On-demand rabies

A self-inactivating rabies virus enables long-term tracing of neural networks without toxicity.

Neural networks, circuits of neurons that fire together to perform a function, represent a fundamental unit in the brain. Questions aimed at elucidating the role of specific networks require tools that allow tracking and manipulating the circuits. G-deleted rabies virus has served this purpose, although with limitations. The virus, which spreads retrogradely (i.e., in the opposite direction of the neurotransmitter) between neurons, enables genetic tracing of the network but is very toxic to the cells, and so its use is restricted to about two weeks.

With the goal of creating a system that would allow control over the transcriptional activity of the rabies virus and eliminate its cytotoxicity, Marco Tripodi and colleagues at the MRC Laboratory of Molecular Biology in Cambridge generated several constructs

in which a rabies virus protein is fused to a PEST proteasome-targeting domain via a tobacco etch virus (TEV) cleavage site. In the presence of TEV protease, the PEST domain is cleaved off and the virus is active. But in the absence of the protease, the viral protein is degraded, and the rabies virus becomes transcriptionally silent shortly after infection. The approach results in a virus with an ON and OFF switch and the ability to spread retrogradely to another neuron in the network and enable genetic manipulation while showing no sign of cytotoxic potential.

To target specific regions of the brain, the rabies virus is injected locally in the brain of mice. In addition, cell-type-specific expression can be achieved by using mouse lines expressing Cre recombinase in a particular neuronal subset. Supplying the TEV protease under the control of a doxycycline-inducible promoter in an adeno-associated virus adds an additional layer of temporal control that

allows researchers to turn the rabies virus ON and OFF by administering doxycycline.

The approach will be particularly useful for research on “anything that requires training of the mice,” says Tripodi. “The simplest training is beyond the time frame of what rabies would enable.” For example, “it will now be possible to ask, ‘if you train an animal to do a task and then you re-train it to do something else, how do the networks change?’” And “you also have the security of knowing you are recording from healthy neurons,” adds Tripodi.

Developing the system was not trivial and required considerable trial and error, says Tripodi. The usefulness of the tool suggests it was worth it.

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Ciabatti, E. *et al.* Life-long genetic and functional access to neural circuits using self-inactivating rabies virus. *Cell* **170**, 382–392.e14 (2017).