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EDITORIAL

In the Enteric Nervous System, It's All About Connections

In business, in politics, and in the enteric nervous system (ENS) it's all about connections. Being able to communicate with others is essential to accomplishing project goals. Nowhere is this proven more elegantly than in the study by Nestor-Kalinoski et al[1] on the architecture, connectivity, and signaling between myenteric neurons of the colon in this month's issue of *Cellular and Molecular Gastroenterology and Hepatology*. Tremendous progress in understanding the neural circuitry of the ganglia that make up the ENS has been made by groups that focused either on imaging neural connections, interrogation of signaling, or pharmacologic studies.[2,3] Nestor-Kalinoski et al[1] integrated all of these approaches to gain novel insights into distinctions among myenteric ganglia in the proximal, mid, and distal colon. Their results offer greater insights into the colonic ENS connectome, identify novel marker expression, reveal previously unappreciated synaptic connections within ganglia, and demonstrate a regional propensity for colonic motor complex induction.

The team undertook a combinatorial approach for their spatial mapping efforts and derived unmatched detail in their assessment of enteric neuron class location and visualization of nerve processes along distinct regions of the mouse colon. Using multiple cre transgenic lines in combination with a barrage of immunohistochemical reagents they classified enteric neurons in proximal, mid, and distal colon regions spatially subdividing each segment further. The careful documentation of regionality offers a framework for reproducibility by ENS scholars that will facilitate comparisons across studies in the future. Prior studies have detected regionality for some types of enteric neurons in the colon; however, the high-resolution spatial mapping and comprehensive nature of this study provide novel insights into ENS architecture. In their effort, quantitative measures of ganglia patterning and interganglionic fiber tract connectivity along the colon are related to the distribution of distinct neuron classes. Moreover, the group thoughtfully integrated their results with prior human ENS structural mapping studies.[4] The evaluation shows comparable distributions for nitrergic and cholinergic neuron classes that provides reassurance regarding the use of mice as surrogates for human studies. Interestingly, the study also identifies unexpected expression of CGRPα among infrequent neurons in proximal and midcolon that may represent a new intrinsic primary afferent neuron subtype.

One of the exciting aspects of the study is the identification of intraganglionic connectivity, synaptic formation between adjacent neurons within the same ganglia, revealed by 3-dimensional reconstruction of neural projections. The extensive number of synaptic sites seen on individual neurons suggests potential for signal modulation and has tremendous implications for regulating intestinal motility. Prior studies of ENS development have focused on whether neurons were present with many investigators targeting the genes and mechanisms that predispose to Hirschsprung disease. Yet the current study suggests that it is equally important to consider how the neurons are connected. A single report previously documented roles for planar cell polarity genes Celsr3 and Fzd3 in enteric neurite formation during longitudinal and radial migration,[5] but much remains to be understood about synaptogenesis in ENS development, maintenance, and aging. Whether enteric intraganglionic connectivity forms like that of the central nervous system through a sequence of synaptic formations and subsequent pruning is unknown. This study also raises intriguing questions about the plasticity of such intraganglionic connections given recent evidence of postnatal enteric neuron birth and replacement.[6,7]

Nestor-Kalinoski et al[1] further related density of neural connectivity with initiation of spontaneous colonic motor complexes by using genetically engineered calcium indicators (GCaMP6) as an indirect measurement of cell activity. The team observed that although all neurons required Ca2+ signal summation to reach a threshold before spontaneous colonic motor complex initiation, the ease of summation and frequency of spontaneous colonic motor complex activity was elevated in proximal ganglia that exhibited more intricate connectivity. Their data suggest that connectome complexity plays a role in initiation of spontaneous colonic motor complexes as a result of the greater density of neuronal connections in proximal colon. This finding agrees with prior work demonstrating connectivity within neuronal clusters for colonic motor functions,[8] and further implies that proximal ganglia are the major initiators of colonic motility. Because interneurons and intrinsic primary afferent neurons are more abundant in proximal regions, increased connectivity may not be the sole reason for heightened spontaneous colonic motor complex activity. Future studies are necessary to determine whether proximal colonic ganglia increase activity of any affiliated motor neuron. And, importantly, whether transplantation of compensatory neurons into these proximal ganglia is sufficient to recover or improve function in cases of colonic dysmotility.

A final element of the Nestor-Kalinoski study is the mapping of vasculature in relation to enteric ganglia. Their images are breathtaking and capture capillary beds that seemingly travel between and through some ganglia along with vascular nets around calretinin-expressing neurons and other yet to be identified neuron types. Although early ENS development is unreliant on enteric vasculature,[9] the presence of these nets around specific neurons raises obvious questions regarding their function. Whether these

capillary nets function in ENS-immune interaction or feedback from blood nutrient levels is open to future study.

Understanding normal ENS architecture, connectivity, and signaling offers an essential framework for comparison with disease states. The studies by Nestor-Kalinoski et al[1] reveal that the colonic "connectome," those functional connections that enable neurons to signal one another, is more elaborate than previously recognized. Their findings and approaches will be valuable going forward as diverse neuron types identified by single-cell RNA-sequencing[10–12] must be integrated into the framework of the ENS. In the end, success at propelling luminal contents through the colon depends on who you talk to.

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Conflicts of interest
The authors disclose no conflicts.

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