ACS Chemical Neuroscience

Viewpoint

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Genetically Encoded Tools: Bridging the Gap between Neuronal Identity and Function

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ABSTRACT: Genetically encoded tools are positioned to serve a unique and critical role in bridging the gap between the genetic identity of neurons and their functional properties. However, the use of these tools is limited by our current understanding of cell-type identity. As we make technological advances that focus on capturing functional aspects of neurons such as connectivity, activity, and metabolic states, our understanding of neuronal identity will deepen and may enable the use of genetically encoded tools for modulating disease-specific circuits for therapeutic purposes.

KEYWORDS: *Neuronal identity, genetically encoded tools, optogenetics*

From early on, the large diversity of cells in the central nervous system has been recognized as an important feature for its function. As a result, considerable efforts have been made to describe neuronal identity systematically. In recent years, through the advancement of genomics, we have gained significant insights into the genetic diversity of neurons. Gene expression patterns of anatomically defined neurons have been systematically studied in humans using microdissection combined with microarray analysis (Allen Brain Atlas, http://www.brain-map.org/) and in mice through the creation of transgenic mouse lines using bacterial artificial chromosome vectors (Gene Expression Nervous System Atlas (GENSAT), http://www.gensat.org/index.html). These ongoing studies are shaping our understanding of how to classify neurons and to define their identity. It seems that a generally applicable definition of a neuronal cell type is a cell or group of cells that have distinct expression patterns of a set genes that dictate their functional properties.[1] This is a powerful way of defining cell types, since it allows us to reconcile new findings with classical studies that focused on a few characteristic genes. It also provides data-driven explanations and predictions of cell-type specific functions.

However, it is also becoming clear that genetically defined neuronal identity does not always capture information provided by context. Circuit-level studies have demonstrated that interconnected neurons show emergent properties such as synchrony and oscillation—properties that cannot be deduced outside their context. Therefore, the genetic identity of a neuron needs to be mapped onto its anatomical structure (as provided to some extent in the above studies), as well as linked to its activity profile through electrophysiological and neurochemical characterization. This information will form a critical starting point for understanding how neuronal activity is transformed into complex functions such as perception and memory. In this respect, a logical next step is to perform large-scale systematic analysis of neural connectivity and activity, as we have been successfully doing for genomic analysis. The current drive for developing breakthrough technologies to map large-scale brain activity[2] is motivated by the disconnect in our knowledge between neuronal identity and function.

Discontinuities between knowledge of neuronal identity and functional properties set the stage for genetically encoded tools—proteins that function as reporters or actuators of cellular processes—to play essential roles. Being able to express these tools reliably in genetically defined population of neurons enables us to assess the function of specific cell types in a given circuit and to compare function across different conditions. Creative use of fluorescent proteins for reading out various cellular states including calcium dynamics, electrical activity, and biochemical signature (Figure 1) is particularly advantageous for bridging the gap between neuronal identity and functional properties, since optical imaging enables viewing a system with multiple levels of resolution, thus enabling population level analysis with single-cell resolution. Moreover, we now have technologies to manipulate neuronal activity in defined populations, extensively demonstrated through the use of light-activated ion channels and pumps and engineered G-protein-coupled receptors (Figure 1). These tools have opened a floodgate of new capabilities for revealing the role of specific cell types in circuit activity and behavior. Considering the wide range of functional diversity of these tools (Figure 1), it is certain they will help speed our goal of mapping neural activity and adding new dimensions to these data, for example, through biochemical analysis of neurotransmitters and metabolites.

Nonetheless, our ability to probe neuronal function using these tools is bound by our existing knowledge of cell-type identity. For example, large-scale genetic studies have found that reproducing endogenous expression pattern of genes requires the entire transcription unit and all associated regulatory factors, which are unknown in many cases, and can be hard to include for large genes (>200 kb).[3] Gene expression can be spatially refined by local injection of viral vectors, but they are limited in the size of genetic payload, restricting their use with relatively short promoters. Moreover, even if we can target these tools accurately, their expression

Special Issue: Monitoring Molecules in Neuroscience 2014

Received: January 7, 2015
Published: January 9, 2015

DOI: 10.1021/acschemneuro.5b00008
ACS Chem. Neurosci. 2015, 6, 14−15

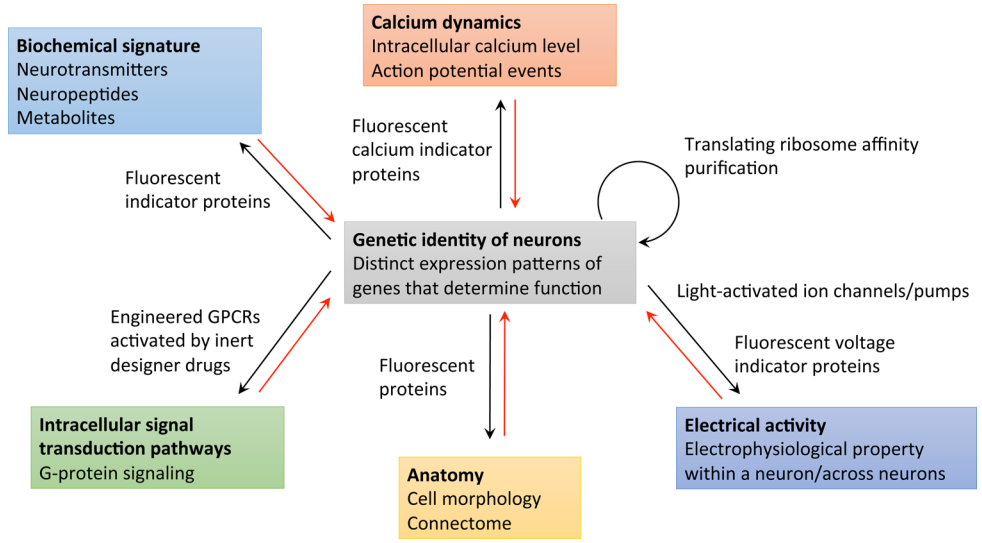

Figure 1. Genetically encoded tools bridge the gap between neuronal identity and functional properties. Using genetic targeting strategies, these tools can be repeatedly expressed in specific cell types to probe their function (black arrows). As we make technological advances that focus on measuring neuronal function, approaches to trace back genetic information based on functional characteristics (red arrows) are likely to become increasingly important.

level may not be sufficient for the desired functionality to take effect, potentially biasing our analysis toward higher expressing neurons. At a more fundamental level, we may not be able to capture the individuality of neurons known to occur through stochastic expression of genes.[1]

In this regard, approaches that rely on genetic targeting to probe neuronal function need to be complemented by approaches that do not rely on a priori knowledge of genetic identity. Recently, it was demonstrated that large-scale single-cell RNA sequencing can be used to classify neurons and predict their function solely based on their anatomical location.[4] Single-cell mass spectrometry now allows quantitative assessment of metabolites in isolated neurons.[5] These "bottom-up" approaches that focus on functional properties of single cells can capture neuronal individuality, and are free of potential bias that could be introduced by our current knowledge of genetic identity of neurons.

In turn, as we make further technological advancements to measure functional properties, it will become increasingly important to trace back the genetic profile of neurons from their functional characteristics (Figure 1). Promising techniques are being developed that enable profiling genetic identity of neurons based on their activity.[6] Future development is necessary in this direction including methods to obtain the genetic profile of neurons based on calcium dynamics, intracellular signaling pathways, and biochemical signatures. These approaches will continue to deepen our understanding of neuronal identity, and may in the future refine our ability to target genetically encoded tools to disease-specific circuits, enabling circuit-based therapies that currently cannot be achieved using pharmacological agents.

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Notes

The authors declare no competing financial interest.

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DOI: 10.1021/acschemneuro.5b00008
ACS Chem. Neurosci. 2015, 6, 14−15