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Looking beyond the intervertebral disc: the need for behavioral assays in models of discogenic pain

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Abstract

Orthopedic research into chronic discogenic back pain has commonly focused on aging- and degeneration-related changes in intervertebral disc structure, biomechanics, and biology. However, the primary spine-related reason for physician office visits is pain. The ambiguous nature of the human condition of discogenic low back pain motivates the use of animal models to better understand the pathophysiology. Discogenic back pain models must consider both emergent behavioral changes following pain induction and changes in the nervous system that mediate such behavior. Looking beyond the intervertebral disc, this review describes the different ways to classify pain in human patients and in animal models. We describe several behavioral assays that can be used in rodent models to augment disc degeneration measurements and characterize different types of pain. We review rodent models of discogenic pain that employed behavioral pain assays and highlight a need to better integrate neuroscience and orthopedic science methods to extend current understanding of the complex and multifactorial pathophysiology of discogenic back pain.

Keywords

intervertebral disc; discogenic pain; behavior; rodent model; nervous system

Introduction

Low back pain (LBP) is an extremely common musculoskeletal disorder and a leading cause of disability worldwide.[1,2] It is defined as pain, muscle tension, or stiffness localized to the region of human body below the costal margin and above the inferior gluteal fold, either with or without leg pain.[3–5] LBP affects more than 70–85% of the population at some time in their life.[6] LBP can also induce psychological problems, including depression, anxiety, stressful responsibility, job dissatisfaction, and mental stress at work.[6] Consequently, the U.S. economic costs for back and neck pain are approximately $200 billion, with healthcare spending estimated at $87.6 billion and additional costs attributed to lost economic productivity from missed work and lower wages.[7–9]

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Competing interests

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Anatomically, the source of LBP may arise from any innervated structures at the lumbar spine, including vertebrae, ligaments, muscles, fasciae, facet joints, and intervertebral discs (IVDs). Among these structures, the IVD is the most prevalent source of LBP,[10] and degeneration of the IVD was shown to be highly associated with LBP.[11–14] Pain originating from a damaged IVD is commonly referred to as *discogenic pain*, and in this review, we use the term to refer to nonspecific back pain associated with degenerated IVDs without larger structural defects (such as nucleus pulposus herniations).

Importantly, in both animal models and human patients, pain is a behavior. In order to perceive pain, there must be cortical activity.[15] That is, while pain may arise from damage in the periphery, a brain is required in order to feel it. Thus, while *in vitro* and *ex vivo* models can help us better understand changes within a degenerated IVD or a single neuron, *in vivo* models are essential for studying higher-order behavior. Behavior is an emergent property that arises from complicated neural circuits and must not be inferred from lower-order changes but instead studied in its own right, for attempting to reduce it will limit our understanding.[16]

The IVD is the hydrated fibrocartilaginous soft tissue between vertebrae along the spine. Morphologically, the IVD can be divided into three major components: nucleus pulposus (NP), annulus fibrosus (AF), and cartilage endplate (CEP). The NP is a highly hydrated and proteoglycan-rich structure at the center of the IVD surrounded by the AF.[17] The AF is an angle-ply and lamellar tissue.[17] The CEP is a thin layer of hyaline cartilage at the superior and inferior margins of the IVD. The NP and AF establish the biomechanical properties of the IVD, including hydrostatic pressure to maintain IVD height and flexibility to allow spine motion, whereas the CEP mainly regulates the transportation of nutrients, cytokines, and waste products between the IVD and adjacent vertebrae.[17] The structural, biochemical, and biomechanical properties of the IVD change with degeneration.[17]

The causes of IVD degeneration are complex and multifactorial.[17] Mechanical loading, traumatic injury, inadequate nutrient supply, intradiscal inflammation, and aging are all major risk factors for IVD degeneration.[17,18] Although risk factors for IVD degeneration have been widely studied, the relationship between IVD degeneration and nonspecific discogenic back pain is still not fully understood. Degeneration accumulates in human IVDs over many years and usually exhibits dehydration of the NP,[19] disorganization of the AF lamellae,[19] undistinguished NP–AF boundary,[19] defects in the endplates,[17] and a loss of IVD height.[20] Degenerated IVDs may also develop annular fissures,[17] which can lead to NP herniation and CEP injury. In the degenerated IVD, the balance between anabolism and catabolism is lost. There is significant downregulation of proteoglycans, water, and collagen content (except type I collagen in NP) and an increase in matrix-degrading enzymes and proinflammatory cytokines,[17,21] such as tumor necrosis factor α (TNF-α),[22] interleukin-1 β(IL-1β),[22] and chemokine C-C motif 2.[23] TNF-α may play a more important role in discogenic pain, while IL-1β may be more critical for the progression of disc degeneration.[24,25] The structural defects and loss of proteoglycan have been considered to create a permissive environment for the ingrowth of nerves and vessels in this otherwise largely avascular and aneural structure of the IVD.[26,27] Additionally, both vascular endothelial growth factor (VEGF) and nerve growth factor (NGF) are increased in

degenerated IVDs,[28–30] further enabling neurovascular ingrowth. Ingrowth of capillaries and nerves from the peripheral AF to the inner AF or even NP are observed in painful IVD degeneration, which may be a result of matrix breakdown and structural failure.[27,31]

Here, we describe different pain classifications and how they are measured using behavioral assays in rodent models and review existing rodent models that employ some of these behavioral assays. We also discuss the importance of studying the nervous system when modeling pain and provide some future considerations when utilizing animal models for the study of discogenic pain.

Low back pain in the clinic

Chronic discogenic LBP can be difficult to diagnose and treat. Numerous imaging studies attempted to determine a definitive association between IVD degeneration and LBP. IVD degeneration is strongly associated with LBP, and degenerative disc disease is the most common diagnosis in back pain patients.[6,13,14,32] However, IVD degeneration is not a sufficient diagnosis for pain development, as evidenced by large numbers of asymptomatic patients with abnormal findings on MRI or CT. Using MRI, IVD herniations are seen in 22–67% of asymptomatic adults and spinal stenosis in 21% of asymptomatic adults over 60, and CT evidence of spinal facet joint osteoarthritis was shown to have no correlation with LBP.[33–36] In addition to structural defects, a 1990 MRI study by Boden *et al.* found IVD degeneration in approximately one-third of asymptomatic subjects.[34] A 7-year follow-up further concluded that abnormal findings on MRI scans were not predictive of the development or duration of LBP.[37] Thus, spine pathology can be observed in the absence of LBP and should not be used as a proxy for LBP in research.

While IVD degeneration may be found in asymptomatic patients, the severity of IVD degeneration as measured by MRI has been shown to correlate with the severity of LBP. Takatalo *et al.* found that lumbar IVD degeneration correlated with pain severity independent of other degenerative findings.[38] Additionally, in asymptomatic individuals, increasing IVD degeneration score from MRI is predictive for developing future first-time LBP episodes.[39] Therefore, while it is inappropriate to use IVD degeneration as a proxy for chronic LBP, it is likely a major contributing factor.

Not only is IVD degeneration seen on imaging studies not indicative of LBP, but patients with discogenic back pain are also poorly indicated for surgery. In IVD degeneration, painful conditions are difficult to associate with specific anatomical and radiographic findings, in contrast to nucleus pulposus herniations or spinal stenosis. In 2007, a Medicare advisory committee concluded that the effectiveness of lumbar spinal fusion surgeries for treating chronic LBP was uncertain, owing to conflicting evidence and large variations in surgical technique.[40] In a prospective Swedish cohort study, while fusion was found to be superior to nonsurgical treatment, only 63% of patients showed pain improvement after fusion surgery, and pain significantly increased from 1 to 2 years after surgery.[41] Identifying which patients are likely to benefit from fusion surgery is difficult: a systematic review found that immobilization, provocative discography, and temporary external fixation were not useful in predicting which patients would benefit from fusion surgery.[42] Given the difficulties in

determining who will benefit from surgery, the American College of Physicians recently updated their LBP treatment guidelines, recommending noninvasive, nonpharmacologic treatments as the first line of therapy.[43] However, nonsurgical treatments also have mixed results. TNF-α has been considered a promising target, as it is associated with painful IVD degeneration in rodents,[44,45] and expression of the TNF-α receptor TNFR1 in the nucleus pulposus correlates with pain in human patients.[46] Given the success of TNF-α inhibitors, such as infliximab, in treating pain in patients with rheumatoid arthritis, they have been considered as therapy for discogenic pain. Yet, a 2014 meta-analysis of TNF-α inhibitors as treatment for sciatica by Wang *et al.* found that they did not significantly improve LBP, leg pain, or rates of return to work at short-term, middle-term, or long-term follow-up.[47] There is a significant clinical need to better understand how IVD degeneration may lead to chronic LBP and how to best classify this pain, so that we may better tailor our treatments to combat the progression from degenerative changes to chronic discogenic pain.

Types of pain

The International Association for the Study of Pain (IASP) defines pain as "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage."[48] This broad definition can be further divided into different types of pain on the basis of mechanism, chronicity, spontaneity, and stimulus intensity required.

Neuropathic versus nociceptive pain

Pain can be classified on the basis of its underlying mechanism as either nociceptive or neuropathic. Nociceptive pain is the transmission of painful stimuli resulting from an injury to a non-neural tissue. Inflammation-related pain, which is observed in osteoarthritis or ankylosing spondylitis, is an example of nociceptive pain. Harmful stimuli, including specific neuropeptides, proinflammatory cytokines, and mechanical insult, stimulate peripheral nociceptive neurons, which then transmit the pain signal to the central nervous system.[49] It is important to note that nociceptive pain is a protective mechanism to discourage the use of damaged tissues and allow for healing. Neuropathic pain, on the other hand, is pain resulting from injury to the nervous system itself. In this mode, a nerve is injured or impinged upon, resulting in pain. The damage can be focal, such as in the case of IVD-related radiculopathy, wherein the NP herniates and directly impinges the nerve root. Neuropathic pain can also be a diffuse systemic pathology, such as in painful diabetic neuropathy, wherein neurons are damaged throughout the body. However, it is important to note that these pain modalities are not exclusive, and LBP is likely to be a mixed pain with both nociceptive and neuropathic elements. Both nociceptive and neuropathic pain can arise from the same pathology, such as IVD degeneration, where innervating neurons are sensitized by intradiscal inflammation and degeneration-related stenosis directly impinges nerves. Distinguishing whether pain is nociceptive or neuropathic is important for determining treatment. Opioids are the standard of care for nociceptive pain[50] but are not helpful for treating neuropathic pain.[51] For patients with neuropathic pain syndromes, drugs that act on the neurotransmitter γ-aminobutyric acid (GABA) receptors, such as gabapentin and baclofen, are typically prescribed.[52,53]

Acute versus chronic pain

Pain is often classified on the basis of its duration as either acute, subacute, or chronic. Acute pain typically occurs in response to tissue trauma and is defined when pain onset and recovery occur within 1 month.[54] Acute LBP is usually self-limiting, with 90% of patients recovering within 6 weeks.[5] However, following acute injury, 2–7% of cases will progress to chronic pain.[5] When this persistent pain lasts 4–12 weeks, it is classified as subacute, and pain that lasts longer than 12 weeks is classified as chronic pain. The American Pain Society defines chronic pain in two ways: (1) pain that extends beyond the period of healing (3–6 months), with levels of identified pathology that often are low and insufficient to explain the presence and/or extent of the pain, and (2) persistent pain that disrupts sleep and normal living, ceases to serve a protective function, and instead degrades health and functional capability.[55] Chronic pain is not uncommon and is believed to affect 20–30% of the population.[56,57]

Evoked versus spontaneous pain

Pain may also be classified according to spontaneity as either spontaneous or evoked. Spontaneous pain, sometimes referred to as clinical pain, is seen in chronic pain disorders.[58] It is not stimulus-dependent, and in animal studies spontaneous pain is described as voluntary behavior and can be measured by assessing the behaviors of animals in unrestrained condition [59]. Unlike spontaneous pain, evoked pain is stimulus dependent and can be measured across different sensory modalities, including mechanical and thermal stimuli. As different sensory modalities utilize different neural pathways, correlation between pain sensitivity across modalities is variable and may be differentially modulated by interventions.[58] Since evoked pain requires a provocative stimulus, it is sometimes referred to as experimental pain. Evoked pain measurements are often used to assess changes in pain thresholds in animal studies and to diagnose human pathology.[59] For patients presenting with LBP, evoked pain tests, such as the straight leg raise, or provocative discography are commonly used to determine if the pain is discogenic in origin.[60,61]

Hyperalgesia versus allodynia

Pain can be classified clinically by the intensity of the stimulus required to produce a pain response as either hyperalgesia or allodynia. IASP has clear definitions for both hyperalgesia and allodynia. Allodynia is defined as pain in response to a stimulus that does not normally provoke pain in healthy subjects, whereas hyperalgesia is defined as increased pain from a stimulus that normally provokes pain in healthy subjects.[48] While allodynia and hyperalgesia are clinical definitions, not mechanistic ones, they are believed to arise from changes in different types of peripheral nerve fibers. Hyperalgesia is thought to arise primarily from sensitization of Aδ- and C-fibers, which are peripheral nerve fibers responsible for normal pain sensation.[62] Thus, the normal pain pathway is overactive and generates a greater response to already painful stimuli. In addition to sensitization of peripheral nerve endings, the heightened behavioral responses of hyperalgesia may also involve sensitization of the central nervous system.[62] In allodynia, peripheral Aδ-fibers, which normally respond to nonpainful touch, are thought to undergo a phenotypic switch to become more similar to pain-sensing C-fibers.[63] In addition, Aβ input to the superficial

dorsal horn of the spinal cord (an important component of the pain pathway) increases in models of neuropathic pain, thus amplifying the pain signal input to this region, and further demonstrating the change in the role of Aβ-fibers in allodynia.[64–66]

Measurements of pain in rodent models

For human patients with LBP, the 10-point visual analog scale is commonly used to assess the severity of LBP. However, as rodents cannot communicate their pain status, we must employ alternative methods. Fortunately, there are multiple validated assays to determine whether an animal is experiencing pain (Fig. 1). This review focuses on the application of behavioral assays in rodent models, as these rodent behavioral pain assays have been well studied, are less technically challenging in rodents compared with other animal models, and have been shown to be sensitive to pain associated with IVD degeneration. While some behavior assays are tested directly on the spine to assess LBP, many of these behavioral assays are tested on the plantar surface of the hindpaw. The plantar surface of the rodent hindpaw is innervated primarily by the tibial nerve, which is composed of spinal nerve roots from L4 to S2.[67] Thus, increased pain sensitivity that refers to the plantar surface of the hindpaw is considered a measure of LBP.

Evoked pain tests

Evoked pain tests are those in which an experimenter must expose an animal to a painful stimulus. Such stimuli may be processed across various sensory modalities: in this section, we will examine evoked pain tests used to measure mechanical pain and thermal pain (both cold and heat).

Mechanical allodynia is most commonly measured using the von Frey assay. In this assay, rodents are placed in wire mesh–floored cages, allowed to acclimate, and then tested with calibrated microfilaments. These filaments are calibrated such that they will buckle when the appropriate amount of mechanical force has been transmitted. Typically, von Frey filaments are applied in ascending force to the plantar surface of the hindpaw with sufficient strength to cause buckling of the filament, although they can be used at other locations, such as the tail, lower back, or face. The most common application of the von Frey assay is using the up–down method described by Chaplan *et al.* Hindpaws are probed a prescribed number of times, and a positive response is defined as brisk withdrawal of the probed foot. Once a positive response is seen, the previous filament is applied. If positive, the lower filament is determined to be the 50% paw-withdrawal threshold. If negative, the next ascending filament is applied, and if that next filament provokes a positive response, the original filament is considered to be the 50% withdrawal threshold. If the next ascending filament is negative, further ascending filaments are applied until a response is provoked.[68,69]

Mechanical hyperalgesia, sometimes referred to as pressure hyperalgesia, can be measured using an algometer.[70,71] An algometer is an applied force gauge that can be applied to a localized region, such as the hind paw or posterior lumbar spine.[72] The applied force is gradually increased until an audible vocalization is elicited to determine the pressure-pain threshold.[55,56] The algometer is a useful assay in rodent models, as it can be similarly

applied in human LBP patients to measure mechanical hyperalgesia in the musculature of the lower back.[57]

Thermal pain sensitivity can be measured as either a sensitivity to heat or to cold, as the two stimulus modalities activate different populations of neurons.[73,74] Tests for sensitivity to heat-provoked pain have been performed for over half a century.[75] One of the first tests for heat hyperalgesia was the hot-plate test. In this assay, developed in the 1940s, a mouse or rat is placed on a hot plate in order to evoke a behavioral response to heat-induced pain, and the latency to the first behavior is measured.[76] In mice, these behaviors include hindpaw licking, brisk hindpaw withdrawal, and jumping; in rats, hindpaw licking or brisk hindpaw withdrawal may be seen in the presence of pain.[75] The hot plate is typically set between 50 ºC and 55 ºC for both mice and rats.[77] Importantly, this temperature range is well above the 42–43 ºC threshold for the transient receptor potential cation channel subfamily V member 1 (TRPV1) receptor, responsible for the sensation of noxious heat.[78] As a result, naive animals will still experience pain at this temperature, but will have increased latency to respond, compared with animals in pain. However, the development of dynamic hot plates, in which the temperature can be steadily ramped up from a nonpainful temperature, enables the assessment of heat allodynia owing to its initially reduced stimulus intensity.[75]

While the hot-plate test can test heat hyperalgesia in the paws, the tail-flick test is used to test the same sensitivity in the tail. Developed around the same time as the hot-plate test, in this assay, a rat's tail is exposed to heat either via immersing it in hot water or by a radiant infrared beam, and the latency until the animal flicks its tail is measured.[79,80] This test is typically performed on rats, as it can be difficult to perform on mice.[81] While the tail-flick test may be helpful in assessing heat hyperalgesia in the tail, immersing the tail in water may have other effects, and one should not equate water immersion and radiant-heat tail-flick tests, as the surface area exposed to the stimulus may vary significantly.[80]

More recently, a radiant heat test known as the Hargreaves' test was developed. Named after the Hargreaves *et al.* paper in which it was first described, this test uses radiant heat to stimulate rodent hindpaws, and latency to response is measured.[82] Because an infrared beam is used, the experimenter can independently evaluate the left and right paws, allowing internal controls for lateralized pain models, such a sciatic nerve injury.[81] The Hargreaves' test enables more specific targeting of the heat stimulus but takes longer to test and requires more elaborate experimental equipment than the hot-plate test.

Following the understanding that heat pain and cold pain are not processed by the same populations of neurons, tests for cold-provoked pain have become more popular. Two of the most common tests for cold hyperalgesia are the cold-plate test and the acetone test. In the cold-plate test, a mouse or rat is placed on a cold plate, and stereotyped pain behaviors are measured similarly to the hot-plate test [83,84]. However, in the cold-plate test, both latency to first pain behavior and total number of pain behaviors during a prescribed time frame are often measured, as there is considerable behavioral variability in cold nociception.[81] Cold plates are usually set between 5 ºC and −5ºC.[85] This is well below the firing threshold for transient receptor potential cation channel subfamily M member 8 (TRPM8), the receptor responsible for responding to both menthol and noxious cold.[86] The threshold for TRPM8 is

20 ºC, but noxious cold is not felt until below 15 ºC.[86] This may explain why the cold-plate test is more technically challenging than the hot-plate test, as the sensation of cold pain appears to exist on more of a gradient than heat pain. While static cold plates measure cold hyperalgesia, dynamic cold plates in which the temperature ramps down from a non-painful temperature can be used to evaluate cold allodynia.[75]

Cold hyperalgesia can also be measured using the acetone test. In this test, a drop of liquid acetone is touched to the hindpaw of a mouse or rat, where it quickly spreads. The evaporation of the acetone causes a cold sensation, and the latency to hindpaw licking or withdrawal and number of these behaviors are measured.[87,88] The acetone test has an advantage over the cold-plate test because it can be applied to a single hindpaw, allowing for the use of internal controls. However, acetone evaporation is considered to be not only a measure of thermal hyperalgesia but also a measure of sensitivity to chemical-induced pain.[89] This lack of specificity, as well as the inability to measure the reaction to a specific temperature, makes this assay variable.

Spontaneous pain tests

Spontaneous pain tests do not require an experimenter to expose an animal to a nociceptive stimulus, and instead rely on observing voluntary behaviors. Spontaneous pain can be difficult to evaluate in rodents, because, as prey animals, they hide signs of injury or pain.[90] However, a variety of measurements have been developed.

One such measurement is the analysis of weight bearing following injury. Unrestrained animals are placed on a sensor plate, and distribution of weight on each paw is assessed. This test can be performed statically or dynamically and is sometimes part of gait analysis.[59] Importantly, analysis of weight bearing is only relevant in unilateral injury models, for weight bearing can be compared between injured and uninjured sides, and changes in weight bearing are unlikely in symmetrical injuries.

Another test for spontaneous pain is the open-field test. This test was first developed as a measure of anxiety-like behavior or “emotionality” and involves placing the rat or mouse in a plexiglass square and measuring exploratory behavior.[91] Briefly, the rodent is allowed to move freely about the space, and the time spent in each region of the box is quantified. Typically, the square is virtually divided into 16 equally sized square regions so that there is a clear center region. The number of central squares visited, the time spent in the central squares, and overall locomotion can be quantified. Both the number of central squares visited and the time spent in the central squares are markers of exploratory behavior, which is reduced in rodents exhibiting anxiety-like behavior.[92] While this test is traditionally used to assess anxiety-like behavior, it has also been adapted as a non-reflexive pain assay. As animals experiencing chronic pain may also exhibit anxiety-like behaviors, both the traditional measurements of the open field test and additional measures of rearing behaviors can be used to evaluate pain behavior without stimulation.[92,93]

Spontaneous pain in rodents can also be measured by evaluating ultrasound vocalizations in a sound-free environment. Rodents are capable of producing both audible and ultrasound vocalizations, so ultrasound vocalizations may be measured when audible vocalizations are

not present.[94] Adult rats emit different types of ultrasound vocalizations depending on their environment and affective state: 22-kHz vocalizations are produced in anticipation of aversive stimuli, and 50-kHz vocalizations are produced when the rat has a positive affective state.[95] Thus, when measuring ultrasound vocalizations, it is important to note not only the presence or absence of such vocalizations but also their frequency. Using ultrasound vocalization measurements to assess pain is somewhat controversial. Technically, it can be difficult to maintain a sufficiently sound-free environment, making measurements unreliable. Additionally, it is unclear whether rodents will reliably produce ultrasound vocalizations when in pain. In a study of neuropathic pain in mice, ultrasound vocalizations were increased in mice with neuropathic pain and reduced when they were given analgesic drugs.[96] However, in a separate study of acute pain in mice, 65% produced no vocalization, and in those that produced ultrasound vocalizations, audible vocalizations were also produced, rendering the ultrasound measurements redundant.[97]

Lastly, spontaneous pain can be measured via video observation. Such observation is divided into observations of facial expressions and observations of paw behaviors. For evaluation of facial expressions, both a rat and a mouse grimace scale exist. The rat grimace scale assess rats across four action units: orbit tightening, nose/cheek flattening, ear changes, and whisker changes. An automated software may be used to photograph rat facial expressions, and then expressions are manually scored using the rat grimace scale. This scale has been shown to reliably and accurately quantify spontaneous pain across a variety of pain models.[98] Similar to the rat grimace scale, the mouse grimace scale assesses orbital tightening, nose bulge, cheek bulge, ear position, and whisker change.[99]

In addition to facial expressions, paw behaviors may also be observed. For this assay, rodents are placed on a room-temperature plate enclosed in a plastic box or under a plastic dome, and the number of hindpaw lifts not related to locomotion are recorded.[87] However, unprovoked paw lifts may not reliably measure spontaneous pain, as they are only seen in some pain models. Spontaneous paw lifting is seen in the spared nerve injury model and a modified spinal nerve ligation model (in which the L5 spinal nerve is ligated and axotomized and the L4 nerve is loosely ligated), but does not occur in the traditional spinal nerve ligation model.[100,101]

Existing models of painful IVD degeneration

In vivo animal models of IVD degeneration exist in a wide variety of species, including rodents, rabbits, ovine, canine, and primates.[102] Mechanical and structural methods have been used to induce IVD degeneration experimentally, and some species develop IVD degeneration with age and have been extensively reviewed previously.[102–104] However, given the practical limitations of housing large animal species, such as sheep and cattle, and the ethical considerations of the use of others, such as canines and nonhuman primates, small animals have often been the model organism of choice for IVD degeneration research. The use of rodent models is further supported by the existence of validated practical assays for probing pain.[20]

The relationship of IVD degeneration to pain is frequently cited as a motivation for investigating IVD degeneration models, yet few studies have directly measured pain in a non-herniation lumbar IVD degeneration model (Table 1). An increase in spontaneous behaviors associated with pain was found up to 3 weeks after performing a facetectomy, puncturing the L4/5 IVD, and inducing NP leakage in a rat model, suggesting increased pain.[105] However, the facetectomy alters the whole motion segment biomechanics so may not be a true mimic of isolated IVD degeneration. A significant increase in pain behavior—as measured by algometer, von Frey assay, and gait analysis—was found after L4/5 and L5/6 IVD injury and nucleotomy using a 0.5-mm diameter microdrill in a rat model.[72] A transient increase in intradiscal TNF-α, IL-1β, IL-6, and substance P at the gene level was reported but returned to preoperative levels within 1 month. A significantly impaired gait was found after L5/6 IVD puncture using a 24-gauge needle in another rat model, but rats returned to normal gait 4 weeks after injury.[106] Another group found an increase in mechanical and heat-induced pain behavior after facetectomy and posterior L4/5 puncture but not anterior puncture without facetectomy.[107]

Other models have used an inflammatory bolus in addition to an IVD puncture injury. A puncture with an injection of an adjuvant adds an additional intradiscal inflammatory component. A significantly decreased pain threshold—as measured by vocal responses to microfilaments applied to the spinous process—was found up to 7 weeks after puncturing the L5/6 IVD ventrolaterally with a 26-gauge needle and injection of 10 μL complete Freund's adjuvant (CFA) with an anterior approach in a rat.[108] Our group has developed a model of painful IVD degeneration wherein an anterior IVD puncture with TNF-α injection induces a decreased mechanical hindpaw withdrawal threshold.[44] We believe the use of TNF-α is more physiologically relevant than CFA, as TNF-α has been extensively implicated in IVD degeneration and may better lend itself to understanding the underlying pathophysiology of IVD degeneration–related pain in an animal model.

Any intentional anatomical alterations to induce spinal injury or instability should parallel the human condition. The IVD is one component of a three-point spinal joint; the other two are the posterior facet joints. Although the facetectomy is a convenient mode to access the IVD and allow for puncture, this fundamentally changes the biomechanics of the spinal joint. Even in a well-controlled study, a facetectomy mimics a situation unlike what is seen in the human patient. The human degenerated IVD is still part of a three-point joint, so models of the condition should aim to preserve the interrelated anatomy. The anterior IVD puncture, which is done via a ventral approach through the abdomen, both preserves the complex spinal joint anatomy and creates a degeneration-inducing injury and is therefore a more physiologically appropriate model of the human condition.

A lone genetic model of IVD degeneration has assayed associated pain behaviors. A genetically modified *Sparc*$^{-/-}$ (secreted protein, acidic, and rich in cysteine) mouse model also has been found to yield spontaneous IVD degeneration with age[109] with evidence of IVD degeneration–related pain.[110] A drawback of this model, however, is degeneration occurs in all IVDs, which is dissimilar to humans, and it is not clear that the effects of *SPARC*$^{-/-}$ are restricted to the IVDs.

IVD degeneration and the nervous system

As illustrated by Boden, IVD degeneration is not sufficient for pain development.[34] Similar findings are observed in preclinical models. For example, two animals with equally degenerated IVDs[111,112] can have vastly different pain behaviors (Fig. 2). In this example, IVD degeneration was induced using anterior puncture and injection as described previously.[20] Both animals had a reduced mechanical paw-withdrawal threshold immediately following injury, illustrating that IVD injury and degeneration can induce painful conditions. However, the paw-withdrawal threshold of one rat slightly recovered 2 weeks postsurgery, while a low threshold was maintained in the other rat throughout the 6-week experiment. Accordingly, these two rats serve as an example of why analyzing the IVD in isolation is insufficient for identifying the structural and molecular bases of IVD degeneration–related pain: these rats may have differences in their nervous systems that underlie the observed differences in their pain behavior. Discogenic pain signals are transmitted from the IVD and adjacent structures via peripheral afferent nerve fibers whose cell bodies lie in the dorsal root ganglia and synapse with projection neurons in the dorsal horn of the spinal cord to multiple brain regions that comprise the “pain matrix” (Fig. 3). The specific molecular basis of painful IVD degeneration in the context of the peripheral nervous system is an important area of investigation that has been reviewed elegantly elsewhere[113–115] and is beyond the scope of this review. Understanding the neural pathway of painful IVD degeneration and regulation will inform tissue targets for both analysis and intervention.

Peripheral nerve endings at the IVD

Neural innervation into the IVD has long been hypothesized to play an important role in discogenic pain. In the healthy IVD, nerves innervate the outer AF.[116] In degenerated IVDs, nerve innervation is much more extensive, and these fibers are predominantly nociceptive Aδ and C-fibers.[113,117,118] Importantly, nerve endings in the inner AF and NP were found more often in painful IVDs than non-painful IVDs,[31] suggesting that the extent of neural ingrowth is important for pain development. Nerve growth into the IVD may lead to pain through neuropathic mechanical (i.e., direct nerve impingement) or nociceptive sensitization (inflammation-related) mechanisms, or more likely a combination of the both.

Dorsal root ganglia

Sensory signals transmitted from the periphery to the spinal cord are carried by a single neuron, and the cell bodies of these neurons form the dorsal root ganglia (DRG).[114] As such, any changes in gene and protein expression in the nerves innervating the IVD that follow repeated stimulation and peripheral sensitization are expected to be found within the DRG, and the DRG has rightly been a popular target for investigating neural involvement with IVD degeneration. Previous rodent discogenic pain studies have shown that injury-induced degeneration and pain are associated with upregulation of intradiscal proinflammatory cytokines and pain-related neuropeptides in the DRG (Table 1). A series of experiments by Ohtori *et al.* using retrograde and anterograde tracers demonstrated that L5/6 IVD in a rat, which corresponds to the human L4/5 IVD, is innervated by T13–L2 DRGs.[119,120] As such,

changes in the nervous system are likely to be found in DRGs several levels cranial to the IVD of interest rather than the IVD-adjacent DRG.

Dorsal horn of the spinal cord

The peripheral primary afferent fibers synapse in the dorsal horn of the spinal cord with both small interneurons and projection neurons that travel to the brain.[121] Of the 10 lamina of the spinal cord, lamina I and II are most important for pain, as this is where Aδ and C fibers primarily synapse,[121] as well as where Aβ fibers may synapse in allodynia states.[64–66] Just as repeated stimulation of peripheral nerves by painful stimuli may cause gene and protein expression changes in the DRG, similar changes farther down the pain pathway would be expected to be seen in the dorsal horn of the spinal cord, either in the projection neurons, interneurons, or both. Lee *et al.* found increased calcitonin gene–related peptide in the dorsal horn following IVD injury and degeneration in a rodent model (Table 1). Importantly, the dorsal horn is a key site of pain modulation.[121–126] The small interneurons of the dorsal horn may inhibit or reduce pain signaling locally[127] or in response to top-down modulation from higher brain structures.[123] In top-down modulation, efferent signaling, predominantly from the rostral ventromedial medulla in the brain stem to the dorsal horn of the spinal cord, is able to modulate pain signals such that less pain is perceived, independent of intensity of signal from the periphery.[121,122] This descending system is believed to play a role in the placebo effect.[126]

Brain

From the dorsal horn of the spinal cord, projection neurons target multiple brain regions, primarily in the brain stem and thalamus.[121] More specifically, brain stem regions where dorsal horn neurons synapse include the caudal ventrolateral medulla,[128,129] the nucleus of the solitary tract,[130] the parabrachial area,[129,131] and the periaqueductal gray matter (PAG).[126,132] Importantly, the PAG is involved in the psychological modulation of pain, as it plays a role in the top-down modulation of pain in the dorsal horn and is implicated in depression.[126,133,134] Thalamic nuclei implicated in pain include the ventral posterolateral and posteromedial nuclei,[135,136] the posterior group,[135] the ventral posterior parvicellular nuclei,[135] and the posterior triangular nucleus.[135,137] From these initial projections, pain is processed in the brain across several brain regions, which are sometimes referred to as the pain matrix. In addition to the thalamic and brain stem nuclei, the pain matrix includes components of both the limbic system and the cortex. Limbic structures involved in pain include the hippocampus[124,138–140] and the amygdala.[126,134] At the cortex, the pain matrix includes the prefrontal cortex,[124,141] insular cortex,[124,134,142] somatosensory cortex,[124,134] and the anterior and posterior cingulate cortices.[124,134,143] As pain processing occurs across a network of brain areas, changes in the brain from chronic pain may be widespread. Luchtmann *et al.* investigated the morphometric changes in the brain in patients suffering from chronic LBP and IVD herniation using MRI and found that patients with herniated IVD exhibited significant changes in both gray and white matter volumes throughout the brain, predominantly in regions of the pain matrix.[133,134]

Important considerations for future studies

A strong and growing body of research is investigating the etiology and potential therapeutic interventions for IVD degeneration in the setting of LBP. However, the complex mechanisms by which IVD injury and degeneration may lead to pain remain poorly understood. Given the importance in associating pain with IVD injury and degeneration, we propose several considerations for study design to ensure that animal models of discogenic pain are applicable to the human condition and to advance our understanding of the mechanism of such pain.

The obvious goal of animal models of IVD degeneration is to closely mimic the human condition of chronic discogenic pain. This raises the question of what is truly considered chronic pain in both human patients and animal models. The current literature presents two different definitions. The first is that chronic pain involves a change in cognitive and emotional cortical areas.[125,134] Chronic pain cannot be completely explained by identifiable somatic pathology and involves structural brain changes.[144] Thus, it is likely that the transition from acute to chronic pain reflects a change from a protective response due to tissue damage to a pathologic change within the nervous system. Using this definition, an experiment would need to establish that an induced IVD injury resulted in long-term brain changes. A more practical means of ensuring a chronic pain model is by considering the duration of an experiment. If the experiment is too short, the pathology may reflect an acute IVD injury rather than chronic degeneration, especially in an injury-induced model. While chronic pain in humans is defined as pain lasting greater than 3 months, in rodents, it is thought that 2–8 weeks is appropriate to establish chronicity, depending on the model.[125,145] Specifically, in the spared nerve injury model, hyperalgesia and allodynia are first seen about 2 weeks after injury,[146] but mood-related symptoms of chronic pain may not be evident until around 8 weeks.[145] As such, experiments investigating pain in a rodent IVD injury model should last for at least 2 weeks, but ideally longer, in order to assess a more complete picture of the chronic pain state. Alternatively, chronic pain may be achieved when pain assays reach a sustained maximum value, with models adjusted accordingly.

Additionally, both male and female animals should be used in future studies. The National Institutes of Health (NIH) has strongly recommended using both sexes in preclinical research studies in order to identify and evaluate any sex-dependent differences.[147] An additional benefit of this is the ability to evaluate the effects of sex in the absence of confounding gender effects that may be seen in human populations. Assessing sex as a biological variable in the study of discogenic pain is particularly important, as differences are seen between males and females in both spine pathology and mechanisms of pain. Spine impairment is more common in women (70.3 per 1000 population) than in men (57.3 per 1000 population).[6] Women typically exhibit lower pain thresholds across multiple modalities.[139] However, this may be a product of physiological factors (due to sex) or psychosocial factors (due to gender/culture) or an interaction of both.[148] In addition, at the cellular level, males and females may use different immune cells and molecular pathways in the perception of pain.[149,150] Investigating sex effects in IVD degeneration models may elucidate pathophysiological differences and inform sex-specific therapeutic strategies.

Considering the complexity of the pain pathway and the nervous system's essential role in pain pathogenesis, animal models of discogenic pain must evaluate both pain behaviors and cellular and molecular changes within the nervous system. In order to determine whether an animal is in pain, we must employ behavioral assays. Further, these assays can be used to more precisely define the type of pain the animal is experiencing and to suggest which neural pathways may be involved. Once pain in an animal model of IVD degeneration has been more clearly defined, the knowledge gained from behavioral measures may be used to inform investigations of cellular and molecular changes within hubs in the pain pathway found in both the peripheral and central nervous system. Examining such changes in the nervous system at a more granular level may enable both better understanding of mechanisms of discogenic pain and treatment precision.

Summary

IVD degeneration is highly associated with LBP, but the complex relationships between the two are unclear, as IVD injury and degeneration does not always result in LBP. Thus, animal models are necessary to probe the underlying pathophysiology of discogenic pain. The majority of animal models on IVD degeneration provide precise anatomical, biomechanical, biochemical, and radiographic measures to quantify the extent of IVD degeneration. A notably small number of animal model studies on IVD degeneration have directly assayed pain, despite the existence of a variety of different pain behavioral assays exist to measure pain, each assessing different pain modalities. Furthermore, long-term behavioral change likely implies adaptations of the central nervous system, for this is the network from which behavior arises. In addition to incorporating diverse behavioral pain assays into studies of discogenic pain, careful consideration must be made to design models that reflect the chronicity and mechanisms of the human clinical condition. Additionally, models should utilize both male and female animals to account for possible sex differences in LBP. Beyond the IVD, future studies should consider the behavioral changes and the nervous system changes that lead to them for a more complete picture of the ways in which IVD injury and degeneration can lead to discogenic pain.

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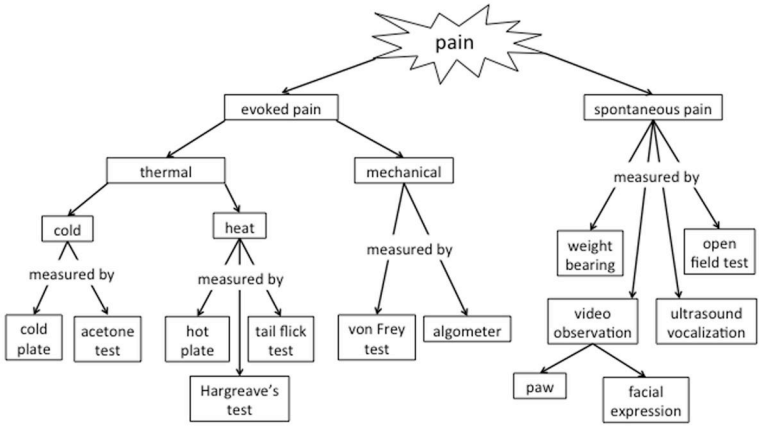

Figure 1.
Pain types and how they are measured in rodent models.

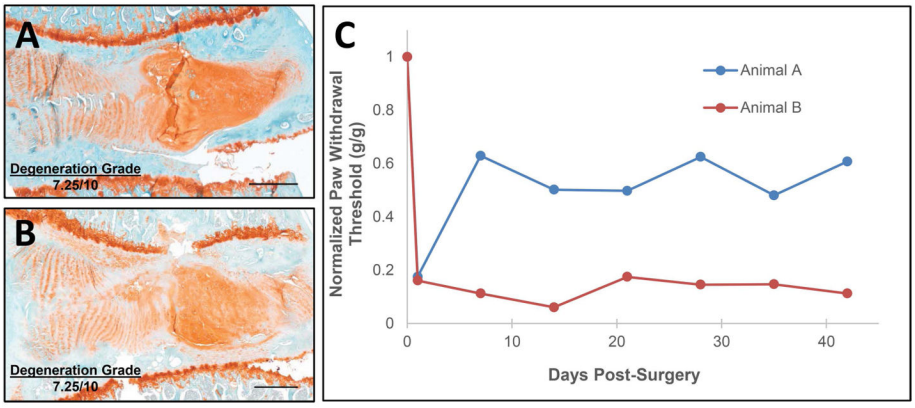

Figure 2.

IVD degeneration–related pain has greater variance than IVD degeneration. IVD degeneration and pain do not have a linear relationship, as demonstrated by two animals with similarly degenerated IVDs having vastly different mechanical paw-withdrawal thresholds. Both animals exhibited reduced mechanical paw withdrawal thresholds during the acute postoperative period, but animal A recovered to a greater extent than Animal B. (A and B) Mid-sagittal sections of rat lumbar IVDs stained with safranin-O/light green exhibited equal degeneration grades as determined by a semiquantitative histological grading scale of a total grade (0–10, least to most degenerated) of AF integrity, AF/NP border definition, NP cellularity, NP matrix condensation, and CEP regularity made by two graders at two time points. (C) Mechanical paw-withdrawal thresholds of corresponding rats normalized to presurgery values exhibited variability. Pain behavior evaluated using the von Frey assay. Scale bars = 250 μm.

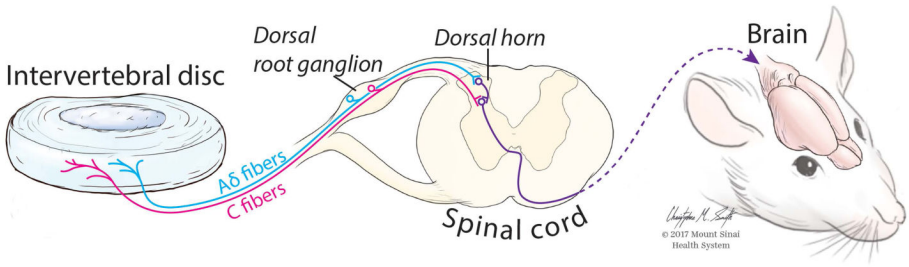

Figure 3.

Pain pathway from the intervertebral disc to the brain. Following IVD degeneration, pain may be evoked by a variety of possible mechanisms, such as nerve root irritation, neurovascular ingrowth, sensitized peripheral nerves, and/or abnormal concentrated stresses. Peripheral Aδ and C fibers transmit pain signals from the IVD and adjacent structures to the central nervous system. The cell bodies of these afferent peripheral nerves form the dorsal root ganglia. Within the spinal cord, Aδ and C fibers synapse with ascending neurons in the dorsal horn, which carry the pain signal to the brain. Thus, modulation of the pain pathway can occur at multiple hubs in the pain pathway: at the site of injury, in the dorsal root ganglion, in the dorsal horn of the spinal cord, or within the brain.

Table 1

Existing models of IVD degeneration

Model type	Species/age	Sex	Intervention	Experiment duration	Biochemical and structural assays	Behavior assays	Reference
Structural	Rat/not specified	Female	L4/5 L facetectomy + L4/5 posterolateral puncture + induced nuclear leakage	21 days	N/A	**Spontaneous:** Stereotypical behavior instances	Olmarker et al.[90]
	Rat/adult	Not specified	L4/5, 5/6 anterior puncture + nucleotomy	7 weeks (behavioral) 9 weeks (molecular)	X-ray Histology qPCR (DRG)	**Mechanical:** von Frey (hindpaw) Algometer (lumbar)	Kim *et al.*[56]
	Rat/8 weeks	Male	L5/6 anterior 10× puncture	4 weeks	IHC (DRG)	**Spontaneous:** Gait analysis	Miyagi *et al.*[91]
	Rat/not specified	Male	L4/5 anterior puncture OR L4/5 L facetectomy + L4/5 posterior puncture	6 weeks	MRI Histology IHC (NP) IHC (DRG) qPCR (NP) Western blot (NP)	**Mechanical:** von Frey (hindpaw) **Heat:** Hot plate (hindpaw)	Li *et al.*[92]
Structural + inflammatory	Rat/not specified	Male	L5/6 anterior puncture + 10μL CFA	8 weeks	Histology IHC (spinal cord, IVD) qPCR (DRG)	**Spontaneous:** Weight loading **Mechanical:** von Frey (hindpaw, lumbar) **Cold:** Tail flick **Heat:** Withdrawal (tail)	Lee *et al.*[93]
	Rat/4 months	Male	L3/4, 4/5, 5/6 anterior puncture + 0.25ng TNF-α or NGF/VEGF injection	6 weeks	X-ray Histology IHC (IVD, DRG)	**Mechanical:** von Frey	Lai *et al.*[94]
Genetic	Mouse/3 months and 9 months	Male	*SPARC*$^{-/-}$	N/A	N/A	**Mechanical:** von Frey (hindpaw, lumbar) **Cold:** Acetone test (hindpaw, back) Withdrawal (tail) **Heat:** Withdrawal (hindpaw, tail)	Millecamps *et al.*[96]

NOTE: DRG, dorsal root ganglia; IHC, immunohistochemistry