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Peripheral and Spinal Mechanisms of Pain and Dry Needling Mediated Analgesia: A Clinical Resource Guide for Health Care Professionals

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Abstract

There are a number of biochemical, biomechanical, endocrinological and neurovascular mechanisms underpinning the anti-nociceptive and anti-inflammatory effects of dry needling (DN). While myofascial trigger points likely play a role in peripheral pain, a diagnostic tool for localizing them has not been validated, and DN studies that have targeted trigger points to elicit localized twitch responses have reported mixed results. Therefore, the mechanism responsible for DN-mediated analgesia may be more complicated. DN activates opioid-based pain reduction, mediated by endogenous cannabinoids and the sympathetic nervous system, and non-opioid pain relief via serotonin and norepinephrine from the brain stem. DN also triggers the hypothalamic-pituitary-adrenal axis centrally and the corticotropin releasing hormone-proopiomelanocortin-corticosteroid axis locally to inhibit cox-2, reducing inflammatory cytokines. Recent studies demonstrate that DN combined with mechanical and/or electrical stimulation may reverse PKC-mediated peripheral hyperalgesic priming by normalizing nociceptive channels, to include TRPV, ASIC, TTX and P2X/Y. Electrical DN (EDN) stimulates immune cells, fibroblasts and keratinocytes to release CGRP and substance-P, altering the stimulation of TTX receptors to reverse hyperalgesia. It also encourages the supraoptic nucleus to release oxytocin to quiet ASIC receptors peripherally and stimulate opioid interneurons spinally. Moreover, EDN inhibits ERK1/2 kinase pathways of inflammation in the spinal cord and stimulates Aδ fibers and N/OFQ to reverse C-fiber mediated central changes. Mechanotransduction of fibroblasts and peripheral nerves via TRPV1 and P2X/Y-mediated intracellular Ca^{2+} wave propagation and subsequent activation of the nucleus accumbens inhibits spinal pain transmission via glycinergic and opioidergic interneurons. The increased ATP is metabolized to adenosine, which activates P1 purinergic receptors, events considered key to DN analgesia and rho kinase-based tissue remodeling. Mechanotransduction-mediated release of histamine further explains analgesia secondary to needling points distal to pain. DN-mediated analgesia is dependent on a number of synergistic physiologic events involving biochemical and mechanical processes in neural, connective and muscle tissue.

Introduction

Over the last 20 years, the number of Americans seeking acupuncture treatment has continued to rise. While less than 1% of the U.S. population sought acupuncture treatment in the early 90's, over 12 million Americans received acupuncture in 2010 [1]. Moreover, 2007 NHIS data suggests that the vast majority of Americans seeking acupuncture do so for pain-related conditions, primarily arthritis, and other types of musculoskeletal pain, headaches, and fibromyalgia [1]. Another intervention that uses thin filiform needles to penetrate the skin is dry needling (DN). A procedure commonly used by Western-based, health care professionals (i.e. physicians, osteopaths, chiropractors, physical therapists, etc.), DN has also gained popularity in the United States for the treatment of neuromusculoskeletal conditions over the past 10 years. As Dunning et al. suggested, while the terminology, philosophy, and theoretical constructs differ between Western-based DN and traditional Chinese acupuncture, the procedure of inserting monofilaments into the body is essentially the same [2]. Therefore, manual DN and electrical DN (EDN) are used synonymously with manual acupuncture and electroacupuncture, respectively, throughout this narrative review to describe needling procedures without medicine or injectate that penetrate the skin to varying tissue depths. Furthermore, given the number of clinical trials authored by acupuncturists not claiming to move "*qi*" along channels or meridians for the purpose of treating traditional Chinese medicine (TCM) diagnoses such as blood stagnation and *bi* syndrome, practitioners of DN should not ignore or abandon the acupuncture literature [2]. On the contrary, evidence-based, health care practitioners should pay attention to large-scale trials that describe location, depth and stimulation of needles, and treatment duration used to successfully treat neuromusculoskeletal conditions, regardless of profession, while carefully considering the biochemical, biomechanical, endocrinological, neurovascular, supraspinal and segmental mechanisms underlying needling treatments without injectate [2,3]. While the former is manageable, the latter is often overlooked. The purpose of this narrative review is to provide a convenient, clinical resource tool for Western-based, health care providers on the peripheral and spinal mechanisms responsible for pain reduction following DN.

The Physiology of Pain Related to Myofascial Trigger Points

The "integrated hypothesis" of myofascial trigger point (MTrP) formation was originally proposed by Travell and Simons in 1993 and later expanded by Gerwin et al. [4]. The hypothesis suggests that excessive acetylcholine (ACh) and subsequent Ca^{2+} release initiates a continuous cycle of localized muscle contraction [4]. The increased Ca^{2+} release from sarcoplasmic reticulum (SR) is likely the product of muscle overuse or contracture, [5-7] while extracellular Ca^{2+} may represent sarcolemma damage via muscle overload or trauma [8,9]. Importantly, a number of studies have recorded a significant increase of end plate noise in MTrP vs. non-MTrP locations, which seem to correlate with patient irritability [8,10,11]. The localized hypertonicity begins to block blood flow to the muscle, resulting in a shortage of oxygen and nutrients and leading to ischemia and hypoxia [4,8].

Local ischemia and hypoxia lead to the release of a number of chemicals responsible for propagating pain and inflammation such as bradykinin, prostaglandins, serotonin, calcitonin gene-related peptide (CGRP) and substance P (SP) along with a number of inflammatory cytokines, such as tumor necrosis factor-α (TNF- α), interleukin-1β (IL-1β, interleukin-6 (IL-6) and interleukin-8 (IL-8) [4,12]. There is also a significant release of H^+ ions and adenosine triphosphate (ATP) [12]. The extracellular increase in H^+ ions results in a drop in pH, which directly affects the action of acetylcholinesterase, an enzyme required to remove ACh from the neuromuscular junction. The acidic environment exaggerates the release of CGRP, which inactivates acetylcholinesterase, while the low pH directly inhibits it [4,12,13]. Gerwin et al. further reported that CGRP intensifies the response of muscle fibers to ACh by increasing the sensitivity and synthesis of receptors at the neuromuscular junction [4]. The increased metabolic demand further depletes any remaining intracellular ATP, effectively shutting down the Ca^{2+}-ATPase pump's ability to reuptake Ca^{2+} back into the SR [14]. Inhibited acetylcholinesterase allows hypersensitive ligand-gated receptors on the sarcolemma continuous access to ACh, and improperly functioning Ca^{2+}-ATPase pumps permit Ca^{2+} to continuously bind with troponin, collectively resulting in hypertonicity and propagating the metabolic crisis. Notably, Shah et al. found a significant increase in H+ ions, bradykinin, CGRP, SP, TNF-α, IL-1β, serotonin and norepinephrine in patients with active trigger points compared to patients with latent and no trigger points in their upper trapezius muscles [12,15]. The continuous presence of factors of pain and inflammation sets the stage for peripheral hyperalgesia.

Vessel compression resulting in an ischemic or hypoxic environment could also be due to the formation of scar tissue in the subacute and chronic patient [16]. Biomechanical deficiencies such as scoliosis, leg length discrepancy, pelvic torsion and facet joint dysfunction could also serve as primary catalysts [17,18]. In the case of facet joint dysfunction, the muscles that attach to or are associated with the displaced or fixated vertebrae would be tonically pulled, stretched and/or irritated [19-21]. This would invariably activate muscle spindles, located in the intrafusal aspect of the muscle. The result is activation of type 1a and type II afferents, which stimulate alpha and gamma motor neurons, respectively [22]. Alpha motor neurons increase muscle tension to counter the stretch, while gamma motor neurons maintain the sensitivity of the muscle spindles [22]. In the case of an irritated facet, the continuous activation of α motor neurons by muscle spindles could certainly propagate a cycle of hypertonicity, catalyzing a metabolic crisis. Interestingly, however, there is no evidence of persistent alpha motor neuron activity to account for this hypertonicity [23]. Rather, the sustained muscle contraction quickly fatigues, and the metabolic crisis results in factors of pain and inflammation, which stimulate chemo sensitive type III and IV muscle spindle afferents [24,25]. Type III and IV afferents inhibit alpha motor neurons [26] while stimulating beta and gamma motor neurons in a positive-feedback loop [22]. While beta and gamma motor neurons continue to sensitize muscle spindles, propagating the cycle of pain and inflammation, beta motor neurons are able to directly stimulate extrafusal muscle fibers, resulting in a sustained, silent contraction more commonly referred to clinically as a palpable taught band [22]. Thus, muscle spindles seem to play a primary role in the formation of MTrPs.

Interestingly, Hubbard and Berkhoff originally hypothesized that the electrical noise coming from active trigger points in the upper trapezius was due to muscles spindles [11]. However, Simons found an increase in motor endplates and not muscle spindles in the vicinity of the trigger point [27]. Moreover the waveform of the noise in active trigger points was consistent with motor endplate and not muscle spindle activity [27]. Traditionally, electrophysiological recordings of end plate noise consist of endplate potentials, caused by spontaneous ACh release from neuromuscular junctions, and end plate spikes, the result of needle irritation of the neuromuscular junction [22,28,29]. However, more recent studies suggest that the electrical noise is produced by extrafusal neuromuscular junctions, while the combination of noise and spikes demonstrates intrafusal activity in the vicinity of the motor endplates [22]. Moreover the spikes may be unique to intrafusal activity, representing the activation of gamma and beta motor neurons by muscle spindles [22,30,31]. These findings again strongly implicate the role of muscle spindles in trigger point formation and maintenance.

The pain-spasm-pain model of hypertonicity also lends itself well to the cycle of pain typically associated with trigger points. Originally proposed by Travell et al. [6] and developed by Johansson and Sojka [32], the model suggests that, regardless of the mechanism, hyperactive muscle produces an increase in metabolic by-products such as lactic and arachidonic acid, eventually resulting in vascularly compromised tissue, which further drops the pH through the release of H^+ ions. Interestingly, Shah found that even in absence of muscle damage, acidity was the primary driving force leading to mechanical hyperalgesia [12,15]. The pain stimulates groups II, III and IV muscle afferents, which provides continuous excitatory projections to γ motor neurons in laminae-9 in the ventral horn of the spinal cord [22]. Since γ motor neurons control the sensitivity of muscle spindles, their stimulation increases the probability that muscle spindles will activate α motor neurons, thereby propagating the cycle of hypertonicity [33]. Interestingly, Lund et al. recently confirmed the presence of pain receptors within muscle spindles [34]. As the authors suggest, activation of these nociceptors may allow spindles to auto-sensitize and directly propagate the "γ-gain" cycle, further implicating their role in the formation of MTrPs [34].

The model of "γ -gain" has been demonstrated in a number of studies of joint pain. In particular, facet joint pain has been shown to activate γ motor neurons and thereby increase the sensitivity of muscle spindles [35-37]. In addition, numerous studies have demonstrated that spinal manipulation increases the afferent discharge from mechanoreceptors and subsequently quiets α motor neurons, resulting in reduced EMG activity of paraspinal muscles [38]. Notably, one of the most published and well-recognized researchers from the acupuncture profession, Chang-Zern Hong, found that he could

reproduce trigger point pain in the rhomboid by adding pressure to the C4-C5 facet joint, but he could not reproduce pain in the facet joint by placing a needle in the trigger point [39]. As such, Hong concluded that needling patients with paraspinal dysfunction is not justified until they receive other non-invasive treatments such as spinal manipulation, as the trigger point is likely the product of facet dysfunction. Therefore, and as Hong suggests, spinal manipulation and needling techniques may work well additively to treat trigger point related dysfunction [39].

The Treatment of Myofascial Trigger Points with Dry Needling

While the above explanation provides an excellent theoretical construct for how MTrPs form and ultimately lead to pain, it does not necessarily imply that trigger points are the primary target for DN procedures [2]. Presently, there is a lack of robust, empirical evidence validating the clinical diagnostic criteria for MTrPs [40] proposed by Travell and Simons [41,42] and Fischer [43,44]. In a systematic review by Myburgh et al., "tenderness" was the only criteria found to be moderately reliable for trigger point identification and only in the upper trapezius [45]. That is, examiners were able to agree that the upper trapezius was tender because of a trigger point but not on the actual location of the trigger point within the muscle. Moreover, while the intra and inter-rater reliability of identifying a muscle with a MTrP (not the location of the trigger point within the muscle) seems to improve with clinical experience and knowledge of the impairment, experts in myofascial pain syndrome and fibromyalgia were unable to reliably identify taught bands, active trigger points and localized twitches when blinded to patient condition [46]. According to Sciotti et al., the inter-examiner error associated with identifying the location of trigger points in muscle ranges between 3.3 and 6.6 cm [47]. It is therefore unlikely that clinicians are able to accurately target trigger points in a reliable fashion with monofilament needles between .025 mm and 0.35 mm wide [47].

The Physiology and Relevance of the Localized Twitch Response

Based on the expanded integrated hypothesis, the primary objective of needling MTrPs is to clear excessive ACh from neuromuscular junctions and facilitate Ca^{2+} reuptake back into the SP [4]. By repeatedly tapping a needle onto sensitive loci within MTrPs via a technique commonly referred to as "pistoning", nociceptive afferent information is thought to be sent to the spinal cord, resulting in the activation of α motor neurons [48,49]. This results in a reflexive localized twitch response (LTR) that may be analogous to blowing up the neuromuscular junction [49]. The LTR seems to be reproducible until the excess ACh at the neuromuscular junction is depleted. Moreover, a number of studies have demonstrated a reduction in endplate noise following the LTR [48,50]. Shah et al. further reported a reduction in CGRP and SP following a LTR in the upper trapezius and a subsequent drop in pain and disability [12].

While clearing excessive ACh from neuromuscular junctions seems advantageous, the results of controlled trials that aim to target trigger points and elicit a LTR are inconsistent with respect to their ability to reduce pain and disability. Two recent studies found no correlation between LTR and treatment success rate after needling the upper trapezius [51] and brachialis muscle [52]. It is not presently known whether the reduction of CGRP and SP is the product of the LTR or

simply a "wash-out" effect of an increase in blood flow secondary to needling [53]. Also, while the LTR is able to produce immediate results in pain and disability, the improvements are often not long-term [54,55]. Moreover, the repeated pistoning of the needle required to elicit a LTR has been shown to be traumatic to muscle tissue, resulting in added inflammation and discomfort [56]. Interestingly, Qerama et al. injected botulinum toxin into the vicinity of trigger points in the infraspinatus, significantly reducing the activity of the end plate potentials. However, the injection did not influence the patient's pain intensity and mechanical pain threshold, raising the question whether the LTR is a clinically relevant phenomenon [57]. After injecting 312 painful sites throughout the body, Lewit found that needles, not the injectate, resulted in immediate analgesia in 86.8% of his patients [58]. Notably, only 2 of the 14 most common treatment sites were muscle tissue [58]. That is, 12 of the most common structures targeted by Lewit did not have neuromuscular junctions required to cause a LTR. Similarly, much of the work by Baldry further suggests that needling the subcutaneous tissue overlaying a MTrP provides enough depth for a successful outcome [59]. The fact that the needles never entered the muscle tissue suggests that the LTR may not be a crucial component of the treatment.

The Cellular Correlates of Peripheral Pain

Peripheral nerve endings contain a number of channels and receptors that recognize various types of pain information [60]. There are a number of intra-cellular signal-transduction mechanisms that process and integrate the information via protein kinase enzymes [60]. Interestingly, a number of studies have suggested that protein kinase C-epsilon (PKC-ε) may be a commonality among the major cellular mechanisms of peripheral hypersensitivity that provides a switch separating acute conditions from central mediated chronic pain, a concept more formally referred to as hyperalgesic priming [61,62-64]. Rats that were injected with carrageenan (low dose prostaglandin) had an inflammatory response, likely mediated by voltage gated tetrodotoxin (TTX) currents via EP3 prostaglandin receptors, which lasted 4-hours and then resolved [61]. However, if the rat was again injected with carrageenan, the resulting inflammation was more severe and lasted for at least 24-hours [61]. During the initial injection, a PKA inhibitor was enough to inhibit the hyperalgesia, but after the second injection, the PKA inhibitor was no longer sufficient. Rather, the hyperalgesia ended only after treatment with a PKC-ε inhibitor. Repeated painful stimuli have also been shown to result in the conversion of previously insensitive, unresponsive, or silent sensory afferents into active nociceptors [65]. It is therefore not surprising that Hong found a significant increase of sensitive loci in the area of MTrPs. It is likely the increase in sensitive loci that Hong is referring to is not the creation of new loci but the "awakening" of previously silent nociceptors [66]. Thus, repeated pain stimuli likely activate cellular cascades mediated by PKC-ε, leading to peripheral hypersensitivity.

The Transition of Peripheral Pain to Central-mediated Chronic Dysfunction

The cell bodies of first-order nociceptive neurons are located in the dorsal root ganglia (DRG). These cells are pseudo-unipolar, as each neuron has a single axon that branches into two parts. One part extends to the periphery to become a nerve ending, while the second part moves centrally to connect with second order nociceptive neurons in the dorsal horn of the spinal cord. C-fiber mediated pain is received by the cell bodies of the DRG and subsequently results in release of
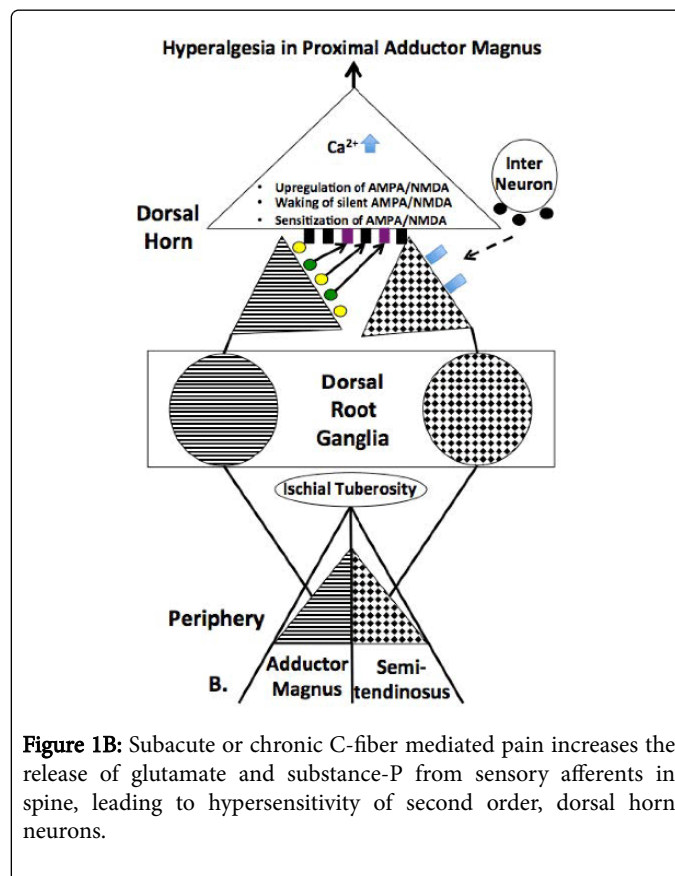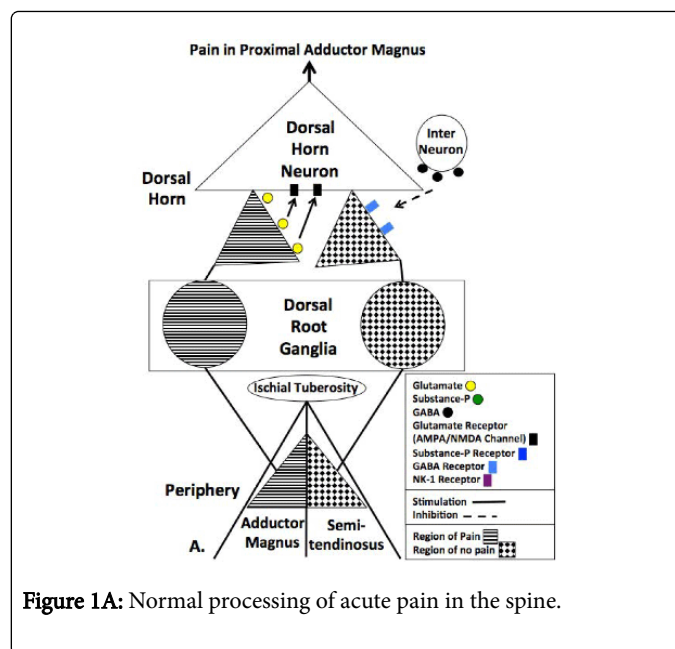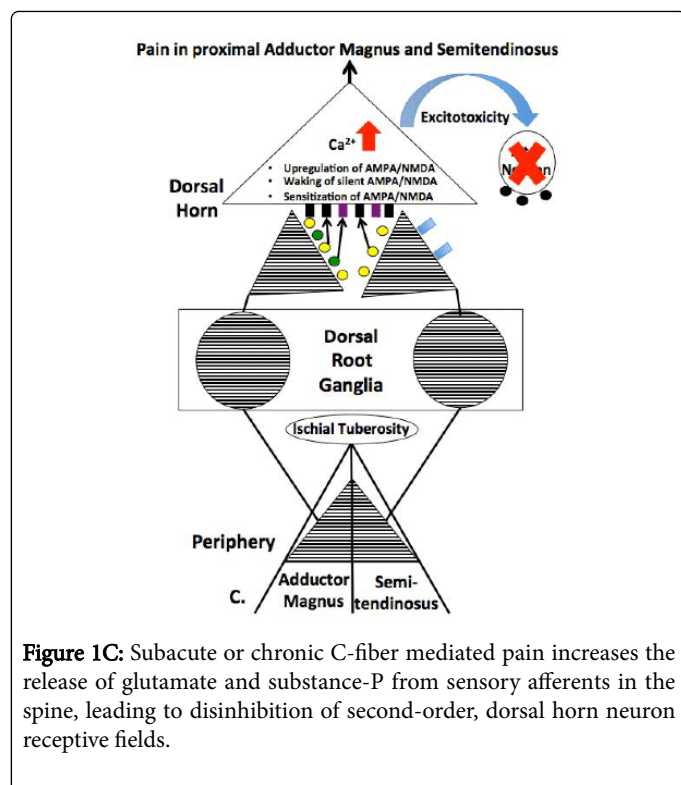
glutamate and SP from the presynaptic terminal in the dorsal horn of the spinal cord [12,67]. The glutamate is initially received by ligand-gated, NMDA and AMPA receptors. While the glutamate allows Na^{2+} and K^+ to enter the postsynaptic terminal, it is not enough to open NMDA channels secondary to an Mg^{2+} block [67]. However, when a sustained amount of glutamate is present, enough Na^{2+} and K^+ are able to move through AMPA receptors to depolarize the postsynaptic membrane and unblock the Mg^{2+} [67]. At the same time, substance-P activates NK-1 receptors, further phosphorylating NMDA receptors and facilitating the movement of Ca^{2+} through the NMDA channels [67]. The influx of Ca^{2+} results in a number of metabolic and genetic changes such as: 1. Production of Cox-2, an enzyme responsible for speeding up the production of prostaglandin [12], 2. Greater sensitivity of existing NMDA channels [67], 3. Synthesis of proteins required for new channels [66] and 4. Activating previously dormant NMDA channels [12,67,68]. Through this process, pain information is more efficiently relayed to the spinothalamic tract, a condition more commonly referred to clinically as hypersensitivity or hyperalgesia.

The over activation and subsequent opening of AMPA and NMDA channels leads to a toxic increase in Ca^{2+} activated enzymes in the postsynaptic neuron, resulting in cell death [12,67]. As the cell disintegrates, it causes a cytotoxic environment, which ultimately results in interneuron apoptosis [12,67]. This is significant, as interneurons manage the receptive fields of dorsal horn neurons. When disinhibited, dorsal horn neurons may therefore become stimulated by previously silent, ineffective or unused synapses from first order nociceptors [12,67]. For example, while both the semitendinosus and the adductor magnus have the same proximal attachment on the ischial tuberosity, nociceptors from the two muscles correspond with their own unique dorsal horn neuron or group of neurons. However, there are also likely a number of silent or unused synapses from first order semitendinosus nociceptors on adductor magnus dorsal horn neurons and vice versa that are typically managed by interneurons.

In the event of an injury resulting in chronic proximal inflammation of the adductor magnus whereby interneurons are lost, the previously silent or unused synapses may become active, resulting in the perception of pain over the adductor magnus and semitendinosus. Given that dorsal horn neurons receive afferents from the skin, muscle and viscera, these synapses are also susceptible to sensitization due to interneuron apoptosis. Importantly, dis-inhibition of the dorsal horn via interneuron apoptosis is a primary mechanism of peripheral referred pain [12,67]. SP, glutamate, and ATP from apoptotic cells also directly stimulate glial cells in the dorsal horn such as astrocytes and microglia to produce cytokines, thereby propagating spinal hyperalgesia [67] (Figures 1A-1C).

The resultant decrease in pain threshold and expansion of sensory receptive fields is more formally referred to as central sensitization [12]. In this case, the repeated C-fiber stimuli, known as wind-up pain, are thought to be the mechanism responsible for central sensitization [69]. Previous studies have demonstrated increased sensitivity to mechanical stimulation and receptive field expansion in dorsal horn neurons following injection of bradykinen into the lumbar multifidus [69,70]. By recording pain threshold following repeated stimuli to peripheral C-fibers, a number of studies have also reported wind-up related, central sensitization in patients with low back pain [71-73] TMJ pain [74] shoulder pain [75] and fibromyalgia [76].

Figure 1A: Normal processing of acute pain in the spine.

Figure 1B: Subacute or chronic C-fiber mediated pain increases the release of glutamate and substance-P from sensory afferents in spine, leading to hypersensitivity of second order, dorsal horn neurons.

Figure 1C: Subacute or chronic C-fiber mediated pain increases the release of glutamate and substance-P from sensory afferents in the spine, leading to disinhibition of second-order, dorsal horn neuron receptive fields.

The Physiology and Relevance of Needle Mechanotransduction

Through a series of elegant studies, Langevin et al. found that a greater pullout force is required to remove a needle from tissue when the needle is wound in one direction compared to when it is wound in both directions [76,78]. Moreover, there was a greater pullout force following uni- and bidirectional winding compared to needle insertion without manipulation [77,78]. By using trichrome staining, Langevin further demonstrated that pullout force is due to the mechanical coupling of collagen fibers to the needle [77]. The mechanical coupling directly pulls on collagen fibers, resulting in better alignment of collagen bundles, and stimulates cells via mechanotransduction [79]. Importantly, Langevin noted that, "acupuncture needle rotation (either uni- or bidirectional) may be important to initiate needle grasp, but other types of needle manipulation such as pistoning may also effectively transmit a mechanical signal to cells once needle grasp has been initiated" [77]. That is, pistoning in the absence of winding is not justified to elicit mechanotransduction. Consistent with this finding, a recent study by Zhang et al. found that needle rotation resulted in significantly greater C-fiber activation, distal superficial and deep mechanoreceptors and stretch receptors compared to lifting, thrusting, scraping, shaking, and flicking [80].
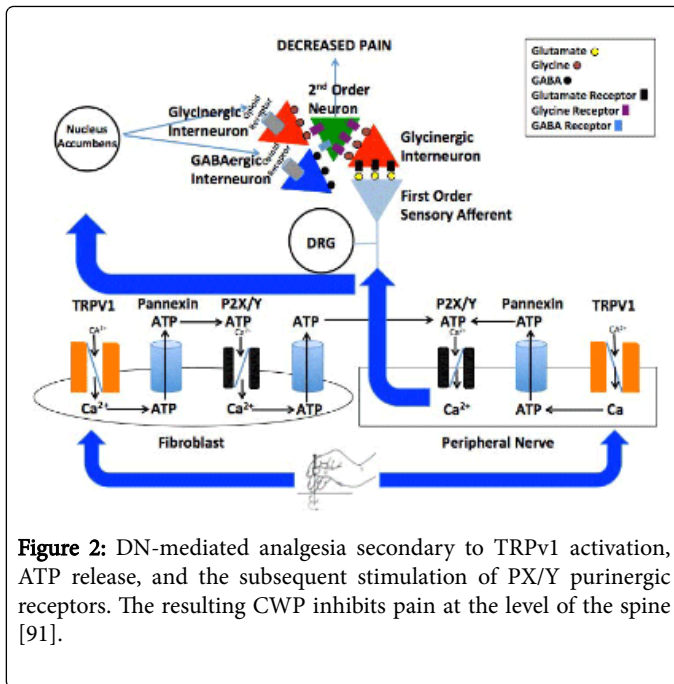
The Effect of Dry Needling Mechanotransduction on TRPv1 Receptors

Of the 4 primary channels/receptors that play a role in peripheral hyperalgesia, TRP receptors are arguably the most intriguing given their possible role in DN-mediated analgesia [81,82]. Interestingly, TRPv1 receptors, TRPv4 receptors and ASIC3 channels are sensitive to changes in PH and/or temperature, but these channels are also

responsive to mechanical stimulation [81,82]. The mechanical stimulation provided by DN may therefore have the ability to influence the very same channels responsible for sensing pain and inflammation and mediating hypersensitivity. Wu et al. used immunofluorescence to demonstrate the presence of more TRPv1 receptors and ASIC3 channels at ST36 (proximal tibialis anterior) compared to a non-acupoint location [82]. However, pharmaceutical stimulation revealed that only the TRPv1 agonist reproduced the analgesic effects of DN at ST36 [82].

According to Wu et al. [82] the mechanotransduction of tissue stimulates TRPv1 receptors on both neural and non-neural cells, resulting in intracellular calcium wave propagation (CWP). The CWP causes the release of ATP from pannexin-1 hemi-channels. As the ATP catabolizes to ADP, it stimulates P2X and P2Y purinergic receptors, respectively, on the same neuron or other neurons. Interestingly, TRPv1 stimulation of non-neural cells [83,84] also results in CWP and ATP release, likely amplifying the stimulation of P2X and P2Y receptors on neurons, a process that has previously been demonstrated in intestinal fibroblasts [85] and keratinocytes [86] following tissue mechanotransduction.

Interestingly, the stimulation of neuronal P2X and P2Y receptors results in brief pain and is therefore a possible correlate of *de qi* (subjective report of a deep pressure or spreading warmth upon needle insertion and manipulation). In both cases, the intercellular CWP is amplified, leading to alteration of C-fiber afferents and, subsequently, the release of glutamate from first order sensory afferents [87]. According to Zhou et al. the altered C-fiber excitation stimulates glycinergic interneurons in laminae-2 of the spinal cord to release glycine [87]. When glycine receptors on postsynaptic dorsal horn neurons receive glycine, they attenuate pain transmission [87]. The noxious stimulus-induced analgesia (NSIA) from DN also likely stimulates the nucleus accumbens, resulting in opioid release. The subsequent stimulation of μ and κ opioid receptors on interneurons in the dorsal horn results in the release of GABA and glycine, further inhibiting the transmission of pain information from sensory afferents to second order dorsal horn neurons [82,88] Thus, the stimulation of TRPv1 receptors via tissue mechanotransduction may help explain the physiologic mechanism responsible for DN-mediated peripheral analgesia. Given this mechanism, it is perhaps relevant to point out that most acupuncturists consider *de qi* to be a crucial parameter in effective needling treatments for pain [89,90]. Also, stimulation of the "acupuncture responding channel" TRPv1 only achieved analgesia when the needle was manipulated at ST-36. When the needle was inserted but not manipulated, the treatment outcome was no better than sham (Figure 2) [82].
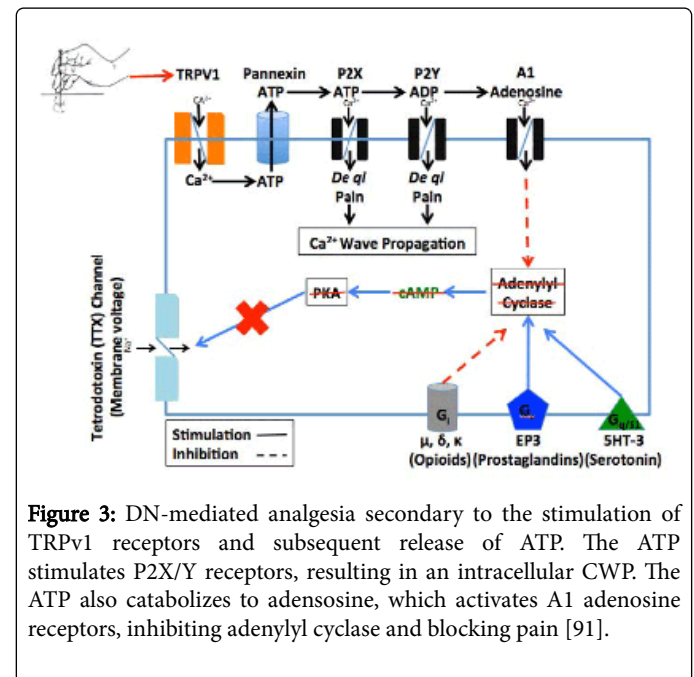
Figure 2: DN-mediated analgesia secondary to TRPv1 activation, ATP release, and the subsequent stimulation of PX/Y purinergic receptors. The resulting CWP inhibits pain at the level of the spine [91].

The Effect of Dry Needling Mechanotransduction on P1 Purinergic Receptors

Dry Needling has been shown to attenuate hyperalgesia via the P1 purinergic receptor family in models of both neuropathic and inflammatory pain [92]. Goldman et al. found a significant release of ATP, ADP and adenosine in the interstitial fluid following 30-minutes of manual DN at ST36, a phenomenon that is likely mediated via stimulation of TRPv1 receptors and subsequent CWP [92]. The level of ATP significantly increased but returned to normal after 30-minutes, while the level of adenosine increased during the 30-minute period 24X and remained at 60-minutes post treatment [92]. Importantly, mechanical stimulation of the needle via rotation every 5-minutes is thought to initially stimulate TRPv1 receptors, facilitating ATP release from sensory afferents via Pannexin-1 channels. The ATP is catabolized to ADP and eventually adenosine, stimulating P2X, P2Y and A1 receptors, respectively [92-95]. While genetically normal mice suffering from inflammation or neuropathic pain experienced significant improvements in mechanical and heat analgesia secondary to DN, A1 receptor knock-out mice did not [92]. Moreover, only normal mice experienced significantly less activation in the anterior cingulate cortex, an area of the brain related to the experience or emotional aspects of pain. In addition, Goldman et al. found that administering deoxycoformycin [92] inhibited enzymes responsible for extracellular adenosine breakdown, resulting in accumulation of adenosine and prolonging the analgesic effect of the DN [92].

Takano et al. also performed DN at ST36 for 30-minutes, during which the needle was rotated bilaterally to elicit *de qi* every 5-minutes and found a significant increase in interstitial adenosine [96]. Previous studies have linked activation of A1 adenosine receptors located on nerve endings [97], afferent nerves [98] and pre-synaptic DRG terminals [99] with anti-nociception. As G-protein coupled receptors, A1 receptor activation is thought to work by inhibiting adenylyl cyclase, attenuating Camp and phospholipase C [96]. Since an increase in Camp is associated with chronic pain, the inhibition of adenylyl

cyclase is noteworthy [96]. Given that opioids also inhibit adenylyl cyclase, there may be a link between adenosine A1 receptors and opioid receptors, suggesting that activation of one may result in analgesia support from the other [100]. Thus, adenosine also seems to play a key role in DN-mediated analgesia. However, consistent with Wu et al., Takano et al. noted that ATP release secondary to DN is not observed without needle manipulation. In the absence of ATP, intracellular CWP and A1 adenosine receptor stimulation is not possible, and the analgesic effects of DN are lost [96]. These findings support the commonly held belief that needle insertion is not enough to relieve pain (Figure 3) [96].

Figure 3: DN-mediated analgesia secondary to the stimulation of TRPv1 receptors and subsequent release of ATP. The ATP stimulates P2X/Y receptors, resulting in an intracellular CWP. The ATP also catabolizes to adenosine, which activates A1 adenosine receptors, inhibiting adenylyl cyclase and blocking pain [91].

The Effect of Dry Needling Mechanotransduction on Mast Cells

The number of mast cells (MCs) located at acupoints, to include ST36, SP6, GB34, LI4, LI11 and PC6 has been shown to be 50% higher than at non-acupoint locations [101]. A number of studies have linked the physical stimulation of needles with the activation of stretch-sensitive chloride channels on MCs, resulting in degranulation [83,102-104]. Some factors initially released during degranulation such as cytokines, serotonin and SP result in discomfort and may add to the perception of *de qi* [80,102]. However, given that MCs are primarily located in loose connective tissue, the cells effectively connect sensory afferents to blood and lymphatic vessels and therefore play a role in vasodilation by releasing histamine, heparin, leukotrienes and NO [80,102,105]. While MC degranulation also directly results in ATP release [102,105], histamine further stimulates ATP release from subcutaneous fibroblasts, amplifying P2X/P2Y and adenosine mediated pain reduction [83].

Notably, blocking MC degranulation by disodium chromoglycate abolished the analgesic effects of DN [106]. Also, the degranulation of MCs further emphasizes the importance of needle manipulation. By comparing 3 types of needle manipulation, a deep needling group, a superficial needling group and a group in which the needle was bilaterally rotated, Choi et al. found that rotation lead to greater

improvement in pressure pain threshold compared to the two groups in which the needle was simply inserted, regardless of depth [107]. Moreover, there was a significant correlation of needle sensation (*de qi*) secondary to needle rotation and improvement in pain pressure threshold [107].

Dry Needling Mechanotransduction and Tissue Restructuring

The mechanical stimulation via winding or twirling of needles and application of electricity leads to mechanotransduction of fibroblasts secondary to their adhesion to collagen fibers, resulting in a significant increase in the elongation, cell perimeter, and cross sectional area along with increased lamilapodia formation and upregulation of mechanical signaling markers such as Rho and Rac kinases [79,108]. Importantly, these structural and genetic changes have been shown to occur in the vicinity of and distal to the needle insertion site [108]. In addition, mechanotransduction has been shown to cause ATP release from keratinocytes [93] fibroblasts [93] MCs [109] and other tissue types [93]. The extracellular ATP is quickly broken down into adenosine, mediating the reduction in pain and inflammation typically associated with DN. Before doing so, however, the ATP is recognized by purinergic P2X/P2Y receptors on fibroblasts, resulting in an influx of cytosolic Ca^{2+} [110]. The CWP leads to the transient disassembly of polymerized actin and the subsequent decrease in fibroblast stress fibers [110]. Simply put, a reduction of fibroblast stress fibers changes the viscoelastic properties of the cells, allowing them to be more easily remodeled. Interestingly, this process requires mechanotransduction, as using cyclopiazonic acid to increase intracellular Ca^{2+} was not enough to alter the actin cytoskeleton in the absence of P2X/P2Y receptor activation [110]. Moreover, the inhibition of rho kinase, a product of DN-mediated mechanotransduction, prevented viscoelastic changes in fibroblasts [110,111].

Langevin et al. found that fibroblasts actively contribute to viscoelastic nature of the entire tissue in which they are found via a rho-dependent mechanism [112]. Scar tissue and fibrotic tissue are typically painful, structurally thicker and less elastic than healthy tissue [112]. However, DN mediated mechanotransduction of fibroblasts may be able to help remodel painful tissue by dampening tissue tension via actin polymerization [110]. A number of studies have demonstrated the use of DN to reduce pain associated with scar [58,113] and fibrotic tissue [114] further supporting this possibility.

Dry Needling Mechanotransduction and Tissue Healing

DN is thought to improve tendon healing via a three-pronged approach. Inflammation typically reduces type 1 collagen synthesis, but DN has been shown to decrease cytokines responsible for inflammation via activation of toll-like receptors on fibroblasts [115,116]. This action sets an appropriate environment for collagen remodeling and production. DN-mediated mechanotransduction stimulates rho/rac kinases, disrupting the actin cytoskeleton, and allowing the tissue to be reorganized [108,115]. Finally, mechanical stimulation of fibroblasts results in collagen synthesis via activation of mitogen activated protein kinases (MAPK). MAPK, the most prominent kinase activated by mechanotransduction, initiates the extracellular signal regulated-kinase ½ (ERK½) pathway [117]. ERK stimulates the production of Type-1 collagen fibers via transcription factors such as activator protein-1 [115,117]. Interestingly, a recent study by Park et al. found that DN at GB34 turned on 236 genes secondary to the ERK cascade [118]. Moreover, blocking the ERK pathway inhibited the anti-nociceptive effects of DN, suggesting that ERK may be the biochemical hallmark of DN analgesia [118].

The Physiology and Relevance of Electrical Dry Needling

A number of studies have also demonstrated a direct effect of electrical dry needling (EDN) on P2X2 and P2X3 purinergic receptors [119,120]. Despite their role in peripheral hyperalgesia following inflammation and neuropathic pain, the literature has only reported on the use of EDN to affect P2X2 and P2X3 receptors exclusively in neuropathic pain models [119,120]. Following a chronic constriction injury to rat hind legs, the number of P2X3 receptors in the L4-L5 DRG increased, corresponding with mechanical and thermal hyperalgesia. After 7 days of ipsilateral or contralateral EDN to GB34 and ST-36 X30 minutes, however, there was a significant reduction in P2X3 expression [119], attenuation of ATP stimuli [119] and an improvement in thermal and mechanical pain thresholds [120].

Calcitonin Gene Regulated Peptide and Substance-P

Evidence from the acupuncture literature suggests that EDN results in a significant increase in peripheral CGRP and SP [80,121], a finding that is counterintuitive given that the primary function of SP and CGRP is to propagate pain and inflammation [67,122]. While the discomfort associated with DN likely leads to the release of SP and CGRP-β from peripheral nerve terminals, the mechanical stimulation also causes release from non-neural sources. Previous studies have shown that more than 50% of SP may be released in peripheral tissue from monocytes, macrophages, keratinocytes, fibroblasts, lymphocytes and platelets, especially in pathologic conditions [80]. There are two primary theories for the early increase in SP and CGRP following DN. First, the additional neuropeptides provided by non-neurologic sources could provide negative feedback onto auto receptors located on nerve endings [80]. Second, the increase in SP may function to regulate peripheral levels of CGRP [123]. The latter is particularly intriguing, given that CGRP has been shown to propagate inflammation in high quantities but provides potent [123-125] anti-inflammatory actions in low concentrations via TTX channel inhibition.

Consistent with the proposed mechanism of hyperalgesia priming, the effect that CGRP has on TTX channels is PKA and PKC dependent [126] and repeated or sustained exposure to CGRP could tip the balance toward PKC-ε and flip the cellular switch from an acute to a chronic pain response [127,128]. It is also interesting to note that while a series of high-dose injections of CGRP are required to stimulate PKC-ε, a sustained low-dose CGRP managed by SP and stimulated by EDN may be able to flip the switch in the opposite direction [124]. Therefore, the analgesic effects of EDN may be due to partial depletion of peripheral CGRP stores followed by a low-level, sustained release of CGRP. As for SP, EDN eventually results in reduction of SP release from the dorsal horn and peripheral nerve endings [129-131].

Thus, while manual stimulation of acupuncture needles initially increases peripheral levels of CGRP and SP, the addition of electricity may allow clinicians to maintain appropriate levels of SP and CGRP to block cellular correlates of hyperalgesia, thereby inhibiting pain and breaking the cycle of chronicity. It is perhaps worth pointing out that RCTs from the acupuncture literature achieve better analgesia by adding electricity than by brief or intermittent manual stimulation alone [132]. While only one systematic review has directly compared

the outcomes of EDN to manual DN, the use of EDN for joint osteoarthritis was superior (Figure 4) [133].
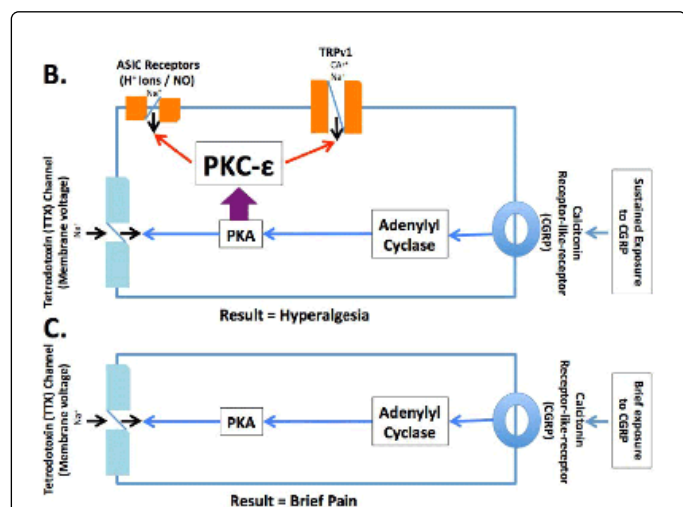
Figure 4A: EDN-mediated analgesia secondary to potentiation of TTX channels. A. Needle manipulation plus EDN facilitates a sustained release of low-dose CGRP, leading to analgesia.
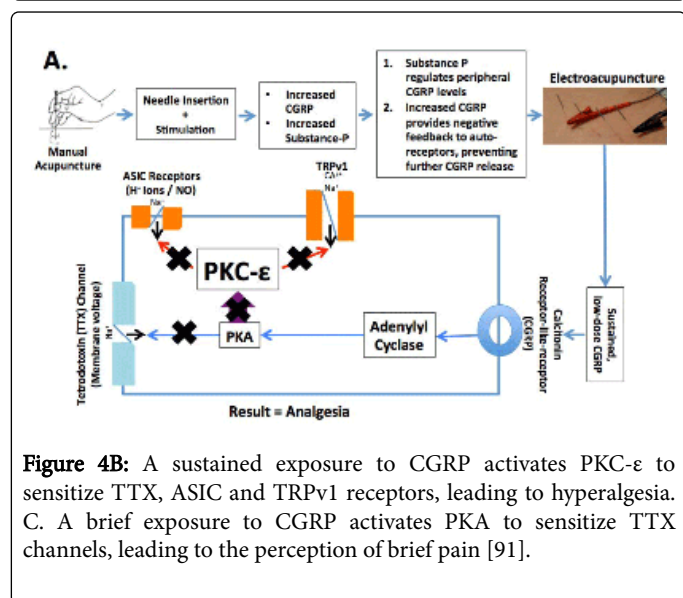
Figure 4B: A sustained exposure to CGRP activates PKC-ε to sensitize TTX, ASIC and TRPv1 receptors, leading to hyperalgesia. C. A brief exposure to CGRP activates PKA to sensitize TTX channels, leading to the perception of brief pain [91].

Electro-Dry Needling for Joint Osteoarthritis: Underlying Mechanisms

The fact that CGRP promotes inflammation acutely is somewhat of a paradox, as the ability of CGRP to increase blood flow through vasodilation has been shown to contribute to tissue healing [134]. A recent study demonstrated that DN is able to promote tendon healing via angiogenesis and fibroblast migration via CGRP release from sensory nerve endings and mechanical stimulation of collagen fibers, respectively [135,136]. In general, CGRP causes vasodilation by binding to CGRP1 receptors on vascular smooth muscle, resulting in the increase of PKA. The subsequent opening of K channels and reduction of Ca^{2+} results in smooth muscle relaxation and vasodilation [134]. The increase in PKA also stimulates nitric oxide (NO) synthase,

an enzyme responsible for producing NO, thereby enhancing the response [134]. Zhang et al. further hypothesized that DN may directly stimulate the sympathetic nervous system to release NO by creating an "axon reflex" within densely innervated tissue [80].

The role of CGRP in joint OA is particularly counterintuitive, as the pain associated with joint OA has been linked to an upregulation of vascular and neural tissue (with CGRP and substance P) in the vicinity of joint structures that are typically aneural [137,138]. However, given that DN has been shown to improve pain in osteoarthritic joints using traditional acupoint and non-acupoint locations, [139] most researchers believe that the general effect of DN may be due to improved blood flow. Following EDN to the vastus medialis, Lazaro et al. measured a significant increase in arteriolar diameter and a persistent increase in mean arterial pressure of arterioles supporting the knee joint [140]. Since the vasodilation disappeared upon application of L-name, a NO synthase inhibitor, the resulting change in vasodilation was likely due to an increase in NO. Whether CGRP also plays a role has yet to be determined, but given the numerous studies that report an increase of CGRP post DN and the potential for CGRP to mediate NO release from endothelial cells, [124,134] it is also a likely player in the vasodilation. Importantly, blocking neuromuscular junctions with succinylcholine, a nicotinic Ach receptor blocker, also inhibited vasodilation, suggesting that muscle contraction via EDN may be required for CGRP and NO-mediated vasodilation [140].

The increased blood flow likely has three primary effects on joints with OA. First, while the role of microvascular restriction is unclear, a recent study by Hussain et al. found that patients with retinal arteriole narrowing are twice as likely to develop knee OA and require a knee replacement [141]. This finding suggests that reduced microcirculation of the knee is likely a factor in the development of OA, to include the muscles that surround the joint [141,142]. Similarly, Biberthaler et al. noted less microcirculation in degenerative rotator cuff lesions [143]. In this regard, EDN may be able to improve vascularity of the joint via CGRP and NO, thereby stopping and/or reversing symptoms associated with osteoarthritis. Second, a number of studies have shown a significant reduction of inflammatory cytokines in the synovial fluid of osteoarthritic joints post DN [144,145]. The increased blood flow likely facilitates the recruitment of opioid producing immune cells required to reduce the level of inflammatory cytokines. Ahsin et al. reported a significant increase in plasma β-endorphin levels after EDN to local points at the knee that correlated with reductions in pain, stiffness and disability, which is likely due to vasodilation [144]. EDN further blocks the local release of IL-1 β and TNF-α in the synovia of osteoarthritic joints [145] and the systemic release of IL-1 β and TNF-α by inhibiting melanocortin-4 in the periaqueductal gray of the brain stem [146]. Third, there is limited evidence suggesting that DN may stimulate an increase in hyaluronic acid, allowing the synovial fluid to better lubricate the joint [147].

Bajaj et al. also reported a significant increase in the number of latent MTrPs in the muscles surrounding osteoarthritic joints [148]. Moreover, the number of latent trigger points corresponded to positive findings of osteoarthritis on radiographs. However, EDN-mediated vasodilation may help combat the physiology associated with MTrPs by reversing the hypoxic environment mediating the energy crisis. Given that Cagnie et al. reported an immediate improvement in blood flow and oxygen saturation following DN in the upper trapezius, DN may also have therapeutic value for muscles associated with joint OA [149].

Stimulation of the Neuroendocrine System via Dry Needling

EDN may also help to reduce and/or normalize systemic inflammation via activation of the hypothalamic-pituitary-adrenal (HPA) axis. Li et al. measured a significant increase in corticotrophin releasing hormone (CRH) from the paraventricular nucleus, adrenocorticotropic hormone (ACTH) from the anterior pituitary and corticosterone (cortisol in humans) from the adrenal cortex in response to inflammation via complete Freund's adjuvant (CFA) [150]. Interestingly, EDN exaggerated the response of the HPA axis in inflamed rats but not in healthy controls, suggesting a unique role of EDN in the presence of inflammation [150]. While ACTH was found to play no role in EDN mediated analgesia, CRH stimulated beta-endorphins from the paraventricular nucleus [150]. Corticosterone also prevents the production of inflammatory cytokines by inhibiting TNF-α and cox-2 [151]. Following an injection of CFA, EDN was able to control the resulting edema by stimulating a 10-fold increase in corticosterone. When corticosterone receptors were chemically blocked, however, the anti-inflammatory effects of DN were lost [152].

Corticotrophin Releasing Hormone Proopiomelanocortin Corticosteroid Axis

Much like the HPA axis, human dermal fibroblasts have a CRH-POMC-corticosteroid axis that responds to local stressors such as solar, thermal and mechanical stress [153] to include EDN [154]. Human dermal fibroblasts have the ability to produce CRH and express CRH receptors. When the CRH receptor is activated, it stimulates proopiomelanocortin (POMC) gene and protein expression via the second messenger cAMP, leading to the production and release of ACTH [155]. Unlike the HPA axis, in the CRH-POMC-corticosteroid axis, both CRH and ACTH stimulate the production of corticosterone from fibroblasts [155]. Importantly, CRH has also been shown to directly stimulate opioids release from immune cells, and CRH antagonists have been shown to block EDN's ability to inhibit pain in inflammatory tissue [156]. Corticosterone locally inhibits inflammatory cytokines, prostaglandin and cox-2, thereby optimizing opioid-based pain reduction [146,151,154]. The CRH-POMC-corticosteroid axis provides another mechanism by which EDN may be able to have an effect peripherally via non-neural cells and may further explain the positive effects of superficial DN.

ASIC Channels

A number of studies have linked ASIC3 to mechanical but not heat hyperalgesia following carrageenan-induced inflammation and a series of acid injections [157,158]. Chen et al. further noted an up regulation of ASIC3 channels in DRG neurons following carrageenan and CFA models of inflammation in mice, a phenomenon stopped and reversed by DN at ST36 [158]. At first glance, these findings suggest that DN may exploit the mechanical aspects of the receptor to reverse hyperalgesia. Unlike TRPv1 receptors, however, there is little evidence to support that down regulation of ASIC channels is a result of tissue mechanotransduction [82]. Rather, in the case of ASIC channels, the down regulation seems to be mediated by oxytocin. According to Yang et al., DN results in elevated oxytocin in the supraoptic nucleus but, surprisingly, not the paraventricular nucleus of the thalamus [159]. Yang et al. further demonstrated a dose-dependent, enhanced analgesic effect of DN with stimulation of the supraoptic nucleus and a minimal analgesic when the nucleus was ablated [160]. According to Qiu

et al. the activation of vasopressin (V1A) receptors by oxytocin released form the posterior pituitary is thought to trigger calcineurin-dependent phosphorylation of ASIC channels, decreasing the amplitude of ASIC currents, acid-evoked membrane excitability, and the depolarization amplitude secondary to acid stimuli [161]. In addition, oxytocin has been shown to inhibit pain directly by binding to oxytocin receptors (OXTR) on first order sensory afferents, second order neurons and GABAergic interneurons in the spinal cord, inhibiting the transmission of pain [162]. Thus, the stimulation of oxytocin from the supraoptic nucleus may help account for the physiologic mechanism responsible for DN mediated peripheral analgesia (Figure 5).
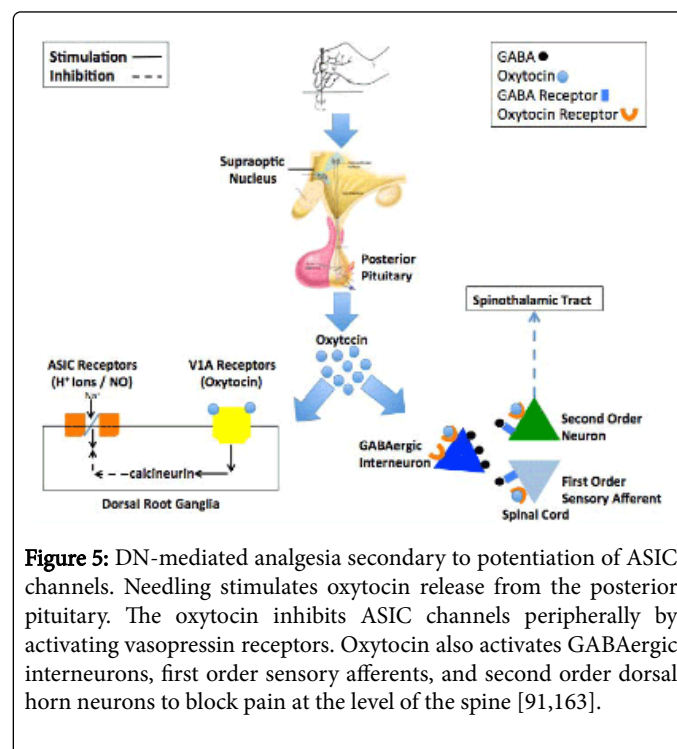
Figure 5: DN-mediated analgesia secondary to potentiation of ASIC channels. Needling stimulates oxytocin release from the posterior pituitary. The oxytocin inhibits ASIC channels peripherally by activating vasopressin receptors. Oxytocin also activates GABAergic interneurons, first order sensory afferents, and second order dorsal horn neurons to block pain at the level of the spine [91,163].

The Physiology of Aβ fibre Stimulation Secondary to Dry Needling

The interneurons of the dorsal horn also play a primary role in managing pain information being relayed from the peripheral to the central nervous system in the spinal cord. According to Melzack and Wall, inhibitory interneurons reside primarily in lamina II and III of the dorsal horn, a region more commonly referred to as the substantia gelatinosa (SG) [164]. Afferents provided by small myelinated Aδ and unmyelinated C-fibers synapse with second order dorsal horn neurons in lamina V, inhibiting interneurons in the SG and relaying pain information to the CNS. When large-diameter Aβ fibers are activated via non-nociceptive stimuli, interneurons of the SG release GABA. The GABA was originally thought to inhibit presynaptic pain fibers, but it is now thought that GABA may also block pain post-synaptically [165]. This should explain why it is often useful to "rub" the skin over the site of an injury to make it feel better or why interferential current may relieve pain in patients. While Aδ and C fibers open the pain gate, Aβ stimulation attempts to keep it closed.

In the case of chronic pain, the gate is continually left open. It is interesting to note that while most GABAergic interneurons of the SG

maintain local connections, some have axons 15 mm-41 mm in length and project 1-2 levels via Lissauer's tract, [166] suggesting that inhibition of the SG at one level can result in disinhibition of dorsal horn neurons at unrelated levels. That is, in the event of SG inhibition, Aδ and C fiber pain would have greater access to dorsal horn neurons at the spinal level associated with the pain and at unrelated levels, propagating both pain and referred pain. However, multiple systematic reviews have demonstrated increased Aβ fiber activation with DN and EDN [167-169]. DN may, therefore, be able to directly stimulate the SG, slamming the door shut on pain traveling from the periphery to CNS and halting symptoms of referred pain.

The Physiology of Aδ fiber Stimulation Secondary to Dry Needling

Boal and Gillette further speculate that the stimulation of Aδ afferents via therapeutic techniques such as spinal manipulation and DN may result in a limited influx of Ca^{2+} through NMDA channels of second order, dorsal horn neurons [69]. The resulting long-term depression could result in down regulation of AMPA receptors, thereby reversing the effects of central sensitization [69]. Gilette et al. also demonstrated decreased receptive field size and mechanical sensitivity in the lateral dorsal horn of the spinal cord following "intense mechanical force" (Aδ stimulation) [170]. While a number of studies have demonstrated a reduction in wind-up pain in humans pre-post spinal manipulation [71-73], future studies should explore this possibility using DN. Like Aβ fibers, a number of systematic reviews have reported Aδ stimulation with DN and EDN [167-169]. Moreover, investigations of DN have produced a more potent analgesic affect following Aδ stimulation than Aβ stimulation [168,171].

The Role of the Endocannabinoid System in Dry Needling-mediated Analgesia

Dry needling is thought to stimulate endogenous opioids (e.g. dynorphin, enkephalins, and endorphins) from immune cells such as neutrophils, eosinophils, basophils, lymphocytes, monocytes and macrophages, which subsequently stimulate μ, δ and κ opioid receptors expressed on peripheral nerve terminals [146,172,173]. Studies that have pharmaceutically blocked endogenous opioids and opioid receptors have been shown to prevent mechanical and thermal analgesia in animal models secondary to DN [146,174].

Peripherally, DN increases the number of opioids via the endocannabinoid system. The endocannabinoid system primarily consists of two types of receptors, cannabinoid CB1 and CB2 receptors. By employing AM251 and AM630 to block CB1 and CB2 receptors, respectively, Gondim et al. demonstrated that both receptor types mediate the anti-nociceptive and anti-inflammatory effects of EDN [175]. However, inflamed tissue has been shown to express 10-100 times the number of CB2 receptors compared to CB1 receptor mRNA, suggesting that EDN may act primarily through CB2 receptors [176,177]. CB2 receptor mRNA is found primarily on immune cells, to include mast cells, T lymphocytes, leukocytes, natural killer cells and macrophages [176,177].

DN also activates the sympathetic nervous system, enhancing the expression of intracellular adhesion molecule-1 (ICAM-1) in blood vessels, thereby increasing the recruitment and migration of immune cells with CB2 receptors to inflamed tissue [146,178]. Zhang et al. further reported an upregulation in the expression of CB2 receptors on macrophages, T-lymphocytes and keratinocytes already present in inflamed skin tissue [179]. In addition, DN stimulates the release of endogenous anandmide, a CB2 receptor ligand and an analogue of tetrahydrocannabinol (THC) [172,173]. A recent study by Su et al. found that EDN resulted in an increase in mRNA levels of POMC, a β-endorphin precursor, and protein levels of β-endorphin in keratinocytes, macrophages and T-lymphocytes [172]. However, sham EDN and the combination of EDN and AM630, a CB2 antagonist, significantly diminished the effects [172]. Thus, by creating an environment rich with CB2 receptors and stimulating endogenous anandmide, EDN may be able to affectively amplify the production and subsequent release of opioids to block pain.

Interestingly, Zhang et al. reported that inflammation and EDN are able to independently increase the number of CB2 receptors in affected tissue [173]. Moreover, the combination of inflammation and EDN result in greater CB2 expression than either alone [173]. Thus, the purpose of CB2 expression following EDN is a bit unclear. Perhaps the stimulation provided by EDN initially propagates and exaggerates CB2 receptor increases already initiated by the cycle of inflammation [173]. That being the case, the therapeutic effects of EDN may be due more so to its ability to increase levels of endogenous anandmide than to recruit and stimulate expression of CB2 receptors.

Complicating the issue further, Whiteside et al. suggested that the anti-nociceptive effects of EDN might not be dependent on endogenous opioids [180]. Interestingly, blocking both peripheral and central opioid receptors with naltrexone in an animal model did not prevent the anti-nociceptive effect of the CB2 agonist GW405833. Instead of stimulating the release of opioids, some authors suggest that the activation of CB2 receptors by endogenous anandmide results in anti-nociception and anti-inflammation by blocking the production and release of inflammatory cytokines such as TNF-α, IL factor and NGF [180]. Cannabinoids have been shown to inhibit the release of pro-inflammatory cytokines such as TNF-α, IL-4, IL-6, IL-8, and IL-10 from immune cells [146,181]. Su et al. further used EDN to decrease mRNA and protein levels associated with IL-1B, IL-6, and TNF-α in inflamed skin tissue via activation of CB2R [181]. Thus, while the exact mechanism responsible for the anti-nociceptive and anti-inflammatory effects of EDN are still not fully realized, the stimulation of CB2 receptors by endogenous anandmide likely results in a combination of opioid release and inflammatory cytokine inhibition to block pain and inflammation, respectively.

The up regulation of CB2 receptors on keratinocytes is particularly interesting, given their non-immune function and location in the epidermis. Remarkably, keratinocytes also have the ability express opioid receptors and release opioids [172,173,182]. A recent study by Moffett et al. (2012) used pulsed radio frequency energy to increase precursor mRNA for enkaphalin and dynorphin in both human dermal fibroblasts and keratinocytes, suggesting that both non-neural cell types have the ability to create and release opioids [183]. While the exact role that non-neural cells such as keratinocytes and fibroblasts play in EDN is still unknown, these cells may account for some of the positive benefits of superficial needling.

The Role of the Sympathetic Nervous System in Dry Needling Mediated Analgesia

EDN also activates the sympathetic nervous system (SNS), which seems to work additively with the endocannabinoid system to reduce pain and inflammation. In addition to upregulating ICAM-1 [146,178] the SNS also releases norepinephrine. When norepinephrine activates

B2-adrenergic receptors on immune cells, it stimulates the release of B-endorphins and blocks inflammatory cytokine production [146,184]. The SNS also activates the HPA axis, stimulating cortisol release [150,152]. As previous mentioned, corticosterone inhibits cytokines, prostaglandin and cox-2, thereby blocking inflammation in rats [151]. Since Cox-2 metabolizes endogenous anandmide, cortisol also affectively conserves endogenous anandmide, prolonging the pain and inflammation reducing effects of the endocannabinoid system [146].

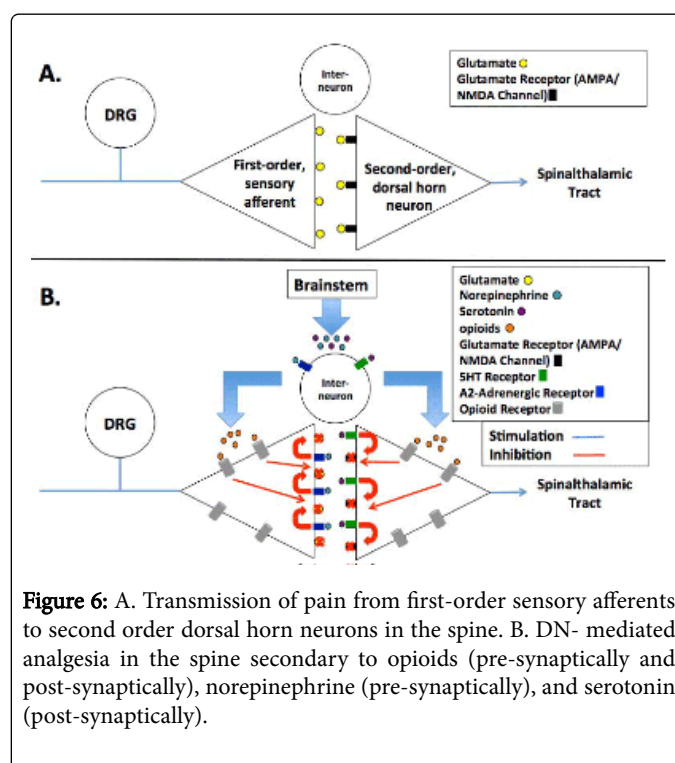## The role of Spinal Opioids/Non-opioids in Dry Needling Mediated Analgesia

Spinally, DN needling has been shown to activate the descending pain modulatory system, which is mediated by a synergic relationship between opioids and non-opioids, including serotonin and norepinephrine [185]. Guo et al. found that EDN results in the reciprocal stimulation of β-endorphin containing neurons in the arcuate nucleus (ARC) and periaqueductal grey (PAG) via glutamate transporter-3 [186]. While stimulation of the ARC enhances the analgesic effects of DN, lesioning of the nucleus almost abolishes it [168,187]. Similarly, injection of ARC and PAG with the opioid receptor antagonist naloxone significantly inhibits the analgesic effects of DN [168,187-189]. Activation of ARC and PAG results in the release of dynophorin and enkaphalin down into the spinal cord. At the same time, nociceptive afferents activate the spinothalamic/spinoreticular tract, which stimulates the locus coeruleus and raphe nucleus to release norepinephrine and serotonin, respectively [189,190]. Surprisingly, while the ARC-PAG-NRM-dorsal horn pathway stimulates the raphe nuclei, it inhibits the locus coeruleus [168]. However, this may reflect the fact that norepinephrine has opposite affects in the brain and the spinal cord [168]. While norepinephrine potentiates EDN analgesia in the spine, it inhibits it in the brain [168,191]. In fact, EDN has been shown to decrease brain norepinephrine [192] while increasing spinal norepinephrine [193]. Thus, the activation of the descending pain modulatory system results in increased opioids, serotonin and norepinephrine in the spine and decreased norepinephrine in the brain [168].

Spinal Serotonin and Norepinephrine

Both serotonin and norepinephrine travel down the dorsal lateral funiculus to inhibit pain in the dorsal horn of spinal cord. Norepinephrine and serotonin can directly or indirectly affect the communication between first order and second order neurons in the dorsal horn of the spinal cord. Recent work by Zhang et al. used immunohistochemical staining to demonstrate that α2-adrenergic receptors are positioned on CGRP-containing primary afferents, while 5-hydroxytryptamine (HT) rectors are located on second order neurons containing NR-1, a subunit of NMDA receptors [194]. As previously mentioned, the transmission of pain information requires the release of glutamate from first order sensory afferents and the subsequent receipt of glutamate from second order dorsal horn neurons. A number of authors have suggested that the activation of α2-adrenergic receptors by norepinephrine work to presynaptically decrease glutamate release from primary sensory afferents [195]. At the same time, activation of 5-HT receptors prevents the phosphorylation of NR-1 subunits, decreasing the ability of NMDA receptors to post-synaptically receive glutamate [196]. Notably, studies that have pharmaceutically blocked α2-adrenergic and 5HT receptors in the spine reported an inhibition of the analgesic effects of EDN [146,197].

Spinal Opioids

Indirectly, norepinephrine and serotonin also influence the transmission of pain in the dorsal horn of the spinal cord via interneurons. Enkephalinergic interneurons have both α2-adrenergic receptors and 5HT receptors [194,198]. In the presence of norepinephrine and/or serotonin, the interneurons release enkephalin, which is recognized by μ and δ opioid receptors pre and post synaptically to inhibit pain transmission. Notably, EDN results in the stimulation of pre and post-synaptic μ, δ and κ receptors during acute pain conditions but only μ and δ receptors during chronic pain conditions [199,200]. This may be due to the adaptive nature of δ receptors, leading to reduced sensitivity [146]. However, the fact that opioids mediate the anti-nociceptive effects of DN suggests that DN may be used to reduce the need for opioid medications (Figure 6) [201].

Figure 6: A. Transmission of pain from first-order sensory afferents to second order dorsal horn neurons in the spine. B. DN- mediated analgesia in the spine secondary to opioids (pre-synaptically and post-synaptically), norepinephrine (pre-synaptically), and serotonin (post-synaptically).

Nociceptin/Orphanin FQ

While opioids, serotonin and norepinephrine all play a part in reducing pain and inflammation secondary to EDN, the role of nociceptin/orphanin FQ (N/OFQ) is perhaps the most intriguing. N/OFQ is an opioid related peptide and endogenous agonist of opioid receptor-like receptor (ORL-1). The actions of N/OFQ depend on its location in the body [202]. While supraspinal and peripheral N/OFQ leads to increased nociception, its spinal actions have a powerful anti-nociceptive effect that mimics opioids [202]. In as little as 3-hours following the induction of inflammation with carrageenan in a rat model, Carpenter et al. noted increased spinal ORL-1 receptor density, which correlated with decreased pain behavior [202]. Similarly, EDN at GB30 and GB34 also increased spinal N/OFQ and ORL-1 receptors density, and N/OFQ antagonist blocked the analgesic effects of EDN [203].

According to Carpenter et al. the purpose of the added ORL-1 receptors may be to amplify the effects of N/OFQ in the spinal cord [202]. Interestingly, a number of studies have demonstrated that spinal N/OFQ inhibits C-fiber pain signals while preserving Aδ pain [204,205]. The fact that N/OFQ and ORL-1 receptor are manufactured in DRG neurons and expressed in the DRG and spinal cord further suggest that the actions of N/OFQ may specifically target presynaptic sensory afferents rather than postsynaptic neurons [206]. As such, N/OFQ is perfectly positioned to inhibit C-fiber pain, thereby preventing and/or blocking central mediated sensitization [202,204]. Previous research has demonstrated that N/OFQ is able to pre-synaptically block the release of glutamate [207] and substance-P [208] preventing the up regulation of glutamatergic receptors on dorsal horn neurons and the disinhibition of receptive fields. Carpenter et al. further suggests that N/OFQ may provide negative feedback to postsynaptic neurons, facilitating a down regulation of glutamatergic receptors [202].

The Physiology and Relevance of Dry Needling Distal Points

The intimately connected but distinct physiology responsible for the perception of itch and pain may provide the justification for needling points outside the site of discomfort. Huang et al. found that the analgesic effect of DN was inhibited if histamine receptors (H1) were blocked by clemastine [209]. While histamine also mediates the perception of itch, there is evidence to suggest that the perception of itch travels from the periphery to the CNS via a pathway that is independent of pain [209,210]. According to Schmelz et al., a unique subgroup of mechano-insensitive C-fibers is responsible for initiating itch in humans [211,212]. Likewise, Liu et al. reported unique MrgprA3 and GRPR / MOR1D-positive second order neurons in the DRG and the dorsal horn, respectively that are responsible for processing itch [210]. These neurons independently project through the ventromedial thalamic nucleus to the anterior cingulate cortex, left inferior parietal lobe, dorsal insular cortex, and supplementary motor area [213]. The latter typically results in the desire to scratch [213]. The purpose of the scratch is to introduce pain stimuli at the site of the itch, as GRPR and MOR1D-negative, dorsal horn neurons are able to inhibit GRPR and MOR1D-positive itch neurons in the presence of pain via Bhlhb5 positive interneurons [210]. Notably, topical capsaicin has been shown to prevent histamine-induced itch experimentally, and it is widely used by dermatologists clinically to treat various disorders associated with itch [214].

While perception of pain clearly has the ability to inhibit the perception of itch peripherally, this is not necessarily the case supraspinally. Sensory afferents associated with itch stimulate the histaminergic system in the tuberomammillary nucleus of the hypothalamus [215]. These neurons send axonal projections throughout the CNS. More specifically, histamine receptors (H1 and H2) have been found in the limbic system, to include the hippocampus, the periaqueductal grey and raphe nuclei [216]. General intracerebralventricular injections and specific microinjections of histamine into the periaqueductal grey and raphe nuclei all had anti-nociceptive effects in animal models [215]. Tamaddonfard et al. further demonstrated a synergistic effect of morphine and histamine in the hippocampus [215].

Taken together, the degranulation of MCs and subsequent release of histamine at an acupoint close to the site of pain (i.e. the MTrP) may be less advantageous, as the pain would likely dominate TRPv1 channels and mitigate the analgesic effects of the histamine in the brain [209]. However, needling an acupoint offset or distal from a trigger point (>10 cm) could stimulate the release of histamine from mast cells in the absence of pain, thereby facilitating histamine-mediated pain reduction supraspinally [209]. This is one of the primary justifications for including distal points as part of evidence-based DN treatment. It also provides direct evidence that trigger points should not be the only target of DN treatment (Figure 7).
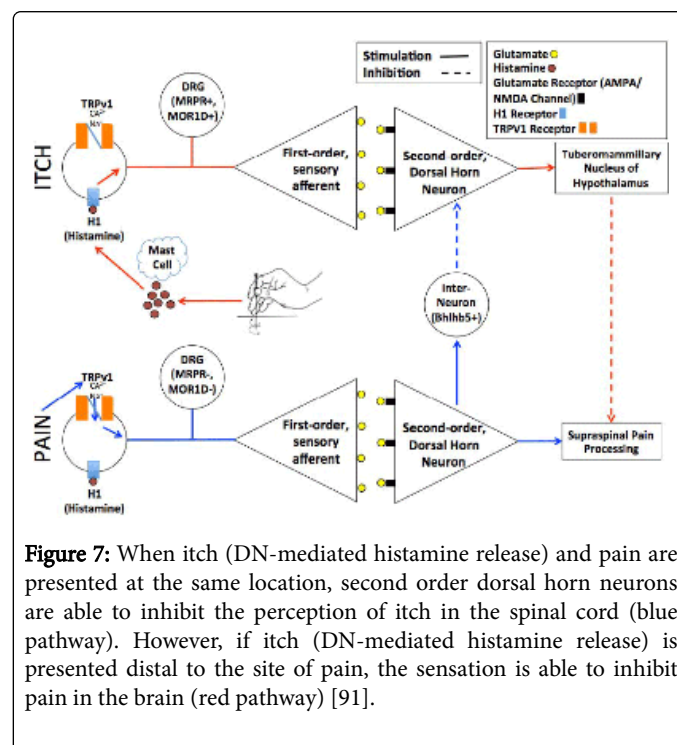
Figure 7: When itch (DN-mediated histamine release) and pain are presented at the same location, second order dorsal horn neurons are able to inhibit the perception of itch in the spinal cord (blue pathway). However, if itch (DN-mediated histamine release) is presented distal to the site of pain, the sensation is able to inhibit pain in the brain (red pathway) [91].

Conclusion

Dry needling has gained increased popularity in Western-based medicine over the past 20-30 years. Physicians, osteopaths, chiropractors and physical therapists presently use needling modalities to treat muscles, ligaments, tendons, subcutaneous fascia, scar tissue and peripheral nerves for the management of a number of neuromusculoskeletal conditions. As specialists in the treatment of neuromusculoskeltal conditions, DN has become particularly popular in the U.S. within the physical therapy profession over the past decade. Interestingly, despite significant evidence to the contrary, many within the profession have confined their use of DN to only targeting trigger points in muscle. Importantly, this narrow philosophy is likely due to the general exclusion of the acupuncture literature from the PT Profession [217] which ironically is conducted in the main by physiotherapists, [218] medical physicians [219-222] and PhDs, [223,224] not traditional Chinese acupuncturists. Certainly the terminology, theoretical constructs, and underlying science surrounding the insertion of needles without injectate is different among traditional Chinese acupuncture and Western-based DN communities; however, the actual technical delivery and the analgesic mechanisms underpinning such have many similarities between professions [2,225]. The complexity of this topic cannot be underestimated, and more, high-quality research must be conducted to fully appreciate the potential of needling therapies for the management of neuromusculoskeletal conditions.

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