

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 axon³ 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 resident¹⁰ 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 injury¹⁶ and wind-up phenomena commonly associated with trigeminal neuralgia.¹⁷ Although activity in non-nociceptive 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 A β -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

Accepted for publication March 30, 2010. The author is not supported by, nor maintains any financial interest in, any commercial activity that may be associated with the topic of this article. Supported by the National Institutes of Health (Bethesda, Maryland).

◆ This Editorial View accompanies the following article: Gemes G, Rigaud M, Koopmeiners AS, Poroli MJ, Zoga V, Hogan QH: Calcium signaling in intact dorsal root ganglia: New observations and the effect of injury. ANESTHESIOLOGY 2010; 113:134–46.

of the Ca^{2+} transient). In the context of previous data indicating that (1) afferent activity alone is sufficient to drive changes in gene expression²⁰ 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 heat²⁹ and cooling³⁰), 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 channels³² 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.

Michael S. Gold, Ph.D., Departments of Anesthesiology and the Pittsburgh Center for Pain Research, University of Pittsburgh, Pittsburgh, Pennsylvania. msg22@pitt.edu.

References

1. Backonja MM, Stacey B: Neuropathic pain symptoms relative to overall pain rating. *J Pain* 2004; 5:491–7
2. Gemes G, Rigaud M, Koopmeiners AS, Poroli MJ, Zoga V, Hogan QH: Calcium signaling in intact dorsal root ganglia: New observations and the effect of injury. *ANESTHESIOLOGY* 2010; 113:134–46
3. Janig W, Grossmann L, Gorodetskaya N: Mechano- and thermosensitivity of regenerating cutaneous afferent nerve fibers. *Exp Brain Res* 2009; 196:101–14
4. Gu JG, MacDermott AB: Activation of ATP P2X receptors elicits glutamate release from sensory neuron synapses. *Nature* 1997; 389:749–53
5. Gold MS, Chessell I, Devor M, Dray A, Gereau RW, Kane S, Koltzenburg M, Louis J-C, Ringkamp M, Treede RD: Peripheral nervous system targets. Rapporteur report, Emerging Strategies for the Treatment of Neuropathic Pain. Edited by Campbell JN, Basbaum AI, Dray A, Dubner R, Dworkin RH, Sang CN. Seattle, IASP Press, 2006, pp 3–36
6. Sukhotinsky I, Ben-Dor E, Raber P, Devor M: Key role of the dorsal root ganglion in neuropathic tactile hypersensitivity. *Eur J Pain* 2004; 8:135–43
7. Hu SJ, Xing JL: An experimental model for chronic compression of dorsal root ganglion produced by intervertebral foramen stenosis in the rat. *Pain* 1998; 77:15–23
8. Ma C, LaMotte RH: Multiple sites for generation of ectopic spontaneous activity in neurons of the chronically compressed dorsal root ganglion. *J Neurosci* 2007; 27:14059–68
9. Michaelis M, Devor M, Janig W: Sympathetic modulation of activity in rat dorsal root ganglion neurons changes over time following peripheral nerve injury. *J Neurophysiol* 1996; 76:753–63
10. Ohtori S, Takahashi K, Moriya H, Myers RR: TNF-alpha and TNF-alpha receptor type I upregulation in glia and neurons after peripheral nerve injury: Studies in murine DRG and spinal cord. *Spine (Phila Pa 1976)* 2004; 29:1082–8
11. White FA, Sun J, Waters SM, Ma C, Ren D, Ripsch M, Stefflik J, Cortright DN, Lamotte RH, Miller RJ: Excitatory monocyte chemoattractant protein-1 signaling is up-regulated in sensory neurons after chronic compression of the dorsal root ganglion. *Proc Natl Acad Sci U S A* 2005; 102:14092–7
12. Peters CM, Jimenez-Andrade JM, Jonas BM, Sevcik MA, Koewler NJ, Ghilardi JR, Wong GY, Mantyh PW: Intravenous paclitaxel administration in the rat induces a peripheral sensory neuropathy characterized by macrophage infiltration and injury to sensory neurons and their supporting cells. *Exp Neurol* 2007; 203:42–54

13. Cherkas PS, Huang TY, Pannicke T, Tal M, Reichenbach A, Hanani M: The effects of axotomy on neurons and satellite glial cells in mouse trigeminal ganglion. *Pain* 2004; 110: 290-8
14. Vit JP, Jasmin L, Bhargava A, Ohara PT: Satellite glial cells in the trigeminal ganglion as a determinant of orofacial neuropathic pain. *Neuron Glia Biol* 2006; 2:247-57
15. Neubert JK, Maidment NT, Matsuka Y, Adelson DW, Kruger L, Spigelman I: Inflammation-induced changes in primary afferent-evoked release of substance P within trigeminal ganglia in vivo. *Brain Res* 2000; 871:181-91
16. Amir R, Devor M: Functional cross-excitation between afferent A- and C-neurons in dorsal root ganglia. *Neuroscience* 2000; 95:189-95
17. Devor M, Amir R, Rappaport ZH: Pathophysiology of trigeminal neuralgia: The ignition hypothesis. *Clin J Pain* 2002; 18:4-13
18. Lu SG, Zhang X, Gold MS: Intracellular calcium regulation among subpopulations of rat dorsal root ganglion neurons. *J Physiol* 2006; 577:169-90
19. Fuchs A, Rigaud M, Hogan QH: Painful nerve injury shortens the intracellular Ca^{2+} signal in axotomized sensory neurons of rats. *ANESTHESIOLOGY* 2007; 107:106-16
20. Klein JP, Tendi EA, Dib-Hajj SD, Fields RD, Waxman SG: Patterned electrical activity modulates sodium channel expression in sensory neurons. *J Neurosci Res* 2003; 74: 192-8
21. Fields RD, Lee PR, Cohen JE: Temporal integration of intracellular Ca^{2+} signaling networks in regulating gene expression by action potentials. *Cell Calcium* 2005; 37: 433-42
22. Ørstavik K, Weidner C, Schmidt R, Schmelz M, Hilliges M, Jorum E, Handwerker H, Torebjork E: Pathological C-fibres in patients with a chronic painful condition. *Brain* 2003; 126:567-78
23. Amir R, Argoff CE, Bennett GJ, Cummins TR, Durieux ME, Gerner P, Gold MS, Porreca F, Strichartz GR: The role of sodium channels in chronic inflammatory and neuropathic pain. *J Pain* 2006; 7:S1-29
24. Devor M: Unexplained peculiarities of the dorsal root ganglion. *Pain* 1999; (suppl 6):S27-35
25. Michaelis M, Blenk KH, Vogel C, Janig W: Distribution of sensory properties among axotomized cutaneous C-fibres in adult rats. *Neuroscience* 1999; 94:7-10
26. Michaelis M, Vogel C, Blenk KH, Janig W: Algesics excite axotomized afferent nerve fibres within the first hours following nerve transection in rats. *Pain* 1997; 72:347-54
27. Blenk KH, Michaelis M, Vogel C, Janig W: Thermosensitivity of acutely axotomized sensory nerve fibers. *J Neurophysiol* 1996; 76:743-52
28. McCarter GC, Reichling DB, Levine JD: Mechanical transduction by rat dorsal root ganglion neurons in vitro. *Neurosci Lett* 1999; 273:179-82
29. Cesare P, McNaughton P: A novel heat-activated current in nociceptive neurons and its sensitization by bradykinin. *Proc Natl Acad Sci U S A* 1996; 93:15435-9
30. Thut PD, Wrigley D, Gold MS: Cold transduction in rat trigeminal ganglia neurons *in vitro*. *Neuroscience* 2003; 119:1071-83
31. Gold MS: Ion channels: Recent advances and clinical applications, Proceedings of the 11th World Congress on Pain. Edited by Flor H, Kaslo E, Dostrovsky JO. Seattle, IASP Press, 2006, pp 73-92
32. Carr RW, Pianova S, Fernandez J, Fallon JB, Belmonte C, Brock JA: Effects of heating and cooling on nerve terminal impulses recorded from cold-sensitive receptors in the guinea-pig cornea. *J Gen Physiol* 2003; 121:427-39