# Getting it through your thick skull

Ikuko T Smith & Spencer L Smith

**Neural activity up to 3 mm deep in mouse brain can now be inhibited optogenetically through the intact cranium with a red-shifted opsin called Jaws.**

Optogenetics has revolutionized our ability to remotely control neurons. With this technique, neurons expressing opsin-based ion channels and pumps can be excited or inhibited by light with high temporal precision. It is not, however, without its limits. The light scattering properties of mammalian brain tissue impose a requirement for closely positioned fiber optics for effective light delivery to activate subcortical brain regions *in vivo*[1]. This restricts experiments to a small population of neurons in close proximity to the fiber optic probe. An ideal optogenetic tool would respond to light delivered at a distance, perhaps even through the skull. In this issue of *Nature Neuroscience*, Chuong *et al.* introduce a new optogenetic inhibitor that provides exactly that[2].

Halorhodopsins, light-activated chloride pumps that suppress neural activity, have been used as counterparts to activator channelrhodopsins[3,4]. Halorhodopsins are found in halophilic archaea, halobacteria, which live in waters with extremely high concentrations of salt. A growing number of halorhodopsins have been isolated from various halobacterial strains. Each strain of halobacteria has its own slightly different version of halorhodopsin, and it is this biodiversity that held the key to the innovation presented by Chuong *et al.*[2].

Refraction and scattering thwart efforts to deliver light of sufficient intensity to deep structures. Mammalian brain tissue is a thicket of convoluted plasma membranes from the sinuous processes of neurons and glia. Refraction occurs where each membrane surface abuts an aqueous intracellular or extracellular compartment. Scattering is most pronounced at shorter wavelengths of the optical spectrum, where absorption of light by hemoglobin creates an additional issue. At longer wavelengths, on the other hand, scattering is better mitigated, but absorption by water becomes an issue. Fortuitously, there is a region in the near infrared that is relatively amenable to deep tissue penetration (**Fig. 1a**). These longer wavelengths can penetrate large volumes of brain tissue to activate opsins in widely distributed neurons and deep brain structures, obviating the need to insert fiber optic probes into the brain. Thus, shifting the activation spectra of optogenetic tools from the blue and yellow range into the red enables less invasive manipulations in optogenetic experiments.

To gain further quantitative insight into the wavelength dependence of large-volume optogenetic stimulation, the authors performed a highly detailed study of light scattering through mouse skull and neural tissue. The model involved empirical data, including X-ray computed tomography scans to measure skull thickness, and predicted a marked increase in transcranial light penetration to deep neural tissues at a red wavelength compared with a green wavelength. Armed with this quantitative model, they revisited the biodiversity found in halorhodopsins in search of a silencer with a red-shifted activation spectrum that could provide a suitable starting point for further protein engineering. Halo57 stood out with its red-shifted action spectrum. Halo57 is one of the relatively recently discovered halorhodopsins found in *Haloarcula* (*Halobacterium*) *salinarum*

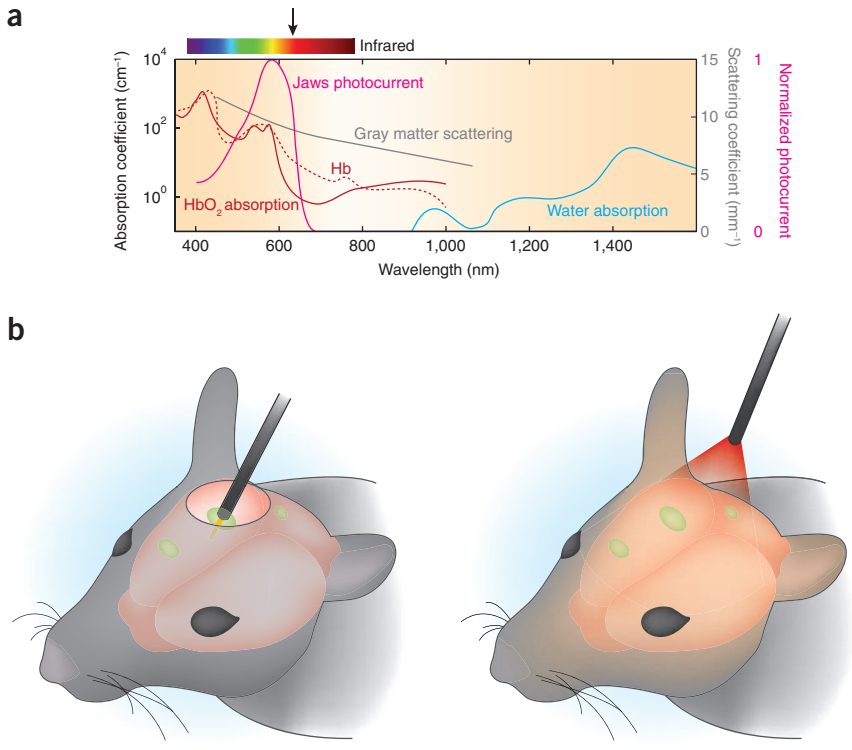


**Figure 1** The red-shifted action spectrum of Jaws allows transcranial optical inhibition of neurons in deep tissue. (**a**) At around 632 nm (arrow), in the optical window of wavelengths between strong absorption by hemoglobin and water, Jaws photocurrents are near maximal. Scattering in gray matter also decreases at longer wavelengths[13–15]. (**b**) When targeting deep brain regions for optogenetic manipulations, conventional approaches (left) with yellow light require invasive insertions of fiber optic probes into the brain. Red light delivered transcranially (right) can activate Jaws and suppress neurons up to 3 mm deep in the brain. The improved sensitivity of Jaws not only permits less invasive optogenetic manipulations, but may also enable remote control of multiple target regions simultaneously.

Ikuko T. Smith and Spencer L. Smith are at the Neuroscience Center, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA. Spencer L. Smith is also at the Department of Cell Biology and Physiology and the Carolina Institute for Developmental Disabilities, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA.
e-mail: slab@unc.edu

(strain Shark), isolated from the crude solar salts of the commercial saltern in Shark Bay, Australia.

Chuong *et al.*[2] subsequently optimized Halo57 using two point mutations in the sixth transmembrane domain (helix F) that increased the conductance of photocurrents. These point mutations were derived from structure-function studies on bacteriorhodopsin and halorhodopsin dating back to the 1980s[5–7]. The resulting mutant Halo57 was further modified for effective membrane trafficking in eukaryotes by appending endoplasmic reticulum transport and Golgi export sequences. Recalling the strain of archaea from which Halo57 was isolated, the new construct was playfully named Jaws.

Jaws exhibited an activation spectrum that was red-shifted by 14 nm relative to the widely used halorhodopsin from *Natronomonas pharaonis*. Under conventional yellow light illumination, Jaws was an improvement over existing constructs. But where Jaws really shone was with red light illumination, which drove robust silencing mediated by Jaws, but not by other leading opsins. The rapid drop-off in efficacy at the red end of the activation spectrum for many optogenetic inhibitors means that small red shifts can have large effects. With 632-nm light, currents were three times as large with Jaws as with eNpHR3.0, a leading silencer.

The combination of higher current density and red-shifted activation spectrum provided just the performance increases needed to permit transcranial silencing of neural activity. Simultaneous electrophysiological recordings confirmed the suppression of spontaneous neural activity in awake mice up to 3 mm below the surface of the brain. This opens the door to less invasive optogenetics experiments, as well as to simultaneous targeting of cells in spatially separated areas (**Fig. 1b**), such as multiple subcortical nuclei or bilateral brain structures.

One neural structure that is an obvious target for therapeutic optogenetic control is the retina. Although channelrhodopsin-based activators can be expressed in retinal ganglion cells, this approach bypasses the complex signal processing circuitry of the retina and cannot restore normal vision. In retinal degenerative diseases such as retinitis pigmentosa, resensitizing photoreceptors may be a preferable approach. Given that photoreceptors are hyperpolarized by light, rather than activated, the options for optogenetic constructs are limited. Enter Jaws. In a mouse model of photoreceptor degeneration, Jaws provided a recovery of light-evoked responses that was superior to that of previous optogenetic silencers.

With every advance, it is natural to wonder what the next step might be. The red shift in the action spectrum of Jaws is a modest 14 nm. Although this was effective, further red-shifted silencers could provide even better transcranial activation. Effective use of Jaws may require pulse shaping, decreasing the light intensity near the end of an inhibition period, to mitigate strong rebound depolarization. This should only modestly affect its temporal performance. On another front, Jaws is a chloride pump, which has appealed to neuroscientists in comparison with the light-activated proton pump bacteriorhodopsin, which can subject neurons to changes in pH. A red-shifted chloride channel may provide even more effective inhibition than a chloride pump such as Jaws by letting many ions flow during a photon-triggered opening, rather than a single ion per photon through a pump[8,9].

This report is an important step into the next generation of optogenetics. Jaws joins several existing optogenetic excitation tools with red-shifted activation spectra[10,11], including one that provides transcranial neural activation[12]. Bidirectional transcranial optogenetic manipulation of large volumes of mammalian cortex is important not only for rodent models, but in larger species as well. In the wake of recent successful optogenetic manipulations in nonhuman primates, it is clear that the ability to manipulate larger volumes of tissue will be beneficial for generating robust behavioral responses. Optogenetic engineering is expanding the range for remote neural control, and thereby the horizons for neuroscience research.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Aravanis, A.M. *et al. J. Neural Eng.* **4**, S143–S156 (2007).
2. Chuong, A.S. *Nat. Neurosci.* **17**, 1123–1129 (2014).
3. Zhang, F. *et al. Nature* **446**, 633–639 (2007).
4. Han, X. & Boyden, E.S. *PLoS ONE* **2**, e299 (2007).
5. Hackett, N.R., Stern, L.J., Chao, B.H., Kronis, K.A. & Khorana, H.G. *J. Biol. Chem.* **262**, 9277–9284 (1987).
6. Mogi, T., Marti, T. & Khorana, H.G. *J. Biol. Chem.* **264**, 14197–14201 (1989).
7. Rüdiger, M. & Oesterhelt, D. *EMBO J.* **16**, 3813–3821 (1997).
8. Wietek, J. *et al. Science* **344**, 409–412 (2014).
9. Berndt, A., Lee, S.Y., Ramakrishnan, C. & Deisseroth, K. *Science* **344**, 420–424 (2014).
10. Zhang, F. *et al. Nat. Neurosci.* **11**, 631–633 (2008).
11. Klapoetke, N.C. *et al. Nat. Methods* **11**, 338–346 (2014).
12. Lin, J.Y., Knutsen, P.M., Muller, A., Kleinfeld, D. & Tsien, R.Y. *Nat. Neurosci.* **16**, 1499–1508 (2013).
13. Prahl, S. Optical absorption of hemoglobin. http://omlc.ogi.edu/spectra/hemoglobin/index.html (1999).
14. Yaroslavsky, A.N. *et al. Phys. Med. Biol.* **47**, 2059–2073 (2002).
15. Hale, G.M. & Querry, M.R. *Appl. Opt.* **12**, 555–563 (1973).



# Sleep replay meets brain–machine interface

Kenneth D Harris

**Brain–machine interfaces provide not only potential therapies, but also new tools for studying neuronal processing. A study now uses them to investigate how learning affects sleep activity in motor cortex.**

The biological role of sleep has long fascinated neuroscientists. There is still no consensus on this question; however, most of the proposed functions of sleep can be divided into two broad categories, both of which may turn out to be important. The first category consists of metabolic functions, such as the repair of low-level wear and tear on neurons and synapses, that may only be possible during the reduced electrical activity levels found during sleep (for example, ref. 1). The second category consists of plasticity functions, meaning the coordinated adjustment of synaptic weights in brain circuits. Examples of such plasticity processes could include a general decrease in synaptic strength to compensate for strengthening as a result of daytime experience[2] and/or selective consolidation of synaptic changes corresponding to salient memories or task learning[3]. In this issue of *Nature Neuroscience*, Gulati *et al.*[4] use a brain–machine interface task to investigate the relationship between sleep and learning in motor cortex.

Many studies have shown that sleep helps subsequent performance of learned tasks[5],

Kenneth D. Harris is in the UCL Institute of Neurology and UCL Department of Neuroscience, Physiology, and Pharmacology, University College London, London, UK.
e-mail: kenneth.harris@ucl.ac.uk