Dendritic Spines in the Spinal Cord: Live Action Pain

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ABSTRACT: Dendritic spines are microscopic protrusions on neurons that house the postsynaptic machinery necessary for neurotransmission between neurons. As such, dendritic spine structure is intimately linked with synaptic function. In pathology, dendritic spine behavior and its contribution to disease are not firmly understood. It is well known that dendritic spines are highly dynamic in vivo. In our recent publication, we used an *intravital* imaging approach, which permitted us to repeatedly visualize the same neurons located in lamina II, a nociceptive processing region of the spinal cord. Using this imaging platform, we analyzed the intravital dynamics of dendritic spine structure before and after nerve injury–induced pain. This effort revealed a time-dependent relationship between the progressive increase in pain outcome, and a switch in the steady-state fluctuations of dendritic spine structure. Collectively, our in vivo study demonstrates how injury that leads to abnormal pain may also contribute to synapse-associated structural remodeling in nociceptive regions of the spinal cord dorsal horn. By combining our live-imaging approach with measures of neuronal activity, such as with the use of calcium or other voltage-sensitive dyes, we expect to gain a more complete picture of the relationship between dendritic spine structure and nociceptive physiology.

KEYWORDS: Dendritic spines, pain, nerve injury, dorsal horn, intravital imaging, 2-photon microscopy

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More than 90% of excitatory neurons in the central nervous system (CNS) have dendritic spines. Dendritic spines are microscopic protrusions that house the synaptic machinery necessary for neurotransmission between neurons.[1,2] As such, dendritic spine structure is intimately linked with synaptic function. In the context of cortical learning and memory, dendritic spines are of great interest as a fundamental aspect of *adaptive*, activity-dependent circuit remodeling and "hard wiring." In pathology, however, much less is known about dendritic spine behavior and its contribution to disease. Over the past decade, emerging evidence from spinal cord studies has revealed a common structural motif of dendritic spine morphology strongly associated with neuropathic pain.[3-5] Specifically, in injury- or disease-induced pain conditions, dorsal horn neurons exhibit (1) increased dendritic spine density; (2) redistributed spines on dendritic branch regions located closer to the cell body, which has a weighted impact on excitatory potential propagation; and (3) increased spine head surface area.[2,6]

Up to this point, studies of dendritic spines in the spinal cord relied on analyses of postmortem tissue. Although informative, these histological studies only provided a snapshot of the actual dendritic spine remodeling processes. In vivo dendritic spines are highly dynamic structures, often appearing and disappearing and changing shape in the order of minutes to hours, depending on the condition.[7,8] To further understand the contribution of dendritic spine changes in neuropathic pain, we set out to observe them in a living system. To do this, we adapted an *intravital* imaging approach developed by Fenrich et al,[9] which permitted us to repeatedly visualize neurons from lamina II in Thy1-YFP neuron-specific reporter mice. Using this imaging platform, our longitudinal study analyzed the *in vivo* dynamics of dendritic spines before and after spared nerve injury (SNI)-induced neuropathic pain.[5]

Our investigation revealed a *time-dependent relationship* between the progressive increase in pain outcome, and a switch in the steady-state, dynamic fluctuations of dendritic spine structure. For example, the onset of injury-induced pain coincided closely with an increase in fluctuations of dendritic spine length and spine head-width, demonstrating that injury can directly alter nociceptive circuit structure. Similar to previous postmortem work, we also detected an increase in the overall density of mushroom-shaped spines 7 days after SNI, suggesting an increase in synaptic strength within the superficial dorsal horn. Interestingly, our unique ability to visually track the same neuron over time revealed that dendritic spine turnover (eg, a ratio measure of spine appearance to disappearance) was dependent on the progression of pain severity. In our study, we observed that as pain sensitivity increased—from 3 to 7 days after SNI—thin-shaped spine elimination decreased, and mushroom spine formation increased. This dynamic switch in dendritic spine behavior triggered by injury likely has significant physiological implications. In cortical memory formation studies,[10] mushroom-shaped spines represent more mature, stronger synapses, ie, with improved neurotransmission efficacy, as compared with thin-shaped spines. As such, our intravital observations strongly suggest that injury or disease that leads to abnormal pain also

contributes to a strengthening of synaptic circuits within the nociceptive regions of the spinal cord dorsal horn.

Interestingly, in hippocampal studies, activity-dependent late-phase long-term potentiation (LTP) is marked by an increase in the de novo formation and maturation of dendritic spines, contributing to the maintenance of synaptic efficacy and an increase in postsynaptic excitability. In the context of nociception, LTP induction in the superficial spinal cord dorsal horn through exogenous electrical stimulation, ie, a tetanus, injury, or inflammation, can lead to central sensitization and pain.[11-13] Although these LTP-pain studies did not investigate changes in dendritic spines in the spinal cord, they support the premise that chronic pain and memory may share similar biological mechanisms.[14] As we and others have shown, more stable mushroom-shaped spine morphologies can have a greater impact on neuronal excitability as compared with thin-shaped spines.[15,16] Taken together, our in vivo dendritic spine study in the spinal cord supports the novel concept that the change in steady-state dynamics of dendritic spines in the nociceptive dorsal horn may represent the "locking-in" of clinically intractable neuropathic pain.

How to Develop a "Window" Into the Spinal Cord

To visualize dendritic spines in the spinal cord, we used 2-photon in vivo imaging. Although intravital imaging has been used to observe dendritic spine changes in the cortex, our study provided the first look into living dendritic spine changes within the spinal cord gray matter. The optimization of a window implantation method first pioneered by Fenrich et al[9] permitted us to visualize neurons located up to 250 microns below the dorsal spinal cord surface (lamina II). To maximize the imaging depth and quality, we custom developed and 3-dimensionally (3D) printed an animal-holder gimbal stage with 3 degrees of freedom in the x-, y-, and z-axis (Figure 1A). Two manual rotation mounts (PRM1 and PRM05, Thorlabs, Newton, New Jersey, USA) were built into the stage to allow fine adjustment of the animal-window position under the 2-photon optical lens. In an unpublished prototype, we developed a new gimbal stage with an integrated, automated, and motorized rotational mount (KPRM1E/M, Thorlabs) to permit fine adjustment throughout the imaging session without touching any part of the microscope or animal. With a gimbal stage–holder system, we have been able to precisely position the animal to optimize the linear path for laser excitation of reporter-expressing neurons in transgenic Thy1-YFP mice. To avoid movement artifacts in our images, our gimbal stage also dorsally suspends the animal with an implanted scaffold around the window, which minimizes dorsal-ventral thoracic deflections, eg, breathing, during an imaging session. The approach and results of using this window imaging platform are shown in Figure 1A and B.

Future Perspective

Two outstanding questions arose from our study.[5] First, although we observed altered steady-state spine dynamics associated with increased pain sensitivity, the trigger or cause for

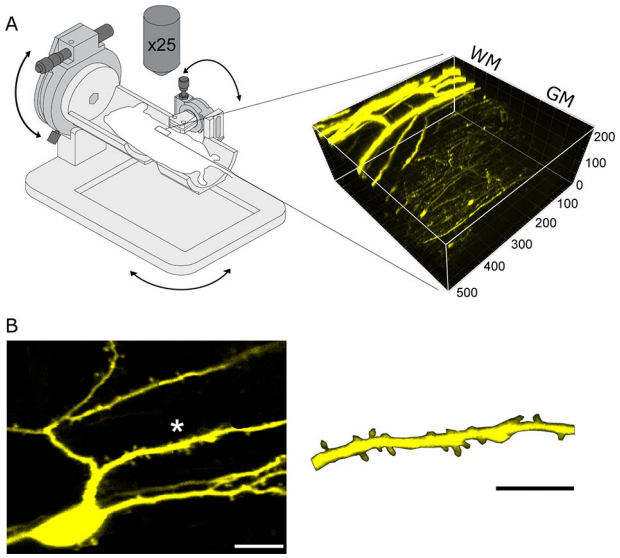

Figure 1. In vivo imaging of superficial spinal cord neurons: (A) Design of custom 3D printed gimbal imaging stage. Glass windows are implanted over the lumbar region of the surgically exposed spinal cord, which permits imaging of 3D volumes of the dorsal horn (WM; white matter, GM; gray matter). (B) Intravital captured image of a dorsal spinal cord neuron with dendritic spines. Adjacent panel (right) shows a magnified image of the dendritic branch region denoted by * (left). Scale bars in (B) are both 10 µm. Source: Images adapted from Benson et al.[5]

these changes following injury is not known. There are multiple factors that can influence dendritic spine plasticity, including inflammation, presynaptic terminal reorganization, and glial interactions.[17-19] Our intravital imaging platform provides a unique opportunity to investigate possible pathological mechanisms associated with dendritic spine and synaptic plasticity. For example, a specific advantage is the ability to visually image the same circuits or neurons before and after a drug intervention. As we have shown previously in postmortem histological investigations, pharmacological treatments that can attenuate pain also appear to modify dendritic spine structure within the dorsal horn.[2,20] Second, RNA expression studies in the dorsal spinal cord revealed multiple populations of excitatory and inhibitory neurons.[21] The Thy1-YFP reporter line we used in our study expresses the YFP protein randomly in dorsal horn neurons, which made it difficult for us to identify the specific type of cells we imaged. More research is needed to understand how different neuronal populations respond to injury or disease, and whether dendritic spine profiles differ across cell types.

To date, live-imaging studies of the spinal cord have largely ignored the structural changes of neurons and have instead focused on neuronal activity at the population level, eg, using calcium imaging. These highly informative studies have shown how mechanical and thermal stimuli are encoded in the dorsal horn.[22,23] Our study showed a switch in spine dynamics between 3 and 7 days after SNI, resulting in an increased number of mature mushroom-shaped spines in the dorsal horn. This descriptive profile is in agreement with our previous pain and dendritic spine studies and suggests that the affected circuit has entered a state of increased excitability (ie, development of central sensitization).

By combining our structure-based live-imaging approach with measures of neuronal activity, such as with the use of calcium or other voltage-sensitive dyes, we expect to gain a more complete picture of the relationship between dendritic spine structure and nociceptive physiology.

In summary, we demonstrated the powerful use of an intravital imaging platform for tracking the live dynamic changes of dendritic spines in the spinal cord. Imaging the same neurons over the course of a week before and after injury-induced pain revealed significant structural anomalies in the steady-state fluctuations of spines.[5] Our findings further support the concept of a "pain memory" in the spinal cord,[3,14,24] and that abnormal dendritic spine dynamics is an empirical correlate of hyperexcitability disorder. Intravital dendritic spine profiling in the spinal cord may be a useful structural bioassay for the study and treatment of disease and injury.

Author Contributions

CAB and AMT contributed to the original work. CAB, MLR and AMT wrote the first drafts of the paper. AMT edited the final copy.

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