fast the basic proof of concept was developed into a versatile, easy-to-use, widely accessible technology that it is today. 10 years is an awfully short time, and if we use my personal time metric, we could say that it is only 2 NIH grant cycles (a cycle defined as a typical 5-year R01 grant). But what made the rise of optogenetics so fast? I believe it was more than just the evident usefulness of the technology itself. Indeed, in my opinion, it is to the credit of Deisseroth and Boyden that they had recognized early that by freely sharing the reagents and methods they can make optogenetics as much of a basic necessity in neuroscience labs as PCs, iPhones and iPads came to be in the lives of everyday citizens. This is a part of their genius that made optogenetics spread like wildfire. The open-source philosophy that they adopted stands in stark contrast to numerous other techniques where the developers tightly control all material and procedural aspects of their methodology for short-term gain, which in most, albeit not all, cases has proven to be a rather penny-wise, pound-foolish attitude in the long run.

Ivan Soltesz

Yukiko Goda: My first encounter with optogenetics and a glimpse into the future was hearing a talk by Gero Miesenböck. It was so striking to see a fly being controlled like a mechanical toy of sorts simply with a beam of light. The visual impact was so strong that one could almost intuitively grasp the significance of the technology for coming years. This was in contrast to the knockout mouse technology. Although being of equal or greater importance in advancing broad areas of life sciences, including neuroscience, its impact was less immediate and required more academic thoughts.

Yukiko Goda

Christian Lüscher: I was curious, but it took us 2 years to get started and try ourselves. The issue for us was the virus that would not express well initially. We also played around with different light sources, using expensive lasers, until we realized that many of the cheaper LEDs had enough power. Once everything was in place, it took us another 6 months to see the first photocurrents… and ever since there was no stopping. I certainly did not foresee the full extent of the transformation that optogenetics would bring to neuroscience, but the concept was so clear that there was no doubt that everybody would use the technique.

Gero Miesenböck: The timeline implied by your question is incorrect. Optogenetics did not suddenly come "onto the scene 10 years ago"; on the contrary, all the core concepts of optogenetics were established well before the Boyden *et al.* paper appeared. A paper published in January 2002 showed that light acting on an ectopically expressed opsin could be used to stimulate neurons (Zemelman, B.V. *et al. Neuron* **33**, 15–22 (2002)) and demonstrated that genetically targeting the photoreceptor allowed one to control specific neuronal populations. A paper published in April 2005 showed that optogenetic activation of different circuits in the brain could change specific aspects of an animal's behavior (Lima, S.Q. & Miesenböck, G. *Cell* **121**, 141–152 (2005)).

Gero Miesenböck

These precedents do not diminish the practical importance of Boyden *et al.* Swapping ChR2 for the opsin used earlier (because the molecular identity of ChR2 was still unknown at the time) made optogenetics more effective and much simpler to use. The alignment of simplicity, ease of use and efficiency catalyzed the rapid spread of optogenetic technology throughout neuroscience. Although I knew from the moment I had the original idea that optogenetics would be transformative, the field didn't explode until the currently dominant version of the technology was introduced in September 2005. But it also took a while for the fundamental concept to sink in. One of the reviewers of the first optogenetics paper (Zemelman, B.V. *et al. Neuron* **33**, 15–22 (2002)) asked whether it wouldn't be better to study the retina instead, "which conveniently has light-sensitive cells already built in."

Georg Nagel: There are good reasons to argue that optogenetics came onto the scene before 2005. Gero Miesenböck published 'chARGe' in 2002 (Zemelman, B.V. *et al. Neuron* **33**, 15–22 (2002)), a combination of three proteins that made neurons light sensitive, and Kramer, Trauner and Isacoff applied a chemical optogenetic approach in 2004 (Banghart, M. *et al. Nat. Neurosci.* **7**, 1381–1386 (2004)) to silence neurons. *Current Biology* published the first truly non-invasive light-manipulation of animals: *C. elegans* in 2005 (Nagel, G. *et al. Curr. Biol.* **15**, 2279–2284 (2005)) and *Drosophila* in 2006 (Schroll, C. *et al. Curr. Biol.* **16**, 1741–1747 (2006)).

Georg Nagel

But optogenetics is not restricted to neurons, and therefore our demonstration of a heterologously expressed light-sensitive proton channel in 2002 (Nagel, G. *et al. Science* **296**, 2395–2398 (2002)), but even more so the strong light-induced depolarization of several animal cells, including human embryonic kidney (HEK293) cells, via Channelrhodopsin-2

in 2003 (Nagel, G. *et al. Proc. Natl. Acad. Sci. USA* **100**, 13940–13945 (2003)), was a demonstration of optogenetics.

Coming back to the question, I did not believe that Channelrhodopsin would have such a transformative impact on neuroscience, but I believed already in 2002 that Channelrhodopsin has great power and potential, therefore we (Ernst Bamberg, Peter Hegemann, Georg Nagel) applied for a patent in the EU and US before publication of Channelrhodopsin-1.

Roger Tsien: Certainly Francis Crick foresaw the transformative impact much earlier, writing "The tendency in neuroscience (and I'm hoping that this will change) is to say, 'Yes, I'd love to have new tools, but will someone else please develop them'?"… "One of the next requirements is to be able to turn the firing of one or more types of neuron on or off in the alert animal in a rapid manner. The ideal signal would be light, probably at an infrared wavelength to allow the light to penetrate far enough. This seems rather far-fetched but it is conceivable that molecular biologists could engineer a particular cell type to be sensitive to light in this way."


Roger Tsien

This was published in 1999 (Crick, F. *Phil. Trans. R. Soc. Lond. B. Biol. Sci.* **354**, 2021–2025 (1999)), but he had been saying more or less the same idea for decades until colleagues such as myself persuaded him to make a publishable citation.

I wrote to Peter Hegemann an e-mail explicitly linking the involvement of *Chlamydomonas* opsins (see **Box 1**).

What types of studies or approaches do you think represent the most effective usage of optogenetics in neuroscience research?

Gero Miesenböck: I feel that optogenetics is used most productively in two situations: when one knows very little about neural mechanisms and when one knows a lot. In the first situation, optogenetics can help identify the important players (much like conventional forward genetic screens can), and in the second, it can be used to test hypotheses.

Kay Tye: At this point, optogenetics is just another tool in our arsenals that can be applied in conjunction with readouts for naturally occurring neural dynamics to test hypotheses about causal relationships. There is nothing wrong with using optogenetic approaches to validate long-standing hypotheses, although at this point in the field, it is a powerful strategy for identifying completely novel roles for neural circuit constituents.

Silvia Arber: I think any study that makes very careful use of optogenetics and, in particular, is aware of the fact that optogenetic activation assesses what a neuron can do, but not what a neuron does do. I often compare optogenetic activation technology to methods removing a cell at early developmental stages from its context in the embryo in order to challenge it to differentiate into something else in a dish. Also there, such manipulations reveal potential, but not the normal fate of a cell.

Jaideep Bains: Visualizing hypothalamic network dynamics for appetitive and consummatory behaviors from the Stuber lab (Jennings, J.H. *et al. Cell* **160**, 516–527 (2015)) or Neurons for hunger and thirst transmit a negative-valence teaching signal from the Sternson lab (Betley, J.N. *et al. Nature* **521**, 180–185 (2015)). Papers like these are probing and discovering new circuits and linking specific cell populations to behaviors. It's important to point out that the papers pushing the boundaries now don't rely solely on optogenetics, but use it in combination with chemogenetics and *in vivo* imaging. Optogenetics is one (very important) tool in the toolbox for dissecting circuit function.


Jaideep Bains

Has there been a major breakthrough in our fundamental understanding of brain function that could not have been possible without optogenetics?

Christof Koch: Not yet.

Michael Häusser: Many important discoveries have been made using optogenetics, but if one sets the bar high for defining a major breakthrough, then the answer is "not yet." That does not mean that it hasn't illuminated almost every corner of neuroscience and transformed the way we do experiments.


Gina Turrigiano

Gina Turrigiano: I would say that to date the outcomes have been somewhat modest. The technology, combined with advances in molecular genetics, has allowed circuit-breaking to be done more precisely than previously possible. On the other hand in my view many of the 'gee wiz' publications that get the media all hepped up—that is, 'remote control this and that'—were pretty obvious from what we already knew.


John Huguenard

John Huguenard: Functional studies of specific long-range projections were not possible before. Whether the insights obtained are a breakthrough or not remains controversial because there are challenges in relating mouse behavior to humans. Our studies on thalamocortical connectivity in the *Gria4*$^{-/-}$

BOX 1

Date: Sun, 10 Oct 1999 22:41:11 -0700
To: peter.hegemann@biologie.uni-regensburg.de
From: "Roger Y. Tsien" <rtsien@ucsd.edu>
Subject: algal rhodopsins

Dear Professor Hegemann: I have been interested for some time in potential methods by which mammalian neurons might be transfected with a gene whose product would permit light-triggering of depolarizations and action potentials. Eventually I came across your outstanding pioneering work on the light-activated conductances and opsins of *Volvox* and *Chlamydomonas*. However, I cannot find any papers on heterologous expression of these opsin genes, especially in the systems more commonly used in electrophysiology, such as *Xenopus* oocytes or HEK293 cells. Has heterologous expression been seriously attempted? If it has, but no light-activated currents were detectable, is it known whether the problem was in (a) getting enough protein expressed on the plasma membrane, (b) incorporating retinal or (c) finding a partner channel, if the opsin itself proved not to be the channel?

paper (Paz, J.T. *et al. Nat. Neurosci.* **14**, 1167–1173 (2011)) enabled in a way the study of Bo Li on selective attention. Driving of parvalbumin cells to produce gamma would not have been possible, and although predicted from earlier theoretical studies, this function, and the ability to modulate it by light, is a breakthrough.

Sheena Josselyn: At first, the studies using optogenetics were mostly confirmatory (verifying lots of what we already knew about how the brain worked from decades of lesion/pharmacology and genetic studies). I think this is true of most new technologies (such as fMRI). Scientists needed to be convinced that it works as advertised before they started thinking about how to do really interesting and innovative experiments to take advantage of the power offered by this new tool. I think the field has now begun to design experiments that take full advantage of this tool. But again, it is only a tool and should not be used to drive the experimental question.

Ann Graybiel: The use of optogenetics has allowed for the first time the manipulation of specific neural cells in real time in awake, behaving animals. Prior use of electrical microstimulation allowed rapid manipulation, but failed to allow manipulations with cell-type specificity. Because neurons with different functional characteristics are intermixed side by side, manipulations without cell-type specificity could not reveal the extent of functional specificity of microcircuits in the mammalian brain. The combination, in optogenetics, of fast kinetics and cell-type specificity has led to a major breakthrough in our identification of, and understanding of, this feature of neural circuit design: within any given circuit, there is extraordinary cell-by-cell microcircuit specificity, whereby intermingled neurons with different patterns of connectivity can influence ongoing and future behavior in strikingly selective ways. Such circuit design was, of course, posited before, and was familiar especially to neuroscientists working on invertebrates, but before optogenetics, this selectivity could rarely be examined systematically at the experimental level in mammals. As an example, this feature of optogenetics has allowed neuroscientists to uncover specific functions of interneurons never before open to experimental analysis. It further has allowed the discovery of a level of online control of behavior never before identified by previous methods.


Ann Graybiel

One point that is tremendously important, but very difficult to quantify, is the emergence of a sort of can-do attitude thanks to the co-development of optogenetics and genetic engineering. No problem is too difficult to tackle, no mystery too opaque to be penetrated. This brave new attitude alone makes optogenetics, along with genetic engineering, an extraordinary game-changer in our field.

Rob Malenka: 'Fundamental understanding' may be too strong a phrase. Optogenetics has certainly advanced our understanding of brain function in very important and even astounding ways. But it has not caused a true paradigm shift (using the term correctly as Kuhn intended) in how neuroscientists think about brain function. We still think about circuits and how they function. We can just explore circuit function and define novel circuits in ways that we could not do and were in fact, unimaginable, without optogenetics.


Rob Malenka

How has the advent of optogenetics changed the types of scientific questions you ask and your approach to solving them?

Christof Koch: We can move from observing the brain to interfering in it. Given the sheer inexhaustible multiplicity of causal factors responsible for any one action in the nervous system, inferring which ones are actually responsible will not be easy even though we now have the technology.


Christof Koch

Silvia Arber: Working on questions of motor control, the temporal resolution that optogenetics offers is very valuable. It is now possible to study at millisecond resolution how changing neuronal activity of defined neuronal populations influences motor behavior. That allows us to make the link between genetically/developmentally defined neuronal populations and their precise function in an animal *in vivo*.


Silvia Arber

Jaideep Bains: I think we can be more ambitious with our questions.

Patricia Janak: Optogenetics has vastly increased the precision with which systems neuroscientists can make causal connections between a given circuit and a given behavior. It has been a game-changer for our research. We are in the phase where this precision allows us to take apart circuits little by little. In the future, we will need to use optogenetics in new ways, along with other approaches, to then determine how multiple parts of a circuit work together in an integrated fashion, *in vivo*—this will be a fascinating challenge!


Patricia Janak

Richard Tsien: It has greatly heightened our interest in attaching functions to particular cell types, and in moving from questions of single neurons to circuits. It makes it much more honorable to be a toolkit inventor, building on the precedent of Roger Tsien, Erwin Neher and Bert Sakmann.

Christian Lüscher: Optogenetics is the beginning of causal neuroscience! For the first time it has become easier to manipulate the brain than to observe its function. I am convinced that if optogenetics is used carefully, it will help us understand how neurons generate behavior. This, however, will require that we develop new observation techniques that have the same spatial and temporal resolution as optogenetics (for example, imaging activity of ensembles using genetically encoded calcium indicators) such that the manipulations can be tailored to closely mimic physiological neural activity.

Antonello Bonci: Before optogenetics, my laboratory used electrophysiology in combination with molecular and behavioral approaches to study drug and reward-dependent synaptic plasticity, but we didn't have any tools to understand which brain pathways were relevant to process/modulate these behaviors. When we started using optogenetics, we could finally start addressing which pathways matter and for which behaviors. Furthermore, it allowed my team to address another fundamental question: the relationship between


Antonello Bonci

synaptic strength and drug-dependent and reward-related behaviors. Optogenetics offered us the opportunity of major leaps forward in our understanding of these complex behaviors. Yet this isn't the best part about optogenetics. Optogenetics allowed my lab to attack the most relevant question that has haunted me since I became a scientist: how to translate quickly and effectively our rodent studies into treatments for patients.

Jessica Cardin: The ability to manipulate targeted cell classes on a fine temporal scale has dramatically changed the way we pose questions about network interactions. Rather than compiling extensive observations and making inferences about the impact of a particular cell class, we tend to ask the causal questions at the beginning of a series of experiments and examine both causal and observational data in parallel. Rapid iteration of optogenetic and viral tools has also led us to make fewer up-front assumptions about experimental limitations.


Jessica Cardin


Michael Häusser

Michael Häusser: It has completely changed how we do experiments in cellular and systems neuroscience. It has provided us with powerful tools for making causal links between elements of neural circuits and behavior—in that we can prove both necessity (by inactivating neuronal populations) and sufficiency (by activating the same neurons). It is progressively replacing conventional pharmacological experiments, in that now one can directly identify the contribution of a particular neurotransmitter pathway, rather than just the involvement of a receptor for a neurotransmitter. And it has meant that we are progressively abandoning stimulating and recording electrodes, the tools that I grew up with as an electrophysiologist.

How does the advent of optogenetics compare to other technological breakthroughs such as the patch-clamp recording or magnetic resonance imaging?

Rob Malenka: I think it has had a much bigger impact than patch-clamping because patch-clamping remains a highly specialized method that can only be used to address a very circumscribed, finite group of questions. Prior to patch clamping, we still had traditional intracellular recording techniques that worked for many of the questions that CNS electrophysiologists wanted to address. Of course, patch-clamping allowed biophysicists to look at single channel properties, but that topic interests a small number of neuroscientists. In contrast, optogenetics opens up new types of experiments that a broad array of neuroscientists are interested in performing—ranging from hardcore cell biologists and electrophysiologists to behavioral neuroscientists working in species ranging from flies to monkeys. Optogenetics also has a broader impact on basic neuroscientists than MRI because MRI needs such highly specialized and expensive equipment and technically is only understood by a small group of investigators. Thus, it's accessible to a small number of working neuroscientists. Plus the temporal and spatial resolution of fMRI approaches really limit what it is able to tell investigators.

Yukiko Goda: From my perspective, optogenetics thus far have been powerful in characterizing brain circuits at the mesoscopic scale. This is somewhere between MRI, which has been revolutionary in functional mapping of macroscopic brain regions, and patch-clamp recording technique, which has had an enormous impact on neurophysiology at molecular and cellular levels. Not to mention, though, optogenetic tools have also been useful for addressing questions at the cellular level.

Anatol Kreitzer: Optogenetics has been the most influential breakthrough in neuroscience during my scientific career. It is at least as important as any major technical development in the past century, including Golgi staining, voltage clamp, patch clamp, GFP, calcium indicators and two-photon microscopy.

Richard Tsien: Interesting that you happen to draw comparisons with methods that are largely devoted to measuring activity rather than merely manipulating it. Optogenetics is way up there in the way it has captured the imagination of experts and lay public alike. But I have to chuckle when newcomers think that practically every problem in neuroscience calls for an optogenetic approach. But no question that the methods are very powerful.


Richard Tsien

Ivan Soltesz: I think it is definitely comparable. However, in a way, it was more useful because, unlike patch-clamp or MRI, optogenetics is a control technology that allows the investigator to interfere with and rationally manipulate the neuronal system in a causal manner *in vivo*, and not 'just' record/describe the activity or structure. Furthermore, patch-clamping and MRI, to remain with these specific examples, never really spread beyond the labs that had already been doing electrophysiology or imaging, whereas optogenetics found applications in virtually all aspects of neuroscience. It is a very rare technology that can impact so many fields so fast and so thoroughly.

Sheena Josselyn: Optogenetics is right up there with these other transformational technologies. The wonderful thing about optogenetics though is that both labs interested in patch-clamp and labs interested in MRI can now incorporate optogenetics into their arsenal. This technique is agnostic to the type of question being asked. It can be used in slices, behaving rodents and non-human primates. Plus, the ease with which optogenetics can be set up and used makes it attractive to non-aficionados. I'm amazed by how many labs have incorporated optogenetics into their research without a huge investment in training (unlike patch-clamp) or equipment (unlike MRI). The way the optogenetics tools have been shared also makes it easy for new labs to use this technique. The brain, for whatever reason, seems highly forgiving. That is, large behavioral effects are observed when largely undefined groups of neurons are synchronously driven by ChR2. Although the early studies did not even attempt to recapitulate the precise temporospatial firing properties that the brain normally uses to communicate, nonetheless, the standard 20-Hz stimulation typical of the early experiments produced large, reliable behavioral responses. It's as if this manipulation was sufficient to nudge the circuits into a different state.

Thomas Insel: The importance of optogenetics is that it brings neuroscience closer to causality. The first 50 years of neuroscience have been mostly observational and correlational. Because optogenetics allows us to turn on and turn off function with cell-specific, millisecond precision, we can begin to identify the activity that is both necessary and sufficient to link neural function to behavior. There are limits, but this is a transformative technology for neuroscience. One other important insight from this technique is that the fundamental advance—using an opsin—simply borrows from an experiment of nature. Indeed, some of the best science of the last few decades has come from studies in arcane species where natural adaptations have revealed

extraordinary opportunities. Neuroscientists who become naturalists, poking around in the broad world of biology for new tools, almost always find something interesting.

Having witnessed the proliferation of optogenetics, should tools be developed to answer specific scientific questions, or should technological innovation come first and the scientific questions follow?

Dan Johnston: Technical innovations always lead the scientific questions, and this has certainly been true with optogenetics. Given the right tools, clever scientists will always come up with novel questions that can be answered with the new techniques. They might not have even thought of the questions without them.

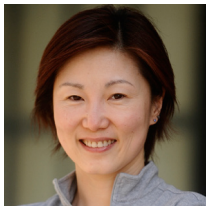

Dan Johnston

Christof Koch: Both will happen simultaneously, one driving the other in a continuous loop.

Scott Sternson: Technology, including optogenetics, is developed to address a scientific need that is relevant to many questions. Before optogenetics, it was clear that approaches to manipulate the activity of specific neuronal cell types were needed, and there had been various attempts at this before optogenetic methods were achieved. Channelrhodopsin, in particular, turned out to be remarkably easy to use and was quickly adopted by neuroscientists working on all types of questions.

Gero Miesenböck: The scientific question must always take center stage. I get dismayed when postdoc applicants want to join my lab to work on optogenetics, or when search committees approach me to suggest faculty candidates in optogenetics. Imagine 30 years ago there had been a hiring spree of faculty working on PCR. What would these people have been doing since then?

Yang Dan: I think it goes both ways, people are motivated to develop new techniques when they see major technical road blocks for a particular field, but sometimes a technique finds surprising application in another field that the developer is not initially aware of.


Yang Dan

Anatol Kreitzer: Scientific questions should always be primary. If you are following technological innovation, it's akin to the 'streetlight effect' where you are searching where it's easiest (under the streetlight), but not necessarily where it's most productive (where you lost your keys). In the case of optogenetics, it has enabled a vast number of experiments that are yielding critical new information about brain function. If it enables you to address long-standing questions, that's great. If you want to use it only because it's an exciting new tool that everyone is using, that is absurd.

Gyuri Buzsáki: Carl Ludwig, the inventor of the kymograph, advocated that "Methode is Alles." While there is truth in this wisdom, the history of science shows that there is no simple recipe. For example, holography and its possible implications were outlined early, but practical applications had to wait for fast and reliable lasers. On the other hand, single-cell PCR and GFP-labeling methods fertilized thinking and brought about numerous discoveries not only not possible without them, but which were not even conceived before the existence of those methods. Just as nature exploits whatever substrate or mechanism is available for novel solutions, the scientific community also quickly jumps on novel methods because they provide new windows on existing problems and new windows offer new views. Novel techniques always undergo an evolution. The initial, hyper-enthusiastic phase is often mixed with outrageous claims about the novel method's power and specificity. In the maturational stage, the claimed super-specificity and super-sensitivity issues are reduced and replaced by more sober understanding of the objective and reliable values of the method. In the third phase, the innovation is adopted by a large community and combined with other methods. This is typically the stage when major breakthroughs are expected. Optogenetics is currently quickly transitioning between the first and third stages, but the hard work needed for the maturation stage needs to be invested sooner or later as well.


Gyuri Buzsáki

Krishna Shenoy: Neuroscience tools are extremely important, just as in all other areas of science and engineering. I'm very happy that President Obama's BRAIN initiative has highlighted the importance of this. And of course optogenetics is a shining (no pun intended) example of this. Tools can lead, tools can follow and tools can be developed hand in hand with science. Of course, a tool is never enough, it must be used to build understanding and/or build treatments.


Rui Costa

Rui Costa: Both go hand in hand: the technology is enabling for the prepared mind. If it would have arrived earlier before optics was so easy and before circuit mapping tools and transgenesis and genetic tools were less developed, it is unclear if it would have taken off so fast. The field was ready and in need.

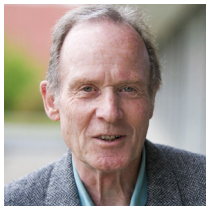
Sheena Josselyn: Both must be done in concert. New tools can inspire research—reminds me of Maslow's hammer (that if all you have is a hammer everything starts looking like a nail). Tool development can allow scientists to ask entirely new questions. With the BRAIN initiative and similar global funding mechanisms, the development of tools has been garnering a large amount of attention (and resources). However, the value of a tool only becomes apparent when it is put to use in service of an important biological question.


Sheena Josselyn

Here's a funny story about the subtle scientific pressure to adopt the latest tools. A few years ago, I gave a presentation where I showed our labs' data on overexpressing a transcription factor and examining the effect on memory. As soon as I finished, one audience member's hand shot up and the listener queried the 'non-physiological' way in which I had manipulated the brain. He suggested that we instead use a more natural approach. Feeling slightly annoyed by this, I concurred that our manipulation was indeed non-physiological. What we really needed to do was to artificially express a protein from a bacteria in neurons in the brain, implant an optical fiber and then shine a light on these neurons. I asked my inquisitive colleague if this approach would be more physiological. He nodded his head in agreement. It just goes to show how the field has really embraced optogenetics and now feels it is a standard (must-use) technique.

Ernst Bamberg: This question is difficult to answer because, depending on the situation, both approaches can be helpful; in other words, this is not a clear alternative. I like to cite Max Planck: "Knowledge precedes application."

The optogenetic tool ChR was found exactly according to the motto by Max Planck because we wanted to know how this molecule works, which afterwards was applied to optogenetics. Now optogenetics opens from its beginning completely new possibilities for new scientific questions, which requires for many problems the development of appropriate tools.

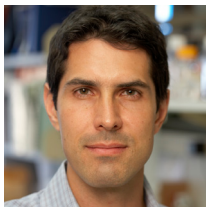

Ernst Bamberg

Richard Tsien: I am troubled by the word 'should', used twice in this sentence. If it's 'should' as in 'ought to be', then who gets to define this imperative? Funding agencies, peer reviewers, individual scientists or perhaps even the general public? Here, I prefer the possibly chaotic patterns of an open system, without too much top-down direction. Clearly, funding needs to be balanced for tools invented on spec and funding for goal-driven studies that invent new tools as they go along. Having spoken with Hodgkin and Huxley, heroes for many of us, after their landmark work on excitability, I feel strongly that there's a lot of room for those that invent their own tools for biological studies and those that are happy to use the tools of others. Cole invented the voltage clamp, but Hodgkin and Huxley knew much better what to do with it. Both contributions were essential.

What do you feel are major conceptual and/or technical limitations in how optogenetics is used in the lab today?

Rob Malenka: For many uses, we're still limited by the availability of Cre driver lines or, more generally, the genetic access to many important subsets of neurons. We're also limited by the light-delivery systems, although engineers are working hard on this and I think it is likely that in a few years injectable LEDs or similar light sources that are controlled remotely will be available.

Scott Sternson: Optogenetics needs to be used primarily to assess the functional significance of activity patterns measured *in vivo* during behavior. Most studies do not do this yet, but, with improved deep-brain imaging capabilities, it will become increasingly common. However, even if the activity patterns are known, we can seldom reproduce these patterns in the exact subset of neurons using optogenetic activation or silencing. Most approaches today involve synchronous activation or silencing of the neurons. Improved fidelity to the endogenous activity patterns would truly realize the potential for optogenetics to test the causal relationship of neuron activity patterns in the brain to behavior.


Scott Sternson

Gero Miesenböck: I see three principal limitations. The first is the difficulty of gaining selective and comprehensive genetic access to the neurons of interest. The second is the difficulty of tailoring optical control signals to individual cells in a population rather than the population as a whole. This is not only a technical problem, but also an intellectual one. What types of activity pattern should we play back to the brain if we had the ability to do so? This brings me to the third, most fundamental difficulty: the lack of a theoretical underpinning for much of neuroscience. We don't understand most neural systems well enough to articulate and test clear hypotheses.

Kay Tye: Right now we need to a priori know the genetic features and have a handle for expressing optogenetic tools in specific neuronal populations. What we can't really do yet is target cells based on specific functions. Although immediate early gene promoters have done a lot in this vein, the windows for tagging are still very imprecise, many orders of magnitude greater than the functional speed of information transfer in a neuron. Furthermore, just to say that a cell was 'active' during a window is not the same as being able to identify specific patterns of behavior within a subset of cells and label only those populations. So right now, we are not able to selectively manipulate neurons with highly specific functionality, and we are limited by the existing genetic tools and/or large temporal windows of activity. Furthermore, selective playback of diverse, specific patterns across large populations of neurons also represents an ongoing challenge. This has been a problem that can be tackled in relatively small populations of opsin-expressing neurons with two-photon imaging, but there are limitations in how many neurons can be controlled in this manner. Finally, the biggest limitation or challenge with optogenetics is light delivery. Light is great because it is temporally precise and has many specific wavelengths, but it does not penetrate through fatty tissues very well, and has been a challenge when translating to larger brains, like primates. Pharmacogenetic approaches address this issue, but come with the tradeoff of much slower timescales for onset of activation/inhibition, and these are limited by drug delivery, binding affinity, etc. Another idea that several groups have initiated effort toward, but still requires development, is the use of magnetism to activate genetically encodable proteins because it could be highly penetrant, non-invasive and could be relatively fast.

Peter Hegemann: In reality, light is not a good medium to activate proteins in large animal brains; light is more suitable for transparent model organisms like *C. elegans*, *Drosophila* and Zebrafish.

What we need for mammalian brains are proteins that are sensitive to other media as ultrasound, teraherz radiation or magnetic fields that penetrate the brain much better than light. What we also need is improved light control of DNA editing, gene expression and light-sensitive enzymes, which not elaborated well enough yet but bear a tremendous potential for future applications in the neurosciences and developmental biology.


Peter Hegemann

Anatol Kreitzer: One of the biggest technical limitations of optogenetics is the current lack of tools for local control of axons and presynaptic terminals. A number of questions in systems neuroscience necessitate the control of specific axonal projections (for example, to Region A, without affecting projections of axon branches from the same neuron to Region B). Selective activation of axons in Region A with channelrhodopsin yields antidromic spikes that may propagate to Region B. Selective inhibition of axons in Region A with current optogenetic tools has been difficult, at best. Developing a tool for temporally precise and reversible optical control of neurotransmission would be a major advance.


Botond Roska

Botond Roska: When using viruses as delivery tools, there is a variation in the expression of optogenetic tools. This is a major limitation since it precludes quantitative interpretation of results. Simultaneous optical actuation with readout and feedback could solve this problem in the future.

Krishna Shenoy: There is one on my mind much these days, and it can be overcome (we are working on it, together with Karl). The

major theme of understanding populations of individual neurons requires, we believe, a dynamical systems perspective that has as a central concept dimensionality of the neural data. For example, if we record from 100 neurons, we often find the motor cortices only appear to use 10–15 dimensions (independent degrees of freedom; see Cunningham, J.P. & Yu, B.M. *Nat. Neurosci.* **17**, 1500–1509 (2014) for a review). But, while optogenetics provides cell type–, temporal- and projection pattern–specific activity modulation, you cannot 'push' neural activity out in certain dimensions. You can push activity up/down in groups of cells, but these cells may well (and generally do) contribute to multiple dimensions. So we need to be able to more precisely and selectively manipulate specific neural dimensions in order to mesh with this other major thrust area of systems neuroscience these days. We think the key is, no surprise, patterned optical illumination so that you can more selectively influence specific dimensions.

Patricia Janak: Scientists need to keep in mind the caveats that may arise in some experimental designs as a result of synchronous optogenetic activation of neuronal populations in bulk that does not mimic natural activity of the neurons or projection fibers in question. Complementary measurements of *in vivo* neural activity are required to fully understand the natural behavior of the circuit in question.

Jaideep Bains: There are two issues that stand out for me. First, *in vivo* activation with light results in (potentially) non-physiological synchronization of neural population firing. Second, its use as a tool in brain slice experiments to examine synaptic function is limited because of widespread expression in membranes throughout the cell that can result in recruitment of calcium release from internal stores.

Do you feel that optogenetics will ever become a clinical tool for treating human disease?

Kay Tye: Regarding diseases of the brain, I would never say never, but there are some major challenges that stand in the way. First, light delivery represents a component that will very likely require it to be an invasive strategy. Optimization could reduce the degree of invasiveness required, but that is one issue. Second, opsin expression is another problem. Right now, in animal research, the main strategy (aside from transgenic animals) is viral transduction. But viral vectors typically do not induce very stable expression, as the protein continues to accumulate over time and with enough accumulation the natural cellular machinery is jeopardized and cells may become sick. Much effort would need to be invested in developing and testing safer opsin expression strategies, and these strategies would need to be tested for years or even decades since we understand the brain far less than we understand some of our peripheral systems. For example, using optogenetics to help blind people see again is in a different category because if opsins are presented to the retina, this does not impose an added risk to the patient since they are already blind. However, if someone has a mental health problem such as depression, it is quite possible to impose an added risk as we could for example also damage circuits important for cognition or sensory processing while trying to treat the depression.

Botond Roska: I hope so. We are working on it.

Anatol Kreitzer: Yes, I believe so, and we may see some early successful applications (for example, in the retina) within a decade. But I suspect it will be longer before it becomes a widely used tool for treating CNS disease. There are many fundamental questions that need to be addressed. Can optogenetic proteins be stably expressed and activated in human brain without inflammation or disruption of cellular processes over decades? How will optogenetic proteins be targeted and expressed in specific cells of the human brain? How will light be delivered uniformly over broad areas deep in the human brain?


Anatol Kreitzer

Ivan Soltesz: I really think and hope so. There is no doubt that optogenetic-based interventions do work in experimental animals in several models of neurological and psychiatric disorders, and by 'work' I mean they do things that are otherwise not possible (in my field of closed-loop control of intractable epilepsies, for example, optogenetics-based intervention can abort a seizure in a way that interferes with only a minimal number and specific types of cells; only a few years ago, nobody thought that one can abort seizures after they have started without shocking the brain with huge currents). Optogenetics have also been implemented in non-human primates, and there are clinical trials with viral vectors, for example, AAVs. In addition, as we understand it today, insertional mutagenesis may be avoided using vectors that remain extrachromosomal, decreasing the possibility for tumor formation. As mentioned above, there are ongoing efforts to apply optogenetics in human brain tissue in several labs using *ex vivo* approaches (that is, resected human brain slices, either acute slices or organotypic slices). There are private companies that try to make the clinical application a reality, fueled by venture capital (for example, Circuit Therapeutics). Exactly where (which disease) will be the one where the first breakthrough will occur in terms of clinical applications is hard to predict, but medically intractable focal epilepsies are definite candidates because, unlike in almost any other application involving the human brain, in the case of the epilepsies the viral vector could be injected and optogenetic control attempted in the specific brain area containing the seizure focus, which will be then surgically resected in any case as part of traditional 'therapy'. Another area where clinical application may take place is pain, where there is usually a well-defined area where intervention can be attempted. Obviously, safe long-term light-delivery devices will need to be adopted or developed, and most likely the first applications will be targeted at brain regions closest to the dura (that is, not located deep in the brain).

Roger Tsien: Once I did not think so, but my mind has been changed by the spectacular progress toward retinal prostheses such as from the group of Duebel in Paris.

Christian Lüscher: Yes, eventually, but not in the next 10 years. There are many obstacles, such as cell type–specific targeting, stability of expression, viral long-term toxicity, etc., that preclude translation for the moment and much development is required to overcome them. I, however, see a window to use optogenetics to develop new deep brain stimulation (DBS) protocols. Characterizing pathological circuit function (with optogenetics) in non-neurodegenerative behavioral diseases may lead to blueprints of manipulations aiming at restoring normal circuit function, thus reversing the pathological behavior (with optogenetics). If such protocols can then be emulated with DBS, clinical trials can be envisioned that test for safety and efficacy. In other words, optogenetically inspired DBS is the 'hic et nunc' translation of optogenetics.


Christian Lüscher

Thomas Insel: Optogenetics was one of the formative technologies that led to the current

BRAIN initiative. Looking back on the tools developed over the past decade, it was clear that the field was ready for a major leap forward. This is a great example of the 'then, now, imagine' approach to launching scientific programs. One slightly simplistic mission of the BRAIN initiative is to imagine we had the tools for human neuroscience that we have today for mice. In reality, the need to deliver an engineered channel and a light source will make optogenetics a tough reach for deep brain stimulation. But an artificial retina, a prosthetic for a cortical pacemaker or another form of chemogenomics (such as designer receptors) are all worth considering for human application. Seeing what this tool has done for studies in mice, optogenetics should persuade clinical neuroscientists to 'think different'.

Antonello Bonci: This is the most extraordinary part about optogenetics, which I consider the best tool that my laboratory has to develop treatments for certain human diseases such as substance use disorders. I personally love its intellectual and technical proximity to therapeutic treatments that have been employed for many years with limited, narrow therapeutic indications. I am referring to brain stimulation techniques such as repetitive Transcranial Magnetic Stimulation (rTMS) and DBS. While the core features of DBS, rTMS and optogenetics differ substantially, they share a similar strength: altering electrical activity in brain regions and pathways in order to ameliorate behavioral symptoms. A concrete proof of my enthusiasm comes from a paper that my group published in *Nature* back in 2013 (Chen, B.T. *et al. Nature* **496**, 359–362 (2013)). In this study, we first showed a marked reduction in prefrontal cortex excitability in compulsive cocaine-seeking rats. We then used *in vivo* optogenetic prelimbic cortex stimulation and observed decreases compulsive drug-seeking behaviors. When I started presenting this data, it immediately caught the attention of clinicians. As early as July 2013, the first treatment-seeking patients were already volunteering to be treated with rTMS stimulation of their frontal cortex, and our initial results, while preliminary (this first study is being submitted as we speak), are remarkably promising. I also find truly extraordinary the fact that, instead of having to wait 15 years to develop a drug target, fellow clinicians could get an optogenetic-based experimental treatment to patients in a matter of months. Given that we have no treatment for cocaine use disorders yet, I find this opportunity truly promising. rTMS has been around for a very long time, and of course our goal now is to run studies on larger populations and standardize this treatment, in order to define how many sessions are required to keep patients free from cocaine use. While this is the first example of such an optogenetic-based rTMS study, I predict that many more will follow, for other types of addiction and behavioral problems.

Michael Häusser: Absolutely. The most likely early prospect is for restoring vision in the retina, which is, after all, the most accessible part of the brain.

Where do you see the use of optogenetics heading in the next 10 years?

Dan Johnston: I would love to see optogenetics extended to other channels, ones that don't just fire or silence cells, but those that have more subtle effects. This could be used as a form of light-activated pharmacology.

Ernst Bamberg: A growing number of optogenetic tools are available. Not only rhodopsin-based tools exist, but also other light receptors such as light-activated cyclases are under study. Still, the main problem is the precise cell-specific expression in living animals. It would be of great interest for cell biologists if these tools could be expressed also in cell organelles. In other words, the major limitation is the lack of appropriate molecular biology.

Gyuri Buzsáki: Methods for interacting with brain circuits at the single-neuron, single-spike level are within reach. Only with such high temporal and spatial resolution techniques will it become possible to 'implant' physiologically relevant synthetic patterns into brain circuits and verify hypotheses based on correlational observations.

Ivan Soltesz: I think that a major breakthrough that will take place will have to do with a much-increased availability of truly cell type–specific genetic lines and viral vectors for opsin expression for which currently there are no good single genetic markers, through either intersectional optogenetics or some other new ways. Ways to control cells based on their specific developmental origin are also going to be increasingly important, and so will the optical control of gene expression. Coupling of the optogenetic intervention to ongoing activity dynamics *in vivo* through the use of miniature imaging and other recording devices and closed-loop, on-demand systems can also be expected to have a transformative impact. Taking it a step further, these technologies may also open up the way to use ongoing activity dynamics to predict future behaviors and trigger optogenetic interventions to modulate that future behavior; for example, in my own field, I expect to have seizure prediction-based (as opposed to 'just' seizure detection-based) optogenetic control of intractable epilepsies within the next 10 years, that is, to use activity dynamics that predict the future occurrence of the epileptic seizure to trigger the optogenetic intervention and prevent it from ever taking place. Another major breakthrough can be expected through the development of wireless communications and wireless powering of the electrophysiological and optogenetic recording and delivery devices in freely moving animals. There are several excellent bioengineering labs that are pushing the envelope on this key technology that will make the need for tethered *in vivo* recordings from behaving animals history. The need is clear; for example, in my lab, where we do 24/7 video-EEG monitoring and closed-loop optogenetics in mice, the tethered nature of the recordings is a huge challenge, especially since the animals are having robust behavioral seizures, so it would be truly transformative to have wireless recording and light-powering technology that would negate the need for long wires and optical cables that can twist and break. Of course, the technology has to be dependable and affordable for it to have a significant impact.

Anatol Kreitzer: The future of systems neuroscience is large-scale, non-invasive, all-optical control and recording. This will be obtained first in zebrafish and *C. elegans*, but it should eventually be possible within regions of the mammalian brain as well. It will require new tools and technology (next-generation voltage sensors, wide-field objectives, new imaging modalities), but it will eventually happen.

Michael Häusser: Even though optogenetics has reached the status of a standard tool in neuroscience, being applied in thousands of labs world-wide, its true potential is only beginning to be tapped. The ability to perform 'all-optical' interrogation of neural circuits will be truly transformational, allowing us to perform precisely targeted and calibrated interventions in the spatiotemporal dynamics of neural circuits on the scale of natural patterns of activity, and should help us to crack the neural code and pinpoint how circuits are altered during disease.

Botond Roska: (Fortunately) One cannot predict the future.

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