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Chronic non-inflammatory muscle pain: central and peripheral mediators

Joseph Lesnak and Kathleen A Sluka

Conditions with chronic widespread non-inflammatory muscle pain, such as fibromyalgia, have complex etiologies with numerous proposed mechanisms for their pathophysiology of underlying chronic pain. Advancements in neuroimaging have allowed for the study of brain function and connectivity in humans with these conditions, while development of animal models have allowed for the study of both peripheral and central factors that lead to chronic pain. This article reviews the current literature surrounding the pathophysiology of chronic widespread non-inflammatory muscle pain focusing on both peripheral and central nervous system, as well as immune system, contributions to the development and maintenance of pain. A better understanding of the mechanisms underlying these conditions can allow for improvements in patient education, treatment and outcomes.

Address
Department of Physical Therapy and Rehabilitation Science, Neuroscience Institute, Pain Research Program, University of Iowa, 1-242 MEB, Iowa City, IA 52252, USA

Corresponding author: Sluka, Kathleen A (kathleen-sluka@uiowa.edu)

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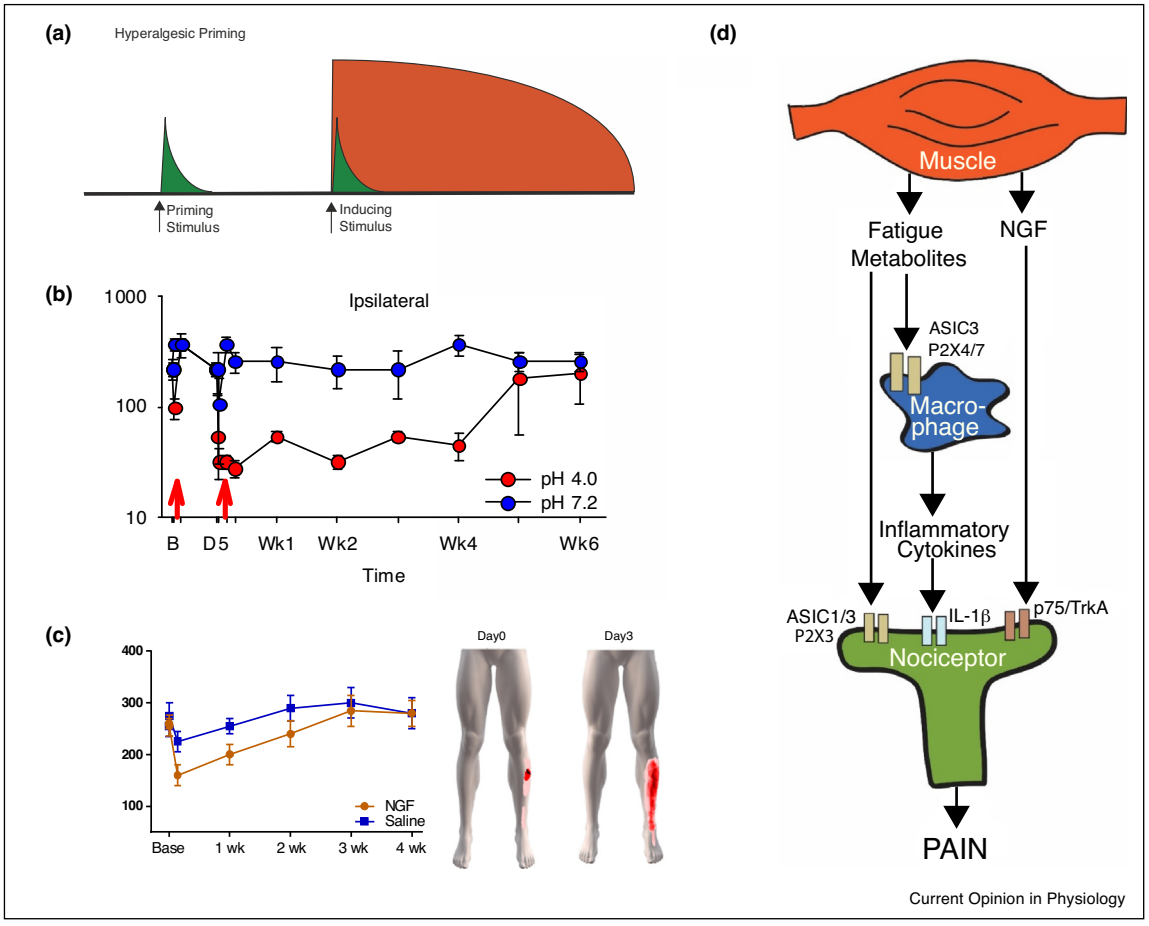
Introduction

Chronic pain is a significant health problem affecting over 100 million Americans—more than diabetes, cancer, and heart disease combined, with musculoskeletal pain affecting between 13–47% of the population [1]. Pain that persists beyond tissue healing time and without overt damage to peripheral tissues is common and difficult to treat. Clinically, localized pain is less common with only 20% of those surveyed reporting pain in one area [2]. Participation in social, physical, and daily activities progressively decrease in relation to the number of pain areas [2]. Women have a greater incidence of musculoskeletal pain [3], are more likely to have five or more areas of pain (23%) compared with men (11%) [2], and infusion of acidic buffer into muscle results in more referred pain in women (80%) compared with men (40%) [4]. Chronic pain can be defined as pain that persists past normal tissue healing time, in the absence of nociceptive input, or that is out of proportion to the injury. Animal models to mimic this type of chronic pain have been developed which allow researchers to investigate potential mechanisms of chronic widespread pain. This review will focus on mechanisms underlying chronic widespread muscle pain.

Models of non-inflammatory widespread pain

Induction of long-lasting, widespread hyperalgesia occurs in response to multiple stressors or insults to the animal [5–15]. These insults can include multiple muscle insults such as inflammation, acute fatiguing exercise, and decreases in local pH; or can include stress, obesity, and sedentary activity levels combined with muscle insult. The most common models involve repeated insults to somatic tissue, including muscle, and have been referred to as hyperalgesic priming. Hyperalgesic priming uses an initial priming stimulus, followed by a second inducing stimulus to result in a longer-lasting and enhanced hyperalgesia (Figure 1a). The priming stimulus has varied and includes inflammatory stimuli such as carrageenan, interleukin-6 (IL-6), or prostaglandins, and non-inflammatory stimuli such as acidic saline and/or fatiguing stimuli [5–7,9,10,16]. After the hyperalgesia resolves from the priming stimulus, a second stimulus is given, which results in a longer-lasting hyperalgesia with minimal to no tissue damage. For example, two injections of pH 4.0 saline into the gastrocnemius muscle, given 2–5 days apart, results in widespread, long-lasting hyperalgesia (weeks); a single injection of pH 4.0 saline has only short-term effects (hours) [5] (Figure 1b). Similarly, injection of PGE2 after carrageenan-induced muscle inflammation has resolved results in long-lasting hyperalgesia (weeks); a single injection of PGE2 has only short-term effects (hours) [9]. In parallel studies, in animals exposed to stressful conditions, an injection of PGE2 results in long-lasting hyperalgesia (weeks) [17,18]. Fatiguing exercise induced by whole-body fatigue or by local electrical stimulation of a single muscle combined with a subthreshold muscle insult also results in widespread and long-lasting hyperalgesia (weeks); fatigue or the muscle insult alone has no effect [6,7,16,19,20]. Interestingly, the hyperalgesia that develops after fatigue combined with muscle insult is greater in female mice compared with male mice, is more widespread in female mice, is easier to induce in female mice, and is longer lasting in female mice [6,7]. Thus, there are several animal models that can result in a long-lasting, widespread and exaggerated hyperalgesia.

Figure 1



(a) Illustration of hyperalgesic priming. A single priming stimulus produces a short-lived hyperalgesic response that resolves (green). A second inducing stimulus, given within a critical window, will then produce a longer-lasting and more robust hyperalgesia (orange). For comparison the initial priming response is overlaid on the enhanced response. Copyright by Dr Kathleen Sluka. **(b)** Intramuscular injection of pH 4.0 produces a short duration decrease in withdrawal threshold to mechanical stimulation of the paw. A second injection five days later produces a greater and longer lasting decrease in withdrawal threshold. Reproduced with permission from Ref. [5]. **(c)** Injection of NGF into human masseter (MA) muscle caused a decrease in masseter muscle pressure pain thresholds (PPT) at day one and day seven following injection. This effect was localized to the masseter muscle since no decrease in PPT at the masseter muscle was seen following injection of NGF into the temporalis (TA) muscle. Muscle contraction produces pain over the tibialis anterior muscle after NGF injection that results in a greater area of pain after several days. Reproduced with permission from Refs. [33,34]. **(d)** Model of peripheral mediators implicated in the development of pain. Fatigue metabolites (H+, ATP) and nerve growth factor (NGF) secreted from muscles activate nociceptors by binding to their receptors. Fatigue metabolites also activate macrophages to increase the production of inflammatory cytokines which work to activate nociceptors. Copyright by Dr Kathleen Sluka.

Peripheral drivers of chronic pain

Nociceptors

Nociceptors are sensory receptors that relay mechanical, thermal, or chemical signals of damaging or potentially damaging stimuli to the central nervous system. Nociceptors are located throughout the body and have peripheral terminals in tissues such as skin, muscle, joint, and visceral organs. Nociceptors have the potential to undergo plastic changes in response to repeated stimuli resulting in sensitization allowing the organism to avoid damaging stimuli [21,22]. However, in the case of chronic pain, this plasticity can outlast the tissue damage or threat, and thus could not serve for a protective purpose [23–26]. We will discuss mediators and systems that can sensitize nociceptors and produce long-lasting, widespread hyperalgesia including fatigue metabolites, neurotrophins, and cells and factors associated with the immune system.

Fatigue metabolites

Fatigue metabolites are released from contracting muscle and can activate receptors located on immune cells or nociceptors to sensitize primary afferent fibers. These metabolites include adenosine triphosphate (ATP), lactate, or decreases in pH which can activate purinergic receptors or Acid Sensing Ion Channels (ASICs), respectively. Combined intramuscular injection of protons,

lactate, and ATP reduces muscle withdrawal threshold in rats while lactate or ATP alone does not reduce the withdrawal threshold [27]. Group III and IV muscle nociceptors are activated by fatigue metabolites mixtures of protons, lactate, and ATP [28]. In DRG neurons, combinations of these fatigue-metabolites synergize to enhance intracellular calcium release and currents [29,30]. In both muscle nociceptors and DRG neurons two subgroups were found to respond to differing metabolites, low and high [29]. In human subjects, intramuscular injection of the combined metabolites produces a synergistic response in a dose-dependent manner with low-dose metabolites producing a fatigue-sensation and high dose metabolites producing a pain-sensation [31]. Thus, decreases in pH, and increases in lactate and ATP produce a synergistic activation of nociceptors to produce hyperalgesia [27,31].

Decreases in pH and increases in lactate activate ASICs and both ASIC3 and ASIC1 are involved in development of chronic widespread pain. In a hyperalgesic priming model using two injections of pH 4.0 saline, mechanical hyperalgesia does not develop in ASIC3 knockout mice or if a non-selective ASIC antagonist is given before the second injection; however, ASIC1 knockout mice still develop hyperalgesia similar to wild-type mice [32–34]. Repeated acid injection produces a bilateral spread of receptive fields of dorsal horn neurons that does not occur in ASIC3 knockout mice [32]. Similarly, using muscle fatiguing stimuli combined with a subthreshold acidic saline injection (2, pH 5.0 saline injections), genetic or pharmacological blockade of ASIC3 prevents development of hyperalgesia [33,35]. Interestingly, in this fatigue-induced pain model, pharmacological blockade of ASIC1 prevents development of hyperalgesia and alters the kinetics of heteromeric ASICs [36••]. Thus, both ASIC1 and ASIC3 mediate the effects of hyperalgesic priming by acid and fatiguing stimuli.

Adenosine Triphosphate (ATP) is a metabolite that is implicated in the development of the muscle hyperalgesia. ATP binds to purinergic receptors (P2X and P2Y), activates group III and IV muscle nociceptors, and produce hyperalgesia [37–40]. ATP-mediated mechanical hyperalgesia is facilitated through activation of P2X while ATP-mediated thermal hyperalgesia is induced through the activation of P2Y and TRPV1 [41–43], showing that ATP is involved in development of multiple pain mechanisms.

Nerve growth factor

The neurotrophin, nerve growth factor (NGF), has been extensively studied for its role in muscle pain. NGF is synthesized by skeletal muscle [2], is increased in individuals with chronic migraine headaches and fibromyalgia (FM) [44,45], and injection of NGF into human subjects reduces pain thresholds to mechanical stimuli both pressure and muscle contraction, but does not produce spontaneous pain [46••,47] (Figure 1c). Recordings from C-fibers in rats show intramuscular injection of NGF produces long-lasting sensitization in female mice [48]. NGF activates approximately 60% of Group IV muscle nociceptors [49], and sensitizes nociceptors to heat and mechanical stimuli [50,51]. Mechanical hyperalgesia is induced by NGF injection into rat muscle [48], and this hyperalgesia is prevented by blockade of the tropomyosin related kinase A (TrkA) [48] or p75 neurotrophin receptor [52]. Peripheral injection of NGF activates the majority of dorsal horn neurons, recorded intracellularly, as evidenced by excitatory post-synaptic potentials (EPSPs), and results in increased responsiveness to noxious and innocuous mechanical stimuli [49]. Thus, animal studies parallel findings in humans.

NGF can alter neurotransmitter expression on nociceptors to make them more excitable. For example, there is increased expression of the NR2B subunit of *N*-methyl-D-aspartate (NMDA) receptors in dorsal root ganglia (DRG) neurons innervating muscle after injection of NGF that is longer-lasting in females [53]. However, blockade of NMDA receptors in muscle reverses hyperalgesia in male, but not female mice [53], suggesting NR2B changes are not involved in hyperalgesia in females. Indeed, females show increases in the neuropeptide Substance P in NR2B positive muscle DRG that does not occur in males [53]. Thus, NGF is involved in the development of hyperalgesia which is sexually dimorphic in animal models.

Immune system

Resident macrophages are located in most tissues including muscle, play a role in protecting against disease [54,55] and recent studies show a role for macrophages in chronic muscle pain models. In the acid-induced and fatigue-induced priming models there are increases in the number of macrophages in the injected muscle, depletion of macrophages in muscle prevents development of hyperalgesia, and blockade of the immune cell receptor toll-like 4 (TLR4) during a priming injection prevents development of hyperalgesia after the second injection [35,56••]. Macrophages produce their effects through release of cytokines. In sedentary animals, the M1 macrophage phenotype releases inflammatory cytokines that can subsequently activate and sensitize nociceptors [57,58]. The inflammatory cytokines, IL-1β and IL-6, sensitize group IV muscle nociceptors [59,60], induce mechanical hyperalgesia [61], increase the number of macrophages in muscle [56••,61], and prime the animal to subsequent stimuli like acid or prostaglandin [9,56••]. In contrast, the M2 macrophage phenotype secretes anti-inflammatory cytokines such as IL-10 which prevents development of hyperalgesia [62]. Physically active animals show an increase in the proportion of M2 macrophages in the muscles and the acid-induced priming

model does not develop; this analgesia is prevented by blockade of IL-10 [62]. Thus, peripheral macrophages within the muscle are involved in the generation of chronic muscle pain through inflammatory cytokines (Figure 1d).

Central drivers of chronic muscle pain

Multiple central pathways have been discovered to play a role in the generation and maintenance of chronic widespread pain [63–66]. In humans, imaging studies have extensively examined cortical sites and connectivity, while in animals, studies have examined multiple sites, neurotransmitter systems, and intracellular pathways involved in the generation of widespread pain [65,67–69].

Imaging

Advancements in neuroimaging have led to a better understanding of brain mechanisms involved in chronic pain. Functional magnetic resonance imaging (fMRI) shows that individuals with FM have greater brain activity in pain processing regions in response to peripherally applied noxious stimuli when compared to healthy controls [70–72]. More recent studies show increased connectivity between the default mode network (DMN), a network of the brain active during resting state, and the insula in individual's with FM that is associated with ongoing pain [73] (Figure 2a). Simultaneously, there is decreased activation of the rostral anterior cingulate cortex (ACC), periaqueductal grey (PAG), rostral ventromedial medulla (RVM), and thalamus during pain states in individuals with FM, all of which are key locations in the descending pain modulatory system [74–76,77••]. Further, there is decreased grey matter volume in multiple cortical areas including ACC, PAG, amygdala, insula, prefrontal cortex, cingulate, striatum, and parahippocampal gyri [77••,78–80], with the decreases in grey matter in the PAG associated with decreased efficiency of descending pain modulation [77••]. Thus, imaging studies show changes in multiple systems that are consistent with enhanced central excitability and reduced central inhibition in individuals with chronic widespread pain.

Central nervous system glial activation in pain processing is well-established in animal models of pain [81]. Although not studied extensively in animal models of chronic non-inflammatory muscle pain, recent imaging studies show increased glial activity in individuals with FM. In individuals with chronic pain, FM and low back pain, positron emission tomography (PET) using a glial activation marker, translocator protein (TPSO), show enhanced activity in the cingulate cortices; this activity correlates with fatigue [82,83••]. Interestingly, FM subjects with a genetic polymorphism for TSPO conferring high binding affinity report high pain intensity; this effect was modulated by the serotonin transporter gene [83••,84] (Figure 2b).

Glutamate/NMDA

Glutamate is an excitatory amino acid which binds to *N*-methyl-D-aspartate (NMDA) receptor and is crucial in the development of muscle pain. In human subjects with FM, proton magnetic resonance spectroscopy shows increased levels of glutamate are found in the right amygdala [85], posterior insula [86], posterior gyrus [87], and posterior cingulate cortex [88]. The levels of glutamate correlated with FM-symptoms including pressure pain thresholds, function, and pain catastrophizing [86–88].

In the acid-hyperalgesic priming model, the second injection increases release of the excitatory neurotransmitter glutamate in the spinal cord dorsal horn and brainstem rostral ventromedial medulla (RVM) [89,90], key areas involved in nociception. In the RVM and spinal cord, blockade of NMDA receptors both prevents and reverses development of acid-induced priming hyperalgesia [89,91–93]. The NR1 subunit of the NMDA receptor is required for NMDA receptor expression at the synapse, and when phosphorylated increases trafficking to the synapse and increases NMDA currents [94,95]. In the acid-induced and fatigue-induced model, there is increased phosphorylation of the NR1 subunit (p-NR1) in spinothalamic tract cells in the spinal cord, and increased p-NR1 in the RVM [16,96]. Further, overexpression of NR1 in RVM neurons produces mechanical hyperalgesia, while downregulation of NR1 in the RVM prevents acid-induced priming hyperalgesia [93]. Thus, glutamate and NMDA receptors play a significant role in development and maintenance of chronic muscle pain in the cortex, brainstem, and spinal cord.

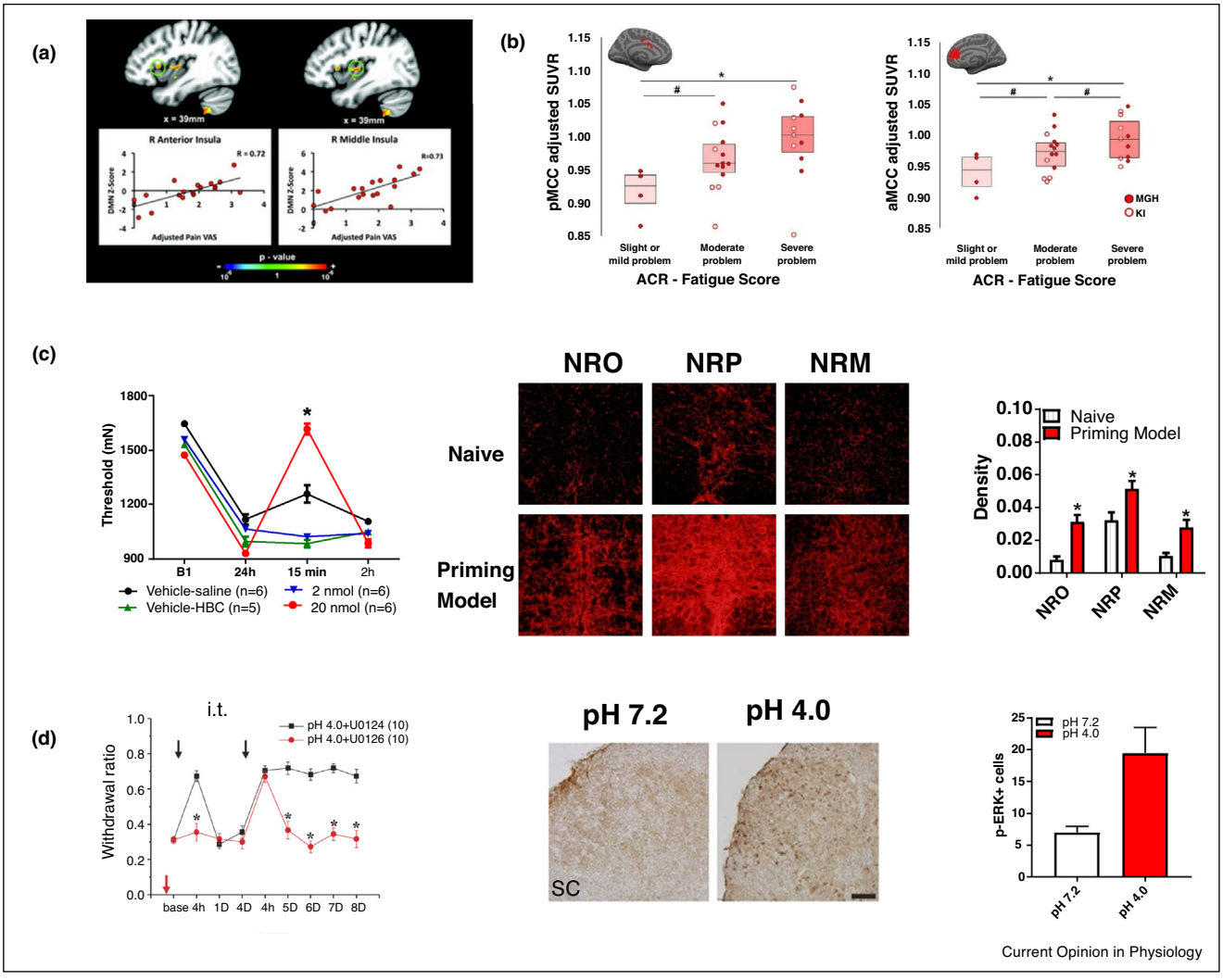
Serotonin

Serotonin (5-HT) has a complex role in pain processing showing both pro-nociceptive and anti-nociceptive effects depending on the location and receptor subtype it is acting on. Reuptake inhibitors are associated with reductions in pain in individuals with FM [97], and reduce hyperalgesia in animal models of muscle pain [98], suggesting a role for serotonin in maintaining the hyperalgesia. In the RVM, increases in serotonin are analgesic [99,100] while depletion of serotonin, using reserpine, produces widespread hyperalgesia [98,101]. Endogenous activity of 5HT is mediated through the serotonin transporter (SERT) which controls the reuptake of 5HT. In both the acid-induced and fatigue-induced hyperalgesic priming models there is enhanced expression of SERT while pharmacological blockade of SERT in the RVM reverses hyperalgesia [102,103] (Figure 2c). In human subjects with FM there is a higher proportion of individuals with a SERT polymorphism that confers lower serotonin tone [104]. Thus, the serotonin system, and in particular SERT, may play a critical role in development of widespread hyperalgesia.

Intracellular messengers

In the acid-induced hyperalgesic priming model, there are increases in phosphorylation of the transcription factor

Figure 2



(a) Higher degree of intrinsic connectivity is seen between the default mode network (DMN) and the insula in people with fibromyalgia (FM). Intrinsic DMN connectivity to the right (R) anterior and middle insula was correlated greater spontaneous pain intensity on a visual analog scale (VAS) at the time of the fMRI. Red circles represent individuals with FM. Reproduced with permission [73]. **(b)** Increased activity in the anterior and posterior middle cingulate cortex (MCC) measured by standardized uptake value ratio (SUVR) was found for individuals with FM reporting higher levels of fatigue on the American College of Rheumatology (ACR) fibromyalgia diagnostic criteria. There were no other significant associations between SUVR at these cortical sites and any other clinical variable. Reproduced with permission from Ref. [83••]. **(c)** Blockade of the serotonin transporter by 20 nmol of fluoxetine injected in the rostral ventromedial medulla (RVM) 24 hours after the second acid injection resulted in a reversal of hyperalgesia measured by muscle withdrawal thresholds in the acid hyperalgesic priming model. Reproduced from Ref. [102]. Immunohistochemical staining of SERT in the nucleus raphe obscurus (NRO), nucleus raphe pallidus (NRP) and nucleus raphe magnus (NRM) in naïve and WT mice 24 hours after induction of activity-induced hyperalgesia priming model. There was an increase in the SERT immunoreactivity in the NRO, NRM and NRP of the RVM in WT mice following induction of the model. Reproduced with permission from Ref. [103]. **(d)** Blockade of ERK with the MEK inhibitor during the priming injection prevented development of hyperalgesia after the second injection. Photomicrographs show immunohistochemical staining for phosphorylated extracellular signal-related kinases (pERK) in the dorsal horn of the spinal cord 2 hours after the acid priming injection. The bar graphs show a significant increase in the number of cells labeled with p-ERK after the first priming injection compared to pH 7.2 injection. Reproduced with permission from Ref. [106••].

extracellular signal related kinase (pERK) in the central nociceptive pathways: spinal cord, amygdala and the paraventricular thalamic nucleus anterior in response to the first [105,106••,107,64] and second injection [107]. Blockade of ERK in the spinal cord (it), but not peripherally (im) or supraspinally (icv), before the first injection prevents, while blockade of ERK in the amygdala reverses, the hyperalgesia. Similarly, blockade of protein kinase C in the spinal cord, which phosphorylates ERK, also prevents hyperalgesia [106••]. The priming

acid injection enhanced long-term potentiation in the spinal cord; this was prevented by pharmacological blockade of ERK [106••]. In the amygdala, an area involved in the affective dimension of pain, there is increased synaptic transmission between the parabrachial nucleus and the central amygdaloid nucleus after second acid injection that is prevented by pharmacological blockade of ERK (Figure 2d). Together these data suggest that the transcription factor ERK play a role in the generation and maintenance of chronic widespread hyperalgesia at multiple sites involved in nociceptive transmission.

Conclusion

Chronic widespread muscle pain in mediated through both peripheral and central factors. Peripherally, fatigue metabolites, neurotrophins, intracellular messengers, and the immune system can sensitize nociceptors leading to hyperalgesia. In the central nervous system, changes in neurotransmitters and intracellular pathways can lead to hyperalgesia, and imaging studies have identified specific cortical site alterations associated with chronic pain. These changes in both the periphery and the central nervous system can lead to the development of chronic widespread muscle pain.

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Conflict of interest statement

Nothing declared.

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