

Overview

The Study of Pain in Rats and Mice

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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.

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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. Nociceptive 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

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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 (A δ) 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 area¹³¹ constitute what is broadly referred to as the “inflammatory soup”: prostaglandins, cytokines, nerve growth factor, lipids and lipoxigenase 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 A β fibers are thought to be mainly involved in the perception of allodynia, while activation of A δ 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 pathway¹⁵⁶ 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 handling¹¹ 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 midair⁴⁷ or in actual pulling force exerted on a bar¹²² 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 changes¹³⁸ in animals such as “wet-dog” shakes,^{27,187} but further research discovered that it also produces cold sensations lasting about 15 min¹⁸⁸ when injected intraperitoneally or applied to skin by triggering activation of TRPM8.^{27,93,191} Applying droplets of *acetone*⁵⁶ to the foot will induce behavioral responses when cold allodynia is present. To assess cold tolerance, an animal may be placed on a pre-chilled 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
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 pain⁸⁴ 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 developed¹²⁴ 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 humans⁹⁸ 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 intracolonicly 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 secondary to inflammation	carrageenan	Winter and colleagues 1962
	acetic acid writhing test	Collier and colleagues 1968
	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 secondary to nerve injury	sciatic nerve ligation (chronic constriction injury)	Bennett and Xie 1988
	partial sciatic ligation	Seltzer and colleagues 1990
	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 postoperative	colonic balloon	Ness and Gebhart 1988
	plantar incision	Brennan and colleagues 1996
	skin/muscle incision and retraction	Flatters 2008
constellation cancer	pancreatic cancer	Ornitz 1987
	osteolytic bone cancer	Wacnik and colleagues 2000
constellation migraine	CGRP injection	Recober and colleagues 2009
	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 low back	SPARC-null	Millecamps and colleagues 2011 and Millecamps and colleagues 2012
	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-used¹⁹⁴ 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^{187,142} 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 down-regulation 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 calcaneus¹⁸¹ 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 neuropathies¹⁶⁹ 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 5HT_{1B} receptors on blood vessels and 5HT_{1D} 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. Dilatation 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 non-cerebral 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 CGRP¹⁴⁷ 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-averse behavior presumably a correlate to photophobia. Delivering CGRP slowly in anesthetized mice induced an elevation in light-averse behavior and an increase in resting behavior under dark conditions.¹⁴⁶ When CGRP was given peripherally by intraperitoneal injection, it also induced light-averse 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 proportion 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 assay¹⁶⁸ and the grip force assay⁸⁹ 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 populations 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.

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