NEWS & VIEWS

NEUROSCIENCE

Controlling neural circuits with light

Michael Häusser and Spencer L. Smith

Two light-sensitive proteins from unicellular organisms have been harnessed to rapidly activate or silence neurons. This optical remote control allows precise, millisecond control of neural circuits.

The prospect of direct brain control has long been a dream of philosophers, science-fiction writers and dictators. Neuroscientists have also indulged in this dream, because observation alone is insufficient to find out how the brain works. Rather, it is necessary to manipulate specific sets of neurons, stimulating or silencing them, to determine their role in brain function. A powerful approach for controlling neural circuits is now described by Zhang and colleagues on page 633 of this issue[1].

The ideal tool for manipulating the activity of neurons would allow targeting of genes in specific neurons, be innocuous in the absence of stimulation, be fully reversible, have a rapid onset and offset, allow bidirectional control, and act at a distance. Such a tool would allow the experimenter to effortlessly manipulate and replay natural patterns of activity in neural circuits.

The traditional approach for controlling neural circuits uses the trusty stimulation electrode, wielded by generations of distinguished neurophysiologists from Charles Sherrington to John Eccles to Bernard Katz. This device generates a local electric field that is highly effective at triggering action potentials in neurons — the wave of electrical depolarization, or 'spike', that ultimately activates the release of neurotransmitter at the synapses between neurons. Although the timing of electrode stimulation is very precise, its specificity and spatial control are poor because all neurons within range are activated. Furthermore, silencing neural activity is as necessary as stimulating it when deciphering brain function, as it is useful to functionally remove cells from a circuit, particularly those that exhibit substantial spontaneous activity. However, silencing is difficult to achieve using a stimulation electrode.

An alternative to electrical stimulation has been the use of chemical activation or inactivation of neurons, by directly applying excitatory and inhibitory neurotransmitters. Recently, 'photo-uncaging' of caged neurotransmitters has made this approach more sophisticated because the use of localized, patterned light yields higher spatial resolution. But the temporal and spatial resolution remains limited by the properties of the cage and the diffusion of neurotransmitter, and specificity cannot be guaranteed. Refinements of this approach, such as two-photon uncaging[2] or restricting receptors for caged neurotransmitters to specific neurons[3], have provided improvements. Furthermore, new genetic tools for silencing neurons[4–6] and specific neural connections[7] have helped to address the specificity problem, but at the expense of temporal resolution.

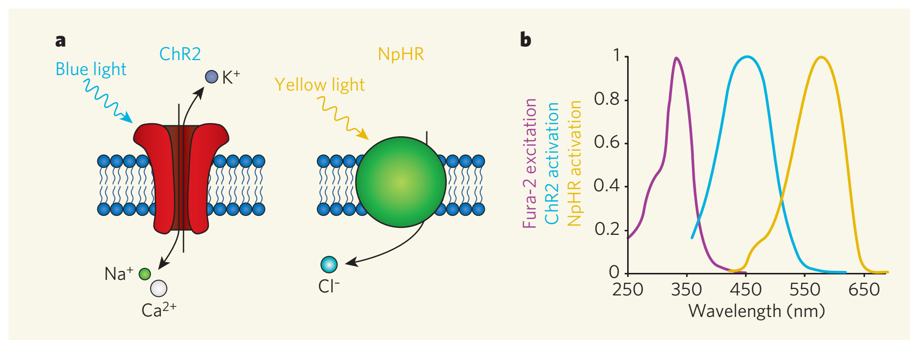

Figure 1 | Light activation of the membrane proteins ChR2 and NpHR. a, Blue light activates ChR2, a cation channel, which depolarizes the cell and causes action potentials. Yellow light activates NpHR, a chloride pump, which causes hyperpolarization and prevents action potentials. **b**, The absorption spectra for these two light-activated proteins are sufficiently separated to ensure that one protein can be activated by light without also activating the other. Furthermore, there is plenty of spectral bandwidth left over to use a fluorescent indicator of intracellular calcium (such as fura-2), which can be used to report neural activity optically.

A pair of light-activated membrane proteins present in nature have now come to the rescue. Zhang *et al.*[1] — a collaboration between neuroengineers at Stanford University and membrane biophysicists at the Max Planck Institute of Biophysics in Frankfurt — have harnessed the power of these proteins to genetically engineer neurons to be controlled by light. In earlier work[8] they used a light-activated ion-channel protein called channelrhodopsin-2 (ChR2) from green algae (Box 1, overleaf). When neurons were genetically engineered to express ChR2, blue light rapidly and selectively triggered action potentials in the neurons (Fig. 1a). This at last gave neuroscientists a genetically encodable, remote-control 'on' switch for neurons.

But that is only half of the story. In the new paper[1] the same team presents the all-important complement to ChR2: *Natronomonas pharaonis* halorhodopsin (NpHR; Box 1). The NpHR protein generates a chloride flux when activated by light. When it is expressed in neurons, activation of this protein by yellow light causes rapid and reversible hyperpolarization, thus preventing spikes (Fig. 1a). As with ChR2, the effect of NpHR is fast and rapidly reversible, despite being an ion pump instead of an ion channel. This rapid-onset hyperpolarization is a dramatic advance over a previous genetically encodable optical membrane probe that relied on the activation of a slower chemical cascade[9].

Thanks to spectacularly good fortune, the excitation spectrum of NpHR is neatly shifted with respect to that of ChR2 (Fig. 1b), such that coexpression of both proteins in the same neuron allows different wavelengths of light to be used to selectively activate or inactivate the same cell. In effect, the system provides the experimenter with a two-knob remote control for increasing or decreasing the activity of specific neurons using different colours of light. A former member of the collaboration has just published an independent study reporting the same technology applied to cultured neurons[10].

To demonstrate the power of the approach, Zhang *et al.*[1] used it in combination with a fluorescent calcium indicator to simultaneously monitor calcium signals associated with activity and manipulate that activity in the same neurons. Activation of ChR2 with

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blue light caused large increases in calcium, but when both blue light and yellow light were presented at the same time, no calcium increase was observed. This shows that the ChR2–NpHR system can be combined with fluorescence-based methods for monitoring activity, allowing light to be used to both observe and control neuronal activity. Future studies could employ a fluorescent protein reporter of activity[11], in place of the dye, to create a fully genetically encodable battery of tools for exploring and mapping neural circuits. In a tantalizing taster of the future prospects of the technique, Zhang *et al.* genetically targeted NpHR and ChR2 to specific types of neuron in the worm *Caenorhabditis elegans*. The results showed that it is possible to rapidly and reversibly silence or activate a particular neuronal subtype with light (Fig. 2a), and simultaneously observe the effect on behaviour.

This is a stunning advance. Nevertheless, there is room for improvement. The cation channel of ChR2 allows Ca^{2+} entry into the cell, which triggers many other signalling pathways that could confound some experiments. Although NpHR could be used to mimic inhibition of synapses (for example locally, in the neuronal dendrites or at the spike-initiation site in the axon), it works by activating a pump, not a channel, and thus its biophysical mechanism of action is different. It also remains to be seen whether the ChR2–NpHR system allows efficient two-photon excitation[12], which would allow precise targeting of light deep within brain tissue, or subcellular targeting of ChR2–NpHR activation (for example to axonal boutons or dendritic spines). Even with these issues, which will probably be addressed by protein engineering, the overwhelming advantages of this new approach should revolutionize the field.

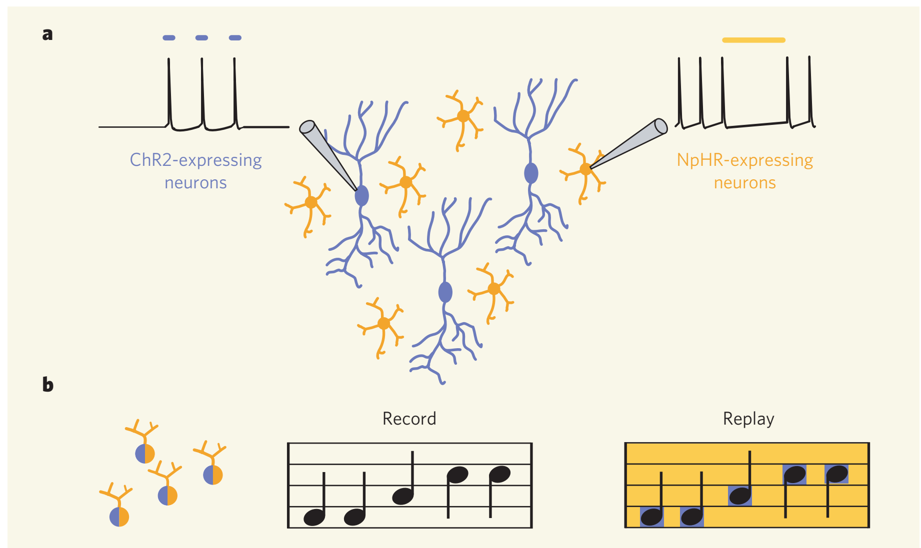

Figure 2 | Remote control of designer neurons[1]. **a**, Selective control of different neuron populations in neural networks. One type of neuron (the normally silent principal cells) is genetically engineered to express ChR2 and fire spikes when activated with blue light. Another type (the spontaneously firing interneurons) express NpHR and are silenced with yellow light. **b**, Precise control of neuronal firing in a neural network coexpressing ChR2 and NpHR, here shown as a musical analogy. First, the pattern of activity in the network is recorded (for example using calcium dyes). Next, the identical pattern of activity is replayed into the same populations of cells using yellow light to suppress spurious spikes and blue light to activate spikes. Such an experiment can help to demonstrate causality of patterns of activity for behaviour.

Proving causality is often an elusive goal in neuroscience, particularly when drawing connections between neural activity and behaviour. The ChR2–NpHR system offers an elegant solution for proving necessity and sufficiency, which together allow causality to be inferred. Patterns of neural activity during stimulus-evoked behaviour could be recorded using existing optical methods. Then, to demonstrate sufficiency, these same patterns could be played back to the neuronal circuit, using ChR2 and NpHR, to elicit the same behaviour in the absence of the original stimulus (Fig. 2b). Changing the precise temporal pattern of spikes in such a replay experiment could also allow one to assess the importance of precise spike timing in behaviour. Subsequently, NpHR activation alone could silence stimulus-evoked neuronal activity to block behaviour, thus demonstrating necessity.

Beyond the laboratory, the ChR2–NpHR system has potential for treating neurological and psychiatric disorders in humans. For example, diseases such as epilepsy, which involves hyperactivity or altered excitation–inhibition balance in neurons, might be ameliorated by activating NpHR and/or ChR2 targeted to specific cell types. With implanted fibre-optic stimulation, these optical tools could also provide a more specific and durable form of deep-brain stimulation, which has shown some success in the treatment of Parkinson's disease. Finally, one can envisage using this approach to create new types of optical neural prosthetic, such as in the retina[13] or the sensory or motor cortex, for improving sensory perception and control of movement. Our new mastery of neural circuits using light might thus eventually allow us to master our own brains — and behaviour. ■

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Box 1 | A tale of two opsins

ChR2 and NpHR are both opsins that are related to rhodopsin, the light-sensitive protein in mammalian vision. But unlike rhodopsin, which activates a chemical cascade that eventually results in neural activity, ChR2 and NpHR are self-contained, light-sensitive proteins that produce a direct electrical signal (depolarization or hyperpolarization, respectively).

Like all opsins, though, they require a vitamin-A-based chromophore cofactor in order to be activated by light. Conveniently, mammalian neural tissue normally contains enough of this cofactor, which means the experimenters did not need to add any.

ChR2 was discovered in the green alga *Chlamydomonas reinhardtii*, where it triggers photo-orientation movements that allow the alga to find optimal conditions for photosynthesis. Its potential as a light-sensitive depolarizer for cells did not escape the attention of the Max Planck group that sequenced the gene[14] (and which led to their collaboration on Zhang and colleagues' study[1]).

The most commonly studied variant of *C. reinhardtii* was isolated in Massachusetts. NpHR, on the other hand, was found thousands of miles away in a dry salt lake in the Sahara desert. In this extreme environment, the archaeum *Natronomonas pharaonis* fills itself with chloride using a pump (NpHR), probably for generating an electrochemical gradient for proton entry that in turn is used to drive ATP synthesis for energy production. This amazing protein can pump chloride into the cell to concentrations 50 times higher than that typically found in neurons.

Apart from some minor optimization of nucleic-acid sequence to increase their levels of expression in mammalian neurons, the ChR2 and NpHR proteins were essentially used in their natural forms for the present work[1]. They will be attractive targets for protein engineers eager to improve and expand on these proteins' already useful properties. **M.H. & S.L.S.**

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ATTOPHYSICS

Tunnel vision

Jonathan P. Marangos

The tunnelling of a bound electron out of an atom in a laser field is a well-known quantum-mechanical process. But it happens very quickly, and it takes some fast work with X-rays and lasers to see it in action.

Advances towards making measurements with attosecond time resolution have caused much excitement in recent years. An attosecond is 10^{-18}s, and this is the timescale over which electron states in matter evolve. Such evolution drives many processes that are of interest to quantum scientists, among them chemical reactions, changes in material structures, optical responses and quantum coherence. Elsewhere in this issue, Ferenc Krausz and colleagues (Uiberacker *et al.*)[1] extend a technique that brings us within sight of these fast-moving phenomena.

Measurements in the attosecond domain are based on the use of high-intensity laser pulses that comprise just a few wave-cycles of electrical field and have a precisely defined form[2]. This strong, oscillating electrical field can be used to extract an electron from an atom, in a quantum effect termed tunnel ionization, and then drive it back in with increased energy. This process is signalled by a short burst of coherent X-rays, typically a few hundred attoseconds long[3], that is perfectly synchronized with the laser field's cycle. Such X-ray bursts have been used in various ways to investigate, for example, the movements of protons in small molecules[4], and the decay of states in atoms where several electrons have been excited[5].

Krausz and his colleagues at the Max Planck Institute for Quantum Optics in Garching, Germany, were pioneers of these methods. They use a precisely controlled laser field that has a cycle time of around 2,700 attoseconds to produce, in a gas jet, a synchronous X-ray burst some 250 attoseconds long and with a photon energy of around 90 electronvolts. This X-ray burst is delivered to the sample to be measured, followed, after a carefully controlled delay, by the original laser pulse. In previous experiments[5], the researchers had clocked the emission time of X-ray-excited electrons with a precision of around 100 attoseconds by measuring the effect of the delayed laser field on the energy distribution. This set-up, termed attosecond streaking spectroscopy, worked well for excitation processes in which an electron is eventually ejected. But in many cases, the electron does not leave the sample. A new technique was needed to measure the dynamics of these more deeply bound states.

In their latest paper (page 627)[1], the researchers describe how they have used the same basic tools to look into such deep states in neon (Ne) atoms (Fig. 1). The 90-eV photons of the X-ray burst had enough energy to free one electron from the atom immediately and excite a second to a higher, but still bound, energy level, creating an excited ionic state, Ne^{+*} (Fig. 1a). When the subsequent laser electrical field is near a maximum, it can strongly distort the potential that binds the electron. The excited electron encounters a finite barrier through which, with high probability, it can tunnel to freedom[6]. An appropriate maximum of the laser field occurs twice per optical cycle (Fig. 1b). The signal of Ne^{2+} ions thus accumulates over time in steps.

The accumulated yield of Ne^{2+} ions depends on the delay between the X-ray burst and the laser pulse. The number of field maxima that can contribute to the Ne^{2+} signal, and so the Ne^{2+} yield, changes in steps as the delay is varied. The steepness of the steps indicates the duration of the tunnel ionization. This behaviour shows that the X-ray excitation and ionization processes of these excited states are indeed very rapid, and must occur in a combined time of less than 300 attoseconds. Although this temporal confinement of the laser-induced ionization had long been suspected, it had not been directly confirmed before. The measurements also show that tunnel ionization becomes the principal ionization process at surprisingly low intensities.

Because the tunnelling from the bound states is so rapid and so exactly synchronized to the X-ray burst, the yield of ions from this process is a suitable probe of their electron dynamics.

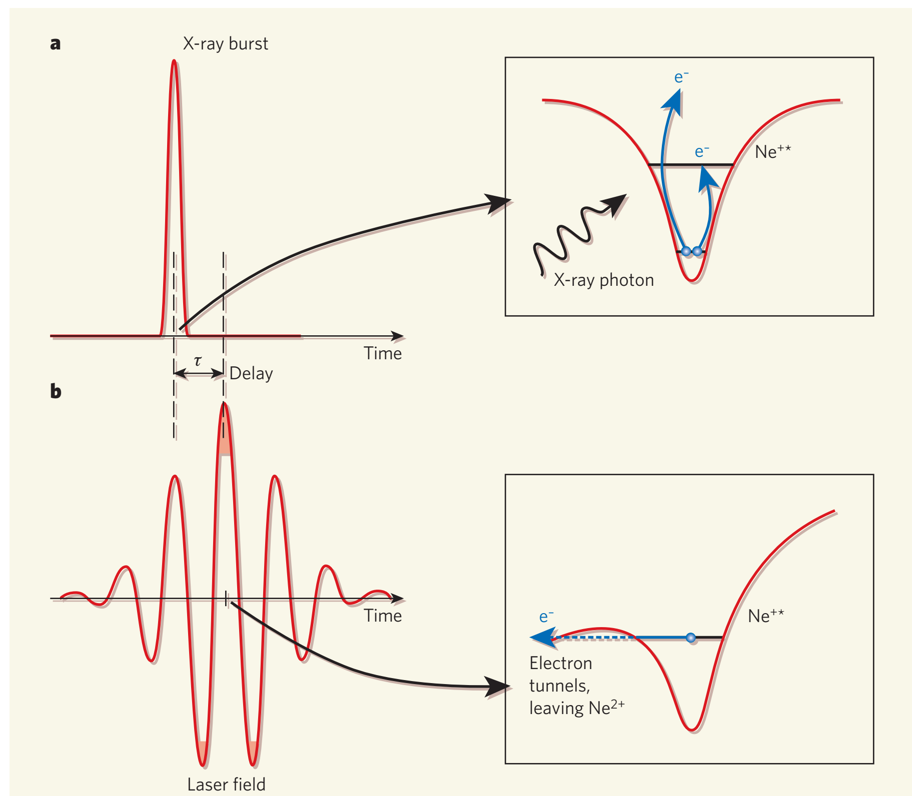

Figure 1 | Attosecond probe. In the experiments of Uiberacker *et al.*[1], the X-ray burst and the laser electrical field have a relative delay, τ, between their centres that can be varied. **a**, The X-ray burst excites two electrons in a neon (Ne) atom. One of these leaves directly, and the other is excited into a bound state of the Ne^+ ion, Ne^{+*}. **b**, The following laser field can, when near one of its maxima (in one of the shaded regions), significantly perturb the Ne^+ ion potential, allowing the second electron to tunnel away quantum-mechanically, resulting in a significant degree of ionization to Ne^{2+}. As the delay τ is changed, the X-ray burst scans across the laser-field profile. The number of field maxima that can contribute to the Ne^{2+} signal, and so the Ne^{2+} yield, changes in steps as τ is varied.

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