Physiology of Chronic Spinal Pain Syndromes From Animal Models to Biomechanics

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Study Design. The literature and current research related to spinal pain mechanisms were reviewed, as well as animal models related to its study.

Objectives. To provide a pragmatic discussion of spinal pain that both reviews relevant research and coherently synthesizes the existing body of literature related to pain, nociception, animal modeling, and injury biomechanics.

Summary of Background Data. A detailed body of literature suggests that spinal pain mechanisms are quite complicated and involve a host of different processes (*e.g.*, genetics, gender, neurophysiology, and biomechanics) that may contribute to clinical manifestations and symptoms.

Methods. Both a review of the literature and a presentation of current and ongoing laboratory research are presented. Specific findings from the authors' laboratory using a rodent model of lumbar radiculopathy are presented to elucidate the role of local nerve root biomechanics in initiating and maintaining behavioral symptoms of nociception and pain.

Results. For an understanding of chronic pain, a bidirectional-translational approach that incorporates crossdisciplinary methods such as *in vivo* biomechanical techniques is required. A conceptual model of chronic spine pain is proposed that details the dynamic and integrated roles of injury, biomechanics, and nociceptive physiology.

Conclusions. Areas of continued research are highlighted that may help guide the management of painful spine symptoms and syndromes. [Key words: animal model, biomechanics, nociception, pain] **Spine 2002;27**: **2526–2537**

Millions of people in every country live and die in needless pain. In 1953, Albert Schweitzer said, "We must all die. But that I can save [a person] from days of torture, that is what I feel as my great and ever new privilege. Pain is a more terrible lord of mankind than even death himself."^{28A}

Pain is a major health problem in the United States, where at least 50 million Americans are partially or totally disabled by intractable pain. It has been estimated that approximately 45% of all Americans seek care for persistent pain at some point in their lives (American Pain Society). Chronic pain often is untreated or mistreated, leading to tragic and costly consequences that include long-term disability, depression, and overuse of diagnostic services and procedures, hospitalizations, surgery, and inappropriate medication.

Headache and low back pain are the most common forms of chronic pain. Fortunately, there are many efficacious therapeutic agents for the treatment of tension and migrainous headache. The same cannot be said for low back pain, or more specifically, chronic spinal pain syndromes. For this reason, elucidation of neural mechanisms that produce and maintain chronic pain is desperately needed. The authors posit that the best way to study these mechanisms is through a bidirectionaltranslational approach, in which basic science findings are applied to clinical diagnoses and treatments, with the data from clinical studies used to provide better models and designs for future cellular–molecular–animal systems experiments (Figure 1).

In this article, the authors first summarize the differences in nociceptive processing between physiologic and pathologic chronic pain. They next discuss animal models of nociception and explain how data obtained from these models may translate into improved treatment of clinical pain. Although there are many animal models of physiologic and chronic pain, the models of low back pain, unfortunately, are limited. Because of the heterogeneous etiology of back pain, it is very difficult to mimic these clinical scenarios effectively in animal models. For this reason, the current animal models of low back pain are directed at injury to nerve roots or dorsal root ganglions (DRGs). The various models of radiculopathy and DRG injury are briefly discussed, followed by a more in-depth review of one rodent model of radiculopathy highlighting neuroimmune alterations and local nerve root biomechanics.

The intent of this article is twofold: 1) to give a concise and cogent summary of existing knowledge regarding nociceptive processing and animal models of chronic pain, and 2) to point out areas of future research in animal model design and basic science studies. The aim is to see translational research in basic science processed toward the improvement of outcomes in clinical care for such disorders as chronic nonmalignant spinal pain syndromes (Figure 1).

Mechanisms of Pain Processing: Physiologic Versus Pathologic Pain

Physiologic Pain Processing. Clearly, it is recognized that acute, physiologic pain is a necessary sensation for the survival and well-being of organisms ranging from paramecium to complex organisms such as humans. In the

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Figure 1. Clinical and experimental knowledge are integrated in a scientific understanding of low back pain and lumbar radiculopathy. This understanding is modified and expanded through continued model development and refinement based on clinical input and experimental findings. The product of evolving low back pain knowledge is returned to the clinical community for the development of better treatments and clinical management.

book, Pain: The Gift Nobody Wants, Paul Brand and Philip Yancey² discuss how our culture demands complete pain relief, usually not recognizing the benefits of enhanced sensitivity after surgery or acute injury. Mechanisms of physiologic pain transmission follow simple cartesian processing principles (e.g., noxious injuries activate peripheral nociceptors: myelinated Aδ and unmyelinated C fibers). Peripheral or nerve root injury sets into play the synthesis and release of numerous inflammatory mediators that act in concert not only to induce inflammation and edema as part of the healing process, but also to sensitize nociceptors and recruit new nociceptors to enhance the pain.^{12,13} These mediators include bradykinin, substance P, histamine, 5-HT, glutamate, Ach, ATP, cholecystokinin, and eicosanoids such as PGE2, PGI2, and LKB4. Also, at the periphery, nociceptive input can be inhibited by action of peripheral opioid receptors.²⁶ Opioids retard the release of substance P and inhibit the synthesis of cyclic AMP.¹³

Primary afferent fibers terminate on neurons in the dorsal horn of the spinal cord. Synaptic transmission between nociceptors and dorsal horn neurons is mediated by a number of chemical neurotransmitters such as the amino acid glutamate, and neuropeptides such as substance P. This nociceptive information then is transmitted from the spinal cord to supraspinal sites, such as the thalamus and cerebral cortex, by ascending pathways. More recently, the role of the cortex in pain processing has been recognized and studied using technology such as positron emission tomography (PET).

By definition, pain is a complex perception influenced by prior experience and by the context in which the noxious stimulus occurs. Thus, unlike nociception, pain is not a simple physiologic response to injury. Studies performed using PET show that activity in the anterior cingulate cortex reflects a subject's perception of pain's un-

pleasantness. In one elegant study performed by Bushnell et al, a volunteer immersed a hand in a 47 C hot water bath.¹⁷ The researchers then suggested that the experience was either less unpleasant or more unpleasant than it actually was. Using PET for analysis, the cingulate cortex was more active when the volunteer believed that the stimulus was more painful. This study clearly underscored the complexity of pain processing, confirming that there is not one specific, localized pain region or even a single mediator of pain that can be manipulated to alleviate pain. Recent studies have illuminated descending inhibitory and facilitory pathways that further modulate interneurons at the spinal level.53 The existence of descending facilitory pathways further highlights the complexity of pain processing and multilevel dimensions of modulation.

Basic Strategies of Pain Control. When considering basic strategies for pain control, the clinician should consider the three sites of pain modulation discussed earlier: peripheral, spinal, and supraspinal. The attenuation or blockade of nociception through intervention at the periphery is accomplished by the use of NSAIDs, regional analgesia, or in extreme situations, ablative procedures. The activation of inhibitory processes that "gate" nociception at the spinal cord and brain includes the use of opioids, α -2 adrenergic agonists, tricyclic antidepressants, anticonvulsants, and perhaps acupuncture. Finally, interference with the actual perception of pain at supraspinal sites can be accomplished using psychotherapy, biofeedback, and other psychological pain modulatory mechanisms.

Interestingly, morphine, a commonly used opioid, acts at each site (peripheral, spinal and supraspinal). This may explain why opioids are considered the gold standard of analgesia, and why the effectiveness of potential new analgesics is compared with the efficacy of morphine. As discussed earlier, opioids have a peripheral action of inhibiting the activity of nociceptors. They also act spinally and supraspinally at opioid-specific receptors in the substantia gelatinosa of the dorsal horn of the spinal cord, periaqueductal gray, and rostral ventral medulla, and have an additional effect on the sensorium that may alter the perception of pain.

Pathophysiology of Chronic Pain. The cartesian pathway, described in 1664 by Rene Descartes as a simple loop from injury to reflex, is far too simplistic for an understanding of pain transmission. This is particularly relevant regarding chronic pain that has extended beyond the period of normal tissue healing. Persistent pain is not a simple extension of acute pain. A cascade of changes initiated by tissue or neural damage elicits a collection of synaptic, neurotransmitter, and modulatory events that mimics synaptic plasticity and remodeling similar to that seen in learning and memory. A large body of evidence has accumulated to indicate that sensitization in the central nervous system is largely responsible for the development of persistent pain states.^{7,16,47,60} However, de-

spite this plethora of data, the exact players in central sensitization and the specific ways of attenuating this process have thwarted investigators so far.

Local pathophysiology at the nerve injury site has implicated a host of potential mediators. Recently, sodium channel subtype accumulation has been in the limelight of pain research. Results have demonstrated increased excitability and spontaneous activity of DRG neurons after axonal injury. Thus, because sodium channels are key elements in action potential generation, blockade of these channels and channel expression have been studied intensely. The subtypes SNS/PN3 and SNS/NaN were found to accumulate at sides of nerve injury in animals and humans with neuropathic pain.8 In addition, using antisense oligodeoxynucleotide technology and knocking down SNS/PN3 but not NaN channel gene expression, prevented hypersensitivity after nerve injury in rats.⁴¹ Therefore, these sodium channel subtypes are considered to be novel therapeutic targets for the treatment of neuropathic pain.

The local production of proinflammatory cytokines through immune cell activation also has been implicated as a potential player in enhanced nociceptor activity. Epineural application of TNF- α produces behavioral and neural hypersensitivity,⁵⁰ and antibodies to TNF- α receptor 1 administered at the site of injury reduced thermal hyperalgesia in a rat model of neuropathy.⁴⁸ These local changes spread to the sensory cell bodies and into the spinal cord. They were displayed as increases in immediate early gene products such as c-jun and c-fos. Certainly, glutamate signals through both ionotropic and metabotropic receptors, as well as tachykinins such as neurokinin receptors, prostaglandins, cytokines, and growth factors, all could be candidates for inducing transcriptional processes that lead to central sensitization. The authors' laboratory has focused on the role of centrally produced proinflammatory cytokines, glial activation, and leukocyte trafficking in an L5 lumbar radiculopathy rodent model discussed further as applicable in a later section.^{6,15,43,51,56} Other factors that should be considered as possible mechanisms for the enhanced neural activity of the injury nervous system include deficits in inhibitory endogenous mechanisms such as GABAergic or opioid transmission, possible apoptosis of inhibitory spinal neurons after nerve or nerve root injury,⁶² and an enhancement of pronociceptive endogenous mediators such as dynorphin.40,54

The authors hypothesize that nerve root injury initiates a cascade of events. The sequencing of these events is not linear. The early and late effects are dynamic and may be host dependent. Early on, there is a robust spinal neuroimmune activation and neuroinflammation that induces central sensitization either directly or indirectly by inhibiting inhibitory interneuron activity at the dorsal horn level. It has been shown that glial or neuronal proinflammatory cytokines can sensitize peripheral nociceptive fields²¹ and sensitize DRGs.³⁸ In addition, spinal cytokines induce the expression of algesic mediators such as prostaglandins, substance P, nitric oxide, and glutamate, which then can in turn create a vicious cascade of sustained elevation and action at inhibitory neurons. In relation to the upstream role of proinflammatory cytokines in producing spinal sensitization, there is mounting evidence that cytokines induce release or expression of cyclooxygenase-2 (COX), inducible nitric oxide (NO) synthase, and substance P, and enhance capsaicin sensitivity.^{18,20,30,46} Similarly, activated glial cells synthesize proinflammatory cytokines, proteases, NO, excess glutamate, superoxide anions, hydrogen peroxide, eicosanoids, and other toxins that act by way of the N-methyl-D-aspartate (NMDA) receptors.^{3,22,31} Therefore, cytokines have the capacity to create downstream modulation of the CNS milieu that may indirectly enhance spinal sensitization.

This summary of research over the past 15 years in chronic pain models can be extrapolated to the clinical scenario of chronic spinal pain syndromes. The focus in the clinical community has been mainly on local surgical, manipulative, and biomechanical interventions. Further understanding of spinal and supraspinal mechanisms and mediators of central sensitization may lead to improved treatment and even prevention paradigms. Realizing the dynamics of these living and integrated relations helps researchers to dissect some of the possible cause-and-effect relations.

Animal Models of Nociception: What Can They Tell Us?

Understanding of the neural mechanisms for some chronic pain syndromes has been accelerated by the use of animal models. Before a discussion of these animal models, it is important to address other aspects of animal models of nociception including: ethical issues, behavioral testing, sensitivity of the testing, clinical relevance, and genetic factors.

Ethical Issues. With any biomedical research involving animals, ethical issues associated with their use must always be considered. This applies even more with the study of pain. These ethical issues create alarm in both lay people and scientists because of the anthropomorphic context of these studies. Pain is defined by the International Association of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage." Researchers and ethics committees make large efforts to minimize extreme discomfort or stress in animal nociception studies because obvious distress compromises the science while raising ethical concerns. The IASP has established guidelines that are reviewed by Institutional Animal Care and Use Committee (IACUC) committees, which mandate that both lay community members and scientists be members. Table 1 outlines the guidelines used by IACUC members, pain researchers using animals, and journals. Zimmerman⁶¹ redefined the IASP definition of pain for animals as "an aversive sensory experience caused by actual or potential injury that

Table 1. International Association for the Study of Pain Guidelines for the Use of Animals in Pain Studies

Experiments involving the study of pain on conscious animals must be reviewed beforehand by scientists and lay persons, and the potential benefit of these experiments must be obvious.

As far as possible, the scientist must test the painful stimuli on him- or herself.

The scientist should assess all behavioral and physiological changes in the animal and report them in publications.

One cannot use animals paralyzed with a neuromuscular blocking agent without a general anesthetic.

The duration of the experiments must be as short as possible and the number of animals involved must be kept to the minimum.

elicits progressive motor and vegetative reactions, results in learned avoidance behavior, and may modify species behavior." For chronic pain animal models used currently, the lesions created are focal, so the animal's behavioral sensitivity often is restricted to only the innervated body part. Investigators measure the absence of vegetative behavior, normal grooming and eating, weight gain, and lack of aggression and use these measurements as indicators of a minimally distressed animal.

Behavioral Testing. Behavioral testing approaches can be divided by the method of stimulation (thermal, chemical, or mechanical) and by the type of stimulus (noxious versus nonnoxious). The two behavioral tests used most often in chronic pain studies are hyperalgesia (increased sensitivity to a noxious stimulus) and allodynia (increased sensitivity to a nonnoxious stimulus). Given the controversy over the use and definitions of these terms, it may be more descriptive to use the terminology of thermal or tactile hypersensitivity associated, respectively, with the Hargreaves method of thermal hyperalgesia¹⁴ and methods for detecting nonnoxious mechanical sensitivities (e.g., the use of von Frey filaments). Reactions produced by a noxious stimulus can fall into one of two categories: 1) responses organized by lower hierarchical areas of the CNS such as withdrawal reflexes and cardiovascular changes, or 2) more integrated complex responses requiring supraspinal input such as tactile hypersensitivity or learned conditioned responses.³⁷

When considering any existing or novel behavioral test of nociception, it is imperative to consider certain parameters. This is especially relevant to the development of future animal models of chronic spinal pain syndromes, which is the focus of this article. Table 2 highlights important components for animal models of nociception.

Genetic Susceptibility. Genetic susceptibility to chronic pain has recently come to the forefront of basic science pain research. For example, it was shown in one study that 10 strains of mice had marked differences in behavioral responses to chemical or peripheral nerve injury.²⁹ These genetic differences also may help to explain disparate findings of drug efficacies among laboratories. The authors' laboratory has observed similar differences among behavioral responses in an L5 radiculopathy

model using two genetically different strains of mice. Preliminary results have shown outcomes similar to those for a peripheral nerve injury. For example, Balb/j mice showed more sensitivity to a nerve root lesion than C57BL6 mice (data in progress).

Animal Models of Chronic Pain

The neuropathic pain models developed so far involve surgical manipulation of the sciatic nerve or spinal nerves, or injury to the spinal cord itself. The models that include direct manipulation of the sciatic nerve include sciatic cryoneurolysis¹⁰; sciatic nerve section, ligation, or crush⁴²; partial nerve injury⁴⁵; and chronic constriction injury.¹ These models mostly display numerous common features that have generated an enormous amount of data culminating in a plethora of publications.

A similar model, but one that has some advantages over the sciatic nerve injury models, is L5–L6 spinal nerve ligation. This model produces early robust tactile hypersensitivity and, to a lesser extent, thermal hyperalgesia. The authors' laboratory has modified this procedure to an L5 spinal nerve transection, in which they observe decreased sham surgery sequelae and more reproducible and reliable behavioral and neurochemical outcomes.⁶ All of these neuropathic pain models should be studied and assessed when models of chronic spinal pain syndromes are considered. An enormous amount of information regarding behavioral testing, drug effects, and mechanisms of persistent pain can be garnered and applied to the treatment of chronic pain.^{25,62}

Models of Chronic Spinal Pain Syndromes

During the past decade, there has been increasing interest in the mechanisms of low back pain. Several useful animal models of lumbar nerve root injury have been developed and used to begin addressing the etiology of painful radiculopathy.^{15,23,24,28,33,35,36,49} These models have established two specific mechanisms at the injury site level: 1) mechanical deformation of the nerve roots and 2) biologic or biochemical activity of a herniated disc tissue. Over the past 5 years, the authors' laboratory has exploited one model of L5 nerve root injury in a fourfold fashion: 1) to address continually the reproducibility, the

Table 2. Parameters to Consider in the Development of Animal Models of Nociception and Chronic Pain

- The input specificity: The stimulus should provoke nociceptive mechanisms. In chronic pain models, this is determined by performing baseline tests on pre-injury animals.
- The sensitivity of the test: It must be possible to quantify the behavior and demonstrate attenuation, such as with pharmacologic interventions.
- Validity of the test: The response must not be contaminated by other factors. This relates to the pharmacologic studies, the drug cannot induce motor deficits that may then mask as analgesic or antihypersensitivity.
- Reliability: Consistency of data measurements when animals are retested.
- Reproducibility: The results obtained from the behavioral test must be reproducible among different experimenters and laboratories.

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Figure 2. Models of lumbar radiculopathy for nerve root injury are schematically depicted. The panel on the left illustrates the clinical injury of a disc herniation producing a mechanical component caused by direct nerve root impingement and a chemical component caused by inflammatory disc material contacting the neural tissue. In contrast, the panel on the right shows a schematic of a rodent model of nerve root injury by ligation (mechanical component) using chromic gut (chemical component) suture material. Although it does not mimic the injury event in vivo exactly, this rodent model does produce low back pain sequelae similar to those in the clinical scenario.



reliability, and most importantly, the clinical relevance of rodent models of radiculopathy; 2) to use this model for testing novel therapeutic agents to determine whether they have efficacy in reducing behavioral hypersensitivity and to investigate physiologic pain mechanisms; 3) to begin addressing the hypothesis that pain resulting from nerve root injury has a central neuroimmune component that may be manipulated to develop novel treatments for chronic spinal pain disorders; and 4) to address *in vivo* the role of local biomechanical factors of nerve root injury in the initiation of behavioral hypersensitivity.

Role of Local Biomechanics in Radicular Pain: In Vivo Animal Model Approach

Biomechanical Considerations for In Vivo Models. It is commonly accepted across the orthopedic and basic science communities, that a mechanical component is involved in most of the injuries leading to chronic spinal pain. For this reason, many animal pain models incorporate this injury component in their modeling approaches to produce pain. It is important to recognize in this discussion that although painful spinal injuries may involve a mechanical component, such pain models may not necessarily require that this modeling component be biomimetic. That is, the injuries applied in the context of producing clinically relevant pain models (producing symptoms that mimic those observed in humans) may not actually have to be true mimics of the clinically observed tissue injuries (Figure 2). For example, in the case of painful radiculopathy resulting from disc herniation, the human injury scenario often includes focal compression of the nerve root. This focal nidus often produces tactile and thermal hypersensitivity, but not always. The impinging disc material may cause the nerve root to be tethered during motion of the spine, thereby introducing a tensile load component as well. In contrast, the previously described rodent model of lumbar radiculopathy uses a ligation with chromic gut (inflammatory) material to apply compression directed inwardly and radially to the L5 nerve roots. Interestingly, such applied injury produces behavioral symptoms that mimic those observed in patients, thereby providing a useful context in which to study the physiologic mechanisms of chronic pain. Separate from the use of mechanics to model clinical injuries, the question still remains: what role do biomechanics at injury or subsequent to injury play in chronic pain mechanisms? The remainder of this section begins to address this question.

Despite many advances in the collective understanding of pain mechanisms, there remain many areas of conflicting hypotheses and uncertainty regarding painful radiculopathy. This lack of a cohesive working mechanistic understanding of chronic pain resulting from nerve root injury is particularly evident in the inconsistency observed clinically in the relation between nerve root deformation and the incidence of painful symptomatology. For example, it remains undetermined why patients with no evidence of nerve root deformation may present with clinical symptoms of radicular pain despite the absence of detectable tissue damage. Likewise, it is equally intriguing that in other patients, imaging studies may document a great deal of nerve root impingement without any or with only minor associated pain. This example demonstrates a complicated clinical picture in which a better understanding of the relations and properties of the different tissues (e.g., nerve, disc, bone, ligament) involved in these painful clinical scenarios can help to guide clinical practices.

Injury Biomechanics and Functional Responses. Mechanical deformation of most biologic tissues produces a host of physiologic changes. However, deformation or loading of neural components has the potential for robust and potentially deleterious physiologic effects given the unique nature of the neural tissue and its crucial role in sensation and functioning. Such injuries are potentially responsible for various pain symptoms. Studies have described how mechanical loading to peripheral nerves produces a direct effect on neurologic functioning *via* the mechanisms described in the Mechanisms of Pain Pro-

cessing section.4,5 Additional research has used mechanical loading to multilevel lumbar nerve roots in the cauda equina and described the resulting physiologic changes.^{32,34,39,44} These changes include altered electrophysiology via decreased thresholds for stimulation, increased amplitude of electrical signaling response, and spontaneous discharge.^{27,32,34,39} From a biomechanical standpoint, many aspects of the injurious loading event such as magnitude, rate of application, and duration all are associated with modulating the nature of these electrophysiologic changes. Nerve root edema also has been noted in these multilevel compression studies using applied compression pressures of 50 and 300 mm Hg, with greater degrees noted for rapid onset times.³⁴ Collectively, these studies provide evidence of local injury and suggest a link between physiologic function or impairment and injury. Functional changes are important with regard to painful injuries, and the electrophysiologic changes have been associated with changes leading to central sensitization of the spinal cord. Although these physiologic changes suggest a potential mechanism by which chronic pain may be initiated, this relation remains uncharacterized in these models because they lack assessment of behavioral sensitivity relevant to pain symptomatology.

Local Biomechanics and Behavioral Hypersensitivity. Whereas *in vivo* animal modeling using cauda equina compression provides useful insight into the functional responses of these such pathologies, it fails to address fully, for example, all the mechanistic issues related specifically to the establishment of persistent pain in association with lumbar radiculopathy. As previously mentioned, with the radiculopathy model used in the authors laboratory, nerve root injury is produced using ligation of the L5 dorsal and ventral nerve roots, which produces behavioral patterns mimicking the hypersensitivity reported in clinical descriptions of painful lumbar radiculopathy⁵⁶ (Figure 2). With continual ongoing adaptation of this injury model, adjustment of ligation severity permits the effects of injury severity on behavioral sensitivity to be investigated.^{15,57,58} As expected from a mechanical standpoint, the more severe the injury (the tighter the applied nerve root ligation), the greater the resulting behavioral hypersensitivity.^{15,57,58} This finding is consistent with the grading of electrophysiologic responses by injury intensity described by Rydevik et al.⁴⁴ It also suggests a specific mechanism by which differential clinical symptoms may be observed. Not only is the nature of the behavioral response dictated by a comparison of injury intensity between "tight" and "loose" silk ligations, but more quantitative methods of injury evaluation have led to increased understanding of this pain model. In vivo image analysis is used to describe the specific injury ligation magnitude by estimating nerve root deformation alone and quantifying compressive tissue strains at injury. For tissue strains of 46% at injury, the amplitude of behavioral sensitivity is significantly higher than that observed in animals receiving tissue compression of 22%.⁵⁷ Most simply, the amount of behavioral sensitivity (measured by mechanical allodynia) produced in this lumbar radiculopathy model, in the absence of any additional inflammatory agents, is directly correlated with the degree of nerve root deformation imposed.⁵⁷ Notably, the complete extreme of this tissue deformation may be considered the transection of the nerve root, by which behavioral sensitivity also can result.

These findings relating injury magnitude and behavioral sensitivity suggest a variety of implications that merit discussion with regard to chronic pain and lumbar radiculopathy. Most obviously, it is suggested that there can be a direct relation between pain-associated behaviors and initial injury severity. Although this finding is somewhat intuitive, the aforementioned in vivo image and correlation analysis is the first to quantify nerve root injury magnitude and mechanical allodynia simultaneously point by point for individual animal subjects. Such analysis is particularly effective in this study using rodent models of pain because rodents appear to be more susceptible to nerve injury-related behavioral sequelae than humans: most animals develop hypersensitivity after injury. In contrast, the human clinical enigma remains, in which imaging studies fail to demonstrate nerve root compression for patients despite their reports of pain symptoms in the face of significant compression and a paucity of symptoms. In these cases, it is possible that a transient deformation or impingement of the affected nerve root occurred before the imaging series and thus was not documented.

Although biomechanical studies are able to document the initial nerve root compression at injury, they are only beginning to delineate the exact relation between injury magnitude and the mechanism through which such injury leads to the onset and maintenance of pain. For example, although a clear relation exists between injury and pain behaviors in this model, it is not currently known whether the removal of the compressive injury consequently lessens behavioral sensitivity or the mechanical injury is simply an inciting event after which local biomechanics become less important. However, in the current radiculopathy model, it is technically difficult to isolate and remove the ligature because of the fibrotic lesions that form over time surrounding the nerve root.

Although experimental study has shown definitively that the magnitude of nerve root compression determines the magnitude of the pain-associated behavioral responses, it remains to be determined whether the injury magnitude is both necessary and sufficient to evoke such a behavioral response. Nonetheless, the integrative implementation of *in vivo* biomechanical techniques in conjunction with animal modeling of pain provides a unique and useful approach for initial examination of chronic pain and the current animal models used for its study (Figure 1).

Whereas the inciting injury event causing nerve root impingement may not itself be an inflammatory event, it is commonly accepted that such an injury produces local inflammation in the area of injury. Histologic experiments using the multilevel cauda equina injury model have reported local edema in nerve roots after their compression.³⁴ It has been hypothesized further that this local cellular response then leads to a swelling of the nerve root structure. Although edema and swelling may be indicators of a previous injury, the current study did not specifically link the incidence of swelling to nociception. Moreover, it remains to be determined whether the biomechanical milieu of the local nerve root actually is a direct modulator of the existence or persistence of pain. In the current model, a temporal description of local swelling in the injured nerve root also has been quantified using in vivo imaging.58 Regional changes in the areas of injury both adjacent and remote from it showed different patterns of swelling for the more severe ligation injuries than for the less severe ones. The overall nerve root swelling profile was more uniform for the less severe cases, yet showed a highly variable regional pattern in the scenarios of greater ligation. Although this study cannot definitively address the relation of swelling to nociceptive mechanisms, it remains to be seen whether the relation is causative or more simply only correlative.

In an attempt at better defining the relation between local biomechanical changes in the nerve root and pain, more recent study has been performed to define the temporal changes in nerve root swelling. This study has shown that the magnitude of the swelling response does not explain the temporal patterns observed for behavioral sensitivity.⁵⁹ Whereas initial swelling at an early stage appears to be dependent on the applied injury, this relation decreases at later stages and does not show a sensitive relation or correlation with initial injury magnitude or behavioral sensitivity. Undoubtedly, a local cellular and structural response will occur in the injured tissue, yet such a response may not be responsible for the maintenance of nociceptive changes as previously hypothesized. Instead, this study suggests that a complicated and multifaceted interplay between mechanics and physiology likely drives chronic pain mechanisms. Moreover, central and cortical roles in these complicated responses cannot be overlooked.

Nerve Root Biomechanics and Central Nervous System Neuroimmune Activation. In working toward an understanding of the effects that mechanical loading have on chronic spinal pain, it is important not to limit this discussion to only one pain behavior. Equally important to understanding the role of biomechanics in persistent pain is characterizing its role in the nociceptive physiologic changes associated with these chronic pain states. As increasing research is being pursued to describe the neuro-immune changes of the central nervous system in persistent pain states,¹¹ it is imperative to begin delineating whether and how mechanics alter the nature of these changes: their magnitudes of responses, temporal

changes in the CNS, and differential responses, if applicable.

Recent interest in pain research has focused on the neuroimmune changes in the central nervous system that contribute to the onset and maintenance of persistent pain.^{6,9,11,56} This collective body of experimental research has documented a robust response of glial cells, microglia, and astrocytes, which become activated in response to peripheral nerve or nerve root injuries. Activation of these cells has been shown to lead to the upregulation and release of numerous pro- and antiinflammatory cytokines, chemokines, and cellular adhesion molecules.9,11,51 Recent studies have documented the infiltration of immune cells from the periphery into the CNS in association with both painful neuropathy and radiculopathy.^{43,52} The role of CNS neuroinflammatory and neuroimmune activation responses in persistent pain is well documented, and has been proposed as one potential mechanism through which central sensitization can occur in the CNS. Whereas the neuroimmune and electrophysiologic responses of the CNS likely work together to affect behavioral hypersensitivity, it is hypothesized that local biomechanics at the injury site actually may modulate both such response cascades.

Indeed, research in the authors' laboratory has focused not only on understanding the role of biomechanics at injury in behavioral responses, but also on understanding how the mechanics at the site of injury modulate the neuroimmune cascades of the CNS in association with these pain behaviors. Recently, such study has focused specifically on the upregulation of cytokines in the spinal cord in the context of injury severity. Using RNase protection assays to detect spinal mRNA in a panel of cytokines (TNF- α , IL-1 α , IL-1 β , IL-6, IL-10) after nerve root injury, a statistically significant correlation was found between the amount of mRNA present and the degree of imposed tissue deformation at injury on postoperative day 7 (Figure 3).57 These findings indicate a modulatory effect of injury magnitude on one aspect of spinal neuroimmune changes of nociception. In an earlier study, using immunohistochemical techniques and a more general grading of injury severity according to a "tight" or "loose" categorization, spinal expression of the proinflammatory cytokine IL-1 β was found to be more intense for "tight" ligation injuries,¹⁵ suggesting that this modulatory effect at injury is preserved at both the message and protein levels for the spinal cytokines in chronic pain responses. This study has initiated the integration of biomechanics in understanding central nociceptive responses. It also has highlighted the need for continued integrative and multifaceted research approaches.

Because the relation of the CNS neuroimmune responses is extremely complicated, examination of one component alone, such as cytokine upregulation, is not sufficient for understanding the mechanism or mechanisms by which mechanics may play a role in initiating and sustaining chronic pain. The study of Hashizume et al¹⁵ using immunohistochemistry techniques allows for

Figure 3. Spinal cytokine mRNA levels show a direct relation to tissue deformation resulting from applied silk ligation. The left panel shows a typical RNase protection assay of injuries rated in terms of severity as normal (n), sham (s), low (l), or high (h). The right panel shows the relative amounts of spinal mRNA for each cytokine. The expression levels significantly correlated with injury strain are indicated by the asterisk (*) in the plot. Other cytokines in rCK-1 probe set not yet detected include TNF- β , IFN- γ , IL-2, IL-3, IL-4, and IL-5. Data from Winkelstein BA et al.57



the *in situ* localization of relevant anatomic and cellular responses in the CNS, but in light of mechanical injury, it also is important to focus attention on the role of biomechanics in glial cell activation responses. Consistent with the grading of behavioral responses and spinal cytokine expression levels according to injury severity, ^{15,57,58} spinal microglial activation, as assessed using staining for OX-42 (CR2/CD11b), is more intense for greater nerve root deformation at injury.^{15,55} In contrast, astrocytic activation, as measured using glial fibrillary astrocytic protein (GFAP), does not show any dependence on injury mechanics at any postoperative time point assessed (days 1, 3, 7, 14).⁵⁵ Although the OX-42 immunoreactivity findings appear at first examination to be consistent with the other changes in the CNS, on closer review in conjunction with the corresponding behavioral sensitivity, the "clinical" picture becomes more complicated to understand.

Although OX-42 staining for microglial activation may provide a highly sensitive marker of cellular activation in response to injury, it may not be the most appropriate or selective marker for pain. For instance, it should be noted that for this painful lumbar radiculopathy model, sham surgeries produce OX-42 staining in the spinal cord in the absence of any pain behavioral hypersensitivity, GFAP staining, or other physiologic changes.^{15,55} Despite the behavioral data and OX-42 staining responses, both showing a graded intensity based on the initial injury severity, the matched behavioral data of each animal on the specific day of OX-42 assessment did not show a correlation with its OX-42 staining. Whereas behaviors decreased over time, microglial activation did not.

This study was a simple investigation, yet its findings raise a number of interesting points with regard to persistent pain and its mechanisms. For example, it suggests that microglial activation, as assessed by OX-42, may not explain behavioral patterns because it may not be directly responsible for them. It is known that subpopulations of activated glia express membrane proteins believed to play a role in persistent pain,^{43,52} and that proteins such as MHCII or CD4 potentially may provide a better explanation of behavioral sensitivity patterns and nociception and may be more directly and finely modulated by mechanical factors. Moreover, these findings also highlight the fact that biomechanics at injury in lumbar radiculopathy models may differentially modulate some neuroimmune responses and not others. In other words, it may be possible that beyond a certain degree of nerve root deformation, the cellular activation responses do not differ, whereas cytokine production and release responses are modulated differentially. This suggests a threshold for activation above which either certain subpopulations of cells are activated or cascades lead to maintenance *versus* the onset of a pain response.

Biomechanical Thresholds for Pain. In light of all the contributing factors presented in this article, it is possible to begin determination of such a threshold or "tolerance" value for tissue loading and persistent pain in the current model. First, however, this discussion must address the particular context of such a threshold number. For nociceptive responses, it is appropriate to consider two such relevant criteria of interest: a threshold for initiation of nociceptive responses and one for maintenance of such a response. The maintenance threshold is of particular interest for clinical management of chronic pain. In acute pain syndromes, the physiologic responses (some of which have been discussed earlier) are "somehow resolved," as is behavioral sensitivity. However, the chronic cases are those that require more consideration of appropriate management and treatments.

In this regard, the current *in vivo* biomechanical techniques for quantifying applied tissue deformations and radial strains are very useful for determining what degree of nerve root tissue strain is responsible for which types of responses. For example, using this technique, it has been determined that the nerve root compression in sham procedures, wherein no nerve root injury is applied and no behavioral sensitivity produced, is 0.5%. Therefore, this value represents an error measurement of this particular biomechanical approach.⁵⁷ For a ligation injury categorized qualitatively as "very loose," with a mean compressive strain of 12.5%, the behaviors also remain unremarkable, within the range of those produced in sham surgeries (unpublished data). This study suggests a lower boundary for such a threshold for pain initiation. In contrast, for "tight" ligation strains with a mean value of 22%, pain-associated behaviors are significantly elevated over those of shams and maintained for at least 7 days. For even more severe, applied ligations (45.6% mean strain at injury), the behavioral responses are yet again significantly more elevated over those of the tight ligation group and the sham group. Although these tissue strain values provide upper limits to initiation and maintenance of pain behaviors, they do not fully define such a threshold value. Nonetheless, it can be hypothesized at this point that nerve root deformations somewhere in the range of 12% to 22% result in the initiation and persistence of a behavioral pain response. More study is needed to define specifically which value in this range is required to produce pain behaviors, then which other threshold of tissue strain, if any, is required to maintain these behaviors in a chronic condition. Moreover, it is worth noting that because of inherent host (individual) differences, the thresholds are most likely different across a range of individuals in a population. Yet there may in fact be a series or cascade of events that determine or predict most cases.

Further complicating this effort to define thresholds of tissue injury for pain in these models, it must be recognized that the study described so far in this section has involved the case of applied mechanical injuries only, in the absence of any additionally applied inflammatory injury. For the clinical scenario of nerve root impingement caused by disc herniation, it has been widely accepted that such an injury has both a mechanical component (compression of nerve root) and a chemical component (inflammatory nucleus material extruded and contacting the nerve root). Study has been initiated using similar biomechanical techniques for ligations with chromic gut material. In this way, direct comparisons can be made with the silk ligation model, in which the mechanics are preserved, and only chemically irritating material is added to the injury scenario. Chromic ligations decrease the threshold of the tissue strain and deformation required to evoke behavioral sensitivity (unpublished data). It has been shown that in the absence of any mechanical injury, chemical agents (*i.e.*, TNF- α and IL-1) alone can elicit electrophysiologic changes³⁴ in both nerve roots and behavioral hypersensitivity.¹⁹ Therefore, it is not surprising that the chemical injury produces a greater behavioral response in combination with a mechanical injury.

Although the chromic ligation findings again may be intuitive, particularly interesting is the finding that unlike the significant positive correlative relation between magnitude of tissue strain and mechanical allodynia observed in the silk injury model, no such positive relation exists for the chromic ligation injury model (unpublished data). In fact, when applied strain at injury using a chromic ligation exceeds 5%, the degree of behavioral sensitivity is not sensitive to the applied injury magnitude, suggesting that in this scenario, dependence on injury magnitude is less important than in the absence of inflammation. Returning to the clinical conundrum associated with impingement and pain symptoms, this finding may explain some of the uncertainty associated with larger impingements and less pain as well as smaller impingements and more pain. In cases of nucleus pulposus herniations, the pain symptoms may be much less sensitive to the mechanical component of injury and may result from both "small" and "large" impingements. Regardless, this study has highlighted an area of research that needs further investigation for a fuller understanding of the factors affecting the mechanism of chronic pain, and for useful information that can be interpreted properly in the context of the clinical problem.

Conceptual Model of Events Linking Abnormal Mechanics to Spine Pain

By combining the findings discussed in the preceding sections, it is possible to begin synthesizing a working conceptual model of events (injury and physiology) that links local mechanics to spine pain (Figure 4). At injury, mechanical and chemical injuries (or mechanical injuries only) initiate a complicated cascade of interrelated events. These initiating mechanical injuries may result from abnormal motions or loads to neural tissue. The initial injury event induces structural tissue changes such as swelling, and material changes such as edema and local cellular changes. These local mechanical responses serve as initiators of the further signaling from the periphery into the CNS. However, local inflammatory and immune responses are initiated simultaneously in the region of injury because of the initial insult. These physiologic responses act in concert with the electrophysiologic signaling responses of neuronal transmission. Whereas these initial events likely each contribute to the perception of injury in the CNS, they also have a very complicated relation with each other. For example, local inflammation may induce neural structural changes that may in turn be directly responsible for modulating local electrical changes in the neural tissue.

Nonetheless, these signals of injury, or even perceived injury, then in turn initiate an equally complicated cascade of nociceptive physiologic responses in the CNS (Figure 4). A neuroimmune response is mounted in the spinal cord, where neurons and glia produce many proinflammatory cytokines, which can in turn induce the expression of numerous algesic mediators that lead to enhanced nociceptive activity, and thus pain. In conjunction with the activation of these cells, cellular adhesion molecules are upregulated and peripheral T cells and macrophages infiltrate across the blood-brain barrier into the parenchyma of the spinal cord. This neuroinflammatory response can itself also enhance the neuroimmune activation responses of the CNS and provide enhancement of algesic mediator production. It is important to recognize also that as these CNS changes are



Figure 4. Proposed conceptual model describing the sequence of events at injury and afterward that lead to chronic pain. Both biomechanical and physiologic responses, as important contributors in this complex cascade of events, have complicated relations with each other and the final pathway that elicits pain.

transmitted to the brain, affecting supraspinal pain mechanisms, the perception of chronic pain itself may play a role in feeding back on the nociceptive responses discussed in this article. Together, the mechanical and physiologic pathways leading from injury to chronic pain are tremendously complicated and involve a highly dynamic process. Although the conceptual model described in this article (Figure 4) is based on injury to neural tissue, it certainly can be broadened to include any tissues of the spine, such as disc and muscle tissue, that have innervation and potential for mechanical loading.

Summary

This proposed conceptual model of chronic spine pain integratively describes the dynamic roles of injury, biomechanics, and nociceptive physiology in chronic pain. However, it also helps to focus and redirect a working understanding of chronic spinal pain. In this way, the bidirectional-translational approach to understanding chronic pain can be modified (Figure 5). This evolving understanding of chronic pain must continue to address important clinical questions while working to refine the existing models of pain. For example, with the incorporation of cross-disciplinary approaches, such as the *in vivo* biomechanical techniques used in this the current study, it is evident that injury model modifications may continue to be necessary when the clinical picture is considered. At the same time, however, research must continue to delineate each aspect of the cascade presented in Figure 4 for better provision of treatment and management options. This aim of this article is to review relevant concepts of nociceptive processing, animal modeling, and tissue biomechanics in the context of painful lumbar radiculopathy. Of course, in humans, it is extremely important to understand cortical activities, and basic research must be directed at this level. The authors, however, are very much aware of the interplay of midbrain and cortical functions with overall behaviors. In the study of pain, there are no lower injury limits. The authors hope that this discussion of the responses important to a better understanding of the mechanisms by which tissue injury leads to pain can help to guide the treatment and management of these symptoms and syndromes.



Figure 5. The integrative approach described in Figure 1 must be ever evolving to incorporate the findings and collective knowledge regarding spine pain. As such, this modified approach is proposed for future work in this area that can lead to an evolving understanding of spine pain and facilitate its clinical management.

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Key Points

• The nociceptive processes of physiologic and pathologic chronic pain are different, and may require differential treatment approaches.

• Using animal models of low back pain and lumbar radiculopathy, a host of specific spinal neuroimmune alterations have been documented in association with measures of behavioral hypersensitivity, and local nerve root biomechanics have been demonstrated to alter both spinal immune and behavioral changes.

• This summary of existing knowledge regarding nociceptive and pain processing in animal models of chronic pain can serve to guide potential areas in future low back pain research.

• A proposed conceptual model of chronic spine pain describes the integrative nature of injury, biomechanics, and nociceptive physiology of such pain.

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