HHS Public Access

Author manuscript

*J Orthop Res*. Author manuscript; available in PMC 2019 May 01.

Published in final edited form as:

*J Orthop Res*. 2018 May ; 36(5): 1305–1312. doi:10.1002/jor.23741.

# Animal Models for Studying the Etiology and Treatment of Low Back Pain

**Changgui Shi**[#1], **Sujun Qiu**[#2], **Scott M. Riester**[3], **Vaskar Das**[4], **Bingqian Zhu**[5], **Atiyayein A. Wallace**[4], **Andre J. van Wijnen**[3], **Fackson Mwale**[6], **James C. Iatridis**[7], **Daisuke Sakai**[9], **Gina Votta-Velis**[8,10], **Wen Yuan**[1], and **Hee-Jeong Im**[10,11]

[1]Department of Orthopedic Surgery, Changzheng Hospital, Second Military Medical University of China, Shanghai, China,

[2]Department of Orthopedic Surgery, Zhujiang Hospital, Southern Medical University, Guangzhou, China,

[3]Department of Orthopedic Surgery, Mayo Clinic, Rochester, Minnesota,

[4]Department of Biochemistry, Rush University Medical Center, Chicago, Illinois,

[5]Departments of Biobehavioral Health Science, University of Illinois at Chicago (UIC), Chicago, Illinois,

[6]Department of Surgery, McGill University and Orthopaedic Research Laboratory, Lady Davis Institute for Medical Research, SMBD-Jewish General Hospital, Montreal, Canada,

[7]Leni & Peter May Department of Orthopaedics, Icahn School of Medicine at Mount Sinai, New York, New York,

[8]Department of Anesthesiology, University of Illinois at Chicago (UIC), Chicago, Illinois,

[9]Department of Orthopaedic Surgery, Tokai University School of Medicine, Kanagawa, Japan,

[10]Jesse Brown Veterans Affairs Medical Center (JBVAMC) at Chicago, Chicago, Illinois,

[11]Department of Bioengineering, University of Illinois at Chicago (UIC), Chicago, Illinois

[#] These authors contributed equally to this work.

## Abstract

Chronic low back pain is a major cause of disability and health care costs. Effective treatments are inadequate for many patients. Animal models are essential to further understanding of the pain mechanism and testing potential therapies. Currently, a number of preclinical models have been

*Correspondence to:* Hee-Jeong Im and Wen Yuan (T: 312.569.7871 [H.-J. I.], +86-21-81885637 [W. Y.]; F: 312.569.8114 [H.-J. I.], +86-21-63520020 [W. Y.]; E-mail: hee-jeong.sampen@va.gov [H.-J. I.], smmuyuanwen@163.com [W. Y.]).

AUTHOR'S CONTRIBUTIONS

CS and SQ were in charge of organizing the review framework, data collecting and manuscript writing and they contributed equally to this work. SMR, VD, BZ, and AAW helped in manuscript editing and revising. AJW, FM, JI, DS and GV helped in the revising and complementing of the manuscript. WY and HJI provided their support in experimental design of all included studies, acquiring project funding, and manuscript editing. All authors have read and approved the final submitted manuscript.

Conflicts of interest: None.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

developed attempting to mimic aspects of clinical conditions that contribute to low back pain (LBP). This review focused on describing these animal models and the main behavioral tests for assessing pain in each model. Animal models of LBP can be divided into the following five categories: Discogenic LBP, radicular back pain, facet joint osteoarthritis back pain, muscle-induced LBP, and spontaneous occurring LBP models. These models are important not only for enhancing our knowledge of how LBP is generated, but also for the development of novel therapeutic regimens to treat LBP in patients. © 2017 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 36:1305–1312, 2018.

**Keywords**

low back pain; animal model; discogenic pain; pain assessment; dorsal root ganglia; spinal cord

Low back pain (LBP) is a serious medical condition that ranks among the most common reasons for patient visit.[1] The estimated cost of LBP in the U.S. is over $100 billion each year.[2] Given the tremendous economic burden of LBP, more effective therapies are much needed. LBP can be caused by various factors including intervertebral disc disease, facet joint osteoarthritis, spinal stenosis, foraminal stenosis, muscle fatigue, and radiculopathy. All of those conditions can be strongly influenced by psychosocial factors.[3] Common treatments for back pain include oral non-narcotic pain medications (e.g., acetaminophen), non-steroidal anti-inflamma-tories, and non-addictive opioids (e.g., tramadol).[4,5] Local anesthetics and corticosteroids can be delivered to pain sites via guided injection procedures. While guidelines for the use of these pharmaco-therapies have been developed for LBP, relatively few high quality clinical trials on nonoperative treatments exist, and these treatments are not tailored specifically for LBP.[6,7]

A major challenge in effective LBP treatment is the development of translational animal models that can simulate LBP observed in human. Animal models that can effectively simulate the histopathology and LBP-related pain behaviors are fundamental for future research. Thus, the aim of this review was to provide an overview of the animal models used to simulate LBP and summarize methods used to evaluate and quantify pain symptoms.

## PAIN PATHOGENESIS

Various anatomic pain generators (e.g., disc, facet joint, muscle, ligament, fascia) could induce LBP.[8] When the generators are stimulated by a noxious stimulus with sufficient intensity, a nerve impulse is created. The pain information from the periphery is then conveyed to the spinal cord dorsal horn via primary afferent sensory neurons. After processing the pain signals, the nociceptive neurons in the dorsal horn provide ascending signals to several brain regions, such as the parabrachial nucleus and thalamus.[9,10] These regions further neuronally connect with the somatosensory cortex, amygdala, prefrontal cortex, insular cortex and anterior cingulate cortex, areas which have shown to be core brain regions ascribed with sensory/discriminative, affective/motivational, and cognitive/evaluative functions during the application of noxious stimuli.[10] Recent studies revealed the structural and functional alterations of the brain in patients with chronic LBP, confirming the role of the brain in LBP condition.[11,12]

Pathways involved in the pathogenesis of LBP include nociceptive, neuropathic, inflammatory, and mixed pain.[13–15] Nociceptive pain does not occur spontaneously and usually is triggered by a painful stimulus (e.g., traumatic injury and burn). It is mediated by the activation of Aδ and C nerve fibers and is typically reversible after stimulus removal and tissue healing.[15,16] Neuropathic pain is caused by nervous system malfunction mediated by direct nerve damage or systemic illness. It is often associated with allodynia or pain sensation caused by a non-painful stimulus. Neuropathic pain is clinically important because it is often chronic and irreversible. Spinal stenosis and radiculopathy are frequent causes of neuropathic pain involving the spine.[14] Inflammatory pain is immunologically mediated and can be caused by inflammatory stimuli (e.g., infection) or autoimmune disorders such as ankylosing spondylitis and rheumatoid arthritis.[17] Mixed pain is caused by multiple pain pathways acting together to generate a pain experience.[18]

## ANIMAL CONSIDERATIONS

Various animal species have been used to study LBP, each with advantages and disadvantages. Ideally, the animal should be cost-effective, able to express pain and mimic human spine anatomy. Primates are ideal for studying LBP, but they are expensive and difficult to manage, particularly during pain assessment. Other large animal models, including rabbit, pig, dog, and goat, have been used successfully to study molecular and morphologic changes in the spine. A major drawback of these models is currently the lack of reliable pain assessment techniques. For instance, rabbits are either insensitive or unable to express the pain that can be quantified using current techniques.[19]

Rodent models are the most widely used animal to study LBP. Compared to other larger animals, they are inexpensive and can express pain that are readily quantifiable. Rat models are large enough to allow for surgical alteration of the back structures to recreate intervertebral disc and facet joint disease. Transgenic mouse models can be used to simulate various spine pathologies.

## PAIN ASSESSMENTS IN LBP MODELS

The methods used to assess low back and radiating pain condition in patients usually include history, physical examination, and self-ratings of pain. The physical examination begins with visual inspection, palpation of the spine, assessment of spinal motion, and then neurologic examination, including muscle strength test, sensation to light touch and pinprick, deep tendon reflexes, and straight leg raising test.[17] Human self-ratings of pain, using both questionnaires and scales, are often used to evaluate the pain intensity and treatment outcome. To date, various pain rating tools have been developed in which the Numeric Pain Rating Scale, Brief Pain Inventory, Pain Disability Index, McGill Pain Questionnaire, and Visual Analog Scale are most commonly cited.[20] Pain assessments in animals are challenging because animals can not report pain in the same way as humans. Pain behavior tests are usually used in animal models as indirect evidence to evaluate the severity and progression of pain. The methodology to quantify LBP behaviors in animal models can be categorized into spinal reflexive and non-reflexive pain tests (Table S-1).

## Spinal Reflexive Pain Tests

Reflexive pain tests are commonly employed to measure altered nociceptive thresholds.[21,22] These pain tests measure the latency to withdraw or escape from a heat, cold, and mechanical stimuli through activating nociceptors at the testing site and inducing localized, stereotyped motor responses.

**Mechanical Hyperalgesia—**Behavioral measures of mechanical sensitivity are commonly used to assess mechanical hyperalgesia and allodynia.[21–23] Mechanical hyperalgesia is evaluated by the hind paw withdrawal threshold in response to probing with von Frey filaments.[24] Most of the models show increased mechanical hyperalgesia in the hind paw.[21–23] Besides hind paw, tail, and low back spinous process have also been used in LBP models. In contrast, tactile allodynia is evaluated by the changes in withdrawal threshold to innocuous stimuli. Sensitivity to non-noxious stimuli is tested by stroking with a cotton wisp at the plantar surface to induce a pain reflex.[25] Vocalization threshold in algometer test can also measure mechanically evoked pain responses. The mechanical pressure is applied to a dorsal skin with an algometer. An audible vocalization is used as the end-point and the threshold in response to applied force is measured to reflect the pain state.[19]

**Thermal Hyperalgesia—**Several techniques can be used to assess thermal hyperalgesia. An early developed thermal testing is the tail-flick test,[26] where the latency to a tail-flick response is measured after dipping the tail into the hot or cold water. Subsequently, the Hot-plate test is developed to measure the latency to either licking the paw or withdrawal of the paw from the hot-plate.[27] Another commonly used heat sensitivity test is the Hargreaves test, which measures the latency to withdrawal of the hind paw from a more localized heat stimulus.[28] The acetone test is usually used to evaluate cold sensitivity.[29] In this test, the total time spent in acetone-evoked behaviors over 1 min is recorded after a drop of acetone applied to the plantar surface of the hindpaw or the dorsal skin.

## Spinal Non-Reflexive Pain Tests

**Spontaneous-Related Behaviors—**Spontaneous behavior changes have been suggested to better relate to the general condition and presence of pain. Olmarker et al.[30,31] are the first to use spontaneous behavior analysis to assess pain discomfort in LBP models. In their studies, the behavioral test was conducted using a home cage monitoring for abnormal behaviors, including altered locomotor activity, rearing, grooming, bending, or “wet dog shaking” behaviors. Other non-reflexive pain measurements include weight bearing test in which the distribution between paws/gait is analyzed, and CatWalk system which is an automated quantitative gait analysis that allows the objective and rapid quantification of individual paw parameters as well as parameters related to inter-limb coordination.[32,33] The advantage of spontaneous pain assessment lies in the absence of the physical experimenter, which decreases the animal’s stress caused by handling or experimenter presence and can lead to a more “natural” pain phenotype produced by the animal.

**Movement-Evoked Hypersensitivity—**Two different pain tests have been used in SPARC (the secreted protein, acidic, and rich in cysteine)-null mouse to examine the

hypothesis that degenerating discs causes back pain.[34] The first test is the tail suspension assay, which involves suspending a mouse tail and observing attempts to support itself by grabbing the base of its tail to relieve axial strain on the spine. The second is the grip force test, which involves stretching a mouse tail back with the mouse forelimb gripping a metal bar and recording the peak force at the point of release.

## ANIMAL MODELS FOR SIMULATING BACK PAIN PATHOGENESIS

Animal models of LBP can be subdivided into the following categories: Discogenic LBP, radicular back pain, facet joint osteoarthritis back pain, muscle-induced LBP, and spontaneous occurring LBP models (Table S-2).

### Discogenic Pain Models

Lumbar intervertebral disc degeneration is the most common cause of LBP. Discogenic LBP occurs in the absence of spinal deformity and signs of neural tension. It arises from the disc itself. A few models have been developed to mimic the clinical features of discogenic LBP.

Olmarker K[30] was the first to report the changes of pain behaviors in a rat lumbar IVD model. The 4th/5th lumbar (L4/5) intervertebral disc was punctured with nucleus pulposus (NP) leakage using a 0.4-mm diameter injection needle through the posterior approach after removing the left facet joint between the 4th and 5th lumbar vertebra. The rats with disc puncture showed significant differences in spontaneous behaviors, including an increased grooming duration for up to 7 days and “wet-dog shakes” behavior for up to 21 days. Those behaviors changes suggested discomfort and pain. However, it is difficult to determine whether the observed changes were induced by the disc injury or by the intraspinal presence of NP. To address this issue, the authors induced a dorsal superficial disc injury (scraping the disc surface) with no penetration to the NP, and placing an allograft NP on the disc surface without simultaneous disc injury. The same injury approach through a ventral side was also used. Behavior analysis demonstrated that application of nucleus pulposus is more likely to induce changes in grooming and “wet dog shakes” than the disc injury alone.[31] These findings suggest that the behavioral changes observed in rats with disc puncture are more likely related to the epidural presence of NP than the disc injury. This study also showed that ventral disc puncture did not induce any behavioral changes as compared with the sham exposure. Similar results were observed by Li et al.[22] who established a discogenic pain model by anterior and posterior annular puncture. The anterior puncture used a ventral abdomen incision approach and the disc injury was induced by inserting a 21-gauge needle into the L4/5 disc for 30 s at a depth of 3 mm. The posterior puncture was induced using the same approach described by Olmarker K.[30] Pain behavior assessments showed that the anterior disc puncture did not induce any pain behavior changes. In contrast, the posterior disc puncture resulted in mechanical allodynia for up to 21 days after surgery. The puncture also resulted in high expressions of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6. The researchers concluded that both cytokine expression and posterior annulus fibrosus (AF) rupture are essential for pain behavior changes in the degenerative intervertebral disc.

On the contrary, Kim et al.[19] developed a chronic discogenic LBP model in rats using an anterior surgical approach to the spine through the abdomen. The L4/5 and L5/6 intervertebral discs were disrupted using a microsurgical drill (0.5–0.8 mm in diameter), followed by removal of the NP. Nine weeks after the surgery, there was apparent X-ray evidence of disc space narrowing and histologic changes marked by infiltration of inflammatory cells and disruption of the IVD architecture. Pain behavior measurement showed that the pressure force (measured using an algometer) needed to generate vocalization decreased significantly for up to seven weeks in the rats with disc drill puncture. The cytokines involved in neuropathic pain pathways were highly stimulated in both the dorsal root ganglia (DRG) and the spinal cord (SC) after injury.

As an alternative to complete removal of the NP, discogenic pain can be induced via injection of various reagents into the intervertebral disc. Lee et al.[21] established a rat chronic discogenic pain model by injecting 10 μl of Complete Freund's adjuvant(CFA) into the L5/6 IVD using a 26-gauge needle through an anterior transperitoneal approach. Pain behaviors were evaluated by mechanical and thermal allodynia tests, as well as weight loading measurement. There was a marked decrease in the withdrawal threshold of the bilateral hindpaws in the CFA rats up to 7 weeks after surgery. This model was also verified by progressive degenerative changes of the discs and upregulation of pain mediator in the DRG and the bilateral SC dorsal horns in the CFA group. However, the results of this model should be interpreted with caution. The injection volume (10 μl) is much higher for the disc space so that the CFA may leak out from the disc and irritate the surrounding tissues.

Lai et al.[35] induced discogenic pain by injecting phosphate buffered saline (PBS) into the nucleus pulposus of the rats. The L3/4, L4/5, and L5/6 intervertebral discs were assessed using an anterior abdominal approach. The researchers injected 2.5 μl PBS into each disc using a 26-gauge needle inserted to a depth of 3 mm. This model reliably induced intervertebral disc degeneration as determined by disc space narrowing measured by radiographs, MRI, and histologic evaluation. In this study, hindpaw mechanical and thermal sensitivity, as well as grooming and gait behavior were used to evaluate the pain behavior changes. The results showed that PBS injection altered pain behaviors with an increased grooming duration, decreased mechanical withdrawal thresholds, and decreased thermal withdrawal latency. In contrast to Kim et al. study,[19] Lai et al.[35] found that mechanical hindpaw sensitivity might be the most sensitive pain measurement.

Still using an in vivo rat lumbar spine injection model, Lai et al.[23] further investigated the roles of inflammatory cytokine and pro-neurovascular growth factors in discogenic pain. In their study, the L3/4, L4/5, and L5/6 discs were injected with 2.5 μl PBS, TNF-α, or nerve growth factor (NGF)/vascular endothelial growth factor (VEGF), using a 26-gauge needle at a depth of 1.5 mm (shallow) or 3 mm (deep). All the injection groups displayed disc degeneration changes such as decreased disc heights, reduced MRI signal intensities, and structure deformity. Pain behavior assessments showed that TNF-α and NGF/VEGF groups had significantly lower mechanical withdrawal thresholds than the PBS group. The rats' painful condition in the deep puncture group was much worse than that in the shadow group. This result was correlated with disc structural changes, in which deep injury induced more severe and accelerated degenerative alterations than the shadow injury. These findings

suggest that the disc inflammation and structural deformity are vital for driving pain-related behavioral changes.

## Radicular Back Pain Models

Many preclinical models have been developed attempting to mimic aspects of clinical conditions that contribute to radicular pain models. They include the application of nucleus pulposus material near the lumbar DRG, chronic compression of the DRG, or localized inflammation of the DRG.[36]

**Nucleus Pulposus Application to the DRG Models—**Intervertebral disc herniation is an important risk factor for LBP and sciatica. An animal model of radicular pain induced by the protrusion or herniated disc was first developed in 1996.[37] The authors performed an L6 lateral laminectomy and implanted allograft materials (NP, AF, and adipose tissue) into the lumbar epidural space. Results showed that the rats with the application of NP and NP plus AF produced mechanical and thermal hyperalgesia after 1 or 2 weeks of surgery. Histology further demonstrated that phospholipase A2 (PLA2) and nitric oxide synthase (NOS) were markedly increased in and around the allograft intervertebral disc materials. However, it is possible that the chemical changes resulted from immune responses to allograft tissue. In order to understand the mechanisms, the same group generated another radicular pain model using autologous intervertebral disc materials.[38] The procedures were performed by exposing left L4 and L5 nerve roots after hemilaminectomies, and then transplanting autologous NP and/or AF obtained from the coccygeal discs on each of the nerve roots. In this model, rats in the NP application group showed mechanical hyperalgesia for 3 weeks after surgery, but not thermal hyper-algesia. On the contrary, rats in the AF application group produced thermal hyperalgesia for 1 week. The mechanical and thermal hyperalgesia were found reduced by the epidural injections of a selective inhibitor for PLA2 or NOS, respectively. These findings indicated that the applied intervertebral disc affected by inflammatory cytokines contributed directly to radiculopathy.

Sasaki et al.[39] then developed a rat model by drilling a partial laminectomy hole (2.5 mm) in the left L5 vertebral arch using a 1-mm drill under a microscope. Nucleus pulposus harvested from a coccygeal disc was subsequently applied at the proximal end of the left L5 nerve root through the laminectomy hole. The rats with NP application presented mechanical hyperalgesia for up to 4 weeks, which could be reduced by anti-TNF-α antibody administration. This model indicated that TNF-α is essential in the production of pain behavior changes induced by application of NP to rats.

To further improve this model, Wei et al.[40] used a simplified surgical procedure in which only left side L5 hemilaminectomy and L5/6 facetectomy were performed to expose the L5 DRG. Autologous NP obtained from the tail was then placed onto the DRG and epidural space. The rats produced a mechanical hyper-algesia response lasting for 4 weeks and expressed a high level of NOS and Cyclooxygenase-2 (COX-2) in DRG. This model elicited a similar duration of hyper-algesia response to Sasaki's[39] model but obviated the use of a 1-mm drill and surgical microscope. Using a similar approach, Shamji et al.[41] exposed the right L5 DRG followed by autologous tail NP implantation. In addition to mechanical

allodynia, the NP-treated rats also showed gait abnormalities characterized by changes in gait symmetry and onset of duty factor imbalance loading the contralateral limb.

To mimic clinical situation, Omarker et al.[42] developed a rat radicular pain model where autologous NP leaked from the adjacent disc. In this model, L4/5 facetectomy was performed to expose the L4 DRG and L4/5 intervertebral disc. A 25-gauge needle was used to puncture the exposed disc and inject some air to produce a small NP herniation. The same needle was used to induce a medial displacement of the L4 DRG. The combination of disc puncture and displacement induced mechanical and thermal hyperalgesia lasting for 18 and 14 days, respectively. This study suggests that both the DRG compression and displacement were major causes of pain.

**Chronic Compression of the DRG (CCD) Models—**Intervertebral foramen stenosis is another important risk factor for LBP and sciatica. CCD is one such model developed for the specific case of radicular pain induced by stenosis. Hu and Xing[43] were the first to establish the CCD model in which the intervertebral foramen at L5 on one side was exposed, and L-shaped stainless steel rods (0.5–0.8 mm) were inserted to beneath the intervertebral foramen. The rods remained in place for the duration of the experiment to produce a steady compression against the L5 DRG. The rods chronic compression produced hyperalgesia of the ipsilateral hind paw to noxious heat stimulus for up to 6 weeks. Using a similar surgical procedure, Song et al.[44] compressed two DRGs (L4 and L5) instead of one, which caused hyperalgesia in responses to mechanical and thermal stimuli for 5 weeks. In this study, a dynamic mechano-allodynia was assessed by drawing a cotton bud cross the skin. The results showed that the foot ipsilateral to the chronic compression elicited a reflex withdrawal to a cotton wisp within 2 weeks. The CCD model has also been implemented in mice using the same surgical procedures as Song et al.[44] The CCD mice displayed dramatic mechanical and thermal hyperalgesia as well as tactile allodynia.[45]

Instead of a metal rod, Gu et al.[46] infiltrated the L5 intervertebral foramen with a hemostatic matrix (SURGIFLO). This novel model resulted in persistent mechanical allodynia and thermal hyperalgesia for up to 4 or 5 weeks after surgery. Upregulation of the N-methyl-D-aspartate receptor and inhibitory factor kb-a within the ipsilateral L5 DRG and spinal cord dorsal horn was also observed.

Nerve roots ligation is another kind of compression model. Kawakami et al.[47] were the first to present this model in which the left L4–L6 DRGs and nerve roots were exposed by laminectomy and facetectomy. The nerve roots were then injured with either chromic gut or silk ligatures. The rats treated with chromic gut ligatures showed thermal hyperalgesia for up to 10 weeks, and mechanical hypoalgesia and motor deficits for 4 weeks postoperatively. Hashizume et al.[48] used the similar procedure and only ligated L5 nerve roots. The results showed that both the chromic gut and silk ligatures induced mechanical allodynia and thermal hyperalgesia, as well as higher expressions of OX-42 and IL-β protein in SC.

**Inflammation of the DRG (LID) Models—**Inflammatory processes are thought to play key roles in LBP and contribute to neuropathic pain. To investigate the role and mechanisms of inflammatory responses within the DRG in the development of chemogenic pathological

pain, Xie et al.[25] developed a new animal model of localized LID model. In this model, DRG inflammation was induced by a single deposit of the immune activator zymosan in incomplete Freund's adjuvant in the epidural space near the L5 DRG via a small hole drilled through the trans-verse process. After a single zymosan injection, rats developed bilateral mechanical hyperalgesia and allodynia for up to 4 weeks. Local inflammation remarkably increased the incidence of spontaneous activity of A- and C-fibers in DRG, and also induced macrophage response and glial activation. Other approaches to locally inflame the lumbar DRG region use CFA and injection of inflammatory soup into the intervertebral foramen.[49,50] CFA application induced mechanical and thermal hyperalgesia, as well as higher levels of inflammatory cytokines in DRG.

## Facet Joint Osteoarthritis Back Pain Models

Facet joint osteoarthritis (FJOA) is another clinically important cause of chronic LBP. FJOA can be generated using chemical agents such as collagenase, plasminogen activator, and monosodium iodoacetate (MIA) that promotes degradation of the articular cartilage surface,[51–53] or through direct mechanical injury to the articular cartilage surface.

Kim et al.[53] developed a FJOA back pain model to evaluate pain behaviors after intra-articular injection of MIA in rats. The researchers performed a posterior midline incision and exposed the left spinal facet joints. A 26-gauge needle was then used to inject0.02 mg of MIA dissolved in 1 μl of normal saline into the left L3/4, L4/5, and L5/6 facet joints. Biochemical assessments and μCT imaging revealed severely damaged facet joint cartilage, proteoglycan loss, and alterations of the subchondral bone structure at 7 weeks post-injection. Pain behaviors were evaluated using the algometer vocalization test and the straight leg raising test. Similar to the studies on discogenic pain model, the authors found the vocalization pressure threshold on the MIA-injected ipsilateral side of the lower back was less than the sham animals, which was correlated with facet joint degeneration.

There have been concerns that chemical agents may cause inflammation and hyperalgesia leading to acute neuropathic pain symptoms not normally observed in chronic LBP. A recent study has shown that osteoarthritis can be induced by simply scratching the articular surface with a needle instead of using a chemical adjuvant.[54] This technique was performed by inserting a 21-gauge butterfly needle accurately to access the joint space through the skin. A microsyringe with a 26-gauge needle was then inserted into the butterfly needle to puncture facet joints (L3/4, L4/5, and L5/6). The percutaneous puncture injury resulted in osteoarthritis-like structural changes in the facet joints cartilage and subchondral bone. Rats with injury developed ipsilateral primary pressure hyperalgesia for over 12 weeks as assessed by the algometer vocalization test. This model suggested that minimally invasive techniques that do not rely on chemical adjuvants may be preferable to traditional osteoarthritis models.

## Increased Intramuscular Pressure-Induced LBP Model

Increased intramuscular pressure (IMP) within the lumbar paraspinal muscles is reportedly a cause of LBP.[55] To elucidate the pathology of LBP accompanied by IMP, Kobayashi et al.[56] developed an experimental rat IMP-induced LBP model by inflating a balloon within the

paraspinal muscles of the lumbar spine (from T13/L1 to L5/6). In this balloon model, the IMP of the paraspinal muscles was significantly elevated with a reduction of intramuscular blood flow in the balloon rats at 1 day after insertion. The expression of substance P, a pain-related neuropeptide, was also significantly increased in the L1 DRG. However, the limitation of that study is no pain-related behavioral tests.

### Transgenic Mouse Models for LBP

Various transgenic mouse models have been developed to evaluate the spinal pathology. Unfortunately, only few have evaluated pain behaviors in spine degeneration. The SPARC-null mouse was shown to reliably develop degenerative disc disease, characterized by disc space narrowing and disc herniation by 6 months.[57] In this model, Millecamps et al. evaluated back pain using tail suspension assay, grip force assay, locomotor capacity, as well as hypersensitivity to mechanical, cold, and heat stimuli.[34,58] The pain tests showed that old SPARC-null mice produced cold allodynia on the plantar surface of the hindpaw and on the lumbar skin. Both young and old SPARC-null animals displayed significant impairment in the grip force test and significantly shorter duration of immobility in the tail suspension test than the wide-type mice. The results indicated that SPARC-null mice experienced significant stretch-induced discomfort, suggesting axial LBP. In another study, the same group noted that SPARC-null mice displayed age-dependent upregulation of the calcitonin gene-related peptide (CGRP) and neuropeptide-Y in DRG, and age-dependent upregulation of microglia, and astrocytes in the SC dorsal horn.[59] However, it is worth noting that the pain behavior changes observed are probably driven not only by disc but other system wide changes due to the global loss of SPARC protein.

Other genetic mouse models for recreating spinal pathology include mice with conditional activation of β-catenin using a Col2a1 driver,[60] and Biglycan deficient mice.[61,62] Studies evaluating pain behavior in these models have been limited. Future investigations studying changes in pain behavior in genetic models such as these would be interesting.

## CONCLUSION

Establishing valid animal models have clinical implications because the models could be used to investigate mechanisms and screen for therapeutic interventions related to LBP. Based on the animal data, clinical trials can be designed more appropriately to test a variety of outcomes. However, research findings in animal models need to be translated to human patients with cautions due to the complex nature of the human condition. Different from animal models, LBP symptoms in human patients often do not reflect the observed degree of pathology. The diversity of causes (injury, infection, and disease), comorbidities, genetic, and psychosocial factors can have a major impact on how pain is perceived and exhibited in patients. Other contributing factors, such as differences in drug pharmacology and behavioral assay methodologies between humans and animals, may also bring challenges to the clinical translation. With these discrepancies in mind, the selection of an appropriate animal model that could mimic as closely as possible the disease phenotype in humans is imperative.

Animal models for LBP are at their early stage of development, and further research should consider the following aspects: Firstly, large animals represent more closely the natural underlying disease in humans and offer greater insight into novel mechanisms responsible for chronic pain. However, very limited exploration in developing LBP models was done in large animals. In fact, large animals have been used to mimic human disc degeneration, including rabbits, pigs, sheep, canine, and monkeys. In addition, large animals have also been used for modeling acute nociception, acute inflammatory pain (post-surgical pain), and chronic inflammatory pain (osteoarthritis).[63] Therefore, the further characterization and development of large animal models for LBP needs to be done. Secondly, efforts are needed to determine the most effective and appropriate methods for evaluating and quantifying pain behaviors. The tests would be reliable and accurate if they could mitigate the experimenter subjectivity, and minimize confounds related to human-animal interactions. Facial grimace, open-field test and rotarod test may work in LBP models and should be tried in future studies. Furthermore, the functional magnetic resonance imaging of the brain could also probably be used for assessing the brain changes in the pre-clinical LBP models. Thirdly, the specific mechanisms of LBP in patients are still unclear. Instead of artificially inducing, perhaps the most useful animal models of pain would be ones in which the etiology of the pain was endogenous. Therefore, the endogenous development of painful disc diseases in animals would be a very important area of interest.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGMENTS

This work was supported by a VA BLD&R Merit Review Award (I01BC002647) to HJI; an NIH NIAMS R01 (AR062136) and R21 grant (AR067935) to HJI; and Arthritis Foundation to HJI.

Grant sponsor: VA BLD&R Merit Review Award; Grant number: I01BC002647; Grant sponsor: Foundation for the National Institutes of Health; Grant numbers: AR062136, AR067935; Grant sponsor: Arthritis Foundation.

## REFERENCES

1. St Sauver JL, Warner DO, Yawn BP, et al. 2013 Why patients visit their doctors: assessing the most prevalent conditions in a defined American population. Mayo Clin Proc 88:56–67. [PubMed: 23274019]
2. Katz JN. 2006 Lumbar disc disorders and low-back pain: socioeconomic factors and consequences. J Bone Joint Surg Am 88:21–24. [PubMed: 16595438]
3. DePalma MJ, Ketchum JM, Saullo TR. 2011 Etiology of chronic low back pain in patients having undergone lumbar fusion. Pain Med (Malden, Mass) 12:732–739.
4. Chou R, Qaseem A, Snow V, et al. 2007 Diagnosis and treatment of low back pain: a joint clinical practice guideline from the American College of Physicians and the American Pain Society. Ann Intern Med 147:478–491. [PubMed: 17909209]
5. National Institute for Health and Care Excellence 2009 Low back pain: early management of persistent non-specific low back pain. London: Royal College of General Practitioners.
6. Lu Y, Guzman JZ, Purmessur D, et al. 2014 Nonoperative management of discogenic back pain: a systematic review. Spine 39:1314–1324. [PubMed: 24827515]

7. Qaseem A, Wilt TJ, McLean RM, et al. 2017 Noninvasive treatments for acute, subacute, and chronic low back pain: a clinical practice guideline from the american college of physicians. Ann Intern Med 166:514–530. [PubMed: 28192789]
8. Allegri M, Montella S, Salici F, et al. 2016 Mechanisms of low back pain: a guide for diagnosis and therapy. F1000Research 5.
9. Tsuda M, Koga K, Chen T, et al. 2017 Neuronal and microglial mechanisms for neuropathic pain in the spinal dorsal horn and anterior cingulate cortex. J Neurochem 141:486–498. [PubMed: 28251660]
10. Farrell MJ. 2012 Age-related changes in the structure and function of brain regions involved in pain processing. Pain Med (Malden, Mass) 13:S37–S43.
11. Kregel J, Meeus M, Malfliet A, et al. 2015 Structural and functional brain abnormalities in chronic low back pain: a systematic review. Semin Arthritis Rheum 45:229–237. [PubMed: 26092329]
12. Ivo R, Nicklas A, Dargel J, et al. 2013 Brain structural and psychometric alterations in chronic low back pain. Eur Spine J 22:1958–1964. [PubMed: 23392554]
13. Nijs J, Apeldoorn A, Hallegraeff H, et al. 2015 Low back pain: guidelines for the clinical classification of predominant neuropathic, nociceptive, or central sensitization pain. Pain Physician 18:E333–E346. [PubMed: 26000680]
14. Harrisson SA, Stynes S, Dunn KM, et al. 2017 Neuropathic pain in low back-related leg pain patients: what is the evidence of prevalence, characteristics, and prognosis in primary care? A systematic review of the literature. J Pain 18:1295–1312. [PubMed: 28619698]
15. Mertens P, Blond S, David R, et al. 2015 Anatomy, physiology and neurobiology of the nociception: a focus on low back pain (part A). Neurochirurgie 61:S22–S34. [PubMed: 25441598]
16. Garcia-Cosamalon J, del Valle ME, Calavia MG, et al. 2010 Intervertebral disc, sensory nerves and neurotrophins: who is who in discogenic pain? J Anat 217:1–15. [PubMed: 20456524]
17. Patrick N, Emanski E, Knaub MA. 2016 Acute and chronic low back pain. Med Clin North Am 100:169–181. [PubMed: 26614726]
18. Konttinen YT, Kemppinen P, Segerberg M, et al. 1994 Peripheral and spinal neural mechanisms in arthritis, with particular reference to treatment of inflammation and pain. Arthritis Rheum 37:965–982. [PubMed: 8024624]
19. Kim JS, Kroin JS, Li X, et al. 2011 The rat intervertebral disk degeneration pain model: relationships between biological and structural alterations and pain. Arthritis Res Ther 13:R165. [PubMed: 21996269]
20. Chapman JR, Norvell DC, Hermsmeyer JT, et al. 2011 Evaluating common outcomes for measuring treatment success for chronic low back pain. Spine 36:S54–S68. [PubMed: 21952190]
21. Lee M, Kim BJ, Lim EJ, et al. 2009 Complete Freund's adjuvant-induced intervertebral discitis as an animal model for discogenic low back pain. Anesth Analg 109:1287–1296. [PubMed: 19762759]
22. Li Z, Liu H, Yang H, et al. 2014 Both expression of cytokines and posterior annulus fibrosus rupture are essential for pain behavior changes induced by degenerative intervertebral disc: an experimental study in rats. J Orthop Res 32:262–272. [PubMed: 24115280]
23. Lai A, Moon A, Purmessur D, et al. 2016 Annular puncture with tumor necrosis factor-alpha injection enhances painful behavior with disc degeneration in vivo. Spine J 16:420–431. [PubMed: 26610672]
24. Chaplan SR, Bach FW, Pogrel JW, et al. 1994 Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 53:55–63. [PubMed: 7990513]
25. Xie WR, Deng H, Li H, et al. 2006 Robust increase of cutaneous sensitivity, cytokine production and sympathetic sprouting in rats with localized inflammatory irritation of the spinal ganglia. Neuroscience 142:809–822. [PubMed: 16887276]
26. D'Amour FE, Smith DL. 1941 A method for determining loss of pain sensation. J Pharmacol Exp Ther 72:74–79.
27. Woolfe G, MacDonald AD. 1944 The evaluation of the analgesic action of pethidine hydrochloride. J Pharmacol Exp Ther 80:300–307.
28. Hargreaves K, Dubner R, Brown F, et al. 1988 A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32:77–88. [PubMed: 3340425]

29. Choi Y, Yoon YW, Na HS, et al. 1994 Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. Pain 59:369–376. [PubMed: 7708411]
30. Olmarker K 2008 Puncture of a lumbar intervertebral disc induces changes in spontaneous pain behavior: an experimental study in rats. Spine 33:850–855. [PubMed: 18404103]
31. Nilsson E, Nakamae T, Olmarker K. 2011 Pain behavior changes following disc puncture relate to nucleus pulposus rather than to the disc injury per se: an experimental study in rats. Open Orthop J 5:72–77. [PubMed: 21566734]
32. Fukui D, Kawakami M, Yoshida M, et al. 2015 Gait abnormality due to spinal instability after lumbar facetectomy in the rat. Eur Spine J 24:2085–2094. [PubMed: 25186827]
33. Miyagi M, Ishikawa T, Kamoda H, et al. 2013 Assessment of pain behavior in a rat model of intervertebral disc injury using the CatWalk gait analysis system. Spine 38: 1459–1465. [PubMed: 23649215]
34. Millecamps M, Tajerian M, Sage EH, et al. 2011 Behavioral signs of chronic back pain in the SPARC-null mouse. Spine 36:95–102. [PubMed: 20714283]
35. Lai A, Moon A, Purmessur D, et al. 2015 Assessment of functional and behavioral changes sensitive to painful disc degeneration. J Orthop Res 33:755–764. [PubMed: 25731955]
36. Strong JA, Xie W, Bataille FJ, et al. 2013 Preclinical studies of low back pain. Mol Pain 9:17. [PubMed: 23537369]
37. Kawakami M, Tamaki T, Weinstein JN, et al. 1996 Patho-mechanism of pain-related behavior produced by allografts of intervertebral disc in the rat. Spine 21:2101–2107. [PubMed: 8893434]
38. Kawakami M, Tamaki T, Hayashi N, et al. 1998 Possible mechanism of painful radiculopathy in lumbar disc herniation. Clin Orthop Rel Res 241–251.
39. Sasaki N, Kikuchi S, Konno S, et al. 2007 Anti-TNF-alpha antibody reduces pain-behavioral changes induced by epidural application of nucleus pulposus in a rat model depending on the timing of administration. Spine 32:413–416. [PubMed: 17304130]
40. Wei M, Mo SL, Nabar NR, et al. 2012 Modification of rat model of sciatica induced by lumber disc herniation and the anti-inflammatory effect of osthole given by epidural catheterization. Pharmacology 90:251–263. [PubMed: 23018204]
41. Shamji MF, Allen KD, So S, et al. 2009 Gait abnormalities and inflammatory cytokines in an autologous nucleus pulposus model of radiculopathy. Spine 34:648–654. [PubMed: 19333095]
42. Omarker K, Myers RR. 1998 Pathogenesis of sciatic pain: role of herniated nucleus pulposus and deformation of spinal nerve root and dorsal root ganglion. Pain 78:99–105. [PubMed: 9839819]
43. Hu SJ, Xing JL. 1998 An experimental model for chronic compression of dorsal root ganglion produced by intervertebral foramen stenosis in the rat. Pain 77:15–23. [PubMed: 9755014]
44. Song XJ, Hu SJ, Greenquist KW, et al. 1999 Mechanical and thermal hyperalgesia and ectopic neuronal discharge after chronic compression of dorsal root ganglia. J Neurophysiol 82:3347–3358. [PubMed: 10601466]
45. Chen RG, Kong WW, Ge DL, et al. 2011 Bilateral mechanical and thermal hyperalgesia and tactile allodynia after chronic compression of dorsal root ganglion in mice. Neurosci Bull 27:233–240. [PubMed: 21788994]
46. Gu X, Yang L, Wang S, et al. 2008 A rat model of radicular pain induced by chronic compression of lumbar dorsal root ganglion with SURGIFLO. Anesthesiology 108:113–121. [PubMed: 18156889]
47. Kawakami M, Weinstein JN, Chatani K, et al. 1994 Experimental lumbar radiculopathy. Behavioral and histologic changes in a model of radicular pain after spinal nerve root irritation with chromic gut ligatures in the rat. Spine 19:1795–1802. [PubMed: 7973977]
48. Hashizume H, DeLeo JA, Colburn RW, et al. 2000 Spinal glial activation and cytokine expression after lumbar root injury in the rat. Spine 25:1206–1217. [PubMed: 10806496]
49. Amaya F, Samad TA, Barrett L, et al. 2009 Periganglionic inflammation elicits a distally radiating pain hypersensitivity by promoting COX-2 induction in the dorsal root ganglion. Pain 142:59–67. [PubMed: 19135800]
50. Song XJ, Gan Q, Cao JL, et al. 2006 Spinal manipulation reduces pain and hyperalgesia after lumbar intervertebral foramen inflammation in the rat. J Manipulative Physiol Ther 29:5–13. [PubMed: 16396724]

51. Shuang F, Hou SX, Zhu JL, et al. 2015 Establishment of a rat model of lumbar facet joint osteoarthritis using intraarticular injection of urinary plasminogen activator. Sci Rep 5:9828. [PubMed: 25892493]
52. Yeh TT, Wen ZH, Lee HS, et al. 2008 Intra-articular injection of collagenase induced experimental osteoarthritis of the lumbar facet joint in rats. Eur Spine J 17:734–742. [PubMed: 18224353]
53. Kim JS, Kroin JS, Buvanendran A, et al. 2011 Characterization of a new animal model for evaluation and treatment of back pain due to lumbar facet joint osteoarthritis. Arthritis Rheum 63:2966–2973. [PubMed: 21953085]
54. Kim JS, Ahmadinia K, Li X, et al. 2015 Development of an experimental animal model for lower back pain by percutaneous injury-Induced lumbar facet joint osteoarthritis. J Cell Physiol 230:2837–2847. [PubMed: 25858171]
55. Styf J, Lysell E. 1987 Chronic compartment syndrome in the erector spinae muscle. Spine 12:680–682. [PubMed: 2961082]
56. Kobayashi Y, Sekiguchi M, Konno S, et al. 2010 Increased intramuscular pressure in lumbar paraspinal muscles and low back pain: model development and expression of substance P in the dorsal root ganglion. Spine 35:1423–1428. [PubMed: 20535049]
57. Gruber HE, Ingram JA, Leslie K, et al. 2004 Cellular, but not matrix, immunolocalization of SPARC in the human intervertebral disc: decreasing localization with aging and disc degeneration. Spine 29:2223–2228. [PubMed: 15480132]
58. Millecamps M, Tajerian M, Naso L, et al. 2012 Lumbar intervertebral disc degeneration associated with axial and radiating low back pain in ageing SPARC-null mice. Pain 153:1167–1179. [PubMed: 22414871]
59. Miyagi M, Millecamps M, Danco AT, et al. 2014 ISSLS Prize winner: increased innervation and sensory nervous system plasticity in a mouse model of low back pain due to intervertebral disc degeneration. Spine 39:1345–1354. [PubMed: 24718079]
60. Wang M, Tang D, Shu B, et al. 2012 Conditional activation of beta-catenin signaling in mice leads to severe defects in intervertebral disc tissue. Arthritis Rheum 64:2611–2623. [PubMed: 22422036]
61. Furukawa T, Ito K, Nuka S, et al. 2009 Absence of biglycan accelerates the degenerative process in mouse intervertebral disc. Spine 34:E911–E917. [PubMed: 19940720]
62. Marfia G, Campanella R, Navone SE, et al. 2014 Potential use of human adipose mesenchymal stromal cells for intervertebral disc regeneration: a preliminary study on biglycandeficient murine model of chronic disc degeneration. Arthritis Res Ther 16:457. [PubMed: 25293819]
63. Henze DA, Urban MO. 2010 Large animal models for pain therapeutic development translational pain research: from mouse to man. Boca Raton, FL: Llc.