Mechanisms and manifestations in musculoskeletal pain

from experimental to clinical pain settings

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ABSTRACT

Musculoskeletal pain is extremely frequent, a major socio-economic burden, and contemporary treatment is inadequate. Acute musculoskeletal pain presents with localised and referred pain as well as localised deep-tissue hyperalgesia, whereas chronic pain often presents with expanded pain areas and widespread hyperalgesia. The mechanistic interplay in the transition from acute to chronic musculoskeletal pain is unclear. Insights can be gained from human mechanistic pain biomarkers combined with experimental human musculoskeletal pain models prolonged for several days. Deep-tissue has the capacity to nociceptively provoke local and/or referred pain and localised hyperalgesia. Referred pain areas expand with prolonged pain. Temporal summation of deep-tissue pain (a pro-nociceptive spinal mechanism) is facilitated in persistent musculoskeletal pain and may partly predict pain sensitivity in asymptomatic participants exposed to long-term pain models, and the treatment outcome in patients with musculoskeletal pain. Anti-nociceptive mechanisms governed by descending inhibitory controls is intriguing as an impairment may explain widespread hyperalgesia. Nonetheless, conditioning pain modulation (an anti-nociceptive mechanism) in experimental pain models and chronic musculoskeletal pain have demonstrated impaired efficacy with inconsistency. Studies of the brain circuitry in musculoskeletal pain have generally demonstrated reorganisation and neuroplastic manifestations in chronic conditions, and experimental pain studies show that cortical adaptations happen immediately or within days. Overall, a trait of the musculoskeletal pain system being more vulnerable to persistent pain could be hypothesised as 1) spinal mechanisms which easily become sensitised, and 2) less flexible cortical reactions when adaptive responses are normally requested (less neuroplastic capacity), thus affecting the control of e.g., the spinal excitability.

Mechanisms and manifestations in musculoskeletal pain: from experimental to clinical pain settings

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Abbreviations: Low back pain (LBP), temporal summation of pain (TSP), conditioning pain modulation (CPM), non-invasive brain stimulation (NIBS), transcranial direct current stimulation (tDCS), repetitive transcranial brain stimulation (rTMS), nerve growth factor (NGF).

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INTRODUCTION

Musculoskeletal pain is an extensive societal challenge, due to the large population affected, an increased expected life span, and the lack of efficient prevention and treatment modalities. Globally approx. 1.3 billion cases of musculoskeletal disorders were reported in the Global Burden of Disease Study 2017, with a higher point prevalence in women than in men, and 95% of prevalent cases were disorders frequently associated with pain such as low back pain (LBP), neck pain, osteoarthritis, rheumatoid arthritis, and other rheumatologic conditions [198]. As a condition it represents a major source of socio-economic burden due to increased healthcare utilisation and lost productivity, not to mention the distress caused for patients and relatives. Information regarding peripheral and central neurophysiological mechanisms is essential to improve diagnosis and therapy. A major knowledge gap is the mechanistic interplay in the transition from acute to chronic clinical pain, and from phasic (seconds) and tonic (minutes) pain to long-term pain (days/weeks) in experimental conditions. The overarching theory is that musculoskeletal pain mechanisms are facilitated due to the continuous peripheral nociceptive barrage and neuroplastic manifestations may cause a change in symptomatology from localised pain and hyperalgesia to expanded pain areas with widespread hyperalgesia.

Based on the clinical manifestations of musculoskeletal pain, the aim of this review is to present the translational aspects of deep-tissue nociception, the unique distribution of musculoskeletal pain and hyperalgesia, disturbed anti-nociceptive and pro-nociceptive modulatory mechanisms, and adaptations of cortical circuitry in musculoskeletal pain. The translational findings are outlined with emphasis on provoking and probing the pain system in human experimental and clinical settings (Fig. 1). Although important for musculoskeletal pain, psychological and motor-control aspects are not within the scope of this review.

Clinical manifestations of musculoskeletal pain

Musculoskeletal pain is frequently expressed as a tight, drilling, diffuse, and radiating sensation with pain referrals away from the pain origin, as well as localised and widespread deep-tissue hyperalgesia [77,78,161]. Deep-tissue pain is therefore difficult to distinguish among pain from various tissues such as muscle, bones, tendons, ligaments and joints. Nonetheless, joint pain is often more localised than myalgia, and bone pain is frequently worse at night but less affected by movements in contrast to e.g., myalgia and joint pain. Deep-tissue hyperalgesia and especially referred pain are unique characteristics for musculoskeletal pain. Clinical cases with referred pain

can be patients with hip osteoarthritis presenting with pain referred to the thigh or knee joint, cervical spondylosis may give referred pain to arm muscles, and lateral elbow pain is sometimes referred from the shoulder region in cases with tendinitis of the supraspinatus muscle. There is often a discrepancy between the degree of tissue damage and the pain intensity e.g., significant joint degeneration in osteoarthritis is not necessarily painful in all patients [62], illustrating that other mechanisms are likely in play. Musculoskeletal pain is categorised as localised, regional, or widespread. Localised pain is found in e.g., joint pain like patellofemoral or osteoarthritis pain whereas localised tenderness and referred pain caused by active myofascial trigger points in the myofascial pain syndrome is an example of a regional muscle pain. Examples of widespread musculoskeletal pain are e.g., fibromyalgia and whiplash associated disorders.

Musculoskeletal pain is defined as nociceptive and recently the nociplastic category was instated which may include the widespread musculoskeletal pain conditions where a pain origin cannot be determined. Neuropathic conditions affecting deeper structures is often neglected, although the German Research Network on Neuropathic Pain includes one parameter assessing the deep-tissue pain sensitivity [195]. A major challenge is to classify musculoskeletal pain with nociceptive, neuropathic and nociplastic mechanistic characteristics [216].

Translational findings on deep-tissue nociception

Muscle

Free nerve endings located around vascular structures and in connective tissue is the basic construct for muscle nociceptors. Polymodal muscle nociceptors related with group III and IV (Aδ and C) afferent nerve fibres can be predominately excited by strong (noxious) mechanical or chemical stimulation and this is presumably associated with perception of acute muscle pain [162].

Provocation of experimental muscle pain in humans has been standardised over the past years. Using physiological stimuli such as exercise or ischemia will endogenously induce muscle pain whereas external stimuli (e.g., injection of algesic substances or intramuscular electrical stimulation) may be used to target specific tissues in a more intensive manner. Pressure stimulation is frequently used to induce deep-tissue pain and to assess the pain sensitivity. Manual pressure algometry (Fig. 2A) has been validated although its variability can be reduced by computer-controlled pressure stimulation, which also allows better standardisation of supra-pain threshold (e.g., tolerance) assessments and establishing stimulus-response functions [86]. Cuff

algometry is a user-independent alternative to pressure algometry compensating for the limited amount of tissue strained and the manual rate of pressure application in manual pressure algometry (Fig. 2B). Using cuff algometry, the pain intensity is related to the computer-controlled inflated pressure in a tourniquet mounted around an extremity [192]. This approach is superior for stimulating deeper tissue than e.g., single point pressure algometry [147] and the user-independent cuff algometry demonstrates good to excellent reliability [87]. Cuff algometry has also been translated to preclinical settings where the firing rates of deep dorsal horn wide-dynamic range neurons were recorded in rats during inflation of the cuff to a comparable pressure intensity as inducing pain in humans (Fig. 2D) [48]. Generally, the pressure pain sensitivity is higher in women compared with men [87,222], and decreases with age for pressure algometry [222] whereas it increases for cuff algometry probably due to the larger volume assessed [87]

Tonic conditions of experimental muscle pain for several minutes can be induced by intramuscular injections of algesic substances (e.g. hypertonic saline). Strong excitation of group III and IV muscle afferent nerve fibres by intramuscular injection of hypertonic saline was demonstrated in animals in contrast to similar stimulations of the thick, fast afferent fibres [33] as well as strong excitation of dorsal horn neurons [98]. Other examples of algesic substances relevant for provocation of tonic muscle pain include acidic buffers, glutamate, capsaicin, and bradykinin shown to excite thin muscle afferent fibres in animal studies; for review [77]. Kellgren initiated in the late 1930's case studies on muscle pain distribution by injection of hypertonic saline [116] which later were followed by larger studies, e.g., [54,77,80–82,85,103,121,208], and also with other substances. For instance, intramuscular injection of capsaicin induced an intense pain sensation [16,18,40,41,83,194,227,228,260,262,264] which also was used in case studies using micro-neurography to verify excitation of thin muscle nerve fibres in humans [149]. Likewise, tonic pain by glutamate injections has been extensively used [4,17,32,34,35,61,215,237]. Moreover, intramuscular infusion of acidic buffered solutions induce pain although with low to moderate intensity [58,67,261] and interestingly in animal studies repeated injections of acidic saline have shown bilateral hyperalgesia [225]. Heated isotonic saline injected into muscle tissue may also induce pain [79,194]. Although not consistent, some algesic-induced tonic muscle pain models (e.g., glutamate) provoke more pain in women compared with men [35,77].

Joint and bone

Group II-IV afferent nerves fibres (Aβ-, Aδ- and C-fibres) innervate the joint [205]. All joint structures are generally innervated by free nerve endings (group III and IV) although not the cartilage. In ligaments and in the fibrous capsule, corpuscular endings related to Group II fibres have been found. In humans, excitation of joint nociceptors can be done by arthroscopic mechanical stimulations [56], electrical needle stimulation [105], or focused ultrasound stimulation [268]. Inflammation and triggering of autoimmune reactions are however counter-indicative of intra-articular stimulation of joint nociceptors in human trials. However, external pressure stimulation has been used for assessing differences in pain sensitivity across the joint structure [14]. Other modalities for exciting joint nociceptors in humans include e.g., intradiscal pressure provocation [207], external facet joint pressure stimulation [217], electrical facet joint stimulation [176], and infrapatellar fat pad injections of hypertonic saline [28,112].

Fundamental mechanisms in bone pain studied by Mantyh and colleagues showed that mineralised bone, periosteum, and marrow are innervated by thin afferent sensory fibres [146,148]. The most densely innervated tissue is the periosteum whereas the bone marrow holds the largest number of sensory fibres. In the bone marrow, nociceptors respond to high intraosseous pressure [169]. In humans, an immediate sharp pain with the highest pain sensitivity was found with direct stimulation of the periosteum compared with similar stimulations of fascia, tendon and ligaments, and muscle as the least sensitive [102]. In line with this, stimulating the surface of the human periosteum by injection of hypertonic saline elicits more pain than intramuscular injections [80]. Mechanical impact stimulation has also been used for induction of experimental bone pain in humans [63].

Connective tissue

In animal connective tissue such as tendon and fascia, innervation of nerve endings of group III and IV afferents have been reported [9,240]. In the animal thoracolumbar fascia, location of calcitonin-gene related peptide and substance P containing free nerve endings illustrate that the outer part is densely innervated. Similar findings were made in a human study [240] suggesting the relevance of fascia in LBP conditions. Electrophysiological studies of thin sensory afferent fibres in fascia have reported responses to heat, mechanical and chemical nociceptive stimulations [239].

Experimental pain provocation techniques directed toward tendon, fascia and ligaments have been targeted in experimental human pain studies [74,75,102,116,181,206,224]. Compared

with saline-induced muscle pain, the saline-induced pain in ligaments and tendon showed more excessive pain [73,102]. Interestingly, saline-induced muscle pain was less intense compared with similar injections of hypertonic saline in fascia [74]. In line with this, hypertonic saline injected into the tendon-bone junction induced more pain compared with its tendon and muscle belly [73], and differences among the pain sensitivity between different tendons have also been shown [103]. NGF-injections into facia compared with muscle caused more long-term hyperalgesia [49,263]. These differences in pain sensitivity between various types of connective tissues challenge the notion of clinical muscle pain per se since it likely has many constituents.

Unique pain distribution of musculoskeletal pain

Referred pain is defined as pain perceived at a distant site or adjacent to the origin of localised pain although the localised pain is not necessarily perceived. A challenge with the referred pain concept is the manifestations of pain spreading to a larger region; in such cases local pain is fused with the referred pain, but this does not exclude involvement of the central mechanism for pain referral. Referred pain from deep-tissue is often felt in other deep structures which is a contrast to visceral pain referral, which is felt both deep and/or superficially. Referred pain has mainly been studied when provoked from muscle tissue [77], but tendon and ligaments may also induce large pain areas likely accommodating referred pain [55,73,103]; in particular injection of hypertonic saline into the long posterior sacroiliac ligament induced large referred pain areas perceived in the low back and down to the lower leg (Fig. 3A) [181].

Interesting studies have demonstrated that it is possible to perceive referred pain from areas with a completely effective regional anaesthetic block when stimulating outside the blocked region [136]. Such findings suggest that a central mechanism is likely involved in the perception of referred pain. Nonetheless, the peripheral sensory information transmitted from the referred pain area might have a role in the intensity of referred pain since it is either reduced or unaffected when referred pain is induced in an area that is completely numb [77].

A likely mechanism for pain referral relates to sensitisation and the synaptic connections to of dorsal horn neurons [96]. Afferent fibres from e.g. different muscles have functional synaptic connections with dorsal horn neurons and latent synaptic connections to other neurons within the same region of the spinal cord [96]. The latent synaptic connections become active following persistent and strong noxious input, and this may allow convergence of input from more than one source. Several features of referred pain may support the above mechanism based on latent

synaptic connections. For instance, there is a correlation between the muscle pain intensity and area of referred pain, there is a delay in the appearance of referred pain (approx. 20-40 s) from perception of local muscle pain [77], and the frequency of referred pain is higher following prolonged mechanical painful stimulation compared with a brief mechanical painful stimulation at the same intensity [73]. Such manifestations could indicate a time-dependent mechanism for referred pain like unmasking of latent synaptic connections [96]. Interestingly, the frequency of referred pain was significantly reduced in healthy participants treated with ketamine (an N-methyl-D-aspartate receptor antagonist targeting sensitisation) compared to opioid and placebo treatment [209], again suggesting involvement of sensitisation of a central mechanism.

The classical demonstration of referred pain is done by saline-induced pain in the brachioradialis muscle causing referred pain to the wrist (Fig. 3B), likewise from the tibialis anterior muscle to the ankle (Fig. 3C), and from the infraspinatus muscle to the shoulder (Fig. 4A) [77]. Tonic pressure pain stimulation on the tibialis anterior muscle [91] or the infraspinatus muscle (Fig. 4BC) [54] have demonstrated similar pain referral patterns to the ankle and shoulder/arm, respectively. Remarkably, assessing the referred pain area after one day with a long-term muscle pain model resulted in enlarged pain areas (Fig. 4D) supporting the involvement of sensitisation (due to long-term pain) in the mechanism of referred pain [54,91]. Recent studies have also shown that asymptomatic participants, who have had a painful joint-related trauma or fracture have larger referred pain areas directing towards the previously injured but now asymptomatic joint (Fig. 4E) [52,53,180] and this may indicate a mechanism underlying memory in the spatial mapping of pain.

Clinically, pain referral patterns following trigger point activation in patients with myofascial pain have been thoroughly mapped [220]. There is notable similarity between pain referral patterns after trigger point activation (palpation) in the tibialis anterior muscle or the brachioradialis muscle in patients with myofascial pain and after intramuscular injection of hypertonic saline in muscles without trigger points [77], indicating that referred pain is a normal finding in healthy participants whereas the development of trigger points in patients is pathological.

Compared with healthy control participants, several chronic musculoskeletal pain groups (e.g., LBP, osteoarthritis, fibromyalgia, and whiplash-associated disorders) show enlarged referred pain areas following saline-induced pain in the tibialis anterior muscle [20,121,177,229]. The pressure-induced referred pain from the infraspinatus muscle [54] may be more applicable in

clinical settings and with this approach expanded pain areas was recently demonstrated in patients with whiplash-associated disorders compared with controls [44]. Expanded saline-induced referred pain areas in patients with chronic musculoskeletal pain may suggest sensitisation of the referred pain mechanism and interestingly the enlargement of referred pain was reduced by ketamine in patients with fibromyalgia [82].

Hyperalgesia in musculoskeletal pain

Localised hyperalgesia

Trauma is frequently associated with localised soreness due to release of multiple endogenous substances sensitising deep-tissue nociceptors [160]. Sensitised deep-tissue nociceptors causes an increased response to mechanical noxious stimuli (hyperalgesia) and/or decreased excitation thresholds (allodynia) for mechanical stimulation. Hyperalgesia and allodynia have not been well mechanistically separated within musculoskeletal pain research and in this review hyperalgesia will unify both concepts.

Short-term deep-tissue hyperalgesia has been induced by injection of capsaicin and glutamate, whereas saline-induced pain does not [77]. Mimicking the clinical persistent pain conditions better, we have developed new models provoking pain and hyperalgesia for several days up to 10-15 days. Using eccentric muscle training (lengthening muscle contractions) will typically induce hyperalgesia and low to moderate pain during function with a maximum pain intensity appearing 1-2 days after the exercise [54,74,154,194,223,270]. Animal studies have found that intramuscular endogenous nerve growth factor (NGF) may be fundamental for the delayed-onset muscle soreness induced by eccentric training [167,248]. Intramuscular NGF injections do not excite dorsal horn neurons, but rather subthreshold excitatory potentials and sensitisation after one day, which is opposite to the effects of intramuscular injections of hypertonic saline [98,238] and hyperexcitability of dorsal horn neurons is more pronounced after 2 NGF injections [97]. This is in line with findings in human studies where intramuscular NGF injections do not provoke immediate pain at injection but rather hyperalgesia and pain the subsequent days, and often repeated NGF injections are used for this prolonged pain model (Fig. 5) [4,5,7,29,37,46,47,60,69,91,123,153,154,171,200,210,214,230,231,235,236,258,263,270]. Likewise, injection of NGF into the infrapatellar fat did not evoke pain at injection but rather hyperalgesia after one day [166]. More intense NGF-induced muscle pain and hyperalgesia have been reported in women compared with men and expression of NMDA-receptors on peripheral

nerve fibres may partly explain the long-term hyperalgesia and pain, particularly in women [4,5,267].

A clinical example of localised hyperalgesia is myofascial trigger points in patients with myofascial pain where discrete sites of hyperalgesic loci are found by palpation in taut bands of muscle fibres [161]. Quantifying the pressure pain sensitivity above trigger points may be done by pressure algometry. However, the coexistence of hyperalgesic trigger points assessed by palpation and pressure algometry is generally poor [8]. Subsequently we have developed pressure algometry methods mimicking pressure palpation by adding rotation and vibration to the pressure probe during pressure stimulation demonstrating a better detection of deep tissue hyperalgesia [1]

Hyperalgesia assessed by pressure algometry across painful structures is regularly reported, e.g. in patients with painful knee and hip osteoarthritis compared with asymptomatic controls [14,88,104,189] as well as in patients with patellofemoral pain [100,101], LBP [157], neck pain [43], and lateral epicondylalgia [164,223]. Detailed analysis of multiple pressure pain assessment sites around the osteoarthritis knee joint demonstrates that not all sites are equally hyperalgesic and interestingly not clearly correlated to MRI-verified synovitis [189]. Following intra-articular injection of analgesia and steroid into the osteoarthritic knee, the peri-patellar pressure hyperalgesia was reduced [111]. In contrast, provoking pain in the infrapatellar fat pad by injecting hypertonic saline, hyperalgesia was found around the knee in patients already receiving osteoarthritic pain relief by intra-articular analgesia and steroid [112], as well as in healthy participants [109]. These findings suggest a clear link between ongoing local pain processes and the localised hyperalgesia. The degree of peri-patellar pressure hyperalgesia in patients with osteoarthritis was associated with the severity of knee pain [14,88,189] suggesting that the pressure stimulation targets the clinical sensitised structures or perhaps more likely that facilitated central mechanisms (e.g., spatial summation) result in hyperalgesia also in tissue not directly affected by the disease.

Spreading and widespread hyperalgesia

Hyperalgesia to pressure outside a painful locus has been demonstrated in conditions where there is no reason to believe that systemic sensitisation of peripheral nociceptors has occurred. In patients with knee and hip osteoarthritic pain compared with control participants, pain thresholds assessed by pressure algometry were reduced at the lower leg and on the arm (i.e., distant from the painful joint) [14,88,104,132]. Likewise, cuff algometry used to record the cuff pressure pain

thresholds on the legs in patients with knee and hip osteoarthritis showed hyperalgesia compared with asymptomatic controls [88,104,185]. Interestingly, the distant hyperalgesia is not normalised when patients with osteoarthritis still have pain even after a revision total knee replacement [221] in contrast to the normalisation seen in patients who experience a significant pain reduction after total knee replacement [88]. Spreading hyperalgesia has also been reported in several other conditions e.g., patients with patellofemoral pain [100,101], LBP [157,177], neck pain [43], lateral epicondylalgia [107,164], and partly in the recovery phase from a tibial fracture [135]. In systemic rheumatologic diseases such as rheumatoid arthritis, pressure pain thresholds are reduced in patients compared with controls, both at the painful joint and occasionally outside [110,137,145].

The spreading hyperalgesia and its temporal characteristics suggests that central mechanisms are sensitised by the nociceptive drive from the painful structure. The spreading hyperalgesia may require pain over a longer time, since 10 min of electrical stimulation of the lumbar facet joints did not cause any distant pressure hyperalgesia [176] whereas it was found after two days with exercise-induced LBP in healthy controls [159] and during the pain flare but not without in patients with recurrent LBP [157]. In NGF-induced long-term pain models, spreading hyperalgesia outside the injection site has also been shown one day or more after the NGF-injection [7,91,230,263].

Compared with asymptomatic controls, widespread hyperalgesia assessed by manual pressure algometry at different body sites is found in patients with fibromyalgia [70,71,76,229] and whiplash pain [44,115,121,232]. Cuff algometry studies have demonstrated similar hyperalgesic findings in patients with chronic widespread pain [59,76,108,139]. Here the aetiology is less understood, but mechanisms other than sensitisation of central mechanisms due to a peripheral nociceptive drive is assumed.

Pro-nociceptive modulatory mechanisms in musculoskeletal pain

Modulatory pain mechanisms acting pro-nociceptively or anti-nociceptively can be assessed by dynamic sensory testing of deep structures. The outcome is less affected by baseline pain sensitivity since the resulting parameter is a relative effect within the same individual.

Temporal summation of pain (TSP) is a pro-nociceptive mechanism, with wind-up of dorsal neuronal activity as the assumed spinal correlate and assessed as the amount of pain increase when repeating phasic noxious stimulation with the same intensity but sufficiently fast. This has been assessed extensively studied by heat, laser, pin-prick and electrical stimulation on the skin.

The first attempt to study TSP in deep-tissue was done by fast repeated intramuscular injections of hypertonic saline inducing progressively increasing pain [81]. Later electrical stimulation via intramuscular needle electrodes was used where the pain perception for one stimulation was lower compared with e.g., 5 stimulations of the same intensity [13,82]. Computer-controlled single point pressure stimulation repeated 10 times (e.g., 1 s stimulation and 1 s pause) provoke a progressive increase in the perceived pain intensity compared to the first stimuli and to a slower repetition protocol [2,171]. Utilising the advantage of stimulating a large tissue volume (i.e., reducing variability) repeated cuff stimulations can also be used to assess TSP (Fig. 2C) with a higher degree of TSP in women compared with men [87,143].

Facilitated summation of pain in patients with musculoskeletal

Enhanced TSP suggests a facilitated central integrative mechanism. Assessing the span in pain thresholds to single and repeated intramuscular electrical stimulations demonstrated facilitated TSP in patients with fibromyalgia compared with controls and this facilitation was normalised by low-dose ketamine [82]. Similarly, in patients with fibromyalgia and whiplash, the electrical threshold evoking the withdrawal reflex to sequential stimulation on the skin is lower compared with controls, indicating a facilitated central mechanism [22].

Temporal summation of pain after repeated pressure stimulation to the knee and lower leg, was facilitated in patients with knee osteoarthritis compared with controls, and even more facilitated in patients with high clinical pain scores compared with moderate pain scores [14]. Using cuff algometry at the legs, facilitated TSP compared with asymptomatic controls have been demonstrated e.g. in patients with knee [132,185] and hip [104] osteoarthritic pain, in adolescents with patellofemoral pain [100], in young females with chronic patellofemoral pain and those who recovered from adolescent patellofemoral pain [101], during pain flare in patients with recurrent LBP [157], and in patients with fibromyalgia [76]. Moreover, increased TSP was reported in patients with neck pain and LBP with radiating pain patterns compared with localised pain patterns [254] and in patients with osteoarthritis undergoing a revision total knee replacement with post-operative pain compared to similar patients without post-operative pain [221]. In a meta-analysis across 27 LBP studies we found facilitated TSP assessed with various modalities although without differences between acute/recurrent and chronic pain conditions [158]. Nonetheless, increased TSP was associated with longer symptom duration in axial spondyloarthritis [165] and knee osteoarthritis [14] suggesting that the sensitisation is a time-

dependent process. Interestingly, a human model of experimental knee pain (saline-induced pain in the infrapatellar fat pad) caused facilitated TSP to repeated pressure stimulation applied locally and to knee-related muscles [109]. Overall, most studies suggest that facilitated TSP is a state phenomenon, as also found in patients with recurrent LBP where facilitated temporal summation is found during a pain flare and not in a pain-free period [157].

Spatial summation of musculoskeletal pain has been studied by relating the difference in pain thresholds with different sized pressure stimulation probes [172] and inflation of one versus two cuffs [192]. The cuff induced spatial summation of pain was higher in women compared with men [138] and in patients with knee osteoarthritic pain, spatial summation of pain assessed on the lower leg was facilitated compared with pain-free controls and this was normalised following total knee replacement [88]. In line with this, facilitated spatial summation assessed on the arm with cuff algometry has been found in patients with lateral epicondylalgia [107].

Musculoskeletal pain summation as predictor for pain progression

A high degree of TSP assessed by manual pin-prick stimulation before total knee joint replacement was associated with the post-operative (12 months) pain intensity [186,190] and similarly was found in another study when cuff-algometry assessed TSP was combined with other central pain mechanisms assessed at baseline [187]. Moreover, in patients with osteoarthritis, facilitated TSP recorded at baseline, was associated with less analgesia following treatment with nonsteroidal anti-inflammatory drugs [11] combined with paracetamol [188]. In other pain interventions, facilitated TSP at pre-treatment was associated with poor outcomes. For example, facilitated TSP at baseline in adolescents with patellofemoral pain was associated with less improvement in pain intensity following several weeks of activity (sport) modification [100]. Furthermore, pain management by physical activity in LBP resulted in pain flares in patients with baseline facilitated TSP [255]. The predictive value of TSP can also be found in experimental studies. Exercise-induced LBP inducing moderate pain for several days did not facilitate the TSP when assessed on the legs, but interestingly the baseline degree of TSP was associated with the pain intensity of the experimentally-induced LBP [159] and similarly when provoking long-term pain in the calf muscle [128].

In summary, summation of pain often assessed distant to the pain locus is generally facilitated in musculoskeletal pain conditions compared with asymptomatic controls or compared with pain-free periods in the same individuals, suggesting that this is a state of the pain system

although with important prognostic value for pain persistence and treatment outcome. However, the TSP may also hold some trait characteristics of the pain system since it also predict the degree of experimental long-term pain conditions in otherwise asymptomatic controls.

Anti-nociceptive modulatory mechanisms in musculoskeletal pain

Contrasting pro-nociceptive mechanisms, conditioning pain modulation and exercise-induced hypoalgesia have anti-nociceptive descending modulatory characteristics and if dysfunctional spinal mechanisms may become hyperexcitable causing e.g. widespread hyperalgesia.

Anti-nociceptive effects on musculoskeletal pain sensitivity

Based on animal work, the term Diffuse Noxious Inhibitory Control (DNIC) was originally used to describe an inhibitory spino-bulbar-spinal loop where noxious stimulation of an extremity led to reduced firing of dorsal horn wide-dynamic range neurons in response to concurrent noxious stimulation of a heterotopic site [27]. The perceptual correlate of DNIC may in part be assessed by the psychophysical protocol Conditioned Pain Modulation (CPM) where a painful test stimulus is applied before and during or immediately after a painful conditioning stimulus applied heterotopically to the test stimulus [193,269]. The CPM-effect is the decreased pain perception of the test stimulus (or the increase in pain threshold) due to the conditioning stimulus. The descending control is under strong cortical influence [23,179] and factors like distraction, expectation, anxiety, and emotional states may impact the CPM magnitude, and therefore DNIC is only a partial reflection of CPM. There is a wealth of preclinical studies on the descending pain modulatory systems [24,25,130] describing its function and pharmacology mainly within neuropathic pain and only few on musculoskeletal pain models.

The descending inhibitory effect on musculoskeletal pain perception has been studied with different test stimulus modalities (often pressure or cuff algometry) and the conditioning stimulus is typically a tonic mechanical stimulus (e.g., pressure) or the cold pressor stimulus (i.e., ice water). In a cohort study with approx. 2000 participants, pressure pain thresholds were increased compared with baseline when applying the cold pressor test as conditioning, although approx. 10% of participants did not show a reduction of pressure pain thresholds [222]. The CPM effect was higher in women than men [222], which may link with the higher prevalence of musculoskeletal pain in women. A user-independent methodology for musculoskeletal CPM assessment is available where cuff algometry on the test leg is used to record cuff pain thresholds

(test-stimulus), and on the contralateral extremity another cuff is automatically inflated providing a painful conditioning during which the cuff algometry on the test leg is repeated (Fig. 6A) [84]. We have recently demonstrated the translational value of cuff algometry for CPM assessment where the response of deep dorsal horn wide-dynamic range neurons to cuff stimulation was reduced when applying a contralateral noxious cuff conditioning (Fig. 6BC) [48]. Methodological parameters regarding the CPM assessments by cuff algometry have been studied demonstrating fair reliability [84] and robustness of fast repetitions of the CPM assessment [93] as well as effects of stress and attention [94,95]. A meta-analysis showed that the inter-session reliability for CPM assessments was fair when using pressure modalities for both test and conditioning [174] and similarly was reported in a systematic review [117].

Reduced anti-nociceptive effects in patients with musculoskeletal

A reduced CPM-effect has been reported in a meta-analysis across different groups of patients with chronic pain compared with controls [140]. In another meta-analysis, CPM was impaired in patients with LBP compared with controls, and the magnitude of impairment was higher in patients with chronic versus acute/recurrent LBP [158]. Moreover, reduced CPM was found in patients with knee [88] and hip [125] osteoarthritis compared with controls, in patients with knee osteoarthritis undergoing a revision total knee replacement with post-operative pain compared to comparable patients without post-operative pain [221], as well as in patients with fibromyalgia [124], and whiplash-associated disorder compared with controls [44,170]. In contrast, several studies have not demonstrated a reduced CPM-effect in patients with osteoarthritic pain [104,132,186,187]. Using cuff algometry for CPM assessment in other patients with musculoskeletal pain, impaired CPM was found in adolescents with patellofemoral pain compared with controls [100], in young females with chronic patellofemoral pain compared with those who recovered from adolescent patellofemoral pain [101], and in patients with recurrent LBP in periods with and without pain compared with controls [157]. Fair to say, other musculoskeletal pain conditions have reported mixed CPM effects, e.g., in patients with temporomandibular disorder pain [126,178]. In an animal model of osteoarthritis, the descending inhibitory control was not affected in the early phase but rather attenuated in the late-stage of osteoarthritis [144] which seems to mimic some of the clinical findings. However, in a cancer bone pain model the descending control was impaired in the early phase and normalised in the later phase [129].

Mixed effects have been reported on the effects of treatment on CPM. A meta-analysis showed no significant change in CPM before and after treatment in patients with knee osteoarthritis, although the degree of CPM normalisation was correlated with the treatment-related pain-reduction [175]. In contrast, a meta-analysis showed CPM improvement by physical therapy in patients with chronic musculoskeletal pain [19]. Thus, the musculoskeletal nociceptive drive may maintain the impaired descending inhibitory control, and an efficient treatment of the nociceptive drive could explain normalisation of the CPM effects. There also exists examples of increased CPM efficacy, e.g., in participants completely recovered from an ankle fracture and now asymptomatic compared with participants who have not experienced an ankle trauma [180].

A major question relates with how much and how long the duration of pain is needed before a CPM impairment occurs. Tonic saline-induced muscle pain or cold pressor pain as conditioning induced generally a higher CPM-effect in males compared with females, but interestingly when applying the two conditioning stimuli at the same time less CPM-effect was observed in males [15]. In a later study where a pain model was applied to the arm for one hour it was demonstrated that the CPM assessed by cuff algometry on the legs was impaired, and immediate experimental reduction or facilitation of the arm pain did not change the CPM attenuation suggesting some plasticity of the descending control system [92]. Nonetheless, although long-term pain was maintained for 1 day the CPM-effect progressed towards a normalisation suggesting that the healthy descending control is adaptive [92]. Likewise, in a model of experimental LBP for several days the CPM was not significantly affected [159].

Of note, better characterisation may be obtained when combining pro-nociceptive and anti-nociceptive mechanisms. Integration of several mechanistic-based parameters (pain sensitivity, TSP and CPM) was useful for characterising individuals with osteoarthritic pain as highly sensitised [12]. Similarly, in a large cohort of patients with chronic musculoskeletal pain, impaired CPM combined with facilitated TSP was associated with widespread pain [249]. The complementary role of CPM and TSP is also shown in an experimental study where painful conditioning mainly reduced the pain response to the first stimulation applied during TSP assessment [99].

Like the CPM protocol, exercise-induced hypoalgesia is reflected in changed pain sensitivity caused by aerobic and resistance exercise. In the healthy population, hypoalgesia generally occurs following exercise [168]. In patients with chronic musculoskeletal pain, exercise-induced hypoalgesia was intact in several patient cohorts, but impaired exercise-induced hypoalgesia or even hyperalgesia has also been reported in other patients with musculoskeletal [253]. In patients

with widespread pain, reduced efficacy of exercise-induced hypoalgesia has also been found compared to controls [226,244]. The non-trivial part of exercise in chronic musculoskeletal pain is the potential pain flare that may reduce the degree of exercise-induced hypoalgesia e.g., in patients with LBP [255]. Moreover, in healthy controls pressure pain thresholds at the head and neck increased when doing repeated arm abductions (i.e., exercise-induced hypoalgesia) but in patients with neck pain, pressure pain thresholds were gradually reduced and pain increased [43]. Recently, reduced exercise-induced hypoalgesia was found in healthy participants exposed to a long-term neck pain model [45]. Interestingly, exercise-induced hypoalgesia caused a reduction of the CPM effect [6]. In contrast, however, the reduction in pressure pain sensitivity due to a cold pressor conditioning (i.e., CPM) and due to exercise (i.e. exercise-induced hypoalgesia) was not significantly correlated [252]. Still, in patients with chronic musculoskeletal pain having high pain sensitivity compared with low pain sensitivity, impaired CPM and attenuated exercise-induced hypoalgesia were found [251]. Such findings suggest that shared inhibitory pathways are recruited in exercise-induced hypoalgesia and CPM.

Reduced anti-nociceptive effects as a predictor for pain progression in musculoskeletal pain

The prognostic value of impaired CPM-effect in musculoskeletal pain conditions is less convincing. In a small study, the CPM-effect in patients with knee osteoarthritic pain before total knee replacement correlated with the pain reduction recorded 6 months after the surgery [250]. However, larger cohort studies in patients with osteoarthritis could not demonstrate a predictive value of impaired CPM per se on chronic postoperative pain [104,132,186,187]. Combining mechanistic pain parameters in the analysis may improve the predictive value. For instance, in pain patients with knee osteoarthritis having impaired CPM and facilitated TSP before total knee replacement, less pain relief was found after 1 year [187].

Cortical manifestations of prolonged and persistent musculoskeletal pain

Chronic musculoskeletal pain is associated with structural and functional cortical reorganisation and maladaptive neuroplasticity [131]. Early pioneering studies demonstrated reorganisation of the primary somatosensory cortex in patients with chronic LBP [66] and similar findings have been reported subsequently [127]. Moreover, reorganisation of the motor cortex was thoroughly studied in LBP [233,246,247]. In the progression from acute to chronic LBP, the cortical representation shifts from sensory-discriminative/nociceptive to emotional circuits [10,90,212]. As

an example, in patients with chronic LBP, the power of gamma oscillations in the prefrontal region reflected the variation in ongoing pain intensity [155]. Moreover, the dynamics of specific brain networks (particularly the default mode network) were changed in patients with LBP and osteoarthritic pain, compared with controls and such modifications correlated with pain duration [21]. Likewise, in a large cohort of patients with LBP, connectivity between sensorimotor, salience and default mode networks were increased compared with controls, with some connections particularly influenced by pain catastrophizing [120]. In line, disruption of whole-brain and local functional connectivity was shown in patients with osteoarthritic pain [26] and various knee osteoarthritis phenotypes were associated with the power of oscillations in different cortical regions [219]. Although, the dynamic changes in connectivity within and between regions is important for chronic pain [119], the following sections will focus on the immediate cortical changes in musculoskeletal pain.

Somatosensory cortical effects of tonic musculoskeletal pain models

Early studies demonstrated that a capsaicin-induced tonic muscle pain evoked similar resting-state electroencephalographic topography patterns as a control injection, but the painful condition resulted in a decreased power of alpha (parietal) and increased power of beta (global) oscillations [41] where the increased power of beta oscillations was in contrast to a similar intradermal pain model [40]. Using a repeated tonic muscle pain model (five injections of hypertonic saline provoking relatively low intensity pain that gradually declined over repetitions), the power of alpha oscillations was reduced in the first two pain epochs, whereas the beta oscillations gradually increased during the five pain epochs [39]. The decreased alpha power was not found if participants were presented to an aversive auditory stimulus suggesting that the pain effect was not due to a general arousal [38]. Similarly, saline-induced tonic muscle pain caused decreased alpha power with a reduced effect if a simultaneous placebo condition was introduced [142] and increased gamma power in case of moderate compared to low intensity saline-induced muscle pain [141]. However, if saline-induced muscle pain was maintained on a steady pain intensity for 5 minutes before electroencephalography was recorded, a long-lasting increase in delta and alpha power was found, possibly illustrating a decreased excitability of the somatosensory cortex [184]. The different findings in alpha power changes in tonic muscle pain models may relate to the required cognitive processing and attention, where the capsaicin pain model induces an immediate high-intensity pain profile, and the saline-induced pain is steadier. Recently, painful

cuff pressure stimulation maintained for three minutes compared with non-painful cuff pressure evoked an increased global electroencephalographic power in several regions (e.g., contralateral anterior cingulate cortex, primary somatosensory cortex, dorsolateral prefrontal cortex, posterior parietal cortex), where the increase in the alpha power may be a result of increased cortical inhibition by the deep tissue pain [259].

The cortical excitability in tonic pain models has also been studied by sensory evoked potentials in electroencephalographic recordings after electrical stimulation of peripheral nerves. Inhibition of early sensory evoked potentials was found following tonic chemically-induced muscle pain, with lasting depression several minutes after the pain vanished [196,197,199,203].

Somatosensory cortical effects of long-term musculoskeletal pain models

The initial transition to chronic pain has been studied by long-term muscle pain models. Reduction of the frontal N30 sensory evoked potential and facilitation of the parietal P45 peak were recorded after 4-5 days with NGF-induced muscle pain [153,154] and similar P45 facilitation was found after days with delayed-onset muscle soreness [152,154]. Such manifestations are interesting because these electroencephalographic recordings are done in participants with daily pain but in resting situations without pain flare, thus different from tonic muscle pain models. Using a long-term pain model (exercise-induced muscle pain), increased alpha power was found within 12 hours, but at peak pain (36 h), no significant changes in the alpha oscillations were observed [191]. These findings suggest that the long-term musculoskeletal pain models also involve cortical adaptations linked with the required cognitive and attentional demands.

Muscle pain for 1-2 weeks induced by NGF injections, demonstrated a negative correlation between the sensorimotor peak alpha frequency of the electroencephalography at baseline and the experimental muscle pain intensity (slow peak alpha frequency associated with higher muscle pain intensity), but the peak alpha frequency was not significantly affected in the 2 week observation period [69]. Using a combined model of exercise-induced muscle pain and NGF-induced muscle pain, a slowing of the peak alpha frequency was demonstrated during the days with pain, but in contrast to Furman et al. [69] the subgroup with highest perceived muscle pain intensity was associated with faster peak alpha frequency at baseline [151]. So far, the peak alpha frequency is still to be recorded in patients with musculoskeletal pain and further clarified as other studies have shown a positive [173] and negative [68] correlation between the tonic thermal-induced pain intensity and the peak alpha frequency before pain induction.

Corticomotor excitability in musculoskeletal pain models

Motor evoked potentials by transcranial magnetic stimulation have been used to assess corticomotor excitability in musculoskeletal pain conditions [57,202,247]. A meta-analysis showed that the corticomotor excitability was depressed during and after induction of tonic deep-tissue pain [31]. The post-pain corticomotor depression seems robust since motor cortex activation by movement [204] or a working memory task (engaging prefrontal and premotor cortices) did not affect the depression [134] although e.g., pre-motor cortex activation (by action observation and motor imagery tasks) may counteract the pain-induced corticomotor depression [133].

In a series of studies, corticomotor excitability was studied in the progression of long-term pain models. In the first studies, facilitated motor evoked potentials [153,154] and expansion of the motor maps (area of the motor cortex inducing a motor evoked potential when doing transcranial magnetic stimulation) were found during the days of peak NGF-induced muscle pain [153,154,200]. However, other studies demonstrated that motor maps were reduced in the days following NGF-induced muscle pain [47], and the reduction was correlated with less motor variability during pain [234]. Moreover, reduced motor maps in NGF-induced long-term pain were found with higher experimental pain intensity, and lower pain causes motor map expansion [211]. Interestingly, the group showing motor map expansion also had lower corticomotor excitability at baseline, suggesting that they are flexible towards pain-related changes.

A meta-analysis found that corticomotor disinhibition assessed by transcortical magnetic stimulation is generally found in patients with chronic pain, but most pronounced in patients with neuropathic pain [182]. Similar findings have been reported e.g., in patients with osteoarthritic pain [218] and fibromyalgia [163]. Likewise, in a long-term muscle pain model, reduced intracortical inhibition and increased intracortical facilitation was found accompanying the expanded motor maps [200], suggesting that such cortical disinhibition is a relatively fast change.

Cortical adaptability in musculoskeletal pain

The fundamental principle that the brain can adapt during various conditions, including musculoskeletal pain, has resulted in studies focusing on mechanisms that support adaptability in the human brain, and a basic construct defined as ‘homeostatic plasticity’ is needed to keep stability in neural networks [30,114]. For instance, the homeostatic modulation may be assessed in

the motor cortex when two blocks of anodal transcranial direct current stimulation (tDCS) and a block of no stimulation in-between are delivered in a priming-test paradigm [243,265,266]. In patients with chronic LBP, such homeostatic modulation was impaired compared to controls [241], indicating reduced adaptability. Using a long-term muscle pain model in healthy participants, the peak impairment of the homeostatic modulation was found after few days of on-going muscle pain [242], suggesting that as pain manifests, the neuroplastic adaptability reduces.

Overall, the neuroplastic capacity may be an important factor in musculoskeletal pain, both as a trait and state. In a study using NGF-induced muscle pain, Seminowicz et al. [213] found that a relative slow baseline peak alpha frequency combined with reduced motor maps when having musculoskeletal pain partly discriminated the most pain sensitive participants from more tolerant participants. In patients with sub-acute LBP compared with controls, early sensory evoked potentials from electrical stimulation on the back were depressed, and the motor maps were reduced [42]. In contrast, less discrete motor maps for back muscles were found in patients with recurrent [245] and chronic [201] LBP compared with asymptomatic controls. Measures recorded in the acute pain phase of LBP may also represent an epiphenomenon, but the reduced sensory evoked potentials were demonstrated as causative of chronic pain after 6 months in contrast to the changes in the motor mapping [106]. Such data indicate that the cortical sensory-discriminative function is impaired in candidates vulnerable to developing chronic musculoskeletal pain. How this links with the sensory-discriminative function in painful stimulations is unknown, although previous studies demonstrated augmented nociceptive evoked potentials (short latency) in patients with chronic LBP [50] and fibromyalgia [51] compared with controls.

Non-invasive brain stimulation in musculoskeletal pain

The cortical manifestations in musculoskeletal pain and, in particular, the predictive characteristics open a therapeutic window for non-invasive neuro-modulatory approaches. Generally, non-invasive brain stimulation (NIBS), such as repetitive transcranial magnetic stimulation (rTMS) and tDCS, has demonstrated efficiency and have been approved for e.g., peripheral neuropathic pain and migraine. So far, the clinical evidence for NIBS in musculoskeletal pain is sparse. The modulatory effects on the cortical excitability have been demonstrated with anodal tDCS [257] and high-frequency rTMS [183] causing increased excitability while cathodal tDCS reduce the excitability [256].

A recent meta-analysis showed that NIBS increased pain thresholds in healthy participants when pooling studies of rTMS and tDCS of the primary motor cortex, but for individual analysis of rTMS and tDCS, this was not significant [72]. Relevant for deep-tissue pain, anodal motor cortex tDCS inconsistently increased pressure pain thresholds in healthy participants [64,65,113,257]. In contrast, the pressure pain thresholds in healthy participants increased after daily sessions of high-frequency rTMS on the left dorsolateral prefrontal cortex [150]. Another approach is the high-density tDCS, where one or more cortical regions can be targeted. In a large sham-controlled design with daily anodal tDCS applied to the primary motor cortex, dorsolateral prefrontal cortex, or both combined, the pain sensitivity (e.g., pressure pain thresholds) was not affected in healthy participants [122].

With anodal high-density tDCS applied daily on three consecutive days to the primary motor cortex and dorsolateral prefrontal cortex in participants exposed to the long-term NGF-induced muscle pain model, some anti-hyperalgesic effects were observed [123]. Moreover, in a sham-controlled design, the long-term muscle pain intensity induced by intramuscular NGF injections was reduced by daily high-frequency rTMS sessions targeting the left dorsolateral prefrontal cortex [210] as well as reversing the pain-induced pressure hyperalgesia, and exerting opposing effects on both cortical somatosensory excitability and corticomotor excitability (Fig. 7) [153]. In a similar long-term pain model, analgesia was demonstrated following high-frequency rTMS to the primary motor cortex but interestingly without any effects on the corticomotor excitability [36]. Across different types of patients with chronic pain, NIBS generally increased pain thresholds [72]. However, in patients with musculoskeletal pain, only few studies exist, e.g., in patients with osteoarthritic pain, active compared with sham anodal tDCS to the primary motor cortex reduced the pressure pain sensitivity [3] and in patients with fibromyalgia, anodal tDCS caused a lasting pain reduction after 10 daily treatment sessions [118].

Studies of NIBS on pro-nociceptive modulatory mechanisms are rare. However, the CPM effects were generally increased by rTMS and tDCS of the primary motor cortex both in healthy participants and patients with chronic pain [72]. Beneficial effect of tDCS was found for CPM assessed by pressure pain thresholds in healthy participants [64,65] and in patients with osteoarthritis [3]. In a long-term pain model (capsaicin), CPM assessed by cuff algometry was reduced in parallel with reduced corticomotor excitability in the sham tDCS, whereas the CPM and corticomotor excitability was not significantly affected upon active anodal high-density tDCS to the motor network [89]. One hypothesis could be that neuroplastic manifestations are needed before

tDCS will be efficient, as also evident in another study where tDCS in patients with LBP having low pain severity had no effect on the pain sensitivity or CPM [156].

Conclusion and future directions

Musculoskeletal pain biomarkers are differentially affected depending on the pain duration and change in the continuum from short-term to chronic pain (Fig. 8): 1) Provoked pain is localised and referred *initially* whereas spreading and extended when *chronic*. 2) Deep-tissue hyperalgesia is localised *initially* and spreading/widespread when *chronic*. 3) The pro-nociceptive modulation has a normal gain *initially*, and a facilitated gain when *chronic*. 4) The anti-nociceptive modulation is more variable but seems to provide an inhibitory function *initially*, with reduced inhibitory efficacy when *chronic*. 5) Cortical circuitries demonstrate adaptations *initially* whereas reorganised and less adaptive when *chronic*. Overall, a trait of the musculoskeletal pain system being more vulnerable to persistent pain could be hypothesised as i) having spinal neuronal mechanisms which become easily sensitised, and ii) having less neuroplastic capacity, being less flexible when a cortical adaptive response is normally requested and thus affecting the down-steam control of the spinal excitability. A similar hypothesis could be proposed for chronic pain (sensitised spinal mechanism and reduced neuroplastic capacity). Although not covered in this review, the lack of adaptability may even extend to psychological and motor-control concepts in musculoskeletal pain. Currently, both hypotheses are not fully supported, but may facilitate novel treatment modalities e.g., using NIBS to modulate the neuroplastic capacity in musculoskeletal pain conditions.

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FIGURE LEGENDS

Figure 1. Provoking, probing, and modulating fundamental mechanisms in musculoskeletal pain at different levels along the neuroaxis and in conditions with different pain durations.

Figure 2. Pressure algometry with a manual increase in pressure intensity until the pressure pain threshold is indicated by the subject (**A**). User-independent cuff algometry with the stimulus-response curve relating the automated increment in cuff pressure intensity with the perceived pain intensity scored by the subject on a visual analogue scale (VAS); cuff pain threshold defined as when the VAS score begins to increase, and tolerance are detected when the subject stops the stimulation (**B**). Temporal summation of pain probed by repeated cuff stimulations at the same painful pressure intensity and the progressive increasing pain intensity is scored on the VAS (**C**). Stimulus-response curve with the cuff pressure intensity and a single unit recording of a deep dorsal horn wide-dynamic range neuron (**D**); based on animal data from [48].

Figure 3. Localised and referred pain by injections of hypertonic saline (cross) in the long posterior sacroiliac ligament (**A**), the tibialis anterior muscle (**B**) and brachioradialis muscle (**C**) in healthy participants. Based on data from [80,181] common pain areas redrawn using Navigate Pain.

Figure 4. Pain distribution from the infraspinatus muscle (cross) when provoked by injection of hypertonic saline (**A**), painful pressure stimulation for 5-s (**B**) and 60-s (**C**), and 60-s pressure stimulation on day-two in a long-term pain model in healthy participants (**D**) and in asymptomatic participants with a history of a shoulder fracture (**E**). Based on data from [53,54] and common pain areas redrawn using Navigate Pain.

Figure 5. The pain distribution across days following nerve growth factor injected in the tibialis anterior (**A**) and brachioradialis (**B**) muscles in healthy participants. Based on data from [154,230] and common pain areas redrawn using Navigate Pain.

Figure 6. Assessment of conditioning pain modulation by cuff algometry in humans, where the cuff pain threshold and tolerance on the left leg increase during conditioning cuff stimulation on the right leg (**A**). Stimulus-response curve with the cuff pressure intensity and a single unit recording of a deep dorsal horn wide-dynamic range neuron without (**B**) and with (**C**) a noxious cuff

stimulation as conditioning on the contralateral calf causing reduced firing; based on animal data from [48].

Figure 7. Daily high-frequency active rTMS sessions targeting the left dorsolateral prefrontal cortex compared with sham rTMS reduced the long-term muscle pain intensity induced by intramuscular NGF injections (**A**), reversed the pain-provoked motor map expansion (**B**), and exerted opposing effects on pain-related changes of the some of the somatosensory evoked potentials (C). Modified from [153,210].

Figure 8. Summarised and generalised findings of the mechanistic pain biomarkers in conditions with different pain durations.

Fig. 1

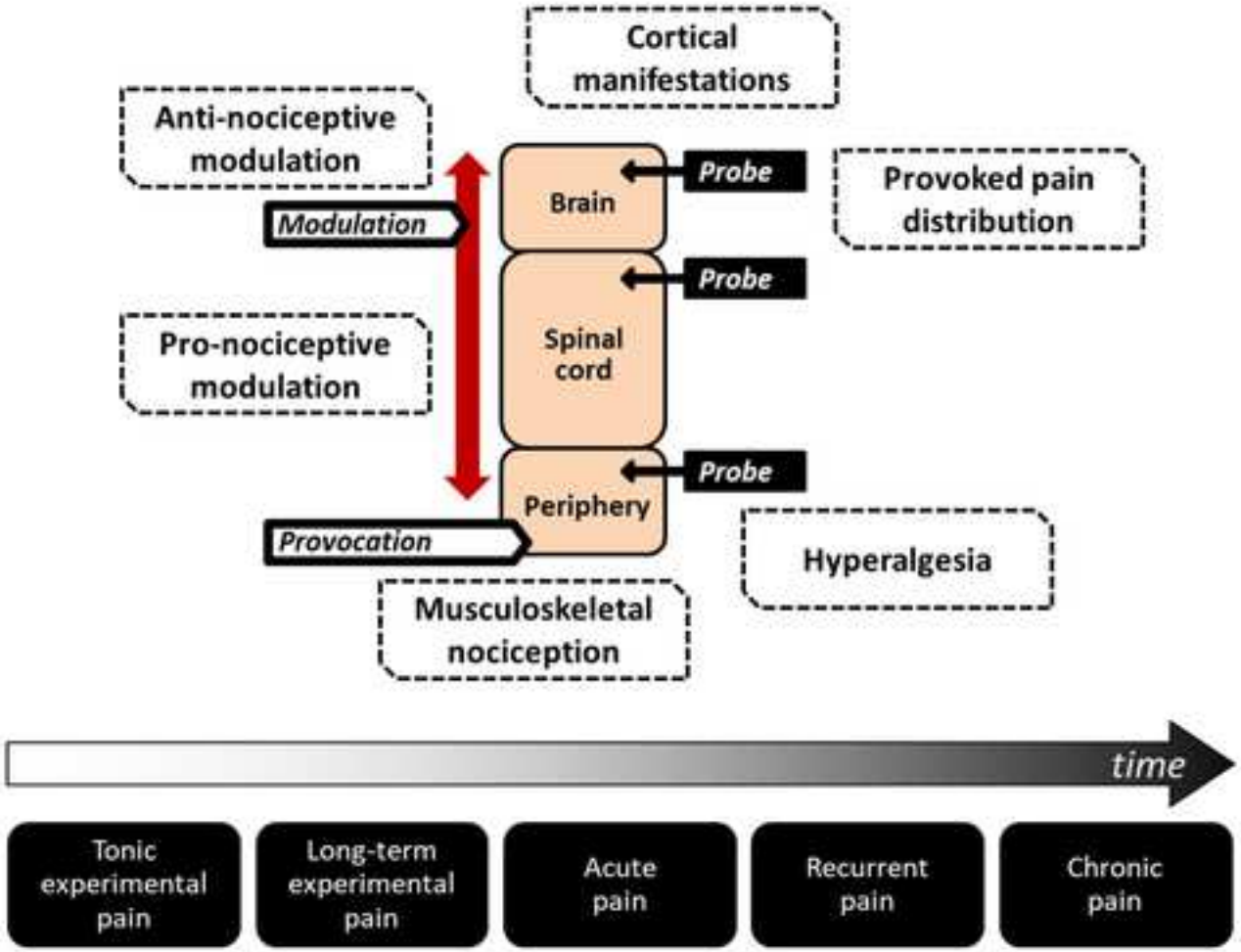


Fig. 2

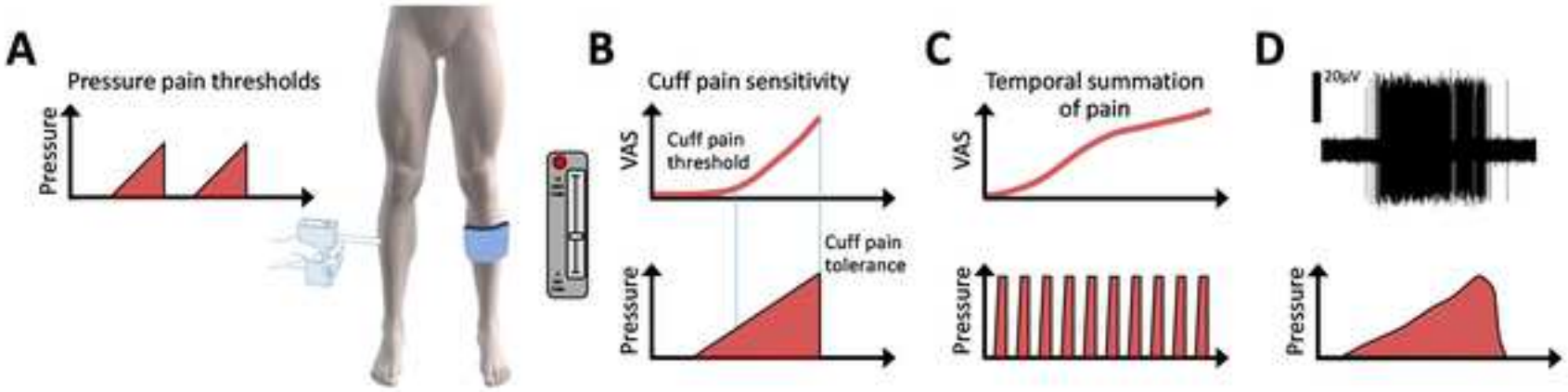


Fig. 3

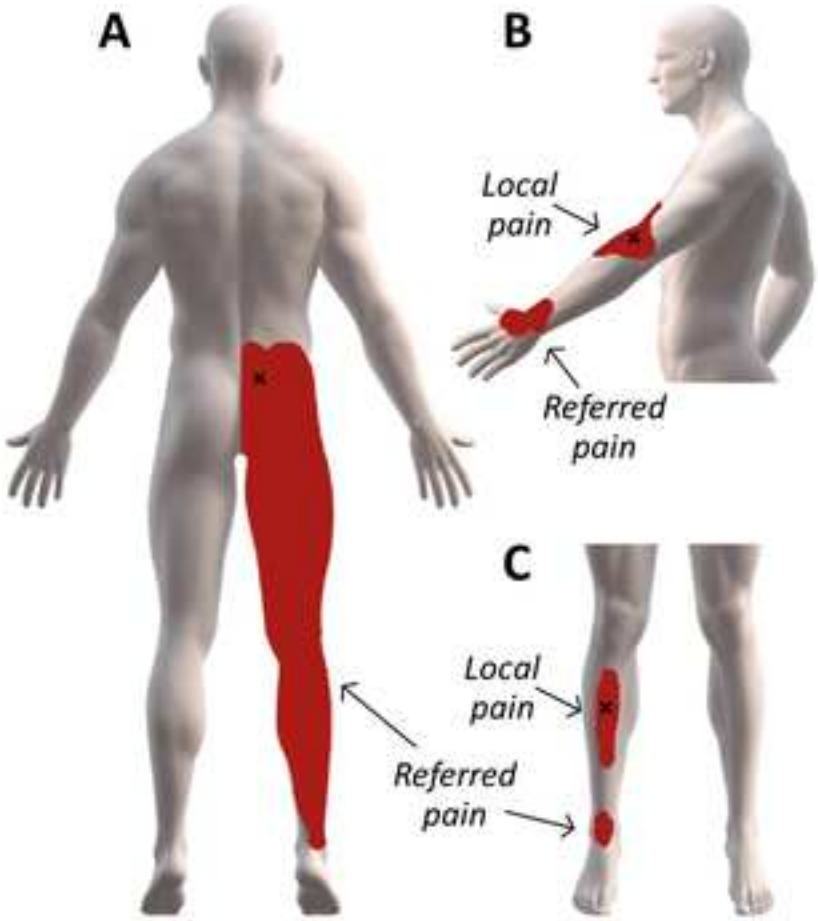


Fig. 4

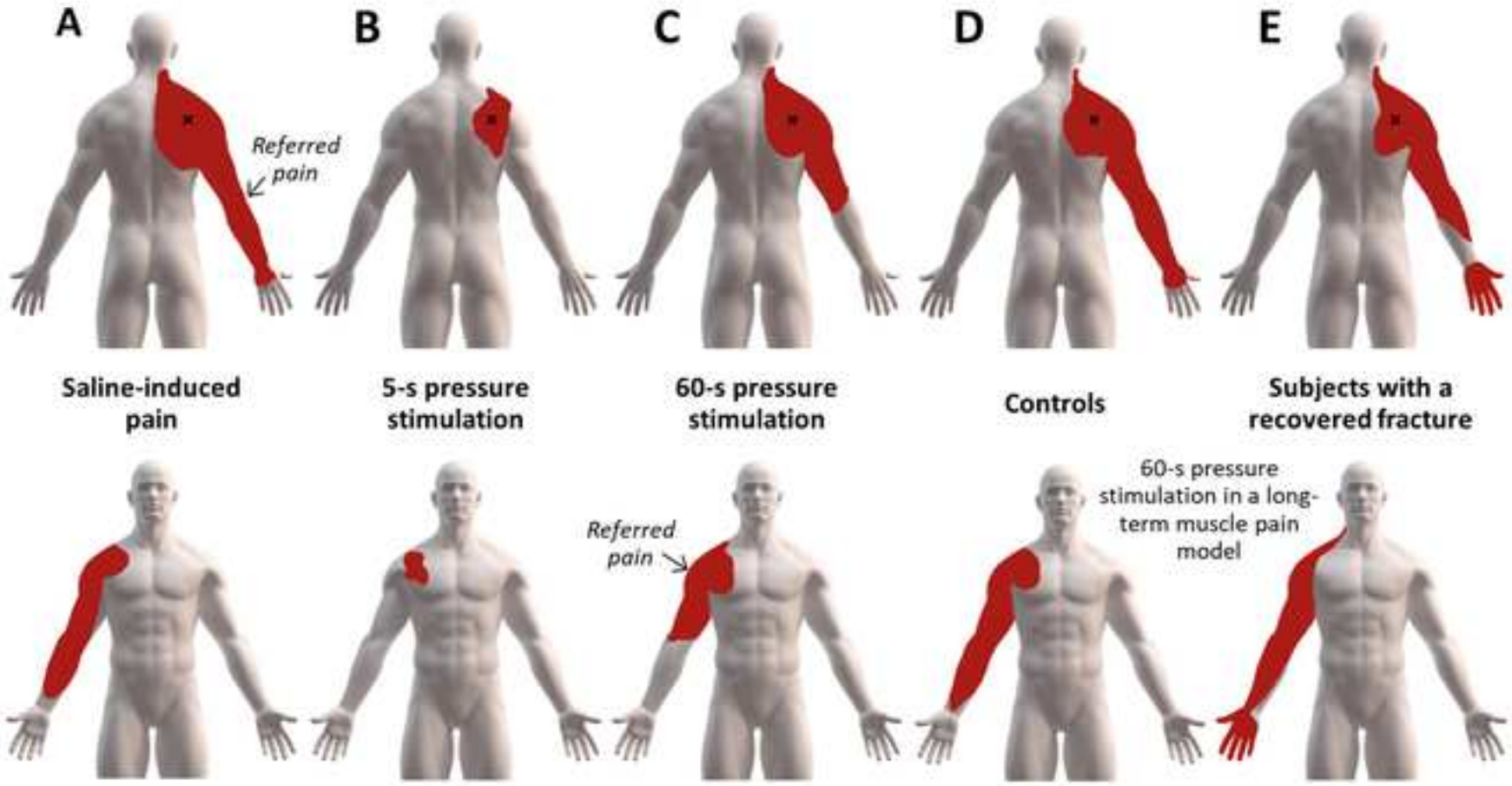


Fig. 5

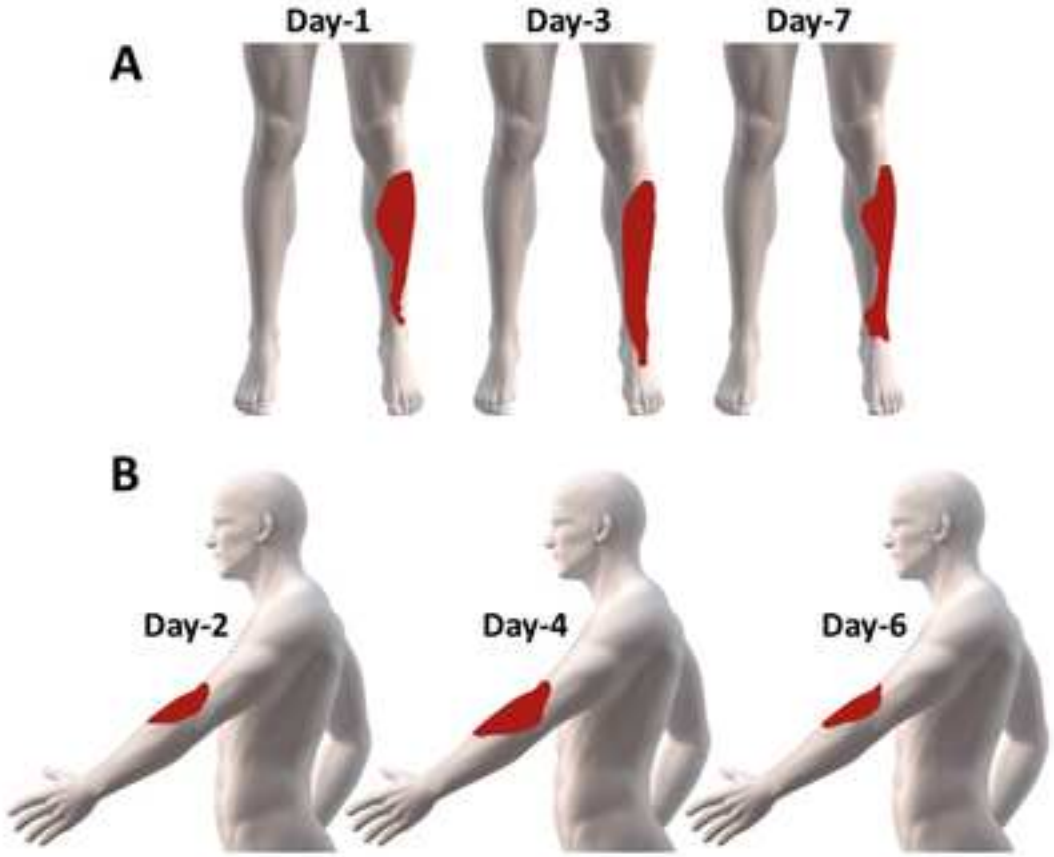


Fig. 6

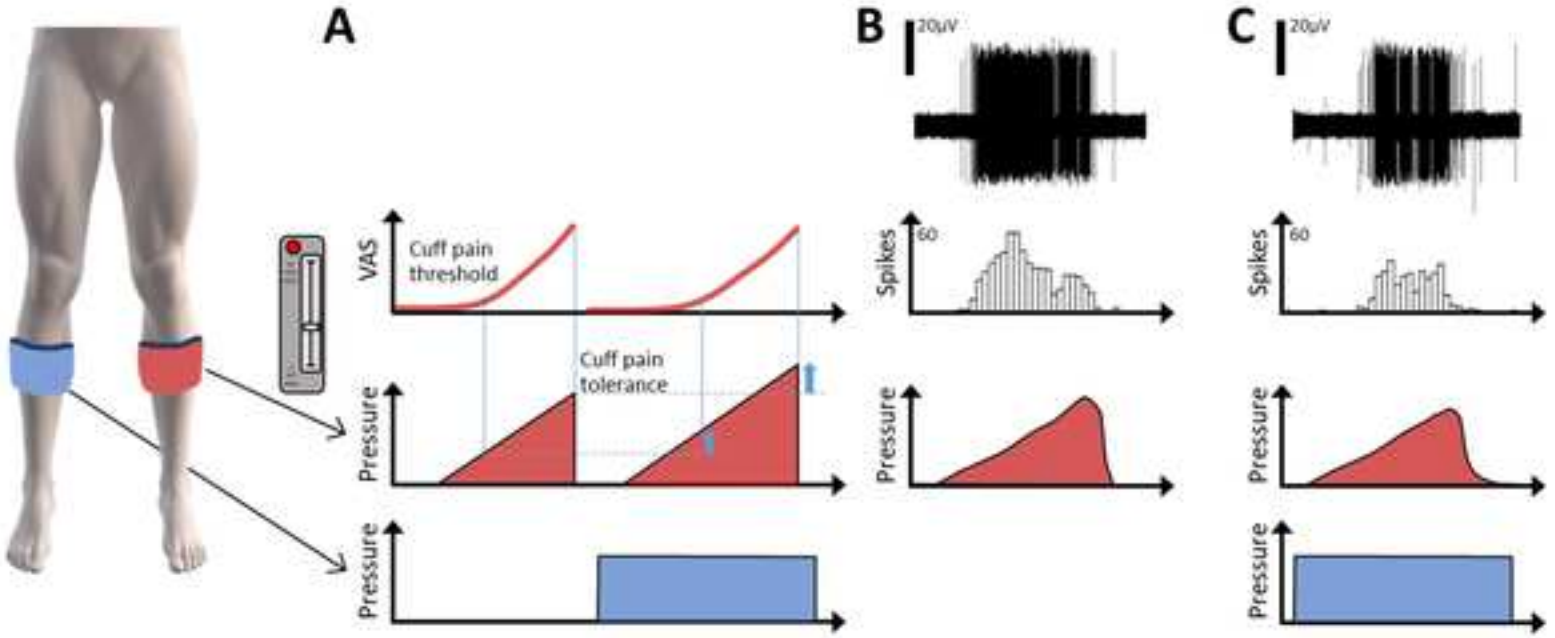


Fig. 7

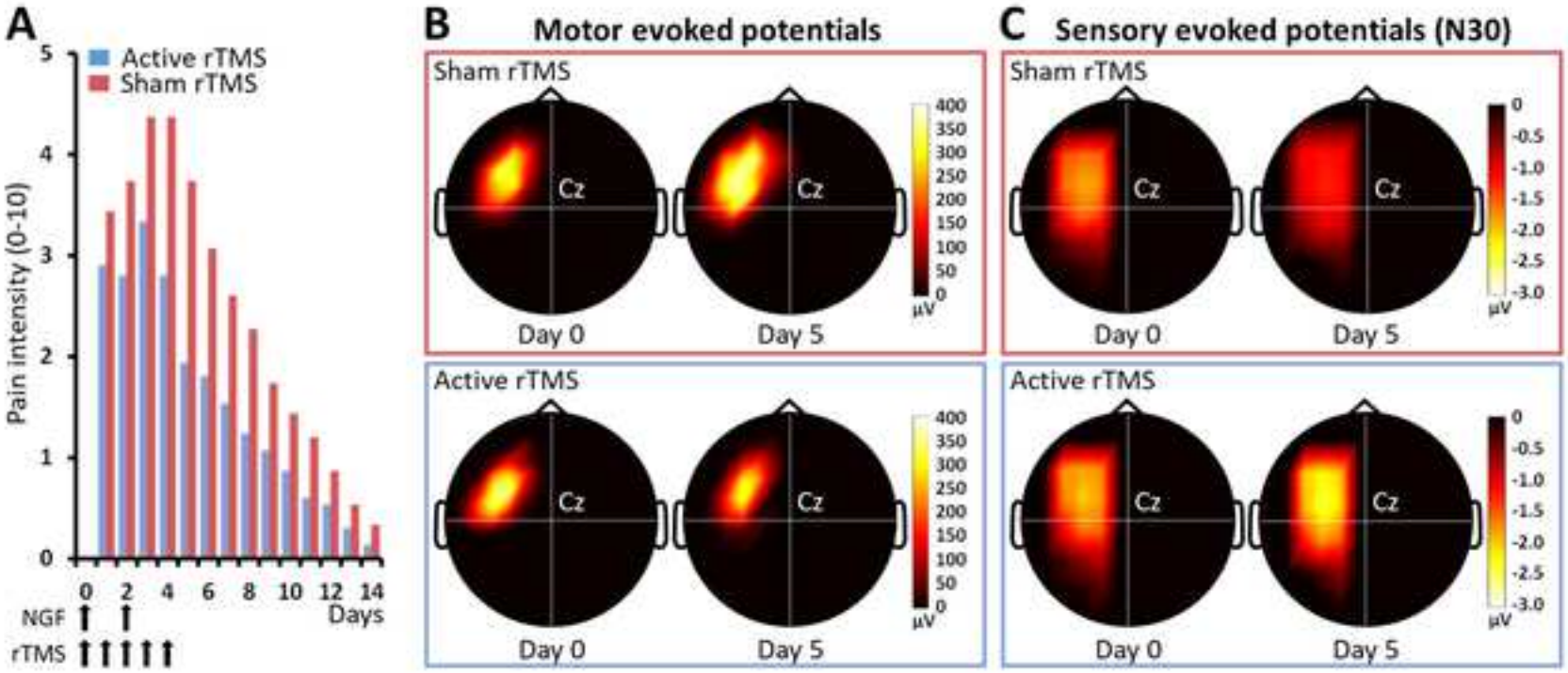
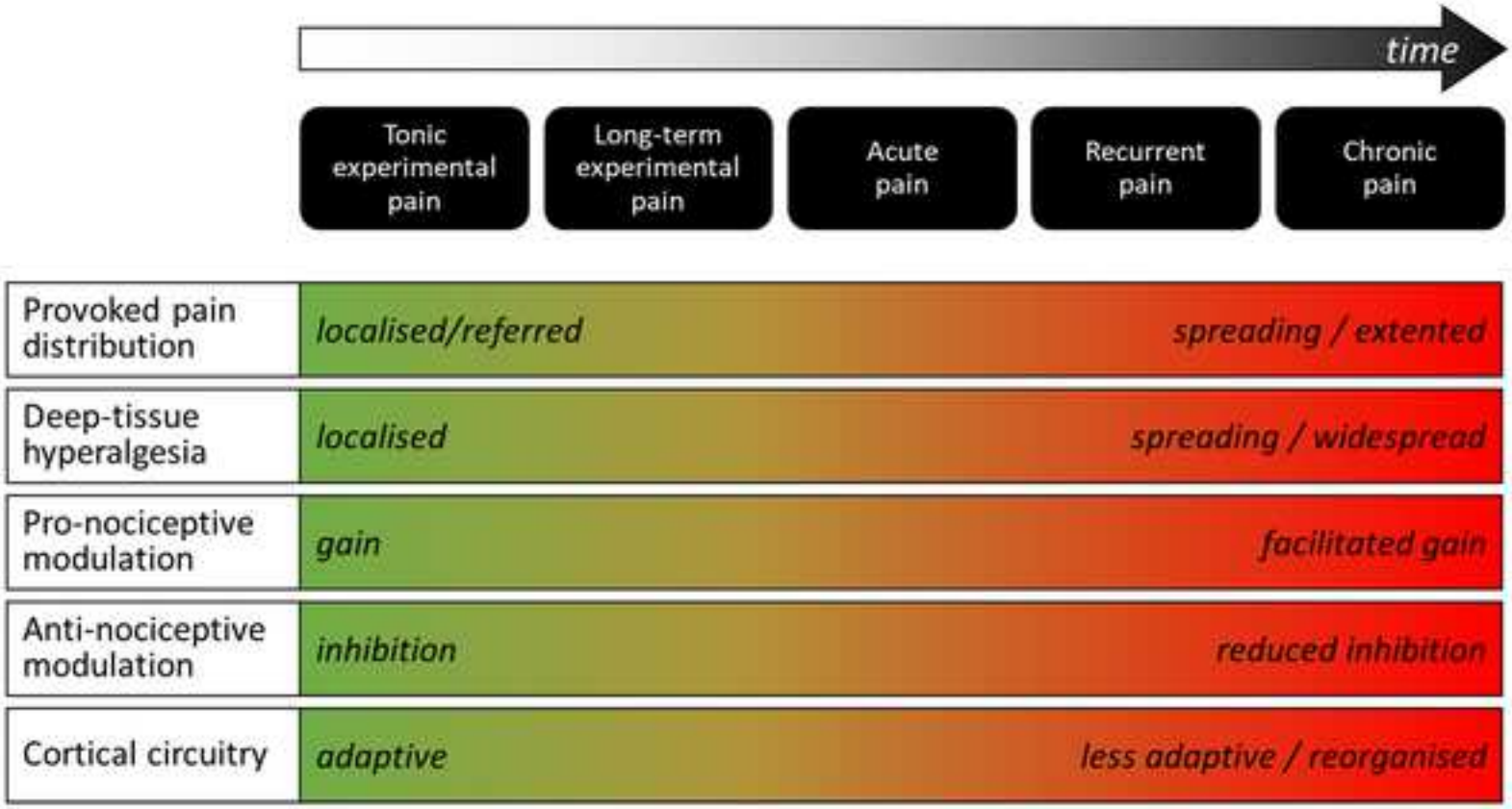


Fig. 8



ICMJE Conflict of Interest Form--REQUIRED from ALL authors of submission at revision stage