NEUROSCIENCE

Dissecting neuronal circuitry

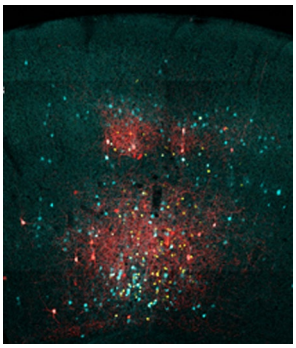
A virus-based tracing approach in combination with recombinase-mediated labeling allows the dissection of neuronal circuit motifs with subtype specificity.

Retrograde monosynaptic tracing has been an important component of the circuit-mapping toolkit in the mouse. In this approach, a neuronal subtype of interest, designated as a starter neuron, is infected with a rabies virus. The virus then spreads to neurons that are presynaptic to the starter neuron. Thus, this approach identifies the input neurons to genetically defined starter neurons. However, it has not been possible to simultaneously label input neurons on the basis of their subtype. The dissection of circuit motifs with full genetic specificity for both starter and input neurons would be important, as the same neuronal subtypes may exhibit different functions and activities depending on their complement of input neuron subtypes.

Hiroki Taniguchi from the Max Planck Florida Institute for Neuroscience and his collaborators addressed this problem by establishing an elegant intersectional monosynaptic tracing (iMT) approach. To do so, they combined the rabies-virus-based monosynaptic tracing approach with Cre- and Flp-dependent intersectional labeling, in which both recombinases have to be present in the same cell to allow the expression of a reporter gene.

The iMT approach involves a number of elements. Genetic specificity in the starter neurons is achieved through the expression of TVA, the receptor for the EnvA-pseudotyped rabies virus, in these cells. Starter neurons also express YFP, as well as the RG protein, a glycoprotein necessary for trans-synaptic propagation of rabies virus. Upon injection of rabies virus carrying Flp and CFP genes, the virus infects TVA-expressing starter neurons and subsequently is transmitted to input neurons of different subtypes, which then express Flp and CFP. Labeling of genetically defined input neurons is achieved through the combination of a subtype-specific Cre driver with a Cre- and Flp-dependent RFP reporter. Taken as a whole, the iMT approach results in YFP expression in starter neurons, CFP expression in input neurons, and additional RFP expression in the desired subtype of input neurons.

Furthermore, the researchers devised an approach for sparse labeling of starter neurons, which is useful to avoid dense labeling of input neurons and potential misassignment of input neurons to the wrong starter neurons. The researchers added an additional layer of control by making use of the residual recombinase activity of the DreER recombinase in the absence of tamoxifen. This additional element allowed them to express TVA and RG in very few neurons in the mouse brain, resulting in a sparse distribution of starter neurons.



Starter principal neurons (yellow) receive inputs from parvalbumin (red) and other (cyan) interneurons. Adapted with permission from Yetman et al. (2019), Springer Nature.

The researchers applied their iMT approach to study differences in the interneuron–principal neuron connectivity in different areas or layers of the mouse cortex.

In summary, iMT constitutes a useful approach for deciphering neuronal connectivity with subtype specificity. The approach is currently limited to structural studies, as rabies virus tools exhibit substantial neurotoxicity, prohibiting meaningful functional analyses. However, when combined with less toxic rabies variants or other tracing tools, the iMT approach could potentially be used to look into the functional properties of different circuit motifs.

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Research papers
Yetman, M. J. et al. Intersectional monosynaptic tracing for dissecting subtype-specific organization of GABAergic interneuron inputs. *Nat. Neurosci.* **22**, 492–502 (2019).