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A Review of Spinal and Peripheral Neuromodulation and Neuroinflammation: Lessons Learned Thus Far and Future Prospects of Biotype Development

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Background: There is increasing literature evidence both clinically and experimentally on the existence of potent, adaptive interactions between the central and peripheral aspects of the neuroimmune system in the genesis and maintenance of chronic neuropathic extremity pain and nociceptive back pain. The neuroinflammatory pathways are modulated by the interaction of pro- and anti-inflammatory cytokines and chemokines, which are released by peripheral immune system-derived cell species (macrophages and leukocytes). This review examines the possible impact of spinal and peripheral neurostimulation on the inflammatory response in the context of acute and chronic pain pathologies of different origin.

Study Design: A narrative review of preclinical and clinical studies addressed to the spinal cord and peripheral nerve stimulation and neuroinflammation.

Methods: Available literature was reviewed on neurostimulation technologies and both acute and chronic low-grade inflammation to identify primary outcome measures and to provide an overview of postulated mechanisms of action of neurostimulation on host inflammatory responses. Data sources included relevant literature identified through searches of PubMed, MEDLINE/OVID, SCOPUS, and manual searches of the bibliographies of known primary and review articles.

Results: A comprehensive review of the literature indicates an alternate or synergistic mechanism of action of neurostimulation, beyond modulating somatosensory pain pathways, in modifying inflammatory response associated with chronic pain, by promoting a systemic anti-inflammatory state with upregulation of anti-inflammatory mediators.

Conclusions: These preliminary findings may have important implications on the potential applications of neurostimulation as an anti-inflammatory therapy and the role of molecular profiling as a preimplant screening modality and post-implant outcome validation. Thus, future targeted clinical and experimental research is highly warranted in this particular novel field of neuromodulation.

Keywords: Biomarkers, chronic back and leg pain, cytokines, neuroinflammation, pain-associated co-morbidities, spinal cord stimulation

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INTRODUCTION

Spinal cord stimulation (SCS) represents an established and adjunctive treatment modality for chronic pain of different origin (1). According to the seminal gate-control theory of pain proposed by Melzack and Wall (2), conventional, tonic SCS paradigm delivered at 40–60 Hz activates dorsal columns to elicit paresthesia that covers patients' painful body regions. This paresthesia-based SCS has proven to be an effective treatment modality for 40–50% of patients with refractory pain conditions, including complex regional pain syndrome (CRPS) and failed back surgery syndrome with predominant neuropathic leg pain (FBSS) (3–7). Within the last five years, newer waveforms (BurstDR SCS) and

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novel ultra-high frequencies (high-frequency SCS HF10) have opened the door of neuromodulation therapy and our current understanding of paresthesia vs. paresthesia-free SCS. Dorsal root ganglion (DRG) stimulation represents a targeted approach for selective neuropathic pain conditions of the lower extremities (CRPS type I and II) (8,9). In addition, recent studies have predominantly sought to explain the molecular effects when stimulating distal sensory nerve terminals via transcutaneous electrical nerve stimulation (TENS) and electroacupuncture (EA) (10–13). However, possible relationships between neurostimulation pain therapies and markers of the neuroimmune axis still remain relatively unexplored in preclinical and clinical trials. Depending on the stimulation target (brain, spinal cord, and peripheral nerve system), there appear to be differences in the observed effects among TENS, EA, SCS, DRG stimulation, subcutaneous stimulation (SQS), and subcutaneous nerve stimulation.

Based upon existing literature, this narrative review attempted to determine the critical role of various stimulation waveforms (traditional tonic, BurstDR, and high frequency) and target of stimulation dorsal column SCS, DRG, and peripheral nerves, in addition to other key parameters in driving mediators of the neuroinflammatory pathway, such as interleukins (IL-1b, IL-4, IL-6, IL-8, IL-10, and IL-13), interferon-γ (IFN-γ), and tumor necrosis factor (TNF-α). We also contend that the studies outlined in this review provide possible areas for future targeted research that will drive alternative mechanisms of action and encourage more universal adoption of neuroinflammatory molecular patterns as potential biomarkers for neuromodulation therapy.

MATERIALS AND METHODS

This review was done using searches of PubMed, MEDLINE/OVID, SCOPUS, and manual searches of the bibliographies of known primary and review articles from inception to present date. Other data included hand searches of publications driven by manuscript authors. Search terms included concepts of the spinal cord and peripheral stimulation, and neuroinflammation with emphasis on both preclinical and clinical studies. Due to the limited number of studies, clinical heterogeneity and methodologic diversity, we felt that a large scale meta-analysis would have limited scope and value to readers and therefore have chosen to present the data as a comprehensive review.

RESULTS

Putting the Inflammatory Response in Context

4-R (Recognition, Recruitment, Response, Resolution) Model of Inflammation

Recognition. The first step to an inflammatory response is recognition. This component of the response is driven by phagocytes and antigen presenting cells. After these cell types recognize an antigen through binding of pattern recognition receptors (PRRs), they will subsequently bind to either pathogen-associated molecular patterns (PAMPS) or lipopolysaccharides (LPS), which results in activation of the proinflammatory response. Proinflammatory cellular material that can be extruded following cellular damage is called damage-associated molecular patterns (DAMPS), and one important example is the high-mobility group box one protein (HMGB1) (14). Interaction of PAMPS and DAMPS with PRRs results in the activation of the proinflammatory cascade, causing the release of proinflammatory cytokines, mainly TNFα, IL-6, and TGFβ (14).

Recruitment. Recruitment mainly involves cellular trafficking by signaling and activation of leukocytes into the involved tissue by cytokines and chemokines, which orchestrate the inflammatory response. The recruitment phase helps to determine the type, severity, and time course of the inflammatory response. Proinflammatory cytokines and chemokines caused by the release of DAMPS and PAMPS can activate leukocytes to produce acute phase cytokines that involve interleukins, leukotrienes, and TNFs. In addition, these acute cytokines cause selective upregulation of selectins and integrins that allow for cellular trafficking.

Response. The response is the capability of the neuroimmune axis to control foreign attacks to antigens that are external, or internal in the case of an autoimmune response. It begins with the process of phagocytosis from antigen presenting cells that then release cytokine mediators resulting in recruitment of alternative innate and adaptive immune cells inclusive of neutrophils and B cells. The response component of the immune response can occur both systemically and locally within the DRG, where further presentation of antigens occurs from phenotypic variations of macrophages, mainly microglial cells. Though there has been a dearth of research in the arena of adaptive immune response driven by T and B lymphocytes, it is important to note that T cell populations of CD4, CD8, T-regulatory cells, and Th1/Th2 play a crucial role in transitioning of acute to chronic pain, specifically through regulating pro- and anti-inflammatory cytokines such as IL-10 (15,16).

Resolution. Resolution is likely the most important area for the healing process, and involves neutrophil apoptosis, follow on tissue remodeling, and scar formation. In many instances of chronic pain, pathogen recognition receptors, cytokines, and adhesion factors that remain elevated for weeks to months are the driving factors in producing inflammatory mediated chronic pain. The 4-R model of inflammation is summarized in Figure 1.

Spinal Cord Stimulation

Tonic Waveform

Several small-scale open-label studies have looked at the interplay of pain intensity, SCS stimulation patterns (frequency), and CSF levels of cytokines for tonic, conventional SCS (17–19). McCarthy et al. assessed the levels of vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), and monocyte chemotactic protein-1 (MCP-1) in the CSF of patients with FBSS in order to determine levels of these cytokines in patients' CSF relative to the neuropathic pain levels (18). For instance, BDNF has been suspected to be involved in the pathogenesis of neuropathic pain, depression, and metabolic disorders. Significantly elevated BDNF, MCP-1, and VEGF levels were detected in FBSS patients with predominant neuropathic leg pain compared to healthy controls. VEGF significantly decreased after tonic SCS treatment and demonstrated a correlation between pain intensity and CSF-VEGF levels (18).

Additional studies have also measured concentrations of glial cell-derived neurotrophic factor (GDNF) in the CSF (lumbar puncture) of nine patients with FBSS experiencing neuropathic pain. In accordance with earlier observations, significantly higher GDNF values were present in patients with FBSS compared to those of

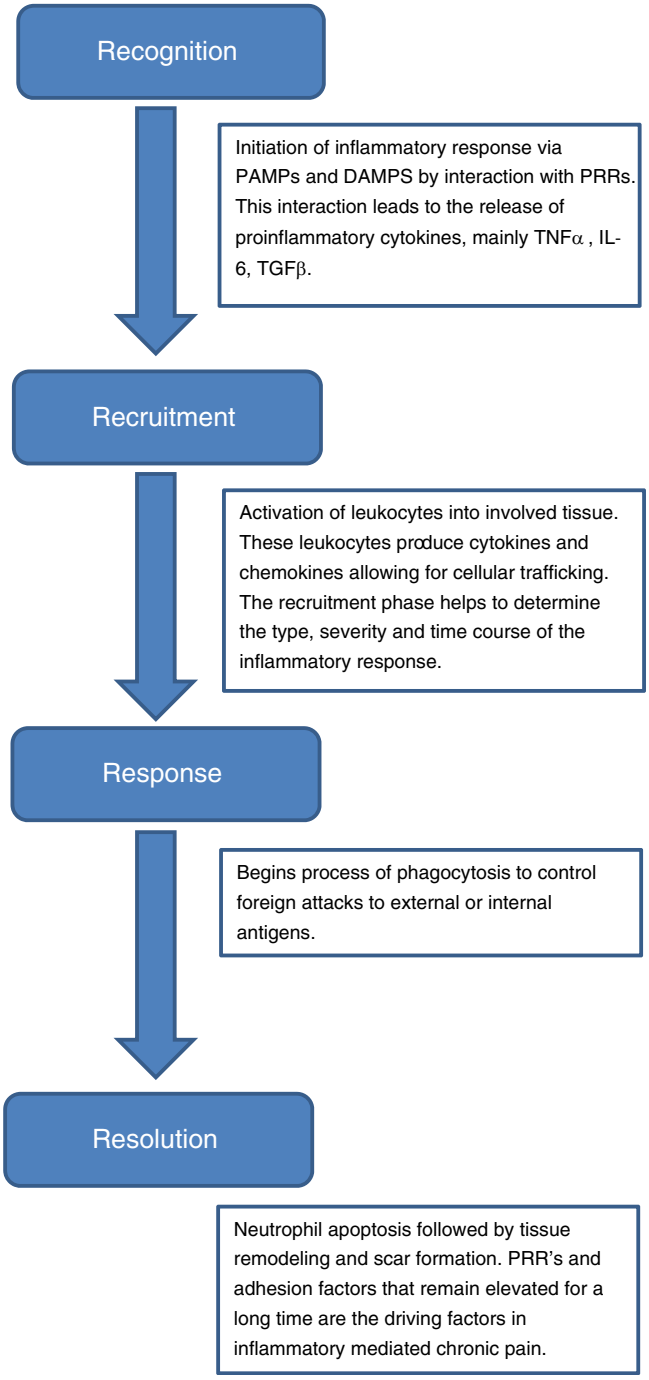

Figure 1. 4-R model for characterizing the inflammatory response with key cellular mediators. [Color figure can be viewed at wileyonlinelibrary.com]

healthy controls. Interestingly, CSF-GDNF levels changed depending on the applied frequencies ranging from 40 to 120 Hz (19).

A recent large-scale mass spectrometry study reported on the effects of conventional SCS (tonic mode 50 Hz) on CSF protein levels in patients with chronic neuropathic pain (17). CSF samples were obtained and concentrations of 86 individual proteins were analyzed by mass spectrometry. The *long-term effects* of SCS stimulation (ranging from 5 months to 10 years) and *acute changes* (after 48 hours of SCS "OFF" condition) were determined for CSF protein patterns and concentrations. Comparing ON/OFF stimulation, a significant change in the CSF protein composition was observed, indicating an SCS-evoked impact on a broad range of proteins previously linked with a variety of nonpain pathways *per se*: Pathways related to neuroprotection, neuronal transmission, synaptic plasticity, and neuroinflammation (17).

Kriek et al. recently did a crossover study in CRPS patients assessing an proinflammatory panel (IL-2, IL-6, IL-12, IL-15, IL-17, TNF-α, and IFN-γ), anti-inflammatory panel (IL-4, IL-5, IL-10, and IL-13), T-cell involvement Th1 (IL-2, IL-12, IL-15, TNF-a, and IFN-γ), Th2 (IL-4, IL-5, IL-6, IL-10, and IL-13), Th17 (IL-17), and Th Regulatory (IL-10). They also looked at chemokines IP-10 and Eotaxin, and the growth factors VEGF, PDGF-β, and basic FGF for the involvement of neuroinflammation and angiogenesis. The crossover study examined the role of tonic SCS in affecting levels of cytokines and chemokines in skin blister fluid. Their main findings showed that after SCS proinflammatory cytokine IL-15 was significantly reduced over time, as well as IL-2, IL-12, and IFN-γ revealed a trend in decreasing levels over time. In the anti-inflammatory panel, there was a trend in decreasing levels over time of IL-4, IL-5, IL-10, as was also the case for growth factor G-CSF, chemokine IP-10 (CXCL10), VEGF, and PDGF-β. These findings collectively suggesting that SCS in CRPS patients attenuates T-cell activation, improves peripheral tissue oxygenation and decreases anti-angiogenetic activity which results in diminished endothelial dysfunction and improved local blood flow (20).

In line with these findings, tonic SCS was found to suppress glial activation in the spinal dorsal horn of the spinal cord in a spinal nerve injury rodent model (21). Specifically, immunohistochemistry was used to identify the spinal cord density of microglia (OX-42) and astrocytes (GFAP and MCP-1), and it was found that glial activation was significantly reduced with low-frequency tonic SCS compared to sham stimulation (21).

BurstDR™ Waveform

Kinfe et al. published a pilot study that showed that BurstDR™ waveform upregulated IL-10 in blood serum predominant back pain patients, a key anti-inflammatory cytokine in the resolution phase of inflammation and regulated by T-regulatory cells and secreted by alternatively activated macrophage phenotype (22). They found a significant increase in the levels of anti-inflammatory IL-10 after burst SCS, while levels of proinflammatory HMGB1 did not change significantly. In addition, metabolic disorders associated mediators, such as leptin, adiponectin, and ghrelin, were found in higher concentrations in FBSS patients as hypertension, diabetes, cardiac ischemia, and disturbance of vascular architecture are highly prevalent in chronic pain patients. The relational analysis was limited due to the uncontrolled study protocol, which points out the need for larger, controlled trials, re-examining the potential usefulness of neuroinflammation assays (22).

Contrary to serum assays in the aforementioned study, it is worth noting that other preclinical and human studies investigating the correlation between neuroinflammation and back pain measured cytokine concentrations in cerebrospinal fluid (CSF), a biofluid compartment closer to the neural interface. In particular, the methodologies used to determine TNF-α values vary, and while some studies done by McCarthy et al. with tonic stimulation demonstrated enhanced plasma levels by LPS-induction other studies failed to confirm these preliminary findings (18,19).

Dorsal Root Ganglion Stimulation

The DRG is the location for the cell bodies of primary sensory neurons that transduce and modulate peripheral nervous

information to the spinal cord and the brain. These pseudo-unipolar cells include Aδ and C neurons responsible for nociception, and Aα and Aβ neurons that mediate proprioception, light touch, and vibration (23). The DRG responds to peripheral afferent injury and inflammation through immune and biochemical processes that lead to abnormal ion currents involving upregulation of A, N- and T-type calcium (Ca^{+}) channels. The DRG is also responsible for alterations in pathways that modulate inward calcium and the up-regulation of cationic currents through vanilloid receptors including TRPV1. This in combination with genetic changes and hyperexcitability both within the DRG and upstream in the central nervous system results in allodynia, hyperalgesia, and chronic neuropathic pain (24). The DRG is a promising therapeutic location for neurostimulation because of its central role in the pathogenesis of chronic pain and advantages compared to dorsal column stimulation and precise anatomic targeting.

Following peripheral nerve injury, the inflammatory response in the DRG is directed by immune cells including lymphocytes, satellite cells, and macrophages. Inflammation activates glial cells and increases spinal IL-1β expression, leading to neural growth factor (NGF) release. NGF is transported retrograde to the DRG cell body, resulting in changes in genetic expression, notably impacting production of transient receptor potential (TRP) channels, which ultimately leads to peripheral sensitization (25). Microglial invasion, in particular, is a well-documented immune process that in conjunction with increased immune cell trafficking to the DRG persists well beyond an initial nerve transection injury. Furthermore, the neuropathic pain state involves satellite glial cells enveloping the DRG, which along with neighboring Schwann cells, secrete TNF-α, growth factors, and chemokines. Subsequently, TNF-α, IL-1β, and IL-6 promote the spontaneous discharge of action potentials, possibly through the nociceptor related upregulation of tetrodotoxin-resistant sodium channel currents (26). The interaction of the autonomic nervous system with the inflammatory cascade and immune cells is an area of controversy and still pending ongoing research (27).

High-quality preclinical evidence for understanding the mechanism underlying DRG neurostimulation is still poor, although evidence for some hypotheses has been documented (28). Neurostimulation has been shown to alter DRG neurophysiology and stabilize neuronal hypersensitivity (29). Koopmeiners et al. demonstrated reduced action potential generation of the sensory nerve somata, and reduced action potential propagation, likely at the T-junction in the DRG following exposure to 60-Hz stimulation pulses (29). It is hypothesized that changes in DRG neuronal activity in turn reduce activation of microglia and subsequent dampening of the proinflammatory cytokine response, as evidenced by changes in T-cell junction activity (30). In a light-induced injury model of microglia *in vitro*, electrical stimulation inhibited IL-1β and TNF-α expression. In the same study, however, electrical stimulation also increased brain-derived neurotrophic factor (BDNF), which was found to be neuroprotective, but interestingly in other studies, demonstrate a possible mechanism to produce chronic pain, mood alterations and metabolic disorders (31,32). Increasing evidence point to the capacity of neurostimulation to alter growth factor expression, a cytokine-induced process that mediates neuropathic pain. NGF and BDNF are associated with DRG sympathetic sprouting following chronic constriction injury while glial cell-line derived neurotrophic factor, a protective protein in the setting of nerve injury, was shown to reverse thermal and mechanical hyperalgesia in animal models (32–35).

In a collagen-induced arthritis rat model, neurostimulation of the DRG with continuous 20 Hz pulse rate reversed inflammatory arthritis (36). In this model, following DRG stimulator implantation and injection of collagen type II incomplete Freund's adjuvant, rats for which electrical stimulation was activated exhibited reduced paw thickness, foot volume, arthritis score, sensitivity to cold and mechanical hyperalgesia, compared to those that were not stimulated. Furthermore, histologic examination of the tibiotarsal joint did not show characteristic inflammatory changes of the marrow spaces, periarticular soft tissues, and joint spaces as well as loss of either bone or cartilage. The authors hypothesized that the observed results related to disrupting dorsal root reflexes. They also showed a process of neurogenic inflammation mediated by the release of substance P and calcitonin gene-related peptide believed to contribute to peripheral inflammatory disease processes (36). These findings emphasize the need for clinical translation and the need for ongoing clinical trials with DRG stimulation.

Vagal Nerve Stimulation

The vagus nerve, also known as cranial nerve X, is a well-established mediator of the autonomic nervous system. More recently, the vagus nerve has also been identified as a key modulator of the innate immune response and inflammatory pathways (37). The recently described pathway termed the "cholinergic anti-inflammatory pathway," is mediated by the α7-nicotinic acetylcholine receptor (α7nAChR) found on circulating macrophages (38,39). The feedback loop is directed by the afferent and efferent components of the vagus nerve to directly modulate the synthesis of proinflammatory cytokines (40).

The afferent vagus nerve fibers originate in the nucleus tractus solitarius (NTS) and project to the parabrachial nucleus (with subsequent projections to the amygdala, hypothalamus, and limbic system) and the dorsal motor nucleus (41,42). The cholinergic anti-inflammatory pathway originates in the dorsal motor nucleus of the vagus, connects to the celiac-superior mesenteric plexus ganglion, and reaches the spleen through the splenic nerve (43). Activation of afferent fibers of the vagus nerve by cytokines and/or pathogen-derived products stimulates the hypothalamic–pituitary–adrenal axis and sympathetic nervous system (44). Efferent activity leads to acetylcholine (ACh) release, which interacts with nicotinic ACh receptors on tissue macrophages to modulate the release of tumor necrosis factor (TNF), interleukin-1β (IL-1β), IL-18, high mobility group box 1 (HMGB1) protein, and other cytokines from the lipopolysaccharide-stimulated macrophages (45,46).

The indirect downstream effect evoked by vagus nerve stimulation is likely mediated by the release of adrenocorticotropin peptides (hypothalamic–pituitary-axis) and impact cardiac and digestive functions, and inflammatory pathways (45,46). The "reflex pathway" can be centrally or peripherally mediated by either chemical or electrical stimulation (47). Classically, increased efferent vagus nerve activity has been associated with reduced heart rate, increased gastric motility, and vasodilation, however more recent studies have found a significant effect on the inflammatory pathway as well (45).

The earliest studies of vagal nerve stimulation (VNS) found that intermittent electrical stimulation causes modulation of neural processes by altering brain activity (48). Since that time VNS has been used in the management of refractory seizures as well as depression, obesity, memory, arthritis, and neurogenesis (49). More recent data has affirmed that cervical VNS inhibits inflammatory cascades resulting in reduced levels of TNF, IL-1β, IL-8, HMGB1, and other cytokines (40). Clinically, both invasive and

noninvasive VNS (nVNS) have demonstrated a reduced response to pain with effects on central and peripheral nociceptor function in humans (50,51).

The effects of VNS have also been studied in the treatment of other pain-inducing conditions such as migraines, osteoarthritis, and Crohn's disease. In animal studies, VNS decreased pain-induced activation of neurons in the trigeminal nucleus caudalis in addition to reducing pain behavior and trigeminal allodynia (52). Case report data from patients with invasive VNS, uncontrolled small-scale clinical studies and larger RCT's (EVENT, PRESTO) investigating nVNS have shown a reduced number of headaches per day and decreased pain intensity scores (preventive use). Furthermore, nVNS was associated with an abortive effect as adjunctive rescue intervention. While abortive effects may likely be the result of direct inhibition of ipsilateral trigemino-nociceptive transmission, the preventive impact of nVNS may be due to neural activity changes in both hemispheres. Human trials assessing possible changes of inflammatory markers are lacking (53–55). Data from animal studies also support the anti-inflammatory effects of VNS in collagen-induced osteoarthritis with a significant reduction in joint inflammation, pannus formation, cartilage destruction, bone erosion, and overall histologic arthritis score (56). Furthermore, delivery of VNS therapy in patients with rheumatoid arthritis lead to significant improvements in disease severity scores after 42 days (57). Finally, in a clinical pilot study of VNS for Crohn's disease, 71% of patients were in endoscopic remission after 6 months of therapy, further supporting the global suppression of chronic inflammation (58).

Peripheral Nerve Stimulation

Early animal models of nerve injury show that compression of a peripheral nerve leads to edema, ischemia, and increased vascular permeability. The result is disordered excitation conduction which proceeds in a stepwise fashion from thicker fibers with larger diameter (e.g. A fibers) to thinner fibers with smaller diameter (e.g. C fibers) (59,60). The decrease in A fiber transmission is believed to increase the sensitivity of mechanoreceptive neurons in the spinal cord to C fibers, a mediator for hyperalgesic symptoms (59). These early studies began to reveal the connection between peripheral nerve stimulation with inflammation. Nam et al. demonstrated that C-fiber response in the dorsal horn following peroneal nerve and tibial nerve stimulation (3 pulses of 50 Hz) was attenuated in both normal cats and those injected with carrageenan, an inflammatory polysaccharide. However, only in the inflammatory model did naloxone fully reverse this effect. These findings suggest that the analgesic response is more heavily dependent on the opioid pathways under inflammatory conditions (61).

Electroacupuncture

Peripheral Mechanisms

As reviewed extensively in a recent paper by Ulloa et al., transdermal stimulation of sensory afferent nerves with electroacupuncture modulates pain through inflammatory pathways (13). Electroacupuncture employs stimulation frequencies 2–100 Hz delivered subcutaneously at specific points related to Chinese medicine meridian theory but are all with rare exception located next to neuronal networks (13). Endogenous opioid pathways mediated by peripheral inflammation play an important role in analgesia for this treatment approach. Multiple pathways are implicated in the increased local levels of opioids following an inflammatory insult. In particular, the sympathetic nervous system evokes upregulation of adhesion molecules in the blood vessels, which attracts immune cells and directly stimulates adrenergic receptors on the cell surface to release β-endorphins (62,63). EA activates the sympathetic nervous system (64). Additionally, it contributes to local increases in opioid level through the release of a corticotrophin-releasing factor, which stimulates opioid release from fibroblasts (65), cannabinoid CB2 receptor up-regulation (66), and the interference of endocannabinoid metabolism via corticosterone induced COX-2 inhibition (67). There is evidence that endogenous opioids are critical in the peripheral response to an inflammatory insult, as an opioid receptor antagonist, intraplantar naloxone methiodide, and a β-endorphin neutralizing antibody has been shown to eliminate the hyperalgesia negated by electroacupuncture in an inflammatory rodent model (68) Other EA peripheral analgesic mechanisms include PGE2 reduction from COX-2 inhibition, downregulation of pronociceptive factors tumor necrosis factor-alpha, IL-1B and IL-6 (69), and increased adenosine-mediated activation of anti-nociceptive ascending pathways (70).

Spinal Mechanisms

Fos protein, a marker and research tool for neuronal activation following painful stimuli, is expressed in multiple areas of spinal laminae in response to peripheral inflammation (71,72). EA exerts anti-nociceptive effects at the level of the spinal cord via inhibition of Fos protein expression and interacts with the neural transmission of analgesia via glutamate, cytokines, signal molecules, and norepinephrine (64). Electroacupuncture appears to induce differential modulation of opioid pathways (delta, kappa, and mu) depending upon the frequency of treatment and inflammatory state. For example, in uninjured rodents, tail flick response was negated in response to noxious thermal stimuli through different receptor subdomains (mu pathways at 2 Hz vs. kappa at 100 Hz). However, when intraplantar capsaicin or complete Freund's adjuvant were injected into rodents as models following inflammatory insult, an increase in mechanical pain threshold or paw withdrawal latency through kappa was not observed following EA at either 2 Hz or 100 Hz (64). These studies suggest that kappa may play a less important role in high-frequency EA in chronic inflammation or states of compromised immunity.

Norepinephrine activation of alpha-2a receptors and serotonergic pathways also contribute to EA anti-nociception at the spinal level (73). The serotonergic receptor subtypes that are activated appear related to the frequency of stimulation and nature of injury (64). Norepinephrine, serotonin, and endogenous opioids may not only exert analgesic effects independently but also through a common NMDA pathway. CFA-induced inflammation has been shown to upregulate subunits of NMDAR (GluN1, GluN2A, GluA1) in both the DRG and dorsal horn (74,75). EA related alpha-2a receptor and serotonin 5-HT1A receptor activation diminishes the phosphorylation of GluN1 (64). An experiment using mu-opioid and delta-opioid agonists produced inhibition of NMDA evoked cellular responses in the medullary dorsal horn, which was reversed with mu and delta antagonists (76).

Astrocyte and microglial cells mediate pain transmission through cytokine secretion. EA was observed to decrease glial cell activation and IL-1β, IL-6, and TNF-alpha in the spinal cord. IL-1β enhance the phosphorylation of GluN1 while TNF-alpha promotes NMDA activity in spinal lamina II neurons (64). Opioids are linked to this pathway, as substance P mediated activation of glial cell activity is blocked by mu and delta opioid receptor

selective agonists (77). EA also activates nociceptive/orphanin FQ, another peptide implicated in pain modulation, that functions to down-regulate cytokines, likely through opiate receptor-like 1 receptor activation (64).

p38 mitogen-activated protein kinase (p38MAPK) is involved in intracellular signaling pathways that promote the transcription of TNF-alpha, IL-1, and COX-2. Phosphorylated p38MAPK upregulation in the spinal dorsal horn, periaqueductal gray and rostral ventromedial medulla is attenuated in CFA rodent models treated with electroacupuncture (78). In a fibromyalgia mouse model involving the injection of acid injection into the gastrocnemius muscle, Lin et al. demonstrated the upregulation of TRPV1 and phosphorylation of pERK in the DRG (79). TRPV1 is a mediator of hyperalgesia activated under inflammatory conditions, capsaicin, acidemia, heat, and anandamide. TRPV1, TRPV4, pERK have all been associated with hypersensitivity of nociceptive neurons and central sensitization (79).

Transcutaneous Electrical Nerve Stimulation and Subcutaneous Electrical Stimulation

TENS involves the application of variable frequencies, typically 1–100 Hz, directly to the surface of the skin. TENS produces analgesia at multiple levels including local, spinal, and supraspinal pathways. At low frequency (<10 Hz) analgesic effects are observed through mu-opioid, GABA, serotonin, and muscarinic receptors (80). Higher frequency stimulation operates through delta-opioid receptors and increased levels of blood and CSF methionine-enkephalins and beta-endorphins (80). Serotonin release and the blocking of adverse cardiopulmonary effects of pain have also been suggested as mechanisms (81). Multiple rat models demonstrated the reduction of secondary mechanical hyperalgesia in joint inflammation with TENS. These findings persist when applying TENS to contralateral sides of injury, enforcing central mechanisms of action (10). In addition to analgesic benefits, TENS has been studied in the setting of wound healing due to observed effects on temperature and blood flow. In a rat model for wound healing, proinflammatory cytokines IL-1, IL-6, and TNF alpha were reduced with TENS therapy (11).

Subcutaneous electrical stimulation involves the placement of electrodes in the subcutaneous space likely targeting sensory nerve ending branches much like TENS. Vera-Portocarrero et al. demonstrated behavioral hypersensitivity in rats following inflammatory or neuropathic insults and showed some differences between SQS and TENS depending on the nature of the painful stimulus (12). Specifically, rats exposed to carrageenan and kaolin injection to induce muscle inflammation in response to mechanical painful stimuli demonstrated increasing hypersensitivity over time when treated with TENS, suggesting the development of tolerance. The opposite occurred in SQS rats; mechanical hypersensitivity was reduced with increasing efficiency over time. An advantage of SQS over TENS in mechanical hypersensitivity was also observed in rats exposed to selective nerve injury. No differences were found with respect to thermal painful stimuli in both inflammatory and neuropathic models. The authors hypothesize the difference may relate to the activation of large Aβ fibers in SQS treatment due to deeper anatomic placement compared to the preferential activation of more superficial Aδ fibers in TENS treatment. Opioid-mediated mechanisms and subsequent tolerance issues are linked to Aδ fibers, whereas non-opioid mediators are released with Aβ fiber activation (12). The mechanism underlying differences in treatment efficacy between inflammatory and neuropathic models for both treatment modalities warrant further investigation.

CONCLUSION

This review covered tonic SCS, BurstDR SCS, DRG stimulation, vagal nerve stimulation and peripheral nerve stimulation (TENS, electroacupuncture) and their respective effects on neuroinflammation. Table 1 highlights the key mediators with regards to anatomic location, waveform and associated cytokine marker elevation within the context of the 4R model of inflammation. At this stage, such objective and reliable measures are not available and appear to be urgently needed to quantify neurostimulation outcomes for different stimulating waveforms and at various neuroanatomic targets. This concept can be extended to other stimulation waveforms, as important cytokine mediators may play an essential role in both mechanism of action and predicting response to therapy.

Potential Limitations

There is considerable variation in the *patterns* of circulating systemic inflammatory mediators (neuroinflammation) relative to

Table 1. Key Mediators with Regards to Anatomic Location, Waveform and Associated Cytokine Marker Elevation.

Therapy	Involved Cytokines	Role of Cytokines	Effect of Therapy
Tonic spinal cord stimulation	IL-15, IL-2, IL-12	Proinflammatory	Reduction in pro-inflammatory cytokines after SCS in CRPS patients (20)
	IL-4, IL-5, IL-10	Anti-inflammatory	Reduction in anti-inflammatory cytokines after SCS in CRPS patients (20)
Burst spinal cord stimulation	IL-10	Anti-inflammatory	Increase in level of IL-10 after burst SCS in back pain patients (22)
Dorsal root ganglion stimulation	IL-Iβ, TNF-α	Proinflammatory	Inhibition of pro inflammatory expression *in vitro* using light-induced injury model of microglia (31)
Vagus nerve stimulation	TNF, IL-1β, IL-8, HMGB1	Proinflammatory	Reduction in proinflammatory cytokines in cervical VNS in humans (50,51)
Peripheral nerve stimulation	IL-1B, IL-6, IL-1β	Proinflammatory	Reduction in proinflammatory cytokines with electroacupuncture in inflamed skin tissues (69)
TENS and subcutaneous electrical stimulation	IL-1, IL-6, TNF-α	Proinflammatory	Reduction in proinflammatory cytokines in a rat model (11)

co-morbidities (e.g., sleep, mood, fever), metabolic status, ongoing interventional therapies (medications, neurostimulation parameters, etc.), and time-of-day (circadian rhythmicity). Clinical studies addressing the compositional status of systemic circulating inflammatory mediators and any possible relations with chronic pain conditions should consider that chronic pain represents a complex multisystem disease of the brain affecting functionally different circuits. The ultimate goal will be to identify a specific molecular pattern rather than one specific marker allowing to create a comprehensive and individualized molecular mapping.

Proposal for a Roadmap Towards a Personalized Neurostimulation Therapy for Pain

Despite the observed impact of peripheral and spinal neurostimulation approaches for pain, the underlying inflammatory mechanisms behind these therapies are poorly understood. Given the inconsistent findings in earlier studies and poor level of study concepts published (Class IV), future targeted inflammation—neurostimulation research should in general integrate molecular pattern assays relative to neurostimulation responsiveness under controlled study conditions. The question remains about whether molecular inflammatory profiling is a suitable tool in the roadmap to a personalized and predictive neurostimulation therapy for pain (PPN). The findings of our review indicate a current lack of evidence, but the pragmatic potential and useful integration of PPN in everyday neurostimulation pain practice represent a novel and undefined roadmap given the substantial open questions that still remain.

The PPN roadmap consists of two essential steps: 1) the establishment of a mechanistic model (biotyping), and 2) subsequent exploration of the effects of standard neurostimulation patterns on predefined biotypes. Of note, biotype-based PPN does not reflect or predict the outcome on an individual level but rather represents an intermediate solution in the field of biomarker development. In a first step, distinct subsets of clustered patients (subset of homogeneous patients with similar inflammatory measure profile within a nosocomial defined pain condition) will have to be characterized by using advanced statistical models (categorical multidimensional driven construct) in order to obtain a mechanistic model. Other suitable PPN screening modalities like electrophysiology (EEG, TMS, and MEG), imaging (structural/functional), computational modeling, epi(genetics) and digital phenotyping are currently under investigation and will certainly support the accomplishment of quantitative measure research in the field of applied neurostimulation for pain. The second step will be the integration and quantification of standardized stimulation relative to suitably preclassified biotypes, which can promote a personalized and predictive system for a subset of patients (biotype). Certainly, this is a long and ambitious roadmap, that in the author's point of view will help to better reveal pain phenotype variations (proper patient selection), neurostimulation responsiveness (definition of responder vs. non-responder) and stratification of applied neurostimulation treatment.

Authorship Statement

All authors contributed equally to the conception, design, and writing of the manuscript.

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COMMENT

Picking up on the contemporary explosion of information relating to the role' of cytokines and like substances in the inflammatory response, the authors in their review have addressed the very important aspect by which these can be modulated in its favor by neurostimulation. Just as changing the basic parameters of neurostimulation to achieve functional effects has occupied researchers for decades, the

more recent results of altering waveform architecture and frequency have already realized clinical benefits. The other more immediate scientific data revealed from studies discussed in this paper are the manner in which inflammatory and inhibitory cytokines can be monitored in different body compartments under the influence of neurostimulation - providing as a bonus, at least some of the many mechanisms of action. The bibliography is pertinent, extensive and borderless. The authors have discussed the inflammatory response under a 4-R (Recognition, Recruitment, Response, Resolution) model, clearly explained in their single figure. The data presented in this paper is information urgently needed to substantiate the bonafides of neurostimulation as a vital clinical therapeutic tool.

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Comments not included in the Early View version of this paper.

