Spine

SPINE Volume 36, Number 25S, pp S218–S225

BIOLOGICAL FEATURES

How Can Animal Models Inform on the Transition to Chronic Symptoms in Whiplash?

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Study Design. A nonsystematic review of the literature.

Objective. The objective was to present general schema for mechanisms of whiplash pain and review the role of animal models in understanding the development of chronic pain from whiplash injury.

Summary of Background Data. Extensive biomechanical and clinical studies of whiplash have been performed to understand the injury mechanisms and symptoms of whiplash injury. However, only recently have animal models of this painful disorder been developed based on other pain models in the literature.

Methods. A nonsystematic review was performed and findings were integrated to formulate a generalized picture of mechanisms by which chronic whiplash pain develops from mechanical tissue injuries.

Results. The development of chronic pain from tissue injuries in the neck due to whiplash involves complex interactions between the injured tissue and spinal neuroimmune circuits. A variety of animal models are beginning to define these mechanisms.

Conclusion. Continued work is needed in developing appropriate animal models to investigate chronic pain from whiplash injuries and care must be taken to determine whether such models aim to model the injury event or the pain symptoms.

Key words: spine, whiplash, pain, neck, animal model. **Spine 2011;36:S218–S225**

This review describes the current understanding of how chronic pain develops, with a focus on whiplash-related pathomechanisms. Background on pain modeling is introduced with relevance to symptoms and sensitivity. Several working hypotheses describing mechanisms of pain symptom development and maintenance are presented, including concepts of the neurophysiology of pain initiation from injury and longer-term signal processing and the central nervous system's (CNS) neuroimmunologic involvement. A schema is presented highlighting the cellular and molecular processes for acute pain, as well as for the transition to a persistent pain state. Specific findings in a variety of animal model systems are also summarized that inform on the pain mechanisms originating from tissues relevant to whiplash injury. Finally, potential considerations for animal modeling and interpretation of findings for a human disease state are presented.

ANIMAL MODELING OF PAIN SYMPTOMS

In vivo models of injury have provided the major platform to define relationships between the time course and extent of activation of the nociceptive cascade and development and maintenance of pain symptoms. A broad range of animal models has been developed to study several pain states, including neuropathic, inflammatory, peripherally mediated and centrally induced. Behavioral assessments enable pain measures in those models, covering different modalities, anatomic regions and paradigms of testing (Figure 1). Behavioral metrics are defined in the context of the applied stimulus and are described in terms related to the clinical symptoms. *Hyperalgesia* is an increased response to a stimulus which is normally painful, and includes all conditions of increased pain sensitivity.[1] *Allodynia* is a particular case of hyperalgesia in which a stimulus that is usually not painful becomes noxious, reflecting that the stimulus and responses differ.[1] Both of these responses are gauges of hypersensitivity in both humans and animals, and so, serve as important and useful quantitative outcomes for modeling of pain symptoms.[2] Various animal models of pain have been developed to mimic the symptoms and the syndromes of acute and chronic pain with an equally broad range of inciting pathologies. Indeed, that body of work is quite extensive and is beyond the scope of this review; several other reviews cover this topic in more detail.[2–4]

PAIN MECHANISMS—DETERMINING THE TRANSITION FROM ACUTE TO CHRONIC STATES

An inciting event that stimulates nerve fibers initiates local responses and can lead to central signaling events that include neuronal and immune responses (Figure 2). In acute or nociceptive pain, these cascades resolve; however, when chronic pain develops, CNS modifications lead to hypersensitivity

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Acknowledgment date: June 9, 2011. First revision date: July 27, 2011. Second revision date: August 8, 2011. Acceptance date: September 19, 2011.

The manuscript submitted does not contain information about medical device(s)/drug(s).

Institutional and Foundation funds were received to support this work. No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

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DOI: 10.1097/BRS.0b013e3182387f96

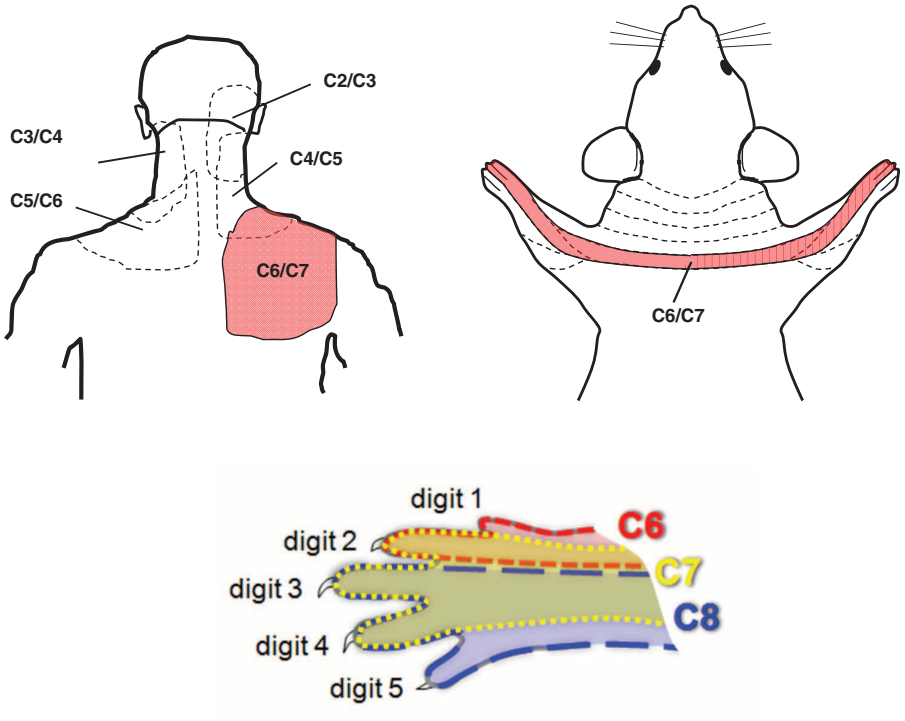

Figure 1. Maps of the regions of referred pain in the human and evoked-pain in the rat enable comparisons between these two species and interpretation of common behavioral tests of sensitivity in the rodent in the context of clinical symptoms in the human. Top left: the distribution of spontaneous whiplash pain in human patients, modified from Lord *et al*.[97] Top right: distributions of evoked-pain regions are also observed in the rat indicating the similar "coathanger" distribution. Bottom right: dermatomes indicating spinal nerve innervations of the forepaw of the rat.

and central sensitization in which the spinal neural circuits respond with a heightened response to stimuli.[2,5] The CNS also mounts a widespread and sustained neuroimmune response further contributing to neuronal plasticity and pain maintenance (Figure 2).

Acute pain results from noxious stimuli that activate small-diameter unmyelinated C-fibers or thinly myelinated Aδ sensory neurons in the periphery.[6] Many different neurotransmitters communicate from the peripheral afferents to spinal cord neurons. Nociceptive pain protects against tissue injury and continues *only* in the presence of sustained noxious stimuli.[5] After a tissue injury, local nociceptors become sensitized and exhibit both lower thresholds for firing and increased firing rates when exposed to a previously nonnoxious stimulus.[5,7,8] This peripheral sensitization is due largely to the production and peripheral release of neurotransmitters and/or other factors from neurons and nonneuronal cells that can infiltrate the injured area (Figure 2).[7,9] Multiple mediators, including bradykinin, amines, prostanoids, NGF, and protons, act rapidly via receptors on nociceptor terminals to sensitize these neurons.[9] This generalized nociceptive pain response encompasses the typical cascade of an acutely painful episode, where the balance of injury, repair and healing is achieved and the collection of electrophysiological and chemical events resolves. However, for persistent pain the local, spinal, and supraspinal cascades become pathologically and permanently altered.[7]

The contribution of the local and widespread inflammatory response plays a large role in the transition from acute to chronic pain. Persistent pain is due, in large part, to the initiation and maintenance of spinal sensitization via plasticity in the neuronal circuits. Although the exact mechanism by which the spinal cord transitions to a hyperexcitable state remains somewhat unknown, many hypotheses have emerged. These theories are highlighted briefly here; more extensive discussions can be found elsewhere.[5,9–11] Simultaneously, different processes occur that induce this central sensitization. The low-threshold Aβ afferents, which normally do not transmit nociceptive signals, are recruited to transmit spontaneous and movement-induced pain.[10] The central hyperexcitability is further exacerbated by a "wind-up" response of repetitive C-fiber stimulation, expanding receptive fields, and spinal neurons taking on properties of wide dynamic range neurons.[12,13] Ultimately, Aβ-fibers stimulate the postsynaptic neurons, whereas these Aβ-fibers previously transmitted only to nonpainful innocuous stimuli. Amplification of noxious signals from afferents at the synapse in the spinal cord can occur and this long-term potentiation can exacerbate pain.[11]

Although the neuronal responses in the nervous system contribute to the onset of pain, neuroimmune responses throughout the nervous system also potentiate persistent pain (Figure 2).[14–17] Tissue injury leads to the release of many chemical mediators that enhance neuronal activity and also initiate a neuroimmune cascade in which many peripheral nonneuronal cells are activated (Figure 2).[7,14,15] These resident cells, including mast and Schwann cells, release mediators such as histamine, prostaglandins, cytokines and chemokines, that recruit other infiltrating immune cells (Figure 2). The cytokine cascade is responsible for the infiltration of immune cells to the site of injury.[8,14,15] Proinflammatory cytokines trigger the release of other inflammatory mediators that sensitizes nociceptors and further maintains neuronal excitability and sensitization, which leads to pain[8,14] (Figure 2). However, some cytokines, such as TNF-α and IL-1β, can also directly sensitize nociceptors during inflammation because sensory neurons express their receptors.[8,18]

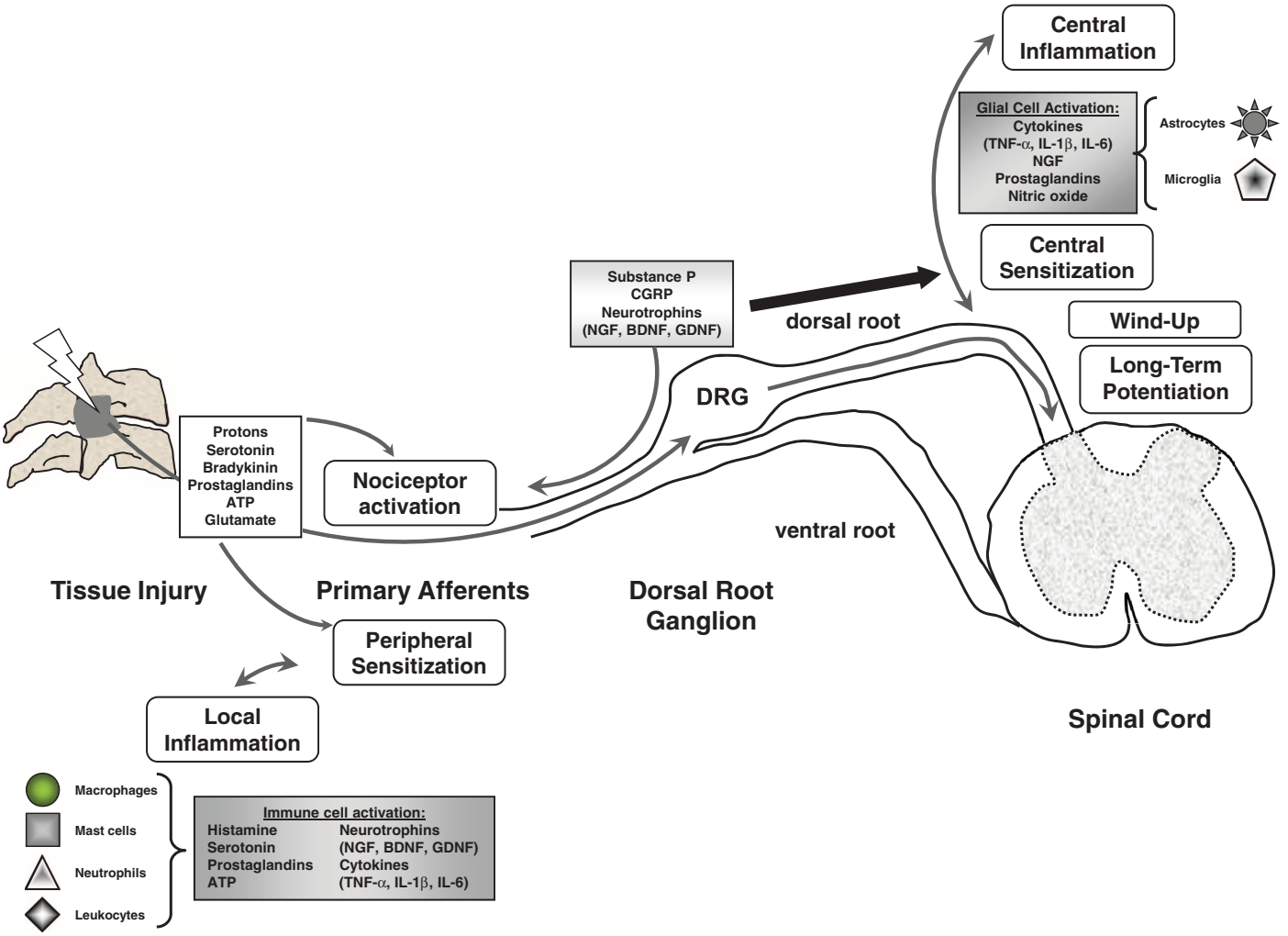

Figure 2. Trauma in the periphery induces the release of chemical mediators that can activate local nociceptors, lead to spinal sensitization, and produce pain. Nociceptor activation leads to neurotransmitter and neurotrophin synthesis in the DRG. In addition, local inflammation involves the recruitment and stimulation of many nonneuronal cells to release inflammatory mediators that modulate neuronal excitability. Both of these neuronal and immune responses can induce central sensitization in the spinal cord that increases the excitability of neurons in the dorsal horn. Spinal glial cells are activated and release inflammatory mediators that further potentiate the neuronal and glial responses, contributing to persistent pain.

Neurotrophins and growth factors (NGF, in particular) are important mediators and modulators of pain.[19–22] Although neurotrophic factors are necessary for the long-term survival of neurons, there is emerging evidence for their roles as crucial mediators of persistent pain. NGF is upregulated in many chronic pain conditions and modulates pain through BDNF, which is released from the spinal terminals of a subset of nociceptors.[21,23] BDNF acts through the trkB receptor and leads to central sensitization by potentiating glutamate neurotransmission in the dorsal horn and promoting the synaptic rearrangement of the Aβ-fibers.[14,21,24] Neurotrophins also have indirect actions on neurotransmission because they are synthesized and released by several types of immune cells,[14] which themselves interface with neurons and can modulate synaptic activity.

Activation of peripheral nociceptors also activates glial cells in the CNS. Microglia are the resident macrophages in the CNS and astrocytes help with maintaining homeostasis at neuronal synapses. Both types of glial cells respond to injury by changing their morphology, proliferating, and releasing inflammatory mediators.[17,25–27] Spinal microglia are activated early after peripheral injury by some neuromodulators, including excitatory amino acids, substance P and others[27,28]; when activated they release several proinflammatory cytokines (IL-1β, TNF-α, IL-6), as well as nitric oxide, prostaglandins and NGF.[16,25,29] In addition, BDNF released from stimulated microglia modulate neuronal signaling in the superficial dorsal horn.[30] These mediators, in turn, induce the exaggerated release of neurotransmitters from presynaptic neurons, sensitize the postsynaptic membrane, activate neighboring astrocytes and enhance microglial activity.[16,25] This positive feedback sustains the continued further release of pain mediators, facilitating the development of neuronal hypersensitivity and leading to persistent pain.

RELEVANCE TO WHIPLASH—CONSIDERATIONS FOR ANIMAL MODELING

There is growing physiologic evidence that various structures in the neck may generate pain and models have been developed for their injury.[2,18] Although the exact pain mechanisms for whiplash remain open to debate, there is consensus on the potential sites of injury and how pain can originate from them. Recent research for cervical spine pain has focused particularly on understanding mechanisms of acute and chronic whiplash injury. These findings are discussed with regard to injury biomechanics and the nociceptive mechanisms related to pain generation and maintenance.

The Facet Joint

Many of the hypotheses of facet-mediated whiplash pain imply that "abnormal" motion patterns develop in the spine, that induce local injury or dysfunction to the specific anatomical components of the facet joint. During these injuries, the lower cervical spine undergoes extension whereas the upper spine flexes, modifying the local biomechanical environment of the facet joint by inducing: (1) compression of the facet joint, and/or (2) excessive capsular ligament strain.[31–35]

The potential for tissue loading and pain generation is primarily relevant to the ligamentous capsule that encloses the facet joint.[18,35,36] Many engineering studies demonstrate the facet capsule to be stretched more during whiplash than in normal physiologic motions.[37–43] However, frank rupture of the cervical facet capsule has not been reported for any whiplash kinematic.[38,39,44] Even transient tensile loading of the cervical facet capsule can activate both short and long-lasting pain pathways.[45–48] For example, short-duration capsule loading activates and saturates mechanoreceptive and nociceptive afferents,[45] suggesting an immediate mechanism to initiate pain. Interestingly, in a rat model of a single exposure of the facet joint to tension loading that results in similar distortions in the capsule that saturate nerve afferents also produces persistent sensitivity.[47,49–53] The capsular biomechanics associated with those responses are very similar to those reported for the human capsule during whiplash simulations.[34,38,39,54] Using animal model systems, it has been further determined that those same transient painful joint loading scenarios are also associated with the production of collagen fiber disorganization,[55,56] and axonal swelling and altered morphology,[48] which provide a potential means to initiate pain maintenance.

Transient facet capsule loading that produces persistent pain also leads to a complicated cellular cascade in the affected afferents in the dorsal root ganglia (DRG) and spinal cord. Both neurons and accessory cells in the DRG and spinal cord exhibit modified phenotypic responses to painful joint loading (Figures 2 and 3).[47,53,57] In particular, painful facet capsule tension increases expression of a marker of the activated stress response in DRG neurons to attempt to reestablish protein homeostasis that can be altered by neuronal damage.[53,58] Gene transcription of substance P in afferents is also induced[52] suggesting that painful loading may induce axonal dysfunction that subsequently disrupts normal protein production.

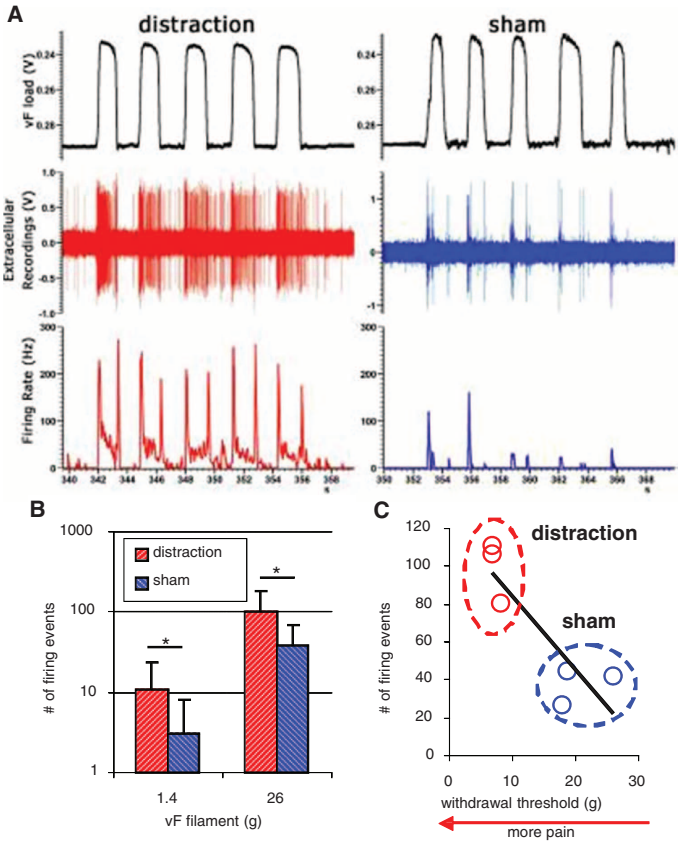

Figure 3. (**A**) Representative extracellular voltage recordings and neuronal firing rate of spinal neurons during repeated stimulation of the forepaw with a 26 g filament after a painful facet capsule injury (distraction) or sham. (**B**) Evoked spinal neuronal firing is significantly (*) elevated after painful injury. (**C**) Neuronal firing upon noxious paw stimulation is strongly correlated with the magnitude of stimulus to elicit paw withdrawal (a lower threshold is more sensitivity).

Those afferent modifications are complemented by increased spinal activity of the glutamatergic system and neuronal hyperexcitability that are sustained in association with persistent pain.[47,57] In contrast, spinal substance P is detected to decrease early and may be a consequence of increased protein usage in the spinal cord and/or injury-induced axonal dysfunction that can affect protein transport.[52] Substance P can regulate the spinal glial responses[28] which has an important impact on pain because spinal glia are critical in the onset and maintenance of *chronic* pain[17,25–27] and are associated with persistent sensitivity in that same model.[49,59] Cytokines are also modulated in both the DRG and spinal cord after loading of the facet joint.[50] Cytokine expression parallels spinal glial activation and both of these responses increase after painful subfailure capsule loading; however, neither may specifically drive *chronic* pain because joint loading that produces greater injury does not independently produce more behavioral sensitivity.[50,59] This apparent disconnect between the central immune response and facet joint loading indicates there may be other cellular processes that maintain facet-mediated persistent pain.

The Nerve Root and DRG

The potential for nerve root and DRG trauma exists because of the flexibility of the cervical spine, coupled with the neck's potential to undergo loading due to motions of the head and torso. The cervical nerve roots can be at particular risk for injury due to increased hydrostatic pressure that can be established during rapid head and neck motions.[60–63] Although there is growing research in these areas, most of the current understanding of chronic pain developing from nerve root injury is from a large body of work in the lumbar spine. However, a unique porcine model was developed to study the potential mechanisms of whiplash-induced pain.[60,61]

Aldman's group hypothesized that pressure gradients in the CNS from blood flow resistance can be generated during rapid spinal motions such as whiplash.[60,61] The spinal vasculature regulates blood volume to accommodate any changes in spinal canal size. Yet, during rapid head/neck motions, resistance to blood flow can generate pressure gradients in and outside the spinal canal.[60–63] These gradients can directly apply pressure to the nerve roots and spinal ganglia that can induce cellular injury.[61,63] Coupling these hypothesized pressure-wave effects with the direct mechanical compression and tethered stretching of the neural tissues that can occur by abnormal rapid spinal motions, several painful injury mechanisms emerge.[60,62] In fact, plasma membrane breakdown of spinal ganglia cells has been induced in an *in vivo* porcine model of rapid head-neck extension.[60–63] Although it suggested a potential mode of injury to neuron cell bodies, that work did not provide any direct measure of pain.

Emerging research in persistent radiculopathic pain has highlighted the influence of injury on the neurophysiologic responses that lead to the establishment of chronic pain. The injury profile at the time of nerve root injury modulates neuropeptide expression in the DRG and the spinal cord, associated with development of persistent pain.[64–66] Cervical root compression that produces persistent sensitivity shows greater allodynia at later time points after more severe dorsal root compression.[64,66] There is a corresponding depletion of substance P in the DRG that also depends on the injury severity, suggesting its synaptic release and utilization may be increased in persistently painful injuries.[67,68] Spinal expression of CGRP also decreases significantly with increasing nerve root loading and pain.[64,66] Further, both the extent of axonal damage and Wallerian degeneration in the compressed nerve root are directly related to the likelihood of developing persistent allodynia.[66,68]

Much of what is understood about the development of persistently painful radiculopathy comes from models of lumbar radicular pain.[69–73] It is clear from studies of lumbar and cervical nerve root compression that direct nerve root trauma (even transient) can induce long-lasting pain and a constellation of physiologic responses throughout the nervous system.[64,65,68,69,74–76] The onset of behavioral hypersensitivity is immediate after trauma and parallels a host of widespread neuronal and neuroinflammatory responses, including endoneurial edema, axonal membrane leakage and Wallerian degeneration.[73,77–79] In response to axonal damage, macrophages infiltrate the injury site, phagocytose myelin debris, and induce the release of IL-1 and TNF-α.[67] By blocking the TNF-α pathway using a soluble TNF receptor at the time of injury, behavioral sensitivity is reduced after injury along with decreases in both TNF-α and IL-1β mRNA.[29,80,81] Axonal degeneration and inflammation can also increase electrical activity in adjacent intact axons,[72,82] leading to spinal sensitization and the immune sequelae that further exacerbate sensitization (Figure 2). Spinal glial cells become activated and modulate other immunologic changes leading to persistent pain, via cytokine and growth factor production.[15,80,83,84] Spinal glial activation is also sensitive to the severity of loading and the presence of chemical irritation in radicular pain,[74,76,85] with greater degrees of nerve root compression inciting greater activation of spinal astrocytes.[71,74] The differentiation in activation is apparent early and is sustained in parallel with persistent pain symptoms,[76] suggesting that astrocytes may directly respond to the changes in the dorsal horn that are induced by damaged primary afferents.[86,87] Spinal microglial activation is also induced and seems to be directly related to the *onset* of pain.[76] However, spinal microglial proliferation induced early after cervical nerve root compression may only indicate tissue trauma and not be because of nociceptive signaling and pain.[88]

LESSONS FROM ANIMAL MODELS OF PAIN AND IMPLICATIONS

Given the extreme complexity of the mechanisms of neck injuries that do and do not result in chronic pain, it must be recognized that there are a variety of factors that have important roles. For example, many biomechanical factors have been shown to alter neuronal function: rate, loading duration, and load magnitude, among others.[47,51,52,57,59,64,74,75,89,90] The factors, which confound the injuries themselves, and the physiologic milieu at the time of injury can also potentially directly modulate the severity and extent of pain.[70,76,81,85,88,91] For example, it can be hypothesized that injury after an already preexisting minor tissue injury may produce a more severe nociceptive response than in the absence of any preexisting damage. It is not unlikely that an initial injury produces the local inflammatory changes discussed earlier, which in turn may lower that tissue's threshold for mechanical injury that can lead to symptoms (Figure 2). In fact, the mechanical insult required to produce behavioral hypersensitivity in the presence of an inflammatory insult is less than that required in its absence, despite producing the same degree of behavioral sensitivity,[70,76,81,85,88] corroborating clinical studies in which patients with preexisting spinal degeneration experience more severe and longer lasting neck pain symptoms.[92,93] It is possible that degeneration can contribute to inflammatory changes in the facet joint that increase its susceptibility to whiplash injury so when its tissues undergo loading that may not normally be painful, the nerve fibers may be presensitized and have lower thresholds for response than those previously required to initiate nociception or that may induce a more widespread central response and chronic pain. Nonetheless, an integrated approach is required to fully understand the complicated disease of chronic pain, especially in the context of whiplash injury.

Although animal models are integral and necessary to define mechanisms of painful injury and those factors that drive the transition to chronic pain, there are several important caveats about using such platforms, in particular to understand a mechanically based injury such as whiplash. Anatomic geometry and scaling are paramount among these considerations. For example, most animal models of pain have been developed in quadrupeds that do not have the same mechanical demands, anatomic relationships and locomotion demands as the bipedal human. This must not be ignored particularly for spine-based pathologies in which the tissue loading can be substantially different among species. However, the neurologic function, tissue responses and relationships between them have been well documented for a number of species. Nonetheless, care must be taken when developing such models and interpreting results.

It is also important to consider whether an animal model is serving as a proxy to model the pathology/injury or the symptoms that develop. Within that context it is also important to note that the current animal models of whiplash pain measure only evoked pain and do not capture the spontaneous pain that is reported clinically. In this sense, animal models could be enhanced by the development of assessments for behavioral outcomes that measure the pain in the referred pain areas observed clinically. Certainly, the behavioral and physiological evidence that is already available strongly suggests spontaneous pain may be induced in these models, but additional work is needed to develop relevant and sensitive assays to detect it. In fact, animal models of whiplash injury and chronic pain offer great promise because they represent a "reduced" model system in which many of the confounding factors that are present in the clinical problem—such as litigation status, compensation issues and psychosocial factors—can be controlled or avoided altogether. Conversely, the complexity of the clinical presentation, which includes a variety of physical function and stress responses,[94] also is not fully captured in the laboratory model.

The complexity of the clinical syndrome of chronic whiplash pain is further extended by the recent reports that genetics play a key role in pain persistence, accounting for many discrepancies observed for seemingly similar injuries.[95] The utility of animal models is both highlighted and hampered by this. Although the clinical picture of persistent pain in humans is indeed intricate, similar genetic specificity has been defined for different strains of rats exhibited different responses to a standardized painful tissue injury.[96] Moreover, such diversity could be exploited in a rat model to determine if and which genetic variants may be more predictive of chronic pain from whiplash. Such studies are needed and would undoubtedly provide additional valuable insight into the clinical management of this syndrome. Indeed, it may be possible to induce behavioral outcomes that model the clinical picture but it is imperative to consider then the pathology, tissue origin and pathways being mapped—the question to consider becomes "is it a model of the injury or the symptoms?"

➢ Key Points

- ❑ Animal models provide utility for defining injury and pain mechanisms in whiplash.
- ❑ Mechanisms by which pain becomes chronic involve the integration of peripheral and central neuronal and immune responses.
- ❑ The development of chronic pain from whiplash injury depends on a variety of factors.
- ❑ Animal modeling of painful injuries, like whiplash, must be interpreted carefully in the context of the goal of modeling the injury event or the resulting symptoms.

Acknowledgments

The author gratefully acknowledges the National Institutes of Health (NIAMS; Grant #AR056288) and the Catharine Sharpe Foundation for support.

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