REVIEW

What Did We Learn About Fracture Pain from Animal Models?

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Abstract: Progress in bone fracture repair research has been made possible due to the development of reproducible models of fracture in rodents with more clinically relevant fracture fixation, where there is considerably better assessment of the factors that affect fracture healing and/or novel therapeutics. However, chronic or persistent pain is one of the worst, longest-lasting and most difficult symptoms to manage after fracture repair, and an ongoing challenge remains for animal welfare as limited information exists regarding pain scoring and management in these rodent fracture models. This failure of adequate pre-clinical pain assessment following osteotomy in the rodent population may not only subject the animal to severe pain states but may also affect the outcome of the bone healing study. Animal models to study pain were also mainly developed in rodents, and there is increasing validation of fracture and pain models to quantitatively evaluate fracture pain and to study the factors that generate and maintain fracture pain and develop new therapies for treating fracture pain. This review aims to discuss the different animal models for fracture pain research and characterize what can be learned from using animal models of fracture regarding behavioral pain states and new molecular targets for future management of these behaviors.

Keywords: pain, fracture, mouse, behaviours

Introduction

Bone fractures from trauma and osteoporosis are a very common clinical problem affecting 3.6% of the UK population every year[1] and this incidence is similar worldwide.[2,3] Fracture occurrence is increasing due to the aging population and the incidence of diabetes, which largely contributes to these fragility fractures.[4] While bone fractures typically heal well with appropriate treatment, a feared complication is the possibility of pain. Complex Regional Pain Syndrome (CRPS) can also be triggered by fracture injury and is a serious complication associated with severe pain.[5]

Three stages of pain are associated with bone fracture: acute, sub-acute, and chronic pain. Acute pain occurs immediately after the fracture, but typically dissipates with time, while the manifestation of sub-acute pain can continue for weeks.[6] Sub-acute pain is thought to be associated with soft tissue injury and muscle weakness. Chronic pain is evident after healing is complete and may be associated with several tissue elements including peripheral nerve damage or the development of scar tissue.

Chronic or persistent neuropathic pain (NP) is one of the worst, long-lasting and most difficult symptoms to manage after fracture repair. It is likely that some of the pathophysiologic mechanisms leading to NP propagate early after injury, leading to the opportunity for early interventions. NP associated with bone fracture originates from a lesion affecting the peripheral nervous system and may be associated with abnormal sensations called dysesthesia or from normally non-painful stimuli (allodynia). The condition may have continuous and/or episodic (paroxysmal) components, with the latter resembling stabbing pain or electric shocks. Conditions associated with bone fracture-associated NP (BFNP) include traction neuropathy, nerve compression from soft tissue oedema, bone fragment, hardware and hematoma.

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Much of the advances in bone fracture research are due to the disposal of animal models, particularly rodents, because they are easy to use, cheap and fracture healing occurs in a few weeks. The availability of genetically modified mice has contributed to favour the use of this species, although the bones are very small for fracture fixation. This has led to the development of reproducible models of fracture with more clinically relevant fracture fixation where there is considerably better assessment of the factors that affect fracture healing and/or novel therapeutics.[7] An ongoing challenge remains for animal welfare as limited information exists regarding pain scoring and management in rodent fracture models.[8] This failure of adequate pre-clinical pain assessment following osteotomy in the rodent population may not only subject the animal to severe pain states but may also affect the outcome of the bone healing study.[9]

Interestingly, the animal models developed to study pain were also mainly rodents and various models of somatic, inflammatory and neuropathic pain were described.[10] It is however very difficult to measure pain in rodents and most of the measurements of pain are indirect assessing naturalistic behaviours and nociceptive reflexes. Including these pain measurements in animal models of fracture may lead to both better management after fracture[11] and understanding of the mechanisms contributing to the mechanistic transition from acute and subacute to chronic pain states.

This review aims to discuss the different animal models for fracture pain research and characterize what can be learned from using animal models of fracture regarding behavioral pain states and new molecular targets for future management of these behaviors.

Animal Models Mostly Used to Study Fracture Pain
Choosing a Model

Building the most suitable animal model of fracture is based on the availability of species, age, sex, choice of the bone, the type of fracture and the mechanism of bone repair. A robust and consistently reproducible model is required, but a standardized model has been a challenge to date as different disease modelling systems have different needs and study design. In addition to reproducibility, relevance to the clinical case needs to be considered as the model should closely recapitulate the most critical aspects of the clinical scenario. When choosing a model for skeletal pain research, a high resemblance between the human and the animal model of choice in terms of skeletal biology and nociception/nervous system is required.

Bone repair models have been covered in depth in previous reviews.[12–16] Many variables are in play: 1) the type of fracture repair, eg normal/delayed union/non-union; 2) the aetiology of fracture, eg osteotomy creating a clean fracture/impact, device creating a more traumatic fracture scenario but less replicable; 3) open versus closed fracture; 4) choice of species and ultimately 5) the fixation method, ie using a pin/external plate/using parallel bone technique, the latter being less reliable, due to potential deformities/differences in bone repair.

Large animals, such as pigs, are preferred as models of human fracture due to the presence of Haversian systems. In contrast, molecular and genetics tools make small animal models such as mice more versatile and suitable. A downside to the bone fracture models utilizing rodents is that bones continue to grow slowly with age and there is a lack of osteonal remodeling in cortical bone.[17] Sexual dimorphism and age also need to be taken into account when modelling fracture. Female bone repair is slower than males due to reduced mesenchymal stem cell numbers.[18] Inter-strain differences in murine models also influence fracture reproducibility in addition to other physiological aspects of the mice such as age and weight.[19] Therefore, it is important to be aware of strain-specific characteristics that may bring limitations to the model system and hinder variability within results in fracture healing studies.

The use of rodent models is accompanied by the complication of size. Fracture healing in long bones can easily be studied using the tibia or the femur due to the relatively short time of complete healing of about 4 weeks in mice[20] and 5 to 6 weeks in rats,[21,22] allowing us to study all stages of healing. However, short bone fractures present a challenging difficulty, due to fixing mechanisms. For instance, in osteoporosis, the most commonly occurring fractures are of the wrist, vertebrae and femoral neck. Whereas models of diaphyseal and metaphyseal fractures[23] have been proposed, vertebral fractures are still difficult to model.

Models of Fracture Pain

While preclinical models using rodents have yielded insights that appear relevant to mechanisms responsible for bone fracture pain, they are limited in scope. The vast majority of studies examining pain behaviours after fracture were conducted in the Einhorn closed fracture model.[24,25] Specifically, most studies are based on mice or rats using a closed mid-diaphyseal fracture of the left femur or tibia 14 days or 21 days after pin placement.[26–29] Alternatively, some groups have utilized a rat or mouse tibial bone which is fractured using pliers with an adjustable stop followed by hindlimb casting. Following four to six weeks of immobilization, the cast is removed.[30–36] This later model was mainly used as a model of complex regional pain syndrome (CRPS), a condition that develops after a range of injuries including fractures. Fracture-induced pain in animal models has not been studied thoroughly, but most studies of pain behaviours following fracture have been characterised in these models of closed fracture and CRPS.

Formerly known as reflex sympathetic dystrophy syndrome, CRPS is a condition that commonly affects a single limb and is accompanied by excruciating pain, changes in skin temperature and swelling or a combination of those symptoms. However, the model is not directly comparable to non-pathological fracture-induced pain due to the immobilisation of the limb, which induces physiological changes such as oxidative stress, vascular and inflammatory alterations that contribute to the pain phenotype observed which are not directly attributable to the fracture itself.[37] Most studies of fracture pain used mice, but the rat model of closed femur fracture model has the advantage of a more accessible central nervous system for electrophysiology studies of pain compared to the mouse.[38]

Fracture Rodent Model Complications That Affect Pain

Factors that influence fracture pain, in addition to the type and care of the fracture, depend on the fixation methods. A stainless steel intramedullary pin can be used to stabilize a fracture. This method is very simple and can be achieved by inserting a syringe needle in the intercondylar notch allowing the study of bone healing. It has been previously used for the molecular characterization of fracture callus in aged and osteoporotic mice,[39,40] the effects of chronic inflammation on fracture healing[41] and the effect of brain injury on bone healing.[42] However, this is less suitable for the study of pain as the procedure itself could lead to a source of pain and confounding results due to damage to the intramedullary cavity. Additionally, the lack of rotational and axial stabilities in this model disadvantages histological analysis due largely to the disruption created by the removal of the pin following euthanasia. These complications could arise in spite of refinements of this method such as the use of locking nail, which improves stability while maintaining the simplicity, the low cost and minimal invasiveness of this technique.

To bypass the potential complications and pitfalls associated with this model, transverse osteotomy models stabilised with an external fixator have been developed. The osteotomy can be fixed with an external composite material plate with four pins, which allows the fixation of the bone in a reproducible manner, with little variation in bone healing positioning. The degree of elasticity of this model allows for endochondral and intramembranous ossification, which is the closest to the physiological healing of a fracture. The mechanism allows for different degrees of flexibility up to complete stability fixation of the osteotomy.[43–45] The advantage over the internal fixation is that the external plate allows for a closed fracture surgery, while the implant does not touch the bone but offers the same level of stability.[45] This model was recently used to study pain behaviours after fracture.[46] In this model, the pain-like phenotype was maintained 6 weeks after fracture surgery.

The internal fixation model functions similarly to the external fixation, except it uses a rigid internal plate, which allows for a closed surgery[47] and metaphyseal fixation[48] that closely replicates the clinical fracture localisation in the femur. This is a fixed model, not allowing for modifications in the distance between the two bone ends, which may be necessary in some non-union fracture studies or even in secondary fracture studies. In spite of its relatively invasive nature and complex surgical technique, this model has the advantage of no medullary cavity damage and allows for a closer replication of the clinical scenario of a hip fixator.[49] The model with internal fixation was however not employed to study fracture pain.

These models of fracture pain have been very useful to characterise acute and subacute pain after fracture. Pain can persist when a fracture does not heal and central sensitization can amplify the perception and the severity of pain. This

has been less studied after fractures. In most studies, fracture healing occurs and mice and rats are kept for a few weeks after fracture, making the study of chronic pain after fracture difficult.

Available Methods to Measure Fracture Pain in Rodents

In humans, pain reports are validated by questionnaires, qualitative sensory testing and pain scale methodology, which cannot be extended to animals. Most often the criteria used are subjective and based on a general clinical impression of the animal's appearance and demeanour. However, using mouse models of fracture, we learned which techniques are useful to examine pain and what can we use them for.[29] Many of the methods quantify "pain like" behaviours in rodents via testing responses to an external stimulus or measuring spontaneous behaviour.[8] However, these pain measurements are likely indirect assessments of pain due to the lack of direct stimulation of bone nociceptive nerve fibres. Moreover, many studies investigating fracture pain also utilize changes in weight distribution and activity levels.[27,38] Adequate validation of bone fracture pain states subsequently necessitates the use of several independent measures and ascertains correlations between them.

Standard tests that are used are studying both evoked and non-evoked naturalistic behaviours.

Evoked-Pain Behaviours

Evoked pain behaviours have been thoroughly characterised and reviewed before[50] and they include von Frey,[51,52] cold plate,[53–55] static weight-bearing,[56,57] CatWalk[58] and Laboras.[59] Mechanical and thermal hyperalgesia were reported to be maintained up to 6 weeks after fracture surgery.[46] These tests require interaction between the animal and the experimenter and can be subjective. When measuring evoked behaviours, consistency of environment, experimenter and other controllable variables is important,[50] in addition to proper randomisation of animals.[60]

Spontaneous Pain

Non-evoked behaviours are used as a way to non-specifically measure changed behaviour in rodents and are a measure of spontaneous pain. It has been shown that naturalistic behaviour measurements in rodents can capture behaviours related to pain, such as changes in activity and rearing[61] using monitoring systems that reduce observer bias. Handling of rodents has a significant effect on their behaviours[62] and naturalistic behaviours are less affected when observed by monitoring systems. Guarding and flinching are commonly used measurements of an ongoing or spontaneous fracture-induced bone pain.[27] Observation periods are often two minutes during which time the number or duration of behaviour is counted.[63] Jimenez-Andrade et al[27] showed that guarding, flinching and weight-bearing had returned to baseline levels in both young and old rats 14 days after fracture, or close to baseline in adult rats. It is therefore questionable whether these naturalistic behaviours can capture the later phases of fracture-induced pain. The burrowing test is increasingly used to quantify spontaneous behaviour and represents a valuable test for assessing skeletal pain.[64] Combinations of these methods rather than individual pain assessments improve reliability. Conditional place preference test is not routinely used to assess spontaneous pain but is a valuable method for analgesic drug assessment against chronic pain.[65]

What Did We Learn About Fracture Pain from Animal Models

The study of pain to identify the mechanisms underlying it and the treatments to alleviate it has relied extensively on animal models. Early pain work was often performed on companion animals, but overtime, the research was conducted in mice and rats due to the wide variety of genetically modified strains that became available and ethical considerations. Using these pre-clinical models of fracture pain in rodents described above, we learned more on acute pain after fracture, changes in bone innervation, nociception processes and pharmacological treatments.

Identification and Molecular Phenotype of Nerves in the Bone

Immunocytochemistry on bone sections taken from animals as well as animal models of fracture and pain have largely contributed to our knowledge of bone innervation and its changes with age and skeletal diseases. Since earlier work demonstrated that bone is densely innervated,[66,67] several studies have described the phenotype of nerve fibres in bone.[68] Both autonomic and sensory nerve fibres were identified in bone.[69] Sympathetic adrenergic neurons expressing tyrosine

hydroxylase are abundant in bone but there is still no evidence for parasympathetic innervation, although cholinergic fibres were identified in the periosteum, mainly derived from postganglionic sympathetic neurons.[69] Sensory nerve fibres in the bone were most described. They are small unmyelinated C fibres and myelinated Aδ axons that are mainly peptide-rich, expressing neuropeptides such as Calcitonin Gene Related Peptide (CGRP) and substance P (SP). Most express neurotrophic tyrosine kinase receptor 1 (TrkA) a receptor for nerve growth factor (NGF) and this differentiates them from nerve fibres expressed in the skin.[70] These nerves play a role in fracture healing as repair is affected by denervation[27,71,72] and neuropeptides expressed in bone.[73] Complete denervation of bone tissue is known to impair fracture healing, while the presence of primary afferent sensory innervation contributes to fracture healing.[74] In contrast, we showed that treatment with propranolol, an antagonist of the beta-adrenergic system and associated sympathetic fibres, fails to affect fracture healing and callus strength.[75] It also appears that fracture repair requires TrkA signaling or calcitonin-gene related peptide release by skeletal sensory nerves.[76,77]

Peripheral nerves associated with bone also change with age and diseases. Although the findings from several studies suggest that the density of bone innervation does not change with age,[78,79] there does appear to be a correlation with the amount of bone present.[80,81] In contrast, ectopic nerve sprouting occurs after fractures[82] but does not appear to exist following successful bone union. Moreover, a mouse model of diaphyseal fracture with an external fixator fails to exhibit enhanced number and density of nerve fibres in bone when assessed 6 weeks after fracture.[46] In contrast, excessive nerve sprouting is maintained in non-healed fractures and may contribute to chronic skeletal pain.[83]

Nociception After Fracture

Due to the limited access to bone biopsies in humans, the understanding of the mechanisms leading to nociception after fractures is largely limited to the use of animal models and techniques, such as immunocytochemistry, gene expression analyses, imaging, anterograde tracing and nerve conduction studies.[84]

After fractures, several mechanisms have been proposed to induce skeletal pain and they can interact with each other. The distortion of the rich-innervated periosteum during injury precipitates a series of events immediately after fracture. The mechanosensitive sensory nerve fibres A-delta and C are rapidly activated and movement and loading of the fracture bone becomes painful.[24] Activation of small diameter unmyelinated C nerve fibres is likely to generate the slow burning bone pain. There is an immediate release of various signalling molecules neurotransmitters, neuropeptides, cytokines and neurotrophins from cells or peripheral nerve processes at the fracture site.[73] These molecules contribute to the stimulation and sensitization of nociceptors. They can also induce ectopic nerve sprouting as demonstrated for NGF.[24] Many inflammatory mediators discharged after fractures contribute to fracture pain sequela. This inflammatory phase together with bone resorption activity necessary to remove bone debris create a state of fracture tissues acidosis which can effectively activate acid-sensing channels expressed on nociceptors such as acid-sensing ion channels (ASICs) and transient receptor potential vanilloid (TRPV) channels, further contributing to bone pain.[85–88]

The advantage of mouse models is that it allows the use of genetically modified mice. This possibility has also largely contributed to our knowledge of pain after fracture. The role of kinins (Bradykinin) in inflammation and fracture pain, for example, was demonstrated using mice that are deficient in kinin receptors.[89] These receptors are highly expressed at the fracture site and mice deficient in B1 and B2 receptors demonstrate a reduction in post-fracture pain sensitivity. Both receptors were shown to contribute to fracture pain via COX-1/COX-2 signalling.

A number of small animal models of fractures also allows the examination of different cell interactions at the fracture site. For example, Li et al demonstrated that neurotrophic factors such as brain-derived neurotrophic factor (BDNF) or neuropeptides such as Substance P (SP) released from afferent C-fibers can stimulate bone formation during fracture healing.[90] Glia cells also contribute to the healing process by myelination and pain signals transmission.[91] Following bone fracture, mechanical injury to nerve fibres may also occur, generating neuropathic pain states.[92]

Animal models also offer a good description of neurochemistry and anatomy. In bone, retrograde labelled sensory experiments in rats have shown anatomical connections between sensory neurons innervated the periosteum and the medullary cavity and ipsilateral DRG spanning lumbar regions L1 to L6.[93] As it is thought that nociceptive input can induce a prolonged increase in excitability and synaptic efficacy of neurons, nerve fibres sensitization following fracture

may serve to amplify pain perception and severity.[94] These attributes are, however, poorly studied in animal models of fracture.

Pathophysiological Neurochemical Changes

Animal models should provide a basis for the pathomechanisms of human diseases. As in humans, it was shown that fracture-induced pain behaviours peak at 2 days post fracture in rodents and decline with soft callus formation.[95] Other studies have, however, shown longer pain behaviours after fracture. Using a mouse model of diaphyseal fracture with external fixator, we showed that the fracture group had persistent mechanical and thermal hypersensitivities six weeks after fracture, indicative of a chronic pain-like phenotype.[46] Animal models of fracture make it easier to correlate these pain behaviours with the healing of fracture, bone remodelling and neurochemical changes in the DRGs or spinal cord.

Comparison of Drugs to Alleviate Fracture Pain

Rodent fracture models, when standardised, allow pain treatment comparison and can identify potent and effective drugs that have the potential to be effective in clinic. While many studies have examined the effects of drugs on fracture healing, more recently, these fracture models also allowed the examination of several therapies on fracture-associated pain such as the anti-nerve growth factor (anti-NGF) therapy, opioids and NSAIDs. The most studied is the anti-NGF and several studies using a rat tibia fracture model of complex regional pain syndrome have demonstrated that anti-NGF antibodies elicit strong anti-nociceptive effects.[26,36] More recently, anti-NGF administered before and weekly after surgery alleviated fracture-induced mechanical nociception in a femoral fracture model with external fixation.[46]

The use of opioids raises many concerns related to dose-dependent increases in long-term pain and major side effects. Morphine was shown to reduce spontaneous pain and increase weight bearing 7 days post fracture in rats.[38] Buprenorphine is commonly used as an analgesic treatment for post-operative pain in small animals.[96] However, emerging evidence suggests that opioid use immediately after trauma worsens outcomes as it impairs nociceptive recovery and alters postfracture expression levels of several genes.[97]

The use of non-steroidal anti-inflammatory drugs (NSAIDs) is common in a number of trauma-induced inflammatory pain conditions in both pre-clinical and clinical studies and has been shown to lower pain scores and reports. However, the use of NSAIDs in bone healing is limited as there are some fears that they may affect bone health and repair.[98] The beneficial effects of bisphosphonates on the reduction of skeletal pain were mainly studied in the context of bone cancer wherein there was an apparent ability to inhibit pain but they produced bone loss in a rat tibia fracture model of complex regional pain syndrome.[32] Anti-sclerostin therapy also prevents painful-age related fractures,[99] although it is still not known if it can directly affect nociception.

Limitations of Fracture Pain Studies in Animals

Although laboratory animals have been extensively used to model human diseases, they have several disadvantages when studying fracture pain. First, pain is often associated with musculoskeletal diseases that are frequent in the elderly population. While life expectancy in the elderly population can reach over 80 years, mice which are often used to study skeletal pain only live for two years. With age, there are different changes in the brain and in the somatosensory system that are very different in humans and mice.[100] Second, patients are not similar, and there are variations in their individual response to pain due to different mechanisms and sensory profiles.[101] This variation is impossible to model in mice which have been highly selected and bred. The same strains of mice are used extensively, for example, the strain C57BL/6J, and utilised as a single representative of the species, limiting the interpretation of the data and the conclusions drawn.[102]

Differences in Physiological Mechanisms Between Humans and Animals

Many aspects need to be considered when using animal models to study fracture pain. One major physiological difference between animals and humans is related to their size.[7] There are distinct interspecies differences with regard to bone composition, density and mechanical competence.[103,104] Furthermore, bone remodelling was shown to be partly controlled by the brain[105] and we cannot exclude that the size of the brain may affect the central control of bone mass. It may also shape pain processing, although it was shown that animals share similar mechanisms of pain detection than

humans and have similar areas of the brain involved in processing pain. Not many controlled studies have compared mechanisms of acute and chronic pain in mammal studies considering size, sex and age. Previous literature has found that studying pain neurophysiology can be very different depending on the animal models. Pain research in larger mammals is rare due to welfare issues, but pain is highly studied in dogs, for example, for bone cancer pain, using a scale for guessing pain severity. Dogs are interesting as it was shown that they approximate human properties the best in terms of bone quantity and quality.[103] Similarities and differences in nociceptors between human and rodents have shown molecular and physiological differences between pain pathways, illustrating some difficulties in translating some of the findings in mice into humans.[106] Efficacy of pain treatments on fracture healing also illustrate differences between animal models. This was shown, for example, with the effects of NSAIDs on fracture healing, which were effective on small animals but not on large animals, such as dogs and goats.[98]

Genetics

It is not easy to study pain in humans as it is influenced by many factors including genetics. Many inter-individual differences in pain are reported that could be explained by changes in DNA, gene expression and epigenetics.[107] Pain sensitivity is also heritable in rodent models of pain, and this was particularly shown by selective breeding of mice. Mice are often used to study pain, mainly due to their inexpensive maintenance, easy breeding, the fact that there are many strains available but also due to the advantage of using transgenic mice available in this species. However, the use of knockout mice means that most studies examining pain are comparing genotypes which differ greatly from each other on a large variety of pain-related traits.[108] The background strain has a significant influence on nociception, and the pain response can be very different to other inbred and outbred mouse strains.

Pain Perception and Psychology

Pain is a subjective experience and involves both emotional and social experiences.[109,110] Assessing pain in humans then requires performing tools that analyse all aspects of pain experience such as the Mc Gill pain questionnaire or the Brief pain inventory. Humans can define the highly complex nature of their pain experience after fractures.[63] Similarly, pain in animals has been defined as "an aversive sensory experience caused by actual or potential injury that elicits protective motor and vegetative reactions, results in learned avoidance behaviour, and may modify species specific behaviour including social behaviour".[111] Defining reliable pain scales in animals is difficult due to the large variations in pain responses. Most pain assessments in animal models do not measure cognitive appraisal or integrate the animal's emotional response.[112] Clinical fracture pain is a perception that is influenced by psychological and experiential factors. Interestingly, humans have been shown to be the best species to heal due to their psychology,[113] suggesting that recovery after fracture may be sensitive to social cues. It is not possible to fully recapitulate this emotion and psychology in an animal fracture model.[114] Emotional experience is what distinguishes true pain from nociception, which is the sensory nervous system's process of encoding noxious stimuli and processing signals to the brain via the spinal cord. This experiential component is likely to vary from animals to humans and between animals of the same species. Pain in animals is likely to be adaptive in an evolutionary sense, animals having evolved methods of suppressing responses to pain to enhance survival.[115] This phenomenon is likely to exist in prey animals such as rodents.[111] Pathophysiologic stress is also very important when assessing pain in both animals and humans. This stress is displayed differently between species. In humans, it can lead to depression and anxiety altering the perception of pain. In laboratory animals, distress can manifest in abnormal respiration, reduced grooming, piloerection, hunching, absence of alertness. The investigation of placebo effects in animal pain research is difficult because animals cannot report their pain and rate their experience. Placebo analgesia in rodent pain research is, however, increasingly used and there is hope for new developments.[116]

Assessing Long-Term Chronic Pain

Most studies of pain in animals have investigated acute pain states such as observed post-surgery. Persistent pain is common after fracture surgery in humans[117] but assessing chronic pain which outlasts bone healing is more difficult to perform in animals, firstly due to the paucity of chronic pain models and the lack of reliable tools. There is limited

information of chronic pain in free-living animals, which may be due to the poor survival of animals with chronic pain, the lack of observations and behaviours assessments. Central sensitization was shown in rodent studies, but behaviours associated with this pain are difficult to assess and may be mainly related to avoidance of pain exacerbation.[118] Nociceptive withdrawal reflex has been suggested as a useful biomarker for central sensitization in rodents.[119] In addition, due to ethical considerations, it is increasingly difficult to keep animals for long-term studies. Most fracture pain experiments in rodents are performed in a few weeks and do not examine the long-term effects of pain.

Imaging Technology

Many advances in our understanding of pain in humans are the results of the development of functional neuroimaging techniques. They can measure changes in neuronal activity by assessing alterations in blood flow, oxygen and glucose metabolism, but also neurotransmitter uptake and binding to its receptor.[120] Functional changes in the pre-clinical brain due to chronic pain have not been fully elucidated, as brain imaging techniques including functional magnetic resonance imaging and positron emission tomography (PET) require the use of anesthesia to suppress movement. However, some insights into possible reflection of pain states have recently been described for neuropathic pain imaging in awake animals and include enhanced neural activity in several brain regions.[121]

Weaknesses Associated to Pain Measurements in Rodents

Most reported measurements of pain in rodents are made during the light phase, not taking into account the natural circadian rhythm of the animal as rodents are nocturnal. This is likely to impair pain assessments. Furthermore, the most commonly used endpoint in assessing skeletal pain in rodents is skin hypersensitivity assessed by von Frey. This does not reflect the clinical situation of a patient with skeletal pain, as skin hyperalgesia is rarely the major pain complaint but rather the spontaneous pain that arises from the bone under usage or localised in the immediate region around the fracture site.

Conclusions

Because pain is one critical factor that controls bone loading required for recovery after fracture, developing appropriate models to study fracture pain is essential. While the choice of animal models to study fracture repair is vast, the study of pain in these models requires very reproducible models that are the least invasive, enabling the analysis of pain due to the osteotomy itself and not pin placements. To date, the models of choice are 1) the closed mid-diaphyseal femoral fracture 21 days after pin placement, which is less invasive and allows for the surgical pain to resolve before fracture; 2) the open femoral fracture stabilised with an external fixator which is standardised and very reproducible but more invasive. None of these models are, however, suitable to study the pain associated with osteoporotic fractures as the fracture is performed in the femoral diaphysis, a site with no osteoporosis, and other models need to be developed. To improve the efficacy of translation of scientific data collected from animal models of fracture pain into the production of clinically affective analgesic agents, we will also need to develop more behavioural animal models and ideally better spontaneous measurements of pain. Although animal models can be valuable to select new compounds with an improved profile, they may be less helpful when assessing novel mechanisms. If we want to make progress in our understanding of fracture pain, both human and animal studies are complementary.

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References

1. Donaldson LJ, Reckless IP, Scholes S, Mindell JS, Shelton NJ. The epidemiology of fractures in England. *J Epidemiol Community Health*. 2008;62:174–180. doi:10.1136/jech.2006.056622

2. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. *J Bone Miner Res*. 2007;22(3):465–475. doi:10.1359/jbmr.061113

3. Hernlund E, Svedbom A, Ivergård M, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden. *Arch Osteoporos*. 2013;8(1–2):136. doi:10.1007/s11657-013-0136-1

4. Ferrari SL, Abrahamsen B, Napoli N, et al. On behalf of the Bone and Diabetes Working Group of IOFDiagnosis and management of bone fragility in diabetes: an emerging challenge. *Osteoporos Int*. 2018;29(12):2585–2596. doi:10.1007/s00198-018-4650-2

5. Rand SE, Basu S, Khalid S. Complex regional pain syndrome: current diagnostic and treatment considerations. *Curr Sports Med Rep*. 2019;18 (9):325–329. doi:10.1249/JSR.0000000000000633

6. Nencini S, Ivanusic JV. The physiology of bone pain. How much do we really know? *Front Physiol*. 2016;7:157. doi:10.3389/fphys.2016.00157

7. Auer JA, Goodship A, Arnoczky S, et al. Refining animal models in fracture research: seeking consensus in optimising both animal welfare and scientific validity for appropriate biomedical use. *BMC Musculoskelet Disord*. 2007;8:72. doi:10.1186/1471-2474-8-72

8. Deuis JR, Dvoraka LS, Vetter I. Methods to evaluate pain behaviour in rodents. *Front Mol Neurosci*. 2017;10:284. doi:10.3389/fnmol.2017.00284

9. Lang A, Schulz A, Ellinghaus A, Schmidt-Bleek K. Osteotomy models-the current status on pain scoring and management in small rodents. *Lab Anim*. 2016;50(6):433–441. doi:10.1177/0023677216675007

10. Walker K, Fox AJ, Urban LA. Animal models for pain research. *Mol Med Today*. 1999;5:319–321. doi:10.1016/S1357-4310(99)01493-8

11. Carbone L, Austin J. Pain and laboratory animals: publication practices for better data reproducibility and better animal welfare. *PLoS One*. 2016;11(5):e0155001. doi:10.1371/journal.pone.0155001

12. Garcia P, Histing T, Holstein JH, et al. Rodent animal models of delayed bone healing and non-union formation: a comprehensive review. *Eur Cell Mater*. 2013;26:1–12. doi:10.22203/eCM.v026a01

13. Haffner-Luntzer M, Kovtun A, Rapp AE, Ignatius A. Mouse models in bone fracture healing research. *Curr Mol Biol Rep*. 2016;2(2):101–111. doi:10.1007/s40610-016-0037-3

14. Holstein JH, Garcia P, Histing T, et al. Advances in the establishment of defined mouse models for the study of fracture healing and bone regeneration. *J Orthop Trauma*. 2009;23:S31–8. doi:10.1097/BOT.0b013e31819f27e5

15. Mills LA, Simpson AH. In vivo models of bone repair. *J Bone Joint Surg Br*. 2012;94-B(7):865–874. doi:10.1302/0301-620X.94B7.27370

16. Schindeler A, Mills RJ, Bobyn JD, Little DG. Preclinical models for orthopedic research and bone tissue engineering. *J Orthop Res*. 2018;36 (3):832–840. doi:10.1002/jor.23824

17. Jilka RL. The relevance of mouse models for investigating age-related bone loss in humans. *J Gerontol A Biol Sci Med Sci*. 2013;68 (10):1209–1217. doi:10.1093/gerona/glt046

18. Strube P, Mehta M, Baerenwaldt A, et al. Sex-specific compromised bone healing in female rats might be associated with a decrease in mesenchymal stem cell quantity. *Bone*. 2009;45(6):1065–1072. doi:10.1016/j.bone.2009.08.005

19. Bartnikowski M, Bartnikowski N, Woloszyk A, Matthys R, Glatt V. Genetic variation in mice affects closed femoral fracture pattern outcomes. *Injury*. 2019;50(3):639–647. doi:10.1016/j.injury.2019.02.012

20. Hiltunen A, Vuoro E, Aro HT. A standardized experimental fracture in the mouse tibia. *J Orthopaed Res*. 1993;11(2):305–312. doi:10.1002/jor.1100110219

21. Claes L, Blakytny R, Göckelmann M, Schoen M, Ignatius A, Willie B. Early dynamization by reduced fixation stiffness does not improve fracture healing in a rat femoral osteotomy model. *J Orthop Res*. 2009;27(1):22–27. doi:10.1002/jor.20712

22. Einhorn T. The science of fracture healing. *J Orthop Trauma*. 2005;19(10):S4–S10. doi:10.1097/00005131-200511101-00002

23. Inoue S, Otsuka H, Takito J, Nakamura M. Decisive differences in the bone repair processes of the metaphysis and diaphysis in young mice. *Bone Rep*. 2017;24(8):1–8.

24. Mitchell SAT, Majuta LA, Mantyh PW. New insights in understanding and treating bone fracture pain. *Curr Osteoporos Rep*. 2018;16 (4):325–332. doi:10.1007/s11914-018-0446-8

25. Bonnarens F, Einhorn TA. Production of a standard closed fracture in laboratory animal bone. *J Orthop Res*. 1984;2:97–101. doi:10.1002/jor.1100020115

26. Koewler NJ, Freeman KT, Buus RJ, et al. Effects of a monoclonal antibody raised against nerve growth factor on skeletal pain and bone healing after fracture of the C57BL/6J mouse femur. *Bone Miner Res*. 2007;22(11):1732–1742. doi:10.1359/jbmr.070711

27. Jimenez-Andrade JM, Bloom AP, Mantyh WG, et al. Capsaicin-sensitive sensory nerve fibers contribute to the generation and maintenance of skeletal pain. *Neuroscience*. 2009;162:1244–1254. doi:10.1016/j.neuroscience.2009.05.065

28. Thompson AL, Largent-Milnes TM, Vanderah TW. Animal models for the study of bone-derived pain. *Methods Mol Biol*. 2019;1914:391–407.

29. Minville V, Laffosse J-M, Fourcade O, Girolami J-P, Tack I. Mouse model of fracture pain. *Anesthesiology*. 2008;108(3):467–472. doi:10.1097/ALN.0b013e3181649333

30. Guo T-Z, Offley S, Boyd E, Jacobs C, Kingery W. Substance P signaling contributes to the vascular and nociceptive abnormalities observed in a tibial fracture rat model of complex regional pain syndrome type I. *Pain*. 2004;108(1–2):95–107. doi:10.1016/j.pain.2003.12.010

31. Guo T-Z, Shi X, Li -W-W, Wei T, Clark JD, Kingery WS. Sex differences in the temporal development of pronociceptive immune responses in the tibia fracture mouse model. *Pain*. 2019;160(9):2013–2027. doi:10.1097/j.pain.0000000000001592

32. Wang L, Guo T-Z, Wei T, et al. Bisphosphonates inhibit pain, bone loss, and inflammation in a rat tibia fracture model of complex regional pain syndrome. *Anesth Analg*. 2016;123(4):1033–1045. doi:10.1213/ANE.0000000000001518

33. Gallagher JJ, Tajerian M, Guo T, et al. Acute and chronic phases of complex regional pain syndrome in mice are accompanied by distinct transcriptional changes in the spinal cord. *Mol Pain*. 2013;9:40. doi:10.1186/1744-8069-9-40

34. Li WW, Guo TZ, Shi X, et al. Autoimmunity contributes to nociceptive sensitization in a mouse model of complex regional pain syndrome. *Pain*. 2014;155:2377–2389. doi:10.1016/j.pain.2014.09.007

35. Wei T, Guo TZ, Li WW, Kingery WS, Clark JD. Acute versus chronic phase mechanisms in a rat model of CRPS. *J Neuroinflammation*. 2016;13:14. doi:10.1186/s12974-015-0472-8

36. Sabsovitch I, Wei T, Guo T-Z, et al. Effect of anti-NGF antibodies in a rat tibia fracture model of complex regional pain syndrome type I. *Pain*. 2008;138(1):47–60. doi:10.1016/j.pain.2007.11.004

37. Guo TZ, Wei T, Huang TT, Kingery WS, Clark JD. Oxidative stress contributes to fracture/cast-induced inflammation and pain in a rat model of complex regional pain syndrome. *J Pain*. 2018;19:1147–1156. doi:10.1016/j.jpain.2018.04.006

38. Freeman KT, Koewler NJ, Jimenez-Andrade JM, et al. A fracture pain model in rat: adaptation of a closed fracture model to study skeletal pain. *Anesthesiology*. 2008;108(3):473–483. doi:10.1097/ALN.0b013e3181649351

39. Li WW, Wang K, Liu Z, Ding W. HIF-1α change in serum and callus during fracture healing in ovariectomized mice. *Int J Clin Exp Pathol*. 2015;8(1):117–126.

40. Wehrle E, Liedert A, Heilmann A, et al. The impact of low-magnitude high-frequency vibration on fracture healing is profoundly influenced by the oestrogen status in mice. *Dis Model Mech*. 2015;8(1):93–104. doi:10.1242/dmm.018622

41. Timmen M, Hidding H, Wieskötter B, et al. Influence of anti-TNF-alpha antibody treatment on fracture healing under chronic inflammation. *BMC Musculoskelet Disord*. 2014;15:184. doi:10.1186/1471-2474-15-184

42. Tsitsilonis S, Seemann R, Misch M, et al. The effect of traumatic brain injury on bone healing: an experimental study in a novel in vivo animal model. *Injury*. 2015;46(4):661–665. doi:10.1016/j.injury.2015.01.044

43. Histing T, Holstein JH, Garcia P, et al. Ex vivo analysis of rotational stiffness of different osteosynthesis techniques in mouse femur fracture. *J Orthop Res*. 2009;27(9):1152–1156. doi:10.1002/jor.20849

44. Rapp AE, Kroner J, Baur S, et al. Analgesia via blockade of NGF/TrkA signaling does not influence fracture healing in mice. *J Orthop Res*. 2015;33(8):1235–1241. doi:10.1002/jor.22892

45. Röntgen V, Blakytny R, Matthys R, et al. Fracture healing in mice under controlled rigid and flexible conditions using an adjustable external fixator. *J Orthop Res*. 2010;28(11):1456–1462. doi:10.1002/jor.21148

46. Magnusdottir R, Gohin S, Ter Heegde F, et al. Fracture-induced pain-like behaviours in a femoral fracture mouse model. *Osteoporos Int*. 2021;32(11):2347–2359. doi:10.1007/s00198-021-05991-7

47. Matthys R, Perren SM. Internal fixator for use in the mouse. *Injury*. 2009;40:S103–9. doi:10.1016/j.injury.2009.10.044

48. Kauschke V, Schneider M, Jauch A, et al. Effects of a pasty bone cement containing brain-derived neurotrophic factor-functionalized mesoporous bioactive glass particles on metaphyseal healing in a new murine osteoporotic fracture model. *Int J Mol Sci*. 2018;19(11):3531. doi:10.3390/ijms19113531

49. Orth M, Baudach J, Scheuer C, et al. Erythropoietin does not improve fracture healing in aged mice. *Exp Gerontol*. 2019;122:1–9. doi:10.1016/j.exger.2019.04.005

50. Minett MS, Eijkelkamp N, Wood JN. Significant determinants of mouse pain behaviour. *PLoS One*. 2014;9(8):104458. doi:10.1371/journal.pone.0104458

51. Bonin RP, Bories C, De Koninck Y. A simplified up-down method (SUDO) for measuring mechanical nociception in rodents using von Frey filaments. *Mol Pain*. 2014;10:26. doi:10.1186/1744-8069-10-26

52. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods*. 1994;53:55–63. doi:10.1016/0165-0270(94)90144-9

53. Allchorne AJ, Broom DC, Woolf CJ. Detection of cold pain, cold allodynia and cold hyperalgesia in freely behaving rats. *Mol Pain*. 2005;1:36. doi:10.1186/1744-8069-1-36

54. Minett MS, Quick K, Wood JN. Behavioral measures of pain thresholds. *Curr Protoc Mouse Biol*. 2011;1:383–412. doi:10.1002/9780470942390.mo110116

55. Yalcin I, Charlet A, Freund-Mercier MJ, Barrot M, Poisbeau P. Differentiating thermal allodynia and hyperalgesia using dynamic hot and cold plate in rodents. *J Pain*. 2009;10:767–773. doi:10.1016/j.jpain.2009.01.325

56. Bove SE, Calcaterra SL, Brooker RM, et al. Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. *Osteoarthr Cartilage*. 2003;11:821–830. doi:10.1016/S1063-4584(03)00163-8

57. Buys MJ, Alphonso C. Novel use of perineural pregabalin infusion for analgesia in a rat neuropathic pain model. *Anesth Analg*. 2014;119:481–488. doi:10.1213/ANE.0000000000000291

58. Bozkurt A, Deumens R, Scheffel J, et al. CatWalk gait analysis in assessment of functional recovery after sciatic nerve injury. *J Neurosci Methods*. 2008;173:91–98. doi:10.1016/j.jneumeth.2008.05.020

59. Quinn LP, Stean TO, Trail B, et al. LABORAS: initial pharmacological validation of a system allowing continuous monitoring of laboratory rodent behaviour. *J Neurosci Methods*. 2003;130:83–92. doi:10.1016/S0165-0270(03)00227-9

60. Hirst JA, Howick J, Aronson JK, et al. The need for randomization in animal trials: an overview of systematic reviews. *PLoS One*. 2014;9:e98856. doi:10.1371/journal.pone.0098856

61. Majuta LA, Longo G, Fealk MN, McCaffrey G, Manthy PW. Orthopedic surgery and bone fracture pain are both significantly attenuated by sustained blockade of nerve growth factor. *Pain*. 2015;156:157–165. doi:10.1016/j.pain.0000000000000017

62. Alvarez P, Green PG, Levine JD. Neonatal handling produces sex hormone-dependent resilience to stress-induced muscle hyperalgesia in rats. *J Pain*. 2018;19:670–677. doi:10.1016/j.jpain.2018.01.009

63. Santy J, Mackintosh C. A phenomenological study of pain following fractured shaft of femur. *J Clin Nurs*. 2001;10:521–527. doi:10.1046/j.1365-2702.2001.00506.x

64. Sliepen SHJ, Diaz-DelCastillo M, Korioth J, et al. Cancer-induced bone pain impairs burrowing behaviour in mouse and rats. *In Vivo (Brooklyn)*. 2019;33:1125–1132. doi:10.21873/invivo.11582

65. Turner PV, Pang DSJ, Lofgren JLS. A review of pain assessment methods in laboratory rodents. *Comp Med*. 2019;69(6):451–467. doi:10.30802/AALAS-CM-19-000042

66. Serre CM, Farley D, Delmas PD, Chenu C. Evidence for a dense and intimate innervation of the bone tissue, including glutamate-containing fibers. *Bone*. 1999;25:623–629. doi:10.1016/S8756-3282(99)00215-X

67. Mach DB, Rogers SD, Sabino MC, et al. Origins of skeletal pain: sensory and sympathetic innervation of the mouse femur. *Neuroscience*. 2002;113:155–166. doi:10.1016/S0306-4522(02)00165-3

68. Alves CJ, Net E, Souza DM, et al. Fracture pain-travelling unknown pathways. *Bone*. 2016;85:107–114. doi:10.1016/j.bone.2016.01.026

69. Brazill JM, Beeve AT, Craft CS, Ivanusic JJ, Scheller EL. Nerves in bone: evolving concepts in pain and anabolism. *J Bone Miner Res*. 2019;34 (8):1393–1406. doi:10.1002/jbmr.3822

70. Castaneda-Corral G, Jimenez-Andrade JM, Bloom AP, et al. The majority of myelinated and unmyelinated sensory nerve fibres that innervate bone express the tropomyosin receptor kinase A. *Neuroscience*. 2011;178:196–207. doi:10.1016/j.neuroscience.2011.01.039

71. Apel PJ, Crane D, Northam CN, Callaghan M, Smith TL, Teasdall RD. Effect of selective sensory denervation on fracture healing. *J Bone Joint Surg Am*. 2009;91:2886–2895. doi:10.2106/JBJS.H.01878

72. Aro H, Eerola E, Aho A, Pentinen R. Healing of experimental fractures in the denervated limb of the rat. *Clin Orthop Relat Res*. 1981;155:211–217. doi:10.1097/00003086-198103000-00034

73. Sun S, Diggins NH, Gunderson ZJ, Fehrenbacher JC, White FA, Kacena MA. No pain, no gain? The effects of pain-promoting neuropeptides and neurotrophins on fracture healing. *Bone*. 2020;131:11509. doi:10.1016/j.bone.2019.115109

74. Wang XY, Guo X, Qu SX, Weng J, Cheng CY. Temporal and spatial CGRP innervation in recombinant human bone morphogenetic protein induced spinal fusion in rabbits. *Spine*. 2009;34(22):2363–2368. doi:10.1097/BRS.0b013e3181b04e52

75. Smitham P, Crossfield L, Hughes G, Goodship A, Blunn G, Chenu C. Low dose of propranolol does not affect rat osteotomy healing and callus strength. *J Orthop Res*. 2014;32(7):887–893. doi:10.1002/jor.22619

76. Li Z, Meyers CA, Chang L, et al. Fracture repair requires TrkA signalling by skeletal sensory nerves. *J Clin Invest*. 2019;129(12):5137–5150. doi:10.1172/JCI128428

77. Zhang Y, Xu J, Ruan YC, et al. Implant-derived magnesium induces local neuronal production of CGRP to improve bone fracture healing in rats. *Nat Med*. 2016;22(10):1160–1169. doi:10.1038/nm.4162

78. Jimenez-Andrade JM, Mantyh WG, Bloom AP, et al. The effect of ageing on the density of sensory nerve fiber innervation of bone and acute skeletal pain. *Neurobiol Ageing*. 2012;33(5):921–932. doi:10.1016/j.neurobiolaging.2010.08.008

79. Chartier SR, Mitchell SAT, Majuta LA, Mantyh PW. The changing sensory and sympathetic innervation of the young, adult and ageing mouse femur. *Neuroscience*. 2018;387:178–190. doi:10.1016/j.neuroscience.2018.01.047

80. Burt-Pichat B, Lafage-Proust MH, Duboeuf F, et al. Dramatic decrease of innervation density after ovariectomy. *Endocrinology*. 2005;146 (1):503–510. doi:10.1210/en.2004-0884

81. Tomlinson RE, Christiensen BA, Giannone AA, Genetos DC. The role of nerves in skeletal development, adaptation and ageing. *Front Endocrinol*. 2020;11:646. doi:10.3389/fendo.2020.00646

82. Li J, Ahmad T, Spetea M, Ahmed M, Kreicbergs A. Bone reinnervation after fracture: a study in the rat. *J Bone Miner Res*. 2001;16 (8):1505–1510. doi:10.1359/jbmr.2001.16.8.1505

83. Chartier SR, Thompson ML, Longo G, Fealk MN, Majuta LA, Mantyh PW. Exuberant sprouting of sensory and sympathetic nerve fibers in non-healed bone fractures and the generation and maintenance of chronic skeletal pain. *Pain*. 2014;155(11):2323–2336. doi:10.1016/j. pain.2014.08.026

84. Mogil JS, Davis KD, Derbyshire SW. The necessity of animal models in pain research. *Pain*. 2010;151:12–17. doi:10.1016/j.pain.2010.07.015

85. Slosky LM, Largent-Milnes TM, Vanderah TW. Use of animal models in understanding cancer-induced bone pain. *Cancer Growth Metastasis*. 2015;23(8 Suppl1):47–62.

86. Yoneda T, Hiasa M, Nagata Y, Okui T, White FA. Acidic microenvironment and bone pain in cancer-colonised bone. *Bonekey Rep*. 2015;4:690. doi:10.1038/bonekey.2015.58

87. Yoneda T, Hiasa M, Nagata Y, Okui T, White F. Contribution of acidic extracellular microenvironment of cancer-colonised bone to bone pain. *Biochem Biophys Acta*. 2015;1848:2677–2684. doi:10.1016/j.bbamem.2015.02.004

88. Deval E, Noel J, Lay N, et al. ASIC3, a sensor of acidic and primary inflammatory pain. *EMBO J*. 2008;27:3047–3055. doi:10.1038/emboj.2008.213

89. Minville V, Mouledous L, Jaafar A, et al. Tibial post-fracture pain is reduced in kinin receptors deficient mice and blunted by kinin receptor antagonists. *J Transl Med*. 2019;17:346–358. doi:10.1186/s12967-019-2095-9

90. Li WW, Guo TZ, Shi X, et al. Substance P spinal signaling induces glial activation and nociceptive sensitization after fracture. *Neuroscience*. 2015;310:73–90. doi:10.1016/j.neuroscience.2015.09.036

91. Dodo Y, Chatani M, Azetsu Y, et al. Myelination during fracture healing in vivo in myelin protein zero (p0) transgenic medaka line. *Bone*. 2020;133:115225. doi:10.1016/j.bone.2020.115225

92. Jimenez-Andrade JM, Martin CD, Koewler NJ, et al. Nerve growth factor sequestering therapy attenuates non-malignant skeletal pain following fracture. *Pain*. 2007;133(1–3):183–196. doi:10.1016/j.pain.2007.06.016

93. Ivanusic JJ. Size, neurochemistry and segmental distribution of sensory neurons innervating the rat. *J Comp Neurol*. 2009;517(3):276–283. doi:10.1002/cne.22160

94. Graven-Nielsen T, Arendt-Nielsen L. Assessment and mechanisms in localised and widespread musculoskeletal pain. *Nat Rev Rheumatol*. 2010;6(10):599–606. doi:10.1038/nrrheum.2010.107

95. Bove SE, Flatters SJL, Inglis JJ, Mantyh PW. New advances in musculoskeletal pain. *Brain Res Rev*. 2009;60:187–201. doi:10.1016/j. brainresrev.2008.12.012

96. Roughan JV, Flecknell PA. Buprenorphine: a reappraisal of its antinociceptive effects and therapeutic use in alleviating post-operative pain in animals. *Lab Anim*. 2002;36:322–343. doi:10.1258/002367702320162423

97. Li -W-W, Irvine K-A, Sahbaie P, et al. Morphine exacerbates post-fracture nociceptive sensitization, functional impairment and microglial activation in mice. *Anesthesiology*. 2019;130(2):292–308. doi:10.1097/ALN.0000000000002495

98. Pountos I, Georgouli T, Calori GM, Giannoudis PV. Do nonsteroidal anti-inflammatory drugs affect bone healing? A critical analysis. *Sci World J*. 2012;606404. doi:10.1100/2012/606404

99. Thompson ML, Chartier SR, Michell SA, Mantyh PW. Preventing painful age-related bone fractures: anti-sclerostin therapy builds cortical bone and increases the proliferation of osteogenic cells in the periosteum of the geriatric mouse femur. *Mol Pain*. 2016;12:1744806916677147. doi:10.1177/1744806916677147

100. Yezierski RP. The effects of age on pain sensitivity: pre-clinical studies. *Pain Med*. 2012;13(2):S27–S36. doi:10.1111/j.1526-4637.2011.01311.x

101. Bannister K, Kucharczyk M, Dickenson AH. Hopes for the future of pain control. *Pain Ther*. 2017;6(2):117–128. doi:10.1007/s40122-017-0073-6

102. Gash AP, Payseur BA, Pool JE. The power of natural variation for model organism biology. *Trends Genet*. 2016;32(3):147–154. doi:10.1016/j.tig.2015.12.003

103. Aerssens J, Boonen S, Lowet G, Dequeker J. Interspecies difference in bone composition, density and quality: potential implications for in vivo bone research. *Endocrinology*. 1998;139(2):663–670. doi:10.1210/endo.139.2.5751

104. Egermann M, Godham J, Schneider E. Animal models for fracture treatment in osteoporosis. *Osteoporos Int*. 2005;16:S129–S138. doi:10.1007/s00198-005-1859-7

105. Haberland M, Schilling AF, Rueger JM, Amling M. Brain and bone: central regulation of bone mass. A new paradigm in skeletal biology. *J Bone Joint Surg Am*. 2001;83:1871–1876. doi:10.2106/00004623-200112000-00016

106. Rostock C, Schrenk-Siemens K, Poble J, Siemens J. Human versus mouse nociceptors-Similarities and differences. *Neuroscience*. 2018;387:13–27. doi:10.1016/j.neuroscience.2017.11.047

107. James S. Human pain and genetics: some basics. *Br J Pain*. 2013;7(4):171–178. doi:10.1177/2049463713506408

108. Wilson SG, Mogil JS. Measuring pain in the (knockout) mouse: big challenges in a small mammal. *Behav Brain Res*. 2001;125:65–73. doi:10.1016/S0166-4328(01)00281-9

109. Harrison A. Assessing patient's pain: identifying reasons for error. *J Adv Nurs*. 1991;16(9):1018–1025. doi:10.1111/j.1365-2648.1991.tb03361.x

110. Mediati RD, Vellucci R, Dodaro L. Pathogenesis and clinical aspects of pain in patients with osteoporosis. *Clin Cases Miner Bone Metabol*. 2014;11(3):169–172.

111. Zimmerman M. Physiological mechanisms of pain and its treatment. *Klinische Anasthesiol Intensivther*. 1986;32:1–19.

112. Whittaker AL, Howarth GS. Use of spontaneous behaviour measures to assess pain in laboratory rats and mice: how are you progressing? *Appl Anim Behav Sci*. 2014;151:1–12. doi:10.1016/j.applanim.2013.11.001

113. Humphrey N, Skoyles J. The evolutionary psychology of healing: a human success story. *Curr Biol*. 2012;22(17):R695–698. doi:10.1016/j.cub.2012.06.018

114. McVeigh LG, Perigini AJ, Fehrenbacher JC, White FA, Kacena M. Assessment, quantification and management of fracture pain: from animals to the clinic. *Curr Osteoporos Rep*. 2020;18:460–470. doi:10.1007/s11914-020-00617-z

115. Dawkins MS. Evolution and animal welfare. *Q Rev Biol*. 1998;73:305–328. doi:10.1086/420307

116. Keller A, Akintola T, Colloca L. Placebo analgesia in rodents: current and future research. *Int Rev Neurobiol*. 2018;138:1–15.

117. Friesgaard KD, Gromov K, Knidsen LF, Brix M, Troelsen A, Nikolajsen L. Persistent pain is common one year after ankle and wrist fracture surgery: a register-based questionnaire study. *Br J Anesthesia*. 2016;116(5):655–661. doi:10.1093/bja/aew069

118. Williams AC. Persistence of pain in humans and other mammals. *Phil Trans R Soc B*. 2019;374:20190276. doi:10.1098/rstb.2019.0276

119. Kelly S, Dobson KL, Harris J. Spinal nociceptive reflexes are sensitized in the monoiodoacetate model of osteoarthritis pain in the rat. *Osteoarthr Cartilage*. 2013;21:1327–1335. doi:10.1016/j.joca.2013.07.002

120. Morton DL, Sandhu JS, Jones AKP. Brain imaging of pain: state of the art. *J Pain Res*. 2016;9:613–624. doi:10.2147/JPR.S60433

121. Inami C, Tanihira H, Kikuta S, et al. Visualisation of brain activity in a neuropathic pain model using quantitative activity-dependent manganese magnetic resonance imaging. *Front Neural Circuits*. 2019;13:74. doi:10.3389/fncir.2019.00074