New optogenetics tools to decipher the neuronal connectivity underlying behavior.

Alfonso Martin-Peña. PhD
Department of Neurology, Center for Smell and Taste, McKnight Brain Institute, University of Florida, Gainesville, FL 32610-0015, USA
Email: alfonso.m.pena@ufl.edu

Abstract
Optogenetic tools have revolutionized the field of neuronal physiology and behavior, however the low penetration power of blue lights through the cuticle of flies limited its use in this model. The development of red-shifted rhodopsins is breaking down that barrier.

Keywords: channelrhodopsin, courtship, *Drosophila*, neuronal connectivity, optogenetic

The human brain is the most complex biological structure when looked at from the number of components it is made of, neurons, and the possible connections between them, synapses. Nowadays, neuroscience is focused on obtaining a brain activity map to identify the functional connections of each neuron and their contribution to specific behaviors [1,2]. The vinegar fly, Drosophila melanogaster, offers an arsenal of genetic tools to investigate these connections, their physiology, and the behavioral outcome resulting from their activity. The latest generation of genetically-encoded activity indicators –the ultrasensitive GCaMP6- enables investigators to register neuronal activity with single action potential resolution in living animals [3]. These tools together with fly genetics allow simultaneous recording of large neuronal populations in vivo. Optogenetic (box) manipulation of neuronal activity, however, has been limited by certain constraints. Current microbial opsins (box), channelrhodopsin (box), are activated by blue light, and light at these wavelengths has a low penetrance power to go through the cuticle of flies. Thus current investigations that employ channelrhodopsins to investigate neuronal activity are restricted to studying the functionality of the nervous system in developmental stages or to peripheral circuits of adult animals. These restrictions displaced the study of neuronal circuits in intact animals to manipulation of neuronal activity with thermogenetics, which is the control of neuronal activity by genetically-encoded temperature-dependent proteins involved in neurotransmission, and bringing all the additional constraints related to temperature variations. Inagaki et al. resolved this issue by using a novel channelrhodopsin, the red activable ChR (ReaChR), which exhibits a shifted excitation wavelength in the red region of the spectrum [4]. Light at this wavelength has higher penetrance index through the cuticle [5], thus enabling application of optogenetic techniques in adult flies.

Glossary box:

Optogenetics is the use of genetically encoded light-sensitive proteins that control or monitor the activity of neurons.

Opsins are a group of light-sensitive receptors associated to the cellular membrane. Their light-induced conformational changes activate a phototransduction cascade signaling the photostimulation inside the cell.

Channelrhodopsin are light-gated ion channels in the subfamily of retinylidene proteins (rhodopsins). Originally, they are sensory photoreceptors in unicellular green algae that have been further modified to be utilized as

Indeed, the work demonstrated that blue opsins

performed poorly because of a low penetrance of the light through the cuticle (~1%), while the red-shifted opsins are much more efficient due to a much higher penetrance of green and red lights (5-10%). Of the two red-shifted opsins examined, one exhibited low levels of expression, probably due to cytotoxic effects or low-efficiency of translation. The second opsin (ReaChR), free of those constrains, was used to investigate the neuronal networks governing wing extension during courtship songs. A set of interneurons, P1 or pMP4, connecting synaptically to downstream projection neurons, pIP10, were analyzed for their role in controlling wing extension during courtship. When P1 interneurons were ReaChR-activated with LED-lights, the onset of wing extension was not time-locked to the initiation of the illumination. There were variable latencies for the onset of wing extension, but the duration of each bout was always short (~1s) per 30s stimulation. In contrast, stimulation of the downstream projection neurons, pIP10, displayed a wing extension behavior that was strongly time-locked to the initiation and termination of illumination. The stochastic control of wing extension, but not the deterministic one mediated by pIP10 stimulation, was modulated by social behavior. Isolation of males increases courtship behavior, and this is in part by increasing the excitability of P1 interneurons that directly increase wing extension. Although pIP10 dendrites contact with P1 neurons, this P1-modulated courtship behavior occurs through a different downstream pathway and is independent of pIP10 neurons [5].

The new optogenetic tool, ReaChR, described in this study [5], enables manipulation of specific neurons, even in the deep brain, of intact adult animals. Activation of this opsin can be regulated easily by adjusting the intensity and frequency of illumination; this supposes a major advance because it allows accurate control of neuronal activity without complex manipulation or invasive surgical procedures. With further optimization, this approach can provide basis for designing a full set of optogenetic tools to control neuronal activity in vivo. The development of opsins with different kinetics, especially faster ones, as well as the addition of inhibitory or modulatory variants would complete the full repertoire of red-shifted ChR. Inagaki *et al* reported for first time a set of neurons that integrates information about the flies' history of social experiences, and this could set the beginning of a new era for identifying neurons tracking internal or emotional states.

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