

# **Pain and the immune system: Emerging concepts of autoimmune pain**

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## **ABBREVIATIONS**

AChR, acetylcholine receptor; AQP4, aquaporin, AMPA;  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CPB, cardiopulmonary bypass; CGRP, calcitonin gene-related peptide; CNS, central nervous system; CRPS, complex regional pain syndrome; CRMP5, collapsing response mediator protein 5; CASPR2, contactin-associated protein 2; CASPR1, contactin-associated protein 1; EMG, electromyography; GABA,  $\gamma$ -amino-butyric acid; GAD, glutamic acid decarboxylase; GLY, glycine; Ig, immunoglobulin; IVIg, intravenous immunoglobulin; LGI1, leucin-rich glioma-inactivated 1 protein; NGF, nerve growth factor; NMO, neuromyelitis optica; NF155, neurofascin 155; PERM, progressive encephalitis with rigidity and myoclonus; SPSP, stiff person spectrum disorder; TRPV1, transient receptor protein vanilloid 1; TLR, toll-like receptor; VGCC, voltage-gated calcium channel; VGKC, voltage-gated potassium complex

## ABSTRACT

The immune system has long been recognized important in pain regulation through inflammatory cytokine modulation of peripheral nociceptive fibers. Recently, cytokine interactions in brain and spinal cord glial as well as dorsal root ganglia satellite glial have been identified important in pain modulation. The result of these interactions is central and peripheral sensitization of nociceptive processing. Additionally, new insights and the term “autoimmune pain” have emerged through discovery of specific IgGs targeting the extracellular domains of antigens at nodal and synaptic structures, causing pain directly without inflammation by enhancing neuronal excitability. Other discovered IgGs heighten pain indirectly by T-cell mediated inflammation or destruction of targets within the nociceptive pathways. Notable identified IgGs in pain include those against the components of channels and receptors involved in inhibitory or excitatory somatosensory synapses or their pathways: Nodal and paranodal proteins (LGI1, CASPR1, CASPR2); glutamate detection (AMPA-R); GABA regulation and release (GAD65, amphiphysin); glycine receptors (GLY-R); and water channels (AQP4). These disorders have other neurological manifestations of central/peripheral hyperexcitability including seizures, encephalopathy, myoclonus, tremor and spasticity, with immunotherapy responsiveness. Other pain disorders, like complex regional pain disorder, have been associated with IgGs against  $\beta$ 2-adrenergic receptor, muscarinic-2 receptors, AChR-nicotinic ganglionic  $\alpha$ -3 receptors, and calcium channels (N and P/Q types), but less consistently with immune treatment response. Here, we outline how the immune system contributes to development and regulation of pain discussing specific IgG mediated pain disorders, and approaches to therapy. Biological agents to treat pain (anti-calcitonin gene-related peptide and anti-nerve growth factor) are also discussed.

## INTRODUCTION

The immune system's involvement in pain was described by Celsus in the first century whereby redness (rubor), warmth (calor), swelling (tumor) were recorded as accompanying pain (dolor).<sup>1</sup> Pain and its immune mediated accompaniments are healthy responses to prevent initial injury and accelerate tissue recovery. However, when pain is out of proportion to injury, a pathologic process is implied. Severity of pain is determined by complex peripheral and central integration of spinal, limbic and cortical pathways, all with potential immune mediation.<sup>2</sup> Psychosocial factors, mood, and age can all influence pain perception.<sup>3</sup> According to the World Health Organization, more than a quarter of the population is suffering chronic pain, defined as pain lasting more than three months. Traditional analgesic drugs are often not efficacious in this population. For patients with chronic neuropathic pain, daily scheduled opioids have a major negative impact through increased morbidity, without clear improved functional outcomes.<sup>4</sup> Phenotypic classifications based on pain generators form the basis for the current pain classifications.<sup>5</sup> Neurologists are involved when pain is thought to be neuropathic and associated with a lesion or disease of the somatosensory nervous system. Guidelines have been created to help determine whether pain is neuropathic.<sup>6</sup> Important in that consideration is whether there are: (1) sensory loss; (2) sensory gain with hyperalgesia and/or allodynia in a neuranatomically plausible distribution and whether investigations confirm a lesion of the somatosensory nervous system. When no pain-generator is found, a somatoform pain disorder is commonly diagnosed.<sup>7</sup>

Recognizing patients with "autoimmune pain" linked to direct IgG disruption of excitability/synaptic circuitry is important as these syndromic disorders often respond to immunotherapies. These patients are relatively rare, and have other neurological accompaniments, some with paraneoplastic etiology.<sup>8</sup> Reviewing the temporal onset, localization, and associated neurological and psychological accompaniments can help identify patients suited for laboratory testing of these autoantibodies. Characteristics such as subacute onset or multifocal neurological manifestations should raise suspicion of an autoimmune component. Similar to other autoimmune or inflammatory immune disorders, asymmetric onset and patchy involvement of different neurological systems are typical. Specific neural involvements which should be sought, including (1) large fiber sensory (vibration and

proprioception) and/or small fiber sensory (heat, pain, cold) involvements, whether they are symmetric, asymmetric or patchy; (2) autonomic nerve dysfunction (e.g. orthostatic/cardiogenic and/or gastrointestinal and/or sweating abnormalities); and (3) encephalopathy and/or personality changes and/or seizures or paroxysmal spells frequently without MRI or EEG equivalents. Neurophysiological studies (EEG, nerve conductions, and needle EMG) and autonomic testing (GI motility-gastric emptying, sudomotor sweat tests and cardiovascular changes) can all help in making the diagnosis, also for use as outcome measures. Sural nerve biopsies are generally not helpful, apart from excluding other pain mechanisms related to destructive injury of peripheral nerves i.e. vasculitis, amyloidosis. Dermal/epidermal skin biopsies can help objectify loss of small unmyelinated nerve fibers, and quantification of those losses.

The aim of this review is to explore the immune-related mechanism in pain disorders, discuss well-known autoimmune neurological pain disorders, and highlight important shifts in how we diagnose and treat certain pain patients.

## **Immune Mediated Triggers and Effectors of Pain**

### **Innate immunity and pain**

Activation of neurogenic inflammation through innate immunity plays a key role in initiating and maintaining neuropathic pain.<sup>9</sup> Afferent nociceptive nerves communicate with the immune system to produce integrative healing of injured tissues. Tissue injury releases endogenous danger signals, termed *alarmins*, which are recognized by toll-like receptors (TLRs), triggering neurogenic inflammation via nociceptive nerve fibers.<sup>9</sup> These fibers are also activated through cytokine receptors, danger receptors, and pathogen-associated molecular receptors (Fig. 1). This response drives an efferent release of neuronal mediators to increase blood flow (vasodilatation), heighten pain sensitivity (through reduced nerve membrane firing thresholds and slowed membrane repolarization), and stimulate both chemotaxis and T-cell priming at the site of injury. The major neuronal mediators are neuropeptides such as calcitonin gene-related peptide (CGRP), and Substance P. These neuropeptides can also induce pain by stimulating proximal effectors to sensitize nociceptors.<sup>10</sup> Cytokines are the main effectors of the response

to tissue injury with two opposing phenotypes: the pro-inflammatory, IL-1 $\beta$ , TNF, IL-6, IL-15, IL-17, IL-18, and IFN- $\gamma$ ; and anti-inflammatory, IL-4, IL-10 and TGF- $\beta$  (Table 1). In neuropathic pain modulation, cytokines are either predominantly algescic or analgesic. In chronic pain, there is often an imbalance between the major cytokine algescic and analgesic mediators released by activated immune and immune-like glial cells.

Another important factor in pain stimulation are microglia, the resident macrophage cells of the central nervous system (CNS) (Fig. 2).<sup>11</sup> Recent studies demonstrate microglial activation is important in chronic pain modulation.<sup>12</sup> Chronic pain can also lead to brain microglia proliferation and activation at the cortex, thalamus, amygdala and hypothalamus, resulting in central pain sensitization.<sup>2</sup> Spinal microglia are activated in the very early phase (within 4 hours) after peripheral nerve injury. The injured afferents are able to release colony stimulating factor-1, which will trigger microglial proliferation in the spinal cord and ATP which activates the P2RX4 expressed on microglia resulting in them adopting a pro-inflammatory phenotype.<sup>13-15</sup> Supraspinal microglia can also be activated by peripheral nerve injury and modulate chronic pain. Activated microglial cells are able to release a variety of diffusible factors including pro-inflammatory cytokines, brain derived neurotrophic factors, and proteases. These microglia-derived factors can signal to astrocytes and neurons which enhance neuronal firing through both direct and indirect mechanisms.

Microglial cells directly contribute to central sensitization however, glia within the peripheral nervous system also have a role in the sensitization of nociceptive signaling (Fig. 2).<sup>11</sup> Dorsal root ganglia glial satellite cells are activated by sensory neuron release of ATP. Satellite glial cells are thereby stimulated to release IL-1 $\beta$ , ATP, nerve growth factor, metallo-proteases, CGRP to sensitize sensory neurons eliciting spontaneous action potential discharges. Next, resident macrophages and T-cells in the DRG releasing chemokines, trigger more macrophage and T cell recruitment. Macrophages and T lymphocytes increase synthesis and release of cytokine IL-1, IL-6 and tumor necrosis factor (TNF). TNF increases the density of tetrodotoxin-resistant Na<sup>+</sup> channel currents in nociceptors (sensory neurons). Macrophages pass through the satellite cell sheath around the primary sensory neuron in attempt to clear damaged neurons. As a result, extracellular K<sup>+</sup> levels of sensory neurons are dysregulated, leading to neuronal hyperexcitability.

## **Adaptive immunity and pain**

Pain can also occur as a result of direct IgG-induced injury of nociceptive fibers via *molecular mimicry*, either post infectious (e.g. pain with Guillaine-Barré Syndrome from *Campylobacter jejuni* infection) or with sterile antigen exposures which may break immunological tolerance (e.g. aerosolized porcine neural tissue exposure and cancers in paraneoplastic syndromes, Table 2).<sup>8</sup> Integral in the generation of antigen-specific IgGs are the initial innate interactions of neutrophils, eosinophils, macrophages and T cells (Fig.1). The specific neuronally derived signaling molecules help drive the adaptive T cell response and propagation of specific IgG reactions. The process begins with the detection, engulfment and phagocytosis of a specific antigen by an antigen-presenting cell (macrophage, dendritic cell, B-cell, others), with subsequent presentation to naive T and B cells. Naive T cells then recognize these processed antigens on MHC molecules and mature into different subtypes driving the immune response. Depending on the type of antigen and the specific cytokine reaction, naive T cells mature into different subtypes driving the immune response to propagation and/or tolerance of the targeted antigen.

Poor correlation of pain with extent of injury, as measured by location and extent of axonal loss raises questions as to cause of many painful conditions. The fact that IgG complexes can directly bind Fc-gamma receptors on nociceptive fibers in the dorsal root ganglia or their soma, causing direct nociceptive excitation, and pain is emerging as an important concept in pain regulation, first recognized in animal models.<sup>16</sup> Specifically, immunoglobulins can stimulate nociceptive receptors by either binding the constant region (Fc) or antigen-binding (Fab) regions without inflammation at nodal or soma structures.

## **Autoimmune IgG Mediated Neurological Pain Disorders**

IgG-mediated pain disorders have overlapping pain symptoms, including cramps, spasms, burning, paresthesia, lancinating pain, and itch. Below are specific examples from diverse antigen-specific forms. We don't include painful disorders where specific IgGs and mechanistic antigens in pain are not yet known, such as vasculitis, contact and atopic

dermatitis, herpes zoster, nor disorders where axonal loss from direct immunoglobulin destruction of nociceptive fibers occurs like in CRMP5 painful neuropathy<sup>17</sup>.

### **Pain in VGKC complex (LGI1-IgG, CASPR2-IgG) and CASPR1-IgG autoimmunity**

The voltage gated potassium complex (VGKC)-IgG disorders provided an initial example of “autoimmune pain”.<sup>18,19</sup> VGKC-IgG autoimmunity was first described with painful muscle cramps, spasms, and fasciculations with electrographic EMG hyperexcitability with myokymia (repetitive regular firing bursts of motor units, 20-80 Hz), cramp discharges and neuromyotonia (rapid firing long continuous firing motor units, 150-300 Hz) in Isaacs syndrome (acquired neuromyotonia with hyperhidrosis) and Morvan syndrome (neuromyotonia, dysautonomia, limbic encephalitis and sleep disturbance). Subsequently, it was realized that leucine-rich glioma-inactivated 1 protein (LGI1) and contactin-associated protein-2 (CASPR2) are the primary antigen targets within the VGKC-IgG complex (Fig. 1).<sup>20,21</sup> Other neurological hyperexcitability features, apart from those involving pain somatosensory pathways, most commonly are limbic encephalitis, seizures, faciobrachial dystonic seizures (LGI1-IgG specific), paroxysmal dizzy spells, and dysautonomia including gastric dysmotility.<sup>20-22</sup> Cancer is actually uncommon but when found, most likely is thymoma, likely with CASPR2-autoimmunity.<sup>22,23</sup>

In these patients, pain is often described beyond those areas of motor hyperexcitability recordable by EMG. Hence, small nociceptive fiber hyperexcitability and dysfunction were theorized and objectified by thermoregulatory sweat testing, quantitative sudomotor reflex screens (showing hyperhidrosis) and quantitative sensory testing (showing heat pain hyperalgesia).<sup>22</sup> Patients typically describe burning and stinging pain in the extremities, less commonly the trunk and face. Pathologic skin biopsies, epidermal laser evoked potentials, and whole sural nerve biopsies from CASPR2-IgG and LGI1-IgG seropositive patients demonstrate no (or limited) interstitial abnormalities and often normal fiber counts, supporting primary nociceptive hyperexcitability as the cause of pain.<sup>24,25</sup> Among 256 patients who were LGI1-IgG or CASPR2-IgG seropositive (or both), neuropathic pain was documented in 21% with LGI1-IgGs and 46% with CASPR2-IgGs.<sup>22</sup> Of patients undergoing EMGs, 24% of the LGI1-IgG positive patients and 75% of CASPR2-IgG positive had electromyographic cramps,



fasciculations, myokymia, or neuromyotonia. Pain was often severe, and opioid use was common (38% of LGI1-IgG and 86% of CASPR2-IgG–positive pain patients). Pain rehabilitation referral was requested for 25% of patients. In this series and earlier series, the immune responsiveness of pain and the other neurological features is often dramatic; however, many need long term immunotherapy and/or antiepileptic medications.<sup>19,23,24,26</sup> Varied immunotherapies have been used successfully including steroids, IVIG, methotrexate and others in open trials.

The understanding for molecular mechanism of how antibodies in the VGKC-complex cause pain is increasing.<sup>27</sup> CASPR2 belongs to the neurexin family, localizes to the juxtaparanode and interacts with contactin-2 of myelinated axons in both peripheral and central nervous systems. It is not restricted to the axons, also present at the soma membrane of DRG cells. Earlier work with sera from VGKC-IgG seropositive, Issacs syndrome patients (presumed CASPR2-IgG) showed neuronal culture suppression of voltage-gated outward K(+) current, with hyperexcitability associated with an observed decrease in VGKC channel density at the node.<sup>28</sup> Passive transfer of CASPR2 antibodies from patients into mice demonstrates direct antibody binding to the afferent cell bodies in the dorsal root ganglia with resultant decreased expression and function of Kv1 channels at the soma membrane and reduced nodal localization. These results link CASPR2 antibodies to enhanced nociceptor hyperexcitability and pain.<sup>29</sup> Additionally, CASPR2 antibodies have also been implicated in causing central microglia reactivity following exposure in utero (but not in adulthood when the blood brain barrier is intact).<sup>30</sup> These findings may have neurodevelopmental implications in terms of central sensitization abnormalities mediated by excess microglial activation. LGI1 prevents rapid Kv1-channel inactivation within the neuronal presynaptic membrane and is highly expressed within brain and spinal cord. The locus of action of antibodies directed against LGI1 is not yet known, but pain could be mediated by potentiation of synaptic transmission within somatosensory pathways.

Contactin-associated protein 1(CASPR-1) exists separately from the VGKC complex at the paranodal region, (Fig. 1). It has recently been described as a rare novel antigen with autoimmunity and neuropathic pain in association with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) and Gullain-Barré Syndrome (GBS).<sup>31</sup> Sensory deficits and

severe pain in the back and feet can develop at the onset in CASPR1-IgG-positive patients. Serum from patients with CASPR-1 autoantibodies bound to small-diameter fibers without reduction of intraepidermal nerve fiber density, suggesting a functional mechanism for the pain. Complement binding was not evident, consistent with the characteristics of IgG4 subclass. This group of patients responded poorly to IVIG, but resolved completely after treatment with rituximab (B cell depletion treatment).<sup>32</sup>

The protein complex formed by CASPR1, contactin-1 (both axonal) and neurofascin 155 (NF155; Schwann cell) organizes as a septate-like tight junctions at the paranodal loops of the node of Ranvier and is essential to maintain molecular organization of the node of Ranvier in all myelinated fibers (Fig 1.). Interestingly, pathogenic antibodies against contactin-1 and NF155, occur in a subset of CIDP patients without neuropathic pain.<sup>33,34</sup> Because CASPR1 regulates AMPA receptors centrally<sup>35</sup>, central sensitization could play a role in this selective pain component with CASPR1 autoimmunity but this has not directly been studied.

### **Pain and Neuromuscular Hyperexcitability in Other Ion Channels (N and P/Q VGCC-IgG, Nicotinic and Muscarinic AChR-IgG)**

P/Q- and N-type voltage-gated calcium-channels (VGCC) are well-known targets in Lambert-Eaton syndrome. They are also associated with other neurological phenotypes, including pain with Stiff Person Syndrome (SPS) and neuropathy with tumors besides small cell lung cancer which is most common.<sup>36-38</sup> Neural tissue loss is not apparent (or minimal) relative to pain severity. In a review of different ion-channel antibodies in 77 patients with neuromuscular hyperexcitability (35 neuromyotonia, 32 cramp-fasciculation syndrome, 5 rippling muscle syndrome, and 5 focal neuromuscular hyperexcitability) vs 85 controls, the P/Q and N VGCC were found in 12% vs 0% controls. Also seen was 35% with VGKC, 12% with ganglionic nicotinic AChR antibodies (alpha-3), and 16% muscle AChR antibodies. The majority had painful neuromyotonia. The calcium channels are expressed by DRG cells and localize presynaptically within laminae 1 and 2 of the dorsal horn of the mouse spinal cord, where they modulate the influx of calcium during the release of neurotransmitters such as glutamate and substance P, and these channels have important role in promoting the excitability of nociceptive neurons.<sup>39</sup> Therefore, although VGCCs, ganglionic nicotinic AChR, muscle AChR

are an unproven cause of pain, there are theoretical reasons to continue considering their potential role in autoimmune pain syndromes.

## **IgG targets in painful cramps and spasms**

Painful cramps and spasms can occur with immune-mediated peripheral or central hyperexcitability. These are paroxysms, in specific muscle groups, e.g. symmetric “plantar flexor spasms” most commonly central in origin; or in isolated asymmetric muscles such as calves, finger flexors, oris oculi, which are more commonly peripheral in origin. Cramp discharges, (either peripheral or central) are resultant from neural or muscle fibers firing simultaneously.

*Stiff Persons Syndrome and Progressive Encephalitis with Rigidity and Myoclonus (GAD65, GlyR, Amphiphysin-IgGs)*

Antibodies to glutamic acid decarboxylase (GAD) are associated with forms of Stiff Persons Syndrome Spectrum Disorders (SPSD).<sup>40,41</sup> SPS is a rare, acquired, autoimmune neurological entity characterized by progressive fluctuating muscle stiffness (rigidity): 59.4% with cramps or spasms and 33.3% with pain.<sup>42</sup> Pain, especially in the lower back or legs, may be the initial experience. Painful spasms may occur spontaneously or be triggered by a sudden noise or light physical contact. GAD65 IgG values >200 nmol/L are common and in one large series the median value was 623 nmol/L.<sup>43</sup> For all neurological presentations of GAD65, only ~50% have a good response to immune treatment.<sup>44</sup> Despite many patients not having a response to immune treatment, the  $\gamma$ -aminobutyric acid agent like diazepam can still be symptomatically beneficial, but often at high dose i.e. 40.0 mg/day.<sup>43</sup>

GAD has two isoforms, GAD65 and GAD67, both are CNS gamma-amino-butyric acid (GABA) synthetic enzyme that convert L-glutamate to GABA. GAD65, which has greater autoantigenicity, is expressed in presynaptic GABA-ergic neurons for vesicle release, whereas GAD67 preferentially synthesizes cytoplasmic GABA. Pain in PSD is unlikely caused by peripheral neuropathy, as classic GAD65 antibody-positive peripheral neuropathy is not well substantiated, mainly large fiber neuropathy and commonly coexisting with diabetes mellitus.<sup>45,46</sup> GAD65 antibody inhibit GAD65 activity and disrupt GABA synthesis in vitro.<sup>47</sup> In

rat and mouse models of chronic pain, GAD65 transcription is downregulated, thereby impairing of GABA synaptic inhibition in the brain stem nucleus raphe magus and promoting the excitability of pain-facilitating neurons.<sup>48</sup> Thus, hyper-activated neurons in the CNS nociceptive pathway likely are the direct cause SPS and pain. Antibodies to GAD65 occur in 60% to 70% of SPS cases, but SPS is also associated with antibodies to GlyR (15.9%)<sup>49</sup>, amphiphysin (< 5% cases, in the setting of both small cell lung and breast cancers)<sup>49</sup> and gephyrin (1 case)<sup>50</sup>.

If painful spasms are rapidly progressive, widespread and also involve the brain stem and spinal cord, progressive encephalitis with rigidity and myoclonus (PERM) is considered. Acute or subacute painful spasms and muscle stiffness are also characteristics of PERM. Painful spasms and rigidity of the neck, back and legs are the most common feature of PERM.<sup>51</sup> Recently, GlyR autoantibodies have been detected in serum and CSF of PERM patients. GlyR autoantibody may co-exist with other antibodies, such as those against NMDAR, GAD-65, VGKC-complex, myelin oligodendrocyte glycoprotein, and aquaporin-4. GlyRs are pentameric ligand-gated chloride channels widely expressed in CNS, but transcripts of GLRA3 (encoding GlyR subunit  $\alpha 3$ ) are found only in the superficial layers of the spinal dorsal horn and in the cerebellum and olfactory bulb. The autoantibodies disrupt  $\alpha 3$ -containing GlyRs in the dorsal horn, resulting in reduced glycinergic neurotransmission, disinhibition may therefore explain the increased pain and itch perception in PERM patients who are GlyR-IgG positive.<sup>51,52</sup>

Amphiphysin, which is critical for recycling of vesicles after GABA release, is another presynaptic antigen pertinent to SPS-like disorder. Amphiphysin-IgG autoantibodies are rare, but highly associated with paraneoplastic neurological disorders.<sup>53</sup> Anti-amphiphysin antibodies might induce SPSP by inhibiting the function of GABAergic neurons.<sup>54</sup> The potential immune effector is local reactivation of amphiphysin peptide-specific cytotoxic CD8+ T cells throughout the brainstem, spinal cord parenchyma, and dorsal root ganglion. This may explain the very poor immunotherapy response in these patients.

### **Neuromyelitis Optica and Aquaporin-4-IgGs**

Pain is often a major symptom associated with neuromyelitis optica (NMO), and pain remains intense, even with immunotherapy and lesional burden management, sometimes with nominal neurological deficits.<sup>55,56</sup> The pain is disproportionately high in NMO patients when compared with Multiple Sclerosis patients with similar lesion burden.

Two pain types are characteristic in NMO: 1) Painful tonic spasms, especially in the recovery stage of NMO, affecting up to 1/3rd of patients with anti-AQP4 autoantibodies and; 2) extremity neuropathic pain.<sup>57</sup> Neuropathic pain is particularly associated with persistent thoracic spinal lesions.<sup>58</sup> The AQP4 water channel IgG target is believed to account for the disproportionate extent of pain.<sup>59</sup> Astrocyte injury in NMO is mediated by AQP4 IgG1 antibodies activated complement or the interaction of effector cells and NF $\kappa$ B driving progranulocyte astroglial response, i.e. in part an indirect inflammatory cause of pain.<sup>60,61</sup> The AQP4 channel is distributed widely in descending and ascending nociceptive somatosensory pain modulating structures (Fig. 1).<sup>62</sup> Autoantibodies against AQP4 are believed to disturb the periaqueductal grey (PAG) and rostroventral medulla (RVM) pathways. Glutamate is the principal excitatory neurotransmitter in the first synaptic relay of nociceptive pathways in laminae I and II of the spinal dorsal horn. In established NMO lesions, loss of astrocytes inevitably interrupts the glutamine-glutamate-GABA pathway which is important in regenerating levels of GABA. Furthermore, downregulation of AQP4 and its complexed EAAT2 transported after binding of AQP4-IgG results in an increase of extracellular glutamate. Such excess glutamate accumulation in the extracellular space would induce aberrant neuronal excitation, resulting in pain. Activation of the complement system with resulting production of both C5a and C5b will injure the astrocyte through membrane attack complex formation and create an inflammatory microenvironment, promoting pain (Fig. 3).<sup>63</sup>

Reducing further loss of astrocytes by prophylactic immunotherapy is foremost in NMO. Also anecdotal evidence has suggested that carbamazepine frequently helpful, but how the drug's mechanism of action (prevention of inward flux of neuronal sodium) diminishes pain is unknown.<sup>64</sup>

### *Autoantibodies Associated With Complex Regional Pain Syndrome*

Complex regional pain syndrome (CRPS) is characterized by limb-confined, spontaneous

and/or stimulus-induced pain (burning pain with allodynia and hyperalgesia) which is usually out of proportion to a precipitating minor injury, accompanied by autonomic dysfunction (changes in vascular tone, sudomotor function, skin temperature and edema), motor disturbances (weakness, tremor and muscle spasms), and trophic skin changes with bone demineralization.<sup>65</sup> Biopsies have shown minimal cellular infiltration, although a network of pro-inflammatory mediators (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 and NGF) may be contributory through nociceptor sensitization.<sup>66</sup> Circulating IgG autoantibodies against autonomic nervous system structures have suggested that CRPS may be an IgG-mediated pain disorder.<sup>67</sup> This experience and pathological observations have led others to propose an alternative term for CRPS i.e. “IRAM” (injury-triggered, regionally restricted autoantibody-mediated autoimmune disorder with minimally destructive course).<sup>68</sup>

In CRPS, the profound, sympathetically maintained pain is considered a defect in the sympathetic-sensory decoupling mechanism.<sup>69</sup> IgG autoantibodies with agonistic-like properties on the  $\beta$ 2-adrenergic receptor ( $\beta$ <sub>2</sub>AR) and/or the muscarinic-2 receptor (M2R) or activating antibodies to alpha-1a adrenoreceptors were found in CRPS patients.<sup>68,70</sup> Both  $\beta$ <sub>2</sub>AR and M2R are involved in modulation of pain and in inflammation.<sup>71</sup> Under physiological conditions, activation of M2R on peripheral nociceptors inhibits nerve excitability.<sup>72</sup> M2R autoantibodies are capable of promoting pain through nociceptive hyperexcitability in CRPS.<sup>73</sup> The discovery of these autoantibodies indicated that patients might respond to immunotherapy, but a large (N=108 patients) multicenter randomized blinded placebo controlled trial with 0.5 g/kg IVIG over 6 weeks could not confirm benefit compared to placebo treated patients.<sup>74</sup> It is likely the discovered autoantibodies are not the cause of the syndrome; rather, they may be biomarkers of a complex and poorly understood pathogenic process.

## **Biological and Emerging designer immune therapies for pain**

New designer Immune based biologic therapies are emerging from the increasing understanding of the molecular pathways associated with pain. Antibody therapies are an attractive area for pharmaceutical development where positive/negative effects of a protein are established. Antibody therapies have high specificity with reduced off-target effects, which is

often not possible in non-biologic therapies. The areas where antibody designer therapies are furthest along are in two well-studied molecular pathways: 1) Calcitonin gene related peptide in headache and 2) NGF and inflammatory pain. Antibodies against NGF have multiple potential pain applications but most commonly studied in arthritis (Fig. 2, 3).

Anti-inflammatory therapy aims to repress innate immune mechanisms of pain. Interestingly, many anti-inflammatory drugs that focus on only a part of the cascade can still offer profound relief from pain hypersensitivity, but most of these drugs have yet to be evaluated in clinical trials of pain. Currently clinical available agents without specific pain evaluation include: antibodies targeting B cells (anti-CD20 and CD19 antibodies) or cytokines (IL-1-Anakinra, IL6-Tocilizumab, TNF- $\alpha$ -infliximab, etanercept, and adalimumab); complement inhibition (C1-esterase inhibitors, anti-C5 mAbs), and proteasome inhibitors (bortezomib, carfizomib, ixazomib). Complement interference by soluble human complement receptor type-1 (TP10) has already been tested in phase II clinical trials to treat chest pain complications of cardiopulmonary bypass surgery (CPB),<sup>75</sup> which are associated with complement activation. In the mouse model of neuropathic pain, chronic constriction injury, repeated administration of FP-1, a potent antagonist of toll-like receptor-4, can relieve thermal hyperalgesia and mechanical allodynia.

### **Anti-Nerve growth factor (NGF) therapy**

NGF is a potent neurotrophic factor during mammalian embryogenesis produced in limited amounts by innervation targets and required for the survival and development of nociceptors and sympathetic efferents. In the postnatal period and adulthood, NGF has been found to have important sensitizing effects on nociceptors and administration promotes mechanical and thermal hyperalgesia in rodents and indeed humans.<sup>76</sup> This is important because inflammation either of injured tissue or nerve results in increased expression of NGF and this factor makes a major contribution to inflammatory pain.<sup>77</sup> Its high affinity receptor, tropomyosin receptor kinase A (trkA), is expressed by peptidergic nociceptive afferents and mast cells. Following binding of NGF to trkA, the receptor autophosphorylates and activates multiple downstream signaling pathways in nociceptor terminals, activating key transducers such as TRPV1, a ligand gated ion channel which respond to noxious heat and capsaicin. Furthermore the receptor complex is

internalized at nerve terminals and transported to the dorsal root ganglia where it affects transcription regulation of numerous cell surface receptors and ion channels, important in pain regulation, as well as neuronal interactions. NGF binds to trkA receptors on Mast cells and can drive Mast cell degranulation and release of inflammatory mediators. The downstream effects of enhanced NGF signaling are both peripheral and central sensitization, resulting in chronic pain (Fig. 4).

Immunotherapies trials have focused on targeting circulating NGF with NGF-sequestering agents to prevent NGF from binding to trkA, or to inhibit trkA function.<sup>78</sup> Pre-clinical, phase 1, 2 and 3 clinical trials have been completed for anti-NGF antibodies.<sup>79</sup> A phase-3 study of 7,000 patients with osteoarthritis, chronic low back pain or cancer pain is underway for the drug tanezumab with fast-track designation by the US Food and Drug Administration. ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)) Multiple earlier trials showed encouraging results, with superior performance of this drug and 2 other anti-NGF drugs (fulranumab and fasinumab) over placebo. One large double blinded, placebo controlled study (N=610) showed that tanezumab was superior to nonsteroidal anti-inflammatory drugs and opioids in terms of functional outcomes and pain scale improvements.<sup>80</sup> An unresolved issue is the potential for accelerated osteoarthritis, which appear to progress more rapidly in persons on drug compared to controls. Safety studies did not demonstrate aberrant effect on peripheral nerves. Limited neuropathic pain investigation trials have also been completed with diabetic neuropathy, visceral pain (pelvic floor and prostatitis) and post herpetic neuralgia pain, and all have shown promise.<sup>78,79</sup>

### **Anti-Calcitonin gene-related peptide (CGRP) therapy**

CGRP, part of the calcitonin family of peptide hormones, has 2 isoforms ( $\alpha$  and  $\beta$ ). The  $\alpha$ -isoform is a 37-amino acid peptide that is released in the trigeminal ganglion after migraine triggers (eg, toxic, ischemic, metabolic, and inflammatory insults). Once released, it is a potent vasodilator in the meninges and is associated with release of nitrous oxide. CGRP can promote nociceptive signaling by a number of mechanisms, including 1) stimulation of satellite glial cells (Fig. 2); 2) excitation of second-order neurons in the trigeminal nucleus (central sensitization); and 3) excitation of primary afferent terminals (peripheral sensitization).<sup>81</sup> The



subunit is expressed in keratinocytes and enteric neurons of the gut and is a potential drug target for other pain disorders.

Evidence is growing from migraine clinical trials that monoclonal antibodies to alpha-CGRP may benefit migrainors.<sup>82</sup> There are currently 4 CGRP monoclonal antibodies under phase 3 trials (eptinezumab, galcanezumab, frestanezumab, erenumab), all but erenumab target the CGRP directly. The preceding safety trials showed a modest reduction in the number of days of headache and in daily analgesic intake, supporting an important role for CGRP in the pathogenesis of migraine pain. These early studies showed a safety profile that was similar to a placebo, but the phase 3 trials will more clearly assess safety and efficacy.

## **Practical aspects of pain management in affected patients**

For autoimmune pain disorders, as more mechanistic drugs become available, greater choices will be afforded to physicians. However, we will always have to balance our desire to help patients with the limitations and risks of therapies. This is especially emphasized because the inherent risks of immunotherapy are great. The first step is to ensure the accuracy of a diagnosis that is immune responsive. Internationally, it is now possible to order accurate testing from certified labs for most of the antibodies discussed. Next, it is important to create a standard outcome measure for immunotherapy. It can be difficult in pain, as objective outcome measures are especially difficult for this subjective experience. The visual analogue pain scale (VUS) is validated useful and a 50% improvement is an excellent indication for a meaningful improvement. For patients on high dose steroids, euphoric effect from the drug can impair pain assessment. Nevertheless, starting with high dose steroids, then moving to lower doses and eventual steroid sparing agents is a common approach, especially when other devastating neurological features are present. In patients with LGI1 or CASPR2 autoimmunity, the resultant seizures, encephalopathy, memory difficulties and pain often improve simultaneously during immunotherapy.<sup>19</sup> In contrast, for patients with pain and NMO, immunotherapy for maintaining spinal cord and eye health may not immediately lead to improved pain, and the goal of immunotherapy may be to avoid future spinal cord and optic nerve injury along with further injury along the somatosensory pathway.

It is helpful to realize that an earlier injury in the somatosensory pathway without active autoimmunity may drive ongoing pain, which is more common in AQP4, GAD, amphiphysin

than LGI1 and CASPR2 autoimmunity. In all patients, membrane stabilizing drugs can be very helpful as these drugs have nociceptive membrane stabilizing features.<sup>26</sup> When selecting a specific type of drug, cost, tolerance and efficacy all are important factors. First line agents for neuropathic pain appropriate to these disorders include Gabapentinoids, tricyclic antidepressants and selective noradrenaline, serotonin reuptake inhibitors.<sup>83</sup> Physicians should allow patients to control the rate of escalation to avoid intolerance from commonly somnolence and altered sensorium, within maximal dosing guidelines, before moving to other medications. For NMO patients with cramps and spasms, carbamazepine can provide selective advantage if gabapentin ineffective. Narcotics should only be prescribed sparingly, barring rescue scenarios, given the chronic nature of these disorders and lack of evidence on functional improvements of narcotics for neuropathic pain.<sup>4</sup> Behavioral and psychotherapeutic approaches including coping strategies should all be attempted, and common coexisting depression addressed.<sup>84</sup> Trial and error for various treatment options while constantly weighing risks and benefits, and engaging patients in the decisions are generally appropriate and beneficial.

## **Conclusions**

Autoimmune pain disorders, in which specific immunoglobulin antigen targets are within the nociceptive pathways, are rare but important to recognize because they are often immune therapy responsive, and are typically associated with other neurologic deficits and possibly cancer. Improved understanding of the mechanisms of pain generation is facilitating developments of designer biological medications for pain management. Membrane-stabilizing epilepsy drugs can help patients with relatively limited risk, whether pain is associated with nonspecific inflammation or directed immunoglobulins against different portions of the somatosensory nociceptive pathway. The discovery of specific circulating autoantibodies needs to be critically assessed before immune causality for pain is assumed. International collaborative efforts are needed, with emphasis on conducting multicenter blinded trials and assessing the most beneficial disease management options for patients with these rare disorders.

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Table 1. Predominant cytokine algescic and analgesic neural mediators

Algescic mediators and cell origins	Analgesic mediators and cell origins
<p><b>Immune cells</b></p> <p>Mast cells: hist, 5-HT, LTs, TNF, IL6  Neutrophils: ROS, TNF, IL-1  Macrophages: TNF, IL-1<math>\beta</math>, IL-6, IL-15, IL-18, CatS  Lymphocytes (Th1/17): TNG, IFN<math>\gamma</math>, IL-17</p> <p><b>Glial cells</b></p> <p>Schwann cells: MMP9, TNF, IL-1<math>\beta</math>, PGE2, ATP, NGF-<math>\beta</math>  Satellite glia: TNF, IL-1<math>\beta</math>, NGF- <math>\beta</math>, CGRP  Microglia: TNF, IL-1<math>\beta</math>, IL-6, NO, PGE2, BDNF, LTs  Astrocytes: ATP, NO, Glutamate, PGs, TNF, IL-1<math>\beta</math>, IL-6, IFN<math>\gamma</math>, CCL 2-5-7, CXCL 1, 2</p> <p><b>Chemokines</b></p> <p>Fractalkine, MCP-1, MIP-1m</p> <p><b>Complement System</b></p> <p>C3, C5a</p>	<p><b>Immune cells</b></p> <p>Mast cells" IL-4, IL-10  Neutrophils: Endorphin/Enkephalin  Lymphocytes: IL-4, IL-10(Th2), B7-H1(T cells), TNF-6, IFN</p> <p><b>Glial cells</b></p> <p>Schwann cells: Epo, GDNF  Satellite glia: GDNF  Microglia: GDNF, IGF-1  Astrocytes: GDNF</p>
<p>Abbreviations: chemokine Histamine (Hist), 5-HT (serotonin), leukotrienes (LTs), tumour necrosis factor (TNF), interleukin (IL), reactive oxygen species (ROS), cathepsin S (CatS), interferon-<math>\gamma</math> (IFN-<math>\gamma</math>),matrix metalloproteinase 9 (MMP9), prostaglandins (PGs), adenosine triphosphate (ATP), nerve growth factor-<math>\beta</math> (NGF-<math>\beta</math>), nitric oxide (NO), brain derived neurotrophic factor (BDNF), (C-X-C motif) ligand 1 and 2, chemokine (C-C motif) ligand 2 (CCL2, 5, 7), monocyte chemoattractant protein-1 (MCP-1),macrophage inflammatory protein-<math>\alpha</math>(MIP-1<math>\alpha</math>), transforming growth factor-<math>\beta</math> (TGF-<math>\beta</math>), glial cell line-derived neurotrophic factor (GDNF), erythropoietin (Epo) and insulin growth factor-1 (IGF-1)</p> <p>Modified with permission from Austin PJ et al. Journal of Neuroimmunology 2010.<sup>10</sup></p>	

Table 2. Autoantibodies in chronic pain

<b>IgG Antibody</b>	<b>Antigen</b>	<b>Oncologic associations</b>	<b>Pain and other neural associations</b>
<b>Autoantibodies with strong evidence of pain causality</b>			
Voltage-gated potassium channel complex IgGs	LGI1, CASPR2, CASPR1	Infrequent: Thymoma	Isaacs and Morvan's syndromes includes autonomic and muscle hyperexcitability, pain only
GAD65 IgG	GAD65	Infrequent: thymoma; renal cell, breast, colon, lung	Dysautonomia with and without pain, myelopathy and spasticity (Stiff-Person Syndrome), cerebellar disease
Amphiphysin IgG	Amphiphysin	Frequent: lung, breast, ovarian	Focal or diffuse pain, neuropathy, myelopathy, encephalopathy, cerebellar syndrome
Glycine-receptor IgG	Glycine receptor gated chloride channel receptor (alpha1)	Infrequent: breast, thymoma, Hodgkins	Pain, brainstem, ataxia, seizures, with progressive encephalomyelitis with rigidity and myoclonus (PERM) or Stiff-Person Syndrome
NMO IgG	Aquaporin-4	Infrequent: breast, thymoma, lymphoma	Rare root and muscle involvements, painful spasms
<b>Autoantibodies associated with pain with less certain causality</b>			
Voltage-gated calcium channel IgG	N-type and P/Q-type VGCC	Carcinomas: lung, breast or gynecological (less frequent with P/Q-type)	Hyperexcitable nerve disorders, including cramp fasciculation, myelopathy and varieties of neuropathy (somatic, sudomotor, painful)
Neuronal ganglionic AChR IgG (alpha-3)	Neuronal AChR containing alpha-3 subunits	Adenocarcinoma, thymoma, small-cell carcinoma	Pandysautonomic neuropathy, including with Adies pupil, somatic neuropathies, painful small fiber; hyperexcitable nerve disorders
Adrenergic receptor IgG	b2-adrenergic receptor, muscarinic-2 receptor, alpha-1a adrenoreceptors	Cancer not described with pain only	Complex regional pain syndrome-1
Modified from Klein CJ with permission. Autoimmune Neurology: Autoimmune-mediated peripheral neuropathies and autoimmune pain 2016. <sup>8</sup>			

## Figure 1. Immune Mediators and Autoimmune Targets in Pain Regulation

The targeted antigens and receptors associated with neurogenic inflammation and *autoimmune pain* without inflammation are shown throughout the neuraxis; each is important in neural nociceptive hyperexcitability and pain. **(Bottom)** *Peripheral nociceptive pain sensitization*. Peripheral nociceptive sensitization refers to hyperexcitation of nociceptor fibers by potent or persistent stimuli; hyperexcitation can occur through afferent PAMP receptors (NIRs, IRLs), danger receptors (TRPA1, TRPV1 DAMP) and cytokine receptors (IL-1 $\beta$ R, TNF- $\alpha$ R). The efferent mediators are subsequently released by neurons, including substance P, calcitonin gene-related peptide (CGRP), adrenomedullin, cytokines, and glutamate; resulting in vessel dilation, and lymphocyte, macrophage, and neutrophil recruitment. The neural mediator cytokines are diverse and can directly alter pain fiber firing thresholds. **(Right)** *Clonal expansion of T and B cells in autoantibody generation*. In immunoglobulin based disorders, production of autoantibodies against tumors, infectious agents, and many unknown antigens through clonal expansion can cause nociceptive hyperexcitability. **(Center)** *Synaptic CNS and PNS regulators of pain*. The CNS ascending and descending nociceptive pathways that are important in pain regulation (shown in red) and influenced by multiple synaptic IgG targeted antigens (AQP4, GlyR, GAD, Amphiphysin, P/Q and N-type VGCC) in autoimmune disease. Peripheral synaptic autoantibody targets include the muscle AChR, neuronal ganglionic AChR ( $\alpha$ 3),  $\beta$ 2AR and M2R, which are associated with complex regional pain syndrome (CRPS). **(Left)** *Nodal IgG targets in autoimmune pain*. The strongest evidence for autoimmune pain disorders comes from two IgG discovered to target nodal protein (LGI1 and CASPR2); and others also have been described (Contactin-1, CASPR1).

Abbreviations: PAMP, pathogen-associated molecular patterns; NLRs, nod-like receptors; TLRs, Toll-like receptors ; TRPA1, transient receptor potential cation channel A1; TRPV1, transient receptor potential cation channel, subfamily V, member1; DAMP, danger-associated molecular pattern ; IL-1 $\beta$ R, interleukin 1 $\beta$  receptor; TNF- $\alpha$ R, tumor necrosis factor  $\alpha$  receptor; CRPS, complex regional pain syndromes; EPPs, end-plate potentials; BNB, blood brain barrier; CASPR2, contactin-associated protein -2; VGKC, voltage-gated potassium channel; AQP4, water channel aquaporin-4; NMO, neuromyelitis optica.

## Figure 2. Glial and Immune Cell Regulation of Pain

Satellite glia and microglial cell activation lead to *peripheral and central pain sensitization*.<sup>11</sup>

**(Top right)** The glial-mediated mechanisms that facilitate excitatory synapses include increased glutamate release, glutamate receptor activation and increased Na<sup>+</sup> current. Pain from primary afferent terminals leads to release of ATP, CCL2, MMP2, NRG1, CGRP, resulting in microglial activation. T-cells are recruited and activated by microglia. BDNF is secreted by activated microglia, together with IL-1, ATP, MMP-9 secreted by satellite glial cells, bind to lamina I nociceptive neurons, and trigger a shift of chloride anion gradients through potassium and sodium channels to produce hyperexcitability of the neuron *central sensitization*. The suppressing mechanism in inhibitory synapses includes reduced release of GABA and glycine, inhibition of GABA receptors, and reduced K<sup>+</sup> current. **(Bottom right)** Dorsal root ganglia glial satellite cells activated by pain leads to *peripheral sensitization*. ① Sensory neurons release ATP to activate satellite glial cells. ② Satellite glial cells then release IL-1 $\beta$ , ATP, NGF, and MMP-9 to sensitize sensory neurons, eliciting spontaneous discharge of action potential. ③ Resident Macrophages and T-cells in the DRG release chemokine fractalkine and CCL2, thereby triggering more macrophage and T cell recruitment. Macrophages and T lymphocytes increase the synthesis and release of cytokines IL-1, IL-6 and TNF. TNF increase the density of tetrodotoxin-resistant Na<sup>+</sup> channel currents in nociceptors (sensory neuron). ④ Macrophages pass through the satellite cell sheath around the primary sensory neuron in an attempt to clear damaged neurons. As a result, extracellular K<sup>+</sup> levels of sensory neurons are dysregulated, leading to neuronal hyperexcitability. **(Top left)** Chronic pain can lead to brain microglia proliferation and activation at the cortex, thalamus, amygdala and hypothalamus, with resultant central pain sensitization.

Abbreviations: DRG, dorsal root ganglia; CNS, central nervous system; IL, interleukin; ATP, adenosine triphosphate; TNF, tumor necrosis factor; CCLs, C-C motif ligands; NGF, nerve growth factor; MMP, metalloproteinase; NRG1, growth factor neuregulin-1; CGRP, calcitonin gene-related peptide; BDNF, brain-derived neurotrophic factor; GABA, gamma-aminobutyric acid.

### Figure 3. Pain excitation mechanisms in AQP4 autoimmunity

Pathogenesis of AQP4-IgG-mediated neuromyelitis optica lesions and impactors of pain development. **(Right)** IgG-AQP4-EAAT2 modulation with internalization stimulates extracellular glutamate production, leading to long-lasting pain amplification. Glutamate is the principal excitatory neurotransmitter in the first synaptic relay of nociceptive pathways in laminae I and II of the spinal dorsal horn. Segmental and descending inhibition is also impaired. **(Left)** Antibody-dependent cellular cytotoxicity (ADCC) leads to astrocyte injury and loss of inhibitory synaptic input. Loss of astrocytes, which are an exclusive source of glutamine in the CNS, interrupts the glutamine–glutamate–GABA axis, with neural hyperexcitability theorized. **(Bottom)** Complement activation leads to excitotoxicity and neural activation by cell mediated cytokine chemokine inflammatory processes. The combination of events leads to interruption of the glutamine-glutamate-GABA pathway, which is essential in pain modulation.

AQP4, aquaporin-4; EAAT2, excitatory amino acid transporter 2; GABA,  $\gamma$ -aminobutyric acid; NMDA, N-methyl-d-aspartate; NMDAR, NMDA receptor; TNF, tumor necrosis factor; P2x3, purine receptor 2x3, ATP, Adenosine triphosphate; IL-17, interleukin-17; TNF, Tumor Necrosis Factor; CCL, Chemokine (C-L motif); CXCL, Chemokine (C-X-C- motif).

### Figure 4. Nerve Growth Factor Nociceptive Pain Regulation

NGF is a potent regulator of nociceptive pain thresholds. NGF is released by diverse tissue injuries, including those involving viscera, bones and nerve.<sup>77</sup> Recent work suggests that it is a potential target for biological antibody therapy in the trigeminal sensory nerve pathway of headache but also other pain generators **(Top)**. NGF can cause heightened pain threshold by directly binding to mast cells, causing degranulation and increased NGF production. Local modulation alters ionic transport regulators of pain, leading to membrane excitability **(Bottom)**. Once bound to its receptor (tropomyosin-related kinase A) at sensory nerve terminals, NGF undergoes retrograde transport and cause increased transcription of a number of inflammatory neuropeptides. Some of these neuropeptides undergo anterograde transport back to the distal sensory terminals (A, peripheral sensitization) or rostrally to the spinal cord and brain (B, central sensitization).

Abbreviations: M, motif; NGF, nerve growth factor; 5-HT, **5**-hydroxytryptamine; TrKA, tropomyosin kinase-A; DRG, dorsal root ganglia; CGRP, calcitonin gene related peptide; BDNF, brain derived neurotrophic factor; BR2, bradykinin receptor 2; P75, neurotrophin peptide 75; SP, substance P; TRPV1, transient receptor protein vanilloid-1.