CELL BIOLOGY

Light-stimulated neurons

Expression of an algal protein in neurons provides a means of selectively photostimulating individual neurons with unprecedented speed and reproducibility.

Neuronal signaling in the brain involves different kinds on neurons interconnected in complex signaling networks. Each signal consists of a transient decrease in the voltage potential across the plasma membrane lasting just milliseconds, and this transient voltage spike, or depolarization, is capable of triggering a spike in other neurons that contact the activated neuron. Determining how individual neurons communicate with each other and mapping their networks are fundamental goals of neuroscience research.

Despite scientists' best efforts, these goals have been hampered by the lack of a method to precisely stimulate defined neurons in a noninvasive manner. Karl Deisseroth at Stanford University has been interested in finding a solution to this problem and says, "We've been watching this literature for a long time but were never satisfied with the slow properties of the available methods." These methods could not trigger multiple single spikes on timescales of less than a second as needed to faithfully reproduce the rapidity of signaling seen *in vivo*.

This situation changed when a group of researchers in Germany discovered a light-activated cation channel in green algae called Channelrhodopsin-2 (ChR2; Nagel *et al.*, 2003). These researchers showed that when this protein was expressed in mammalian cells, brief photostimulation was capable of generating inward cation currents. Deisseroth realized that this protein had the potential to fulfill the needs of neuroscientists looking for a selective and noninvasive method to control neuronal signaling.

To see if ChR2 could trigger voltage spikes in neurons similar to those seen *in vivo*, Deisseroth and colleagues used a virus to express the channel in primary neurons taken from rat brain (Boyden *et al.*, 2005). They attached YFP to ChR2 so they could identify the neurons in which the channel was expressed. Illumination of individual neurons with blue light triggered fast voltage spikes indistinguishable

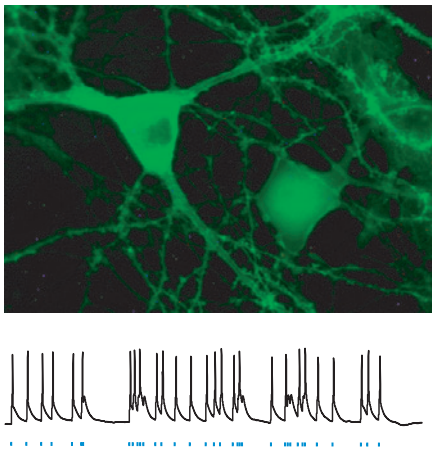

Figure 1 | Neurons expressing YFP-tagged Channelrhodopsin-2 and a voltage trace showing photostimulation-elicited spikes.

GENE REGULATION

ALL SILENT ON THE TRANSCRIPTION FRONT

Peptide nucleic acid (PNA) or RNA directed against the genomic DNA sequence around the transcription start site effectively knock down the expression of the targeted gene and make for an effective addition to the arsenal of gene silencing methods.

If you really want to know the importance of something, try doing without it for a while. Gene silencing by RNA interference has become a routine method in many laboratories to attempt just that; small RNA oligonucleoties bind to complementary sequences on the mRNA and trigger its degradation. Unfortunately, despite its great potential and wide use, RNA interference does not always work effectively. This prompted David Corey and his team at the University of Texas to look for sites of intervention other than mRNA. "We thought that transcription start sites might be a very susceptible place for gene inhibition", he says, and in two recent papers in *Nature Chemical Biology* his group describes the results.

They designed single-stranded PNAs—DNA mimics with an uncharged backbone—and double-stranded RNA oligonucleotides, each recognizing the DNA sequence around targeted transcription start sites. Cells transfected with antigene PNAs or RNAs that were perfectly complementary to the transcription start site showed efficient knockdown of the gene, whereas probes with even a few mismatches did not. These results confirmed to Corey that they were specifically targeting genomic DNA and that the transcription start site was a good point of intervention to achieve gene silencing. His team has since tested antigene RNAs against the transcription start sites of five more genes, all with good knockdown effects, making them confident that their approach works as a general silencing mechanism.

Corey is not advocating that the use of antigene PNAs or RNAs should supplant the targeting of mRNA, but sees the two approaches as complementary. He says, "There should be a side-by-side comparison to see if there is an advantage in targeting the chromosome in the nucleus rather than the mRNA in the cytoplasm." In some cases, targeting the gene—with only two copies per cell—may be more potent than targeting multiple copies of mRNA. Also, designing antigene PNA or RNA probes will be easier than finding targets for RNA interference because they are directed against a defined sequence of DNA and thus do not require complex

from natural spikes. By using light pulses as short as 10 ms, the authors showed they could precisely trigger fast and reproducible trains of spikes. They could also reliably produce subthreshold voltage transients, which are important in signaling but difficult to produce artificially.

Surprisingly the method worked without requiring application of retinal, which is used to form the light-absorbing chromophore in the protein. Presumably this is due to endogenous retinal or the presence of precursors in the culture medium. Deisseroth says, "Some tissues may need retinal supplementation and some may not. The jury is still out on that." The other surprise was the lack of toxicity seen even after expression for a week. "I think the reason for this is that ChR2 really is closed or mostly closed unless it is excited by light, and ambient light is about a thousand-fold too weak to excite it," remarks Deisseroth.

The great potential of the technique, however, will only be realized when it is demonstrated to work in acute brain slices and *in vivo*. It still isn't known whether it will be capable of functioning without the addition of retinal. Ease of use could also be improved with red-shifted variants that could be created by mutagenesis of ChR2. If this method does work well *in vivo*, the ability to express ChR2 in defined subsets of neurons should open up entire new avenues of research into neuronal signaling and functional integration.

Daniel Evanko

RESEARCH PAPERS

Nagel G. *et al.* Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proc. Natl. Acad. Sci. USA* **100**,13940–13945 (2003).

Boyden E.S. *et al.* Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci.* **8**, 1263–1268 (2005).

algorithms to predict which sequence will make a good target for interference.

The usefulness of different silencing approaches is readily apparent in cases in which the phenotype after gene knockdown is more subtle. Corey reasons that if one sees the same phenotype regardless of whether DNA or RNA was targeted, the result is trustworthy and not an artifact of a specific method.

For Corey the excitement is not only in the efficiency of antigene RNAs to silence genes, but also in the underlying mechanism. "The bigger picture" he suggests, "is that in mammalian cells RNA is able to recognize DNA, and the importance of that is only beginning to be appreciated…the method works so well it is hard to believe that it isn't involved in natural regulation." Silencing genes by RNA recognition of transcription start sites may well be an application of a mechanism nature has been using all along, and it remains for us to explore this new type of cellular regulation.

Nicole Rusk

RESEARCH PAPERS

Janowski, B. *et al.* Inhibiting transcription of chromosomal DNA with antigene peptide nucleic acids. *Nat. Chem. Biol.* **1**, 210–215 (2005).

Janowski, B. *et al.* Inhibiting gene expression at transcription start sites in chromosomal DNA with antigene RNAs. *Nat. Chem. Biol.* **1**, 216–222 (2005).

NEWS IN BRIEF

BIOSENSORS

Direct molecular detection of nucleic acids by fluorescence signal amplification

PCR amplification is a typical component of most high-sensitivity DNA detection strategies, but Ho *et al.* present an alternative approach that relies on a specially designed optical polymer for fluorescence-based detection of target DNA sequences. This method is capable of detecting DNA at zeptomolar concentrations within five minutes without enzymatic amplification.

Ho, H.A. *et al. J. Am. Chem. Soc.*; published online 18 August 2005.

GENE REGULATION

Tuning genetic control through promoter engineering

Using error-prone PCR to introduce mutations into a constitutively active promoter, Alper *et al.* demonstrate an approach for the generation and analysis of libraries of promoters with varying transcriptional output levels. The authors suggest this method could be useful for more precise manipulation of gene expression or for studying sequence determinants of promoter activity.

Alper, H. *et al. Proc. Natl. Acad. Sci. USA* **102**, 12678–12683 (2005).

CHEMICAL BIOLOGY

Combinatorial polyketide biosynthesis by *de novo* design and rearrangement of modular polyketide synthase genes

Natural polyketide compounds perform a wide variety of biological functions, and the ability to synthesize novel polyketides could prove valuable. This process is complicated, however, and requires the coordinated action of multiple enzyme modules. Menzella *et al.* describe swappable enzymatic 'cassettes' that promise to simplify the combinatorial synthesis of new polyketides.

Menzella, H.G. *et al. Nat. Biotechnol.*; published online 14 August 2005.

PROTEOMICS

Large-scale identification of yeast integral membrane protein interactions

The difficulties of working with membrane proteins have limited the ability of researchers to study this segment of the interactome. Miller *et al.* performed a split-ubiquitin two-hybrid interaction screen and analyzed their data with a support vector machine 'learning algorithm' in an effort to more confidently assess the associations of integral membrane proteins in yeast.

Miller, J.P. *et al. Proc. Natl. Acad. Sci. USA* **102**, 12123–12128 (2005).

GENOMICS

The transcriptional landscape of the mammalian genome

Researchers from the FANTOM Consortium and RIKEN have collaborated in an extensive analysis of the mouse transcriptome, yielding the surprising finding that the mouse genome seems to contain many more genes than previously predicted: an estimated 181,047 transcripts generated from dense 'transcriptional forests' encompassing more than 60% of the genome.

The FANTOM Consortium *et al. Science* **309**, 1559–1563 (2005).