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Editorial

Editorial for "In vivo spinal cord imaging in health, injury and disease"

Seeing is believing. Since the first publication over 14 years ago, in vivo imaging in the mammalian spinal cord has emerged as a powerful tool for elucidating cellular and molecular mechanisms underlying its structure and function in health, injury and disease. As reviewed in this Special Issue, in vivo imaging has advanced our understanding of sensorimotor processing, axonal degeneration and regeneration after injury, and the mechanisms underlying neuroinflammatory disease. These advances were made possible by key technical developments, including surgical preparations for stable and long-term in vivo imaging, new labeling techniques for structural and functional imaging of specific cells and circuits, and microscopy approaches for high-speed and high-resolution optical interrogations in behaving animals. To share these exciting and ongoing developments with interested scientists in the field and the research community at large, we have assembled a set of ten reviews focused on both current technical approaches and their biological applications in health, injury and disease. These articles critically reflect on strengths and weaknesses of in vivo imaging, scientific discoveries uniquely enabled by this technology, as well as current opportunities and challenges.

The first article by **Cheng et al.** serves as a primer for in vivo imaging methodologies. The authors provide an overview of surgical preparations, fluorescence labeling strategies, and optical techniques for cellular-resolution imaging in the mouse spinal cord (including second- and third-harmonic generation, two- and three-photon, and Coherent Anti-Stokes Raman Scattering or CARS microscopy). In doing so, they highlight the unique challenges of in vivo imaging in the spinal cord as compared to the brain, and discuss several approaches for chronic imaging. They then share their thoughts on future technological advances.

The next two articles apply in vivo imaging to study the physiological responses of spinal cord circuits. **Ran and Chen** discuss their approach for probing the molecular and circuit logic of thermosensation in the mouse spinal cord. Using two-photon calcium imaging, they present evidence for distinct coding schemes for sensing warm versus cold temperatures, and the specific role of TRPV1 channels in warmth sensation. Additionally, they discuss how their calcium imaging approach may be applied to the study of sensory coding more generally, including nociception. **Xu and Dong** extend this line of thought by first reviewing how in vitro and in vivo application of calcium imaging techniques has shaped our current understanding of pain circuits in the spinal cord. They then discuss how new genetic and imaging tools may be combined to further our understanding of pain circuits in the future.

The next set of three articles centers on the theme of axonal responses to injury. **Schaffran et al.** begin by introducing the dorsal column sensory axons as an experimental model and highlight their baseline responses to injury at different time points. They then discuss sensory axon regeneration, including the well-known conditioning lesion effect, and the ability of other neuronal populations to regenerate. They devote the rest of their article to experimental design considerations, including methods for injury induction and chronic imaging, followed by their view on future opportunities. Next, **Zheng et al.** compare and contrast in vivo imaging with conventional histology for studying the basic properties of axon degeneration and regeneration after injury. They then present evidence from in vivo imaging that the location of axonal injury relative to a major branch point markedly impacts degenerative and regenerative responses. Based on this study and other evidence in the literature, they discuss a synaptic suppression hypothesis of axon regeneration and propose a model to explain the graded response to axonal injury depending on the branch structure. **Denecke et al.** further expand on this theme by discussing how pharmacologic and genetic interventions may be combined with in vivo imaging to shed light on the molecular mechanisms underlying axon pathology and outgrowth after spinal cord injury. They first discuss insights gained into baseline degenerative and growth responses of axons along with changes in the environment (e.g., vascular, glial and neuroinflammatory responses). They then present an extensive overview of in vivo imaging studies that have tested molecular interventions and discuss their biological implications.

Another set of three reviews further expands the topic to non-neuronal cells and their role in injury and disease. The article by **Evans et al.** begins by describing the basic setup for in vivo spinal cord imaging, including in cervical and ventral regions. They then discuss special considerations for chronic imaging of immune processes and provide a comprehensive overview of fluorescent labeling strategies to visualize immune and other cells along with their dynamic interactions. **Borjini et al.** review insights gained from imaging peripheral and innate immune cell interactions with spinal cord vasculature with a particular emphasis on blood-brain barrier (BBB) permeability changes in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, and spinal cord injury. They describe how structural and functional imaging has been used to evaluate the efficacy of existing therapeutic approaches, discover biomarkers, or identify new targets for restricting tissue damage or enhancing its repair. Complementing this article's focus on the initiation phase of neuroinflammatory disease, **Schumacher et al.** devote their article on imaging the execution phase. They first discuss strengths and limitations of current animal models for multiple sclerosis and neuromyelitis optica spectrum disorders, and then review findings on phagocytic, glial and axonal responses to neuroinflammatory lesions with a particular focus on immune-mediated axonal damage. They also share their thoughts on future directions, including the need for new biosensors and indicator mice to better understand metabolic state and cell-cell interactions.

Finally, in perhaps the most forward-looking article in this Special Issue, **Nelson et al.** offer their perspective on the current state-of-the-

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art in optically interrogating cellular and microcircuit activity in the spinal cord of behaving animals, followed by a brief review of insights gained so far. They discuss current opportunities for elucidating cellular interactions and spinal microcircuits under naturalistic conditions, as well as current technical challenges and potential solutions.

While this Special Issue is certainly not exhaustive, we feel that it provides a comprehensive overview of the current state-of-the-art. Some overlap in topics was deliberate and in some cases inevitable, as elements such as chronic window preparation, fluorescence labeling and image stabilization are of shared concern. We feel that these different perspectives are both valuable and crucial for methodological and conceptual advances. Most importantly, we hope that the technical approaches and biological findings covered in this Special Issue will spark new ideas for innovative studies geared toward advancing our understanding of the fundamental mechanisms underlying spinal cord function and dysfunction, as well as the development of new therapeutic approaches for spinal cord injuries and diseases.

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