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Editorial

Special Issue of the Journal of Chemical Neuroanatomy "New methods for studying brain connectivity using viral tracing"

1. Introduction

Neurotropic viruses have greatly enhanced our understanding of connectivity in nervous systems, and thanks to powerful methods for engineering their genomes they promise to become increasingly important in the future. Viruses have already been engineered to target specific neuronal subtypes, to direct transport of cargo in either the anterograde or retrograde direction, to cross variable numbers of synapses, to produce Golgi-like fills of targeted neurons and to deliver cargo that can be used to both monitor and manipulate neural activity. However, significant deficits in the neuroanatomical toolbox remain presenting investigators with plenty of opportunities for methodological innovation. This special issue attempts to survey recent advances in exploiting the unique properties of neurotropic viruses to map connectivity and assess its functional significance in a variety of neural structures.

One of the most critical features of any neuroanatomical tracer is the *direction* it travels: so-called *retrograde* tracers are taken up by axon terminals and transported back to the cell body, whereas *anterograde* tracers travel in the opposite direction. When interpreting the results of a neuroanatomical experiment, it is clearly essential to be able to distinguish between the two directions of transport, and a recent survey of non-viral tracers made clear that, while many "classical" tracers have a predilection for one direction or the other, almost none of them are exclusively transported in a single direction (see Table 1 of Nassi et al. 2015).

In this special issue, *Huadong Wang and colleagues* raise a similar concern for a viral tracer, H129, previously thought to be purely an anterograde tracer. They use a replication-deficient mutant of H129 (called 'H306') to show that this Herpes simplex virus can indeed be transported retrogradely and therefore that studies using this tool must be interpreted with caution, especially when survival times are long. Taking a step back, *Kevin Beier* provides an overview of differently directional viruses and strategies for engineering them to either change or improve their directional specificity. Like *Wang et al.*, he provides evidence that the directional specificity of some viral tracers may have been overstated. But, more importantly, he provides a careful analysis of how the biological mechanisms of the virus interact with those of the neuron to produce directional specificity and, in some cases, trans-synaptic spread. He makes it clear that a more thorough understanding of viral replication, trafficking, shedding and infection will be critical for engineering better viral tracers.

Two other contributions to the special issue focus on protocols that will be invaluable to practicing neuroanatomists. The use of G-deleted rabies virus to map the inputs to genetically defined populations of neurons is simple in concept, but, as *Lavin, Jin and Wickersham* remind us, "the devil is in the details." And they provide a detailed protocol to this end, complete with the description of an ideal injection apparatus, instructions on the preparation of reagents, and a step-by-step surgical protocol. In a similar spirit, *Eriko Kuramoto* describes a method for using the Sindbis virus to label and reconstruct single axons and its application to study thalamocortical projection neurons. Though laborious, the method provides unique insights into neuronal circuitry by virtue of the beautifully elaborate reconstructions of axonal arborizations that it permits.

Finally, as a counter-point to the labor-intensive microscopy-based methods required by virtually all existing neuroanatomical approaches, *Justus Kebschull*, reviews a new method that allows mapping the outputs of thousands of individual neurons in a single experiment, so-called "MAPseq." The method uses Sindbis virus to introduce nucleic acid "barcodes" along with the sequence of a presynaptic protein that drags the barcode to the infected neuron's synaptic outputs. Projections from a given neuron at the injection site to a given brain structure are established by DNA sequencing, matching up barcodes at the two locations.

While the five articles in this special issue are by no means a complete representation of virus-based neuroanatomical approaches, they constitute an interesting sample of this space. Importantly, they show the range of what is currently possible—from the anatomical detail of single neuronal projections to high-throughput, sequence-based methods—and they provide insights into approaches for generating the next generation of viral tracing tools.

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