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A bad break: mechanisms and assessment of acute and chronic pain after bone fracture

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Abstract

Pain is one of the primary indicators of a bone fracture and serves both a functional and practical role in guiding recovery. However, fracture pain can persist long after the fracture itself has clinically healed. The neural and molecular mechanisms that drive acute pain postfracture, and how these mechanisms are pathologically usurped to trap patients into persistent, debilitating, and often difficult to treat, chronic pain, are not well understood. The aim of this review is to provide insight into the risk factors for pain persistence after fracture, review the physiological and pathophysiological mechanisms of fracture pain, and critically evaluate the literature around fracture pain assessment techniques/models. Taken together, the concepts covered herein will provide a strong foundation to support the development of more effective treatments to better alleviate postfracture pain.

Keywords: Fracture-related pain, Chronic fracture pain, Preclinical pain models, Innervation of fracture callus

1. Introduction

Fractures, especially those that fail to heal properly, are one of the most common injuries worldwide, with *The Lancet* Global Burden of Diseases reporting 178 million new fractures and 445 million prevalent fractures in 2019.[3] Global instances of nonunion, the failure of a broken bone to heal, are estimated between 8% and 14% with higher instances in patients with concomitant injuries, comorbidities (aging, diabetes, obesity), and those occurring in the lower extremity.[26–28,59] The diagnosis of either delayed fracture healing or nonunion is made clinically after 6 to 12 months based on the persistence of a fracture line on plain radiographs and pain or instability with weight bearing. The growing prevalence of osteoporosis associated with the rapidly aging global population is expected to substantially increase the prevalence of both fractures and nonunion over the next decade.[34,138] The standard treatment to address these scenarios of poor fracture healing is surgical intervention, which is associated with significant medical costs and loss of societal productivity. Comparing quality-of-life scores among patients suffering from chronic health conditions, patients with nonunions scored worse than all the musculoskeletal conditions and as the third worst of all chronic conditions, only behind Parkinson disease and paralysis.[184,208,250]

Pain is a multifaceted coordinator of the physical and biological healing response and is defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage."[1] Reports of the immediate pain experience after a fracture are surprisingly variable, ranging from "little to none" to "severe" pain.[17,100,220] Although patients with lower extremity fractures often present with tenderness at the fracture site and pain during weight bearing,[24,92] clinical pain scores per se are not indicative of the presence of a fracture after injury.[245,276]

Although unpleasant, pain immediately after fracture serves a beneficial role as it discourages activity that may otherwise propagate injury. Patients with congenital insensitivity to pain have experienced lower extremity fractures that go unnoticed, enabling premature weight bearing that has led to further bone damage and subsequent deformities.[88,94,173,207] Normally, as lower extremity fracture healing progresses, there is a concomitant reduction in pain[57] and an increase in weight bearing[112,135] that lead to gradual improvements in gait-related parameters.[253] In fact, this bone loading is a critical catalyst for the anabolic phases of bone formation.[13,239,253,266] Pain during weight bearing beyond the normal healing window is a major clinical indicator of incomplete or delayed healing.[47,250] In this context, if the pain persists beyond 3 months, then it is diagnosed vaguely as chronic pain.[244] To alleviate persistent pain[160] and promote healing,[18] many patients will undergo multiple surgical interventions, which are ranked among the most painful surgical procedures a patient can undergo.[80]

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Opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) are the standard of care treatment for postfracture pain.[61] Nonsteroidal anti-inflammatory drugs effectively relieve musculoskeletal pain and work by inhibiting cyclooxygenase activity upstream of the prostaglandins that trigger inflammation. However, several prominent studies have found that NSAIDs can delay fracture healing.[82,230,255] Despite the fact that other studies find the evidence to be weak or contradictory,[209] in the United States, the perceived risk has led to widespread avoidance of NSAIDs to treat pain during fracture healing. This clinical perception has resulted in opioids becoming the most prescribed and dispensed pain medications used during fracture management. The number and type of opioid prescribed varies by geographical region and injury, but the published numbers are staggering. For example, 1 study found that among 41 different conditions associated with acute pain, fractures had the highest opioid prescription rate in patients with Medicaid (56.3%) and those who are privately insured (44.8%).[180] In patients with ankle or hip fractures that require surgery, over 60% fill prescriptions for opioids, and ~21% of them still filled the prescription at 9 months postop.[50,134] Although the long-term effects of childhood treatment with opioids are still unknown, recent evidence suggests that 37% to 51% of pediatric fractures treated in the emergency department receive an opioid prescription.[143,268]

Although NSAID addiction is exceedingly rare,[83] opioids have garnered significant attention because of the associated risks of substance misuse and long-term addiction, which have contributed to our current opioid epidemic.[21,49,102] In recent years, there has been a concerted effort from professional societies, such as the IASP and the Orthopaedic Trauma Association, to produce comprehensive guidelines and recommendations for multimodal pain management protocols. These protocols are designed to improve acute pain management and include adjunctive therapies to reduce reliance on opioids.[116] To date, however, whether these protocols will transfer to the management of chronic fracture pain has not been well studied. Taken together, current evidence-based protocols for managing the spectrum of acute to chronic fracture pain remain inadequate. The goal of this review article is to motivate the development of novel therapies for managing fracture pain, without inhibition of bone healing. This effort requires a comprehensive understanding of the mechanisms and pathophysiology of the onset and persistence of pain after fracture.

2. Risk factors for chronic postfracture pain

Most often, acute pain subsides within a week or 2 of the fracture.[68] However, clinical studies have found that a substantial percentage of patients (13.4%-90%) enter into a state of chronic pain, even in some instances where there is radiological confirmation of fracture union (**Table 1**).[11,57,73,141,178,237] Chronic postfracture pain can persist and manifest as pain with loading/exercise, sensitivity to innocuous stimuli (mechanical allodynia),[212] and/or shifting temperature sensitivity (ie, thermal hyperalgesia).[91,281] Interestingly, heat sensitivity occurs early after fracture,[181] whereas cold sensitivity predominates at later time points.[162] The reason some fracture pain becomes chronic remains poorly understood; however, both nonmodifiable and modifiable risks factors have been identified. Nonmodifiable factors include age over 65 years,[36,51,139,201] female sex,[57,201] and intensity of acute pain.[36,201,274] Modifiable risks include smoking,[44,139] high body mass index/obesity,[51] time to surgery,[44,274] type of surgical procedure,[51,274] and preoperative opioid use.[139,201,220] Lower socioeconomic status and psychosocial factors, such as high anxiety, depression, and post-traumatic stress disorder, have also emerged as strong predictors of chronic pain.[249]

Despite otherwise normal fracture healing, up to 30% of tibia and radius fracture patients reported complex regional pain syndrome (CRPS).[171] Complex regional pain syndrome, previously called reflex sympathetic dystrophy, which is characterized by severe pain and inflammation accompanied by sensory, autonomic, motor, and nutritional disturbances.[63] The disease can range from mild and self-limiting to chronic with functional impairment in daily living.[97] Complex regional pain syndrome is associated with an abnormal host response to tissue injury and reportedly involves multiple mechanisms. For example, the National Institute of Health reports that over 90% of CRPS cases involve damage and subsequent dysfunction of peripheral nerves, leading to altered cutaneous blood flow and a variety of uncomfortable paresthesia.[197] Altered sympathetic nerve activity, peripheral and central sensitization, elevated local and systemic inflammatory cytokines, and decreased systemic anti-inflammatory cytokines are other proposed mechanisms involved in CRPS.[31,171]

3. Signaling pathways for fracture pain

The skeletal system is intricately innervated, playing a pivotal role in nociception after fracture. This section describes the anatomy and physiology of bone innervation, the pathophysiological mechanisms underlying fracture-associated pain, and the pathways that may cause transition to chronic pain.

3.1. Bone innervation

Bone tissues are innervated by a combination of primary sensory and autonomic nerves (**Fig. 1**).[22,37,60,104,117,124,161,163,233,260] The primary nociceptive sensory Aδ and unmyelinated C fibers are the most abundant nerves in bone.[132,233] Via projections to the dorsal horn of the spinal cord, afferent fibers engage interneurons and projection neurons that conduct nociceptive signals to the brain, where pain perception is generated.[16,118,125,132,179,269] The Aδ and C nerve fibers have distinct electrophysiological and molecular characteristics. Aδ afferents transmit immediate sharp pain[30,210,258] and conduct action potentials at a high conduction velocity (10-20 m/s) as a result of their thin myelination.[147,163,279] These fibers are defined molecularly by the presence of tropomyosin receptor kinase A (TrkA), the high-affinity receptor for a nerve growth factor (NGF), and the intermediate neurofilament portion of the cytoskeleton (neurofilament 200 kD, NF200).[25,64,65,152,193,241] Conversely, the unmyelinated C fibers transmit long-lasting dull or burning pain[30,210,258] with a slow conduction velocity of 1 m/s.[12,147,163,252,279] C fibers can be split into 2 subpopulations, peptidergic and nonpeptidergic.[16] Peptidergic C fibers, like Aδ, have TrkA receptors but are distinguished from Aδ by their synthesis of the neuropeptides substance P (SP) and calcitonin gene–related peptide (CGRP).[12,252,282] These factors contribute to not only nociception but also neurogenic inflammation and vasodilatation.[37,48,126,132,170,200] Specifically, the release of CGRP and SP leads to an influx of proinflammatory cytokines in the innervated area.[16] Bone-innervating nonpeptidergic C fibers can be distinguished by the binding of isolectin B4[124] and their lack of markers commonly expressed in skin innervating counterparts, such as P_2X_3, a receptor channel, and MAS-related GPR family member G (Mrgprd).[132] Nonpeptidergic C fibers have also been shown to contribute to inflammatory-mediated pain after bone fracture through purinergic signaling.[196]

Primary sensory nerves innervate the full thickness of the bone, from the outer layer of the bone (the periosteum), through the dense cortical bone, and into the inner bone marrow.[42,119,132,144,183] The

Table 1

The incidence of chronic postfracture pain.

Incidence of chronic postfracture pain	Type of fracture	Author
13%	Hip fracture with surgery	Dasch et al.[51]
64%	Rib fracture	Fabricant et al.[67]
19%	Wrist or ankle fracture with surgery	Friesgaard et al.[73]
51%	Fragility fracture (vertebrae, hip, shoulder, wrist, other)	Gheorghita et al.[81]
78%	Lower extremity fracture	Griffioen et al.[90]
55%	Tibial fracture with surgery	Khan et al.[141]
20%	Distal radius fracture	MacDermid et al.[159]
52%	Fragility fracture (vertebrae, hip, wrist, other)	Sale et al.[219]
90%	Vertebral body fragility fracture	Suzuki et al.[234]
45%	Lower extremity fracture	Van Wyngaarden et al.[249]
60%	Distal radius fracture with surgery	Yoon et al.[274]

periosteum is a highly bioactive thin fibrous tissue that covers most of the bone surface and is broken with the fracture. Over 90% of Aδ and C fibers are located in the inner cambium layer of the periosteum and are organized into a mesh-like network where they sense mechanical damage or distortion of the underlying cortical bone.[40,132,172,233] Interestingly, this cambium layer also contains osteochondral progenitor cells, suggesting a potential interplay between the nerve fibers and the stem cell niche, an hypothesis that has yet to be fully explored.[14,52,192,241,243,247,269] The cortical bone is similarly innervated by both Aδ and C fibers, which pass through Haversian and Volkmann canals alongside blood vessels.[40,132,172] These fibers are also associated with blood vessels within the bone marrow cavity.[161] A previous study reported that the relative density ratio of sensory nerve fibers across the periosteum, cortical bone, to

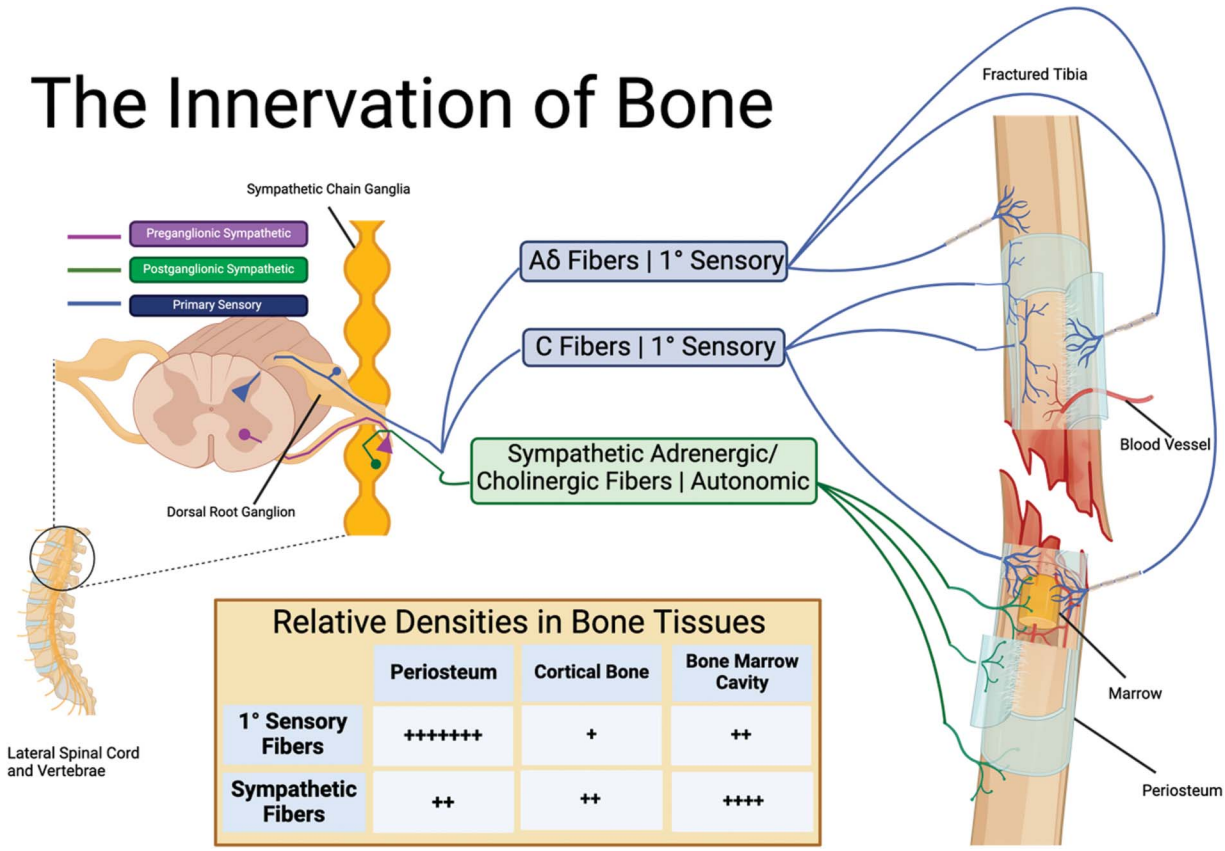

Figure 1. Schematic representation of the neural innervation in bone tissue. The figure highlights the pathways and types of nerve fibers involved in bone innervation, including preganglionic and postganglionic sympathetic fibers (represented in purple and green, respectively) and primary sensory fibers (in blue). The figure illustrates how Aδ and C sensory fibers, as well as postganglionic sympathetic fibers, project to different bone regions, such as the periosteum and medullary cavity, and the distribution of sympathetic adrenergic/cholinergic fibers. The table summarizes the relative densities of these fibers in cortical bone, periosteum, and the medullary cavity, indicating higher densities of primary sensory fibers in the periosteum and sympathetic fibers in the medullary cavity. Created with BioRender.com.

bone marrow cavity is 100:0.1:2.[37] This value implies that the periosteum is particularly sensitive to noxious stimuli because it is innervated by the greatest relative number of sensory nerve fibers.

Bone is also innervated by cholinergic, acetylcholine releasing, and adrenergic, norepinephrine or epinephrine releasing, sympathetic nerve fibers.[60,95,151,269] These postganglionic sympathetic nerves are small, unmyelinated fibers with a slow conduction velocity, similar to that of C fibers.[110] In the periosteum, the sympathetic nerves are far less dense compared with primary sensory nerve fibers.[104,121,161] However, their density in the cortical bone nutrient canals of mice was found to be twice that of the primary sensory fibers.[40] Sympathetic nerve fibers are also closely associated with medullary and periosteal vasculature, spiraling around cortical bone blood vessels.[25,172] Similar to primary sensory fibers, they terminate as free-nerve endings within the bone marrow.

The cholinergic innervation is less abundant in bone tissues compared to the adrenergic innervation.[5,10,40,43,77,105,110,121,161] The cholinergic fibers synthesize acetylcholine and vasoactive intestinal peptide, which regulate local blood flow and inflammatory responses. Adrenergic neurons also regulate bone metabolism and remodeling by modulating osteoblast/osteoclast activity through the release of tyrosine hydroxylase and norepinephrine.[41,60,156]

3.2. Physiological and pathophysiological mechanisms underlying fracture pain

The drivers of fracture pain, both neural and molecular, shift over the time course of healing (**Fig. 2**). Early on, pain results from acute activation of sensory neurons (nerve injury, inflammatory mediators) and may be beneficial to healing, by minimizing weight bearing/movement on the affected bone. Later in the process, sensitization (peripheral and central) of the nociceptive circuits heightens pain, whereas the bone continues to heal. However, fracture pain often continues to persist beyond the point that is beneficial for fracture healing (**Table 1**), transitioning into the pathological form of chronic pain. This section dissects 5 key mechanisms that contribute to fracture pain: (1) activation of Aδ and C-fiber activity, (2) peripheral nerve sensitization, (3) the sprouting of sensory and sympathetic nerve fibers, (4) the onset of central sensitization, and (5) the interpretation of pain by the brain.

3.2.1. Mechanical and inflammatory activation of Aδ and C-fibers sensory nerves

The instant, acute pain felt at the time of fracture is primarily driven by mechanical disturbance of the Aδ and C fibers.[186,189] In isolated preparations of Aδ and C fibers from fracture models, the nerves are activated by mechanical distortion during and after the

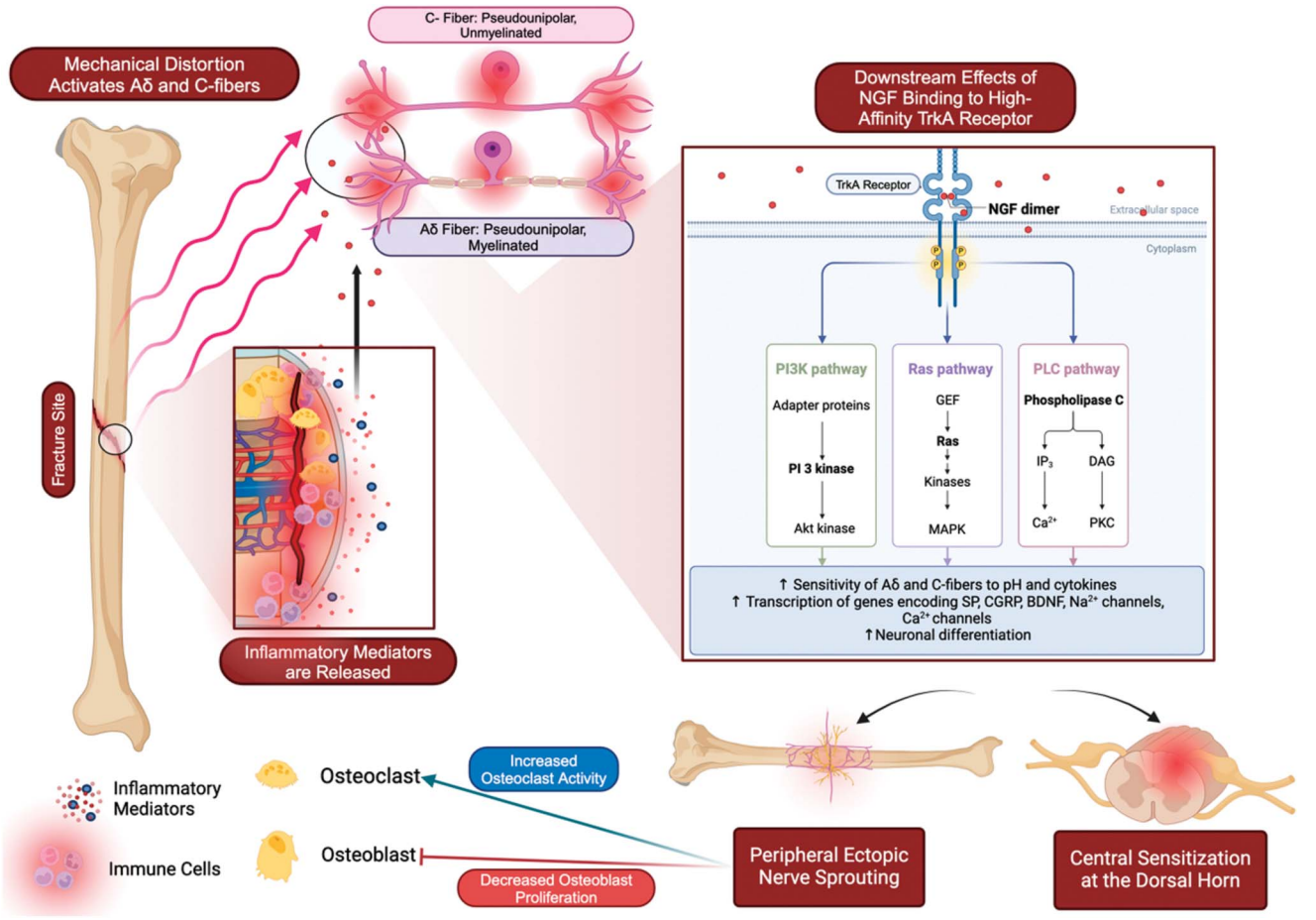

Figure 2. Pathophysiological mechanisms underlying fracture pain and sensitization. Mechanical distortion at the fracture site drives a complex interplay between key neural and molecular factors during the progression of fracture pain and pain sensitization. Damaged bone, soft tissue, and vasculature release inflammatory mediators and attract immune cells to the site of injury. NGF dimers bind to the high affinity TrkA receptor, triggering signaling cascades through the PI3K, Ras, and PLC pathways, and influencing gene transcription and neuronal differentiation. These molecular events contribute to the heightened sensitivity to pain during bone healing and can lead to chronic pain conditions. Further, peripheral ectopic nerve sprouting has been shown to increase osteoclast activity, while decreasing osteoblast proliferation. Created with BioRender.com. NGF, nerve growth factor; PI3K, type I phosphatidylinositol 3-kinase; PLC, phospholipase C; TrkA, tropomyosin receptor kinase A.

fracture then continue to discharge until they are repositioned spatially to their original location.[2,163,177,191,222] Although the precise mechanisms by which fracture-related mechanical stimuli activate the sensory neurons are still unclear, recent studies suggest that piezo-type mechanosensitive ion channel component 2, a mechanically gated ion channel, and Stomatin-like protein 3, a membrane bound scaffolding protein, are significantly involved.[188,194]

The standard treatment after fracture consists of realigning the broken bone ends and then stabilizing them using orthopaedic hardware (plates, nails, screws, wires) and splints. This mechanical stabilization contributes to immediate pain relief, likely associated with reduced mechanical disturbance of the sensory nerves. Specifically preserving the highly innervated periosteum, which supports both nerve and bone repair, also relieves some of the acute pain signals from mechanosensitive neurons.[75] Interestingly, fracture stabilization does not relieve all acute pain.

The intense, sharp pain felt initially gets replaced by more of a dull, aching pain sensation. This subacute pain sensation may be driven more by the inflammatory response, which also contributes significantly to nociceptor sensitization. Both the innate and adaptive inflammatory response are critical components of appropriate fracture healing. This complex and highly coordinated inflammatory response is beyond the scope of this article but has been recently covered in detail by multiple comprehensive reviews.[33,35,46,84] Briefly, the immune response can be thought of as occurring in multiple waves initiated by proinflammatory neutrophils, followed by invading macrophages. More recently, the adaptive immune system has also been shown to significantly contribute to fracture healing.[32,157,203,218] In normally healing fractures, these proinflammatory responses are followed by a proregenerative immune response driven largely by anti-inflammatory macrophages and osteomacs, or tissue-resident macrophages.[71,120,221]

It is likely that this transition in pain sensation is still mediated by both Aδ and C-fiber activation, because chemical ablation of C-fiber terminals was only able to partially alleviate pain post-fracture.[128] Both fiber types are responsive to several known pronociceptive factors, including, prostaglandins, growth factors, neurotransmitters, bradykinin, endothelins, and cytokines.[167,265] These factors are released from damaged bone cells and invading immune cells, which then activate the afferent pain fibers in bone.[15,58,167] Inflammation also leads to significant tissue acidosis.[213] Acidosis of the fracture callus induces bone resorption activity necessary for fracture healing but also heightens the activity of the Aδ and C fibers.

Finally, the activation of nonpeptidergic C fibers by glial cell line–derived neurotrophic factor, neurturin, and artemin after bone fracture has been shown to contribute to inflammatory-mediated pain.[196] Sequestration of these factors using antibodies reduces behavioral endpoints of pain (ie, hindlimb weight bearing ratio) after the injection of complete Freund adjuvant into the bone marrow.[196]

3.2.2. Peripheral nerve sensitization

Within minutes to hours after fracture, continued stimulation of nociceptors drives peripheral pain sensitization, lowering the threshold for activation and thereby amplifying the responses of nociceptors to noxious and innocuous stimuli.[106,168,187,189] A normal response to injury, peripheral pain sensitization produces allodynia (pain in response to normally innocuous stimuli) and hyperalgesia (an exaggerated or prolonged pain experience compared to one's baseline response to a noxious stimulus).[16] Inflammation is a major driver of the sensitization process,[16] partly because of the presence of bradykinin, prostaglandin E2, serotonin, tumor necrosis factor α, colony stimulating factors, as well as factors that activate the protease-activated receptor 2 at the fracture site.[182] This inflammatory milieu drives changes in ion channels, which in turn modifies nociceptor excitability.[154,168]

Although many agents are capable of sensitizing nociceptors in bone, the secretion of NGF after fracture plays a crucial role in coordinating peripheral sensitization through interaction with its high-affinity receptor, TrkA, on nociceptors. Nerve growth factor is released from several inflammatory cells, including mast cells, macrophages, and lymphocytes, as well as fracture callus hypertrophic chondrocytes.[8,9,22,37,195,214,227,231] The binding of NGF to TrkA-expressing nociceptors initiates a molecular cascade that drives nociceptor depolarization and hyperexcitability.[101,140,165,228] This cascade leads to the activation of various intracellular signaling pathways, including the MAPK/ERK and PI3K/Akt pathways.[45] In bone, this has been shown to contribute to the phosphorylation of various ion channels, such as the transient receptor potential vanilloid 1 and acid-sensing ion channel 3 ion channels, which heightens the sensitivity of the sensory nerve fibers to local pH changes, heat, and noxious mechanical stimuli.[62,166,277] Moreover, NGF induces phosphorylation of bradykinin receptors and prostaglandin E2 receptors, causing the Aδ and C fibers to be more receptive to these cytokines.[53,277] The NGF-mediated increase in transcription of various genes involved in nociception also contributes to peripheral sensitization. Specifically, transcription of SP, CGRP, and brain-derived neurotrophic factor were shown to increase after the nuclear localization of NGF-TrkA complexes in clathrin-coated signaling endosomes.[4,53,86,115,137,205,225,271] However, more research is needed to understand the precise mechanisms of NGF's contribution to early peripheral pain sensitization in fracture.

3.2.3. Ectopic sprouting in sensory and sympathetic nerve fibers

In addition to playing a role in peripheral pain sensitization, NGF/TrkA signaling drives nerve sprouting within the fracture.[41,117,184] Recent studies suggest that innervation precedes neovascularization of the fracture tissue, with NGF expression peaking during the proinflammatory and early endochondral phases of healing.[152,214] In addition to its role in driving innervation, NGF has a trophic function during fracture healing, driving cell migration/proliferation and osteogenesis.[214,270] Interestingly, this trophic function may use the p75 pathway in addition to, or perhaps instead of, the TrkA pathway.[267,270]

The sprouting of nerve fibers is hypothesized to be important in guiding the patient to protect the injured limb and avoid overuse until the fracture has healed.[117,248] In a rat model of bone injury, NGF and the associated nerve sprouting were found to contribute to mechanical hyperalgesia during endochondral ossification.[5,202,273] Studies outside of fracture repair also support this premise, with data showing nerve fiber sprouting correlates with pain intensity in bone cancer pain.[41,132,169] As the fracture successfully heals and the callus is remodeled, the newly sprouted nerve fibers regress,[150] and once the bone is completely healed, the bone innervation is restored to its prefracture architecture. Despite the growing body of evidence that NGF/TrkA signaling is involved in nerve sprouting across several skeletal pathologies, no mechanistic studies have been performed to understand the molecular pathways involved with nerve regression after fracture healing. However, during fracture

healing, the resolution of inflammation and the endochondral conversion of hypertrophic chondrocytes to osteoblasts eliminates the primary sources of NGF expression, and this decreased expression level may contribute to the regression of the newly sprouted nerves.

If the normal healing process does not occur, the ectopic nerves do not regress, and fracture-induced pain persists.[129–131,153,169,224,240,251] Vigorous nerve sprouting leading to an increased density of sympathetic and sensory nerve fibers and the formation of neuroma-like structures near the fracture site has been observed in mice with nonhealed fractures.[41,104] Based on these findings, anti-NGF has been explored as a potential therapeutic to reduce pain after fracture healing, however, with mixed outcomes. Reducing ectopic sprouting by blocking NGF/TrkA signaling decreased hyperalgesia and pain behaviors in animal models,[56,129,202,273] and similar treatments have reduced pain in humans. However, the negative outcome of these treatments was the potential to accelerate osteoarthritis[25,109,145,259] and inhibit bone formation.[280]

3.2.4. Central sensitization

Central sensitization is a process that refers to functional changes in central nociceptive circuits provoked by the continued activation of peripheral nociceptors.[262–264] With central sensitization, nociceptive circuits in the dorsal horn of the spinal cord become increasingly excitable by repeated stimulation, have improved synaptic efficacy, and reduced inhibition, resulting in increased activation of spinal circuits even after the stimulus has been removed.[7,105,111,123,168,223,225,229,257,272] This neuronal adaptation has been shown to cause neuropathic and inflammatory pain. Electrophysiological evidence demonstrates that increased signaling from injured nociceptive fibers in bone increases the receptive field of neurons in the dorsal horn of the spinal cord.[105] Importantly, central sensitization can enhance the experience of pain in the context of bone fracture and repair.[184]

Central sensitization is driven by several complex neurobiological changes in dorsal horn neurons, and these processes are detailed in excellent recent reviews.[262–264] At a high level, current evidence suggests that the key trigger of central sensitization is the increase in intracellular calcium (Ca^{2+}) beyond the normal threshold levels. This abnormal intracellular Ca^{2+} is largely driven by overactivation of the N-methyl-D-aspartate receptor, but the calcium permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor and other voltage-gated Ca^{2+} channels may also contribute to the increase. This increase of Ca^{2+} creates a positive feedback loop in which there is (1) further activation of N-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors, (2) recruitment of receptors to the membrane, and (3) activation of protein kinase C, which consequently reduces the inhibitory signals from gamma-aminobutyric acid and glycine neurotransmitter release. Other cellular and molecular mechanisms have been identified as contributing to central sensitization and increased intracellular Ca^{2+}, including the upregulation of endogenous opioid peptide dynorphin,[111,223,225] and microglial activation and astrocyte hypertrophy in the spinal cord.[111,223,227]

In the context of fracture-induced pain, these molecular, genetic, and cellular changes likely contribute to central sensitization, enabling innocuous mechanical inputs to engage nociceptive pathways (mechanical allodynia), cause a spread of sensitivity outside of the area of injury into noninjured tissue (secondary hyperalgesia), and increase the magnitude and duration of the response to repeated inputs (temporal summation).[264] Although it is clear that various skeletal pathologies can effectively trigger central sensitization,[168] an important area of future study will be to specifically correlate fracture characteristics and systemic factors with the acute and chronic pain sensitization patterns that vary from patient to patient.

3.2.5. Modulation of postfracture pain by the brain

The perception of pain is processed within a diverse set of brain regions that are collectively termed the "pain matrix."[242] It is believed that coordinated activity across the regions that comprise the pain matrix drives the pain experience, as opposed to the specific activation of any single region (ie, primary somatosensory or anterior cingulate cortices).[242] In cats, there is evidence of Aδ-driven activation of primary and secondary somatosensory cortices during stimulation of bone-innervating nerve fibers,[124] but how fracture alters cortical activation, and furthermore, how alterations in cortical circuits contributes to chronic postfracture pain, remains unknown. In humans, there are no studies that specifically address how ongoing fracture-induced pain alters brain activity. In 1 particularly relevant brain imaging study, patients with lower back pain were monitored during the transition from the subacute to chronic phases. The authors demonstrated that brain regions associated with the subject's experience of pain shifted from those traditionally involved in pain perception (ie, the pain matrix) to those that are largely emotionally related.[99] Interestingly, patients with particular psychosocial traits (ie, depression, pain catastrophizing) have a greatly increased risk of chronic pain after bone fracture.[85,211,249] This finding raises the possibility that functional brain states before injury prime these patients for increased risk of chronic pain, regardless of the type of injury.[149,256] Developing a mechanistic understanding of how bone fracture alters brain activity to drive chronic pain will aid the progress of much needed treatments for patients whose chronic pain remains even after resolution of the bone fracture, and the local mechanisms that drive pain during healing (eg, inflammation).

4. Assessment of fracture pain: from human to mouse

The molecular and cellular changes that occur at the fracture site, through the spinal cord and into the brain, functionally manifest into the experience of pain. Quantifying fracture-related pain is more challenging than perhaps expected, but recent efforts to address the opioid epidemic have motivated improvement in this area. In this section, we review pain assessment techniques used in a clinical and preclinical setting, with a summary of their pros and cons in **Table 2**.

4.1. Clinical assessment of pain after fracture

Clinical assessments of fracture pain aim to capture a patient's immediate[122,174,204] or recalled[220] subjective experience of pain. Unfortunately, there is no biomarker of pain. Rather, the 11-point (0 = no pain, 10 = worst pain imaginable) verbal numeric rating scale[127] or visual analog scale[136] has historically been used and is generally a reliable and valid measure of pain intensity.[20] For patients who, for various reasons, are unable to express their own pain levels, the Faces Pain Scale-Revised is often used to assess fracture pain.[74] Although often used in clinical practice to assess pain changes during the fracture healing process, it has been suggested that these scales are

Table 2

The summary of pain assessment.

Model	Pros	Cons
Evoked (animal)		
Von Frey test[39,93,148,181,198]	• Useful for assessing mechanical sensitivity and allodynia • Can be manual or electrical • Electrical method is less time-consuming	• Manual method is time-consuming • Requires experienced technique • High interobserver variability • Skin hypersensitivity not necessarily skeletal pain surrogate
Hot and cold plate[66,93,181]	• Useful for assessing thermal hyperalgesia via conductive thermal stimuli across the entire paw • Can elicit a range of behaviors	• Test sites not primary site of injury • Skin hypersensitivity not necessarily skeletal pain surrogate
Hargreaves test[182]	• Allows comparison between fractured and contralateral limb at particular plantar locations • Focused, infrared thermal stimulus	• Primary site of pain is not within the hind paw • Focuses on the withdraw reflex
Spontaneous (animal)		
Weight bearing[89,175,222]	• Allows for dynamic and static analysis • Suitable for various pain types (osteoarthritis, bone cancer, fracture) • Advanced software available for use	• Only suited for unilateral hind limb injuries • Remains unstudied specifically in fracture-related pain
Gait analysis[72,142,220,232]	• Detailed evaluation of behavioral changes	• Rats and mice may not show significant gait changes postfracture • Some parameters more reliable than others • Does not always correlate with other methods
Guarding behaviors and flinching[72,142,220,232]	• Useful indicators of spontaneous nociceptive behaviors • Recapitulates some behaviors seen clinically	• Time-intensive video analysis • Lacks standardized analysis methods
Facial expression, nest complexity score, etc.[133]	• Useful for evaluating spontaneous pain behavior	• Labor intensive
Clinical (human)		
Numeric Rating Scale and Visual Analog Scale[108,127,155,176,199]	• Reliable and valid measure of pain intensity	• Inadequate for assessing chronic pain • Mostly examines intensity, not quality
Faces Pain Scale-Revised[74,103,199]	• Useful for patients unable to express their own pain levels	• Inadequate for assessing chronic pain

only suitable for measuring the acute phase and inadequate for assessing the chronic phase.[199]

In 2004, the National Institute for Health launched a major initiative to create a series of person-centered measures to evaluate and monitor physical, mental, and social health in adults and children by creating Patient-Reported Outcome Measurement Information System (PROMIS) in multiple languages.[38] In the fracture setting, there has been a gradual increase in the use of the PROMIS Pain Interference and Physical Function since 2017; however, they are still far from being incorporated as standard of care. A recent systematic review helps collect and summarize the prospective and retrospective use of PROMIS measures in the orthopedic fracture population.[113] This review, along with some additional more recent publications,[114] highlights that most studies are small cohorts of patients with specific fracture types and that pain patterns remain understudies without clear conclusions or validation.[190] The challenge and heterogeneity of studying pain in human populations supports the integration of preclinical models as an important strategy to study pain and screen analgesics.

4.2. Animal fracture pain models

There are a few widely used preclinical models that are useful for probing fracture-induced pain.[226] They include a cortical drill hole,[185] osteotomy,[19] and the 3-point bending fracture (Einhorn model)[181] of the femur or tibia. Although a cortical hole model is commonly used to evaluate fracture healing, osteotomy and Einhorn models are more relevant to the assessment of fracture pain in rodents.[133,142,146,162,181,184,206,261,281] Osteotomies are typically created using a saw-like device to transect the bone in an open procedure to visualize the bone. After the osteotomy, the bone is usually stabilized with plates, intramedullary nails, or external fixation.[19,133,146,162,206,261,275,281] Alternatively, the Einhorn fracture model creates a closed, traumatic fracture with concomitant muscle injury using a 3-point bending device or guillotine mechanism.[181] The fractures are stabilized percutaneously through intramedullary pinning via a small incision made above the tibial tuberosity.

Bone fracture is studied across a wide variety of animal models, with rodents being used in the vast majority of studies (~85%).[78] Larger animals, such as sheep, dogs, and pigs, are also used to test the efficacy of treatments that can be used in companion animals and humans. In this regard, targeted pharmacological manipulation of the NGF signaling pathway (anti-NGF monoclonal antibody) is now a promising treatment for pain relief in dogs.[62,79] Although, there is no general consensus as to which large animals should be used for investigational new drug-enabling studies regarding safety and efficacy.

4.3. Animal pain behavior overview

The assessment of pain in nonhuman animals is considerably limited because the animal's subjective state cannot be directly probed (ie, verbally) and, therefore, relies on behavioral assays to approximate pain experiences in humans.[54,69] This has led to the development of a number of quantitative methods for assessing pain in preclinical models. These methods are designed to

distinguish between different dimensions of the possible pain experience of an animal,[54,246] such as evoked vs spontaneous pain. Below we provide an overview of how these preclinical assays are used to assess the nature of fracture-induced pain and provide a summary in **Table 2**.

4.4. Evoked pain behaviors for assessing fracture pain

In response to the external application of a noxious stimulus, animals will often produce an evoked behavior, indicative of pain, which may include the withdrawal or shaking of, or otherwise tending to, the stimulated body part.[54] One of the most common methods to measure evoked pain in rodents is through different types of reflexive avoidance assays. In these assays, the hind paw is stimulated through different mechanisms (ie, mechanical, thermal, chemical), and the animal's sensitivity to pain and withdrawal reflex is measured. External stimuli can also be applied to the site of injury or to another site in close proximity to the site of injury, although most often stimuli are applied to the hind paws of a rodent. However, the primary site of the pain is localized at the fracture itself, which confounds the assessment of fracture-induced pain by assessing evoked sensory thresholds of the hind paw skin. Such tests of cutaneous sensitivity may be more useful in assessing referred pain or secondary hyperalgesia because of inflammatory or neuropathic processes that are known to produce clinically relevant changes in cutaneous thresholds (ie, CRPS[217]).

Although none of these assays are well-established in the fracture literature, the von Frey test is the most widely used behavioral assay. In this assay, to determine the mechanical nociceptive threshold, a series of thin, calibrated monofilaments of ascending weights are applied/pressed-into to the plantar surface of hind paw.[87,96,162,181,206,236] The nociceptive threshold is quantified by assessing changes in the frequency or type of physical responses (ie, flinch, withdrawal, shake, lick) in response to the different filaments. For example, in injury models that produce hypersensitivity, the physical response to a particular weight fiber will increase in comparison to baseline/preinjury levels.[39] Ideally, the administration of an analgesic reverses this enhanced sensitivity.[70] Time saving methods for identifying the von Frey threshold include the Dixon Up/Down method[39,55] or Simplified Up/Down Method,[23] which provide an estimate of the fiber weight at which a mouse would withdraw the stimulated paw on 50% of the trials. Although widely used, from the tester's perspective, von Frey measurements of mechanical sensitivity not only are laborious and tedious but can also produce highly variable results between individual laboratories and testers.

Thermal sensitivity testing takes on a few different forms.[54] A calibrated heat stimulus (usually in the form of radiant heat[98] or laser beam[254]) can be selectively applied to the plantar surface of the paw, which produces a behavioral response to the temperature similar to those produced by the von Frey monofilaments. As such, the heat intensity can be calibrated so that latency to response, and its alteration by injury and analgesia, becomes a quantifiable endpoint.[6] Additional thermal assays for assessing preclinical fracture-induced pain include hot plate and cold plate tests, where an animal is placed on a temperature controlled plate and observed for nociceptive behaviors (ie, flinch, withdrawal, shake, lick) and escape behaviors, such as jumping. Quantification of pain response is performed by either measuring the latency to the first sign of nociception and/or quantifying the number of nociceptive behaviors over a set time interval.[54] Importantly, through careful notation of behavioral responses during thermal sensitivity tests, it is possible to dissociate reflexive (ie, spinally mediated) vs affective-motivational (ie, brain-mediated) behaviors. Behaviors such as licking or jumping fit indicate an unpleasant sensory experience to noxious stimuli and the motivation to alleviate or escape from the applied stimulus.[254]

4.5. Spontaneous pain behaviors for assessing fracture pain

In addition to the assessment of evoked pain behaviors, the assessment of spontaneous pain behaviors is also useful to evaluate fracture pain in animals.[54] Weight bearing and gait assays are the most commonly analyzed spontaneous pain behaviors in mice. However, additional behavioral and expression assays have also been developed to complement these assays do not require specific equipment.

Weight-bearing analysis can be divided into 2 major categories: static and dynamic. To evaluate the static weight bearing, the animal is placed in a small container with a tilted floor so that weight is applied to the hindlimbs. Floor sensors measure the weight applied to each hindlimb separately, allowing comparison of weight distribution between the 2 hindlimbs. Similarly, dynamic weight bearing monitors weight percentages to each limb during exercise/movement. These methods have been shown to be effective for osteoarthritis and bone cancer pain and are also applicable to fracture pain.[72,158,164,175,222,238]

Gait analysis, using devices such as CatWalk, can identify changes in gait after fracture.[253] Extracted variables include several parameters depending on the system, including limping, swing speed, stride length, paw print area, paw pressure, stance phase duration, swing phase duration, and interlimb adjustment. Although some studies show clear changes is gait parameters after fracture,[107,261] one study found that gait did not correlate with mechanical allodynia in experimental models of chronic pain.[76]

Another new device that shows promise in detecting pain-related changes in weight bearing and gait is the BlackBox system. Zhang et al.[278] recently published on new data collection and analysis platform that automatically, quantitatively, and objectively measures rodent behavior in nature in an observer-independent and unbiased manner. This technique involves recording a mouse freely moving in the dark for an extended period of time, continuously acquiring 2 parallel video data streams, and automatically extracting and quantifying behavioral features from these data using machine learning techniques. They have identified changes that capture the pain state of rodents in multiple pain models. To date, this method has not been applied to assess pain in a fracture model.

Spontaneous pain behavior can also be evaluated by monitoring guarding behaviors during a specified period of time. Guarding behaviors can include grooming, rearing, and the number of spontaneous paw flinches on the fractured limb. Excessive grooming has been validated and used as an indicator of spontaneous nociceptive behavior specific to fracture pain.[164] Rearing refers to the simultaneous lifting of both front paws and is considered an exploratory behavior that occurs naturally in healthy mice.[235] In general, animals in pain decrease the number of spontaneous rears with loss of interest in the external environment.[29] Furthermore, because rearing involves loading on the hindlimbs, the number of rears is thought to change with the degree of pain and has been used to assess pain in fracture models.[133] Finally, spontaneous paw flinches is defined as "quick and high frequency shaking," and increased in animal with fracture pain.[142] In addition to these guarding behaviors, facial expression,[133] the nest complexity score,[133] explorative

score,[261] and open-field test[206] have been explored to evaluate spontaneous pain behavior in fractured mice.

Tests of affective motivational responses to analgesic treatments can be performed using conditioned place preference assays.[54] In these tests, mice are given the choice to explore 2 distinguishable, yet otherwise equivalent chambers. Then, the mouse is placed in one chamber and paired with drug (eg, an analgesic) but cannot enter the opposing chamber. On a separate exposure, the mouse is placed within the other chamber, but now paired with a control injection, such as saline. On the final testing session, the mouse is again allowed to freely move between 2 chambers, and if a preference for one chamber over another is formed, they will spend more time in that chamber. In this context, an analgesic used in a mouse that is in pain will generate preference for the chamber paired with analgesic over the chamber paired with saline.[215]

4.6. Future directions

As overviewed, a number of methods have been developed to evaluate various pain behaviors in rodents. Taken together, these assays have largely been developed in different fields and there are a limited number of papers using any of these spontaneous pain assays to evaluate fracture-specific pain, making broad conclusion on pain behaviors difficult, but supporting the potential applicability of these measurements in future studies. Further, new pain assessment technologies are actively being developed.[216] For example, Zhang et al.[278] recently published on new data collection and analysis platform that automatically, quantitatively, and objectively measures rodent behavior in nature in an observer-independent and unbiased manner. This technique involves recording a mouse freely moving in the dark for an extended period of time, continuously acquiring 2 parallel video data streams, and automatically extracting and quantifying behavioral features from these data using machine learning techniques. They have identified changes that capture the internal pain state of rodents in multiple pain models. To date, this method has not been applied to assess pain in a fracture model.

5. Conclusions

Fracture prevalence is expected to increase with the aging of society, and this population is at a particularly high risk of poor healing and subsequently chronic postfracture pain. In treating fractures, effective pain management that does not interfere with healing is critical. To this end, it is necessary to better understand the clinical associations of pain with fracture healing progression and, in parallel, study the molecular and cellular mechanisms of pain sensation in preclinical settings. This review helps to summarize the current state of knowledge on mechanisms of postfracture pain, factors associated with persistent postfracture pain, and methods of measuring pain in humans and mice. However, we posit that there remain significant gaps in our understanding of fracture-related pain, the chronification of pain, and the efficacy of established and emerging analgesics. We hope that this article will help motivate the inclusion of pain assays in future fracture studies to advance the field of study and help to search for opioid alternatives for pain management to address the current opioid crisis.

Conflict of interest statement

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