

Noncoding RNAs

New Players in Chronic Pain

Brianna Marie Lutz, B.S., Alex Bekker, M.D., Ph.D., Yuan-Xiang Tao, M.D., Ph.D.

ABSTRACT

Chronic pain, a common clinical symptom, is often treated inadequately or ineffectively in part due to the incomplete understanding of molecular mechanisms that initiate and maintain this disorder. Newly identified noncoding RNAs govern gene expression. Recent studies have shown that peripheral noxious stimuli drive expressional changes in noncoding RNAs and that these changes are associated with pain hypersensitivity under chronic pain conditions. This review first presents current evidence for the peripheral inflammation/nerve injury–induced change in the expression of two types of noncoding RNAs, microRNAs, and Kcna2 antisense RNA, in pain-related regions, particularly in the dorsal root ganglion. The authors then discuss how peripheral noxious stimuli induce such changes. The authors finally explore potential mechanisms of how expressional changes in dorsal root ganglion microRNAs and Kcna2 antisense RNA contribute to the development and maintenance of chronic pain. An understanding of these mechanisms may propose novel therapeutic strategies for preventing and/or treating chronic pain. (**ANESTHESIOLOGY 2014; 121:409-17**)

CHRONIC pain usually caused by inflammation and tissue or nerve injury is a major public health problem worldwide. It is characterized by ongoing or intermittent burning pain, an enhanced response to noxious stimuli (hyperalgesia), and pain in response to normally innocuous stimuli (allodynia). Current treatment for this disorder has had restricted success due to our inadequate understanding of the mechanisms that lead to the initiation and maintenance of chronic pain. It is known that inflammation and nerve injury produce changes in the expression of receptors, enzymes, ion channels, neurotransmitters, neuromodulators, and structural proteins in primary sensory neurons of the dorsal root ganglion (DRG) at both transcriptional and translational levels.^{1–3} Such changes are considered to contribute to chronic pain development and maintenance.^{1–3} However, it is unclear how peripheral inflammation or nerve injury alters the expression of these genes and/or proteins in DRG. Understanding this mechanism may enable the development of new strategies to prevent and/or treat chronic pain.

Recent studies suggest that the mechanism for gene regulation involves widespread noncoding (nc) RNAs.^{4–6} RNA had long been thought to be a simple and intermediary component of gene expression, as it is transcribed from DNA and

then translated into proteins in cells. However, it has become increasingly clear that mammalian genomes encode not only protein-coding RNAs but also a vast number of ncRNA transcripts.⁷ Because the function of each newly identified ncRNA has not been fully elucidated, the common practice is to group ncRNA transcripts based on transcript size: small/short ncRNAs (*e.g.*, microRNAs [miRNAs]; 18 to 200 nucleotides) and long ncRNAs (*e.g.*, native Kcna2 antisense [AS] RNA; >200 nucleotides).^{7–9} ncRNAs have been systematically identified in the mammalian nervous system, including in pain-related regions.¹⁰ They can be regulated and may govern the expression of both protein-coding and noncoding genes. An intriguing association between aberrant expression of ncRNAs and the development of diseases has been demonstrated recently.^{11,12} Previous studies have shown that peripheral inflammation and nerve injury drive changes in the expression of some miRNAs and Kcna2 AS RNA in DRG.^{13–16} These changes might be responsible for inflammation/nerve injury–induced alterations of some pain-associated genes, an increase in DRG neuronal excitability, and behavioral pain hypersensitivity.^{14,16} The evidence indicates that ncRNAs might be new key players in the mechanisms that underlie the development and maintenance of chronic pain.

Submitted for publication December 19, 2013. Accepted for publication March 12, 2014. From the Department of Anesthesiology, Rutgers Graduate School of Biomedical Sciences (B.M.L.), Department of Anesthesiology (A.B.), and Departments of Anesthesiology, Cell Biology and Molecular Medicine, Pharmacology and Physiology, and Neurology and Neuroscience (Y.-X.T.), New Jersey Medical School, Rutgers, The State University of New Jersey, Newark, New Jersey.

Copyright © 2014, the American Society of Anesthesiologists, Inc. Lippincott Williams & Wilkins. Anesthesiology 2014; 121:409–17

In this article, we first review current evidence for the changes of two types of ncRNAs, miRNAs and Kcna2 AS RNA, in pain-related regions, particularly in DRG, after peripheral inflammation and nerve injury. We then discuss how peripheral noxious stimuli induce such changes. We finally explore potential mechanisms of how expressional changes in DRG miRNAs and Kcna2 AS RNA contribute to the development and maintenance of chronic pain. This review provides more up-to-date knowledge regarding the role of ncRNAs in the mechanisms of chronic pain.^{17,18}

miRNAs in Chronic Pain

Formation of miRNAs

Since the discovery of the first miRNA, *lin-4* in *Caenorhabditis elegans*, hundreds of miRNAs have been identified in the nervous system.^{19–21} These miRNAs are coded by specific genes. Generally, a miRNA molecule is synthesized from a long RNA primary transcript known as a pri-miRNA (fig. 1). In the cellular nucleus, pri-miRNA is cleaved by Droscha, an RNAIII endonuclease, to produce a characteristic stem-loop structure of approximately 60 to 70 nucleotides in length, known as pre-miRNA (fig. 1). After pre-miRNA is exported from the nucleus into the cytoplasm, it is cleaved by Dicer, another RNAIII endonuclease, to produce double-stranded mature miRNA (fig. 1). The latter is either unwound *via* an unknown helicase or cleaved by the enzyme Ago2 to lead to a single-stranded miRNA (fig. 1).²² The single strands

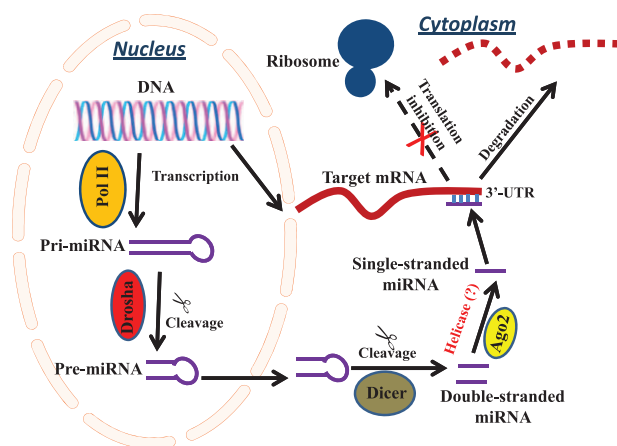


Fig. 1. Formation of mature microRNA (miRNA). miRNA is transcribed from the genome (DNA) *via* RNA polymerase II (Pol II). The resulting pri-miRNA transcript is then cleaved *via* the endonuclease Droscha to create a 60–70 nucleotide long pre-miRNA. This transcript is then removed from the nucleus *via* exportin-5 to the cytoplasm where it is cleaved by Dicer, another endonuclease. The resulting double-stranded mature miRNA is unwound by a helicase or cleaved by Ago2. The single-stranded mature miRNA then acts as the core of RNA-induced silencing complex. This complex guides the miRNA to its target sequence located within the 3'-untranslated region (3'-UTR) of the target messenger RNA (mRNA). Incomplete or complete base-pairing results in degradation of the mRNA or inhibition of translation.

completely or incompletely bind to specific messenger RNA (mRNA) sequences, resulting in degradation or translational repression of target mRNAs.²³ A recent link between miRNA-mediated poly(A)-tail length shortening and mRNA destabilization has been reported, suggesting another potential mechanism of ncRNA-mediated gene regulation.²⁴

Expressional Changes of miRNAs after Noxious Stimulation

Changes in the expression of miRNAs in response to noxious stimulation have been reported. Bai *et al.*¹³ reported a significant down-regulation of mature miR-10a, -29a, -98, -99a, -124a, -134, and -183 in the mandibular division of trigeminal ganglion ipsilateral to complete Freund's adjuvant (CFA)-injected rat masseter muscle in a model of peripheral inflammation (table 1). Such down-regulated miRNAs were observed 4 h after injection and recovered differentially to a normal level or higher than normal level.¹³ Expression and down-regulation of miRNAs occurred in all sizes of trigeminal ganglion neurons (but not in glial cells and other non-neuronal cells) that innervate the inflamed muscle although the miRNA signals varied among neurons (table 1).¹³ Injection of CFA into a hind paw also reduced expression levels of miR-1, -16, -206, and -143 in the ipsilateral DRG neurons,^{25,26} but increased miR-1, -16, and -206 levels in the ipsilateral spinal dorsal horn neurons (table 1).²⁵ These changes clearly correlate to CFA-induced peripheral inflammation. It should be noted that CFA-induced changes in miRNAs may be immune related as CFA also causes immune response. Interestingly, peripheral injection of formalin led to a significant down-regulation of miRNA-124a expression in the neurons of dorsal horn ipsilateral to injection (table 1).²⁷ These studies provide promising evidence of miRNA changes in pain-related regions under inflammatory conditions.

In addition to peripheral inflammation, expressional changes of miRNAs were observed after peripheral nerve injury. L5 spinal nerve ligation (SNL) induced a drastic decrease in the expression of miR-1, -7a, -96, -103, -182, -183, and -206 in the injured DRG^{14,25,28,29} and in the expression of miR-200b and -429 in the nucleus accumbens (table 2).³⁰ L5 SNL also down-regulated the expression of 59 miRNAs in the uninjured L4 DRG (table 2).³¹ Consistently, in the sciatic nerve transection or chronic constriction injury model of neuropathic pain, the injured DRG showed reduced expression of several miRNAs, including miR-10a, -30b, -99a, -100, -143, -582-3p, and -720 (table 2).^{26,32} In contrast, miR-21 in the injured DRG was up-regulated after L5 SNL (table 2).^{14,15} Although these changes in expression may not be ruled out to be related to regeneration, the evidence indicates that the expression of miRNAs is differentially and spatially regulated in pain-related regions after peripheral nerve injury.

Furthermore, expressional changes of miRNAs have also been observed in patients with painful diseases. In bladder

Table 1. miRNAs Associated with Peripheral Inflammation

Inflammatory Models	miRNAs	Change in Expression	Tissue	Target Gene	Reference
CFA into masseter muscle	miR-29a, -98, -99a, -124a, -134, -183	↓	Rat ipsilateral trigeminal ganglion	Unknown	Bai <i>et al.</i> ¹³
CFA into hind paw	miR-1, -16, -206	↓	Rat ipsilateral DRG neurons	Unknown	Kusuda <i>et al.</i> ²⁵
	miR-1, -16, -206	↑	Rat ipsilateral spinal dorsal horn neurons	Unknown	
Formalin injection	miR-143	↓	Murine ipsilateral DRG neurons	Unknown	Tam Tam <i>et al.</i> ²⁶
	miR-124a	↓	Murine ipsilateral dorsal horn neurons	MeCP2; proinflammatory marker genes	Kynast <i>et al.</i> ¹⁸
Bladder pain syndrome	miR-449b, -500, -328, -320; 31 miRNAs	↑ ↕	Human bladder biopsies	Neurokinin-1 Unknown	Sanchez <i>et al.</i> ³³
Complex regional pain syndrome	18 different miRNAs	↕	Human blood	Unknown	Orlova <i>et al.</i> ³⁴
Osteoarthritis	miR-199a, -558	↓	Human chondrocytes	Cyclooxygenase-2	Akhtar <i>et al.</i> ³⁵ ; Park <i>et al.</i> ³⁷
	miR-146a	↑	Human synoviocytes		Li <i>et al.</i> ³⁶
Peritonitis model of self-limiting acute inflammation	miR-21, -146b, -208a	↑	Murine exudates	Unknown	Recchiuti <i>et al.</i> ³⁸
	miR-302d	↓		Unknown	
	miR-142-3p	↓		IL-6, IL-23A, TGFBR1	
	miR-142-5p	↓		c-Fos, ATF2, SRF, CREB	
	miR-203	↑		STAT5, SOCS3, JAK1	
	miR-219	↓		TNF- α , TNF- α R, IL-1, IL-1R accessory protein	

ATF2 = activating transcription factor 2; CCI = chronic constriction injury; CFA = complete freund's adjuvant; CREB = cyclic adenosine monophosphate response-element binding protein; DRG = dorsal root ganglion; IL = interleukin; MeCP2 = methyl-CpG-binding protein 2; miR = microRNA; ncRNAs = noncoding RNAs; SOCS3 = suppressor of cytokine signaling-3; SRF = serum response factor; STAT5 = signal transducer and activator of transcription; TGFBR1 = transforming growth factor β receptor 1; TNF = tumor necrosis factor.

Table 2. Noncoding RNAs Associated with Peripheral Nerve Injury

Neuropathic Pain Models	ncRNAs	Change in Expression	Tissue	Target Gene	Reference
L5 spinal nerve ligation	miR-1, -7a, -96, -103, -182, -183, -206	↓	Injured DRG of mice	For miR-7a, β 2 subunit of voltage-gated sodium channels is a potential target	Sakai <i>et al.</i> ¹⁴ ; Kusuda <i>et al.</i> ²⁵ ; Favereaux <i>et al.</i> ²⁸ ; Aldrich <i>et al.</i> ²⁹
	miR-200b, -429	↓	Nucleus accumbens of mice	Unknown	Imai <i>et al.</i> ³⁰
	miR-21	↑	Injured DRG of mice	Matrix metalloproteinases; endogenous inhibitors of phosphatidylinositol 3-kinase; negative regulators of extracellular signal-regulated kinase	Sakai <i>et al.</i> ¹⁴ ; Sakai and Suzuki ¹⁵
	59 miRNAs	↓	Uninjured L4 DRG in mice	Unknown	von Schack <i>et al.</i> ³¹
	Kcna2 AS RNA	↑	Injured DRG of mice	Kcna2	Zhao <i>et al.</i> ¹⁶
Sciatic nerve transection/CCI	miR-10a, -30b, -99a, -100, -143, -582-3p, -720	↓	Injured DRG of rat	Unknown	Tam Tam <i>et al.</i> ²⁶ ; Brandenburger <i>et al.</i> ³²

CCI = chronic constriction injury; DRG = dorsal root ganglion; miR = microRNA; ncRNAs = noncoding RNAs.

biopsies from patients with bladder pain syndrome (also known as interstitial cystitis), 31 miRNAs were differentially expressed (table 1).³³ An inverse relation was observed in which neurokinin-1 mRNA/protein was down-regulated and four miRNAs (miR-449b, -500, -328, and -320) were up-regulated.³³ Differential expression of 18 miRNAs was

reported in blood from patients with complex regional pain syndrome (table 1).³⁴ In addition, miR-146a, -199a, and -558 may be linked to pain-related pathophysiology of osteoarthritis through regulation of the expression of cyclooxygenase-2 (table 1).³⁵⁻³⁷ It seems that miRNA profiles have the potential to serve as biomarkers of pain.

miRNAs Regulated by Inflammatory Mediators in Chronic Pain

How peripheral noxious stimulation causes the alternations of miRNA expression in pain-related regions is incompletely understood, but it is very likely that miRNA expression may be controlled by inflammatory mediators (fig. 2). Administration of resolvin D1, an anti-inflammatory lipid mediator, counter-regulated the expression of miR-21, -142, -146b, -203, -208a, -219, and -302d in a murine peritonitis model of self-limiting acute inflammation (table 1),³⁸ suggesting at least partial involvement of inflammatory mediators in inflammation-induced changes in miRNA expression. A recent study revealed that stimulation with interleukin (IL)-1 β , an inflammatory mediator, produced a significant reduction in miR-558 in normal and osteoarthritis chondrocytes possibly through IL-1 β -induced activation of mitogen-activated protein kinase and nuclear factor- κ B (table 1).³⁷ IL-1 β also increased the expression of miR-21 in DRG neurons,¹⁵ the insulinoma cell line MIN6,³⁹ and human pancreatic islets.³⁹ Activator protein 1 (AP-1), a transcription factor, may participate in this effect of IL-1 β as the promoter region of miR-21 contains the binding site of AP-1,⁴⁰ and IL-1 β triggers AP-1 activation in DRG neurons.¹⁵ Given that peripheral inflammation and nerve injury increase DRG IL-1 β expression, IL-1 β may be responsible for inflammation-induced down-regulation of miR-558 and nerve injury-induced up-regulation of miR-21 in the injured DRG (fig. 2).^{15,37}

Potential Mechanisms of miRNAs' Effects on Chronic Pain

It has been demonstrated that miRNAs exert their functions through their complete or incomplete sequence homology to the 3'-untranslated region of target mRNAs, resulting in a block in translation or mRNA degradation (fig. 1).²³ Studies on inflammatory pain suggest that miRNAs specifically target pain-related genes (fig. 2). When a miRNA-124a

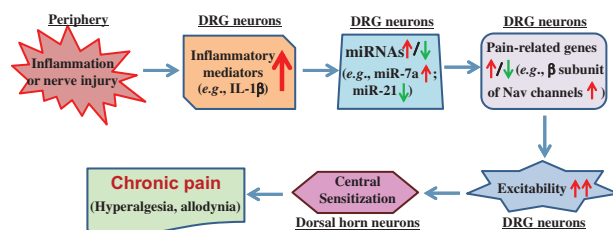


Fig. 2. Proposed model for the mechanism by which microRNAs (miRNAs) contribute to chronic inflammatory and neuropathic pain. After peripheral inflammation or nerve injury, the increase in inflammatory mediators such as interleukin (IL)-1 β causes a change in the expression of miRNAs in dorsal root ganglion (DRG) neurons. This change includes up-regulation of some miRNAs (e.g., miR-7a) and down-regulation of other miRNAs (e.g., miR-21), resulting in an alteration in pain-related genes, such as an increase in β -subunit of voltage-gated sodium channels (Nav), in DRG. Such an alteration leads to an increase in DRG neuronal excitability, spinal central sensitization, and pain hypersensitivity (hyperalgesia and allodynia).

inhibitor was intravenously administered after formalin injection, the down-regulation of miR-124a in the spinal cord was enhanced. This resulted in exaggerated formalin-induced nociceptive behaviors associated with an up-regulation of the pain-relevant miRNA-124a target methyl CpG-binding protein 2 and proinflammatory marker genes in the spinal cord.²⁷ In contrast, blocking formalin-induced down-regulation of spinal cord miRNA-124a through pre-miRNA-124a administration counteracted these effects and reduced nociception by down-regulating these target genes.²⁷ miRNA-181a possesses multiple complementary binding sites for the γ -aminobutyric acid (GABA)_A receptor subunit GABA_{A α -1} gene, *GABRA1*, suggesting a possible target for this miRNA. A neonatally zymosan-induced increase in miR-181a resulted in down-regulation of the GABA_{A α -1} mRNA and protein in the spinal cord.⁴¹ This effect may contribute to neonatal cystitis-induced chronic visceral pain.⁴¹ Identification of the target genes of miRNAs with specific changes in chronic pain may provide insight into the role of miRNAs in chronic pain development and maintenance.

The importance of miRNAs in pain is further validated in a study in which the activity of Dicer, a key enzyme in mature miRNA formation (fig. 1), is eliminated.²³ Conditional knockout of Dicer in DRG Nav1.8 neurons resulted in not only the loss of all mature miRNAs but also the reduced pain-related transcripts including voltage sodium channel (Nav) 1.7, Nav1.8, and Ca²⁺/calmodulin-dependent protein kinase II in the primary sensory neurons.⁴² The conditional null mice failed to display inflammatory mediator-induced enhancement in excitability of Nav1.8 sensory neurons and formalin-induced c-FOS expression in spinal cord.⁴² These mice also exhibited significant inhibition of inflammatory pain after formalin, CFA, and carrageenan injection.⁴² In contrast, Dicer null mice displayed an intact acute nociceptive behavior in response to electrical, mechanical, and thermal stimuli,⁴² indicating that the loss of mature miRNAs in the nociceptors does not affect acute pain transmission to the spinal cord and brain. Therefore, miRNAs may be potential targets for the prevention and/or treatment of chronic inflammatory pain.

The functional role of miRNAs in neuropathic pain has also been observed (fig. 2). Although Dicer null mice exhibited intact SNL-induced pain hypersensitivity,⁴² the role of miRNAs in neuropathic pain cannot be ruled out as deletion of DRG Nav1.8 or most DRG nociceptors had no effect on neuropathic pain.^{43–45} Moreover, nerve injury-induced increases in abnormal ectopic discharges were found primarily in injured myelinated afferents and the corresponding large and medium DRG neurons.^{46,47} Thus, miRNAs expressed in large and medium DRG neurons may be involved in the production of abnormal spontaneous activity and neuropathic pain initiation. Indeed, miR-7a is expressed in small, medium, and large DRG neurons and robustly decreased in the injured DRG in the late phase of neuropathic pain¹⁴ (table 2). Blocking this decrease through

miR-7a overexpression in the injured DRG suppressed up-regulation of the $\beta 2$ subunit protein of voltage-gated sodium channels in the DRG, normalized long-lasting hyperexcitability in nociceptive neurons, and attenuated established neuropathic pain without affecting acute pain and inflammatory pain.¹⁴ Furthermore, mimicking nerve injury-induced down-regulation of DRG miR-7a through intrathecal administration of a specific miR-7a inhibitor increased $\beta 2$ subunit protein levels in the DRG and led to pain-related behaviors in intact rats.¹⁴ Another miRNA, miR-21, is persistently up-regulated in the injured DRG neurons during the late phase of neuropathic pain¹⁵ (table 2). The intrathecal administration of a miR-21 inhibitor (a single-stranded RNA with chemical modifications) alleviated nerve injury-induced mechanical and thermal hyperalgesia.¹⁵ miR-21 may participate in neuropathic pain conditions by down-regulating multiple targets including negative regulators of matrix metalloproteinases (which exhibit increased activity after nerve injury),⁴⁸ an endogenous inhibitor of phosphatidylinositol 3-kinase (that is decreased after nerve injury),⁴⁹ and negative regulators of extracellular signal-regulated kinase.⁵⁰ miRNAs may also be therapeutic targets for intractable chronic neuropathic pain.

Taken together, it is very likely that inflammatory mediators produced by peripheral inflammation or nerve injury act on peripheral nociceptors and then change the expression of DRG miRNAs. These changes may alter pain-related gene expression and lead to an increase in neuronal excitability in DRG, resulting in spinal cord central sensitization and pain hypersensitivity in response to peripheral stimulation (fig. 2).

Native *Kcna2* AS RNA in Chronic Neuropathic Pain

Identification of Native *Kcna2* AS RNA and Its Expression in DRG

Long ncRNAs include AS RNA, double-stranded RNA, and long RNA species. Unlike the study of miRNAs, the study of long ncRNAs is still in its infancy. Although long ncRNAs may be implicated in gene-regulatory roles such as chromosome dosage compensation, imprinting, epigenetic regulation, cell cycle control, nuclear and cytoplasmic trafficking, transcription, translation, splicing, and cell differentiation,^{7,51,52} most long ncRNAs remain uncharacterized and their biological significance underestimated.^{7,52,53} We recently identified a new native RNA that is 2.52 kb in size and contains no apparent open reading frame,^{16,54} indicating that it is a long ncRNA. We named it *Kcna2* AS RNA because most of its sequence is complementary to the voltage-gated K^+ channel *Kcna2* RNA (also known as *Kv1.2* RNA). This AS RNA seems to be transcribed from the opposing DNA strands of the *Kcna2* RNA gene at the same genomic locus.

Under normal conditions, *Kcna2* AS RNA was expressed in pain-related areas, including DRG, from rats, although the signals were weak. It is also observed in DRGs from mouse, monkey, and human specimens.¹⁶ Using *in situ*

hybridization histochemistry, we found that *Kcna2* AS RNA was detected exclusively in DRG neurons. Approximately one fifth of neurons are labeled in the DRG of normal rats. Most are medium-sized although some are small and a few are large.¹⁶ Consistent with this subpopulation distribution pattern, the double-labeling observations showed that the majority of *Kcna2* AS RNA-labeled neurons are positive for neurofilament-200 protein, a marker for myelinated A-fibers and large and medium DRG neurons. Some were positive for P2X3/isolectin B4, the markers for small DRG nonpeptidergic neurons, or for calcitonin gene-related peptide, a marker for small DRG peptidergic neurons. Compared with *Kcna2* AS RNA, *Kcna2* mRNA and protein are highly expressed in DRG. Approximately 70% of the DRG neurons were positive for *Kcna2* protein.^{16,54,55} Most of these positive neurons were large in size.^{16,54,55} Double labeling of *Kcna2* AS RNA with *Kcna2* protein showed a tiny overlap between them.¹⁶ It seems that *Kcna2* AS RNA and *Kcna2* protein have opposing expression and distinct subpopulation distribution in normal DRG neurons.

Myeloid Zinc Finger Gene 1-Mediated Increase of *Kcna2* AS RNA after Nerve Injury

The data from our laboratory^{16,54} and those of others^{55–60} revealed that peripheral nerve injury time-dependently down-regulated *Kcna2* mRNA and protein in the injured DRG. In contrast, the level of *Kcna2* AS RNA was time-dependently increased in the injured DRG after peripheral nerve injury (fig. 3).^{16,54} Such an increase occurred predominantly in large DRG neurons. No changes in the amount of *Kcna2* AS RNA were observed in intact DRG, spinal cord, and other pain-related brain regions. Furthermore, using single-cell

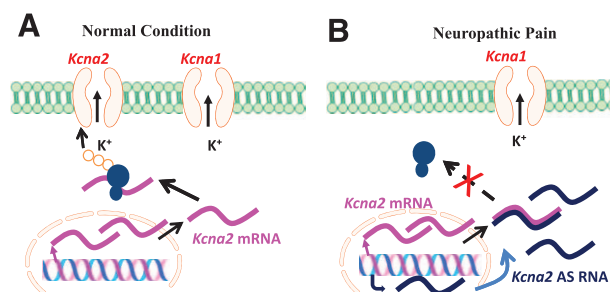


Fig. 3. *Kcna2* antisense (AS) RNA up-regulation specifically and selectively targets *Kcna2* expression in neuropathic pain. (A) Under normal conditions, due to highly low expression of *Kcna2* AS RNA, *Kcna2* messenger RNA (mRNA) that is transcribed from the genome is translated into protein, resulting in the expression of the *Kcna2* channel at the cell membrane. (B) Under neuropathic pain conditions, peripheral nerve injury promotes the expression of *Kcna2* AS RNA that is transcribed from the opposing strand of the *Kcna2* gene. Increased expression of *Kcna2* AS RNA specifically and selectively inhibits expression of *Kcna2* mRNA via extensive overlap of their complementary regions, including the transcription and translation inhibition sites, leading to reduced expression levels of the membrane *Kcna2* channel only, not other *Kcna* family members (e.g., *Kcna1*).

quantitative reverse-transcription polymerase chain reaction, we demonstrated that the ratios of *Kcna2* mRNA to *Kcna2* AS RNA were decreased, particularly in individual medium and large DRG neurons after SNL (fig. 3).¹⁶ These results indicate that expression of *Kcna2* AS RNA, like that of miRNAs, can be induced in the injured DRG after peripheral nerve injury (table 2).

Nerve injury-induced up-regulation of *Kcna2* AS RNA is triggered by myeloid zinc finger gene 1 (MZF1), a transcription factor belonging to the family of zinc finger proteins. The *Kcna2* AS gene promoter contains the consensus MZF1-binding motif. Once bound to this motif, MZF1 promotes transcription of target genes.^{61,62} We found that MZF1 binds to this motif on the *Kcna2* AS gene promoter in the DRG,¹⁶ and SNL time-dependently increases MZF1 expression and its binding activity in the injured DRG.¹⁶ Moreover, MZF1 directly promotes *Kcna2* AS gene transcription and is coexpressed with *Kcna2* AS RNA in DRG neurons.¹⁶ It is very likely that nerve injury-induced up-regulation of DRG *Kcna2* AS RNA occurs specifically in response to the increased MZF1. It is worth noting that the increase in *Kcna2* AS RNA might be induced by other transcription factors and/or caused by increases in RNA stability and other epigenetic modifications. These possibilities will be addressed in future studies.

Kcna2 RNA Specifically and Selectively Targeted by *Kcna2* AS RNA

Nerve injury-induced opposing changes in the expression of *Kcna2* AS RNA and *Kcna2* mRNA/protein in individual DRG neurons suggest that the increased *Kcna2* AS RNA may be responsible for the decreased *Kcna2* mRNA and protein under neuropathic pain conditions (fig. 3). Consistent with this speculation, overexpression of full-length *Kcna2* AS RNA in cultured HEK-293T cells or in cultured DRG neurons markedly knocked down *Kcna2* mRNA, but not *Kcna1* mRNA, *Kcna4* mRNA, *Scn10a* (*Nav1.8*), and their proteins.¹⁶ In *in vivo* experiments, *Kcna2* AS RNA overexpression time-dependently reduced *Kcna2* mRNA in the DRG.¹⁶ No changes were observed in the expression of *Kcna1*, *Kcna4*, and *Scn10a* at the levels of mRNA and protein in the DRGs injected with AAV-*Kcna2* AS RNA.¹⁶ These results suggest that nerve injury-induced DRG *Kcna2* down-regulation is likely caused by a nerve injury-induced increase in DRG *Kcna2* AS RNA (fig. 3). *Kcna2* AS RNA functions as a biologically active regulator of *Kcna2* mRNA and specifically and selectively targets *Kcna2* in primary sensory neurons in neuropathic pain. This effect may be related to the extensive overlap of their complementary regions, including the transcription and translation initiation sites (fig. 3).¹⁶

DRG *Kcna2* AS RNA as a Trigger in Neuropathic Pain Genesis

Although the detailed mechanisms by which nerve injury leads to neuropathic pain are still elusive, it is generally believed that neuropathic pain is induced by abnormal spontaneous

activity that arises in neuromas and the medium and large DRG cell bodies.¹⁻³ Voltage-dependent potassium channels (Kv) govern cell excitability. Application of Kv antagonists to sensory axons and to sites of ectopic afferent discharge facilitates ectopic firing.⁶³⁻⁶⁶ Injection of these antagonists into nerve-end neuromas provokes intense pain in humans.⁶⁷ We found that selective reduction of *Kcna2* expression in DRG by *Kcna2* AS RNA decreased total Kv current, depolarized the resting membrane potential, decreased current threshold for activation of action potentials, increased the number of action potentials in large and medium DRG neurons, and produced neuropathic pain symptoms.¹⁶ Rescuing nerve injury-induced down-regulation of DRG *Kcna2* by blocking nerve injury-induced up-regulation of DRG *Kcna2* AS RNA attenuated induction and maintenance of nerve injury-induced mechanical, cold, and heat pain hypersensitivities.¹⁶

Given that nociceptive neurotransmitters and/or modulators (substance P and calcitonin gene-related peptide) in the injured myelinated fibers and in large and medium DRG neurons are dramatically increased at the early stage after nerve injury,^{68,69} it is conceivable that peripheral nerve injury up-regulates the expression of native *Kcna2* AS RNA through activation of the MZF1 transcription factor in the injured DRG. This up-regulation silences the expression of DRG *Kcna2* mRNA and protein, resulting in a decrease of total Kv current and an increase of ectopic discharge in large and medium DRG neurons. Ectopic discharge triggers the release of nociceptive transmitters and/or modulators in primary afferent terminals, leading to central sensitization in the dorsal horn and major symptoms of neuropathic pain (fig. 4). Thus, *Kcna2* AS RNA may be an endogenous trigger in neuropathic pain development and maintenance. *Kcna2* AS RNA may be a potential target for the prevention and/or treatment of neuropathic pain.

Conclusion

The lines of evidence described above indicate that ncRNAs including miRNAs and *Kcna2* AS RNA in peripheral and central nervous systems are endogenous instigators of chronic

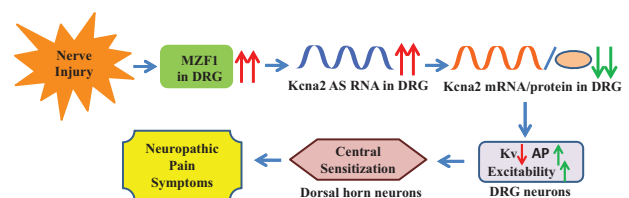


Fig. 4. Proposed model for the mechanism of how *Kcna2* AS RNA is involved in neuropathic pain. Nerve injury leads to an increase in myeloid zinc finger gene 1 (MZF1), a transcription factor that enhances the transcription of *Kcna2* AS RNA, in dorsal root ganglion (DRG). The *Kcna2* AS RNA silences expression of the *Kcna2* messenger RNA (mRNA) and protein. The reduced *Kcna2* protein expression at DRG neuronal membrane results in reduced K⁺ current (Kv), increases number of action potentials (AP) and neuronal excitability in DRG neurons, and produces spinal cord central sensitization and neuropathic pain symptoms (hyperalgesia and allodynia).

pain. miRNAs have been extensively studied in the past decade and may be used as prognostic and diagnostic biomarkers and potential new drug targets for chronic inflammatory pain and neuropathic pain^{17,18}; however, miRNAs have multiple and specific downstream targets due to their small size.^{48–50} This characterization may result in the limited use of miRNAs in chronic pain treatment because they might interfere with other physiological functions and produce potential side effects. Compared with the previous reviews on miRNAs in pain processing,^{17,18} this review updates current knowledge on miRNAs in chronic pain. More importantly, this review summarizes the latest finding on a long ncRNA *Kcna2* AS RNA in chronic pain,^{16,54} which has not been discussed in previous reviews.^{17,18} Although the studies on long ncRNAs are still at the early stage, accumulating evidence indicates that they specifically and selectively target their corresponding gene's expression.^{16,54} As peripheral inflammation and nerve injury alter the expression of many other genes in addition to *Kcna2* in pain-related regions,^{1–3} it is very likely that those genes, like *Kcna2*, are regulated by a corresponding long ncRNAs. Significant regulations of long ncRNA transcription may be a general cellular response to peripheral inflammation and nerve injury and participate in the induction and maintenance of chronic pain. Given that long ncRNAs have the characterization of specifically and selectively targeting the corresponding genes, it is conceivable that the significance of long ncRNAs in chronic pain will become even more apparent in the coming years.

Acknowledgments

This work was supported by grants from the National Institutes of Health, Bethesda, Maryland (grant nos. NS072206, HL117684, and DA033390), and the Rita Allen Foundation, Princeton, New Jersey.

Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Tao: Department of Anesthesiology, New Jersey Medical School, Rutgers, The State University of New Jersey, 185 South Orange Avenue, MSB, F-548, Newark, New Jersey 07103. yuanyang.tao@njms.rutgers.edu. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY'S articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

References

- Campbell JN, Meyer RA: Mechanisms of neuropathic pain. *Neuron* 2006; 52:77–92
- Latremoliere A, Woolf CJ: Central sensitization: A generator of pain hypersensitivity by central neural plasticity. *J Pain* 2009; 10:895–26
- Wang W, Gu J, Li YQ, Tao YX: Are voltage-gated sodium channels on the dorsal root ganglion involved in the development of neuropathic pain? *Mol Pain* 2011; 7:16
- Novina CD, Sharp PA: The RNAi revolution. *Nature* 2004; 430:161–4
- Hannon GJ: RNA interference. *Nature* 2002; 418:244–51
- Willingham AT, Gingeras TR: TUF love for “junk” DNA. *Cell* 2006; 125:1215–20
- Wapinski O, Chang HY: Long noncoding RNAs and human disease. *Trends Cell Biol* 2011; 21:354–61
- Gibb EA, Brown CJ, Lam WL: The functional role of long non-coding RNA in human carcinomas. *Mol Cancer* 2011; 10:38
- Ørom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, Bussotti G, Lai F, Zytnicki M, Notredame C, Huang Q, Guigo R, Shiekhattar R: Long noncoding RNAs with enhancer-like function in human cells. *Cell* 2010; 143:46–58
- Rosok Ø, Sioud M: Systematic identification of sense-antisense transcripts in mammalian cells. *Nat Biotechnol* 2004; 22:104–8
- Fabbri M, Calin GA: Epigenetics and miRNAs in human cancer. *Adv Genet* 2010; 70:87–99
- Farazi TA, Spitzer JI, Morozov P, Tuschl T: miRNAs in human cancer. *J Pathol* 2011; 223:102–15
- Bai G, Ambalavanar R, Wei D, Dessem D: Downregulation of selective microRNAs in trigeminal ganglion neurons following inflammatory muscle pain. *Mol Pain* 2007; 3:15
- Sakai A, Saitow F, Miyake N, Miyake K, Shimada T, Suzuki H: miR-7a alleviates the maintenance of neuropathic pain through regulation of neuronal excitability. *Brain* 2013; 136(Pt 9):2738–50
- Sakai A, Suzuki H: Nerve injury-induced upregulation of miR-21 in the primary sensory neurons contributes to neuropathic pain in rats. *Biochem Biophys Res Commun* 2013; 435:176–81
- Zhao X, Tang Z, Zhang H, Atianjoh FE, Zhao JY, Liang L, Wang W, Guan X, Kao SC, Tiwari V, Gao YJ, Hoffman PN, Cui H, Li M, Dong X, Tao YX: A long noncoding RNA contributes to neuropathic pain by silencing *Kcna2* in primary afferent neurons. *Nat Neurosci* 2013; 16:1024–31
- Kress M, Hüttenhofer A, Landry M, Kuner R, Favereaux A, Greenberg D, Bednarik J, Heppenstall P, Kronenberg F, Malcangio M, Rittner H, Uçeyler N, Trajanoski Z, Mouritzen P, Birklein F, Sommer C, Soreq H: microRNAs in nociceptive circuits as predictors of future clinical applications. *Front Mol Neurosci* 2013; 6:33
- Kynast KL, Russe OQ, Geisslinger G, Niederberger E: Novel findings in pain processing pathways: Implications for miRNAs as future therapeutic targets. *Expert Rev Neurother* 2013; 13:515–25
- Farh KK, Grimson A, Jan C, Lewis BP, Johnston WK, Lim LP, Burge CB, Bartel DP: The widespread impact of mammalian MicroRNAs on mRNA repression and evolution. *Science* 2005; 310:1817–21
- Kim J, Krichevsky A, Grad Y, Hayes GD, Kosik KS, Church GM, Ruvkun G: Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. *Proc Natl Acad Sci U S A* 2004; 101:360–5
- Wheeler G, Ntounia-Fousara S, Granda B, Rathjen T, Dalmay T: Identification of new central nervous system specific mouse microRNAs. *FEBS Lett* 2006; 580:2195–200
- Muljo SA, Kanellopoulou C, Aravind L: MicroRNA targeting in mammalian genomes: Genes and mechanisms. *Wiley Interdiscip Rev Syst Biol Med* 2010; 2:148–61
- Bartel DP: MicroRNAs: Target recognition and regulatory functions. *Cell* 2009; 136:215–33
- Subtelny AO, Eichhorn SW, Chen GR, Sive H, Bartel DP: Poly(A)-tail profiling reveals an embryonic switch in translational control. *Nature* 2014; 508:66–71
- Kusuda R, Cadetti F, Ravanelli MI, Sousa TA, Zanon S, De Lucca FL, Lucas G: Differential expression of microRNAs in mouse pain models. *Mol Pain* 2011; 7:17

26. Tam Tam S, Bastian I, Zhou XF, Vander Hoek M, Michael MZ, Gibbins IL, Haberberger RV: MicroRNA-143 expression in dorsal root ganglion neurons. *Cell Tissue Res* 2011; 346:163–73
27. Kynast KL, Russe OQ, Möser CV, Geisslinger G, Niederberger E: Modulation of central nervous system-specific microRNA-124a alters the inflammatory response in the formalin test in mice. *Pain* 2013; 154:368–76
28. Favereaux A, Thoumine O, Bouali-Benazzouz R, Roques V, Papon MA, Salam SA, Drutel G, Léger C, Calas A, Nagy F, Landry M: Bidirectional integrative regulation of Cav1.2 calcium channel by microRNA miR-103: Role in pain. *EMBO J* 2011; 30:3830–41
29. Aldrich BT, Frakes EP, Kasuya J, Hammond DL, Kitamoto T: Changes in expression of sensory organ-specific microRNAs in rat dorsal root ganglia in association with mechanical hypersensitivity induced by spinal nerve ligation. *Neuroscience* 2009; 164:711–23
30. Imai S, Saeki M, Yanase M, Horiuchi H, Abe M, Narita M, Kuzumaki N, Suzuki T, Narita M: Change in microRNAs associated with neuronal adaptive responses in the nucleus accumbens under neuropathic pain. *J Neurosci* 2011; 31:15294–9
31. von Schack D, Agostino MJ, Murray BS, Li Y, Reddy PS, Chen J, Choe SE, Strassle BW, Li C, Bates B, Zhang L, Hu H, Kotnis S, Bingham B, Liu W, Whiteside GT, Samad TA, Kennedy JD, Ajit SK: Dynamic changes in the microRNA expression profile reveal multiple regulatory mechanisms in the spinal nerve ligation model of neuropathic pain. *PLoS One* 2011; 6:e17670
32. Brandenburger T, Castoldi M, Brendel M, Grievink H, Schlösser L, Werdehausen R, Bauer I, Hermanns H: Expression of spinal cord microRNAs in a rat model of chronic neuropathic pain. *Neurosci Lett* 2012; 506:281–6
33. Sanchez FV, Burkhard FC, Kessler TM, Kuhn A, Draeger A, Monastyrskaya K: MicroRNAs may mediate the down-regulation of neurokinin-1 receptor in chronic bladder pain syndrome. *Am J Pathol* 2010; 176:288–303
34. Orlova IA, Alexander GM, Qureshi RA, Sacan A, Graziano A, Barrett JE, Schwartzman RJ, Ajit SK: MicroRNA modulation in complex regional pain syndrome. *J Transl Med* 2011; 9:195
35. Akhtar N, Haqqi TM: MicroRNA-199a* regulates the expression of cyclooxygenase-2 in human chondrocytes. *Ann Rheum Dis* 2012; 71:1073–80
36. Li X, Gibson G, Kim JS, Kroin J, Xu S, van Wijnen AJ, Im HJ: MicroRNA-146a is linked to pain-related pathophysiology of osteoarthritis. *Gene* 2011; 480:34–41
37. Park SJ, Cheon EJ, Kim HA: MicroRNA-558 regulates the expression of cyclooxygenase-2 and IL-1 β -induced catabolic effects in human articular chondrocytes. *Osteoarthritis Cartilage* 2013; 21:981–9
38. Recchiuti A, Krishnamoorthy S, Fredman G, Chiang N, Serhan CN: MicroRNAs in resolution of acute inflammation: Identification of novel resolvin D1-miRNA circuits. *FASEB J* 2011; 25:544–60
39. Roggli E, Britan A, Gattesco S, Lin-Marq N, Abderrahmani A, Meda P, Regazzi R: Involvement of microRNAs in the cytotoxic effects exerted by proinflammatory cytokines on pancreatic beta-cells. *Diabetes* 2010; 59:978–86
40. Fujita S, Ito T, Mizutani T, Minoguchi S, Yamamichi N, Sakurai K, Iba H: miR-21 Gene expression triggered by AP-1 is sustained through a double-negative feedback mechanism. *J Mol Biol* 2008; 378:492–504
41. Sengupta JN, Pochiraju S, Pochiraju S, Kannampalli P, Bruckert M, Addya S, Yadav P, Miranda A, Shaker R, Banerjee B: MicroRNA-mediated GABA A α -1 receptor subunit down-regulation in adult spinal cord following neonatal cystitis-induced chronic visceral pain in rats. *Pain* 2013; 154:59–70
42. Zhao J, Lee MC, Momin A, Cendan CM, Shepherd ST, Baker MD, Asante C, Bee L, Bethry A, Perkins JR, Nassar MA, Abrahamsen B, Dickenson A, Cobb BS, Merckenschlager M, Wood JN: Small RNAs control sodium channel expression, nociceptor excitability, and pain thresholds. *J Neurosci* 2010; 30:10860–71
43. Abrahamsen B, Zhao J, Asante CO, Cendan CM, Marsh S, Martinez-Barbera JP, Nassar MA, Dickenson AH, Wood JN: The cell and molecular basis of mechanical, cold, and inflammatory pain. *Science* 2008; 321:702–5
44. Akopian AN, Souslova V, England S, Okuse K, Ogata N, Ure J, Smith A, Kerr BJ, McMahon SB, Boyce S, Hill R, Stanfa LC, Dickenson AH, Wood JN: The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways. *Nat Neurosci* 1999; 2:541–8
45. Kerr BJ, Souslova V, McMahon SB, Wood JN: A role for the TTX-resistant sodium channel Nav 1.8 in NGF-induced hyperalgesia, but not neuropathic pain. *Neuroreport* 2001; 12:3077–80
46. Liu CN, Wall PD, Ben-Dor E, Michaelis M, Amir R, Devor M: Tactile allodynia in the absence of C-fiber activation: Altered firing properties of DRG neurons following spinal nerve injury. *Pain* 2000; 85:503–21
47. Tal M, Wall PD, Devor M: Myelinated afferent fiber types that become spontaneously active and mechanosensitive following nerve transection in the rat. *Brain Res* 1999; 824:218–23
48. Gabriely G, Wurdinger T, Kesari S, Esau CC, Burchard J, Linsley PS, Krichevsky AM: MicroRNA 21 promotes glioma invasion by targeting matrix metalloproteinase regulators. *Mol Cell Biol* 2008; 28:5369–80
49. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T: MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* 2007; 133:647–58
50. Mei Y, Bian C, Li J, Du Z, Zhou H, Yang Z, Zhao RC: miR-21 modulates the ERK-MAPK signaling pathway by regulating SPRY2 expression during human mesenchymal stem cell differentiation. *J Cell Biochem* 2013; 114:1374–84
51. Yu W, Gius D, Onyango P, Muldoon-Jacobs K, Karp J, Feinberg AP, Cui H: Epigenetic silencing of tumour suppressor gene p15 by its antisense RNA. *Nature* 2008; 451:202–6
52. Ponting CP, Belgard TG: Transcribed dark matter: Meaning or myth? *Hum Mol Genet* 2010; 19(R2):R162–8
53. Mercer TR, Dinger ME, Mattick JS: Long non-coding RNAs: Insights into functions. *Nat Rev Genet* 2009; 10:155–9
54. Fan L, Guan X, Wang W, Zhao JY, Zhang H, Tiwari V, Hoffman PN, Li M, Tao YX: Impaired neuropathic pain and preserved acute pain in rats overexpressing voltage-gated potassium channel subunit Kv1.2 in primary afferent neurons. *Mol Pain* 2014; 10:8
55. Rasband MN, Park EW, Vanderah TW, Lai J, Porreca F, Trimmer JS: Distinct potassium channels on pain-sensing neurons. *Proc Natl Acad Sci U S A* 2001; 98:13373–8
56. Everill B, Kocsis JD: Nerve growth factor maintains potassium conductance after nerve injury in adult cutaneous afferent dorsal root ganglion neurons. *Neuroscience* 2000; 100:417–22
57. Ishikawa K, Tanaka M, Black JA, Waxman SG: Changes in expression of voltage-gated potassium channels in dorsal root ganglion neurons following axotomy. *Muscle Nerve* 1999; 22:502–7
58. Kim DS, Choi JO, Rim HD, Cho HJ: Downregulation of voltage-gated potassium channel alpha gene expression in dorsal root ganglia following chronic constriction injury of the rat sciatic nerve. *Brain Res Mol Brain Res* 2002; 105:146–52
59. Park SY, Choi JY, Kim RU, Lee YS, Cho HJ, Kim DS: Downregulation of voltage-gated potassium channel alpha gene expression by axotomy and neurotrophins in rat dorsal root ganglia. *Mol Cells* 2003; 16:256–9
60. Yang EK, Takimoto K, Hayashi Y, de Groat WC, Yoshimura N: Altered expression of potassium channel subunit mRNA and

- alpha-dendrotoxin sensitivity of potassium currents in rat dorsal root ganglion neurons after axotomy. *Neuroscience* 2004; 123:867-74
61. Hsieh YH, Wu TT, Tsai JH, Huang CY, Hsieh YS, Liu JY: PKC α expression regulated by Elk-1 and MZF-1 in human HCC cells. *Biochem Biophys Res Commun* 2006; 339:217-25
 62. Luo X, Zhang X, Shao W, Yin Y, Zhou J: Crucial roles of MZF-1 in the transcriptional regulation of apomorphine-induced modulation of FGF-2 expression in astrocytic cultures. *J Neurochem* 2009; 108:952-61
 63. Devor M: Potassium channels moderate ectopic excitability of nerve-end neuromas in rats. *Neurosci Lett* 1983; 40:181-6
 64. Devor M, Govrin-Lippmann R: Axoplasmic transport block reduces ectopic impulse generation in injured peripheral nerves. *Pain* 1983; 16:73-85
 65. Targ EF, Kocsis JD: 4-Aminopyridine leads to restoration of conduction in demyelinated rat sciatic nerve. *Brain Res* 1985; 328:358-61
 66. Targ EF, Kocsis JD: Action potential characteristics of demyelinated rat sciatic nerve following application of 4-aminopyridine. *Brain Res* 1986; 363:1-9
 67. Chabal C, Jacobson L, Russell LC, Burchiel KJ: Pain responses to perineuromal injection of normal saline, gallamine, and lidocaine in humans. *Pain* 1989; 36:321-5
 68. Devor M: Ectopic discharge in Abeta afferents as a source of neuropathic pain. *Exp Brain Res* 2009; 196:115-28
 69. Weissner W, Winterson BJ, Stuart-Tilley A, Devor M, Bove GM: Time course of substance P expression in dorsal root ganglia following complete spinal nerve transection. *J Comp Neurol* 2006; 497:78-87

ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Paul Meyer Wood and His Collectibles: Packing and Moving



During World War I, the U.S. Army Ambulance Corps drilled (*above*) volunteers like young Paul Meyer Wood, through many lessons, including how to pack and move “valuables.” Those lessons would assist him later in life as Dr. Wood shifted anesthesia antiques in his Wood Library-Museum (WLM) from downtown New York City out to Foregger’s boat house in Long Island and upstate to Mrs. Wood’s “Meyer Family Home” in Highland Falls. Dr. Wood never lived to see his namesake museum open formally in Park Ridge, Illinois, on Busse Highway (1963) or to see the WLM’s move within the same town to North Northwest Highway (1992). So, what might the ever-patient Dr. Wood, the master of packing and moving anesthesia antiques, have commented about the most recent move of the WLM and its “mother ship” American Society of Anesthesiologists to Schaumburg, Illinois? Why, “Forward, march!” (Copyright © the American Society of Anesthesiologists, Inc.)

George S. Bause, M.D., M.P.H., Honorary Curator, ASA’s Wood Library-Museum of Anesthesiology, Schaumburg, Illinois, and Clinical Associate Professor, Case Western Reserve University, Cleveland, Ohio. UJYC@aol.com.