



Neuroimaging of pain in animal models: a review of recent literature

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Abstract

Neuroimaging of pain in animals allows us to better understand mechanisms of pain processing and modulation. In this review, we discuss recently published brain imaging studies in rats, mice, and monkeys, including functional magnetic resonance imaging (fMRI), manganese-enhanced MRI, positron emission tomography, and electroencephalography. We provide an overview of innovations and limitations in neuroimaging techniques, as well as results of functional brain imaging studies of pain from January 1, 2016, to October 10, 2018. We then discuss how future investigations can address some bias and gaps in the field. Despite the limitations of neuroimaging techniques, the 28 studies reinforced that transition from acute to chronic pain entails considerable changes in brain function. Brain activations in acute pain were in areas more related to the sensory aspect of noxious stimulation, including primary somatosensory cortex, insula, cingulate cortex, thalamus, retrosplenial cortex, and periaqueductal gray. Pharmacological and nonpharmacological treatments modulated these brain regions in several pain models. On the other hand, in chronic pain models, brain activity was observed in regions commonly associated with emotion and motivation, including prefrontal cortex, anterior cingulate cortex, hippocampus, amygdala, basal ganglia, and nucleus accumbens. Neuroimaging of pain in animals holds great promise for advancing our knowledge of brain function and allowing us to expand human subject research. Additional research is needed to address effects of anesthesia, analysis approaches, sex bias and omission, and potential effects of development and aging.

Keywords: Pain, Imaging, fMRI, MEMRI, PET, EEG, Animals

1. Introduction

Brain imaging of pain involves the use of various techniques to either directly or indirectly image the structure, function, or pharmacology of the brain under painful conditions.^{57,77} These neuroimaging techniques include electroencephalography (EEG), multiple modalities of magnetic resonance imaging (MRI), and positron emission tomography (PET). Previous literature in human neuroimaging has shown that acute pain is usually processed by a main core of brain regions, whereas chronic pain, due to adaptive or maladaptive neuronal plasticity, modulates several brain circuits including somatosensory,

cognitive, affective, motivational, and reward.^{5,13,59,70} Six recent animal functional MRI (fMRI) studies have contributed to the field through the advantages of using longitudinal investigations paired with pharmacological and nonpharmacological interventions.^{17,40,46,61,75,88} In addition, at the end of the imaging protocol, brain or other tissue of interest can be processed to further elucidate mechanisms and correlate changes in neuroimaging with molecular aspects of the tissue.

Previous reviews of animal neuroimaging studies of pain processing in animals have described activations in areas seen in common with human studies.^{5,12,13,59,77,81} The brain areas most commonly activated by noxious stimulation included the primary and secondary somatosensory cortices (S1, S2), insula, anterior cingulate cortex (ACC), and periaqueductal gray area (PAG).^{5,12,13,59,77,81} In addition, pharmacological imaging in animals also shows consistent activation patterns compared with humans with pain and opioid analgesia.^{12,71,77} This review will provide an overview of the current state of the field, as well as innovations and limitations in neuroimaging techniques. We searched for all articles published from January 1, 2016, to October 10, 2018, using the following search terms: animal, pain, brain, diffusion tensor imaging (DTI), EEG, functional MRI (fMRI), manganese-enhanced MRI (MEMRI), PET, and structural MRI. Exclusion criteria for this review were studies that did not include brain imaging and did not assess pain. In that period, 18 animal neuroimaging studies have used fMRI,^{1–3,9,17,22,35,37,40,46,56,61,64,75,84,85,88,89} whereas 10 other

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Table 1**A summary of the features, advantages, and disadvantages of the neuroimaging methods discussed in this review.**

Neuroimaging methods	Studies	Animals	Sample	Setup	Software packages	Advantages	Disadvantages
EEG	2 studies ^{48,72}	Sprague–Dawley rats	n = 5–8	Invasive; anesthesia or awake; intracranial, epicranial or subcutaneous electrodes; freely moving rats or in stereotaxic apparatus under anesthesia; EEG system; physiological monitoring system	Spike2; MATLAB; “purpose-built software” (spectral analyzer, Craig Johnson)	Fast acquisition: 5 minutes recording each condition; high temporal resolution; portable; signals generated directly from neural activity	Acquisition is limited to cortical areas; poor spatial resolution
fMRI	18 studies: rats, ^{1,2,9,17,22,40,56,61,64,75,85,88} mice, ^{37,46,84} and monkeys ^{3,35,89}	Sprague–Dawley, LEW/CriCrij and Long-Evans rats; C57BL/6 and BERK sickle transgenic mice; <i>Macaca fascicularis</i> and <i>Saimiri sciureus</i> monkeys	n = 3–19 for rats, n = 2–10 for mice, and n = 3–8 for monkeys	Noninvasive; anesthesia or awake; MRI scanner; physiological monitoring system	SPM; MATLAB; FSL; AMIRA; Brain Voyager; MagnAn; CONN; brain connectivity toolbox; AFNI; stimulate; R statistical programming environment	High spatial resolution; longitudinal studies	Expensive; poor temporal resolution; indirect measure of neuronal activity
MEMRI	5 studies ^{16,25,41,42,74}	Sprague–Dawley and Long-Evans rats	n = 4–10	In vivo minimally invasive or fixed brains; anesthesia; cannula or pump for MnCl ₂ injection; MRI machine; physiological monitoring system	ParaVision Bruker; MRVision; ImageJ; SPM; MATLAB; ANTs; FSL	High spatial resolution; measures cerebral metabolism; morphometry	Expensive; Mn ²⁺ toxicity limits repeat application
PET	3 studies ^{19,44,78}	Sprague–Dawley rats	n = 3–17	Minimally invasive; anesthesia; radiotracers by intravenous or intraperitoneal injection; PET scanner; physiological monitoring system	AMIDE; SPM; Siemens microPET Manager Software; Imsgrtm	Measurement of cerebral metabolism; drug binding	Expensive; use of radioactive tracer limits repeat application

EEG, electroencephalography; fMRI, functional MRI; MEMRI, manganese-enhanced MRI; SPM, Statistical parametric mapping; PET, positron emission tomography

studies have used less commonly used techniques including 5 with MEMRI,^{16,25,41,42,74} 3 with PET,^{19,44,78} and 2 with EEG.^{48,72} There were no studies using diffusion tensor imaging or structural MRI in that period. Brain activity, connectivity, and receptor-binding capacity have been assessed in these studies. Finally, a brief discussion to encourage and aid future studies how investigate pain processing with neuroimaging tools is also explored.

2. Innovations and limitations in neuroimaging techniques

Neuroimaging studies using animals have investigated changes in brain mechanisms through different pain models over the last decade.^{6,9,26,38,69} We will discuss new approaches and limitations of each of the neuroimaging techniques used, considering the nature and features of the signal, as well as the setup and analysis methods (Table 1).

2.1. Characteristics of the signal

The most commonly used brain imaging method in animals is MRI, which includes fMRI^{1–3,9,17,22,35,37,40,46,56,61,64,75,84,85,88,89} and MEMRI^{16,25,41,42,74}; in the current literature of pain. Functional MRI usually uses the blood oxygenation level–dependent (BOLD) method as an indirect measure of neuronal activity associated with a task or stimulus, or using temporal interdependence of fMRI time series across regions to infer brain connectivity (see Refs. 4 and 80 for reviews). On the other hand, MEMRI uses manganese ions (Mn (2+)) as an MRI contrast agent that can be transported across active synapses and along axons (see Ref. 53 for review), revealing activation related to a task or stimulus across networks. Relatively fewer recent studies have used PET and EEG in pain neuroimaging, probably due to the disadvantages of these techniques, eg, radiotracer use and expense, and non-standard setup for PET and EEG, respectively.^{19,44,48,72,78} Although PET and EEG have poor spatial resolution, they measure more direct neuronal activity than BOLD signal through glucose

metabolism using fludeoxyglucose (FDG)-PET and brain electrical activity using EEG waveforms (see Refs. 18 and 65 for reviews). Efforts have been made to increase temporal and spatial resolution of neuroimaging techniques; however, there are specific limitations of each method (**Table 1**).

Spatial resolution refers to how accurately the measured activity is localized within the brain, and temporal resolution refers to how closely the measured activity corresponds to the timing of the actual neural activity.⁴⁵ Functional MRI has a good spatial resolution (mm) that can be further improved using high magnetic field and scan duration (small animal scanners were 7 to over 11.7 Tesla (T) in recent studies,^{1–3,17,22,35,40,46,56,61,64,75,84,85,88,89} except 2 studies using 4.7 T^{9,37}). However, some methods that increase signal-to-noise ratio can also come at a cost of greater distortion. For example, higher magnetic field strengths improve signal-to-noise ratio and contrast-to-noise ratio yielding higher resolution. Nevertheless, images with higher resolution are more sensitive to motion artifacts³⁶ and the faster cardiac and respiratory rates from rodents.⁴³ Furthermore, high-resolution demands long acquisition times, and motion artifacts can worsen with longer acquisition times.²⁷ A good strategy is to image structures with enough detail and minimal distortion to achieve proper identification.³⁶ The spatial resolution for in-plane resolution and slice thickness, respectively, in the reviewed fMRI articles ranged from 0.2 to 0.4 mm and 0.5 to 5 mm for mice studies, 0.2 to 0.8 mm and 0.5 to 2 mm for rat studies and 1 to 2 mm and 1 to 2.4 mm for monkey studies.^{1–3,9,17,22,35,37,40,46,56,61,64,75,84,85,88,89} By contrast, the temporal resolution of neuronal events is limited through the hemodynamic delay: typically, the BOLD response has a peak occurring ~4–6 seconds after the onset of a brief neuronal event.³² One of the reviewed studies improved the temporal resolution by capturing more points of the hemodynamic response and interpolating those linearly (multishot EPI), which can also reduce the repetition time (TR) of magnetic pulses (500 ms).⁸⁵ However, it is worth mentioning that those procedures cannot change the speed of the hemodynamic response. Manganese-enhanced MRI benefits from the use of T1-weighted images, providing better spatial resolution than fMRI, varying between 150 and 75 μm for rat studies,^{16,25,41,42,74} but the temporal resolution is up to 8 hours to achieve a good signal-to-noise ratio.⁷⁴

The temporal resolution of PET is poor compared with fMRI and EEG, and is limited by the metabolism of the tracer molecule.⁴⁵ Because of the low spatial resolution, the 3 reviewed PET studies reported the use of a structural MRI template to coregister the functional data to better localize the neuronal activity, which is a standard method in PET.^{19,44,78} Although spatial resolution is limited in PET, the method has the unique ability to measure glucose metabolism and receptor-binding levels, such as metabotropic glutamate receptor 5 (mGluR5) and opioid receptors (μ , δ , and κ) in the whole brain.^{19,44,78} However, the temporal resolution of these phenomena is usually low due to the uptake time for the tracer to reach the brain and to achieve the peak of its radiation (minutes).

By contrast, EEG provides far better temporal resolution than other neuroimaging techniques, with capacity to record oscillatory activity in groups of neurons within milliseconds (~3–500 Hz).^{48,57} We review only 2 EEG studies investigating pain in rats.^{48,72} They attempted to optimize the source localization using electrodes over the skull to assess primary somatosensory cortex, subcutaneous electrodes for whole-brain analysis, or craniotomy to implant a screw for prefrontal cortex (PFC) activity. However, the poor spatial resolution remains a challenge in

EEG.^{48,72} Furthermore, the contribution of signals from subcortical structures to the EEG waveforms is debated.^{47,66}

2.2. Setup and analysis

Some characteristics regarding setup for each technique are shown in **Table 1**. Briefly, fMRI in animals is performed by using an MRI scanner, radiofrequency coils, anesthesia, and a physiological monitoring system. Manganese-enhanced MRI requires the same setup as fMRI including the injection of the contrast agent. Practical issues are often faced in the MRI setup and require personnel training, including positioning of the animal and the receiver coil, choice of receiver coils, and monitoring of the anesthesia and physiological recordings. Once these issues are well settled by the research group, the quality of the data is highly improved. Positron emission tomography also uses a specific scanner, anesthesia, physiological monitoring system, and the injection of the radiotracer. By contrast, EEG requires more invasive procedures, such as surgery to place the EEG screws and electrodes in the head. The EEG setup essentially involves the use of electrodes, signal amplifier, and acquisition system. The 2 reviewed EEG articles were performed by different methods, which were recording from freely moving rats with intracranial and epicranial electrodes using tethered system⁴⁸ and recording from anesthetized rats with subcutaneous electrodes and mechanical ventilation.⁷² Magnetic resonance imaging or PET scanners are usually part of a core facility and available for use at an hourly rate. On the other hand, EEG is frequently purchased by individual laboratories at a lower cost.

A potential confound of most neuroimaging studies is the use of anesthesia. Although fMRI can be performed in awake rats, the impact of stress and anxiety associated with the restraint in these studies is a potential confound.^{9,17} Although animals are habituated to the scanning environment, awake rodent fMRI protocols can cause long-lasting changes in physiological and brain responses to pain stimuli that are stress-related.⁵⁰ Isoflurane has been the most common anesthetic source for fMRI,^{1,3,22,37,40,56,61,75,84,85,89} MEMRI,^{25,41,42} and PET.^{19,44} Halothane and sevoflurane are also inhalational anesthetics, but few studies have been used them because of the associated higher rate of liver injury and the decreased cerebral blood flow compared with isoflurane.^{20,28,68,72,78,88} Medetomidine is a sedative-analgesic that was injected by subcutaneous or intravenous infusion in 3 fMRI studies of pain.^{2,46,64} Although, medetomidine is gaining popularity as an anesthetic for rodent fMRI because it is also suitable for longitudinal studies, the BOLD signal changes during forepaw stimulation are similar to those observed under isoflurane, and it can induce bradycardia.⁸⁷ Interestingly, a resting-state fMRI study has shown that combination of isoflurane and medetomidine at lower doses better resembled the connectivity pattern from awake rats compared with either isoflurane or medetomidine alone.⁶² The mechanism of this interaction is not yet understood but might be explained by the different actions of the anesthetics: isoflurane causes vasodilation and medetomidine causes vasoconstriction, which distinctively modulate the BOLD signal.^{62,87} Isoflurane–medetomidine and α -chloralose resembled the awake condition similarly,⁶² but α -chloralose is toxic and thus not suitable for longitudinal use, being used only in terminal preparation.¹⁶ Urethane was also administered mixed with α -chloralose in a MEMRI study with rats, but bradycardia, hyperventilation, and its carcinogenic nature are disadvantages of urethane.²⁹ Anesthetics can induce several side effects; however, those are less relevant to signals from MEMRI and PET than to fMRI. BOLD relies on changes in the

cerebral blood flow and oxygen level during the scan to capture the signal, whereas the $MnCl_2$ contrast or the radiotracer is administered hours to days before the actual scan. An fMRI study with monkeys has used sedation through propofol.³⁵ Propofol is an intravenous anesthetic that can reduce heart rate and cerebral blood flow, which may change the shape of the hemodynamic response function.³⁰ Studies in rats and humans showed that propofol decreases connectivity in the thalamocortical and frontoparietal networks.^{73,79}

An additional limitation in neuroimaging studies is the variety of analysis methods, which perpetuate the challenge of data reproducibility. **Table 1** shows the diversity of software packages recently used in the reviewed articles. Software packages can differ in data input, preprocessing steps, sensitivity to correct motion, image manipulation tools, and statistical approaches and outputs.^{10,55} The most commonly used software packages for neuroimaging analysis are the Oxford Center for fMRI of the Brain (FMRIB) Software Library (FSL) and the statistical parametric mapping.^{1–3,9,17,19,22,25,35,40,46,56,61,74,75,85,88,89} Studies have shown that FSL and statistical parametric mapping perform similarly in fMRI statistical analysis.^{55,63} However, they have some weaknesses and strengths. Several studies have compared the performance of multiple software applications in human brain imaging,^{23,39,55,63} but no studies comparing the performance in animal neuroimaging studies have been published. Furthermore, it is worth mentioning that the preprocessing guidelines for animal data are far less established than for human data, eg, whereas human fMRI studies often apply spatial normalization to a common stereotaxic space, usually the Talairach or MNI templates, animal fMRI studies mostly report results in non-standard space, which make direct comparisons and meta-analysis across studies unfeasible.⁵¹

The analysis of neuroimaging data can be performed using several approaches in software packages. The most common in fMRI studies of pain is the investigation of maps showing whole-brain activity from innocuous and noxious stimuli, which is basically created by modeling a block design paradigm of on and off conditions.^{1–3,9,17,35,37,40,46,61,64,75,84,85,88,89} Other methods have been used to assess specific regions and networks. Region of interest (ROI)-based analysis is the second most used method in recent fMRI studies of pain, which can measure the magnitude of the BOLD signal within ROIs and connectivity between an ROI and the whole brain or other ROIs during noxious stimulation or rest.^{2,22,56,64,75,85,89} Model-free independent component analysis was also used to identify networks related to pain processing.^{1,9} Independent component analysis establishes networks by regions that share the same response pattern without a previous assumption of ROIs.¹⁵ Graph theory,^{37,46} dynamic causal modeling, and band pass-filtered partial correlation analysis⁷⁵ were used by few studies. These approaches can determine various characteristics of a network, such as the number of significant functional connections of an ROI,^{37,46} effective connectivity directly driven by response to stimuli,⁷⁶ and exclusion of signal components directly time-locked to stimuli and shared among network regions.⁷⁵

Functional connectivity was also inferred by MEMRI and EEG studies.^{48,74} Sperry et al.⁷⁴ used a covariance pattern model to infer connectivity through the coactivated regions during pain using MEMRI. LeBlanc et al.⁴⁸ investigated EEG by measuring the power transferred between brain regions suggesting functional connectivity. However, the localization of the brain areas was possible due to the invasive implanted screw electrodes, which could induce pain through infection or inflammation. Furthermore, 2 EEG studies of pain have investigated the different

patterns of neuronal oscillations in the frequency domain, such as the power spectra^{48,72} and F50, which is the frequency below which 50% of the signal power is present and F95, which is the frequency below which 95% of the signal power is present.⁷² Results are discussed below.

Other challenges to be addressed in neuroimaging analysis are noise and statistical power. Noise decreases signal-to-noise ratio, which consequently lowers the power to detect signals related to the task of interest.⁸⁶ Although most of the recent fMRI studies in animals have addressed this issue using physiological parameters, motion, and signals extracted from ventricles and white matter to regress out artifacts and increase signal,^{3,9,17,22,37,40,46,56,75,89} other neuroimaging techniques have not considered these variables in their current protocols.^{16,19,25,41,42,44,48,72,74} For example, non-neuronal physiological fluctuations due to pulsatility of blood flow in the brain and respiration can induce signal variance of the fMRI signal. These events are often comparable with that of the BOLD response during task or at rest, but several approaches are available to regress them out.¹⁴ From a statistical perspective, removal of non-BOLD artifacts improves effect size and statistical power. However, the easiest way of increasing statistical power is to increase sample size.²¹ Small sample sizes can produce highly variable estimates of the size and variance of the desired effect, which require the researchers to carefully justify this factor in protocols.⁵⁸ Sample size can be calculated by developed software packages, such as Neuro Power Tools (<http://www.neuropowertools.org>).

3. Mechanisms proposed by recent neuroimaging studies of pain

3.1. Acute pain

Acute pain models were induced by incision, inflammation, or mechanical, thermal, or electrical stimulation and revealed a core pattern of nociception-evoked activations^{2,3,16,25,35,37,64,72,74,89} (**Fig. 1**). The most commonly activated regions during acute pain were S1, insula, cingulate cortex, thalamus, retrosplenial cortex, and PAG, all of which are part of ascending and descending nociceptive pathways.^{2,3,16,25,35,37,64,89} These findings are similar to those of human studies showing that S1, insula, cingulate cortex, and thalamus consistently respond to acute pain and are believed to play an important role in the sensory discriminative and affective aspects of pain processing.⁵⁷ Although examining common regions identified across studies can be useful in identifying brain areas most associated with acute pain, individual studies can provide more detailed mechanisms. For example, Amirmohseni et al.² examined differential activations in incisional and inflammatory pain using mechanical or electrical stimuli. The BOLD signal changes in cingulate cortex, thalamus, retrosplenial cortex, PAG, and striatum were significantly higher in rats with inflammatory pain than in rats with incisional pain or sham upon mechanical stimulation. Mechanical stimulation also produced a bilateral activation in S1, whereas electrical stimulation caused unilateral activation in S1 in both pain models. The presence of bilateral S1 activity after unilateral mechanical stimulation could result from uncrossed afferent pathways.² Although the findings observed in knockout mice may not be directly correlated with results in physiologically normal animals, another fMRI study also confirmed that multisensory inputs are processed in different ways.³⁷ They investigated the impact of a lack of the voltage-gated sodium channel $Na_v1.8$ on brain structures and their interactions upon the perception of cold and heat noxious stimuli in knockout mice. BOLD signal amplitudes were strongly reduced in thalamus, cingulate cortex, and retrosplenial cortex upon

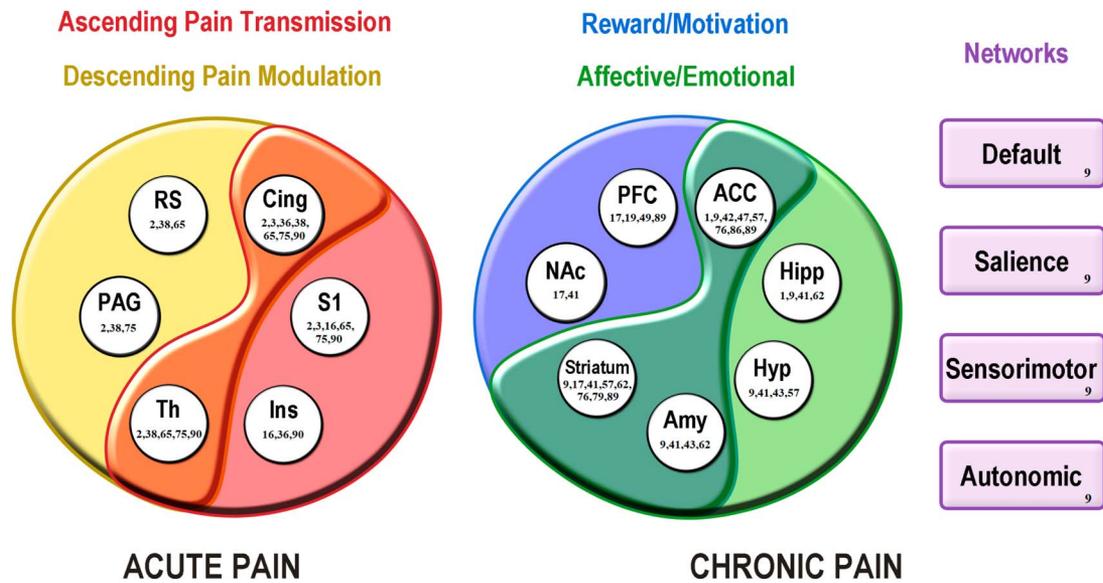


Figure 1. Altered brain activity and connectivity in animal models of acute and chronic pain. This figure summarizes the recent findings in neuroimaging studies of pain from January 1, 2016, to October 10, 2018, as indicated by the reference numbers. The color-coded circles show the proposed brain circuitries for acute and chronic pain. Acute pain is more associated with activation of regions from ascending and descending pain pathways, whereas chronic pain is more associated with regions related to reward/motivation and affective/emotional aspects of pain and altered functional connectivity of several networks. Overlap shows areas involved in both pathways or aspects of pain. ACC, anterior cingulate cortex; Amy, amygdala; Cing, cingulate cortex; Hipp, hippocampus; Hyp, hypothalamus; Ins, insula; NAc, nucleus accumbens; PAG, periaqueductal gray area; PFC, prefrontal cortex; RS, retrosplenial cortex; S1, primary somatosensory cortex; Th, thalamus.

noxious cold stimulation compared with heat stimulation.³⁷ A reduced number of connections between affective and motivational-related regions were affected by the $\text{Na}_v1.8$ knockout under cold but not under heat stimuli, eg, hippocampus and frontal cortex.³⁷ These findings show the impact of $\text{Na}_v1.8$ on noxious cold signaling and suggest its potential application in the treatment of especially cold pain states such as cold allodynia. Thus, it is important to carefully interpret the relationship between brain response and types of noxious stimulation to understand molecular mechanisms and target future treatment approaches.

Developmental study of pain processing has also been performed in rodents.⁷⁴ In accordance with a recent study with human infants,³³ Sperry et al.⁷⁴ demonstrated that both sensory and affective-related regions are activated by pain in rat pups. Using MEMRI, the authors investigated brain activity after formalin injection in 12-day-old rat pups.⁷⁴ The formalin injection induced enhanced activity of medulla, thalamus, sensory cortex, PFC, ACC, amygdala, and hypothalamus. Further research is needed to better understand the effects of development and aging on networks engaged in pain processing.

3.2. Chronic pain

Most recent animal neuroimaging studies have investigated brain mechanisms of chronic pain, including neuropathic pain, arthritis, fibromyalgia, migraine, visceral pain, trigeminal pain, and sickle cell.^{1,9,17,19,25,40-42,44,46,48,56,61,75,78,84,85,88} Despite the variety of pain models, types of stimulation, awake or anesthetic protocols, neuroimaging techniques, and analysis, a group of brain structures seems to be more involved in the later stages of pain (**Fig. 1**). A consistent observation is more widespread cerebral activity and connectivity in chronic pain compared with pain-free animals.^{17,46,48,56,75,85,88} Plastic changes have been primarily reported in S1 and ACC under chronic pain.^{46,48,56,75,85,88} An EEG study showed increased coherence between S1 and PFC at a late, but not early stage of neuropathic

pain, suggesting that chronic pain increases connectivity between regions related to sensory/discriminative (S1) and negative/aversive (PFC) dimensions of pain.⁴⁸ Prefrontal cortex has abnormal structure and function in humans with chronic pain, and studies have shown its potential as a therapeutic target for chronic pain conditions.⁷⁰ Furthermore, increased PFC metabotropic glutamate receptor 5 (mGluR5) modulated negative mood symptoms of neuropathic pain, revealed by PET.¹⁹ Several studies using fMRI and MEMRI reported enhanced activity of PFC, ACC, hippocampus, amygdala, basal ganglia, and nucleus accumbens, but not the S1 in chronic pain.^{1,17,41,42,46,56,61,75,85} Allodynia-related brain activity was not dependent on S1 in rats with neuropathic pain and instead involved the nucleus accumbens and PFC.¹⁷ The participation of basal ganglia in chronic pain can also be demonstrated by the reduced opioid receptor availability in caudate-putamen of rats with chronic neuropathic pain.⁷⁸ A PET study observed the positive correlation between anhedonia/depression-like behavior and the deficit of opioid receptor expression, suggesting the contribution of the opioid system to the comorbid depression in chronic pain.⁷⁸ Regions mentioned above are known to be part of brain circuits related to emotion and motivation.⁵⁹ Onishi et al.⁶¹ observed that activation of the amygdala and hippocampus could be, at least in part, responsible for the persistent electrical hyperalgesia and allodynia seen in a chronic stage of neuropathic pain. Similarly, Jeong and Kang⁴¹ demonstrated that neuropathic pain is transmitted to the S1 and parietal region through the cingulum bundle and limbic system. Furthermore, mechanical hyperalgesia from osteoarthritis pain induced more widespread brainstem and ACC activity, compared with stimulation of the noninjured paw.¹ Thus, data from rodent studies reinforce the idea that chronic pain is associated with altered brain activity in many regions encoding affective, emotional, reward, and motivational contexts of pain.

Connectivity of the limbic system is increased in chronic pain and ACC may be a key region modulating this network.^{46,56,75,85,88}

In addition to receiving afferent nociceptive information, ACC modulates the emotional and motivated behaviors of chronic pain through increased connectivity to striatum, hypothalamus, and mediodorsal thalamus.^{46,56,75,88} Morris et al.⁵⁶ showed that higher ACC connectivity to hypothalamic/preoptic nuclei and the bed nucleus of the stria terminalis correlated with the reduction of motivated behavior, which was represented by burrowing behavior in rats with arthritis pain. Furthermore, allodynia induced by air-puff stimulation increased activity in the ACC and decreased activity in S1 in rats with chronic trigeminal pain.⁷⁵ Anterior cingulate cortex also exerted an increased influence on superior colliculus and striatum, which suggests the role of ACC in the chronification of pain.⁷⁵ Of the studies reviewed, one with migraine model reported altered resting-state fMRI functional connectivity in a number of networks previously identified in humans with the same chronic pain condition.^{9,49} The networks included the default mode, sensorimotor, interoceptive (saliency), and autonomic circuits.⁹ The findings reviewed in this section showed similarities according to the chronic pain model; however, further research is needed to accurately replicate methods, estimate effect sizes, and compare them critically with the results of the original studies. Taken together, these findings suggest widespread modulation of emotional, motivational, and cognitive responses to chronic pain.

3.3. Descending pain modulation

Three studies reviewed the brain circuitry of endogenous pain inhibitory systems.^{22,40,44} In our own fMRI study, diffuse noxious inhibitory controls (DNIC) and brain networks were evaluated with respect to sex differences.²² We reported that the strength of DNIC behavior was higher in males compared with females and males without testosterone, and brain circuitry in the DNIC state is modulated by sex. Descending pain pathways, including connections between PAG, prelimbic cortex, ACC, and insula had stronger connectivity in males during DNIC induction. Females and nontestosterone males had increased connectivity in areas related to emotional and affective components of pain, such as ACC, hippocampus, and thalamus, compared with intact males. Nontestosterone males additionally had stronger connectivity of the nucleus accumbens with prelimbic cortex, ACC, insula, and thalamus. Our findings suggested that testosterone plays a key role in reinforcing the endogenous pain inhibitory system, while circuitries related to reward and emotion are more strongly recruited in the absence of testosterone.²² Human studies showed that females and males potentially differ in pain-related brain activation through different pathways, and testosterone may be a key factor in modulating pain sensitivity through descending circuits.^{11,34,82,83} We also previously investigated the estrogen stress-dependent interactions in response to noxious visceral stimulation in females using fMRI.⁴⁰ Noxious visceral stimulation activated insula, ACC, and amygdala, whereas estrogen dramatically altered this visceral nociceptive processing in the brain after an acute stressor. These alterations included increased activation of amygdala, striatum-pallidum, nucleus accumbens, superior and inferior colliculi, and cerebellum, and decreased activation of the medial thalamus, hippocampus, and brainstem regions. These findings could reflect an upregulation of stress-related circuits and downregulation of descending modulatory circuits.⁴⁰ The findings also support that sex hormones can dramatically alter nociceptive processing in the brain. The final reviewed study investigating descending pain pathways used PET to demonstrate that motor cortex stimulation (MCS) can attenuate neuropathic pain by increasing activity of striatum, thalamus, and cerebellum.⁴⁴ The impact of acute and chronic

pain on reward/motivation circuits gained from preclinical models is also found in the human literature.^{60,90} Furthermore, they performed immunohistochemistry to investigate changes in c-fos and serotonin expression, as well as extracellular electrophysiological recordings. These findings showed increased c-fos activity and amount of serotonin in lumbar levels of spinal cord and PAG, suggesting that MCS may be a descending modulator of neuropathic pain. Serotonin is a major neurotransmitter involved in descending pain modulation, as well as GABA and dopamine.⁷ Dopamine is known to be released by striatum, and MCS in neuropathic rats induced higher activity in this region.⁴⁴ Thus, releases of dopamine by striatum and serotonin by PAG may be responsible for the pain relief achieved by MCS. Unfortunately, the sex of the rats was not reported.⁴⁴

3.4. Interventions

Analgesic effects of pharmacological and alternative treatments have been evaluated using neuroimaging techniques.^{35,41,44,48,64,72,88} An EEG study investigated the effects of pregabalin ($\alpha 2\delta$ -ligand gabapentinoid from the class of anticonvulsants) and mexiletine (nonselective voltage-gated sodium channel blocker from the IB class of antiarrhythmic agents) on EEG power. They showed that pregabalin and mexiletine reversed the changes in EEG power of S1 and PFC and S1-PFC coherence in inflammatory and neuropathic pain models in rats, but similar effects were not observed when ibuprofen was given in an acute capsaicin model. Gabapentin, also an $\alpha 2\delta$ -ligand gabapentinoid, decreased activity in the posterior parietal association area, superior and inferior colliculi, S1, ACC, and cingulum bundle in rats with neuropathic pain who underwent MEMRI.⁴¹ Hama et al. also showed that pregabalin reduced bilateral ACC activation in monkeys with acute post-operative pain during non-noxious pressure stimulation using fMRI. However, morphine was able to reduce both ACC and bilateral insula activation under the same condition.³⁵ This lack of reduced insula activation by pregabalin may represent a specific effect of this drug on ACC, while the morphine may have a broader action on pain-related brain responses. Changes in EEG responses to noxious thermal and electrical stimuli were seen after administration of 2 exogenous opioids, morphine, or opiorphin in rats.⁷² F50 and F95 values were similar to baseline after administration of either compound, which suggests that EEG signal may reflect the modulatory processes induced by opioids.⁷²

Nonpharmacological therapies have also been investigated.^{44,64,88} Motor cortex stimulation improved neuropathic pain by modulating endogenous pain inhibitory system, as discussed above.⁴⁴ An fMRI study showed that dorsal root ganglion electrical stimulation, ie, ganglionic field stimulation (GFS), could reduce activity of brain regions comprising the ascending spinothalamic system, specifically the contralateral thalamic nuclei, S1, and secondary somatosensory cortex (S2). BOLD responses in caudate-putamen, nucleus accumbens, globus pallidus, and amygdala were also reduced with GFS.⁶⁴ Thus, GFS may not only reduce pain intensity but could also modulate the saliency and motivational aspects of acute pain. Wu et al.⁸⁸ have shown longitudinal changes in brain activity of rats with chronic neuropathic pain and electroacupuncture. They performed a sciatic nerve transection model followed by electroacupuncture 5 times per week for 4 months, and fMRI scans acquired every month after treatment. Areas of the somatosensory cortex were more activated in the first 2 months and then deactivated in the following 2 months in the treated group. By contrast, limbic areas were not constantly activated or deactivated, showing a fluctuating pattern in activity during treatment.⁸⁸ All 3 of the above nonpharmacological

treatments had analgesic effects. Taken together, these findings show that efficient pharmacological or nonpharmacological treatments can potentially reverse the maladaptive neuroplasticity of chronic and acute pain.

4. Considerations for future studies and conclusions

The neuroimaging studies discussed in this review aimed to determine brain functional responses to several models of acute and chronic pain. Each technique can be used to address a particular question. For example, fMRI is the gold standard for longitudinal studies because of its noninvasiveness; MEMRI uses the MnCl₂ contrast, which is a calcium analog that can enter excitable cells and provide highly resolved functional and structural information; PET has a variety of radiotracer combinations that can be used to investigate cerebral metabolic processes, including receptor-binding and glucose metabolism; and EEG is the most direct technique to measure neuronal activity but is limited in spatial resolution. The use of multiple neuroimaging techniques can help address some limitations. Nonconventional anesthetic methods are encouraged, such as mixed isoflurane and medetomidine. Neuroimaging studies of awake animals might be limited by induced stress and unknown effects of the training. Subsequently, the variety of analytical methods is a common problem of neuroimaging techniques. It is recommended to use 2 or more software packages to enable higher reproducibility and refinement of the analytical methods in neuroimaging research.

Finally, investigators should take into consideration the problem of sex bias and omission in neuroimaging research of pain. From the 28 recent studies reviewed here, 20 were performed in males, 3 in females, 4 did not report the sex, and 1 was performed in males and females showing sex comparisons. Sex differences in pain perception have been shown in many studies in human subjects,^{34,52,54,67} and women are generally afflicted by chronic pain at higher rates than men.⁸ Furthermore, to the best of our knowledge, effects of aging have not been investigated by animal neuroimaging pain studies. Further research is needed to better understand the multidimensional aspects of pain in the brain. Several newer technologies could be combined with neuroimaging, including transcranial focused ultrasound, to induce neuromodulation in specific brain areas or opening of the blood-brain barrier²⁴ and chemogenetic fMRI to remotely stimulate chemical-specific neurons during fMRI.³¹ To conclude, brain imaging studies in animals are adding important contributions to our understanding of pain processing, modulation, treatment effects, and treatment targets.

Disclosures

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