Mapping Brain Activity onto Molecularly Defined Cells

Alexander Jones[1,2] and Leon G. Reijmers[1,*]
[1]Department of Neuroscience, School of Medicine, Tufts University, Boston, MA 02111, USA
[2]Graduate Program in Neuroscience, Sackler School of Graduate Biomedical Sciences, Tufts University, Boston, MA 02111, USA
*Correspondence: leon.reijmers@tufts.edu
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The brain processes information and generates behavior by employing a wide array of different cell types. In this issue of *Neuron*, Wu et al. (2017) report a novel method that enables the efficient identification of molecularly defined cells that participate in a specific brain function.

The brain has the impressive ability to convert complex combinations of sensory input and internally stored information into optimal behavioral output. The underlying computations are performed by a diverse array of neuronal cell types, with the support of various non-neuronal cell types. For most brain functions, the cell types that perform the underlying computations remain poorly defined. To identify cell types that contribute to a specific brain function, it is necessary to monitor brain activity at single-cell resolution while the animal is performing that function. A classic example is place cells that support the function of spatial navigation, which were discovered by *in vivo* electrophysiology recordings in rats that navigate a maze (O'Keefe and Dostrovsky, 1971).

Brain activity can be monitored at single-cell resolution by imaging immediate-early gene (IEG) expression. IEG expression occurs when cells are activated by certain types of extracellular signals that trigger intracellular signaling to the nucleus. In neurons, sufficient activation of certain neurotransmitter and neuropeptide receptors will induce IEG expression, providing a useful readout of neuronal activation (Flavell and Greenberg, 2008). Imaging IEG expression enables the identification of specific cell types that are activated during the execution of a brain function. This can be achieved by dissecting the brain shortly after the brain function has been executed, followed by double immunohistochemistry, or double *in situ* hybridization, for both an IEG product and a cell-type marker. However, the limited number of validated cell-type markers that may be used for this approach prohibits purely discovery-based studies and may obscure important functional heterogeneity among cell types that appear similar when only one cell-type marker is used.

To circumvent some of the limitations of IEG imaging, Wu and colleagues developed an alternative method called Act-seq (Wu et al., 2017). Act-seq replaces the imaging of IEG expression with single-cell sequencing of IEG-encoded mRNAs. Act-seq starts with dissociating and collecting all the cells from a dissected brain region and then uses the high-throughput method Drop-seq to collect and sequence all the RNA from each collected cell. The resulting RNA-seq data are then used to precisely quantify how much mRNA for each known IEG is present in every collected cell. This is an important advance compared with imaging-based detection of IEG expression, which relies on less precise quantification of only a small number of IEGs using antibodies or *in situ* probes. Since, as Wu et al. show, different cell types express different IEGs when activated, Act-seq makes it possible to identify a larger variety of activated cells than would be possible when imaging the expression of only a few IEGs.

An important challenge that Wu et al. had to resolve was to minimize IEG expression induced during the dissociation and sorting of the cells, as this would introduce noise in the IEG expression patterns that might mask the IEG expression patterns associated with the brain function of interest. They report that adding a high concentration of the transcriptional inhibitor actinomycin D can prevent almost all transcription of IEG genes during the process of cell dissociation and sorting. In addition, they report that lowering the temperature during certain steps also reduces background IEG expression. They validated these modifications by comparing samples from mice at rest with samples from mice undergoing seizure, thereby identifying conditions resulting in maximum signal-to-background levels in both neuronal and non-neuronal cell types. Importantly, the low-IEG-expression background achieved with the optimized protocol makes it possible to detect the activation of cells caused by manipulations that are much milder than seizure, as demonstrated with the identification of cells activated by acute stress.

The sequencing-based method reported by Wu et al. represents a significant advancement from traditional imaging-based methods for mapping brain activity, because it enables truly unbiased discovery of cell types that are active during the execution of a brain function. Wu et al. demonstrate this by using their RNA-seq data to generate a *de novo* molecular taxonomy of all the cells located in the brain region that they dissected from their mice, which was the medial amygdala. This molecular taxonomy includes 16 different types of neurons and three different types of astrocytes. They then determined the location of each activated cell within this taxonomy. This revealed that restraint stress causes the activation of medial amygdala cells that include two different types of neurons. Interestingly, both activated neuronal cell types express the neuropeptide CCK, indicating a role for CCK-expressing medial amygdala neurons in the brain response to restraint stress.

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Future studies could use the Act-seq method for the parallel analysis of activated neurons in all regions of the brain, which previously would have necessitated the generation of large, unwieldy image datasets. A challenge for brain-wide application of Act-seq is that micro-dissecting each individual brain region might take so much time that it leads to deterioration of cell integrity, thereby decreasing the number of intact cells that can be collected. A faster approach would be to quickly cut the brain in a number of arbitrarily placed pieces, immediately followed by cell dissociation. This would require the availability of a brain-wide molecular taxonomy, so that the brain region from which each activated cell originated can be determined post hoc based on its gene expression profile. It might be possible to assemble such a brain-wide molecular taxonomy by using the single-cell gene expression datasets that are being generated for an increasing number of brain regions (Chen et al., 2017; Gokce et al., 2016; Tasic et al., 2016; Wu et al., 2017; Zeisel et al., 2015).

Another future expansion of the Wu et al. method relates to circuit-level analysis. Identifying the location of brain cells activated during a certain brain function, as well as their cell types, is an important first step. However, understanding the computations performed by these cells also requires knowledge of how those cells are connected with each other. Determining the synaptic connectivity of activated neurons with RNA-seq analysis might become possible with the further development of methods that label synaptic connections with RNA barcodes and that can maybe be extended to other types of cellular connections (Peikon et al., 2017).

Finally, the sequencing-based method that Wu et al. developed for generating a brain activation snapshot could one day evolve into methods that can reconstruct the activation history of a brain. Progress is being made with the development of genetic tools that turn a cell into a recording device for defined cellular events, which could include intracellular signaling events in response to cell activation (Frieda et al., 2017; Reijmers et al., 2007; Roquet et al., 2016; Shipman et al., 2016). Sequencing-based readout of these recordings could enable the post hoc identification of multiple activation events that occurred in the same cell at different time points. Though single-cell mapping of the brain's activation history is still far on the horizon, the Act-seq method reported by Wu et al. is showing the way. More immediately, the Act-seq method will greatly facilitate the discovery of novel cell types in the brain that participate in defined brain functions.

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