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Beyond the Lamppost

Approaches to Answering the Questions of Interest

As goes the story of the man found on his hands and knees looking for his lost keys under the light of the lamppost, scientists are often accused of using one model system or another, not necessarily because it is the most appropriate to answer the question of interest, but because it is the easiest to use. This may be particularly true for the study of pain in which, for example, hypersensitivity to punctate mechanical stimuli is widely used as a measure of "neuropathic pain" in preclinical models of peripheral neuropathy because it is so robustly manifest in rodents despite the fact this form of hypersensitivity is not a primary, or even secondary, positive sign of neuropathic pain in patients.[1] So, given that pain associated with tissue damage, the most common source of pain, is generally due to neural activity initiated in the peripheral terminals of nociceptive afferents, it is reasonable to ask why one would want to study the cell body of the primary afferent to learn anything about the mechanisms underlying this neural activity. Gemes *et al.*[2] described a novel approach to study the cell body of primary afferents in the intact ganglia. Importantly, however, they have not developed this approach to learn anything about afferent terminals. Rather, they have described a powerful way to study signaling at the afferent cell body.

The need to study signaling in the afferent somata is based on an increasing body of evidence indicating that peripheral terminals are not the only source of activity leading to afferent input to the central nervous system. And although there is evidence for the emergence of activity arising from sites along an injured axon[3] or even along the central processes,[4] activity arising from within the sensory ganglia (dorsal and trigeminal root ganglia) contributes significantly to the total afferent input, particularly after traumatic nerve injury.[5,6] In fact, activity arising from within the sensory ganglia may be the primary source of activity for some types of injury such as those associated with disc compression.[7,8] Sympathetic–primary afferent coupling was the original focus of this activity.[9] However, subsequent data highlighted the contribution of resident[10] and recruited immune cells,[11,12] the activation of satellite cells,[13,14] release of transmitter from within the ganglia,[15] and even the emergence of mechanical and chemical sensitivity.[7,8] Cross-talk within the ganglia has been suggested to contribute to both the emergence of pain outside an area of injury[16] and wind-up phenomena commonly associated with trigeminal neuralgia.[17] Although activity in nonnociceptive afferents is thought to contribute to paresthesias and dysesthesias associated with nerve injury, activity in nociceptive afferents may contribute to ongoing pain. From a therapeutic perspective, minimally all this activity arising from within the ganglia may contribute to the difficulty in obtaining complete pain relief with a peripheral nerve block, particularly in the case of nerve injury.

Gemes *et al.* described a way to record simultaneously electrical activity and Ca^{2+} transients in sensory neuron somata in the intact ganglia. Even better, with spinal and peripheral nerves left intact as well, the authors were able to assess the effect of neural activity (*i.e.*, propagated action potential) on signaling within the ganglia. The ability to monitor Ca^{2+} will be a critical feature here, particularly in the context of cross-talk within the ganglia because of the importance of Ca^{2+} to transmitter release and the actions of so many receptor-mediated processes. Thus, the authors have described an approach that can be used to begin teasing apart the growing array of processes that may contribute to the emergence of activity from within the sensory ganglia.

The authors used their intact preparation to describe several phenomena, such as differences between C- and Aβ-fibers with respect to the magnitude of evoked Ca^{2+} transients and the effect of nerve injury on the magnitude of evoked Ca^{2+} transients in C-fibers that were largely consistent with results previously obtained in dissociated neurons.[18,19] However, more interesting and potentially more important is their observation that the Ca^{2+} transient in each neuron is determined by a distinct pattern of activity. Although action potential frequency was the only parameter manipulated, frequency was sufficient to differentiate neurons based on the level of activity associated with a maximal increase in intracellular Ca^{2+} (*i.e.*, at some point, that varied between neurons, higher frequencies of stimulation resulted in no greater, and in some cases, a decrease in the magnitude

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of the Ca^{2+} transient). In the context of previous data indicating that (1) afferent activity alone is sufficient to drive changes in gene expression[20] and (2) the pattern of activity can determine the pattern of gene expression,[21] the activity-dependent tuning described in this study has several important implications. First, it increases the possibility, as suggested, that the pattern (*i.e.*, doublets or short bursts) of activity may be even more critical for the "tuning" of the Ca^{2+} transient than frequency alone. This possibility begs for a more detailed parametric analysis of the activity–Ca^{2+} transient relationship in this intact preparation. Second, it opens a whole new avenue for investigation into the differential regulation of gene expression in specific subpopulations of afferents. This issue may be particularly important in light of evidence that specific subpopulations such as the "mechanically insensitive afferent," may play a particularly important role in chronic pain.[22] Third, the "pattern tuning" of afferents increases the intriguing possibility that it may be possible to manipulate the time course of a pain syndrome by manipulating the pattern of activity; that is, if the maintenance of a chronic pain syndrome is dependent on a particular pattern of gene expression which in turn is dependent on a particular pattern of activity, it may be possible to suppress the "problematic" pattern of gene expression with the appropriate pattern of activity. In some populations of afferents, an increase in activity may ultimately have the most beneficial long-term consequences (possibly accounting for some of the therapeutic efficacy of electroacupuncture). Minimally, disrupting activity-dependent patterns of gene expression may also explain the long-term effect of a peripheral nerve block that often far outlasts the duration of the block.[23]

Although Gemes *et al.* developed a powerful approach to the study processes in the intact ganglion and went on to highlight some of the limitations to the dissociated neuron, the authors correctly point out that as much as 99.8% of the volume of a primary afferent neuron is outside the soma.[24] And while signaling within the ganglia is appropriately an area of active investigation because, as noted earlier, the vast majority of pain we experience is due to activity that arises from sites outside the ganglia, it is still important to understand mechanisms underlying the sensitization and activation of nociceptive afferent terminals. The intact preparation, because it is intact, precludes the study of many of these processes, simply because they do not normally occur within the intact ganglia. The observation that nerve injury results in emergence of mechanical and thermal sensitivity at cut ends of the injured fibers[25–27] is evidence that molecules necessary for the sensitization and activation of nociceptive afferents are trafficked out of the ganglia. Furthermore, it is possible to take advantage of this fact by studying the cell body of acutely dissociated sensory neurons because dissociated neurons become responsive to the same stimuli capable of activating and sensitization afferent terminals *in vivo*, including mechanical,[28] thermal (both heat[29] and cooling[30]), and a wide array of chemical stimuli.[31] Thus, although studying the intact terminal would still be an ideal place to learn about pain arising from the periphery given unique anatomical constraints in association with unique distribution of ion channels[32] and other proteins and cellular structures, the dissociated cell body provides a unique window for these events.

What remains true in any scientific endeavor is that one should use the most appropriate tools available to answer the question of interest. Thanks to the work of Gemes *et al.*, we now have a powerful new tool to understand the neurobiology of primary afferents, in particular the effect of signaling within the ganglia. With luck, and the continued application of all the tools available, novel and more effective approaches for the treatment of pain may soon be at hand.

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