

Preview

An atlas for unveiling the functional organization of spinal circuits

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Defining the positional organization of neurons in the spinal cord is critical for understanding their function. In this issue, Fiederling and colleagues present a method to accurately map position and connectivity of neurons in a universal three-dimensional spinal cord reference atlas.

The spinal cord has important roles in motor control, coding of somatosensory information, and regulation of the autonomic nervous system; understanding the organization and function of spinal circuits is therefore a fundamental goal of neuroscience. Recent technological advances are providing powerful tools for high-throughput characterization of neurons at molecular, physiological, and circuit levels. The introduction of single-cell approaches, neuronal activity sensors, and viral tracing technologies comes with the promise of solving the long-standing problem of precisely defining cell identity and function by combining in-depth molecular characterization with profiles of physiological activity and circuit diagrams in the context of detailed behavioral analysis (Lein et al., 2017; Bando et al., 2019; Pereira et al., 2020; Xu et al., 2020). Single-cell sequencing is revealing the molecular heterogeneity of spinal neurons, whereas at an anatomical level, new tracing tools allow the mapping of spinal circuits with unprecedented resolution. These advances along with the introduction of intersectional approaches combining the power of mouse genetic, molecular characterization, and viral tracing are paving the way for studying the physiological roles of defined neuronal subtypes (Osseward and Pfaff, 2019).

Molecular, anatomical, and physiological studies are providing the scientific community with rich datasets describing in details discrete aspects of neuronal identity and function. However, seamless integration of this information can be challenging because experiments are often

conducted at different level of resolution and in the absence of a rigorous spatial framework encompassing the entire spinal cord. This is of particular relevance as several studies aimed at understanding the role of spinal neurons highlighted the role of positional features in the organization of circuits, revealing a predictive link between neuronal location and morphology with connectivity and ultimately function (Tripodi and Arber, 2012). Thus, a comprehensive classification of different features of neuronal identity within the context of a spinal cord three-dimensional model is an important step forward for understanding the functional organization of spinal circuits. The ongoing development of anterograde and retrograde tracing techniques, coupled with the increasing availability of molecular markers is offering the opportunity to specifically access virtually any neuron of choice, thus opening the way for integrated molecular, anatomical, and functional analysis. These advances come with the promise of a system approach toward the characterization of spinal circuits. In this context, a universal reference atlas to annotate the position of molecularly defined subsets of neurons, along with their wiring, physiological, and functional properties in a three-dimensional model will be needed to compare and integrate data across experiments and laboratories. The power of such an approach is exemplified by the success of the brain atlas generated by the Allen Institute (www.brain-map.org), the gold standard in terms of integrating anatomic, genomic, and physio-

logical features of neurons in a common, unified framework (Jones et al., 2009). Although a spinal cord atlas is also offered, only gene expression analysis tools are currently available, thus greatly limiting the breadth of information that can be extracted.

In this issue of *Cell Reports Methods*, Fiederling and colleagues introduce a relatively simple but effective experimental approach for high-resolution three-dimensional analysis of neuronal position and connectivity in the adult mouse spinal cord (Fiederling et al., 2021). First, the authors introduce a low-cost and time-saving solution for serial cryo-sectioning of the entire spinal cord by devising water-soluble supports, called “SpineRacks,” that can be produced by using commercially available three-dimensional printers. SpineRacks are optimized to fit nine tissue segments, allowing the ordered sectioning of the entire adult spinal cord in a single block. In addition, SpineRacks configuration can be easily customized for orderly sectioning of any other tissue or organism. This approach considerably reduces sectioning time and results in the precise arrangement of sections on the slides, thus allowing automated acquisition and ordering of images. Next, the authors built a three-dimensional reference atlas of the adult mouse spinal cord by digitizing and aligning annotated sections from the Allen mouse spinal cord atlas and developed “SpinalJ,” an open-source plugin for ImageJ (Schneider et al., 2012). SpinalJ comprises a series of software tools that are necessary for



image registration, atlas mapping, and analysis of spinal cord sections. To validate the method, the authors took advantage of the well-known stereotyped positional organization of neuronal cell bodies, somatosensory afferents terminals, and descending fibers in the spinal cord, by either labeling ChAT⁺ spinal neurons, IB4⁺ peripheral fibers, or virally labeled long-range projections of the cortico-spinal tract. The authors mapped images obtained from these experiments onto the reference atlas and confirmed the accuracy and reproducibility in the reconstruction of cell body positions or axon terminals in the entire spinal cord volume. Moreover, they tested the ability of SpinalJ of accurately report small differences in relative positioning of similar cell types by faithfully and precisely mapping the location of the FoxP1⁺ subset of cholinergic neurons within the parental population across different samples, thus indicating that the tool can be successfully used to compare the organization of closely positioned neurons.

Altogether, the tools described in this study provide an easily implementable solution for high-resolution comparative analysis of different features of neuronal identity in three-dimensions. The use of SpineRacks and serial sectioning can often be preferable to whole-tissue clearing and imaging methods, which offer advantages in terms of tissue coverage and resolution, but might require lengthy optimization and not be readily available to all laboratories (Weiss et al., 2021). The affordable and rapid workflow for sample processing and analysis make this method suitable for most

laboratories, without the need for expensive equipment and imaging analysis software. It will be desirable in the future to improve the resolution and the scope of the atlas, which is now based solely on the mouse adult spinal cord annotations from the Allen Institute, by including multiple developmental stages and different model organisms. The possibility of integrating into SpinalJ positional datasets from different users offers the opportunity to further refine the quality of the current atlas, as well as to generate new atlases suited for developmental and comparative anatomy studies. These considerations highlight how participation from the scientific community could be a powerful driving force to quickly move toward the common goal of generating a definitive spinal cord reference system. This work offers the opportunity to tackle this challenge by providing researchers with a versatile tool for basic and clinical studies focusing on the organization and function of neural circuits in the spinal cord.

In summary, the recent introduction of high-throughput technologies for identifying neurons and circuits at anatomical level, as well as for molecular and functional profiling, is generating a wealth of information that will greatly benefit by the ability to compare experiments in a unified spinal cord framework. The work of Fiederling and colleagues provides a solid, affordable, and easily implementable solution to achieve this goal and allow comparison of the spatial organization of spinal neurons and circuits across different experimental conditions and laboratories.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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