Comparative Medicine
Copyright 2019
by the American Association for Laboratory Animal Science

Vol 69, No 6
December 2019
Pages 555–570

Overview

The Study of Pain in Rats and Mice

Christina M Larson,[1] George L Wilcox,[2,3,4] and Carolyn A Fairbanks,[2,3,4]*

Pain is a clinical syndrome arising from a variety of etiologies in a heterogeneous population, which makes successfully treating the individual patient difficult. Organizations and governments recognize the need for tailored and specific therapies, which drives pain research. This review summarizes the different types of pain assessments currently being used and the various rodent models that have been developed to recapitulate the human pain condition.

DOI: 10.30802/AALAS-CM-19-000062

Pain has been recognized across the world as far back as written documents exist and once was considered an inevitable and inescapable part of the human condition. Today, pain is defined as "an unpleasant sensory and emotional experience associated with actual or impending tissue damage, or described in terms of such damage";[110] it is frequently the trigger for people to seek medical attention.[59]

The global burden of chronic pain is significant and is broadly recognized as a cause of human suffering and social cost, in terms of health care and diminished productivity. Pain is caused by many sources in a heterogeneous human population, ranging from trauma to cancer to illnesses such as diabetes, and it manifests in a constellation of signs, each of which can occur along a continuum. Not unlike the diverse mechanisms that underlie different forms of cell proliferation that fall under the broad category of "cancer", numerous distinct pathologies and mechanisms result in the emergence of diverse painful conditions that converge on the common general term of "chronic pain." However, distinct from many other health conditions, the sensation of pain frequently accompanies and signals the presence of other diseases. Nociplastic pain differs from nociceptive and neuropathic pain, in that it often arises independently of a separate disease condition related to peripheral or central maladaptive neural plasticity and does not signal impending tissue damage. After many years of advocacy, unrelieved chronic pain is now recognized as a disorder in and of itself.[143]

Pain has been often clinically divided by recency of onset into acute (sudden) or chronic (long-standing). Unfortunately, this distinction may fail to elucidate appropriate analgesic therapy. For the purposes of study and treatment, the International Association for the Study of Pain now divides pain into 3 types, according to mechanistic origin: Nociceptive, neuropathic, or nociplastic.[110] *Nociceptive pain* is associated with actual or impending tissue injury; it occurs acutely and resolves once the tissue heals or the noxious stimuli ceases. *Neuropathic pain* is caused by disease or injury to the somatosensory nervous system and may become chronic in nature. *Nociplastic pain* is associated with changes in the nervous system that cause the body to register pain when no actual or impending tissue injury is present. The pathology of nociplastic pain begins with remodeling of the pain pathway in the central nervous system during injury and continues for an indefinite period. The cessation of pain signaling when noxious stimuli have ceased or when tissues have healed is the primary hallmark that distinguishes nociceptive pain from neuropathic or nociplastic pain. Neuropathic and nociplastic pain are distinguished based on whether a lesion or disease process can be identified in the nervous system; nociplastic pain is essentially an exclusionary diagnosis assigned when no discernable cause can be identified. Time course to resolution of either neuropathic pain or nociplastic pain is not predictable for any individual patient.

The study of pain to identify the neurobiologic and neurophysiologic mechanisms underlying its transmission through the peripheral and central nervous systems has relied extensively on animal modeling for hundreds of years. Early European research on the nervous system was performed in species readily available to anatomists. In the late 18th century, nerves were transected in the dog to study nerve conduction.[73] British and American military surgeons in the Crimean War and the American Civil War, respectively, understood the nervous system in enough detail to be able to recognize that a particular type of pain predictably occurred in regions of the body remote from the site of gunshot injury,[72,120] and understood that it was distinct from pain that occurred at the location of the injury. Their contemporaries in research performed anatomic studies examining compressive injuries; temporary interruption of nerve transmission was assessed by the application of a column filled with mercury on the sciatic nerve of a rabbit, and the magnitude of compression was measured in inches of mercury.[119] By the end of the 19th century, civilian physicians were readily able to identify and evaluate neuropathic pain as part of their follow-up on military injuries.[118]

While early work to define the working of the nervous system and the differences between these types of pain was done in companion animals, over time the species used in pain research have shifted to rats and mice. These species are inexpensive to house, easy to handle, fecund, and quick to mature. As a result, they have become the preferred models for genetic screening and manipulation, resulting in a wide variety of genetically modified strains becoming available in the laboratory mouse

Received: 30 May 2019. Revision requested: 17 Jul 2019. Accepted: 30 Sep 2019.
[1]Comparative and Molecular Biosciences, University of Minnesota College of Veterinary Medicine, St Paul, Minnesota; [2]Departments of Neuroscience, [3]Pharmacology, [4]Dermatology, University of Minnesota Medical School, Minneapolis, Minnesota; [5]Department of Pharmaceutics, University of Minnesota College of Pharmacy, Minneapolis, Minnesota
[*]Corresponding author. Email: carfair@umn.edu

and, to a lesser degree, the laboratory rat. These genetically modified animals are natural choices for research elucidating the role of single-gene knockouts, mutations, and insertions; through these manipulations, the role of individual receptors and neurotransmitters can be defined. Such methods aid researchers in defining the mechanisms of pain neurotransmission and its alteration in the pathologic state, with the unspoken assumption that such primitive processes will be conserved across the animal kingdom. Pain research also encompasses the search for potent analgesics that lack attendant risks of addiction or overdose; this search has driven the development of both simple and complex pain models, as well as many methods of quantifying pain.

This article will review the sensory system, pain assessment methods, and rodent models used to model human pain conditions.

Sensory system

The nervous system contains a multitude of nociceptors, which are first-order sensory neurons dedicated to detecting types of noxious signals and carrying that information to the spinal cord. *Nociception* is the term used to describe this neural processing of noxious stimuli.[110] Nociceptive signaling in these neurons occurs in response to physical signals that can indicate actual or impending tissue damage, such as excess heat or cold, pinprick, excess free hydrogen ions (leading to an acidic state), excessive pressure, or the presence of inflammatory mediators like prostaglandins. Nociceptors can be specific for a certain type of signal or may be multimodal, that is, sensitive to multiple types of stimuli.

In the nociceptive components of the nervous system as described by Kandel and colleagues,[88] nociceptors transduce physical signals from noxious stimuli into electrical signals by using terminals located in the skin, subcutaneous tissues, and mucous membranes (including those membranes lining hollow organs). The nociceptors generate electrical impulses that travel on thinly-myelinated (Aδ) or unmyelinated (C) fibers, past the cell bodies of these neurons housed within the dorsal root ganglion, and terminate in the superficial laminae of the dorsal horn of the spinal cord (primarily laminae I and II). There, these afferent first-order sensory neurons synapse with second-order spinal relay neurons, as well as various interneurons. Some interneurons are known to modulate the signal here, while others extend from this initial synapse to reach the dendrites of motor neurons responsible for protective reflexes. At the initial synapse in the ascending pain pathway, the first-order neurons release neurotransmitters such as glutamate or substance P to carry the signal across the synapse and create an action potential in the second-order ascending neurons. These neurotransmitter signals may also activate a reflex arc that protects the organism. The ascending fibers of second-order neurons ascend to either the thalamus or dorsal column nuclei with collaterals to many other nuclei including the periaqueductal gray matter.

In the brain, second or third order nociceptive neurons synapse with neurons leading to the basolateral amygdala, where a negative affect such as aversion, fear, or unpleasantness is assigned.[39] Additional neurons reach the somatosensory cortex where the signal is consciously perceived.[1,26,31,65] Descending modulation[24,125] of the pain signal is instigated within the rostroventral medulla where ON and OFF cells (nonacronymic terms) project back down the spinal cord to the dorsal horn of the spinal cord to either increase or decrease the firing frequency of the second-order neurons.

Actual or threatened damage to nonneural tissue activates nociceptors; a pain signal arising from these specialized sensory nerves is termed *nociceptive pain*.[110] It is directly triggered by a noxious stimulus being applied to, or occurring within the organism, and it is informative about threats to the organism. An example of this is pain due to a skin incision or superficial laceration. During the pain state, in addition to the nociceptive signal about the noxious stimuli, the organism may also experience allodynia, which is the sensation of pain upon the occurrence of a stimulus that is normally not noxious. Nociceptive pain resolves when the triggering noxious stimulus is no longer present, and thus is typically acute in nature.

Inflammatory pain, frequently identified as a distinct pain state, is a particular subset of nociceptive pain. It occurs secondary to the release of cytokines and other inflammatory mediators from immune cells as well as from the damaged tissue. Inflammation may develop after tissue injury or during the development of tissue pathology or tumor growth. At the site of damage or pathology, nociceptors become sensitized. Inflammation subsequently drives dramatic biochemical and molecular changes along all parts of the neural pain pathways that extend from the peripheral nociceptor to the cerebral cortex.[66,150] Along with mild acidification of the inflamed region, substances known to be released in the local area[131] constitute what is broadly referred to as the "inflammatory soup": prostaglandins, cytokines, nerve growth factor, lipids and lipoxygenase products, and ATP, among others yet to be defined. We are only now beginning to appreciate the complexity of these changes to the neural pathways and to understand the mechanisms that translate tissue injury or tumor development into chronically painful conditions.[56,110,197]

Pain that results from a lesion or disease in the somatosensory system itself is called *neuropathic pain*;[110] common examples of this type of pain are phantom limb pain after amputation and remote pain after damage to the spinal cord. In humans, the neuropathic pain state is diagnosed when patients experience mechanical allodynia, mechanical hyperalgesia, cold allodynia, and/or thermal hyperalgesia without a change in the threshold of tolerated heat.[198] Aβ fibers are thought to be mainly involved in the perception of allodynia, while activation of Aδ and C-fibers leads to mechanical and thermal hypersensitivity.[34]

Neuroplasticity

Pain from some injuries or disease states persists beyond the resolution of inflammation and tissue healing. This change from an acute to chronic nature is driven by neuroplasticity. This is a well-recognized property of the central nervous system, where neurons can alter the quantity and distribution of receptors, neurotransmitters, and intracellular signaling mechanisms to adjust to changes in the environment. It also occurs in the peripheral nervous system at the terminal sensory field of the peripheral nociceptors during inflammation. In the normal state, such neuroplasticity modulates signals and allows for adaptation.

Unfortunately, in some situations neuroplasticity results in allodynia, hyperalgesia (amplification of pain signals), or even ongoing pain signals in the *absence* of stimuli. *Nociplastic pain* is defined as pain that arises from altered nociception, that is occurring in the absence of a disease or lesion of the somatosensory nervous system and also in the absence of clear evidence of actual or threatened tissue damage that would activate peripheral nociceptors.[110] In other words, if pain cannot be differentiated as neuropathic or nociceptive, it is nociplastic pain. It is a maladaptive sensation that occurs without any indication of impending or actual tissue damage.

This sort of pain is a functional derangement of the pain pathway[156] and can occur through alterations in any step of signal processing. Neurotransmitters, neuromodulators, and their respective receptors on neurons can be altered by neuroimmune mediators in presence, absence, or quantity, altering synaptic connectivity and leading to decreased modulation of the ascending pain signal and/or aberrant signaling by peripheral nociceptors. This type of pain signal may occur completely uncoupled from noxious stimuli, and thus lacks a predictable resolution.

The older literature does not clearly distinguish between neuropathic pain and nociplastic pain, but instead simply discusses them together under the heading of neuropathic pain. Similarly, "chronic pain" is often used when neuropathic or nociplastic pain is being discussed.

Measurements

Physicians evaluating human pain rely on subjective pain scales, facial expressions, patient reports, and evoked measurements, while veterinarians rely on owner reports of behavioral changes in addition to a thorough physical exam and evaluation of evoked responses. The evaluation of pain in mice and rats is confounded by the fact that they are prey species normally distressed by handling[11] and that they will mask spontaneous behavioral and postural signs of pain.[29] While spontaneous behaviors and observed measurements are an enticing concept for evaluating the complete experience of pain, evoked measurements of pain remain the gold standard method for confirming the establishment of pain states. Measurements commonly used in rodents are briefly mentioned here (Figure 1); extensive reviews of these assays are published in this volume and elsewhere.[50,68]

Evoked measurements

Evoked reflexive assays are the standard measurement of nociception in the field because these measurements can be quantified, and as evoked measures, can be elicited when needed and tailored to the type of nociception to be assessed. The *von Frey*[35] assay, first described for use in human assessments by Maximilian von Frey in 1896, can be performed to measure tolerated cutaneous point-source pressure on the plantar surface of the paw or on other sensory fields of interest, using either serial measurements with progressively thicker filaments or an electronic probe that quantifies pressure using a force plate within the handle. The paw withdrawal threshold, the point at which the animal no longer tolerates the pressure, does depend to some degree on whether the animal is standing on a wire grate or an opaque flat surface.[135] A method to quantify the tolerable amount of deep pressure applies pressure over the inflamed or injured site by using an instrument capable of delivering calibrated pressure, such as the Randall–Selitto analgesiometer,[145] calibrated forceps, and others.

Muscle function and willingness to exert muscular strength can be measured by allowing the subject to grasp a weighted object while suspended in midair[47] or in actual pulling force exerted on a bar[122] in the *grip force* assay. The mice are presented with a metal bar that they grasp with their forepaws; they are then gently pulled back by the base of the tail until they release the bar. The peak force in grams at the time of release of forepaws is the dependent measure. It has been previously used to assess movement-related hyperalgesia in preclinical models of muscle inflammation of the triceps,[89] and the impact of osteolytic sarcoma introduced to the humerus.[182] In both instances, these conditions result in a reduction in ability of mice to grip the bar. Concurrent with the grasping of the bar by the forepaws, the mice are simultaneously pulled back from the bar by the tail, creating a stretching force along the spine, making it a useful assay for lower back pain. In the *tail suspension assay*,[168] mice are suspended by the tail, and a variety of behaviors directed toward either escaping the suspended state (rearing, self-supporting) compared with effective acceptance of the suspended state (immobility, full extension) are the dependent measures. Depressed mice spend less time performing escape behaviors and increase the time spent fully extended or immobile, suggestive of learned helplessness. One must be careful to assess the pain state with consideration of the effect of depression on the assay.[168] Animals with lower back pain spend less time in extended states and more time engaging in escape behaviors.[112]

Changes to thermal sensitivity can be quantified using a variety of assays. Heat allodynia and hypersensitivity can be distinguished by establishing the precise temperature required to activate a given population of receptors. Temperatures over 42 °C begin to activate the thermoreceptor TRPV1, while painfully hot temperatures over 52 °C activate TRPV2; these and other thermoreceptors are covered in more detail in other reviews.[123,131] *Capsaicin* can also be used to activate TRPV1 and is discussed in more detail in the inflammatory pain models below. Thermal sensitivity may be measured by placing the animal on a *hot plate* and timing the latency to paw lift or paw licking.[7,157,199] *Hargreaves–Dubner*[75] (Hargreaves) testing is similar but applies radiant heat to the plantar surface of the target paw only. The *tail flick*[81] assay applies heat to the tail to evoke a reflexive flick of the tail away from the source of heat. There are many options for the heat source; it can be a light beam,[43] laser,[134] or thermocouple;[76,174] or it may be a heated water bath.[81] While *hot-water tail immersion*[14,69] is performed at 55 °C, the term *warm-water tail flick*[153] describes testing using a water bath warmed to 52.5 °C or less. In older literature the term 'warm water' was applied to a variety of temperatures. The precise name used will depend on publication date and the degree to which the specific nociceptor populations had been identified at that time. These warming or heating tests can be used to distinguish between the populations of thermoreceptors that respond to noxious heat by adjusting the precise temperature to which the skin is exposed. An important consideration is that restraint methods may influence results obtained on the tail flick immersion test due to the stress of handling.[144] Ambient temperature also changes response latency on these tests,[76,134] as does the pigmentation of the skin.[190]

Cold allodynia, the activation of cold nociceptor responses by normally nonpainful cold temperatures, can also be assessed by chemical or thermal methods. Two receptors are involved: TRPM8 receptors respond to cool temperatures less than 25 °C, and TRPA1 receptors respond to noxious cold temperatures less than 17 °C.[131] The TRPM8 response is required for the animal to respond to acute noxious cold.[93] TRPM8 receptors can also be triggered with *menthol*.[179] *Icilin* was initially patented as a chemical that induced behavioral changes[138] in animals such as "wet-dog" shakes,[27,187] but further research discovered that it also produces cold sensations lasting about 15 min[188] when injected intraperitoneally or applied to skin by triggering activation of TRPM8.[27,93,191] Applying droplets of *acetone*[36] to the foot will induce behavioral responses when cold allodynia is present. To assess cold tolerance, an animal may be placed on a prechilled surface in the *cold plate*[18] assay. Adaptation to a drop in temperature can be assessed when a glass plate is chilled while the animal is standing on it in the *cold plantar assay*,[18,23] using wet

Category	Model	Dependent measure	Early references
evoked	tail flick	latency to withdrawal response	D'Amour and Smith 1941
	hot plate	latency to withdrawal response	Woolf and MacDonald 1944
	analgesiometer	grams of force tolerated	Randall and Selitto 1957
	icilin	brisk purposeful withdrawal	Wei and Seid 1983
	tail suspension	duration of escape behaviors; latency to immobility	Steru and colleagues 1985
	cold water tail flick	latency to withdrawal response	Pizziketti and colleagues 1985
	cold plate	latency to withdrawal response	Bennett and Xie 1988
	Hargreaves-Dubner	latency to withdrawal response	Hargreaves and colleagues 1988
	acetone	brisk purposeful withdrawal	Choi and colleagues 1994
	von Frey	maximum grams tolerated	Chaplan and colleagues 1994
	menthol	brisk purposeful withdrawal	Voets and colleagues 2007
	cold plantar assay	latency to withdrawal response	Brenner and colleagues 2012
	grip force	maximum grams of pull	Deacon 2013
spontaneous	force plates	maximum weight on limb	Min and colleagues 2001
	thermal gradient	time	Moqrich and colleagues 2005
	2-temperature choice test	time	Moqrich and colleagues 2005
	vocalizations	occurrence, frequency	Han and colleagues 2005
	burrowing	weight moved	Deacon 2009
	gait analysis	can be analyzed for range of motion, weight bearing, speed, symmetry, etc.	Piesla and colleagues 2009
	rearing	frequency	Benjamini and colleagues 2010
	dolognawmeter	time to chew; time spent chewing	Dolan and colleagues. 2010
	facial grimaces	movements are scored	Langford and colleagues 2010
	running wheel	duration	Cobos and colleagues 2012
	nest building	latency to incorporation of novel material	Jirkof 2014

Figure 1. Selected pain assays and early references, organized by type of assay and then chronologically.

or dry ice applied under the glass to cool it. *Cold-water tail flick*[137] can also be used; it was developed as a parallel model (-10 °C) to the hot-water tail flick assay. Ice baths are the typical means of cooling a surface or an immersion bath; it is a technical challenge to inexpensively and precisely generate cool rather than cold temperatures. Thus, chemical stimulation of cold nociceptors in rodent models may have an advantage due to receptor specificity, even if chemical cold stimuli are not the normal environmental triggers generating pain complaints from humans with neuropathic cold allodynia.

Spontaneous measurements

The primary advantage of spontaneous measurements is that they appear to recapitulate the perception and affective aspects of pain, in addition to nociceptive sensation. The animal chooses whether to display the species-typical behavior, and to what degree. Some assays are performed while the animal is in the home cage, while other assays require moving the animal to a specialized environment. The background frequency, duration, and speed of these behaviors are dependent upon the species and in some cases the age, sex, and strain of the animal, as well as individual differences in curiosity, anxiety, and activity level.

Rodents are well known for their propensity to *burrow* in appropriate substrates.[46,83] Speed of digging, weight moved, and willingness to dig (latency) can be sensitive measures as an assessment of either abdominal pain[84] or foot pain.[196] However, changes in burrowing do not directly correlate with evoked measurements of mechanical hypersensitivity.[6] Mice are known for their instinctive drive for *nest building*, which depends on quality of substrate, social stress, and other factors, in addition to the pain experience.[83] Both of these spontaneous behaviors are complex and require a fully-functioning central nervous system.

Some rodent strains are also known for their nocturnal drive to move; the speed, distance, and timing of this movement can be measured via *running wheel*.[37,67] However, exercise has been shown to be beneficial in reducing pain in both humans and rodents,[136] so these activity levels can be difficult to interpret. Weight-bearing alone can be assessed using *force plates*.[117,202] Sophisticated *gait analysis*[133,160] can be performed, but the findings are not necessarily reflective of a localized pain state nor consistent with evoked measurements. The researcher must consider whether the observed changes in gait are reflective of the pain state; the species, strain, and sex of the subjects; or changes to motor function secondary to alterations in nerve transmission from sedation or neuroplasticity.

Rats and mice can be assessed for their willingness to move, and some of these behaviors can be quantified as indicators of abdominal, joint, or back pain. Both species display a variety of *rearing*[15,172] behaviors during exploration of a novel space or object. This is useful in quantifying pain of the anatomic structures involved in weight-bearing in the rearing position, such as the lower back or hind limbs. It is even possible to evaluate speed of chewing by means of a *dolognawmeter*[53] to quantify pain associated with the oral cavity and jaw; animals with pain in tooth or temporomandibular joint will chew through the bar measurably slower than an animal lacking hypersensitivity. *Hunched* body postures can be excellent indicators of abdominal or visceral pain.[100,192]

The *conditioned place preference paradigm*[177] can be used to determine whether an animal shows a preference for a location where it has received a drug, over the other location where no intervention was administered. When used in studies of analgesia, it can also be used to assess preference for a location where pain was successfully ameliorated. This assay is somewhat dependent on strain, sex, age, and the severity of pain.

To assess thermal sensitivity, spontaneous behavior can be assessed by the *2-temperature choice test* or *thermal gradient*, both of which were originally developed[124] to assess preferences for moderately warm or cool temperatures. An animal with an inflamed or nerve-injured paw is likely to show a preference for walking on a room temperature surface over a cool or warm one, although this will vary with strain and pain model.

Facial expressions associated with pain have been clinically recognized in humans[98] for decades. In both rats and mice, *facial grimaces*[2,96,97,115,116,132,164] can be scored for assessment of generalized pain, although these grimaces are not always expressed, nor are they exclusively displayed in pain states. Clinicians observe that these grimaces, along with piloerection, hunched posture, and the presence of porphyrin in orbital secretions (in the rat) appear when the animal is regionally or generally impacted by the disease state, while significant changes in the facial expression are not usually observed in animals expected to be experiencing very localized pain (for example animals bearing soft tissue tumors or spontaneous skin conditions). As noted above, a further complication is that facial grimace expressions can also appear in affective states of fear or anxiety.[49] Positive affective states can also be recognized in these animals.[63]

Both *audible* and *ultrasonic vocalizations*[74,100] can be quantified as a spontaneous behavioral indicator of pain. However, like facial expressions, vocalizations are not purely generated as expressions of pain and maybe challenging to interpret.[86] Vocalizations are also influenced by affective state[162,200] and may be emitted as aggressive/anxious indicators during social encounters.[178]

Spontaneous behaviors are complex, but the subject is free to choose whether to display the behavior and to what degree. The difficulty with spontaneous behaviors is that in every case they reflect not only the pain state but also the status of the central nervous system and the individual strain,[170] sex,[25] and social status,[20] etc. Voluntary behaviors are often minimally affected by many pain states.[159] To date, no single spontaneous behavior has been identified as an indicator of pain state correlated with evoked measurements of hypersensitivity.

In summary, a variety of assays are useful for evaluating specific nociceptive populations (Figure 1). Selected evoked and spontaneous measures particularly applicable to specific, specialized preclinical models of human pain syndromes will be revisited in the next section.

Pain models

Laboratory rodents live in highly controlled environments, where the risk of natural exposure to illness or injury is very low. In addition, most rodents are used in research before they reach one year of age, so age-related disease or morbidity is uncommon. As a result, researchers must generate pain states to have a timely cohort large enough in size to calculate statistical significance. Many models (Figure 2) have been developed to recapitulate various human conditions, and each has pros and cons.

In all models involving surgery, reproducibility of the pain state and the survival of the subject rest upon good aseptic and surgical techniques and careful selection of appropriate analgesic and anesthetic regimens. Perioperative analgesics such as NSAIDs (such as carprofen and meloxicam) and opioids are used clinically in both humans and animals to prevent development of postoperative pain. Clearly, use of these analgesics will interfere with the native development of a pain state. The question to be answered in each case is what element of the human surgical procedure the rodent model is designed to reproduce. A useful consideration is whether the standard of analgesic care in human medicine can be mimicked or whether it will interfere with the development of the pain state in the model.

Among anesthetic drugs, NMDA antagonists (for example, ketamine and related drugs), local anesthetics (lidocaine), and α_2-adrenergic agonists (for example, xylazine) all have direct analgesic effects or adjunctive effects. The use of these therapies also interferes with the development of the pain state. Most anesthetic drugs, like the majority of opioids, are controlled substances. Currently, inhalant anesthetics constitute the anesthetic regimen of choice for ease of use, ready availability (they are not controlled substances), and minimal interference with the development of the pain state.

Regardless of the model, researchers have an ethical obligation to minimize pain and suffering. It is imperative to promptly carry out the planned activities. Once research subjects develop hypersensitivity, the sensitivity should be quantified as soon as possible. Animals must be treated in a timely fashion with the study agent and/or control analgesics per the IACUC-approved research plan. As soon as the last data point is collected, researchers should humanely euthanize animals without delay.

Nociceptive pain: Inflammatory models

Inflammatory models may be induced using a wide variety of chemical insults. These antigenic chemicals induce an influx of inflammatory cells, triggering the release of cytokines along with other inflammatory mediators (such as prostaglandins and bradykinins) from the inflamed tissue.[41] Chemically-induced inflammation can be generated in a range of specific locations. These agents have been administered into the subcutaneous space within the plantar surface of the paw, as well as the vibrissal pad. They have also been injected into the abdomen, instilled into the bladder or the lumen of the gastrointestinal tract, or injected intraarticularly to cause synovial degeneration that mimics arthritis.

One of the earliest models of inflammatory hypersensitization in the rat used *carrageenan*[195] to create edema by intraplantar injection in a hindpaw. This was used in assays to screen for the effectiveness of novel antiinflammatory drugs, as it is excellent at inducing inflammatory pain mediated by prostaglandins. *Capsaicin*, first isolated in 1846,[173] was also one of the earliest chemical agents used to distinguish nociceptors.[171] It specifically activates TRPV1 receptors,[33] among its other actions,[61] and has played a role in defining the mechanisms of abdominal pain when injected intracolonically to mimic the pain observed in irritable bowel syndrome.[94] *Acetic acid*,[38] when injected intraperitoneally, causes writhing, which is an abdominal constriction movement triggered by release of endogenous inflammatory mediators within the abdominal cavity. *Formalin*[57] has been injected both by intraperitoneal and intraplantar routes; it causes a biphasic response characterized by excitation of nociceptive C fibers and central sensitization of dorsal horn neurons.[51] Another well-defined inflammatory model uses *complete Freund's adjuvant*[165] (inactivated mycobacteria suspended in an oil emulsion). This chemical was originally used to develop a rodent model of arthritis, but it is now commonly used to induce inflammation by intraplantar injection into the subcutaneous space of the hind foot. *Lipopolysaccharide*,[40,62,108] derived from the wall of Gram-negative bacteria, is highly antigenic and induces the release of many of the inflammatory mediators that incite nociception. To create joint degeneration similar to that seen in osteoarthritis, *monosodium iodoacetate*[106,149] has been administered by intraarticular injection and is the preferred model for this

Pain	Model	References
nociceptive	carrageenan	Winter and colleagues 1962
secondary to	acetic acid writhing test	Collier and colleagues 1968
inflammation	formalin	Dubuisson and Dennis 1977
	capsaicin	Szolcsanyi 1977
	collagen-induced arthritis	Holmdahl and colleagues 1986
	complete Freund adjuvant	Stein and colleagues 1988
	lipopolysaccharide	Ferreira and colleagues 1993
	monosodium acetate	Marker and Pomonis 2012
neuropathic	sciatic nerve ligation (chronic constriction injury)	Bennett and Xie 1988
secondary to nerve	partial sciatic ligation	Seltzer and colleagues 1990
injury	spinal nerve ligation	Kim and Chung 1992
	streptozotocin	Calcutt and colleagues 1996
	sciatic nerve transection	Ma and Bisby 1997
	partial sciatic nerve transection	Ma and Bisby 1997
	spared nerve injury	Decosterd and Woolf 2000
	spinal nerve transection	Tsuda and colleagues 2003
	saphenous nerve partial ligation	Walczek and colleagues 2005
	chemotherapeutics	Authier and colleagues 2009
	cobra venom	An and colleagues 2011
	brachial plexus avulsion	Liu and colleagues 2017
	upper brachial plexus avulsion	Liu and colleagues 2017
constellation	colonic balloon	Ness and Gebhart 1988
postoperative	plantar incision	Brennan and colleagues 1996
	skin/muscle incision and retraction	Flatters 2008
constellation	pancreatic cancer	Ornitz 1987
cancer	osteolytic bone cancer	Wacnik and colleagues 2000
constellation	CGRP injection	Recober and colleagues 2009
migraine	nitroglycerin delivery	Bates and colleagues 2010
	direct dural application of migraine-inducing agents	Becerra and colleagues 2017
	noninvasive dural stimulation	Burgos-Vega colleagues 2019
constellation	SPARC-null	Millecamps and colleagues 2011 and Millecamps and colleagues 2012
low back	disc injury	Millecamps and Stone 2018

Figure 2. Selected pain models and early references, organized by type of pain and then chronologically.

condition. *Collagen-induced arthritis*[77] utilizes immunization with type II collagen in adjuvant to create a widely-used[194] chronic arthritic model. Any of these agents may be used in a variety of anatomic locations, although each has differing time to onset of hypersensitivity and duration of hypersensitivity. Inflammation due to the choice of suture material[87,142] was also a component of early models of nerve constriction injury intended to create neuropathic pain, since *silk*[90,161] or *chromic catgut*[18] were originally used. As observed clinically, localized cutaneous inflammatory pain models generally do not cause reduction of food intake or grooming. Quantification of hypersensitivity at the site of inflammation is necessary to provide a granular assessment.

Neuropathic pain: Nerve injury models

Neuropathic pain is defined as pain resulting from a lesion or disease in the somatosensory system itself.[110] Such pain states can be induced by injury or chemical administration. Injury to the brain and spinal cord is not discussed directly here. Injury to the peripheral nervous system can be modeled by peripheral nerve injury. This controlled trauma (transecting, constricting, stretching, or crushing the nervous tissue) causes immune cells to penetrate the nerve tissue. Certain drugs can also cause damage to the peripheral nociceptors either directly (for instance, chemotherapy) or indirectly (for instance, as a side effect from creating a diabetic state).

Development of neuropathic pain requires immunocytes to infiltrate the nerve to release the inflammatory soup of cytokines; without nerve signaling, inflammatory infiltration does not occur and the neuropathic state will not develop.[55,158,163,201] Thus, this injury is permitted to persist in the absence of analgesic therapy to cause changes to the central nervous system, ranging from internalization of nociceptive receptors to downregulation of inhibitory modulatory tone.

Evaluating inflammatory responses and their impact on the neuron can be carried out by a crushing constriction injury, either by direct application of transient pressure, or by ligation with chromic catgut suture. All of, or a portion of, the peripheral nerve may be damaged to induce immunomodulatory changes to the nervous system. Common models to induce inflammation secondary to the crushing injury include *spinal nerve ligation*[18,90] of lumbar dorsal nerve roots such as L5-L6, in a location distal to the dorsal root ganglion; *saphenous nerve partial ligation*;[48,185] *sciatic nerve ligation*;[9,18] or *partial sciatic ligation*.[18,154,161] The term *chronic constriction injury*[18,19] is anatomically nonspecific, but is usually used to refer to ligation of the entire sciatic nerve, using one or more ligatures. Crushing injury results in edema and inflammation which severs some, but not all, nerve fibers.

Additional neuropathic pain models entail surgical transection of the selected nerve. Examples of this method include the *sciatic nerve transection* model,[102-104] or the *spinal nerve*

transection,[90,176] where the L5 spinal nerve and potentially also the L6 spinal nerve are transected distal to the dorsal root ganglia to induce allodynia to mechanical and cold stimuli, heat hyperalgesia, and pain. A related model is global or complete *brachial plexus avulsion*[101,186] where all or some of the spinal nerves from C5-T1 are avulsed by traction from the spinal cord; this model develops long-lasting mechanical and cold allodynia, but it has the disadvantage of distorting or damaging the dorsal root ganglia. Complete axotomy does result in lasting mechanical allodynia, but when performed in a limb, autotomy or self-mutilation of the affected limb is a possible sequela. The likelihood of this undesirable outcome varies by strain and can be minimized by selecting a strain less likely to show this effect. Axotomies often result in motor deficits that can be quantified by gait analysis,[52] although clinically such deficits appear to cause little if any perturbation of the animal's ability to reach food and water.

More refinement brought partial nerve transection to the forefront, allowing for preservation of the limb. The entire targeted nerve is identified during surgery but only some of its branches are transected. In the *partial sciatic nerve transection*,[99,102-104] the nerve fiber bundle is divided just proximal to the branch innervating the biceps femoris and only half is transected. In *spared nerve injury*,[48] 2 of the 3 branches of the sciatic nerve are transected and the remaining branch preserved. In *upper partial brachial plexus avulsion*,[101] the upper of the 3 nerve trunks constituting the brachial plexus is avulsed, while the middle and lower trunks are preserved.

Peripheral neuropathic pain can be induced by chemical means as well. Injection of *cobra venom*,[5] for instance, into the infraorbital nerve has been used to create a model of trigeminal neuralgia, with long-lasting mechanical allodynia on both the ipsilateral and contralateral side.

Diabetic peripheral neuropathy has long been studied in rodent strains developing spontaneous diabetes as well as after high fat or high sugar diets.[148] *Streptozotocin*[32,45,148] is a chemical means of inducing diabetes in rodents, although during the excretion period, the waste of these animals poses a risk to human handlers. Husbandry and management of diabetic rodents in general, can become labor-intensive due to polyuria, increased food consumption, and weight loss. In general, chemically-induced neuropathies, while in some cases direct models of human clinical pain conditions, can cause weight loss and general debilitation to the animal, as well as pose exposure risks to persons handling the animal or its soiled bedding.

There are benefits and drawbacks to each method of peripheral injury. Direct injury to the nerve is relatively easy to perform, and the neuropathy is typically localized to the affected limb or the sensory field of the damaged nerve and its immediate neighboring sensory fields. Aside from the complete nerve transection models, behavioral signs of spontaneous pain appearing during the neuropathic stage are limited or absent after nerve injury. Nerve ligation models can directly mimic elements of the human condition, such as carpal tunnel syndrome, sciatica, and other constrictive nerve injuries. However, constriction injury depends on the degree of trauma from pressure or tight ligation and thus may vary between surgeries and between labs, whereas direct severing of nerves creates a consistent injury. Surgical transection of nerve fibers preserves neighboring tissue structures for histologic evaluation, while crushing, stretching, or constriction injuries limit the value of histology.

Nociplastic pain models

Nociplastic pain arises from altered nociception in the absence of clear evidence that would aid in classifying the pain as nociceptive or neuropathic.[110] By definition, all animal pain models involve an insult to the body, either directly to the tissues of the somatosensory nervous system in the case of neuropathic pain, or to any other tissue in the case of nociceptive pain. Therefore, it stands to reason that there are no pure models of nociplastic pain.

Pain constellations

When analgesic medications are discussed, pain is often described as a singular phenomenon. However, pain describes a variety of syndromes arising from different mechanistic etiologies, akin to a set of stars making up a constellation. Some clinical syndromes associated with pain in the human population arise from multiple deep tissues and structures, the sensory assessments of which are challenging. Such complexity can be difficult to recapitulate in the animal, but progress has been made in developing pain models that directly mimic the human condition as best as it is currently understood. Considering these approaches may provide insight into strategies for modeling other specialized pain conditions of unique pathophysiology.

To provide a human clinical example, recrudescence and subsequent remission of varicella zoster virus in adults can lead to postherpetic pain; in other words, the disease process can trigger neuroplastic changes that cause pain signals to persist after the lesions have healed.[151] In this particular syndrome, pain is thought to be incited by an immune or inflammatory response to the viral reactivation in the nerve. This would fit the definition of nociceptive pain, but the associated neuroplastic changes can cause pain to persist after healing, subsequently transforming the pain into a nociplastic pain.[105] This type of complexity is associated with many chronic pain conditions.

Pain constellations: Postoperative models

In humans, postoperative nociceptive pain may incite lingering nociplastic pain states after surgeries, with prevalence ranging from 10% to a majority of patients.[42,85] Minimizing postoperative pain is important in order to reduce the likelihood of long-term nociplastic pain as a sequela to surgery.

Most surgical procedures can be directly mimicked in the animal model, but difficulty also arises in designing an appropriate sham procedure as a negative control. Anesthesia alone can be used as a sham to control for the effects of the anesthetic drugs on the nociceptive system. However, once surgical trauma encompasses to more than one tissue type, it becomes challenging to tease apart distinct nociceptive signaling. One oft-used method is to create a sham surgery, to control for pain from superficial structures. The usual procedure is to perform the skin and muscle incisions necessary for the complex procedure and then close the surgical site, without disturbance or damage to nerves, organs, or bones. The cohort undergoing sham surgery would experience nociceptive signaling from surface structures but not from deep structures. The study cohort would experience nociceptive signaling from both surface structures and deep structures.

Sham surgery groups become important for evaluating visceral pain, since unlike the nociceptors of skin or eye, visceral nociceptors cannot be touched from the external surface of the body. As discussed above, writhing assays triggered by intraperitoneal injections of irritating substances can be used, but these inflammatory models are limited in their ability to

elucidate mechanical sources of pain. To evaluate mechanonociceptors in visceral pain, a *colonic balloon*[126] can be placed in the descending colon under anesthesia, and once the animal is fully recovered, the balloon can be distended to precise pressures while the animal's behavioral responses are quantified. Control animals would logically be animals that have undergone the same surgery, but either do not have the colonic balloon inflated after surgery or do not have the balloon placed. This model is useful for mimicking the mechanical visceral pain associated with colon cancer, irritable bowel syndrome, and other diseases of the lower gastrointestinal tract.

Overall, the most logical choice in creating a specific postsurgical pain model is to, as much as possible, perform the same surgery in the animal. Major surgeries, even those creating bone defects, are generally well-tolerated by rodents due to their small body mass relative to their strength. As such, the ability to recapitulate the human surgical procedure in the rodent model is limited primarily by the technical skill of the surgeon and the size of the instruments and equipment. An exhaustive list of such models is not provided here. However, an identical procedure is not always practical nor necessary, so most models simplify the surgery.

By interacting with the same type of tissues involved in the human surgery while minimizing the scope of surgical trauma to the animal, a greater number of normal behaviors can be preserved and the postoperative pain state can be consistently reproduced. For example, the *plantar incision*[21,22,139] model, creating a skin incision on the plantar surface of the hind paw, can be used to model the inflammatory nociceptive pain associated with skin incisions, and deeper tissue manipulation can be simulated in the procedure by retracting, stretching, or incising the plantaris muscle in the arch of the foot. To model pain such as might be associated with a hernia repair, the *skin/muscle incision and retraction*[64,139] model can be used, where an incision is created in the medial aspect of the thigh. The gracilis muscle is incised and retracted to hold it open either briefly, or for up to an hour. These models are modest in their overall impact on the animal, yet recapitulate minor to moderately invasive surgeries in humans.

Pain constellations: Cancer pain models

After tissue injury, the development of tissue pathology, or tumor growth, inflammation often occurs at the site. As a consequence, dramatic biochemical and molecular changes occur along all parts of the neural pain pathways, from the newly-sensitized peripheral nociceptor to the cerebral cortex.[66,150] We are only now beginning to appreciate the complexity of these changes, and to understand the mechanisms that translate tissue injury or tumor development into chronically painful conditions.[56,111,197]

The situation becomes more difficult when dealing with cancer pain, because there is a wide range of tumor types and molecular subtypes. Some tumors can be very painful (for example, bone cancer and pancreatic cancer). Other tumor types (such as lipomas, melanomas, enchondromas and many other types of benign tumor) may be painless (depending on size and location). We now know that tumor pain is associated with complex interactions that occur in the tumor microenvironment. An understanding of these interactions is critical to the development of new therapeutic approaches to treat not only tumor-induced pain but also tumor development, growth, and metastasis. A wide variety of cancer models are available, including xenograft models, whereby cancer cells from human patients are implanted into mice with humanized immune systems. However, not all cancer models are widely used as pain models. This review will touch on soft tissue and bone pain in 2 common models; an exhaustive review of cancer models[95,183,184] is beyond the scope of this work.

To model visceral cancer pain, *pancreatic cancer* is commonly used as a model despite its relative rarity in the human patient population (approximately 2% to 3% of annual new cancer cases in the United States).[4,100] In humans, the disease carries with it a substantial burden of visceral pain. A transgenic mouse model was created to spontaneously express exocrine pancreatic tumors.[128] These animals demonstrate vocalization upon palpation and a hunched posture in the late stages of the disease.[100,155] This pain can be exposed earlier in the disease process if the animals are given naloxone.[155]

Bone cancer pain affects approximately half or more of patients diagnosed with cancer.[60] Since this type of pain can cause dramatic reductions in quality of life and several animal models have been developed to investigate cancer-induced bone pain,[16,129] this review will focus on the mechanisms underlying this type of cancer pain. *Osteolytic bone cancer* pain can be modeled in rodents by injecting osteolytic fibrosarcoma cells into any one of the following bones: femur,[78,184] humerus,[184] calcaneus[181] or tibia.[204] A number of factors can contribute to bone cancer pain and include: the release of chemical mediators,[152] the increased pressure within the bone, microfractures, the stretching of periosteum, reactive muscle spasm, nerve root infiltration and compression of nerves by the collapse of vertebrae.[109]

While each of these contributing factors is important, recent evidence suggests that understanding the microenvironment in which cancer, particularly metastatic bone cancer, develops, is critical for appreciating how cancer produces pain.[189,203] When tumor cells of any origin metastasize to bone, they interact with the microenvironment to promote bone destruction through the secretion of osteolytic factors by the tumor cells, and the subsequent release of growth factors and other mediators from the bone.[167] Dynamic interactions occur among tumor cells, hematopoietic stem cells, osteoblasts, osteoclasts, the vascular compartment in bone, inflammatory cells that have invaded the tumor, and the nerve fibers that innervate the bone and bone marrow. Cancer cells, inflammatory cells, and immune cells that reside in bone metastases produce acidic conditions by releasing protons (hydrogen ions), which appear to activate nerve fibers directly by stimulating TRPV1 and ASIC channels located on the nerve fibers.[203] Cancer tissues directly secrete endogenous formaldehyde, which, at low concentrations, contributes to metastatic bone cancer pain by activating TRPV1 channels, especially in the acidic environment near the tumor.[175] In the microenvironment of many cancers, sensory neurons are chronically exposed to nerve growth factor (NGF), which, under normal conditions, is secreted to promote the local growth and survival of afferent sensory neurons.[80,130] The secretion of NGF into the tumor microenvironment by either cancer cells or other cell types within the tumor likely leads to several changes that contribute to pain; the NGF binds directly to TRK receptors on primary sensory neurons.[152] Anti-NGF therapy has been shown to attenuate tumor-induced pain.[82]

In addition to inflammatory and neuropathic pain generated by the aberrant remodeling of tissue and displacement of normal tissues and organs, pain can also develop from the therapies used to address cancer. *Radiation* therapy, often necessary to treat cancer, can also cause local neuropathies[169] in humans, likely due to the death of not only cancer cells but also adjacent cells, releasing cytokines and other inflammatory agents. *Chemotherapeutic*[10,79,140] agents such as paclitaxel, vincristine, and

cisplatin are studied directly in animals to evaluate the phenomenon of chemotherapy-induced peripheral neuropathies. Exposure to these agents, when shed by the animals in feces or urine, poses a direct risk to human handlers.

Pain constellations: Migraine models

Headache afflicts 3 billion (49%) individuals annually; headache disorders collectively are the third most prevalent disorder and migraine alone is the sixth.[54] Migraine also contributes significantly to disability in working and daily life; it is the second most common cause of disability (after low back pain)[54] and the leading disability in those under 50.[166] Over 47 million Americans suffer from migraine.[180] Within the human population, manifestations of headache and of migraine are highly variable with respect to pain intensity, severity, duration, and location, as well as referred allodynia/hypersensitivity of the face, neck, or other regions. Triptan medications, a first-line treatment for migraine, activate both 5HT1B receptors on blood vessels and 5HT1D receptors on peripheral nerve endings and central terminals. However, there is considerable variation in individual responsiveness to treatments and likewise in commonly-experienced adverse side effects (such as flushing, tingling numbness, dizziness, thermal sensations). Such variability drives continued work toward understanding migraine mechanisms to develop new medications.

The established mechanisms underlying migraine include cortical spreading depression and release of the neurotransmitter, calcitonin gene-related peptide (CGRP). CGRP and its receptor are expressed throughout the sensory system. Peripheral nerve endings innervating the dura mater contain CGRP. Release of CGRP activates receptors on the dural blood vessels, leading to subsequent cerebrovascular dilatation. Dilation of these dural vessels can apply noxious pressure to peripheral nerve endings, resulting in pain. An additional component of headache pain involves referred pain. This arises from activation of axonal branches of the trigeminal nerve, which then activates other portions of the nerve. Referred pain also arises from convergence, where trigeminal primary neurons and sensory neurons originating from other regions coactivate cervical dorsal horn sensory neurons. Such circuitry could explain why noncerebral regions become painful under conditions of headache.

CGRP is a signature neurotransmitter associated with the pathophysiology of migraine.[58] CGRP levels are elevated in CSF, serum, and saliva of migraine patients; injection of CGRP can trigger migraine in migraine patients.[146] Based on that mechanism, several preclinical models of migraine have been developed. One example is *CGRP injection.* Direct intracerebroventricular injection of CGRP[147] in unanesthetized mice with an overexpressed constituent of the CGRP receptor (Ramp1) increased the amount of time these mice spent in a darkened chamber, with light–aversive behavior presumably a correlate to photophobia. Delivering CGRP slowly in anesthetized mice induced an elevation in light-aversive behavior and an increase in resting behavior under dark conditions.[146] When CGRP was given peripherally by intraperitoneal injection, it also induced light-aversive behavior and an increase in resting behavior in dark conditions. These changes could be reversed via administration of sumatriptan or CGRP-antibody.[146] Further, application of the facial grimace scale revealed a phenotype of increased eye squint that was alleviated both by antiCGRP antibody and partially reversed by the CGRP receptor antagonist sumatriptan in male CD1 mice.[146] This observation represents a spontaneous pain phenotype induced by CGRP in mice that reflects a pain behavior common in people with migraine.

The vasodilator nitroglycerin, often used to treat angina, is a trigger for headaches in humans; *nitroglycerin delivery* has been used to experimentally induce migraine in humans and rodents. Intraperitoneally-delivered nitroglycerin induces thermal and mechanical allodynia within 30 to 60 min that is reversible by sumatriptan.[12] Another group demonstrated altered light aversive-behavior and increased meningeal blood flow after intraperitoneal administration of nitroglycerin.[107] The migraine state induced by nitroglycerin becomes chronic after repeated intraperitoneal administration of nitroglycerin every other day up to 9 d (5 injections).[141] Such a dosing regimen resulted in development and persistence of tactile hypersensitivity of the hindpaw for several days beyond the period of dosing. Sumatriptan selectively inhibits tactile hypersensitivity of chronic nitroglycerin, but not CFA-treated hindpaws. The antimigraine prophylactic medication toperimate also prevented the development of tactile hypersensitivity arising from repeated dosing of nitroglycerin.[141]

Another model of migraine involves *direct dural application* of an acidic mixture of inflammatory mediators such as bradykinin, serotonin, prostaglandin-E2, and histamine ("inflammatory soup") to the exposed or cannulated dura of a mouse or rat. The inflammatory soup activates the peripheral nerve endings that innervate the dura. Such exposure results in subsequent activation and sensitization of central trigeminovascular neurons in the trigeminal nucleus and sensory thalamus.[13] This sensitization likely leads to cephalic and extracephalic allodynia. This approach requires surgical exposure of the cranium (craniotomy) to access the peripheral nerve fibers that innervate the dura mater. Applying the inflammatory soup yields tactile and thermal sensitization.[30] This exposure results in pain-depressed behaviors such as reduced locomotor and exploratory behavior.

A recently introduced method uses a *noninvasive dural stimulation model* to stimulate the dural peripheral neurons (afferents)[28] without a craniotomy, representing a significant refinement of the direct method used to induce a migraine. Under light anesthesia, allyl isothiocyanate, low pH (6.0) solutions, or interleukin-6 cytokine can be introduced onto the dura. These agents altered facial grimace response thresholds in a sumatriptan-dependent manner.

One of the challenges in developing migraine models is capturing the complete experience, which includes sensory pain, referred pain, affective changes, cognitive changes, and nausea, in addition to the well-known photophobia. As already noted, measuring pain in animals is difficult and often relies on reflex measures of external limbs. In the case of headache, accessing the trigeminal and cerebrovascular circuitry may more be challenging than hindlimb peripheral nerve injury. Dependent measures used to capture these experiences range from assessment of pain-stimulated behaviors (for example freezing, eye-blink, or increased grooming) to pain-depressed behaviors (for example reduction in locomotor activity or rearing). Referred pain is assessed with standard evoked (reflex) measures following tactile (von Frey), thermal, or chemical stimulation.[180] Propensity to seek dark areas can be measured to assess photophobia, while food and water intake is measured as a surrogate for nausea which is otherwise not clinically evident in rodents.[180]

In summary, a variety of preclinical models of migraine based on either trigger initiators or pathophysiology are applied to probe both mechanisms of migraine pathology and analgesic treatments. Assessments include spontaneous, reflex, and locomotor behaviors that reflect diverse human pain and light aversion (photophobia) feature that are associated with migraines. The specificity of pain associated with migraine has

been characterized through the use of analgesic medications (triptans such as sumatriptan, CGRP-antibodies, topiramate) that are specifically used for migraine to reverse the dependent measures.

Pain constellations: Lower back pain models

Chronic pain associated with the lower back is notably a disabling and high burden syndrome frequently associated with other conditions such as depression and sleep disturbance. Chronic low back pain arises from nociceptive processes involving inflammatory activation of nerves that innervate tissues associated with physiology of the back including ligaments, joints, muscle, fascia, and tendons.[112] Chronic low back pain is also driven by neuropathic processes resulting from pathologic changes arising in nerve roots associated with the spine or damaged lumbar discs, and it is associated with structural and functional changes in the brain that may contribute to ongoing pain.[8] Therefore, multiple mechanisms (nociceptive-inflammatory, neuropathic, nociplastic) underlie chronic back pain. Improving our understanding of the mechanisms underlying low back pain will facilitate the optimization of treatment plans. The relatively recent establishment of preclinical models of low back pain is, therefore, greatly needed and strongly justified. While preclinical models of spine function and physiology have been broadly used in a variety of species,[44] establishing a pain phenotype specific to the lower back presents a greater challenge. While axial compressive loads are greater in the bipedal spine, the load on intervertebral discs in the quadrupedal horizontally-aligned spine may actually be greater due to the muscle contraction and ligament tension necessary to stabilize the spine against bending forces.[3,193] The mouse intervertebral disc has been ranked as the disc most geometrically similar in porportion of nucleus pulposus, disc width, and disc height to the human intervertebral disc.[127] Two recent thoroughly characterized models in mice[112,113] are described below.

Degeneration of intervertebral discs is associated with the development of chronic low back pain in humans that manifests as pain confined to the low back region (axial pain) or pain that radiates through the leg (radicular pain). A protein termed "*SPARC*" (Secreted Protein Acidic and Rich in Cysteine) is diminished in human discs during disc degeneration.[70] Engineering a mouse line with deletions of the SPARC protein produced age-related disc degenerative changes and diminution of natural hydration, as expected.[71] Axial pain is established in the SPARC-null mice by 3 mo and cold hypersensitivity by 12 mo. The SPARC-null mouse model represents progressive disc degeneration with age. There is significant variability in degeneration across discs and individual mice, an advantage for its similarity to the clinical condition. However, the deletion of SPARC is not restricted to the spine; as the mice age, other problems such as osteopenia develop, which can complicate interpretation. Because the control animals also remain in the vivarium for up to a year, they too begin to show manifestations of aging. In fact, the controls also eventually develop the cold hypersensitivity that the young SPARC-null mice demonstrated by 3 mo, thus offering a model of age-induced cold hypersensitivity.

In this model, the tail suspension assay[168] and the grip force assay[89] became important assessments for axial pain or pain focused within the low back region. In mice with low back pain, the time spent in escape behaviors is increased and the time in immobility or at full extension is reduced.[112] The reduction of time in immobility in SPARC-null mice is interpreted as the avoidance of gravity-induced stretching of the spine. Pretreatment with antiinflammatory or antineuropathic drugs attenuates the abnormal behavior, suggesting analgesia and confirming the likelihood that the measurement likely reflects a painful condition in the low back. SPARC-null mice demonstrate reduced grip force strength as early as 6 wk of age that persists for up to 7 to 8 mo of age, presumably due to the pain arising during axial stretching of the assay. SPARC-null mice with disc degeneration do not appear to be hypersensitive to tactile or heat stimuli applied to hindpaws. Sole reliance on testing those modalities would be unlikely to detect a pain phenotype. This illustrates the importance of using a broad and rationally-designed stimulation strategy for assessing pain in new preclinical models of pain syndromes.

Assessment of sensitivity to specific classes of analgesics (such as NSAIDs) or antineuropathic drugs (such as gabapentin) makes it possible to parse out the mechanisms underlying the specific pain sensation, rendering this preclinical model particularly valuable for screening analgesic drugs of differing mechanisms in a single preclinical pain model.

Another method of modeling lower back pain is the *disc-injury model,* established to elucidate the distinctions between injury-induced changes and progressive disc degeneration.[113] In this model, anesthetized mice receive a ventral incision to visualize the L4/L5 intervertebral disc, which is slowly penetrated with a 30 gauge needle. After recovery, subjects are tested in the same assays, grip force assay, and tail suspension assay. Between 3 to 9 mo after injury, disc-injured mice demonstrate behavior indicative of avoidance of axial stretch in the tail suspension assay. These responses resolve by 12 mo postinjury. No effect is observed in the grip force assay. No difference in response to tactile stimulation exists between disc-injured and noninjured mice. The subset of mice that specifically display narrowing of the disc and innervation of the dorsal aspect of the disc show elevated responses to acetone-induced cold stimulation.

The best model to use to study lower back pain is likely dictated by the question and the priorities of the study. Certain commonalities in the disc degeneration occur as a sequela to acute disc injury and also from progressive disease (SPARC model), specifically the axial pain and cold hypersensitivity. In contrast, clear differences are evident in terms of variability and duration of pain responses. While mice in either model appear to develop the progressive degeneration, not all disc-injured mice develop the cold sensitivity, an observation similar to the variability seen in clinical presentation. Thus, the disc injury model may offer mechanistic insight that could help differentiate patients and optimize treatment. An advantage of the SPARC progressive disc degeneration model is that the outcomes are robust, enabling clear pharmacological assessments. The SPARC model also reflects a more prevalent clinical problem than does a single disc incision. These models are best viewed as complementary models which address different syndromes associated with low back pain.

Discussion

Pain has been a variable, yet constant, aspect of the human condition, and animals have long been used to decipher the pain condition. Over many, many decades, pain research has uncovered an enormous scope of knowledge. Distinct poplulations of neurons code for different pain modalities. Membrane receptors, neuronal transmitters, and glial transmitters are involved in propagating pain signals. Connections between distinct regions of the brain and spinal cord have been identified which contribute to carrying the pain signal to the brain while also modifying signals from the brain to the spinal circuitry. The

totality of what is known about pain pathways and the related endogenous analgesic systems is due to the efforts by many academic generations of an international pain research community. They remain unwavering in their unified commitment to discovering innovative approaches to alleviate the entire spectrum of acute and chronic pains. Researchers aim to apply preclinical modeling of such types of pain in animal subjects to improve our understanding of pain and develop new medications. These global efforts stand the best chance of success when supported through open communications and collaboration of scientific researchers with laboratory animal veterinarians and veterinary staff members. Each professional brings valuable insight and complementary experience to the table. Including veterinarians in study design and implementation and bringing the pain research community to review research protocols in IACUC settings results in refined approaches to preclinical modeling, not only in pain research but also in other biomedical fields. This cross-disciplinary team approach also enhances considerations for animal welfare while ensuring the scientific goals of the study can be achieved.

Although the methods often used to perform sensory assessment may seem simplistic to those who are reviewing studies and protocols or are seeking to enter the field, the study of pain in animal models is not trivial and requires training as well as careful study design. Even the controlled environment of the research lab has variables that may not be sufficiently constrained to produce pain behaviors consistent between research groups, potentially leading to difficulty validating analgesic findings.[11,121] Because no single model will perfectly recapitulate all human patients in all painful conditions, there is a need to use a variety of models of pain,[19] both the induced pain models established in laboratory animal species reviewed here and the spontaneous or induced pain states in other species.[92] This review has featured the specific methods used to assess pain in preclinical subjects, and strategies to develop preclinical models that feature specific pain conditions. Other excellent reviews[59,68,91] have provided important summaries including additional preclinical pain models and refinements that are beyond the scope of this review. All of these approaches, methods, and models have been developed and refined over decades by scientists trained in sensory assessments, neurobiology, and neuropharmacology. In 2018 the National Institutes for Health significantly increased its investment in pain research and new analgesic development as a response to the opioid epidemic. Such investment has attracted scientists with limited experience in pain research to contribute to building solutions. Therefore, those new to the field and the laboratory animal veterinarians and staff members who support and monitor such research must perform due diligence to understand the nuances of preclinical pain modeling such as are described in this review. Fully understanding the field will educate newcomers entering the field and reduce the risk of replicating common errors. Education and engagement of those experienced with these approaches is essential to avoid such pitfalls and will greatly advance the 3R principles of Reduction, Refinement, and Replacement. Through collaboration, education, and good study design, we can work together toward a future where addiction is no longer a public health crisis and where all people with pain receive appropriate and safe care.

Acknowledgment

Salary support for Dr Larson was provided by the Office of the Director, National Institutes of Health (T32OD010993). The content is solely the responsibilty of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. **Ahmad AH, Abdul Aziz CB.** 2014. The brain in pain. Malays J Med Sci **21:**46–54.
2. **Akintola T, Raver C, Studlack P, Uddin O, Masri R, Keller A.** 2017. The grimace scale reliably assesses chronic pain in a rodent model of trigeminal neuropathic pain. Neurobiol Pain **2:**13–17. https://doi.org/10.1016/j.ynpai.2017.10.001.
3. **Alini M, Eisenstein SM, Ito K, Little C, Kettler AA, Masuda K, Melrose J, Ralphs J, Stokes I, Wilke HJ.** 2008. Are animal models useful for studying human disc disorders/degeneration? Eur Spine J **17:**2–19. doi:10.1007/s00586-007-0414-y.
4. **American Cancer Society**. [Internet]. 2000. Cancer facts and figures. [Cited 21 September 2019]. Available at: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures.html
5. **An JX, He Y, Qian XY, Wu JP, Xie YK, Guo QL, Williams JP, Cope DK.** 2011. A new animal model of trigeminal neuralgia produced by administration of cobra venom to the infraorbital nerve in the rat. Anesth Analg **113:**652–656.
6. **Andrews N, Legg E, Lisak D, Issop Y, Richardson D, Harper S, Huang W, Burgess G, Machin I, Rice ASC.** 2011. Spontaneous burrowing behaviour in the rat is reduced by peripheral nerve injury or inflammation associated pain. Eur J Pain **16:**485–495. https://doi.org/10.1016/j.ejpain.2011.07.012.
7. **Ankier SI.** 1974. New hot plate tests to quantify antinociceptive and narcotic antagonist activities. Eur J Pharmacol **27:**1–4. https://doi.org/10.1016/0014-2999(74)90195-2.
8. **Apkarian AV, Bushnell MC, Treede RD, Zubieta JK.** 2005. Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain **9:**463–484. https://doi.org/10.1016/j.ejpain.2004.11.001.
9. **Attal N, Jazat F, Kayser V, Guilbaud G.** 1990. Further evidence for 'pain-related' behaviours in a model of unilateral peripheral mononeuropathy. Pain **41:**235–251. https://doi.org/10.1016/0304-3959(90)90022-6.
10. **Authier N, Balayssac D, Marchand F, Ling B, Zangarelli A, Descoeur J, Coudore F, Bourinet E, Eschalier A.** 2009. Animal models of chemotherapy-evoked painful peripheral neuropathies. Neurotherapeutics **6:**620–629. https://doi.org/10.1016/j.nurt.2009.07.003.
11. **Balcombe JP, Barnard ND, Sandusky C.** 2004. Laboratory routines cause animal stress. Contemp Top Lab Anim Sci **43:**42–51.
12. **Bates EA, Nikai T, Brennan KC, Fu YH, Charles AC, Basbaum AI, Ptáček LJ, Ahn AH.** 2010. Sumatriptan alleviates nitroglycerin-induced mechanical and thermal allodynia in mice. Cephalalgia **30:**170–178. https://doi.org/10.1111/j.1468-2982.2009.01864.x.
13. **Becerra L, Bishop J, Barmettler G, Kainz V, Burstein R, Borsook D.** 2017. Brain network alterations in the inflammatory soup animal model of migraine. Brain Res **1660:**36–46. https://doi.org/10.1016/j.brainres.2017.02.001.
14. **Ben-Bassat J, Peretz E, Sulman FG.** 1958. Analgesimetry and ranking of analgesic drugs by the receptacle method. Arch Int Pharmacodyn Ther **122:**434–447.
15. **Benjamini Y, Lipkind D, Horev G, Fonio E, Kafkafi N, Golani I.** 2010. Ten ways to improve the quality of descriptions of whole-animal movement. Neurosci Biobehav Rev **34:**1351–1365. https://doi.org/10.1016/j.neubiorev.2010.04.004.
16. **Bennett GJ.** 2010. Pathophysiology and animal models of cancer-related painful peripheral neuropathy. Oncologist **15 Suppl 2:**9–12. https://doi.org/10.1634/theoncologist.2009-S503.
17. **Bennett GJ, Chung JM, Honore M, Seltzer Z**. 2003. Models of neuropathic pain in the rat. Curr Protoc Pharmacol **21:**5.32.1–5.32.16. https://doi.org/10.1002/0471141755.ph0532s21
18. **Bennett GJ, Xie YK.** 1988. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain **33:**87–107. https://doi.org/10.1016/0304-3959(88)90209-6.
19. **Berge OG.** 2011. Predictive validity of behavioural animal models for chronic pain. Br J Pharmacol **164:**1195–1206. https://doi.org/10.1111/j.1476-5381.2011.01300.x.

20. **Bernstein IS.** 1981. Dominance: the baby and the bathwater. Behav Brain Sci **4:**419–429. https://doi.org/10.1017/S0140525X00009614.
21. **Brennan TJ.** 1999. Postoperative models of nociception. ILAR J **40:**129–136. https://doi.org/10.1093/ilar.40.3.129.
22. **Brennan TJ, Vandermeulen EP, Gebhart GF.** 1996. Characterization of a rat model of incisional pain. Pain **64:**493–502. https://doi.org/10.1016/0304-3959(95)01441-1.
23. **Brenner DS, Golden JP, Gereau RW.** 2012. A novel behavioral assay for measuring cold sensation in mice. PLoS One **7:**1–8. https://doi.org/10.1371/journal.pone.0039765.
24. **Brink TS, Pacharinsak C, Khasabov SG, Beitz AJ, Simone DA.** 2012. Differential modulation of neurons in the rostral ventromedial medulla by neurokinin-1 receptors. J Neurophysiol **107:**1210–1221. https://doi.org/10.1152/jn.00678.2011.
25. **Broida J, Svare B.** 1984. Sex differences in the activity of mice: modulation by postnatal gonadal hormones. Horm Behav **18:**65–78. https://doi.org/10.1016/0018-506X(84)90051-5.
26. **Brooks J, Tracey I.** 2005. From nociception to pain perception: imaging the spinal and supraspinal pathways. J Anat **207:**19–33. https://doi.org/10.1111/j.1469-7580.2005.00428.x.
27. **Burford RG, Chappel CI**. 1972. "Wet dog shake" induction in rats by a novel compound AG-3-5, p 33. Fifth International Congress of Pharmacology. San Francisco, California, 23–28 July 1971. The International Union of Pharmacology (IUPHAR).
28. **Burgos-Vega CC, Quigley LD, Trevisan Dos Santos G, Yan F, Asiedu M, Jacobs B, Motina M, Safdar N, Yousuf H, Avona A, Price TJ, Dussor G.** 2019. Noninvasive dural stimulation in mice: a novel preclinical model of migraine. Cephalalgia **39:**123–134. https://doi.org/10.1177/0333102418779557.
29. **Burkholder T, Foltz C, Karlsson E, Linton CG, Smith JM.** 2012. Health evaluation of experimental laboratory mice. Curr Protoc Mouse Biol **2:**145–165. https://doi.org/10.1002/9780470942390.mo110217.
30. **Burstein R, Yamamura H, Malick A, Strassman AM.** 1998. Chemical stimulation of the intracranial dura induces enhanced responses to facial stimulation in brain stem trigeminal neurons. J Neurophysiol **79:**964–982. https://doi.org/10.1152/jn.1998.79.2.964.
31. **Bushnell MC, Ceko M, Low LA.** 2013. Cognitive and emotional control of pain and its disruption in chronic pain. Nat Rev Neurosci **14:**502–511. https://doi.org/10.1038/nrn3516.
32. **Calcutt NA, Jorge CM, Yaksh TL, Chaplan SR.** 1996. Tactile allodynia and formalin hyperalgesia in streptozotocin-diabetic rats: effects of insulin, aldose reductase inhibition and lidocaine. Pain **68:**293–299. https://doi.org/10.1016/S0304-3959(96)03201-0.
33. **Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D.** 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature **389:**816–824. https://doi.org/10.1038/39807.
34. **Challa SR.** 2015. Surgical animal models of neuropathic pain: pros and cons. Int J Neurosci **125:**170–174. https://doi.org/10.3109/00207454.2014.922559.
35. **Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL.** 1994. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods **53:**55–63. https://doi.org/10.1016/0165-0270(94)90144-9.
36. **Choi Y, Yoon YW, Na HS, Kim SH, Chung JM.** 1994. Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. Pain **59:**369–376. https://doi.org/10.1016/0304-3959(94)90023-X.
37. **Cobos EJ, Ghasemlou N, Araldi D, Segal D, Duong K, Woolf CK.** 2012. Inflammation-induced decrease in voluntary wheel running in mice: A nonreflexive test for evaluating inflammatory pain and analgesia. Pain **153:**876–884. https://doi.org/10.1016/j.pain.2012.01.016.
38. **Collier HO, Dinneen LC, Johnson CA, Schneider C.** 1968. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol Chemother **32:**295–310.
39. **Corder G, Ahanonu B, Grewe BF, Wang D, Schnitzer MJ, Scherrer G.** 2019. An amygdalar neural ensemble that encodes the unpleasantness of pain. Science **363:**276–281. https://doi.org/10.1126/science.aap8586.
40. **Cunha TM, Dal-Secco D, Verri WA Jr., Guerrero AT, Souza GR, Vieira SM, Lotufo CM, Neto AF, Ferreira SH, Cunha FQ.** 2008. Dual role of hydrogen sulfide in mechanical inflammatory hypernociception. Eur J Pharmacol **590:**127–135. https://doi.org/10.1016/j.ejphar.2008.05.048.
41. **Cunha TM, Verri WA Jr., Silva JS, Poole S, Cunha FQ, Ferreira SH.** 2005. A cascade of cytokines mediates mechanical inflammatory hypernociception in mice. Proc Natl Acad Sci USA **102:**1755–1760. https://doi.org/10.1073/pnas.0409225102.
42. **Cunningham J, Temple WJ, Mitchell P, Nixon JA, Preshaw RM, Hagen NA.** 1996. Cooperative hernia study. Pain in the postrepair patient. Ann Surg **224:**598–602. https://doi.org/10.1097/00000658-199611000-00003.
43. **D'Amour FE, Smith DL.** 1941. A method for determining loss of pain sensation. J Pharmacol Exp Ther **72:**74–79.
44. **Daly C, Ghosh P, Jenkin G Oehme D, Goldschlager T.** 2016.A review of animal models of intervertebral disc degeneration: pathophysiology, regeneration, and translation to the clinic. Biomed Res Int **2016:**1–14. doi: 10.1155/2016/5952165.
45. **Davidson E, Coppey L, Lu B, Arballo V, Calcutt NA, Gerard C, Yorek M.** 2009. The roles of streptozotocin neurotoxicity and neutral endopeptidase in murine experimental diabetic neuropathy. Exp Diabetes Res **2009:**1–9. https://doi.org/10.1155/2009/431980.
46. **Deacon RM.** 2009. Burrowing: a sensitive behavioural assay, tested in 5 species of laboratory rodents. Behav Brain Res **200:**128–133. https://doi.org/10.1016/j.bbr.2009.01.007.
47. **Deacon RMJ.** 2013. Measuring the strength of mice. J Vis Exp **76**:1–4. https://doi.org/10.3791/2610.
48. **Decosterd I, Woolf CJ.** 2000. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain **87:**149–158. https://doi.org/10.1016/S0304-3959(00)00276-1.
49. **Defensor EB, Corley MJ, Blanchard RJ, Blanchard DC.** 2012. Facial expressions of mice in aggressive and fearful contexts. Physiol Behav **107:**680–685. https://doi.org/10.1016/j.physbeh.2012.03.024.
50. **Deuis JR, Dvorakova LS, Vetter I.** 2017. Methods used to evaluate pain behaviors in rodents. Front Mol Neurosci **10:**1–17. https://doi.org/10.3389/fnmol.2017.00284.
51. **Dickenson AH, Sullivan AF.** 1987. Peripheral origins and central modulation of subcutaneous formalin-induced activity of rat dorsal horn neurones. Neurosci Lett **83:**207–211. https://doi.org/10.1016/0304-3940(87)90242-4.
52. **Dinh P, Hazel A, Palispis W, Suryadevara S, Gupta R.** 2009. Functional assessment after sciatic nerve injury in a rat model. Microsurgery **29:**644–649. https://doi.org/10.1002/micr.20685.
53. **Dolan JC, Lam DK, Achdjian SH, Schmidt BL.** 2010. The dolognawmeter: a novel instrument and assay to quantify nociception in rodent models of orofacial pain. J Neurosci Methods **187:**207–215. https://doi.org/10.1016/j.jneumeth.2010.01.012.
54. **Dorsey ER, Elbaz A, Nichols E, Abd-Allah F, Abdelalim A, Adsuar JC, Ansha MG, Brayne C, Choi J-YJ, Collado-Mateo D, Dahodwala N, Do HP, Edessa D, Endres M, Fereshtehnejad S-M, Foreman KJ, Gankpe FG, Gupta R, Hankey GJ, Hay SI, Hegazy MI, Hibstu DT, Kasaeian A, Khader Y, Khalil I, Khang Y-H, Kim YJ, Kokubo Y, Logroscino G, Massano J, Mohamed Ibrahim N, Mohammed MA, Mohammadi A, Moradi-Lakeh M, Naghavi M, Nguyen BT, Nirayo YL, Ogbo FA, Owolabi MO, Pereira DM, Postma MJ, Qorbani M, Rahman MA, Roba KT, Safari H, Safiri S, Satpathy M, Sawhney M, Shafieesabet A, Shiferaw MS, Smith M, Szoeke CEI, Tabares-Seisdedos R, Truong NT, Ukwaja KN, Venketasubramanian N, Villafaina S, Weldegwergs KG, Westerman R, Wijeratne T, Winkler AS, Xuan BT, Yonemoto N, Feigin VL, Vos T, Murray CJL, GBD 2016 Parkinson's Disease Collaborators.** 2018. Global, regional, and national burden of migraine and tension-type headache, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol **17:**954–976. https://doi.org/10.1016/S1474-4422(18)30295-3.
55. **Dougherty PM, Garrison CJ, Carlton SM**. 1992. Differential influence of local anesthetic upon two models of experimentally induced peripheral mononeuropathy in the rat. Brain Res **570:**109–115. doi:10.1016/0006-8993(92)90570-y.

56. **Dubin AE, Patapoutian A.** 2010. Nociceptors: the sensors of the pain pathway. J Clin Invest **120:**3760–3772. https://doi.org/10.1172/JCI42843.
57. **Dubuisson D, Dennis SG.** 1977. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain **4 Supp C:**161–174. https://doi.org/10.1016/0304-3959(77)90130-0.
58. **Edvinsson L.** 2006.Neuronal signal substances as biomarkers of migraine. Headache **46:**1088–1094. https://doi.org/10.1111/j.1526-4610.2006.00502.x.
59. **Fairbanks CA, Goracke-Postle CJ.** 2015. Neurobiological studies of chronic pain and analgesia: Rationale and refinements. Eur J Pharmacol **759:**169–181. https://doi.org/10.1016/j.ejphar.2015.03.049.
60. **Farhanghi M, Holmes RA, Volkert WA, Logan KW, Singh A.** 1992. Samarium-153-EDTMP: pharmacokinetic, toxicity and pain response using an escalating dose schedule in treatment of metastatic bone cancer. J Nucl Med **33:**1451–1458.
61. **Fattori V, Hohmann M, Rossaneis A, Pinho-Ribeiro F, Verri W.** 2016. Capsaicin: current understanding of its mechanisms and therapy of pain and other pre-clinical and clinical uses. Molecules **21:**1–33. https://doi.org/10.3390/molecules21070844.
62. **Ferreira SH, Lorenzetti BB, Poole S.** 1993. Bradykinin initiates cytokine-mediated inflammatory hyperalgesia. Br J Pharmacol **110:**1227–1231. https://doi.org/10.1111/j.1476-5381.1993.tb13946.x.
63. **Finlayson K, Lampe JF, Hintze S, Würbel H, Melotti L.** 2016. Facial indicators of positive emotions in rats. PLoS One **11:**1–24. https://doi.org/10.1371/journal.pone.0166446.
64. **Flatters SJ.** 2008. Characterization of a model of persistent postoperative pain evoked by skin/muscle incision and retraction (SMIR). Pain **135:**119–130. https://doi.org/10.1016/j.pain.2007.05.013.
65. **Garland EL.** 2012. Pain processing in the human nervous system: a selective review of nociceptive and biobehavioral pathways. Prim Care **39:**561–571. https://doi.org/10.1016/j.pop.2012.06.013.
66. **Gerstner G, Ichesco E, Quintero A, Schmidt-Wilcke T.** 2011. Changes in regional gray and white matter volume in patients with myofascial-type temporomandibular disorders: a voxel-based morphometry study. J Orofac Pain **25:**99–106.
67. **Grace PM, Strand KA, Maier SF, Watkins LR.** 2014. Suppression of voluntary wheel running in rats is dependent on the site of inflammation: evidence for voluntary running as a measure of hind paw-evoked pain. J Pain **15:**121–128. https://doi.org/10.1016/j.jpain.2013.10.001.
68. **Gregory NS, Harris AL, Robinson CR, Dougherty PM, Fuchs PN, Sluka KA.** 2013. An overview of animal models of pain: disease models and outcome measures. J Pain **14:**1255–1269. https://doi.org/10.1016/j.jpain.2013.06.008.
69. **Grotto M, Sulman FG.** 1967. Modified receptacle method for animal analgesimetry. Arch Int Pharmacodyn Ther **165:**152–159.
70. **Gruber HE, Ingram JA, Leslie K, Hanley EN Jr.** 2004. Cellular, but not matrix, immunolocalization of SPARC in the human intervertebral disc: decreasing localization with aging and disc degeneration. Spine (Phila Pa 1976) **29:**2223–2228. https://doi.org/10.1097/01.brs.0000142225.07927.29.
71. **Gruber HE, Sage EH, Norton HJ, Funk S, Ingram J, Hanley EN Jr.** 2005. Targeted deletion of the SPARC gene accelerates disc degeneration in the aging mouse. J Histochem Cytochem **53:**1131–1138. https://doi.org/10.1369/jhc.5A6687.2005.
72. **Guthrie GJ.** 1820. A treatise on gun-shot wounds, on injuries of nerves, and on wounds of the extremities, 2nd ed. London: Burgess and Hill.
73. **Haighton J.** 1797. An experimental inquiry concerning the reproduction of nerves: from the same work. Med Facts Obs **7:**155–169.
74. **Han JS, Bird GC, Li W, Jones J, Neugebauer V.** 2005. Computerized analysis of audible and ultrasonic vocalizations of rats as a standardized measure of pain-related behavior. J Neurosci Methods **141:**261–269. https://doi.org/10.1016/j.jneumeth.2004.07.005.
75. **Hargreaves K, Dubner R, Brown F, Flores C, Joris J.** 1988. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain **32:**77–88. https://doi.org/10.1016/0304-3959(88)90026-7.
76. **Hole K, Berge OG, Tjølsen A, Eide PK, Garcia-Cabrera I, Lund A, Rosland JH.** 1990. The tail-flick test needs to be improved. Pain **43:**391–392. https://doi.org/10.1016/0304-3959(90)90039-G.
77. **Holmdahl R, Jansson L, Larsson E, Rubin K, Klareskog L.** 1986. Homologous type II collagen induces chronic and progressive arthritis in mice. Arthritis Rheum **29:**106–113. https://doi.org/10.1002/art.1780290114.
78. **Honoré P, Schwei J, Rogers SD, Salak-Johnson JL, Finke MP, Ramnaraine ML, Clohisy DR, Mantyh PW.** 2000. Cellular and neurochemical remodeling of the spinal cord in bone cancer pain. Prog Brain Res **129:**389–397. https://doi.org/10.1016/S0079-6123(00)29030-4.
79. **Höke A, Ray M.** 2014. Rodent models of chemotherapy-induced peripheral neuropathy. ILAR J **54:**273–281. https://doi.org/10.1093/ilar/ilt053.
80. **Jankowski MP, Koerber HR.** 2010. Neurotrophic factors and nociceptor sensitization, p 31–50. Chapter 2. In: Kruger L, Light AR, editors. Translational pain research: from mouse to man. Boca Raton (FL): CRC Press/Taylor and Francis.
81. **Janssen PA, Niemegeers CJ, Dony JG.** 1963. The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. Arzneimittelforschung **13:**502–507.
82. **Jimenez-Andrade JM, Ghilardi JR, Castañeda-Corral G, Kuskowski MA, Mantyh PW.** 2011. Preventive or late administration of anti-NGF therapy attenuates tumor-induced nerve sprouting, neuroma formation, and cancer pain. Pain **152:**2564–2574. https://doi.org/10.1016/j.pain.2011.07.020.
83. **Jirkof P.** 2014. Burrowing and nest building behavior as indicators of wellbeing in mice. J Neurosci Methods **234:**139–146. https://doi.org/10.1016/j.jneumeth.2014.02.001.
84. **Jirkof P, Cesarovic N, Rettich A, Nicholls F, Seifert B, Arras M.** 2010. Burrowing behavior as an indicator of post-laparotomy pain in mice. Front Behav Neurosci **4:**1–9. https://doi.org/10.3389/fnbeh.2010.00165.
85. **Johansen A, Romundstad L, Nielsen CS, Schirmer H, Stubhaug A.** 2012. Persistent postsurgical pain in a general population: prevalence and predictors in the Tromsø study. Pain **153:**1390–1396. https://doi.org/10.1016/j.pain.2012.02.018.
86. **Jourdan D, Ardid D, Eschalier A.** 2002. Analysis of ultrasonic vocalisation does not allow chronic pain to be evaluated in rats. Pain **95:**165–173. https://doi.org/10.1016/S0304-3959(01)00394-3.
87. **Kakoei S, Baghaei F, Dabiri S, Parirokh M, Kakooei S.** 2010. A comparative in vivo study of tissue reactions to 4 suturing materials. Iran Endod J **5:**69–73.
88. **Kandel ER, Schwartz JH, Jessell TM, Siegelbaum SA, Hudspeth AJ, editors.** 2013. Principles of neural science, 5th ed. New York (NY): McGraw–Hill.
89. **Kehl LJ, Trempe TM, Hargreaves KM.** 2000. A new animal model for assessing mechanisms and management of muscle hyperalgesia. Pain **85:**333–343. https://doi.org/10.1016/S0304-3959(99)00282-1.
90. **Kim SH, Chung JM.** 1992. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain **50:**355–363. https://doi.org/10.1016/0304-3959(92)90041-9.
91. **King T, Porreca F.** 2014. Preclinical assessment of pain: improving models in discovery research. Curr Top Behav Neurosci **20:**101–120. https://doi.org/10.1007/7854_2014_330.
92. **Klinck MP, Mogil JS, Moreau M, Lascelles BDX, Flecknell PA, Poitte T, Troncy E.** 2017. Translational pain assessment: could natural animal models be the missing link? Pain **158:**1633–1646. https://doi.org/10.1097/j.pain.0000000000000978.
93. **Knowlton WM, Bifolck-Fisher A, Bautista DM, McKemy DD.** 2010. TRPM8, but not TRPA1, is required for neural and behavioral responses to acute noxious cold temperatures and cold-mimetics in vivo. Pain **150:**340–350. https://doi.org/10.1016/j.pain.2010.05.021.
94. **Laird JM, Martinez-Caro L, Garcia-Nicas E, Cervero F.** 2001. A new model of visceral pain and referred hyperalgesia in the mouse. Pain **92:**335–342. https://doi.org/10.1016/S0304-3959(01)00275-5.

95. **Lam DK, Dang D, Zhang J, Dolan JC, Schmidt BL.** 2012. Novel animal models of acute and chronic cancer pain: a pivotal role for PAR2. J Neurosci **32:**14178–14183. https://doi.org/10.1523/JNEUROSCI.2399-12.2012.
96. **Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AM, Ferrari MD, Craig KD, Mogil JS.** 2010. Coding of facial expressions of pain in the laboratory mouse. Nat Methods **7:**447–449. https://doi.org/10.1038/nmeth.1455.
97. **Leach MC, Klaus K, Miller AL, Scotto di Perrotolo M, Sotocinal SG, Flecknell PA.** 2012. The assessment of post-vasectomy pain in mice using behaviour and the Mouse Grimace Scale. PLoS One **7:**1–9. https://doi.org/10.1371/journal.pone.0035656.
98. **LeResche L, Dworkin SF.** 1984. Facial expression accompanying pain. Soc Sci Med **19:**1325–1330. https://doi.org/10.1016/0277-9536(84)90020-0.
99. **Lindenlaub T, Sommer C.** 2000. Partial sciatic nerve transection as a model of neuropathic pain: a qualitative and quantitative neuropathological study. Pain **89:**97–106. https://doi.org/10.1016/S0304-3959(00)00354-7.
100. **Lindsay TH, Jonas BM, Sevcik MA, Kubota K, Halvorson KG, Ghilardi JR, Kuskowski MA, Stelow EB, Mukherjee P, Gendler SJ, Wong GY, Mantyh PW.** 2005. Pancreatic cancer pain and its correlation with changes in tumor vasculature, macrophage infiltration, neuronal innervation, body weight and disease progression. Pain **119:**233–246. https://doi.org/10.1016/j.pain.2005.10.019.
101. **Liu Y, Wang L, Meng C, Zhou Y, Lao J, Zhao X.** 2017. A new model for the study of neuropathic pain after brachial plexus injury. Injury **48:**253–261. https://doi.org/10.1016/j.injury.2016.11.007.
102. **Ma W, Bisby MA.** 1997. Differential expression of galanin immunoreactivities in the primary sensory neurons following partial and complete sciatic nerve injuries. Neuroscience **79:**1183–1195. https://doi.org/10.1016/S0306-4522(97)00088-2.
103. **Ma W, Bisby MA.** 1998. Increase of preprotachykinin mRNA and substance P immunoreactivity in spared dorsal root ganglion neurons following partial sciatic nerve injury. Eur J Neurosci **10:**2388–2399. https://doi.org/10.1046/j.1460-9568.1998.00249.x.
104. **Ma W, Bisby MA.** 1998. Partial and complete sciatic nerve injuries induce similar increases of neuropeptide Y and vasoactive intestinal peptide immunoreactivities in primary sensory neurons and their central projections. Neuroscience **86:**1217–1234. https://doi.org/10.1016/S0306-4522(98)00068-2.
105. **Mallick-Searle T, Snodgrass B, Brant JM.** 2016. Postherpetic neuralgia: epidemiology, pathophysiology, and pain management pharmacology. J Multidiscip Healthc **9:**447–454. https://doi.org/10.2147/JMDH.S106340.
106. **Marker CL, Pomonis JD.** 2012. The monosodium iodoacetate model of osteoarthritis pain in the rat. Methods Mol Biol **851:**239–248. https://doi.org/10.1007/978-1-61779-561-9_18.
107. **Markovics A, Kormos V, Gaszner B, Lashgarara A, Szoke E, Sandor K, Szabadfi K, Tuka B, Tajti J, Szolcsanyi J, Pinter E, Hashimoto H, Kun J, Reglodi D, Helyes Z.** 2012. Pituitary adenylate cyclase-activating polypeptide plays a key role in nitroglycerol-induced trigeminovascular activation in mice. Neurobiol Dis **45:**633–644. https://doi.org/10.1016/j.nbd.2011.10.010.
108. **Meller ST, Dykstra C, Grzybycki D, Murphy S, Gebhart GF.** 1994. The possible role of glia in nociceptive processing and hyperalgesia in the spinal cord of the rat. Neuropharmacology **33:**1471–1478. https://doi.org/10.1016/0028-3908(94)90051-5.
109. **Mercadante S.** 1997. Malignant bone pain: pathophysiology and treatment. Pain **69:**1–18. https://doi.org/10.1016/S0304-3959(96)03267-8.
110. **International Association for the Study of Pain,Task Force on Taxonomy, Merskey H, Bogduk N, editors.** 1994. Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms, 2nd ed. Seattle (WA): IASP Press.
111. **Middlemiss T, Laird BJA, Fallon MT.** 2011. Mechanisms of cancer-induced bone pain. Clin Oncol (R Coll Radiol) **23:**387–392. https://doi.org/10.1016/j.clon.2011.03.003.
112. **Millecamps M, Tajerian M, Sage EH, Stone LS.** 2011. Behavioral signs of chronic back pain in the SPARC-null mouse. Spine(Phila Pa 1976) **36:**95–102. https://doi.org/10.1097/BRS.0b013e3181cd9d75.
113. **Millecamps M, Stone LS.** 2018. Delayed onset of persistent discogenic axial and radiating pain after a single-level lumbar intervertebral disc injury in mice. Pain **159:**1843–1855.
114. **Millecamps M, Tajerian M, Naso L, Sage EH, Stone LS.** 2012.Lumbar intervertebral disc degeneration associated with axial and radiating low back pain in ageing SPARC-null mice. Pain **153:**1167–1179. https://doi.org/10.1016/j.pain.2012.01.027.
115. **Miller AL, Leach MC.** 2015. The mouse grimace scale: a clinically useful tool? PLoS One **10:**1–10. https://doi.org/10.1371/journal.pone.0136000.
116. **Miller AL, Leach MC.** 2015. Using the mouse grimace scale to assess pain associated with routine ear notching and the effect of analgesia in laboratory mice. Lab Anim **49:**117–120. https://doi.org/10.1177/0023677214559084.
117. **Min SS, Han JS, Kim YI, Na HS, Yoon YW, Hong SK, Han HC.** 2001. A novel method for convenient assessment of arthritic pain in voluntarily walking rats. Neurosci Lett **308:**95–98. https://doi.org/10.1016/S0304-3940(01)01983-8.
118. **Mitchell JK.** 1895. Remote consequences of injuries of nerves and their treatment. Philadelphia (PA): Lea Brothers.
119. **Mitchell SW.** 1872. Injuries of nerves and their consequences. Philadelphia (PA): JB Lippincott. https://doi.org/10.1097/00000441-187207000-00024
120. **Mitchell SW, Morehouse GR, Keen WW.** 1864. Gunshot wounds and other injuries of nerves. Philadelphia (PA): JB Lippincott.
121. **Mogil JS.** 2017. Laboratory environmental factors and pain behavior: the relevance of unknown unknowns to reproducibility and translation. Lab Anim (NY) **46:**136–141. https://doi.org/10.1038/laban.1223.
122. **Montilla-García Á, Tejada MÁ, Perazzoli G, Entrena JM, Portillo-Salido E, Fernández-Segura E, Cañizares FJ, Cobos EJ.** 2017. Grip strength in mice with joint inflammation: A rheumatology function test sensitive to pain and analgesia. Neuropharmacology **125:**231–242. https://doi.org/10.1016/j.neuropharm.2017.07.029.
123. **Moore C, Gupta R, Jordt SE, Chen Y, Liedtke WB.** 2018. Regulation of pain and itch by TRP Channels. Neurosci Bull **34:**120–142. https://doi.org/10.1007/s12264-017-0200-8.
124. **Moqrich A, Hwang SW, Earley TJ, Petrus MJ, Murray AN, Spencer KS, Andahazy M, Story GM, Patapoutian A.** 2005. Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. Science **307:**1468–1472. https://doi.org/10.1126/science.1108609.
125. **Morgan MM, Whittier KL, Hegarty DM, Aicher SA.** 2008. Periaqueductal gray neurons project to spinally projecting GABAergic neurons in the rostral ventromedial medulla. Pain **140:**376–386. https://doi.org/10.1016/j.pain.2008.09.009.
126. **Ness TJ, Gebhart GF.** 1988. Colorectal distension as a noxious visceral stimulus: physiologic and pharmacologic characterization of pseudaffective reflexes in the rat. Brain Res **450:**153–169. https://doi.org/10.1016/0006-8993(88)91555-7.
127. **O'Connell GD, Vresilovic EJ, Elliott DM.** 2007. Comparison of animals used in disc research to human lumbar disc geometry. Spine (Phila Pa 1976) 32:328–33.
128. **Ornitz DM, Hammer RE, Messing A, Palmiter RD, Brinster RL.** 1987. Pancreatic neoplasia induced by SV40 T-antigen expression in acinar cells of transgenic mice. Science **238:**188–193. https://doi.org/10.1126/science.2821617.
129. **Pacharinsak C, Beitz A.** 2008. Animal models of cancer pain. Comp Med **58:**220–233.
130. **Pantano F, Zoccoli A, Iuliani M, Lanzetta G, Vincenzi B, Tonini G, Santini D.** 2011. New targets, new drugs for metastatic bone pain: a new philosophy. Expert Opin Emerg Drugs **16:**403–405. https://doi.org/10.1517/14728214.2011.588600.
131. **Patapoutian A, Peier AM, Story GM, Viswanath V.** 2003. ThermoTRP channels and beyond: mechanisms of temperature sensation. Nat Rev Neurosci **4:**529–539. https://doi.org/10.1038/nrn1141.

132. **Philips BH, Weisshaar CL, Winkelstein BA.** 2017. Use of the rat grimace scale to evaluate neuropathic pain in a model of cervical radiculopathy. Comp Med **67:**34–42.
133. **Piesla MJ, Leventhal L, Strassle BW, Harrison JE, Cummons TA, Lu P, Whiteside GT.** 2009. Abnormal gait, due to inflammation but not nerve injury, reflects enhanced nociception in preclinical pain models. Brain Res **1295:**89–98. https://doi.org/10.1016/j.brainres.2009.07.091.
134. **Pincedé I, Pollin B, Meert T, Plaghki L, Le Bars D.** 2012. Psychophysics of a nociceptive test in the mouse: ambient temperature as a key factor for variation. PLoS One **7:**1–18. https://doi.org/10.1371/journal.pone.0036699.
135. **Pitcher GM, Ritchie J, Henry JL.** 1999. Paw withdrawal threshold in the von Frey hair test is influenced by the surface on which the rat stands. J Neurosci Methods **87:**185–193. https://doi.org/10.1016/S0165-0270(99)00004-7.
136. **Pitcher MH.** 2018. The impact of exercise in rodent models of chronic pain. Curr Osteoporos Rep **16:**344–359. https://doi.org/10.1007/s11914-018-0461-9.
137. **Pizziketti RJ, Pressman NS, Geller EB, Cowan A, Adler MW.** 1985. Rat cold water tail-flick: a novel analgesic test that distinguishes opioid agonists from mixed agonist-antagonists. Eur J Pharmacol **119:**23–29. https://doi.org/10.1016/0014-2999(85)90317-6.
138. **Podesva C, DoNascimento J**. [Internet] 2019. 1,2,3,6-Tetrahydropyrimidine-2-one compounds and processes for making them. Patent US3821221. [Cited 15 April 2019]. Available at: https://pubchem.ncbi.nlm.nih.gov/patent/US3821221.
139. **Pogatzki EM, Raja SN.** 2003. A mouse model of incisional pain. Anesthesiology **99:**1023–1027. https://doi.org/10.1097/00000542-200310000-00041.
140. **Poupon L, Kerckhove N, Vein J, Lamoine S, Authier N, Busserolles J, Balayssac D.** 2015. Minimizing chemotherapy-induced peripheral neuropathy: preclinical and clinical development of new perspectives. Expert Opin Drug Saf **14:**1269–1282. https://doi.org/10.1517/14740338.2015.1056777.
141. **Pradhan AA, Smith ML, McGuire B, Tarash I, Evans CJ, Charles A.** 2014. Characterization of a novel model of chronic migraine. Pain **155:**269–274. https://doi.org/10.1016/j.pain.2013.10.004.
142. **Racey GL, Wallace WR, Cavalaris CJ, Marguard JV.** 1978. Comparison of a polyglycolic-polylactic acid suture to black silk and plain catgut in human oral tissues. J Oral Surg **36:**766–770.
143. **Raffaeli W, Arnaudo E.** 2017.Pain as a disease: an overview. J Pain Res **10:**2003–2008. https://doi.org/10.2147/JPR.S138864.
144. **Ramabadran K, Bansinath M, Turndorf H, Puig MM.** 1989. Tail immersion test for the evaluation of a nociceptive reaction in mice. Methodological considerations. J Pharmacol Methods **21:**21–31. https://doi.org/10.1016/0160-5402(89)90019-3.
145. **Randall LO, Selitto JJ.** 1957. A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn Ther **111:**409–419.
146. **Rea BJ, Wattiez AS, Waite JS, Castonguay WC, Schmidt CM, Fairbanks AM, Robertson BR, Brown CJ, Mason BN, Moldovan-Loomis MC, Garcia-Martinez LF, Poolman P, Ledolter J, Kardon RH, Sowers LP, Russo AF.** 2018. Peripherally administered calcitonin gene–related peptide induces spontaneous pain in mice: implications for migraine. Pain **159:**2306–2317. https://doi.org/10.1097/j.pain.0000000000001337.
147. **Recober A, Kuburas A, Zhang Z, Wemmie JA, Anderson MG, Russo AF.** 2009. Role of calcitonin gene-related peptide in light-aversive behavior: implications for migraine. J Neurosci **29:**8798–8804. https://doi.org/10.1523/JNEUROSCI.1727-09.2009.
148. **Rees DA, Alcolado JC.** 2005. Animal models of diabetes mellitus. Diabet Med **22:**359–370. https://doi.org/10.1111/j.1464-5491.2005.01499.x.
149. **Sabri MI, Ochs S.** 1971. Inhibition of glyceraldehyde-3-phosphate dehydrogenase in mammalian nerve by iodoacetic acid. J Neurochem **18:**1509–1514. https://doi.org/10.1111/j.1471-4159.1971.tb00013.x.
150. **Sakurai E, Kurihara T, Kouchi K, Saegusa H, Zong S, Tanabe T.** 2009. Upregulation of casein kinase 1∈ in dorsal root ganglia and spinal cord after mouse spinal nerve injury contributes to neuropathic pain. Mol Pain **5:**1–14.
151. **Sampathkumar P, Drage LA, Martin DP.** 2009. Herpes zoster (shingles) and postherpetic neuralgia. Mayo Clin Proc **84:**274–280. https://doi.org/10.4065/84.3.274.
152. **Schmidt BL, Hamamoto DT, Simone DA, Wilcox GL.** 2010. Mechanism of cancer pain. Mol Interv **10:**164–178. https://doi.org/10.1124/mi.10.3.7.
153. **Schuster DJ, Kitto KF, Overland AC, Messing RO, Stone LS, Fairbanks CA, Wilcox GL.** 2013. Protein kinase Cε is required for spinal analgesic synergy between delta opioid and alpha-2A adrenergic receptor agonist pairs. J Neurosci **33:**13538–13546. https://doi.org/10.1523/JNEUROSCI.4013-12.2013.
154. **Seltzer Z, Dubner R, Shir Y.** 1990. A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. Pain **43:**205–218. https://doi.org/10.1016/0304-3959(90)91074-S.
155. **Sevcik MA, Jonas BM, Lindsay TH, Halvorson KG, Ghilardi JR, Kuskowski MA, Mukherjee P, Maggio JE, Mantyh PW.** 2006. Endogenous opioids inhibit early-stage pancreatic pain in a mouse model of pancreatic cancer. Gastroenterology **131:**900–910. https://doi.org/10.1053/j.gastro.2006.06.021.
156. **Sewell RDE.** 2018. Neuropathic pain models and outcome measures: a dual translational challenge. Ann Transl Med **6 Suppl 1:**1–4. https://doi.org/10.21037/atm.2018.09.58.
157. **Sewell RD, Spencer PS.** 1976. Antinociceptive activity of narcotic agonist and partial agonist analgesics and other agents in the tail-immersion test in mice and rats. Neuropharmacology **15:**683–688. https://doi.org/10.1016/0028-3908(76)90037-X.
158. **Shankarappa SA, Tsui JH, Kim KN, Reznor G, Dohlman JC, Langer R, Kohane DS**.2012.Prolonged nerve blockade delays the onset of neuropathic pain. Proc Natl Acad Sci U S A 109:17555–17560. doi:10.1073/pnas.1214634109.
159. **Sheahan TD, Siuda ER, Bruchas MR, Shepherd AJ, Mohapatra DP, Gereau RW 4th, Golden JP.** 2017. Inflammation and nerve injury minimally affect mouse voluntary behaviors proposed as indicators of pain. Neurobiol Pain **2:**1–12. https://doi.org/10.1016/j.ynpai.2017.09.001.
160. **Shepherd AJ, Mohapatra DP.** 2018. Pharmacological validation of voluntary gait and mechanical sensitivity assays associated with inflammatory and neuropathic pain in mice. Neuropharmacology **130:**18–29. https://doi.org/10.1016/j.neuropharm.2017.11.036.
161. **Shir Y, Seltzer Z.** 1990. A-fibers mediate mechanical hyperesthesia and allodynia and C-fibers mediate thermal hyperalgesia in a new model of causalgiform pain disorders in rats. Neurosci Lett **115:**62–67. https://doi.org/10.1016/0304-3940(90)90518-E.
162. **Simola N, Granon S.** 2019. Ultrasonic vocalizations as a tool in studying emotional states in rodent models of social behavior and brain disease. Neuropharmacology **159:**107420. https://doi.org/10.1016/j.neuropharm.2018.11.008.
163. **Sotgiu ML, Castagna A, Lacerenza M, Marchettini P**. 1995. Pre-injury lidocaine treatment prevents thermal hyperalgesia and cutaneous thermal abnormalities in a rat model of peripheral neuropathy. Pain 61:3–10. https://doi.org/10.1016/0304-3959(94)00120-4.
164. **Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, Mapplebeck JC, Wei P, Zhan S, Zhang S, McDougall JJ, King OD, Mogil JS.** 2011. The rat grimace scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Mol Pain **7:**55.
165. **Stein C, Millan MJ, Herz A.** 1988. Unilateral inflammation of the hindpaw in rats as a model of prolonged noxious stimulation: alterations in behavior and nociceptive thresholds. Pharmacol Biochem Behav **31:**445–451. https://doi.org/10.1016/0091-3057(88)90372-3.
166. **Steiner TJ, Stovner LJ, Vos T, Jensen R, Katsarava Z.** 2018. Migraine is first cause of disability in under 50s: will health politicians now take notice? J Headache Pain **19:**1–4. https://doi.org/10.1186/s10194-018-0846-2.
167. **Sterling JA, Guelcher SA.** 2011. Bone structural components regulating sites of tumor metastasis. Curr Osteoporos Rep **9:**89–95. https://doi.org/10.1007/s11914-011-0052-5.
168. **Steru L, Chermat R, Thierry B, Simon P.** 1985. The tail suspension test: a new method for screening antidepressants in mice.

Psychopharmacology (Berl) **85:**367–370. https://doi.org/10.1007/BF00428203.

169. **Stoll BA, Andrews JT.** 1966. Radiation-induced peripheral neuropathy. BMJ **1:**834–837. https://doi.org/10.1136/bmj.1.5491.834.
170. **Streng J.** 1971. Open-field behavior in 4 inbred mouse strains. Can J Psychol **25:**62–68. https://doi.org/10.1037/h0082368.
171. **Szolcsányi J.** 1977. A pharmacological approach to elucidation of the role of different nerve fibres and receptor endings in mediation of pain. J Physiol (Paris) **73:**251–259.
172. **Tanaka S, Young JW, Halberstadt AL, Masten VL, Geyer MA.** 2012. Four factors underlying mouse behavior in an open field. Behav Brain Res **233:**55–61. https://doi.org/10.1016/j.bbr.2012.04.045.
173. **Thresh L.** 1846. Isolation of capsaicin. Pharm J **6:**941.
174. **Tjølsen A, Lund A, Berge OG, Hole K.** 1989. An improved method for tail-flick testing with adjustment for tail-skin temperature. J Neurosci Methods **26:**259–265. https://doi.org/10.1016/0165-0270(89)90124-6.
175. **Tong Z, Luo W, Wang Y, Yang F, Han Y, Li H, Luo H, Duan B, Xu T, Maoying Q, Tan H, Wang J, Zhao H, Liu F, Wan Y.** 2010. Tumor tissue-derived formaldehyde and acidic microenvironment synergistically induce bone cancer pain (formaldehyde and cancer pain). PLoS One **5:**1–15. https://doi.org/10.1371/journal.pone.0010234.
176. **Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, Inoue K.** 2003. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature **424:**778–783. https://doi.org/10.1038/nature01786.
177. **Tzschentke TM.** 2007. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict Biol **12:**227–462. https://doi.org/10.1111/j.1369-1600.2007.00070.x.
178. **Vivian JA, Miczek KA.** 1998. Effects of μ and δ opioid agonists and antagonists on affective vocal and reflexive pain responses during social stress in rats. Psychopharmacology (Berl) **139:**364–375. https://doi.org/10.1007/s002130050727.
179. **Voets T, Owsianik G, Janssens A, Talavera K, Nilius B.** 2007. TRPM8 voltage sensor mutants reveal a mechanism for integrating thermal and chemical stimuli. Nat Chem Biol **3:**174–182. https://doi.org/10.1038/nchembio862.
180. **Vuralli D, Wattiez AS, Russo AF, Bolay H.** 2019. Behavioral and cognitive animal models in headache research. J Headache Pain **20:**11. https://doi.org/10.1186/s10194-019-0963-6.
181. **Wacnik PW, Eikmeier LJ, Ruggles TR, Ramnaraine ML, Walcheck BK, Beitz AJ, Wilcox GL.** 2001. Functional interactions between tumor and peripheral nerve: morphology, algogen identification, and behavioral characterization of a new murine model of cancer pain. J Neurosci **21:**9355–9366. https://doi.org/10.1523/JNEUROSCI.21-23-09355.2001.
182. **Wacnik PW, Kehl JL, Trempe MT, Ramnaraine LM, Beitz JA, Wilcox LG.** 2003. Tumor implantation in mouse humerus evokes movement-related hyperalgesia exceeding that evoked by intramuscular carrageenan. Pain **101:**175–186. https://doi.org/10.1016/S0304-3959(02)00312-3.
183. **Wacnik PW, Pacharinsak C, Beitz AJ.** 2011. Animal models of cancer pain, p 117–145. In: Ma C, Zhang J-M, editors. Animal models of pain. London (United Kingdom): Humana Press. https://doi.org/10.1007/978-1-60761-880-5_8.
184. **Wacnik PW, Wilcox GL, Clohisy DR, Ramnaraine ML, Eikmeier LJ, Beitz AJ.** 2000. Cancer pain mechanisms and animal models of cancer pain. Pain Res Manag **16:**615–637.
185. **Walczak JS, Pichette V, Leblond F, Desbiens K, Beaulieu P.** 2005. Behavioral, pharmacological and molecular characterization of the saphenous nerve partial ligation: a new model of neuropathic pain. Neuroscience **132:**1093–1102. https://doi.org/10.1016/j.neuroscience.2005.02.010.
186. **Wang L, Yuzhou L, Yingjie Z, Jie L, Xin Z.** 2015. A new rat model of neuropathic pain: complete brachial plexus avulsion. Neurosci Lett **589:**52–56. https://doi.org/10.1016/j.neulet.2015.01.033.
187. **Wei ET.** 1976. Chemical stimulants of shaking behaviour. J Pharm Pharmacol **28:**722–724. https://doi.org/10.1111/j.2042-7158.1976.tb02849.x.
188. **Wei ET, Seid DA.** 1983. AG-3-5: a chemical producing sensations of cold. J Pharm Pharmacol **35:**110–112. https://doi.org/10.1111/j.2042-7158.1983.tb04279.x.
189. **Weilbaecher KN, Guise TA, McCauley LK.** 2011. Cancer to bone: a fatal attraction. Nat Rev Cancer **11:**411–425. https://doi.org/10.1038/nrc3055.
190. **Wen T, Ansonoff MA, Pintar JE.** 2009. The tail pigmentation pattern of C57BL/6J mice affects nociception/pain quantification in the tail flick test. Eur J Pain **13:**564–567. https://doi.org/10.1016/j.ejpain.2008.07.012.
191. **Werkheiser JL, Rawls SM, Cowan A.** 2007. Nalfurafine, the kappa opioid agonist, inhibits icilin-induced wet-dog shakes in rats and antagonizes glutamate release in the dorsal striatum. Neuropharmacology **52:**925–930. https://doi.org/10.1016/j.neuropharm.2006.10.010.
192. **Wesselmann U, Czakanski PP, Affaitati G, Giamberardino MA.** 1998. Uterine inflammation as a noxious visceral stimulus: behavioral characterization in the rat. Neurosci Lett **246:**73–76. https://doi.org/10.1016/S0304-3940(98)00234-1.
193. **Wilke HJ, Rohlmann A, Neller S, Graichen F, Claes L, Bergmann G.** 2003. A novel approach to determine trunk muscle forces during flexion and extension: a comparison of data from an in vitro experiment and in vivo measurements. Spine (Phila Pa 1976) **28:**2585–2593.
194. **Williams RO, Inglis JJ, Simelyte E, Criado G, Sumariwalla PF.** 2005. Analysing the effect of novel therapies on cytokine expression in experimental arthritis. Int J Exp Pathol **86:**267–278. https://doi.org/10.1111/j.0959-9673.2005.00443.x.
195. **Winter CA, Risley EA, Nuss GW.** 1962. Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med **111:**544–547. https://doi.org/10.3181/00379727-111-27849.
196. **Wodarski R, Delaney A, Ultenius C, Morland R, Andrews N, Baastrup C, Bryden LA, Caspani O, Christoph T, Gardiner NJ, Huang W, Kennedy JD, Koyama S, Li D, Ligocki M, Lindsten A, Machin I, Pekcec A, Robens A, Rotariu SM, Vob S, Segerdahl M, Stenfors C, Svensson C, Treede R-D, Uto K, Yamamoto K, Rutten K, Rice A.** 2016. Cross-centre replication of suppressed burrowing behaviour as an ethologically relevant pain outcome measure in the rat: a prospective multicentre study. Pain **157:**2350–2365. https://doi.org/10.1097/j.pain.0000000000000657.
197. **Woolf CJ.** 2010. What is this thing called pain? J Clin Invest **120:**3742–3744. https://doi.org/10.1172/JCI45178.
198. **Woolf CJ, Mannion RJ.** 1999. Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet **353:**1959–1964. https://doi.org/10.1016/S0140-6736(99)01307-0.
199. **Woolfe G, Macdonald AD.** 1944. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J Pharmacol Exp Ther **80:**300–307.
200. **Wöhr M, Schwarting RKW.** 2013. Affective communication in rodents: ultrasonic vocalizations as a tool for research on emotion and motivation. Cell Tissue Res **354:**81–97. https://doi.org/10.1007/s00441-013-1607-9.
201. **Xie W, Strong JA, Zhang JM.** 2009. Early blockade of injured primary sensory afferents reduces glial cell activation in two rat neuropathic pain models. Neuroscience **160:**847–857. doi:10.1016/j.neuroscience.2009.03.016.
202. **Yam MF, Por LY, Peh KK, Ahmad M, Asmawi MZ, Ang LF, Yin DB, Ong SY, Abdulkarim MF, Abdullah GZ, Salman IM, Ameer OZ, Mohamed EA, Hashim MA, Farsi E, Hor SY.** 2011. Development of a stepping force analgesic meter for a rat arthritic model. Sensors (Basel) **11:**5058–5070. https://doi.org/10.3390/s110505058.
203. **Yoneda T, Hata K, Nakanishi M, Nagae M, Nagayama T, Wakabayashi H, Nishisho T, Sakurai T, Hiraga T.** 2011. Involvement of acidic microenvironment in bone pain associated with cancer colonization. Bone **48:**100–105. https://doi.org/10.1016/j.bone.2010.07.009.
204. **Zhang RX, Liu B, Wang L, Ren K, Qiao JT, Berman BM, Lao L.** 2005. Spinal glial activation in a new rat model of bone cancer pain produced by prostate cancer cell inoculation of the tibia. Pain **118:**125–136. https://doi.org/10.1016/j.pain.2005.08.001.