CELL BIOLOGY OF THE NEURON

Losing touch with sodium channels

Cold sores — those annoying blisters that occasionally appear around the mouth, causing tingling, pain and loss of touch sensation — are caused by the herpes simplex virus-1 (HSV-1). We know that this neurotropic virus infects sensory neurons and reduces their excitability, but the molecular mechanism of its action remains poorly understood. Reporting in *The Journal of Cell Biology*, Storey *et al.* show that HSV-1 infection reduces the membrane expression of sodium channels in primary sensory neurons, uncovering a new way in which neuronal excitability can be regulated.

Using voltage-clamp techniques, the authors found that the amplitude of sodium currents in dorsal root ganglion cells was markedly reduced 24 hours after HSV-1 infection. By staining infected neurons with an antibody against the sodium channels, they obtained evidence for a decrease in the amount of channel protein in the plasma membrane. Moreover, blocking membrane internalization, which does not affect viral entry, prevented the reduction of sodium currents.

How does the virus exerts its effect? As a first step to address this issue, Storey *et al.* set out to determine the relevance of the different stages of viral infection to the change in current amplitude. They found that blocking viral envelopment did not stop the loss of sodium currents in the infected neurons. By contrast, blocking the synthesis of viral DNA — a manipulation that eliminates the expression of 'late' but not 'early' viral proteins — prevented the

NEUROTECHNIQUE

A bright idea

The intrinsic brightness of green fluorescent protein (GFP) and the fact that it can be used to make fusion-protein constructs make it an invaluable tool for studying biological processes *in vivo*. However, its value would be significantly greater if it could be used to selectively mark proteins through photoactivation — a goal that has now been achieved thanks to work published by Patterson and Lippincott-Schwartz in *Science*. These authors had the bright idea of making a GFP that remains 'off' until it's switched 'on'.

GFP normally exist in two forms — a 'neutral' and an 'anionic' form, which produce major and minor absorbance peaks, respectively. Intense illumination with ~400-nm light converts GFP mainly to the anionic form, which results in an increase in minor peak absorbance, as well as a subsequent threefold increase in fluorescence after excitation at 488 nm.

Patterson and Lippincott-Schwartz therefore decided to search for a GFP variant with a reduced minor absorbance peak. They hoped that if they could find such a variant, it would mean that photoconversion with ~400-nm light would produce a larger increase in minor peak absorbance, and therefore a more marked increase in fluorescence after 488-nm excitation.

Because a previously reported GFP mutation at threonine 203 resulted in reduced absorbance at 488 nm without affecting the major absorbance peak, the authors studied various substitutions at this position and found what they were looking for in the form of a histidine substitution. They called this stable GFP variant photoactivatable GFP (PA-GFP), because they found that it had virtually undetectable absorbance at the minor peak and that irradiation with ~400-nm light resulted in a large increase in minor peak absorbance. They also found that 488-nm excitation of photoconverted PA-GFP produced an ~100-fold increase in fluorescence.

So, how useful is PA-GFP for studies in living cells? When the authors studied cells expressing PA-GFP, they found that, in contrast to cells expressing wild-type GFP, 488-nm excitation produced very little fluorescence before photoconversion. Furthermore, after photoconversion with ~400-nm light, they saw that 488-nm excitation produced a more than 60-fold increase in fluorescence for PA-GFP-expressing cells, compared with only an ~2.6-fold increase for wild-type-GFP-expressing cells.

Patterson and Lippincott-Schwartz concluded their report by highlighting some of the biological applications of PA-GFP. They showed that it can be used both as a free protein to study protein dynamics (they looked at protein diffusion across the nuclear envelope) and as a chimeric construct to study membrane dynamics (they used it to monitor interlysosomal membrane exchange). Both of these approahes will find many applications in the study of cellular processes in neurons. All in all, the stability and optical contrast of PA-GFP, combined with the fact that signals can be obtained from it rapidly and specifically, mean that searching for PA-GFP was a very bright idea indeed.

Rachel Smallridge,
Associate Editor,
Nature Reviews Molecular Cell Biology

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Jennifer Lippincott-Schwartz's laboratory: http://dir2.nichd.nih.gov/nichd/cbmb/sob/Jennifer_Lippincott_Schwartz.html

Encyclopedia of Life Sciences: http://www.els.net green fluorescent protein

decrease in current amplitude. The authors further established that the late viral protein ICP34.5 is crucial for the effect of HSV-1 on sodium currents, as infection with viruses that lack such a protein did not result in sodium channel loss.

In addition to identifying a mechanism to account for the decrease in neuronal excitability that accompanies HSV-1 infections, the data of Storey *et al.* point to a new way to regulate the availability of sodium channels at the plasma membrane. What intracellular pathways does this process engage? Do neurons use this mechanism under physiological conditions? HSV-1 and, in particular, ICP34.5 will be useful tools to answer these questions.

Juan Carlos López

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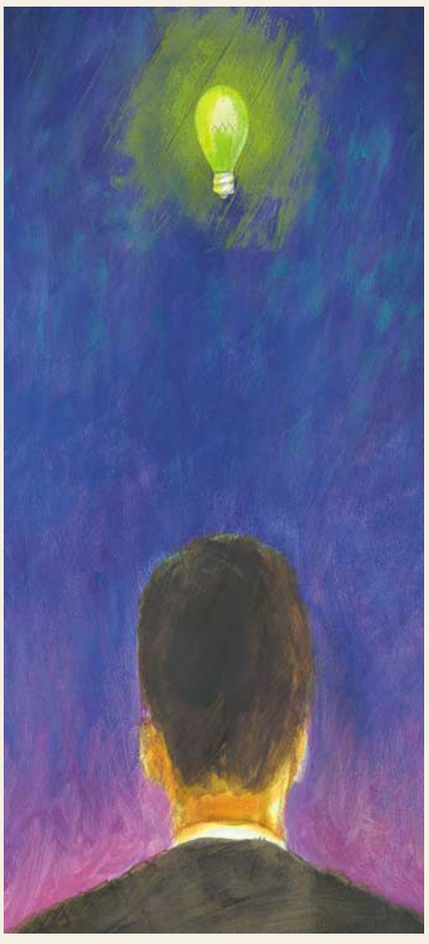

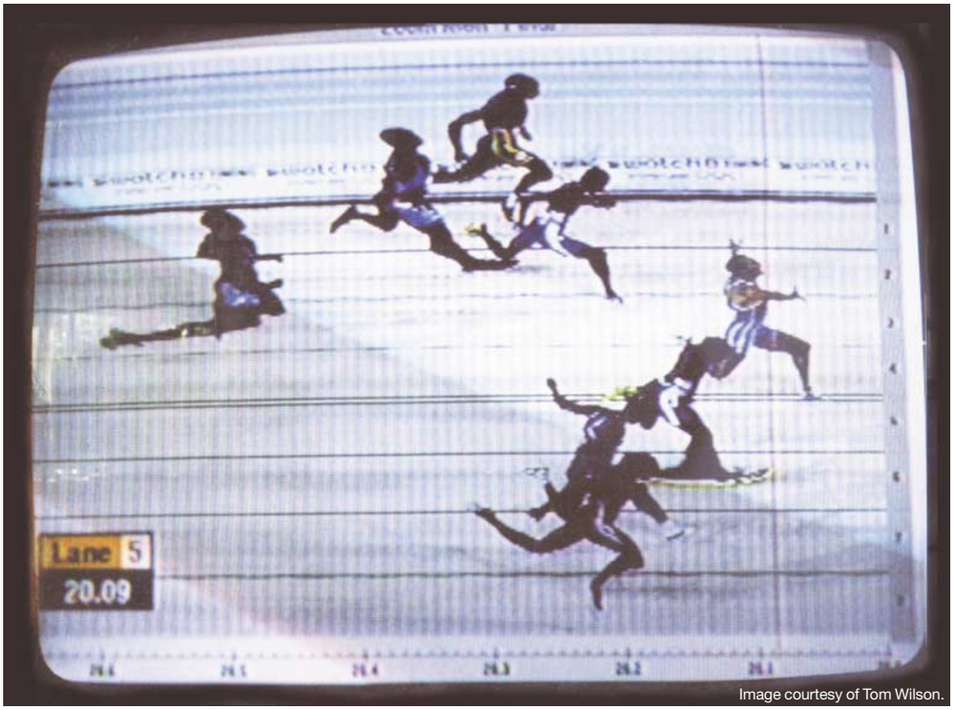

Image courtesy of Tom Wilson.

LEARNING AND MEMORY

Action replay

The fact that we are able to recall previous events in detail, even those from the distant past, indicates that we have a robust neural system for the acquisition and storage of memories. But despite this impressive storage capacity, in the minutes to days after an event, our memory of the experience is prone to disruption. Hoffman and McNaughton argue that this period of lability reflects the way in which new memories are converted into a long-lasting form. Reporting in *Science*, they provide evidence that memory consolidation involves the reactivation of distributed components of 'memory traces' during periods of behavioural inactivity that follow an event.

According to the 'trace-reactivation theory' of memory consolidation, in 'offline' periods after an event — for example, during quiet waking or sleep — neurons in higher-level cortical regions are thought to activate cells in lower-level regions that were also active during the experience. Repeated co-activations of lower-level ensembles result in the formation of connections that are necessary to encode the memory trace. Previous studies have shown that patterns of cortical activity that accompany an experience can be triggered spontaneously during subsequent periods of rest. Hoffman and McNaughton set out to test a second prediction of the theory — that distributed components of the reactivated memory trace should appear concurrently within relevant cortical sites.

The authors implanted a 12-by-12 lattice of electrodes into each of four areas of the primate neocortex — posterior parietal cortex (PP), motor cortex (M), somatosensory cortex (SS) and dorsal prefrontal cortex (PFC). They recorded simultaneously from multiple individual neurons during an initial period of rest (rest 1), a sequential reaching behaviour and a second period of rest (rest 2). Cell-pair firing-rate correlations were then calculated for each epoch. The authors found that, for PP–PP, M–M, SS–SS, PP–M, PP–SS and M–SS cell pairs, firing correlations during the task were more similar to those of rest 2 than to those of rest 1. This indicates that neurons that were co-activated in these regions during the task tended to be activated together afterwards. No such pattern was seen for cell pairs that included neurons of the PFC. Hoffman and McNaughton went on to show that the 'temporal bias' of cell-pair interactions during the task was preserved in rest 2 in the M and SS. So, if one cell tended to fire after another during the task period, then this firing sequence tended to recur in rest 2. This preservation of temporal bias was not observed for PFC neurons.

These data lend support to the proposal that memory-trace reactivation occurs in a coordinated, distributed manner across the neocortex. Interestingly, no evidence was found of trace reactivation in the PFC, which has been implicated in memory retrieval in humans. Further studies will be needed to elucidate the mechanisms that underlie memory-trace reactivation, and to establish whether or not the PFC has a part to play in this process.

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