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Deconstructing Brain Wiring, One Neuron at a Time

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Researchers have long said they won't be able to understand the brain until they can put together a "wiring diagram" – a map of how billions of neurons are interconnected. Now, researchers at the Salk Institute for Biological Studies have jumped what many believe to be a major hurdle to preparing that chart: identifying all of the connections to a single neuron.

In the March 1 issue of the journal *Neuron*, the researchers describe how they modified the deadly rabies virus, turning it into a tool that can cross the synaptic space of a targeted nerve cell just once to identify all the neurons to which it is directly connected.

"We've wanted to do this for a very long time and finally found a way to make it possible," says the study's senior author, Edward M. Callaway, Ph.D., a professor in the Systems Neurobiology Laboratories. "It will offer us an unprecedented view of the brain."

The problem neuroscientists are confronted with "is akin to a computer user who tries to figure out how the machine's electronic chip works by looking down at it; there is no way to figure out how things are connected," Callaway says. "If you were given a wiring diagram, you could begin to understand how the chip moves electricity and how that operates the computer."

Neuroscientists also want to deconstruct the flow of electrical signals in the extraordinarily complex architecture of the brain and then correlate

these neural circuits with such brain functions as perception and behavior. But these circuits are difficult to unravel because dozens of different neuronal types are entangled within a precisely connected network, and even neighboring neurons of the same type differ in connectivity and function.

So, researchers have been trying to figure out the pattern of connections typical of a type of neuron, to see which other cell types they connect with and how those connections are configured. To do this, they need a tracer that can tease apart the chain of directly connecting neurons, identifying them one by one.

Viruses that naturally spread between neurons have previously been used to outline the flow of nerve cell communication, but they have two drawbacks. First, once inside the brain, they keep spreading from cell to cell without stopping. Second, they cross different synapses – the specialized junctions between nerve cells - at different rates, crossing bigger, stronger synapses faster than smaller, weaker ones. Together these attributes make these viruses unable to determine exactly which cells are connected to which. The team of Salk researchers sought to create a modified virus whose spread could be limited to a single synaptic connection.

“The core idea is to use a virus that is missing a gene required for spreading across synapses but to provide the missing gene by some other means within the initially infected cells,” says Ian Wickersham, Ph.D., postdoctoral researcher and lead author on the project.

With the critical gene deleted from its genome, the virus is marooned inside a cell, unable to spread beyond it. However, supplying the missing gene in that same cell allows the virus to spread to cells that are directly connected to it. Since these neighboring cells lack the gene supplied in the first cell, the virus is stuck. Only the cells connected directly to the

original cell are labeled.

The team's second challenge was to find a way of targeting the viral infection specifically to particular cells, so that the virus could be used to map the connections of cell types of interest or even of single cells. The solution came from a conversation between Callaway and John Young, Ph.D., a Professor in the Infectious Disease Laboratory, and co-author of the study. When Callaway described the problem, Young immediately suggested the answer, which is based on an avian viral receptor that Young discovered as a postdoctoral fellow at UCSF. The protein that ordinarily coats rabies virus particles was replaced with its equivalent from a bird virus. This prevented the modified rabies virus from infecting mammalian neurons at all – unless they had been engineered to express the bird virus's receptor, a bird cell surface protein known as TVA. Such neurons will then be "disguised" as bird cells and the rabies virus – now acting like a bird virus – will infect them.

"The bottom line is that you need two genes expressed in the cell or cell type of interest: TVA, to get the rabies virus in, and the missing viral gene so the virus can spread to connected cells," says Wickersham.

Experimenting on slices of neonatal rat brain, the Salk researchers inserted these two genes into selected neurons – as well as a gene that fluoresces red when expressed. Then they applied the modified rabies virus, which had furthermore been given the ability to make infected cells fluoresce green. The result was spectacular: as expected, these red cells were selectively infected by the virus, which spread to hundreds of surrounding cells, turning them brilliantly fluorescent green.

While these experiments were conducted using slices of brain, it is possible to produce transgenic mice that will express specific genes in a targeted class of neurons, Callaway says. "All neurons of the type we select will then express the avian viral receptor and the rabies virus

protein, allowing the modified rabies virus to infect targeted cells and spread only once to connecting cells," he adds. The wiring map can be constructed step by step as subsequent populations of cells are imaged.

The recombinant rabies virus could contain genes for any proteins of interest, Callaway says, and he adds that once scientists can identify a neural circuit, they can then deactivate it, and test for changes in brain function.

Source: Salk Institute for Biological Studies