



Up-and-coming Radiotracers for Imaging Pain Generators

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Abstract

Chronic musculoskeletal pain is among the most highly prevalent diseases worldwide. Managing patients with chronic pain remains very challenging because current imaging techniques focus on morphological causes of pain that can be inaccurate and misleading. Moving away from anatomical constructs of disease, molecular imaging has emerged as a method to identify diseases according to their molecular, physiologic, or cellular signatures that can be applied to the variety of biomolecular changes that occur in nociception and pain processing and therefore have tremendous potential for precisely pinpointing the source of a patient's pain. Several molecular imaging approaches to image the painful process are now available, including imaging of voltage-gated sodium channels, calcium channels, hypermetabolic processes, the substance P receptor, the sigma-1 receptor, and imaging of macrophage trafficking. This article provides an overview of promising molecular imaging approaches for the imaging of musculoskeletal pain with a focus on preclinical methods.

Keywords

- ▶ pain
- ▶ inflammation
- ▶ nociception
- ▶ molecular imaging
- ▶ positron emission tomography

Unmet Clinical Need in Diagnosis and Management of Chronic Pain

Chronic pain is among the most prevalent diseases worldwide, affecting ~ 20.5 to 21.8% of U.S. adults¹ and costing as much as \$635 billion in the United States alone.² It is now the number-one reason for lost days at work and a major driver of the devastating opioid epidemic. Patients with chronic pain are left with lifelong suffering and disability that can largely be attributed to our inability to accurately pinpoint pain generators with current anatomy-based imaging approaches. Up to 96% of asymptomatic patients show magnetic resonance imaging (MRI) abnormalities.³ It can be especially challenging to assess whether inflammatory or degenerative changes shown on imaging are merely a result

of the so-called normal aging process or are actually active relevant changes representing pathology in patients with persistent joint or nerve pain. Additionally, it may be challenging to identify which, if any, of a patient's many imaging abnormalities, such as multiple bulging disks or multilevel facet arthropathy, is the cause of their discomfort. This lack of precision in diagnosis might either indirectly or directly contribute to delayed, ineffective, or even unnecessary surgery because imaging affects therapeutic decisions.

Low back pain (LBP), for example, is a disease that suffers poor diagnostics. Despite LBP being ranked the third most burdensome disease in terms of mortality or poor health,⁴ the etiology of a person's LBP remains undecipherable in the vast majority of cases. It is no surprise that management and

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treatment of LBP is challenging and has relatively limited efficacy because the exact source of LBP is unknown in 80 to 90% of patients.⁵ Many imaging methods (radiography, computed tomography [CT], MRI) have been used for diagnosis, but identified structural or signal abnormalities have not been a reliable marker for pain.

For example, a literature review study showed disk degeneration and signal loss present in ~90% of asymptomatic individuals ≥ 60 years of age.³ This diagnostic inaccuracy results in inadequate pain management that invariably leads to the rampant use of opioids for symptomatic relief because the exact cause of LBP cannot be identified. In fact, LBP ranks among the top conditions leading the prescribed opioid use in primary care,⁶ despite the elevated risk of addiction, complications, and poor surgical outcomes. Additionally, the danger of labeling patients with anatomical diagnoses that may not correspond to the true source of pain and the potential for unneeded procedures with unclear benefits make current imaging potentially detrimental.^{7,8} Moreover, imaging conducted on patients without signs of major underlying diseases did not improve clinical outcomes, and physicians should avoid routine and even urgent lumbar imaging in these patients according to a meta-analysis of imaging techniques for LBP.⁹

Another example of inconclusive imaging occurs in myofascial pain syndrome (MPS). A disorder characterized by the presence of myofascial trigger points (MTrPs) affects ~8 to 10% of the population but also suffers from a lack of tools to diagnose accurately and consistently the precise location of these MTrPs.¹⁰ Correspondingly, post-amputation pain (PAP) is another challenging condition that affects >90% of all amputees and endures management challenges because the offending neuroma is not easily discriminated from nonpainful neuromas in the amputated limb.¹¹ Accurate localization of MTrPs in MPS and painful neuromas in PAP would profoundly improve the outcomes of these challenging disorders.

Better diagnostic imaging methods are needed to enable correct identification of the pain generator and subsequent better targeted personalized treatment. More specifically, an imaging biomarker that tracks heightened pro-nociceptive tissue at the site of initial pain generation in humans is needed because it will allow us to identify the tissues that directly contribute to symptoms. The inflamed or damaged tissue, in which several local cellular and molecular changes have occurred as part of the nociceptive process, is where molecular imaging can find its target. We discuss preclinical molecular imaging techniques that are being used to pinpoint the causes of pain more precisely.

Pathophysiology of Chronic Pain

Chronic pain can be categorized as nociceptive or neuropathic pain. Nociceptive pain is the result of ongoing tissue inflammation or damage. Neuropathic pain is caused by persisting damage to the somatosensory nervous system. More recently, a third category has been added, namely nociplastic pain, with this definition: multifocal pain that is more widespread or intense, or both, than would be

expected given the amount of identifiable tissue or nerve damage, as well as other central nervous system (CNS)-derived symptoms, such as fatigue, sleep, memory, and mood problems.¹²

Nociplastic pain has been described as either a bottom-up response to a peripheral nociceptive or a neuropathic trigger (i.e., often referred to as central sensitization) or a top-down CNS-driven response.¹³ This entity is important to keep in mind because treatment approaches would differ from nociceptive inflammatory or neuropathic pain entities. It is important to note, though, that in clinical practice, placing pain in a certain category is difficult because an individual's pain experience is often a combination of these categories.¹⁴ For instance, ongoing inflammation in an individual with nociceptive pain can eventually have elements of neuropathic pain because chronic inflammation can lead to damaged nerve endings.

Further complicating the diagnostic picture for pain is another important physiologic phenomenon known as the gate theory that implies not all painful stimuli reach or is paid attention to in the brain.¹⁵ Thus there may be multiple potential peripheral sources of pain, but the patient's central nervous processing of all the nociceptive input filters the information to focus on one or a few of the perceived more important pain generators.

Nociceptors are equipped with receptors and ion channels that enable the detection of potential harmful stimuli.¹⁶ Mechanical, chemical, and thermal stimuli may lead to an electrochemical signal resulting in afferent activity in the peripheral and central nervous system.¹⁷ Inflammatory mediators, including prostaglandin E₂, neurotrophic growth factor, bradykinin, chemokines, and cytokines, are generated at the site of tissue damage. These mediators stimulate the terminals of peripheral neurons, activate inflammatory cells for healing, interact with ion channels or neural cell receptors, and change activation thresholds, resulting in improved synaptic transmission efficiency. Ultimately, this situation may lead to pain.¹⁸

"Pain arising as a direct consequence of a lesion or disease affecting the somatosensory system" is the definition of neuropathic pain.¹⁷ It can occur in the absence of a stimulus or at much lower nociceptive thresholds. This scenario often leads to allodynia (pain from a noninjurious stimulus) and hyperalgesia (increased and prolonged pain sensation to a painful stimulus). The nociceptive nerve's excitability is affected by a host of modulatory events, such as neuroimmune mediators, ion channel dysregulation, inflammatory mediators, and macrophage interactions.¹⁹ Closely following these processes are the phenomena of microglial cell activation, central sensitization, and changes in synaptic plasticity, just a few of the maladaptive changes that occur in the brain and spinal cord centers that lead to an environment where pain sensitivity is increased.²⁰

Molecular Imaging for Chronic Pain

Numerous molecular mechanisms are involved in chronic pain that act in parallel.¹⁹ A paradigm for molecular imaging techniques targeting this multitude of receptors and inflammatory

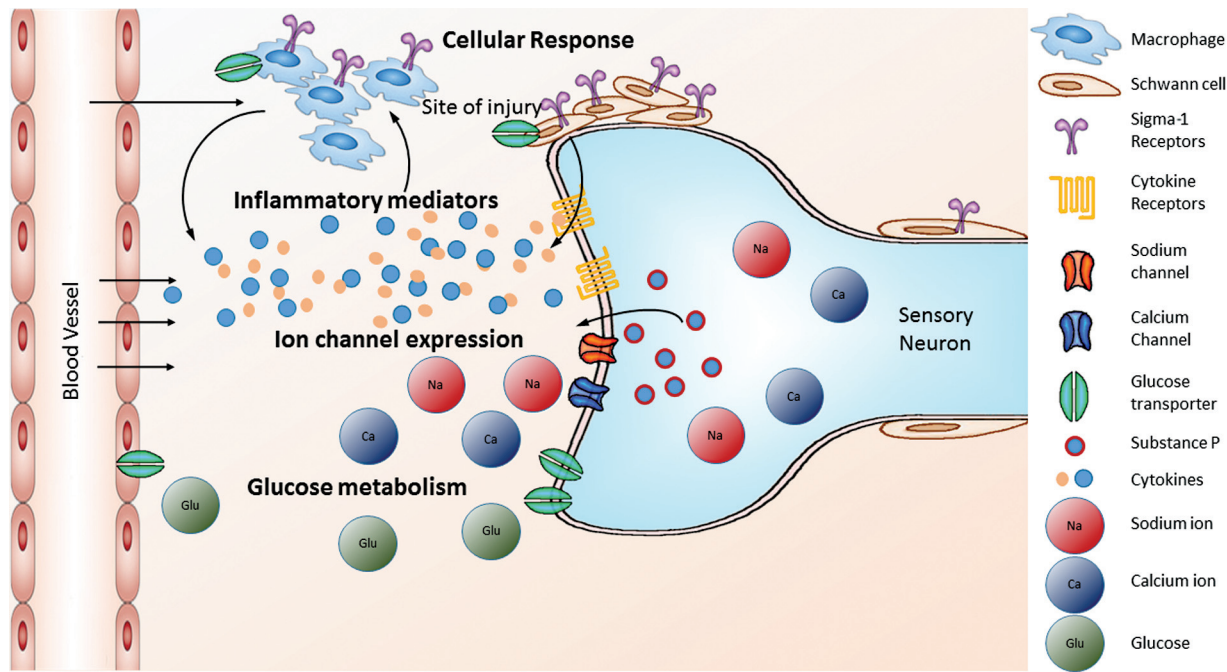


Fig. 1 Four main mechanisms of neuropathic pain imaging. (1) Cellular response to neuropathy: Schwann cell and macrophage imaging by sigma-1 receptor positron emission tomography (PET) and iron oxide magnetic resonance imaging (MRI), respectively. (2) Inflammatory mediators and receptor using radiolabeled neuropeptides and COX-2 tracers. (3) Ion channel expression by utilizing radiolabeled saxitoxins that are specific toward the voltage-gated sodium channels and calcium channel imaging by manganese-enhanced MRI. (4) Metabolic response can be studied by monitoring increased fluorodeoxyglucose utilization by ^{18}F -FDG PET/MRI. (Reproduced with permission from Tung KW, Behera D, Biswal S. Neuropathic pain mechanisms and imaging. *Semin Musculoskelet Radiol* 2015;19(2):103–111)²¹

mediators involved in pain was previously proposed dividing mechanisms into four categories (→ **Fig. 1**): cellular response, inflammatory mediators and receptors, ion channel expression, and metabolic response. For each mechanism, the latest imaging insights are reviewed in the following sections.²¹

Cellular Response

Several types of cells react to tissue damage with the goal of containing and mending the damage. In case of tissue damage, T cells immediately react to start the healing process. In case of nerve damage, Schwann cells multiply quickly in response to the injury, clearing away the dead tissue and building the foundation for axonal regeneration. In the immediate aftermath of injury or inflammation, active macrophages are drawn from the circulation to help the repair process. Both glial cells and macrophages are monocytic derivatives that have a role in both the maintenance of neuropathic pain syndromes and the modulation of chronic pain. Furthermore, the maladaptive remodeling of the nervous system seen in the neuropathic pain phenotype is significantly influenced by activated microglial cells and astrocytes.²² The reaction of various types of cells in the vicinity of the site of tissue or nerve damage, as well as in the CNS upstream of the injury, can be examined using molecular imaging techniques.

Translocator Proteins Positron Emission Tomography Imaging of Activated Glial Cells and Macrophages

Activated macrophages and microglia were discovered to be closely linked to areas of neuronal damage and neuroin-

flammation in conditions of chronic pain.^{23–25} They show dramatically upregulated expression of translocator proteins (TSPOs), a protein with five transmembrane domains and a high hydrophobicity that limits neurosteroid biosynthesis by moving cholesterol from the outer to the inner mitochondrial membrane.²⁶ → **Fig. 2** shows an overview of the TSPO structure and function.²⁷ In a 2022 study, it was shown that TSPO improves autophagy impairment and mitochondrial biogenesis,²⁸ which may provide a new strategy for the treatment of neuropathic pain. TSPO is only moderately expressed in healthy tissues, but it is significantly elevated in inflammatory situations that can be visualized using radioligands to TSPO.²⁹

Thus areas of elevated peripheral and central neuroinflammation and sensitization in relation to increased nociceptive activity can be objectively identified using imaging focused on TSPOs. Multiple TSPO radioligands are on the market and have been used in many human brain studies.²⁷ Among these, to name a few, are 11C-PK11195, 11C-PBR28, 18F-DPA-714, and 11C-DAA1106. For the past few decades, [11C]PK11195 has been among the most widely used TSPO tracers. Increased 11C-PK11195 positron emission tomography (PET) uptake in the spinal cord of mice who underwent partial sciatic nerve ligation was seen in preclinical studies of neuropathic pain.^{30,31} Others have discovered time-dependent increases in TSPO-expressed activated microglial cells in the spinal cord of mouse models of spinal cord injury and complicated regional pain syndrome (CRPS), respectively.³² This indicates that increased radiotracer uptake is associated with increased microglial cell activation, pointing to a

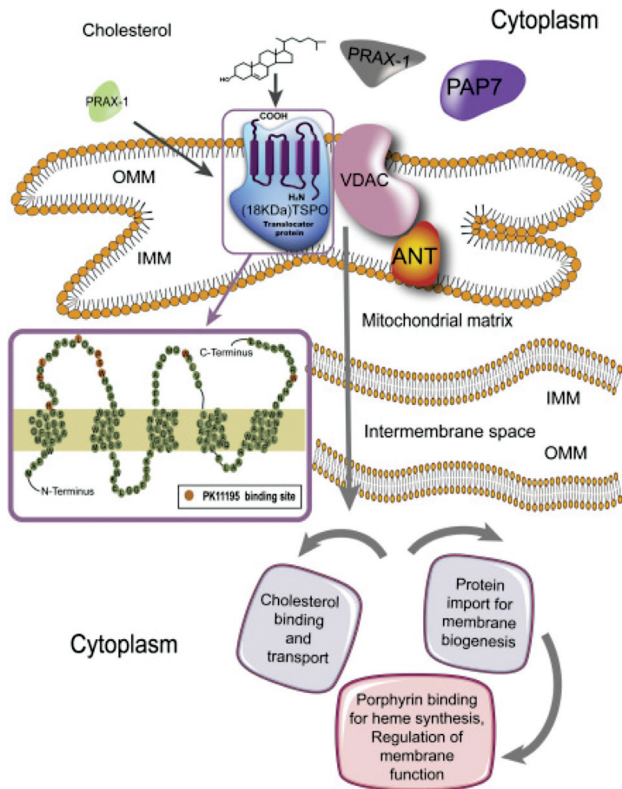


Fig. 2 Translocator protein (TSPO) structure and function. ANT, adenine nucleotide transporter; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; PAP7, protein kinase A regulatory subunit Ri-associated protein; PRAX-1, peripheral benzodiazepine receptor-associated protein 1; VDAC, voltage-dependent anion channel. (Reproduced with permission from Zhang L, Hu K, Shao T, et al. Recent developments on PET radiotracers for TSPO and their applications in neuroimaging. *Acta Pharm Sin B* 2021;11(2):373–393)²⁷

potential use of this tracer in neuropathic pain patients. However, despite providing useful data on a variety of neurologic conditions in humans,³³ its poor brain perme-

ability, high plasma protein binding leading to a relatively low signal-to-noise ratio (SNR), have severely constrained its sensitivity and overall usefulness for brain disorders. Moreover, its short half-life of ¹¹C (20.4 minutes) limits widespread transportation and clinical trials. Second-generation TSPO-PET tracers with enhanced *in vivo* characteristics have been developed, such as [¹¹C]DPA-713 and [¹¹C]PBR28.³⁴ The Loggia laboratory showed increased uptake in the brain of patients with LBP, and Albrecht et al showed increased uptake at the spinal cord using [¹¹C]PBR28.^{35–37}

Even though these novel PET tracers exhibit higher SNR, a limitation due to a single nucleotide polymorphism in the TSPO gene, there is individual variation in TSPO binding potential due to rs6971 polymorphism.³⁸ Third-generation TSPOs have been developed that are insensitive to rs6971 polymorphism, and clinical translation is being studied.³⁹ In addition, TSPO ligands with the ¹⁸F radioisotope have a lower positron energy (650 keV) and a longer half-life ($t_{1/2} = 109.7$ minutes), facilitating extended dynamic PET studies and transportation to more distant facilities.

Imaging of Sigma-1 Receptors

The sigma-1 receptor (S1R), originally believed to be an opioid receptor subtype, is a transmembrane protein that is highly concentrated in specific areas of the CNS and has a known involvement in the onset and maintenance of chronic pain.⁴⁰ These receptors are highly expressed in macrophages and Schwann cells that multiply after brain injury and play a role in inflammation and healing.^{41,42} Both spontaneous nociceptive activity and central sensitization are influenced by proinflammatory mediators generated by immune cells such as macrophages, Schwann cells, and others.¹⁹ S1R density increases significantly as a result of the proliferation of Schwann cells, the attraction of macrophages to areas of nerve inflammation, and potential cellular upregulation of S1R expression. **Fig. 3** shows an overview of the structure

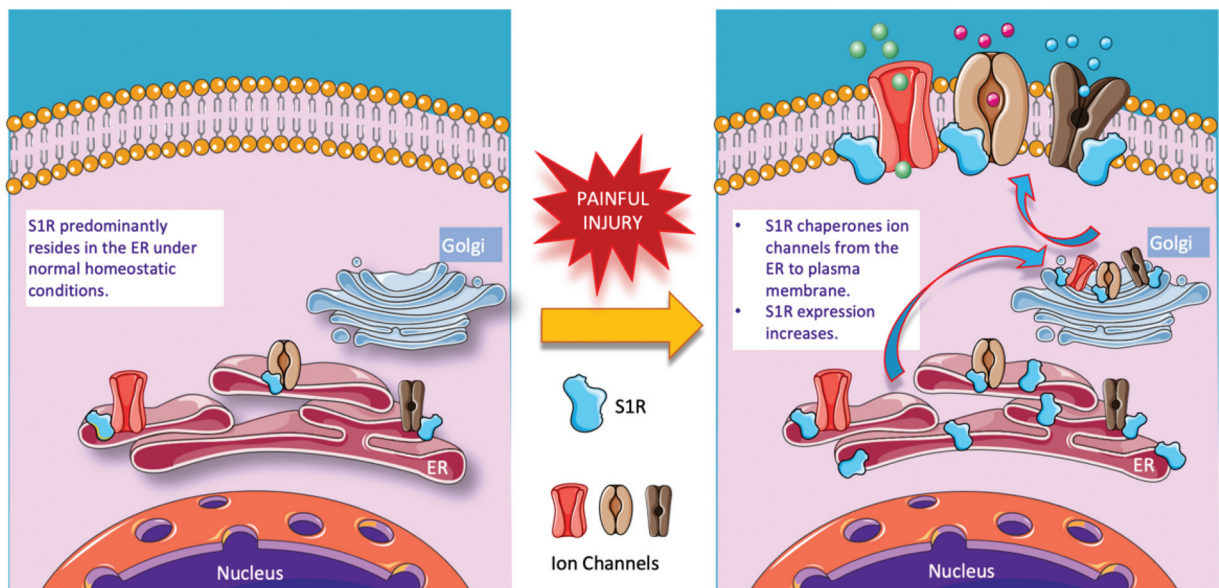


Fig. 3 The sigma-1 receptor (S1R) pathway. ER, endoplasmic reticulum. (Reproduced with permission from Yoon D, Kogan F, Gold GE, Biswal S. Identifying musculoskeletal pain generators using clinical PET. *Semin Musculoskelet Radiol* 2020;24(4):441–450)⁴³

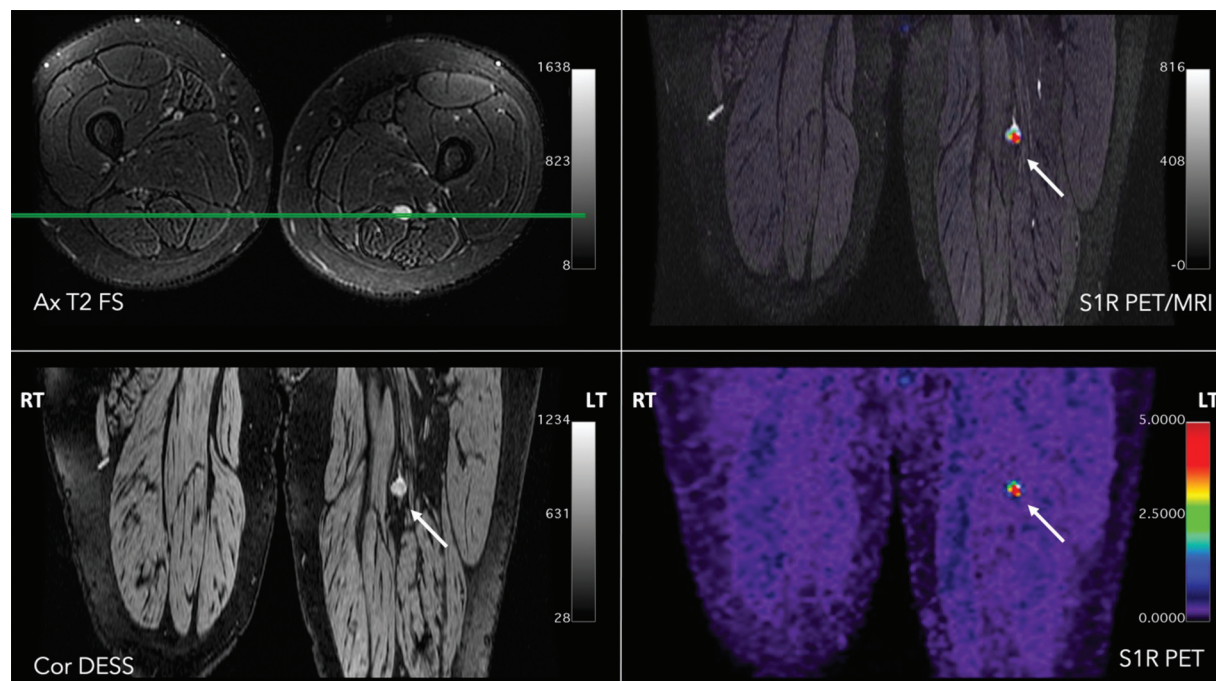


Fig. 4 Patient presented with a painful peripheral nerve sheath tumor (PNST) of the left sciatic nerve. Magnetic resonance image (MRI) (axial T2 with fat saturation (upper left image) and coronal double echo and steady (DESS) image (white arrow, bottom left image) show focal 1.2-cm mass with increased T2 signal in the tibial component of the left sciatic nerve. Corresponding sigma-1 receptor (S1R) positron emission tomography (PET) and S1R PET/MRI show focally increased tracer uptake (white arrow; maximum standardized uptake value [SUVmax] = 5.0). Note radiotracer uptake in contralateral right sciatic nerve (SUVmax = 0.4).

and function of S1Rs.⁴³ Therefore, radiotracers designed to detect the S1R can aid in locating nerve inflammation.

A highly selective radiolabeled S1R ligand, [¹⁸F]FTC-146, has been demonstrated to exhibit enhanced uptake in a neuroma brought on by nerve damage in an animal model of neuropathic pain. According to immunohistochemical results, the enhanced tracer uptake was associated with S1R expression and Schwann cell proliferation. The origins of posttraumatic pain, postsurgical pain, CRPS, and neuro-inflammatory chronic pain syndromes that arise from nerve injury can be identified by employing particular radiotracers to locate the areas of neural damage and other related nociceptive and inflammatory processes.^{44–46}

This tracer has translated to clinical trials (► **Figs. 4** and **5**), and early clinical results are detailed in a dedicated article in this issue of *Seminars*. ► **Fig. 4** shows increased uptake of the S1R radiotracer in an individual with a painful peripheral nerve sheath tumor (PNST) (7–8/10 on the Numeric Rating Scale [NRS]) of the left sciatic nerve (► **Fig. 4**). This PNST was subsequently removed, and the patient reported complete relief. In contrast, a nonpainful neuroma (0/10 on the NRS scale) had developed in another patient following biopsy of the sural nerve. S1R PET/MRI imaging of this lesion demonstrated no significant uptake of the radiotracer in this neuroma (► **Fig. 5**).

Magnetic Resonance Imaging of Macrophage Trafficking

As mentioned earlier, macrophages and microglia are crucial to the process of repairing nerve injury, but inflammatory mediators generated by these cells at the same time contrib-

ute to the etiology of chronic pain by sustaining and potentiating the increased sensitivity of pain-sensing neurons.¹⁹

Macrophages can be tagged with iron nanoparticles and followed using MRI, given that iron has superparamagnetic characteristics. MRI can thus be used to follow the migration of iron oxide nanoparticles after intravenous administration into the body. In a neuropathic pain paradigm, Ghanouni et al used ultra-small superparamagnetic iron oxide (USPIO) MRI to show that macrophages did indeed traffic to sites of nerve injury. USPIO-injected rats displayed lower T2-weighted signal at the site of nerve injury. A reduction in the recruitment of macrophages to the neuroma after the administration of minocycline, which suppresses macrophage and microglial activity, led to a reduction in pain behaviors.⁴⁷

USPIO-loaded macrophages can be used to identify areas of macrophage infiltration along nociceptive pathways when paired with MRI's outstanding anatomical resolution. Additionally, by locating the sources of persistent pain, this technique may help in the creation of new targeted analgesics with more precise macrophage inhibitors. In recent years, promising results were demonstrated with ferumoxytol. Shen et al demonstrated significantly larger changes in T2 before and after ferumoxytol injection in mice that received Complete Freund's adjuvant (CFA) versus saline. In humans, ferumoxytol-enhanced MRI identified the nerve root that corresponded with the patient's symptoms, without any corresponding structural abnormalities.⁴⁸ The clinical use of ferumoxytol has been restricted due to potential safety issues. A recent review showed that ferumoxytol was well tolerated, with no serious adverse events, and only in a

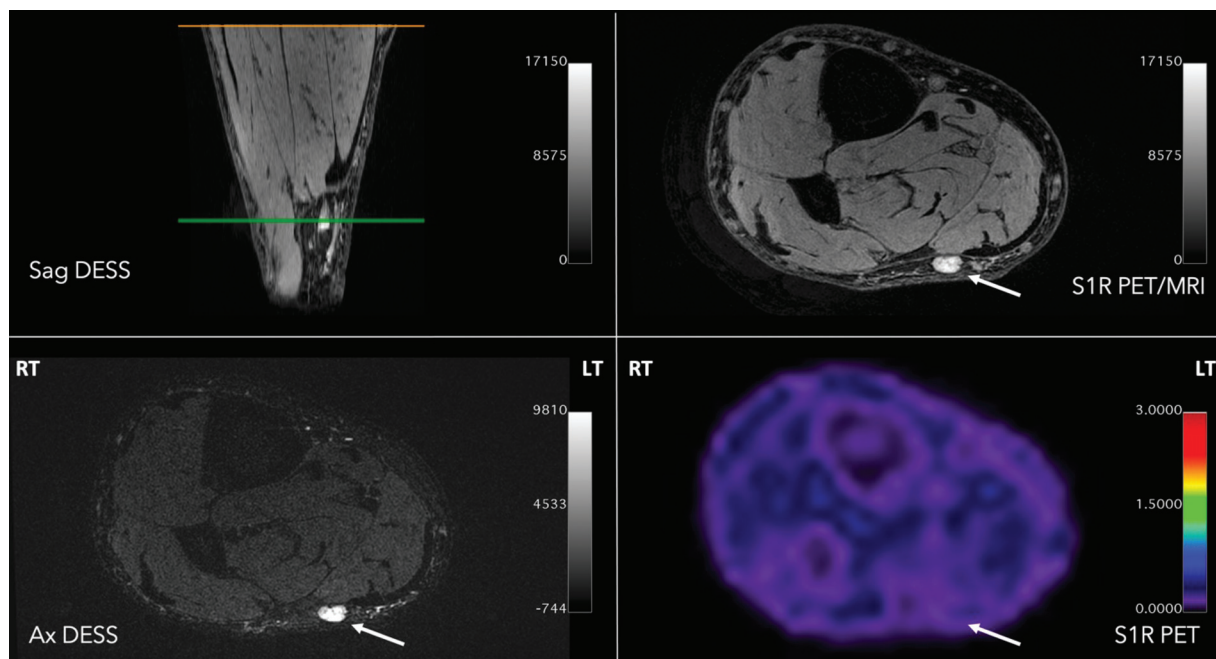


Fig. 5 Patient sustained a neuroma following biopsy of the right sural nerve. Aside from being a small palpable subcutaneous mass in the posterolateral aspect of the distal right lower leg, this mass was asymptomatic (Numeric Rating Scale: 0/10). Sagittal (top left image) and axial (bottom left image) magnetic resonance imaging (MRI) of the lesion demonstrate a discrete focal high-signal lesion in the expected location of the sural nerve, consistent with a sural nerve neuroma, located just lateral to the Achilles tendon. Sigma-1 receptor (S1R) positron emission tomography (PET) (bottom right) and S1R PET/MRI (upper right image) reveal no significant S1R PET tracer uptake in the right sural nerve neuroma (white arrow; SUVmax = 0.2). The green line in the upper left image indicates the location of axial images relative to the sagittal image.

few adverse reactions, indicating that more widespread clinical use is possible.⁴⁹

Inflammatory Mediators and Receptors

Numerous mediators produced by inflammatory cells, as well as Schwann cells and nerve cells, are involved in nerve injury and inflammation.¹⁹ An intuitive method to identify neuropathic nerves is to radiolabel such mediators and attempt to discover an enhanced inflammatory response. Numerous molecules associated with inflammation and pain, including neuropeptide analogs, have been radiolabeled. These substances have potential for imaging neuropathic pain.

Labeled Neuropeptides and Analogs

Numerous cell receptors from different groups have been found to express themselves more frequently during nociception, which results in spontaneous nociceptive activation and increased sensitization. It is believed that upregulation of these receptors plays a significant role in the emergence of hypersensitivity in the pain pathway, leading to a transition from acute to chronic pain states.

Many different animal pain models have been used to study substance P and its receptor, the substance P receptor (neurokinin-1 receptor), also known as the tachykinin receptor-1 (TACR1). **Fig. 6** shows a schematic overview of substance P-mediated signaling and ion channels in the peripheral sensory neurons.⁵⁰ Tachykinins are widely present within the central and peripheral nervous system. In persistent pain models, such as the CFA paradigm, substance

P receptor overexpression in both the dorsal root ganglion as well as the dorsal horn of the spinal cord was shown.^{51–53} It may be possible to locate the sources of the receptor-mediators of chronic pain using radiolabeled neuropeptides. Using [¹¹¹In-DTPAArg1] radiolabeling, substance P and some of its analogs were localized to healthy substance P receptor-positive tissues, such as the salivary gland and arthritic joints in animal models.^{54,55} Preliminary PET scans of the brain in healthy individuals using the substance P antagonist SPA-RQ, which has been radiolabeled with ¹⁸F, demonstrated its predicted striatal localization in a dose-dependent manner.^{55,56}

This substance P receptor antagonist is highly selective to the NK1 receptor and may act as a marker for inflammatory alterations because NK1 receptor expression is elevated in painful, inflamed tissue. However, NK1R antagonists are still rarely used in therapeutic settings despite the widespread expression of the NK1R and the significance of substance P in the regulation of physiologic processes. The potential of many NK1R antagonists was explored during early phase clinical trials, but many were found ineffective or not significantly useful.⁵⁷ Anti-tumor and anti-emetic effect was demonstrated, but infusions of all NK1R antagonists are associated with side effects, including phlebitis/thrombophlebitis and hypersensitivity reactions (such as anaphylaxis).

The reason most NK1R antagonists' clinical trials failed is still a mystery. Some explanations for the gap between preclinical and clinical outcomes have been proposed, including species differences in NK1R distribution and

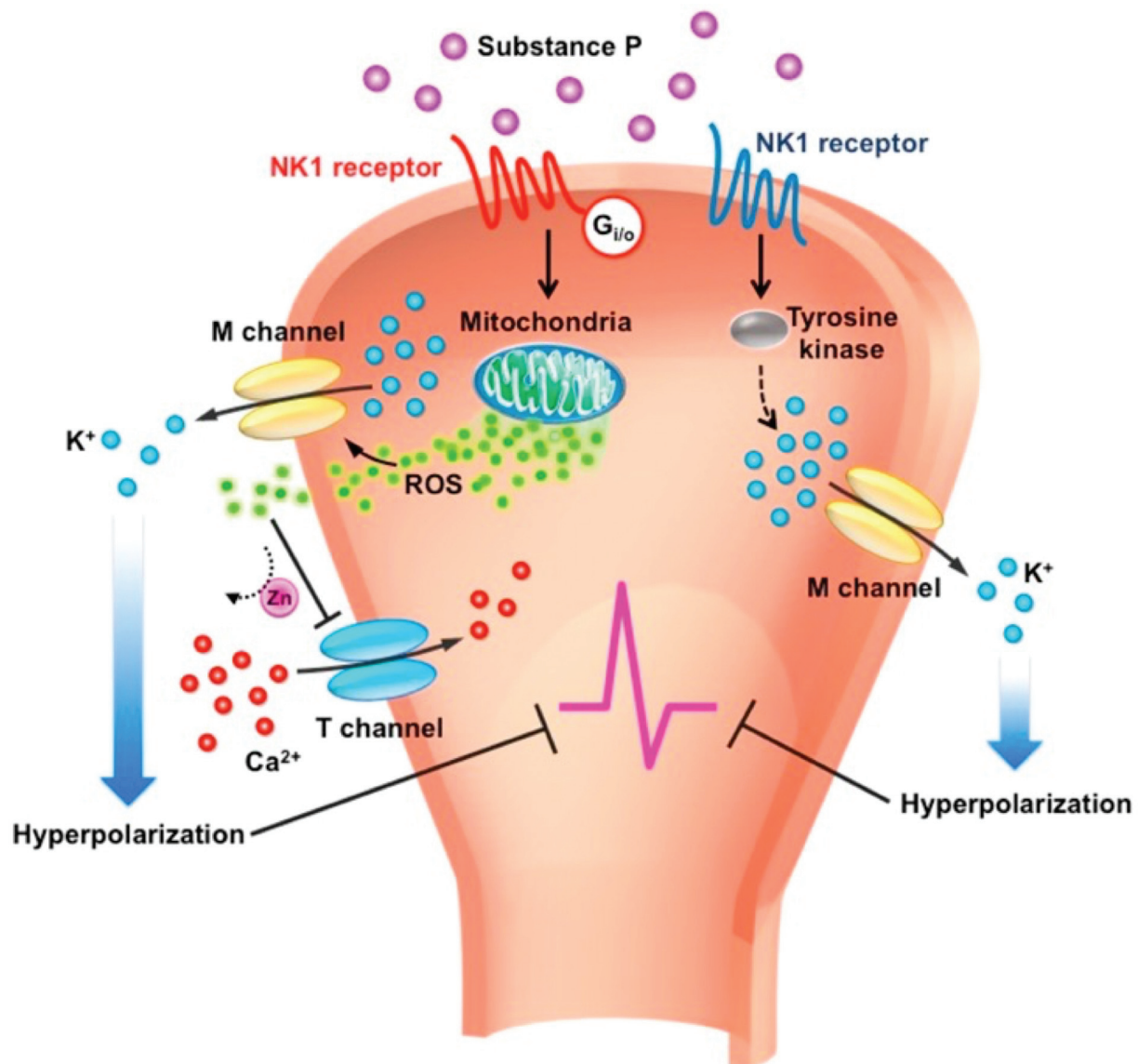


Fig. 6 Schematic diagram of substance P-mediated signaling and ion channels in the peripheral sensory neurons. *Gi/o*, one of the G proteins; NK1, neurokinin-1; ROS, reactive oxygen species. (Reproduced with permission from Chang CT, Jiang BY, Chen CC. Ion channels involved in substance P-mediated nociception and antinociception. *Int J Mol Sci* 2019;20(7):1596)⁵⁰

affinities to NK1R antagonists and the effectiveness of animal models in predicting clinical pain. The failure may also be partially explained by the accumulative finding of substance P's antinociceptive action, which could act via opioid signaling.⁵⁰ Moreover, substance P is more complex with effects on multiple ion channels, and thus more research is needed to establish how NK1R antagonists can be used for pain reduction.⁵⁰

The analgesic properties of opium have been known for many years, and the opioid crisis is obviously worsening each year. However, the function and mechanism of the endogenous opioid system still remains poorly known today. Opioid receptors (ORs) comprise four subclasses: μ (MOR), δ (DOR), κ (KOR), and ORL-1 (opioid receptor-like 1, also termed NOP, nociceptin opioid peptide) receptors (→ Fig. 7).⁵⁸ For years, pain studies focused on opioid receptor ligands and dominated the field of clinical PET research, with MOR the most important

target. In past years, PET tracers were developed with better binding properties in vivo and a shorter half-life when binding to ¹¹C (→ Fig. 7).⁵⁹ NOP receptor ligands are being researched largely as an alternative to MOR opioid analgesics because NOP receptors are found in a variety of areas (including the brain, spinal dorsal horn, and dorsal root ganglion), which are important in transmitting pain.⁶⁰ The first results showed contradictory effects, both antinociceptive and nociceptive, but recently it was antinociceptive effects that were shown in nonhuman primates.⁶⁰

The calcitonin gene-related peptide, bradykinin, serotonin, brain-derived neurotrophic factor, N-methyl-D-aspartate, vanilloid receptor VR1, neurotensin, tyrosine kinase B, and cholecystokinin are other ligand-receptor systems that are typically increased in inflammatory pain and may be involved in modulating pain.⁶¹ These peptides could potentially be exploited for the purposes of imaging pain.

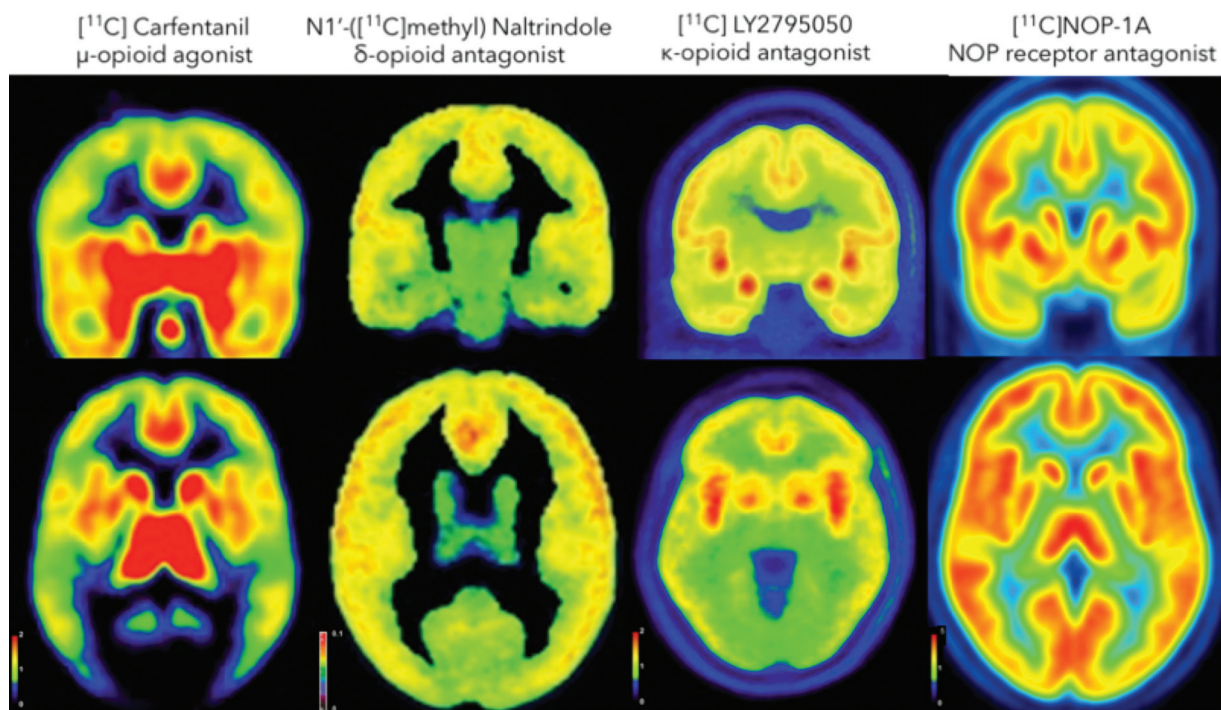


Fig. 7 Human brain opioid receptor positron emission tomography images in coronal (upper row) and axial (lower row) planes. (Reproduced with permission from Pecina M, Karp JF, Mathew S, Todtenkopf MS, Ehrich EW, Zubieta JK. Endogenous opioid system dysregulation in depression: implications for new therapeutic approaches. *Mol Psychiatry* 2019;24(4):576–587)⁵⁸

COX-2 Tracers

The cyclooxygenase-2 (COX-2) enzyme is regarded as a significant stimulator of tissue inflammation by converting arachidonic acid into prostaglandin H, a precursor for prostaglandin (PG) E₂. PGE₂ regulates the common signs of inflammation, such as redness, swelling, heat, fatigue, and hypersensitivity to pain. COX-2 can be inhibited by nonsteroidal anti-inflammatory drugs. COX-2 is the isoform predominantly found in the spinal cord and brain. Inflammation characterized by an elevated COX-2 enzyme level may be imaged with the help of radiolabeled COX-2 inhibitors. Inflammation quickly increases COX-2 levels, which then quickly decline to baseline levels.⁶²

COX-2 is regarded as one of the most specific targets for neuroinflammation PET imaging.⁶³ Unfortunately, the effectiveness of COX-2 as a marker target has not yet been established with ^{99m}Tc-celebex, ¹⁸F-SC51825, and ¹⁸F-desbromo-DuP-697, because there have been no reports of successful localization in inflammation models. Due in part to the very modest level of COX-2 enzyme produced in this condition, imaging with ¹⁸F-desbromo-DuP-697 in an animal paw inflammatory model was unsuccessful.⁶⁴ Other major drawbacks of the previous radiotracers include substantial defluorination in the case of [¹⁸F] tracers, significant defluorination in the case of [¹⁸F] tracers, high nonspecific binding, low COX-2 affinity, and others.^{63,65} More recently, a

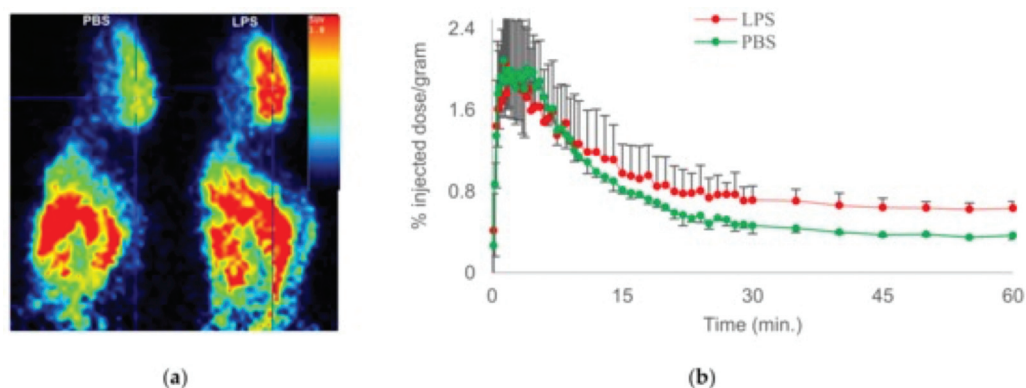


Fig. 8 (a) Representative micro-positron emission tomography (PET) images of [¹⁸F]MTP in phosphate-buffered saline (PBS)- and lipopolysaccharide (LPS)-administered mice. (b) Time activity curves (TACs) of [¹⁸F]MTP in PBS (green)- and LPS (red)-treated mice. (Reproduced with permission from Prabhakaran J, Molotkov A, Mintz A, Mann JJ. Progress in PET imaging of neuroinflammation targeting COX-2 enzyme. *Molecules* 2021;26(11):3208)⁶³

novel cyclooxygenase-2 PET radioligand, [11C]MC1, was developed that did show upregulation in primate neuroinflammation and in human peripheral tissue with inflammation.⁶⁶ Another new radioligand, [18F]MTP, is a potential candidate for COX-2 imaging in clinical studies of neuroinflammation, but more research is needed before clinical translation is possible (►Fig. 8).⁶³

Ion Channel Expression

Ion channels, such as sodium calcium and channels, play an important role in generating action potentials, maintaining the resting membrane potential in neurons and transmitting signals through nerve fibers. Increased calcium and sodium flux across the membrane causes altered excitation threshold, aberrant action potentials, and continuous nerve firing in neuropathy and pain. Thus ion channel expression or activity is another method to detect foci of aberrant activity.

Nuclear Imaging of Voltage-Gated Sodium Channels

The production of action potentials and the transmission of electrical signals down the nociceptive pathway depend heavily on voltage-gated sodium (NaV) channels. NaV chan-

nel density is elevated in damaged or inflamed nerves.^{67,68} Chronic pain has been linked to an increase in sodium channel density as was also shown in preclinical studies.⁶⁹⁻⁷¹ Many commonly prescribed analgesics, including lidocaine, work by blocking NaV channels. They are composed of a pore-forming α subunit and at least one associated β subunit, and they are often characterized by their differential sensitivity to tetrodotoxin. ►Fig. 9 is a schematic representation summarizing the mechanisms modulating NaV channel function and the effect of therapeutic agents in diabetic sensory neuropathy.⁷² Other naturally occurring toxins that bind to select NaV isoforms with single-digit nanomolar affinity are guanidinium toxins, saxitoxin (STX), and gonyautoxin (GTX).

Saxitoxin has been altered and radiolabeled with 18F, enabling nanomolar-affinity binding to NaV channels. In a neuropathic pain animal model using 18F-saxitoxin and PET/MRI, painful neuromas were found to have a 40% increase in PET signal. Increased radiotracer uptake is also seen in the soft tissues next to the neural injury, indicating that [18F] benzamide-STX collects outside of the neuroma, where upregulation of NaVs in uninjured tissues close to the injury may play a role in the emergence of the chronic pain phenotype

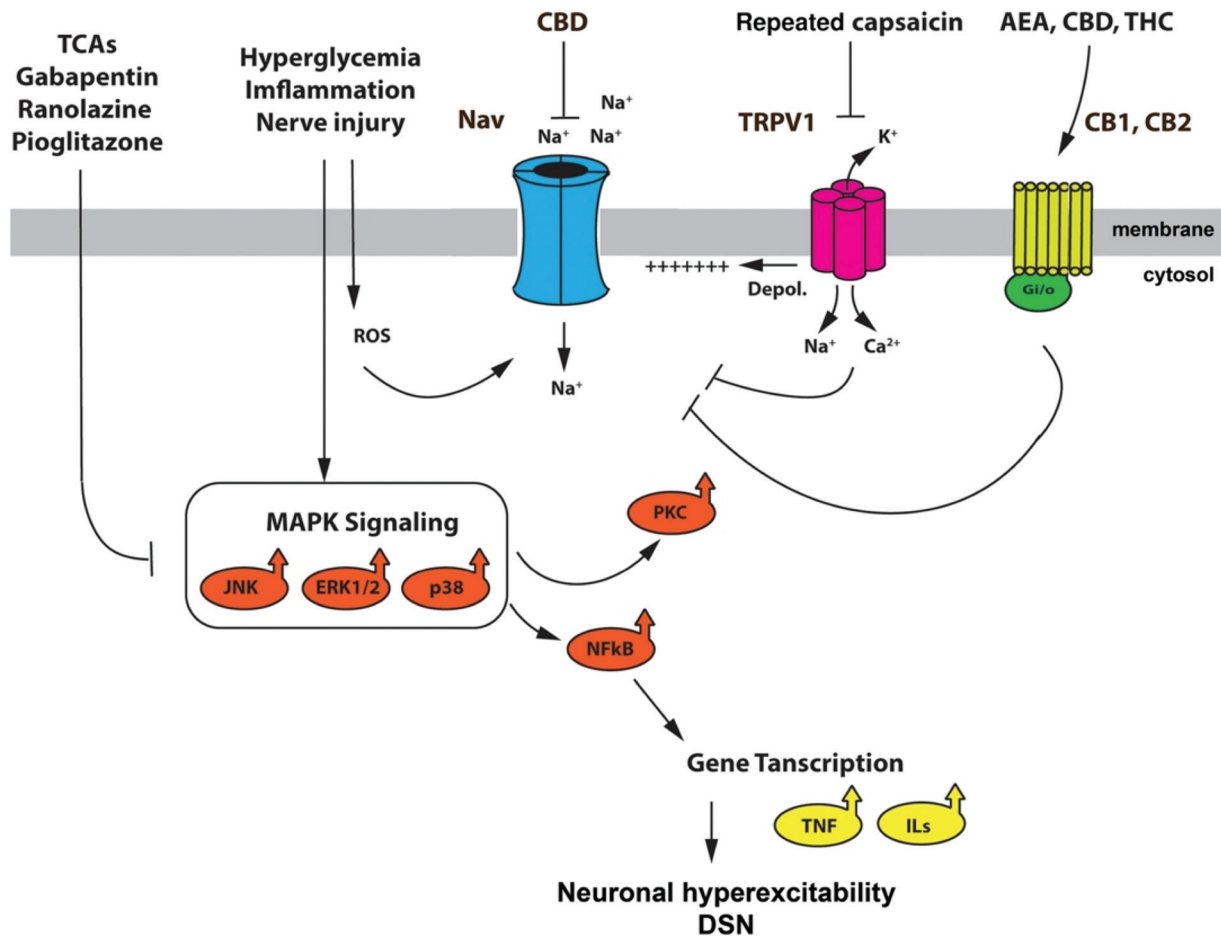


Fig. 9 Schematic representation summarizing the mechanisms modulating voltage-gated sodium channel function in diabetic sensory neuropathy and the effect of therapeutic agents. AEA, N arachidonoyl ethanolamine, CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2. CBD, cannabidiol; ECS, endocannabinoid system; ROS, reactive oxygen species; TCAs, tricyclic antidepressants; THC, Δ 9-tetrahydrocannabinol. (Reproduced with permission from Bigsby S, Neapetung J, Campanucci VA. Voltage-gated sodium channels in diabetic sensory neuropathy: function, modulation, and therapeutic potential. *Front Cell Neurosci* 2022;16:994585)⁷²

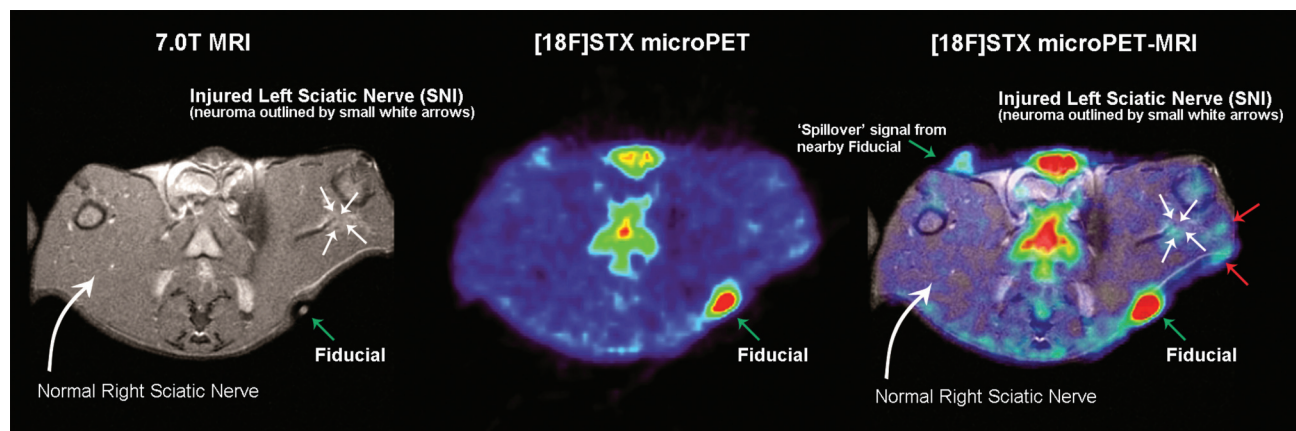


Fig. 10 The [18F]benzamide-STX ([18F]STX) positron emission tomography/magnetic resonance imaging (PET/MRI) of a rat with spared nerve injury (SNI) and neuropathic pain of the left hindlimb. The left image is a transverse small animal MR image through the thigh at the level of the left SNI showing an enlarged neuroma (straight white arrows). The right sciatic nerve is normal in size and caliber, present in its typical normal appearance and nearly obscured by the surrounding muscular tissue (curved white arrow). The central image is the [18F]STX PET image at the same transverse location as the adjacent MR image. The right figure shows the two coregistered images of the PET and MR that can be accurately fused using the fiducial marker (green arrow). Increased [18F]STX PET signal is seen in the neuroma created by the SNI. By comparison, no significant [18F]STX PET signal is seen in the right nerve. An additional interesting finding of this experiment is that radiotracer uptake also appears to be increased in the soft tissues immediately adjacent to the neural injury and at the skin of the animal (red arrows). Although the reason for this observation is speculative, the finding of activity outside of the neuroma may suggest other changes related to the nerve injury that may be an important part of the evolution of the chronic pain phenotype.

(►Fig. 10).⁷³ Although this approach has interesting potential, there have not been any significant developments in recent years, given the potential relative toxicity of this agent.

Another method to image sodium channels was realized by the emergence of ²³Na-magnetic resonance imaging (²³Na-MRI),⁷⁴ which is challenging due to a low SNR, but the development of ultrahigh magnetic field scanners and specialized sodium acquisition sequences show promise for this technique. PET imaging with radiocaine offers a new and exciting PET method to image sodium channels.⁷⁵ Radiocaine is a fluorine-18 radiotracer based on the local anesthetic lidocaine, a nonselective NaV blocker, developed by Hooker's group and previously used for cardiac NaV 1.5 imaging using PET. The details of this relatively new tracer are described in a separate article in this issue.

Manganese-enhanced Magnetic Resonance Imaging of Voltage-gated Calcium Channels

Voltage-gated calcium channels are crucial for pain signaling in both the peripheral and CNS. In the peripheral nervous system, the L, T, N, and P types of voltage-gated calcium channels (VGCCs) facilitate nociception. In peripheral nerves, membrane depolarization activates VGCCs and causes extracellular calcium to flood the cell. Calcium is an integral second messenger facilitating downstream events such as neurotransmitters and neuropeptides release, neuronal plasticity, and gene expression for pain processing. Hyperexcitability in peripheral nerves is hypothesized to be caused by alterations in the gene expression of VGCCs. Calcium channel blockers and channel subunit knockouts were shown to reduce hyperalgesia and allodynia in animal pain models.^{76–78}

In addition to VGCCs, NMDA calcium channels are also hypothesized to play a role in pain hypersensitivity. The

expression of NMDA channels is upregulated, and the channel undergoes chemical modification to remain open to allow more calcium to enter.^{61,79} For many years, researchers have studied calcium signaling in a range of disorders using both traditional chemical fluorescent calcium indicators and protein-based genetically encoded calcium indicators.

The $\alpha 2\delta$ -1 is the voltage-activated Ca^{2+} channel subunit identified to have a leading role in neuropathic pain and serving as a binding site of gabapentinoids for treatment.⁸⁰ A variety of gabapentinoids have been identified as VGCC $\alpha 2\delta$ ligands, of which four are on the market,⁸¹ while investigations into the optimal ligand continues.⁸²

Manganese is a physiologic follower of calcium and a T1-shortening MRI contrast agent. The rate of calcium (and manganese) outflow is very sluggish in hyperactive cells, which causes manganese to build up inside the cell and produce imaging contrast. Manganese-enhanced MRI (MEMRI) with T1-weighted MR imaging has shown potential. It has been able to show the dynamic activation of the lumbar plexus in a spared nerve injury model as well as anti-nociceptive effects on the plexus after opioid administration (►Fig. 11). In this example, the addition of buprenorphine depresses neuronal activation of the plexus after spared nerve injury, and the imaging results of decreased T1W signal in opioid-treated animals correlate with the analgesia that the animals experience following opioid administration.

Although there is eagerness to use this tool to identify nerve injuries and hyperactive neuronal tissues, the translation of MEMRI to humans will be difficult, given the potentially toxic dosages needed for image contrast.⁸³ Recent advances by Engle et al have made radiomanganese radiotracers (⁵¹Mn) available and, in conjunction

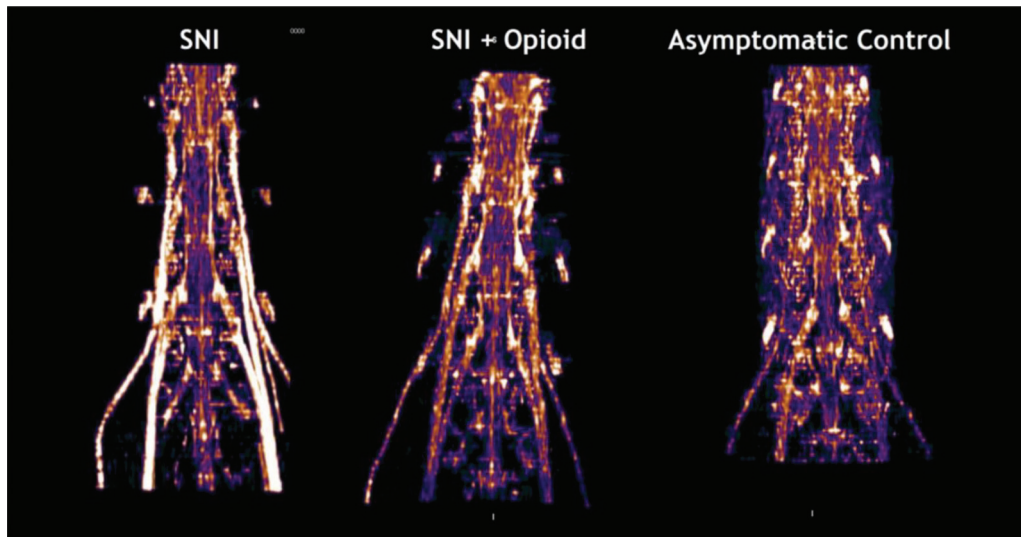


Fig. 11 Manganese-enhanced magnetic resonance imaging (MEMRI) (T1-weighted [T1W] three-dimensional maximum intensity projection [MIP]) of the lumbar cord and lumbar plexus of an animal 1 week after receiving a left sciatic spared nerve injury (SNI) (left image) that creates a model of neuropathic pain involving the hindlimb. Middle image is a T1W three-dimensional MIP image of the lumbar cord and plexus of an SNI animal treated with a dose of an opioid (buprenorphine). The right image is the T1W of an uninjured control asymptomatic animal. Increased T1W signal is observed in the lumbar nerve roots in the SNI animal compared with animals that received an analgesic or received no injury. Images were obtained following a 24-hour intraperitoneal injection of manganese chloride ($MnCl_2$). Addition of buprenorphine depresses MEMRI signal and, correspondingly, neuronal activation of the plexus after SNI. This imaging result correlates with the analgesia the animals experience following opioid administration. Of interest, increased MEMRI signal is seen in bilateral peripheral nerves in the setting of a unilateral injury. Bilateral neurosensory activation is a common phenomenon observed in animal models and reflected here with MEMRI.

with PET/MRI, ^{51}Mn can be safely administered at significantly lower doses, thus facilitating safe clinical translation.^{84,85}

Metabolic Response

Both chronic inflammatory diseases and continuous brain activity are energy-intensive activities. Another method of identifying local abnormalities in the peripheral nervous system is by imaging the rate of energy expenditure or metabolic activity.

Nuclear Imaging of Glucose Metabolism

A well-known radiopharmaceutical PET marker called 18F-fluorodeoxyglucose (18F-FDG) mimics glucose when it enters cells and is held inside them during the glycolytic cycle. The absorption of glucose and FDG is enhanced in tissues with increased glucose, such as infectious, inflammatory, or malignant diseases. In chronic pain syndromes, continuously or spontaneously firing neurons and their associated inflamed tissues also have increased glucose metabolism. In 18F-FDG PET/MRI, this phenomenon is used to depict enhanced metabolism. Recently, brain activity in regions such as the medial prefrontal cortex, primary somatosensory cortex hindlimb region, and the centrolateral thalamic nucleus were shown to be positively correlated with mechanical allodynia-related behavioral changes.⁸⁶ Behera et al used PET/MRI in a rat model to pinpoint increased 18F-FDG uptake in damaged nerves. The absorption of 18F-FDG and behavioral tests for allodynia in the afflicted paw were highly linked. In contrast, control nerves in the subject's contralateral normal leg of control animals with no symptoms displayed considerably decreased 18F-FDG uptake.⁸⁷ Another preclinical study confirmed the

feasibility of FDG PET/MRI to identify peripheral neuropathic pain.⁸⁸

Pain patients have also been found to exhibit 18F-FDG avidity in their peripheral nerves. In a clinical case report, 18F-FDG PET/CT revealed that the lumbar spinal cord and sciatic nerves were rich with 18F-FDG. The biopsies of these neural structures revealed pathologic indicators of chronic neuropathy.⁸⁹ In the past few years, multiple studies were performed by the Biswal group^{43,90} in chronic foot pain,⁹¹ sciatica,⁹² and CRPS.⁹³

► **Fig. 12** is a representative axial 18F-FDG PET/MR image obtained from the cervical spine of a female patient with long-standing right-handed CRPS and no additional insight about the cause of the symptoms after a standard clinical work-up including conventional MRIs. An [18F]FDG PET of her cervical spine demonstrated a highly [18F]FDG-avid right C5 nerve root and dorsal root ganglia as well as increased [18F]FDG uptake in the spinal cord at this level. The MRI component of this study showed a hypertrophic right uncovertebral process that was contributing to significant right C5–C6 neural foraminal narrowing, resulting in impingement of the exiting right C5 nerve root with associated neuropathic and inflammatory changes in this nerve. The caregivers of this patient, based on these imaging findings, decided to perform surgery to decompress the right C5–C6 neural foramen, which subsequently resulted in marked improvement in the symptoms of her right hand.

Thus imaging sites of hypermetabolic neural tissues and neuroinflammation may be a strategy for identifying chronic neuropathic pain generators. The recent Food and Drug Administration approval of FDG for inflammation facilitates large clinical studies. One caveat to bear in mind, however, is

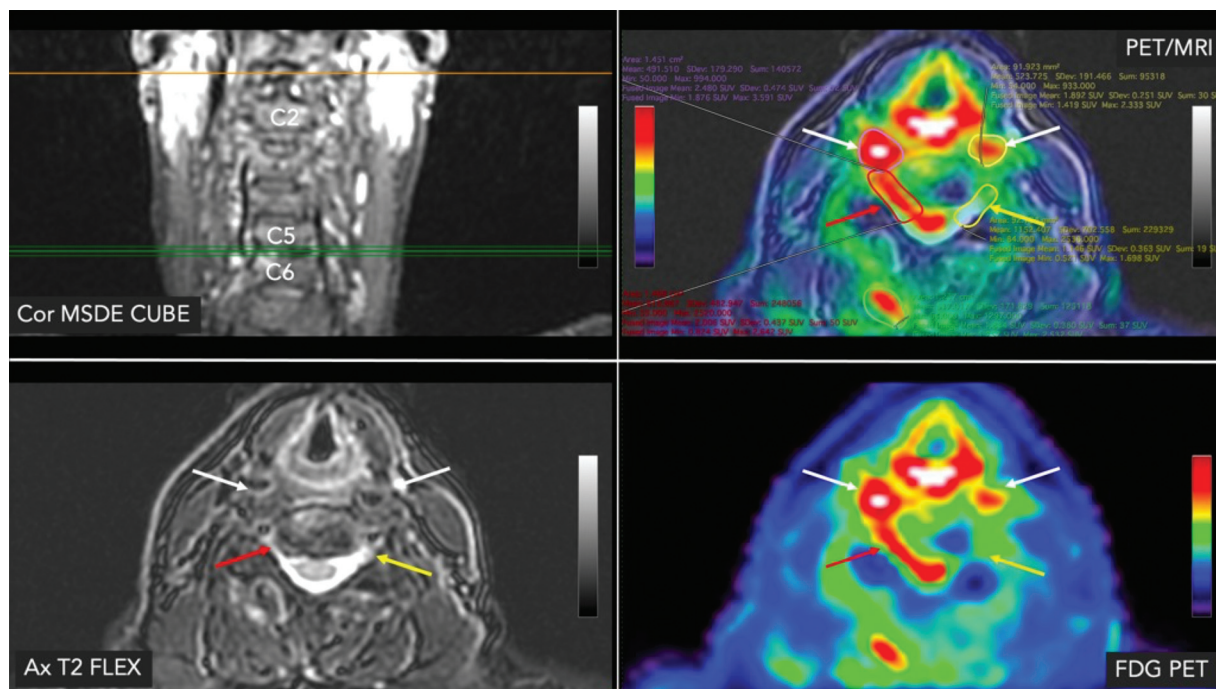


Fig. 12 The [18F]fluorodeoxyglucose positron emission tomography/magnetic resonance imaging (FDG PET/MR) of a patient with right-handed complex regional pain syndrome who, despite regular clinical testing, including conventional MRIs, had no further explanation for the symptoms. Upper right image is a coronal MSDE CUBE sequence shown here for reference; the other three images are axial images acquired at the level of the C5–C6 intervertebral disk (horizontal green line). The patient’s cervical spine’s [18F]FDG PET revealed a right C5 nerve root and dorsal root ganglia that were significantly [18F]FDG-avid (red arrow, lower right and fused upper right images; maximum standardized uptake value [SUVmax] = 2.64) when compared with similar structures on the left (SUVmax = 1.70; yellow arrow). There was also enhanced [18F]FDG uptake in the spinal cord at this level. The right C5 nerve root exiting this research was impinged upon due to a hypertrophy right uncovertebral process that was contributing to a considerable right C5–C6 neural foraminal constriction (red arrow, lower left image), along with concomitant neuropathic and inflammatory alterations in this nerve. Incidentally, FDG PET also shows increased tracer uptake on the right greater than the left longus colli muscles (right SUVmax = 3.59; left SUVmax = 2.33; white arrows) and right semispinalis cervicis muscle (green region of interest) that may represent neuropathic change in these paravertebral muscles. Based on these MRI results, the patient’s caregivers made the decision to approve surgery to decompress the right C5–C6 neural foramen, which later led to a significant noticeable improvement in symptoms.

the nonspecificity of FDG, because it is based on abnormal glucose metabolism that can be abnormal due to biological processes other than inflammation. Another issue could be the high bone marrow uptake that renders image evaluation difficult if potential pain generators are thought to be located in or adjacent to bone.

Current Challenges in Molecular Imaging of Painful Conditions

The importance of imaging a pain-generating pathology-specific molecular biomarker cannot be overemphasized. In spite of the presence of anatomical features that seem normal on currently available imaging techniques, molecular imaging relies on identifying abnormal biological processes. Due to the numerous and intricate biochemical processes involved in the mechanism of pain, finding a common molecular pathway for chronic pain is a difficult, if not impossible, task. The fact that different pain etiologies, such as inflammatory versus neuropathic pain, result from different physiologic and biomolecular changes complicates matters. For example, patients with spinal cord injury pain typically do not respond well to COX-2 inhibitors, in contrast to those with knee osteoarthritis. This shows that whereas

COX-2 is crucial for the management of inflammatory pain, it is far less important for the management of neuropathic pain. As a result, a radiotracer with a label designed to detect high COX-2 levels may only be able to pinpoint the site of inflammatory pain and possibly be limited in its detection of neuropathic pain.

The identification of tissues of interest in neuropathic pain faces challenges with molecular imaging techniques due to its technological constraints. In PET imaging, for example, small structures of interest, such as dorsal root ganglia and peripheral nerves, are extremely vulnerable to patient motion artifacts. Additionally, 18F-FDG, a clinical radiotracer that can image inflammatory processes, may be more greatly absorbed in surrounding tissues than in important structures like neural and perineural tissues. The spill-over activity from surrounding tissues into the smaller structures of interest will confound quantitative measurements in these target tissues of interest. Even with the most advanced PET scanners, it is practically impossible to confidently ascribe any aberration to an anatomical structure without the aid of anatomical imaging.

The inclusion of CT to PET/CT is beneficial to some degree, particularly for spinal imaging. However, the superior soft tissue contrast of MRI enables the radiologist to attribute

PET-detected abnormalities to a specific category of soft tissue, such as peripheral nerves or substructures within small joints. Therefore, the introduction of PET/MRI as a hybrid molecular-anatomical imaging approach has opened previously unexplored avenues and provides significant advantages over PET/CT.

PET will likely provide important localization of nociceptive, neuropathic, or nociplastic sources of pain that can then inform the focused efforts of high-resolution, small field-of-view MRI (or ultrasonography) to perhaps unveil an underlying structural explanation for a patient's pain. Simultaneous imaging with PET/MRI has emerged as a superior clinical imaging entity for a variety of cancers and pediatric applications, and it is likely to play an important role in the advancement of so-called pain imaging. In addition, radiation doses, which are not negligible with PET/CT, are significantly reduced with PET/MRI as PET detectors improve and as artificial intelligence (AI) is utilized to facilitate even lower tracer dose administration. Ongoing research and improvements in motion correction techniques and AI-assisted reconstruction methods to improve SNR will help address current challenges in imaging small structures.

The fact that pain is a subjective experience that varies from person to person and is impacted by affective elements may be one of the biggest challenges in trying to image pain. Developing whole-body imaging assays to simultaneously study the central and peripheral nervous systems in their entirety would be one way to potentially attribute more specificity to an imaging approach in this regard, allowing for the study of a person's pain from a more comprehensive, individualized perspective. Understanding the regions of the body's system with elevated activity as shown by imaging biomarkers may help us better understand how each person experiences pain. The readout of such a whole-body technique may help characterize patients according to their relative contributions from peripheral versus central systems, thereby potentially characterizing patients as more peripherally versus centrally sensitized. Such knowledge could potentially help predict whether an individual patient will benefit from a localized surgery versus avoiding surgery altogether and therefore choosing more systemic or centrally focused therapies. Although this is an exciting prospect of this whole-body molecular imaging approach, significant work lies ahead to make this a reality.

Summary

With > 100 million sufferers, a yearly cost of \$560 to \$635 billion, and several comorbidities associated with it, pain is still the main cause of patient visits to the doctor in the United States. Because morphological aberrations have been shown to have low sensitivity and specificity, current routine imaging diagnosis of pain is currently limited to their detection and identification. Moving away from morphological markers, molecular imaging offers an opportunity to image distinct cell types, molecular receptors, ion channels, and/or inflammatory mediators involved in increased nociceptive activity and would therefore enable the realization of more

biologically, functional, and physiologically relevant imaging of pain generators. This is an area of research that shows promise for the creation of instruments that are more exact and objective for the diagnosis of pain, and it may also open the door for more specialized and targeted treatments.

Implementation of peripheral imaging for chronic pain generators remains difficult, even though imaging research for peripheral pain generators is promising. Methods aimed at ion channels are potentially toxic. For example, techniques that target ion channels may be harmful. The subjective and affective aspects of pain must be considered, as well as the accuracy of preclinical animal pain models when used to simulate the complicated human pain experience. Imaging of small structures of interest, such as dorsal root ganglia and peripheral nerves, is another challenge. However, the detection of pain-generating sources can result in more intelligently directed treatment by combining the sensitivity and specificity of molecular markers with the high anatomical spatial and contrast resolution offered by CT and MRI. If successful imaging and identification of pain were to become a reality, it would have a significant impact on medicine and help millions of people with chronic pain.

Disclosure

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Conflict of Interest

None declared.

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