TECHNIQUES

Light takes a deep dive

> selective activation of target brain areas expressing SOUL could be achieved non-invasively

The advent of optogenetics — using light to activate opsins expressed in selected neuronal subtypes — has revolutionized the field of neuroscience. It has certain limitations, however, in part owing to the poor penetration of light through brain tissue, which necessitates the surgical implantation of optical fibres in order to deliver light to the region of interest at a sufficient strength to activate opsins. The implantation procedure damages brain tissue, creating an inflammatory response, neuronal loss and altered spine turnover — thus creating a non-physiological neural environment. Now, Gong et al. demonstrate the use of a modified step-function opsin (SFO) with extreme light sensitivity in mice and macaques. They show that this SFO can be activated in deep brain structures (with minimal disturbance of brain tissue) and can influence behaviour.

SFOs have certain characteristics that set them apart from other opsins: they have slow off-kinetics, which means they stay open for as long as 30 minutes after activation and, unlike with other opsins (which, when activated, induce neuronal spiking), activation of SFOs induces a subthreshold depolarization that sensitizes neurons to endogenous activation. SFOs, therefore, enable the activity of neuronal ensembles to be increased long after the activation signal has ceased, while preserving endogenous firing patterns across these neuronal ensembles.

Crucially, SFOs are highly light sensitive, and Gong et al. used specific mutations to increase this light sensitivity further to create an SFO with ultra-high light sensitivity (SOUL) that can be photoactivated under extremely low light conditions. SOUL is activated by blue light and can be inactivated using orange light.

The authors developed a Cre-inducible mouse line with a tdTomato reporter that allowed targeted, cell-type specific expression of SOUL.

First, the authors selectively expressed SOUL in the mouse mediodorsal thalamus. The firing rate of neurons in this region increased considerably when light was delivered transcranially, and this scaled with light intensity. The authors then expressed SOUL in the mouse lateral hypothalamus (LH) — one of the deepest areas of the mouse brain (~6 mm below the skull surface) — and were able increase the number of neurons in this brain area expressing c-FOS (a marker of neuronal activation) by delivering light transcranially. These findings suggest that selective activation of target brain areas expressing SOUL could be achieved non-invasively.

Optogenetic stimulation of excitatory neurons in the LH has previously been shown to suppress feeding in food-deprived mice. To test whether SOUL activation in the LH could induce behavioural change, the authors expressed SOUL selectively in calcium/calmodulin-dependent protein kinase II (CaMKII)-positive neurons in the LH of food-deprived mice. Light delivered transcranially, as before, resulted in a marked reduction in food consumption compared with control mice.

For this approach to have utility in the human brain, it is crucial to demonstrate that SOUL can be used on a bigger scale in larger animals. To investigate this, the authors used macaques and delivered light via an external fibre placed outside the dura. To test whether this set-up could successfully activate deep cortical neurons, the authors induced SOUL expression under the control of the human synapsin promoter in a small region of left lateral prefrontal cortex at depths of 0.5–5.6 mm. They recorded single-neuron and multi-unit activity within the injected region. They found that most of the neurons there were activated by blue light, showing an approximately twofold increase in firing rate that was maintained for the 2 minute test period until inactivation by orange light. These effects on neuronal activity were robustly detected at depths of 2–3 mm, which is the thickness of the superficial layers of the macaque cortex, suggesting that this method can be used to activate cortical neurons across all superficial cortical regions.

Interestingly, the authors noticed rhythmic fluctuations in local field potentials (LFPs) recorded by the electrodes following blue light delivery and hypothesized that these might indicate synchronized oscillations in the activated neural population. Indeed, they found that blue light induced, and orange light ceased, an increase in the power of LFP oscillations, especially in the alpha and theta bands.

Together, these findings show that SOUL is a minimally invasive tool for activating specific neuronal subtypes in any mouse brain area and in the macaque cortex using an external light source.

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