Neurobiology of Fibromyalgia Syndrome

DONALD D. PRICE and ROLAND STAUD

ABSTRACT. Accumulating evidence suggests that fibromyalgia syndrome (FM) pain is maintained by tonic impulse input from deep tissues, such as muscle and joints, in combination with central sensitization mechanisms. This nociceptive input may originate in peripheral tissues (trauma and infection) resulting in hyperalgesia/allodynia and/or central sensitization. Evidence for abnormal sensitization mechanisms in FM includes enhanced temporal summation of delayed pain in response to repeated heat taps and repeated muscle taps, as well as prolonged and enhanced painful after-sensations in FM patients but not control subjects. Moreover, magnitudes of enhanced after-sensations are predictive of FM patients' ongoing clinical pain. Such alterations of relevant pain mechanisms may lead to longterm neuroplastic changes that exceed the antinociceptive capabilities of affected individuals, resulting in ever-increasing pain sensitivity and dysfunction. Future research needs to address the important role of abnormal nociception and/or antinociception for chronic pain in FM. (J Rheumatol 2005;32 Suppl 75:22-28)

Key Indexing Terms:
TEMPORAL SUMMATION CHRONIC PAIN FIBROMYALGIA

In our review, the neurobiology of fibromyalgia syndrome (FM) is discussed in the context of what is known about neural mechanisms of nociception and central mechanisms of persistent pain conditions. We present a general view of mechanisms of nociception, central temporal summation, and central sensitization, and as well compare sensory tests that examine these mechanisms in normal pain-free human subjects. We then show how amplification and other alterations of these mechanisms apply to patients with FM.

NOCICEPTION, ACUTE PAIN, PERSISTENT PAIN

Pain is usually related to impulse input that originates from nociceptors in somatic or visceral tissues. The impulses travel in myelinated (A-delta) and unmyelinated (C) peripheral nerves, which first project to dorsal horn nociceptor-specific neurons and wide dynamic range neurons, before these second-order neurons transmit nociceptive information to brain regions involved in pain, including the thalamus, anterior cingulate cortex (ACC), anterior insular cortex, and somatosensory cortex. Nociceptor-specific neurons are so termed because they respond predominantly to specific stimulus intensities that either cause tissue damage or would cause tissue damage if maintained over time. Wide dynamic range neurons respond differentially over a very broad range of stimulus intensities, from very gentle touch to stimuli that cause tissue damage. Brain regions that receive input from nociceptor-specific and wide dynamic range neurons are related to sensory-discriminative, cognitive-evaluative, and affective processing of somatosensory nociceptive input. The activation of these brain regions is associated with pain experience and subsequent reflex and protective behaviors. Importantly, the same brain areas are likely to be involved in both acute and persistent pain conditions.

Reflex and reflective behaviors that are aimed at eliminating acute pain are not operative in chronic pain syndromes including FM. Patients with FM, like most chronic pain sufferers, do not display pain behaviors usually seen in acute pain, including increased perspiration, hypertension, hyperthermia, and tachycardia. FM patients have abnormal pain thresholds (hyperalgesia) and report amplified pain with a variety of nociceptive stimuli, including pressure, heat, and cold. Because no consistent tissue abnormalities have been detected in FM, central pain processing abnormalities need to be considered as important contributors to the heightened pain sensitivity of these patients.

In our review, we also discuss recent evidence that the clinical pain of patients with FM is related to abnormal central temporal summation of pain, or "windup," evoked by repetitive stimulation of peripheral nociceptive afferent neurons. Sensory testing experiments can be used to demonstrate that abnormal windup of FM patients is related to central nervous system (CNS) mechanisms of central sensitization and persistent pain.

As background to the central sensory abnormalities of FM patients, we discuss the normal role of nociceptors and the central consequences of repetitive stimulation of nociceptive neurons, and also describe how these mechanisms appear to be distorted in FM patients.

From the Department of Neuroscience and Department of Medicine, University of Florida, Gainesville, Florida, USA.

The authors have no conflict of financial interest related to the contents of this article.

D.D. Price, PhD, Departments of Oral and Maxillofacial Surgery and Neuroscience, Colleges of Dentistry and Medicine; R. Staud MD, McKnight Brain Institute, University of Florida.

Address reprint requests to D.D. Price, Claude Denson Pepper Center, PO Box 100416, Gainesville, FL 32610-0416. E-mail: Dprice@dental.ufl.edu

ROLE OF DIFFERENT NOCICEPTORS FOR PAIN

Nociception is associated with activation of a heterogeneous group of nociceptors that either express the neuropeptide substance P (SP) and calcitonin gene related peptide (CGRP) or isolectin B_4 (IB_4)[1]. Their sensory neurons terminate in the dorsal horn of the spinal cord, mainly in laminae I and II and to a lesser degree in lamina V. These spinal cord regions also contain postsynaptic neurons that express receptors implicated in nociceptive transmission, such as SP, neurokinin 1, and neurokinin 2, as well as glutaminergic receptors [N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, metabotropic]. TRPV-1 and TRPVL-1 receptors have been recently found to be activated by noxious heat[2], mechanical stimuli, or low pH (acid-sensing ion channels)[3,4]. Although IB_4-positive neurons can express several of these receptors, they are the only ones to display the purino-receptor P2X3[5]. This latter receptor is activated by purines such as adenosine triphosphate, which are frequently released after tissue injury. Very little is known about the receptor expression of neurons that innervate different tissues of the body, but some tissues seem to contain special pain receptors. Much information has been obtained from animal models in which specific pain receptors are lacking. Inbred mice without TRPV-1 receptors show decreased response to noxious heat, but mechanical nociception is normal[6]. Alternatively, mice without P2X3 receptors show no reduced nociceptive behavior using known stimuli.

MECHANISMS OF SLOW TEMPORAL SUMMATION AND CENTRAL SENSITIZATION

Using electrical shocks to a cutaneous nerve of cats, Mendell and Wall found that repeated volleys of action potentials in C-fibers resulted in a progressive increase in the number of action potentials evoked in second-order dorsal horn neurons[7]. Thus, with each successive C-fiber volley, the evoked impulse discharge of second-order neurons had a higher frequency and was more prolonged. This progressive increase in response reflects slow temporal summation and has been termed windup. Windup has been demonstrated to result from central rather than peripheral nervous system mechanisms, mostly because the input from peripheral nociceptors has been shown to decline or stay the same with stimulus repetition[8,9]. Windup of dorsal horn nociceptive neurons involving NMDA receptor mechanisms[10-12] can be attenuated in a dose-dependent manner by NMDA receptor antagonists[11-14].

This important mechanism of pain amplification, which operates at least partly in the dorsal horn of the spinal cord, precisely parallels the psychophysical characteristics of temporal summation of second pain. First pain, which is conducted by myelinated A-delta pain fibers, is often described as sharp and can be readily distinguished from second pain by most subjects. In contrast, second pain (transmitted by unmyelinated C-fibers), which is thought to be related to some chronic pain states, is most frequently reported as dull, aching, or burning[8,9,15-17]. Similar to windup in the dorsal horn, second pain increases in intensity when painful stimuli are applied more often than once every 3 seconds. Windup of both dorsal horn neurons and second pain can be inhibited by application of NMDA receptor antagonists, including dextromethorphan[13] and ketamine[14]. These parallels between windup of dorsal horn neurons and second pain almost certainly relate to the fact that dorsal horn neurons that display windup project to pain-related areas of the brain via several pathways. These pathways include not only the well characterized spinothalamic tract, but also the spinoreticular, spinohypothalamic, and the spinopontoamygdaloid pathways[18]. As a consequence of these multiple central projections, windup in the dorsal horn is likely to be a major cause of windup of second pain. This is critical, because windup is likely to be related to mechanisms of central sensitization and hence some persistent pain conditions[19,20].

Several types of well controlled experimental stimuli can reliably evoke windup of pain when applied to somatic tissues of normal pain-free human subjects[8,21,22], including electrical stimulation of C nociceptors[8], thermal stimulation of C nociceptors[17], and mechanical stimulation of muscle nociceptors[17,21-24]. Figure 1 illustrates the characteristics of windup of second pain evoked in normal pain-free subjects by repeated thermal stimuli at 52°C.

Some persistent pain conditions including FM are thought to be related to central mechanisms of sensitiza-

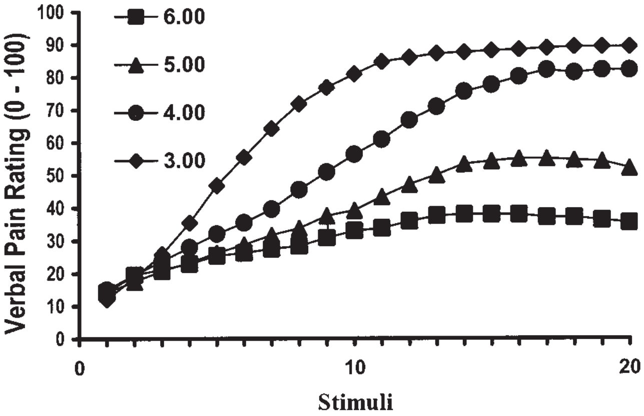

Figure 1. Windup of second pain in healthy control subjects. Twenty repetitive heat stimuli (52°C) were applied to the hand. Contact time was 0.7 s; interstimulatory intervals (ISI) varied between 3 s and 6 s, as shown at upper left. Pain intensity of test stimuli was rated on a verbal rating scale of 0–100 (0 = no pain; 20 = minimal pain; 100 = unbearable pain). Only repetitive stimuli at short ISI (3 s) resulted in a significant increase of pain ratings, compared to long ISI, which produced minimal pain sensations. From Vierck, *et al. J Neurophysiol 1997;78:992- 1002*[17].

tion wherein nociceptive neurons of the dorsal horn become hyperresponsive to nociceptive and sometimes even non-nociceptive somatic stimulation[25,26]. Central sensitization, in turn, is characterized by hyperalgesia and allodynia. It is associated with enlarged receptive fields and is often thought to occur as a consequence of slow temporal summation of dorsal horn neurons to repeated impulses from primary nociceptive neurons. Since this repetitive impulse input can be elicited by experimental laboratory stimuli, central sensitization and windup can be studied in both normal pain-free subjects and in patients with pain conditions such as FM.

NEUROPHYSIOLOGICAL ABNORMALITIES IN FM

Before we discuss the specific abnormalities of windup in patients with FM, it would be useful to consider some general sensory abnormalities and physiological characteristics of these patients.

As described by Mease elsewhere in these proceedings, FM is a chronic pain syndrome characterized by generalized pain, tender points (TP), disturbed sleep, and pronounced fatigue. Pain in FM is consistently felt in the musculature and may be related to sensitization of CNS pain pathways. The pathogenesis of FM is unknown, although abnormal concentration of CNS neuropeptides and alterations of hypothalamic-pituitary-adrenal axis have been described[27-30]. There is a large body of evidence for a generalized lowering of pressure pain thresholds in FM patients[31-35]. This mechanical allodynia of FM patients, however, is not limited to TP, but appears to be widespread[35]. In addition, almost all studies of FM patients showed abnormalities of pain sensitivity while using different methods of psychophysical testing. Most investigations have utilized thermal (heat and cold), mechanical, chemical, or electrical stimuli (single or repetitive) to the skin or muscles.

The most frequently described sensory abnormality in FM is the presence of TP. Eighteen areas have been defined as tender points by the American College of Rheumatology[36]. In addition to chronic widespread pain, the presence of decreased mechanical pain thresholds (tenderness) is required in at least 11 out of 18 TP for the diagnosis of FM. Abnormal tenderness, however, does not seem to be restricted to TP sites in FM but this abnormality is most frequently generalized[33,35]. Most TP are located at tendon insertion areas and have shown few detectable tissue abnormalities. Analysis of algesic substances at TP sites by microdialysis showed no difference between FM patients and healthy controls[37] and magnetic resonance imaging of TP was also unable to detect any specific abnormalities[38]. Although there is evidence for local vasoconstriction of TP areas in FM[39], these findings may mostly reflect physical deconditioning[40].

ABNORMAL WINDUP IN PATIENTS WITH FM

The noninvasive method of summation of second pain or windup has been used for the evaluation of central pain processing in patients with FM[41]. This technique reveals sensitivity to input from unmyelinated (C) afferents and indicates the status of NMDA receptor systems, which are implicated in a variety of chronic pain conditions.

Using a series of repetitive heat stimuli, we assessed temporal summation of second pain in both healthy controls and FM subjects[41]. Although windup pain was evoked in both controls and patients, the perceived magnitude of the sensory response to the first stimulus within a series was greater for patients versus controls, as was the degree of temporal summation within a series (Figure 2). Following the last stimulus in a series, painful windup after-sensations rated at 15 and 30 seconds after the last stimulus also were greater in magnitude and lasted longer in FM subjects compared to controls. These results indicate both augmentation and slower decay of nociceptive input in FM patients and provide convincing evidence for the presence of central sensitization.

The more prolonged after-sensations during windup decay, however, may have been simply related to the fact that greater windup occurred in FM patients. In order to specifically test whether after-sensations are more intense and take longer to decay in FM versus controls, we adjusted the stimulus temperature in a manner that evoked similar windup pain in both groups, as shown in Figure 3. Despite similar temporal summation in both groups, after-sensations were more intense and took more than twice as long to decay in FM compared to control subjects. Thus, the presence of enhanced windup

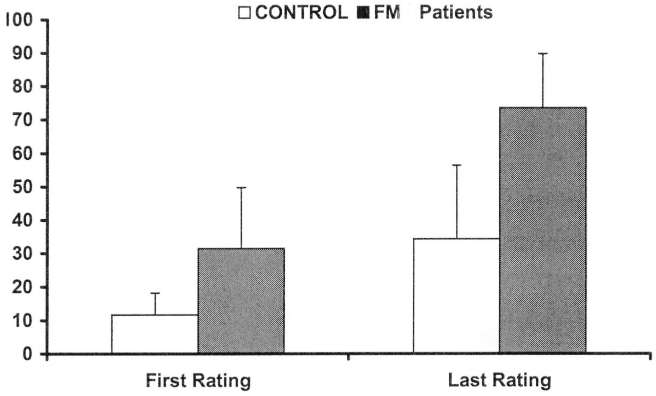

Figure 2. Windup of second pain in 10 female patients with FM and healthy controls, using repetitive heat stimuli of 52°C to the hand. Contact time of each stimulus was 0.7 s and ISI was 2 s. The rating of only the first and last stimulus (n = 20) of each series is shown. Pain intensity of test stimuli was rated on a verbal rating scale of 0–100 (see definition, legend to Figure 1). From Vierck, *et al. J Neurophysiol 1997;78:992-1002*[17].

and prolonged stimulus-evoked after-sensations may be functionally important for the initiation and maintenance of persistent pain conditions such as FM.

Enhanced magnitudes of windup of second pain and enhanced after-sensations are unlikely to be related to a response bias because these characteristics are highly specific. For example, there is no reason why patients with FM would expect enhanced after-sensations when their magnitude of windup of second pain is adjusted to match that of healthy subjects. Further, FM patients do not complain of ongoing heat pain, but rather pain from deep tissues. Thus, it is possible that central sensitization is evoked and maintained by impulses in deep tissues and thereby produces a central sensitized state during which central neurons are hyperresponsive to multiple sensory inputs, including cutaneous heat. There is recent evidence for this kind of peripheral and central interaction in irritable bowel syndrome[42]. Given these considerations, windup of heat-induced second pain may be a valuable diagnostic test in FM patients.

WINDUP MEASURES PREDICT CLINICAL PAIN INTENSITY IN FM PATIENTS

If windup and central sensitization are important mechanisms for FM pain, one should expect robust associations between windup, windup decay, and clinical pain intensity. In order to test the role of central pain mechanisms such as windup and windup decay for clinical pain we evaluated their usefulness as predictors of pain intensity of patients with FM. We found that thermal windup ratings correlated well with clinical pain intensity (Pearson's r = 0.529), thus emphasizing the important role of these pain mechanisms for FM. In addition, a statistical prediction model that included TP count, pain related negative affect, and windup ratings accounted for 50% of the variance in FM clinical pain intensity[43]. Importantly, each of these 3 factors was shown to statistically account for unique amounts of variance in clinical pain intensity. Windup after-sensation, however, was the strongest predictor of clinical pain intensity, accounting for most of the detectable variance (27%).

ABNORMAL MUSCLE WINDUP IN PATIENTS WITH FM

We have proposed that impulse input from deep tissues, particularly muscles, reflects the peripheral source that evokes and maintains central sensitization in FM[23]. This proposal predicts that repeated stimulation of muscle nociceptors would induce windup and central sensitization in FM patients. Accordingly, we conducted a study in which force-controlled mechanical stimulation was applied to the flexor digitorum muscle of the forearm in a series of brief contacts (15 stimuli, each of 1 second duration, at 3 or 5 second interstimulus intervals)[23]. These trains of stimuli were applied to both healthy controls and FM patients, as shown in Figure 4. Similar to cutaneous heat stimuli, FM patients demonstrated much more pronounced windup as well as more intense and

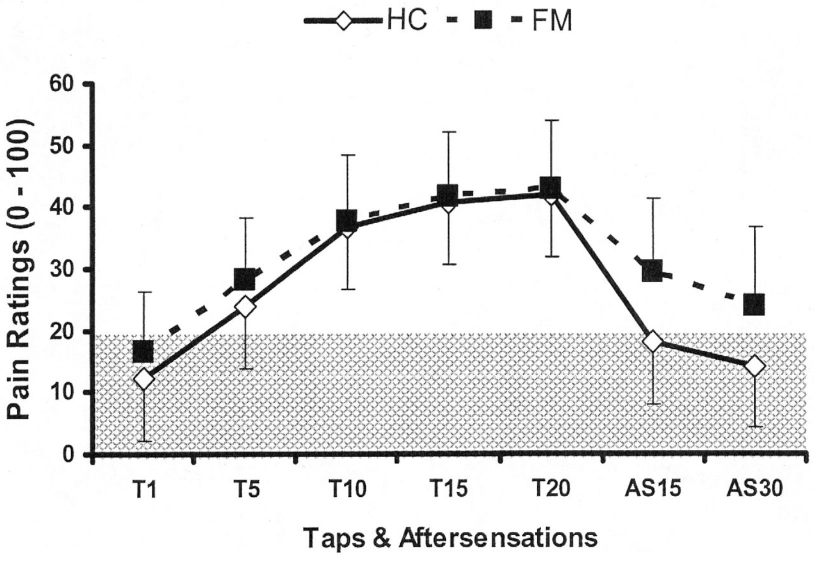

Figure 3. The decay of pain after windup is slower for FM patients compared to healthy control subjects (HC) even when the stimulus temperature is adjusted for the 2 groups to produce the same magnitude of windup. After-sensations at 15 and 30 seconds after last stimulus are painful for FM but not control subjects. The shaded area represents pain threshold of the verbal pain rating scale (0-100) used for this study. Based on data from Staud, *et al*[43]. *Pain 2003;105:215-22.*

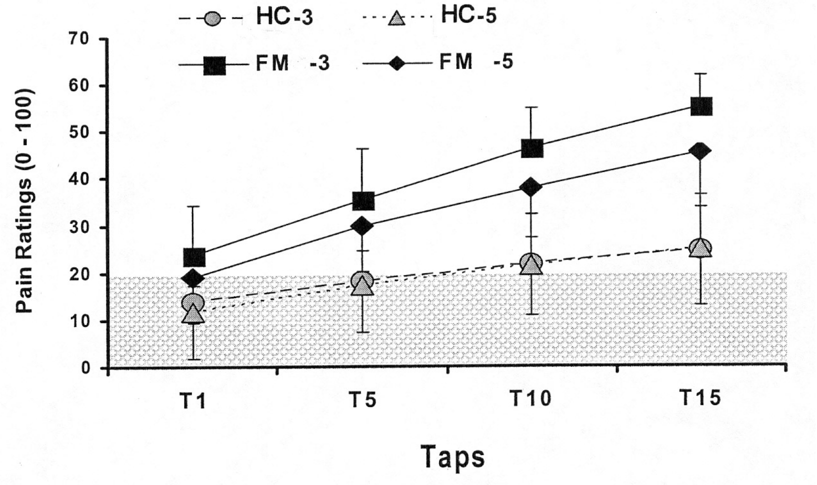

Figure 4. Pain ratings during a series of 15 repetitive muscle taps at ISI of 3 and 5 s, averaged across all series presented to controls (HC) and patients with FM. The shaded area identifies sensation levels below pain threshold (a rating of 20). Based on data from Staud, *et al*[23]. *Pain 2003;102:87-95.*

slower declining after-sensations compared to control subjects.

EFFECT OF EXERCISE ON FM PAIN

The explanation we have proposed thus far is that evoked or ongoing impulse input from deep tissues induces and maintains central sensitization in patients with FM. This explanation raises the question of how this peripheral input is generated. One obvious possibility is that of exercise of muscles associated with abnormal sensitivity (mechanical allodynia). Exercise has been shown to activate endogenous opioid and adrenergic systems, but attenuation of experimental pain by exercise has not been demonstrated consistently. We therefore assessed the antinociceptive effects of exercise on windup, a psychophysical method that is especially sensitive to opioid modulation, in both healthy controls and FM patients[44]. In addition, we determined the effects of exercise on windup after-sensations evoked by repeated thermal stimuli as described above. Temporal summation of late pain sensations was substantially attenuated by strenuous exercise in controls, but enhanced in individuals diagnosed with FM, an effect opposite to that obtained from age/sex matched control subjects. This study indirectly implicates a role of muscle nociceptors in FM pain and also suggests that analgesic effects of exercise may be lacking in FM patients.

Another line of evidence suggests that powerful antinociceptive mechanisms become activated during muscle contraction in healthy control subjects[45]. Specifically, during isometric muscle contraction of control subjects, the mechanical pain threshold increases over the contracted muscles as well as over distal muscle areas[46]. In FM patients, however, the pain threshold decreased over all areas, more pronounced proximal to the muscle contraction compared to distal[47]. This exercise related hyperalgesia may be the result of either sensitization of mechanoreceptors in FM or dysfunction of afferent pain inhibition activated by muscle contraction. These findings may explain some of the increased pain during exertion that is reported by FM patients.

ROLE OF MUSCLES FOR CLINICAL PAIN IN FM

The predominant symptom in FM is muscle pain and stiffness, consistent with our explanation thus far. In fact, many studies have focused on muscle tissue abnormalities in FM[48,49]. Light and electron microscopic evaluations identified moth-eaten and ragged-red fibers, indicating uneven and proliferating mitochondria. This finding suggested hypoperfusion of painful muscle tissues and has led to examinations of muscle microcirculation. Oxygen multipoint electrodes in trapezius muscles identified abnormal tissue oxygen pressures in FM patients. Because microcirculation of muscle tissues is controlled by locally produced metabolites, humoral factors, and the sympathetic nervous system, several investigations focused on these possible mechanisms[48,50,51]. Sympathetic ganglion blockade reversed the abnormal muscle findings. In addition, the amount of SP, a neurotransmitter stored within the afferent nociceptive fibers, was found to be increased in the trapezius muscles of FM patients compared to healthy controls[52].

Skeletal muscles have different fiber types, including type I, type IIA, and type IIB. Type I muscle fibers are associated with static muscle tone and posture. They are slow-twitch, fatigue resistant myocytes that contain high numbers of mitochondria for oxidative phosphorylation. Type II fibers are fast-twitch fibers and have high contraction force over short periods. They fatigue easily, are rich in glycogen, and use anaerobic glycolysis for energy metabolism. Type I muscle fibers can transform into type II fibers depending on demand placed on individual muscles. Therefore, inactivity and pain can be responsible in type II fiber loss/transformation.

Ionotropic and metabotropic nociceptors are found on peripheral unmyelinated sensory afferents in the skin and muscle[53]. These polymodal muscle nociceptors are located along blood vessels, except capillaries[54], and comprise free nerve endings supplied by group III (thin myelinated) and group IV (nonmyelinated) afferents, with conduction velocities of less than 30 m/s. The nerve endings have receptors for algesic substances like bradykinin, serotonin, glutamate, and prostaglandin E_2[55,56], which contribute to the sensitization of muscle nociceptors[57,58]. This sensitization process by endogenous substances that are likely to be released during trauma or inflammatory injury is probably the best established peripheral mechanism for muscle tenderness and hyperalgesia. Although information about responses of muscle nociceptors is largely based on animal studies[59,60], similar findings have also been reported from human studies[61,62].

CONCLUSION

Accumulating evidence suggests that FM pain is maintained by a combination of tonic impulse input from deep tissues, such as muscle and joints, in combination with central sensitization mechanisms. This nociceptive input may originate in peripheral tissues (trauma and infection) and result in hyperalgesia/allodynia and/or central sensitization. Such alterations of relevant pain mechanisms may lead to longterm neuroplastic changes that exceed the antinociceptive capabilities of affected individuals, resulting in ever-increasing pain sensitivity and dysfunction. Future research needs to address the important role of abnormal nociception and/or antinociception for chronic pain in FM.

REFERENCES

1. Kitchener PD, Wilson P, Snow PJ. Selective labeling of primary sensory afferent terminals in lamina II of the dorsal horn by injection of Bandeiraea simplicifolia isolectin B4 into peripheral nerves. Neuroscience 1993;54:545-51.
2. Caterina MJ, Julius D. The vanilloid receptor: a molecular gateway to the pain pathway. Annu Rev Neurosci 2001;24:487-517.
3. Gunthorpe MJ, Smith GD, Davis JB, Randall AD. Characterisation of a human acid-sensing ion channel (hASIC1a) endogenously expressed in HEK293 cells. Pflugers Arch 2001;442:668-74.
4. Krishtal O. The ASICs: signaling molecules? Modulators? Trends Neurosci 2003;26:477-83.
5. Burnstock G. P2 purinoceptors: historical perspective and classification. Ciba Found Symp 1996;198:1-28.
6. Caterina MJ, Leffler A, Malmberg AB, et al. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 2000;288:306-13.
7. Mendell LM, Wall PD. Responses of single dorsal cord cells to peripheral cutaneous unmyelinated fibres. Nature 1965;206:97-9.
8. Price DD. Characteristics of second pain and flexion reflexes indicative of prolonged central summation. Exp Neurol 1972;37:371-87.
9. Price DD, Hu JW, Dubner R, Gracely RH. Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses. Pain 1977;3:57-68.
10. Davies SN, Lodge D. Evidence for involvement of N-methylaspartate receptors in "wind-up" of class 2 neurons in the dorsal horn of the rat. Brain Res 1987;424:402-6.
11. Dickenson AH, Sullivan AF. Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurons following C fibre stimulation. Neuropharmacology 1987;26:1235-8.
12. Dickenson AH. A cure for wind up: NMDA receptor antagonists as potential analgesics. Trends Pharmacol Sci 1990;11:307-9.
13. Price DD, Mao J, Frenk H, Mayer DJ. The N-methyl-D-aspartate receptor antagonist dextromethorphan selectively reduces temporal summation of second pain in man. Pain 1994;59:165-74.
14. Arendt-Nielsen L, Petersen-Felix S, Fischer M, Bak P, Bjerring P, Zbinden AM. The effect of N-methyl-D-aspartate antagonist (ketamine) on single and repeated nociceptive stimuli: a placebo-controlled experimental human study. Anesth Analg 1995;81:63-8.
15. Price DD. Psychologicial and neural mechanisms of pain. New York: Raven Press; 1988.
16. Yeomans DC, Cooper BY, Vierck CJ Jr. Effects of systemic morphine on responses of primates to first or second pain sensations. Pain 1996;66:253-63.
17. Vierck CJ Jr, Cannon RL, Fry G, Maixner W, Whitsel BL. Characteristics of temporal summation of second pain sensations elicited by brief contact of glabrous skin by a preheated thermode. J Neurophysiol 1997;78:992-1002.
18. Bernard JF, Besson JM. The spino(trigemino)pontoamygdaloid pathway: electrophysiological evidence for an involvement in pain processes. J Neurophysiol 1990;63:473-90.
19. Dickenson AH, Sullivan AF. NMDA receptors and central hyperalgesic states. Pain 1991;46:344-6.
20. Price DD, Mao J, Mayer DJ. Central neural mechanisms of normal and abnormal pain states. In: Fields HL, Liebeskind JC, editors. Pharmacological approaches to the treatment of pain: new concepts and critical issues. Seattle: IASP Press; 1994:61-84.
21. Price DD, Dubner R. Mechanisms of first and second pain in the peripheral and central nervous systems. J Invest Dermatol 1977;69:167-71.
22. Yeomans DC, Proudfit HK. Nociceptive responses to high and low rates of noxious cutaneous heating are mediated by different nociceptors in the rat: electrophysiological evidence. Pain 1996;68:141-50.
23. Staud R, Cannon RC, Mauderli AP, Robinson ME, Price DD, Vierck CJ. Temporal summation of pain from mechanical stimulation of muscle tissue in normal controls and subjects with fibromyalgia syndrome. Pain 2003;102:87-95.
24. Verne GN, Robinson ME, Price DD. Hypersensitivity to visceral and cutaneous pain in the irritable bowel syndrome. Pain 2001;93:7-14.
25. Berglund B, Harju EL, Kosek E, Lindblom U. Quantitative and qualitative perceptual analysis of cold dysesthesia and hyperalgesia in fibromyalgia. Pain 2002;96:177-87.
26. Kosek E, Ekholm J, Hansson P. Sensory dysfunction in fibromyalgia patients with implications for pathogenic mechanisms. Pain 1996;68:375-83.

27. Russell IJ, Orr MD, Littman B, et al. Elevated cerebrospinal fluid levels of substance P in patients with the fibromyalgia syndrome. Arthritis Rheum 1994;37:1593-601.
28. Bradley LA, Alarcon GS, Sotolongo A, et al. Cerebrospinal fluid (CSF) levels of substance P (SP) are abnormal in patients with fibromyalgia (FM) regardless of traumatic or insidious pain onset [abstract]. Arthritis Rheum 1998;41 Suppl:S256.
29. Vaeroy H, Helle R, Forre O, Kass E, Terenius L. Elevated CSF levels of substance P and high incidence of Raynaud phenomenon in patients with fibromyalgia: new features for diagnosis. Pain 1988;32:21-6.
30. Neeck G, Crofford LJ. Neuroendocrine perturbations in fibromyalgia and chronic fatigue syndrome. Rheum Dis Clin North Am 2000;26:989-1002.
31. Lautenschlager J, Bruckle W, Schnorrenberger CC, Muller W. Measuring pressure pain of tendons and muscles in healthy probands and patients with generalized tendomyopathy (fibromyalgia syndrome) [German]. Z Rheumatol 1988;47:397-404.
32. Quimby LG, Block SR, Gratwick GM. Fibromyalgia: generalized pain intolerance and manifold symptom reporting. J Rheumatol 1988;15:1264-70.
33. Tunks E, Crook J, Norman G, Kalaher S. Tender points in fibromyalgia. Pain 1988;34:11-9.
34. Mikkelsson M, Latikka P, Kautiainen H, Isomeri R, Isomaki H. Muscle and bone pressure pain threshold and pain tolerance in fibromyalgia patients and controls. Arch Phys Med Rehabil 1992;73:814-8.
35. Kosek E, Ekholm J, Hansson P. Increased pressure pain sensibility in fibromyalgia patients is located deep to the skin but not restricted to muscle tissue. Pain 1995;63:335-9.
36. Wolfe F, Smythe HA, Yunus MB, et al. The American College of Rheumatology 1990 criteria for the classification of fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum 1990;33:160-72.
37. Ashina M, Stallknecht B, Bendtsen L, et al. Tender points are not sites of ongoing inflammation — in vivo evidence in patients with chronic tension-type headache. Cephalalgia 2003;23:109-16.
38. Kravis MM, Munk PL, McCain GA, Vellet AD, Levin MF. MR imaging of muscle and tender points in fibromyalgia. J Magn Reson Imaging 1993;3:669-70.
39. Jeschonneck M, Grohmann G, Hein G, Sprott H. Abnormal microcirculation and temperature in skin above tender points in patients with fibromyalgia. Rheumatology Oxford 2000;39:917-21.
40. Simms RW. Is there muscle pathology in fibromyalgia syndrome? Rheum Dis Clin North Am 1996;22:245-66.
41. Staud R, Vierck CJ, Cannon RL, Mauderli AP, Price DD. Abnormal sensitization and temporal summation of second pain (wind-up) in patients with fibromyalgia syndrome. Pain 2001;91:165-75.
42. Verne GN, Hines NC, Robinson ME, et al. Central representation of visceral and cutaneous hypersensitivity in the irritable bowel syndrome. Pain 2003;103:99-110.
43. Staud R, Robinson ME, Vierck CJ Jr, Cannon RL, Mauderli AP, Price DD. Ratings of experimental pain and pain-related negative affect predict clinical pain in patients with fibromyalgia syndrome. Pain 2003;105:215-22.
44. Vierck CJ Jr, Staud R, Price DD, Cannon RL, Mauderli AP, Martin AD. The effect of maximal exercise on temporal summation of second pain (wind-up) in patients with fibromyalgia syndrome. J Pain 2001;2:334-44.
45. Mense S, Simons DG, Russell IJ. Muscle pain: understanding its nature, diagnosis, and treatment. Philadelphia: Lippincott Williams and Wilkins; 2000.
46. Kosek E, Ekholm J. Modulation of pressure pain thresholds during and following isometric contraction. Pain 1995;61:481-6.
47. Kosek E, Ekholm J, Hansson P. Modulation of pressure pain thresholds during and following isometric contraction in patients with fibromyalgia and in healthy controls. Pain 1996;64:415-23.
48. Yunus MB, Kalyan-Raman UP. Muscle biopsy findings in primary fibromyalgia and other forms of nonarticular rheumatism. Rheum Dis Clin North Am 1989;15:115-34.
49. Bengtsson A. The muscle in fibromyalgia. Rheumatology Oxford 2002;41:721-4.
50. Kalyan-Raman UP, Kalyan-Raman K, Yunus MB, Masi AT. Muscle pathology in primary fibromyalgia syndrome: a light microscopic, histochemical and ultrastructural study. J Rheumatol 1984;11:808-13.
51. Yunus MB, Kalyan-Raman UP, Masi AT, Aldag JC. Electron microscopic studies of muscle biopsy in primary fibromyalgia syndrome: a controlled and blinded study. J Rheumatol 1989;16:97-101.
52. De Stefano R, Selvi E, Villanova M, et al. Image analysis quantification of substance P immunoreactivity in the trapezius muscle of patients with fibromyalgia and myofascial pain syndrome. J Rheumatol 2000;27:2906-10.
53. Cairns BE, Hu JW, Arendt-Nielsen L, Sessle BJ, Svensson P. Sex-related differences in human pain and rat afferent discharge evoked by injection of glutamate into the masseter muscle. J Neurophysiol 2001;86:782-91.
54. Mense S. Nociception from skeletal muscle in relation to clinical muscle pain. Pain 1993;54:241-89.
55. Graven-Nielsen T, Mense S. The peripheral apparatus of muscle pain: Evidence from animal and human studies. Clin J Pain 2001;17:2-10.
56. Svensson P, Cairns BE, Wang KL, et al. Glutamate-evoked pain and mechanical allodynia in the human masseter muscle. Pain 2003;101:221-7.
57. Marchettini P, Simone DA, Caputi G, Ochoa JL. Pain from excitation of identified muscle nociceptors in humans. Brain Res 1996;740:109-16.
58. Simone DA, Marchettini P, Caputi G, Ochoa JL. Identification of muscle afferents subserving sensation of deep pain in humans. J Neurophysiol 1994;72:883-9.
59. Kumazawa T, Mizumura K. The polymodal C-fiber receptor in the muscle of the dog. Brain Res 1976;101:589-93.
60. Mense S. Muscular nociceptors. J Physiol Paris 1977;73:233-40.
61. Graven-Nielsen T, Mense S. The peripheral apparatus of muscle pain: evidence from animal and human studies. Clin J Pain 2001;17:2-10.
62. Sorensen J, Graven-Nielsen T, Henriksson KG, Bengtsson M, Arendt-Nielsen L. Hyperexcitability in fibromyalgia. J Rheumatol 1998;25:152-5.