The voltage-gated sodium channel NaV 1.9 in visceral pain.

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The Voltage-gated Sodium Channel $Na_V1.9$ in Visceral Pain

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ABSTRACT

Background Visceral pain is a common symptom for patients with gastrointestinal (GI) disease. It is unpleasant, debilitating and represents a large unmet medical need for effective clinical treatments. Recent studies have identified $Na_V1.9$ as an important regulator of afferent sensitivity in visceral pain pathways to mechanical and inflammatory stimuli, suggesting that $Na_V1.9$ could represent an important therapeutic target for the treatment of visceral pain. This potential has been highlighted by the identification of patients who have an insensitivity to pain or painful neuropathies associated with mutations in *SCN11A*, the gene encoding voltage-gated sodium channel subtype 1.9 ($Na_V1.9$).

Purpose Here we address the role of $Na_V1.9$ in visceral pain and what known human $Na_V1.9$ mutants can tell us about $Na_V1.9$ function in gut physiology and pathophysiology.

Key words

Irritable bowel syndrome; inflammatory bowel disease; visceral pain; voltage-gated sodium channel; $Na_V1.9$; Nociceptor sensitivity; visceral afferent; enteric nervous system

KEY MESSAGES

- Visceral pain and hypersensitivity are hallmark symptoms of patients with gastrointestinal diseases such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD).

- Recent evidence implicates the voltage-gated sodium channel subtype 1.9 ($Na_V1.9$) as a regulator of primary visceral nociceptor sensitivity and as a contributor to sodium current conductance in neurones of the enteric nervous system.

- Patients with painful and painless phenotypes associated with mutations in $Na_V1.9$ have been identified, which, in some cases, possess complex gastrointestinal disorders.

- These studies have served to confirm a key role for $Na_V1.9$ in visceral pain and suggest that $Na_V1.9$ may contribute to gastrointestinal disorders.

INTRODUCTION

Chronic visceral pain affects millions of individuals worldwide and is a leading reason for presentation to a surgeon or gastroenterologist.(1, 2) The most frequent diagnoses are functional pain disorders, such as irritable bowel syndrome (IBS), although chronic abdominal pain is commonly associated with gastrointestinal diseases such as inflammatory bowel disease (IBD) even during remission.(3) Pain from the gastrointestinal tract is thought to arise either as a consequence of the direct activation of nociceptive afferent nerves or the sensitisation of these nerves to physiological stimuli, such as bowel movements. Immune and inflammatory mediators including cytokines, proteases, ATP, histamine, 5-hydroxytryptamine and prostaglandins are strongly implicated in this process,(4, 5) with central sensitisation and psychological factors also contributing to altered pain thresholds.(6) Other disease pathologies that precipitate aberrant distension or obstruction of the bowel such as functional loss of motility or fibrosis will further trigger nociceptor activation and cause pain.(7) The use of existing analgesics (including non-steroidal anti-inflammatory drugs (NSAIDs) and opioids) to treat chronic visceral pain are often constrained by poor efficacy, risk of dependency and/or adverse gastrointestinal side effects; limiting their effectiveness in a clinical setting. As such, there is a clear need for novel pain therapies to provide benefit to those suffering from abdominal pain.

Pain-sensing nerves or nociceptors are a subtype of extrinsic primary sensory neurones which innervate the gut.(8) They are activated by noxious stimuli, such as high-threshold mechanical distension of the bowel, inflammatory mediators, hypoxia and ischaemia, and relay this signal to the central nervous system (CNS) where it is perceived as pain. Visceral spinal afferents have cell bodies located in the dorsal root

ganglia (DRG) and may project to lamina I, V and X of the dorsal horn.(9, 10) Nociceptors transduce noxious stimuli through the activation of a variety of ion channels, or receptors present on their nerve endings evoking generator potentials. If the stimulus is sufficient, the generator potential produced will trigger an action potential leading to the transmission of painful stimuli along the somatosensory pathway in the form of regenerative action potentials. The probability of a given noxious stimulus initiating action potential firing is dependent on the afferent endings basal excitability, which is plastic and prone to modulation during inflammation.(11-15) One example of this plasticity is the development of mechanical hypersensitivity in "silent" afferents, a subset of nociceptors that are mechanically insensitive under normal conditions, which following inflammation become sensitised to mechanical stimuli.(14, 16) Additionally inflammation also results in the sensitisation of existing mechanosensitive nociceptors making them hyperexcitable to previously innocuous levels of physiological stimuli.(13) These changes in nociceptor behaviour undoubtedly contribute to the development of visceral hypersensitivity to colorectal distension and inflammatory hyperalgesia observed in both rodent colitis models and in the pathogenesis of human GI disorders.(4)

As a consequence, the pharmacological modulation of visceral nociceptor excitability is a key approach to the development of novel visceral analgesics. This is likely to be successful as evidenced by the amelioration of abdominal pain following rectal application of the local anaesthetic lidocaine in patients with irritable bowel syndrome.(17, 18) Although identifying a common mechanism of action able to overcome the potential redundancy in nociceptor activation due to the multiple signalling pathways present on the afferent ending, or the multiple mediators

implicated in nociceptor activation during gastrointestinal disease, represents a significant challenge.

Voltage-gated sodium channels (Na_V) have the potential to act as one such point of convergence in nociceptor activation, being critical to the electrogenesis of excitable cells. In particular, three ($Na_V1.7$, $Na_V1.8$ and $Na_V1.9$) subtypes of the nine $Na_V1.1$-$Na_V1.9$ α-subunits encoded by the genes (*SCN1A*-*SCN5A* and *SCN8A*-*SCN11A*) are strongly expressed in sensory neurones and have been associated with human pain disorders.(19-24) Of these, the recent association of mutations in $Na_V1.9$ with human pain phenotypes (23-25) has led to renewed interest in this channel as a therapeutic approach to the treatment of pain. Alongside $Na_V1.9$ channelopathies, expression in nociceptive sensory neurones and attenuated pain behaviours in $Na_V1.9$ knock-out mice have linked the channel to the transduction of noxious stimuli. This review addresses the role of $Na_V1.9$ in visceral pain and the effect of $Na_V1.9$ channelopathies on gastrointestinal phenotype.

BIOPHYSICAL CHARACTERISTICS OF $NA_V1.9$

The tetrodotoxin (TTX)-resistant $Na_V1.9$ isoform is expressed primarily by small diameter neurones of the peripheral nervous system and is implicated in nociception.(26) The channel possesses unique biophysical characteristics producing a persistent sodium current, with slow activation and inactivation kinetics, and a hyperpolarised voltage-dependent activation, activating close to the resting membrane potential (threshold of activation ~ -65mV).(27-30) The slow kinetics of $Na_V1.9$ activation likely precludes the channel from contributing to the action potential up-stroke.(27) Indeed, the sub-threshold membrane potential activation of $Na_V1.9$ and

significant overlap in activation/inactivation gating at around the resting membrane potential mean that $Na_V1.9$ acts as a contributor to resting membrane conductance.(31-33) It is in this capacity that $Na_V1.9$ is likely capable of regulating resting neuronal excitability and the development of generator potentials in nerve terminals to depolarising stimuli, such as mechanical force, acidity or temperature. In addition to these voltage-dependent roles of $Na_V1.9$, we and others have shown that $Na_V1.9$-mediated persistent sodium currents are greatly enhanced in the presence of inflammatory mediators, leading to more depolarised resting membrane potentials, which may be sufficient to trigger action potential electrogenesis. Therefore in the presence of inflammation, $Na_V1.9$ has the potential to be both a key mechanism by which inflammatory mediators trigger spontaneous action potential firing and an important regulator of afferent sensitivity to external stimuli (see Figure 1).

$Na_V1.9$ IN VISCERAL NEURONES AND BEHAVIOURAL PAIN PHENOTYPES

In line with these proposed roles for $Na_V1.9$ in the modulation of sensory neuronal excitability, several studies have provided evidence for the involvement of $Na_V1.9$ in visceral pain and the development of inflammatory mechanical hypersensitivity.(34-38) Most recently, Hockley *et al*. has shown using a combination of *in situ* hybridisation and immunohistochemistry that approximately half of thoracolumbar DRG neurones back-labelled by tracer injection into the wall of the distil colon express $Na_V1.9$,(36) building on previous qualitative studies.(39-41) Consistent with the expression of $Na_V1.9$ in gut-projecting DRG neurones, the colonic afferent fibre response to a broad range of algogenic inflammatory mediators, such as ATP, PGE_2, adenosine, and bradykinin are greatly attenuated in $Na_V1.9$ -/- mice.(36, 42, 43) Importantly, from a translational

perspective, responses to the application of multiple inflammatory mediators applied at once, either in the form of supernatants from both chronically and acutely inflamed human bowel (e.g. resected tissue from patients with inflammatory bowel disease or appendicitis), or as an experimental inflammatory soup (ATP, PGE_2, bradykinin, histamine & 5-hydroxytryptamine) were also significantly reduced in visceral afferents of $Na_V1.9$ -/- mice.(36, 44) This illustrates $Na_V1.9$ as a down-stream effector of afferent excitability to multiple inflammatory mediators, including those present in human disease, and highlights its value as a target for the treatment of inflammatory visceral pain. Afferent responses to direct nerve activators such as capsaicin or mechanical stimuli, which stimulate pain-sensing nerves through the activation of channels such as TRPV1, TRPV4, TRPA1 or ASIC3 are also significantly reduced in $Na_V1.9$ -/- mice, as was the mechanical hypersensitivity observed following application of inflammatory soup.(36) The translation of these observations into a loss of the perception of pain centrally is supported by behavioural studies that have demonstrated robust visceral phenotypes in $Na_V1.9$ -/- mice to colorectal distension after intracolonic instillation of an inflammatory agent.(45) A reduction in bladder afferent activity to PGE_2 has also been reported in $Na_V1.9$ -/- mice, and thermal and mechanical hypersensitivity following intraplantar injections of inflammatory agents (including carrageenan and complete Freud's adjuvant) is greatly attenuated in $Na_V1.9$-/- mice implicating $Na_V1.9$ in the broader regulation of nociceptor activation by inflammatory mediators.(33-35, 46, 47)

GUT PHENOTYPES ASSOCIATED WITH HUMAN $NA_V1.9$ CHANNELOPATHIES

The recent identification of human $Na_V1.9$ channelopathies associated with congenital insensitivity to pain, episodic pain syndrome and painful neuropathy has led to functional studies of this channel. Familial episodic pain has been associated with point mutations in *SCN11A*, the gene encoding $Na_V1.9$, in two Chinese families.(24) These two separate mutations at Ala808Gly and Arg225Cys cause episodic pain predominantly in the lower distal extremities and was worsened by fatigue (see Figure 2A). Specifically, these mutations possess significantly increased current density and unaltered voltage dependence of activation and inactivation.(24) This leads to increased neuronal excitability, which was not attributed to changes in resting membrane potential or action potential threshold. Instead, increased action potential firing following current injections is likely causal to the observed hyperexcitability of sensory neurones.

Recently eight heterozygous variants of *SCN11A* were identified in 12 patients from a cohort of 393 patients with painful neuropathy.(25) Mutations in *SCN9A* and *SCN10A* were not found in these patients and a detailed functional electrophysiological analysis was carried out on two $Na_V1.9$ mutations (Ile381Thr and Leu1158Pro) possessed by four patients, which were shown to confer gain-of-function characteristics to the $Na_V1.9$ channel (see Figure 2A). Symptoms presented late in life (>50 years of age) and consisted of numbness, tingling and typically dull burning pain in the lower limbs. These symptoms were associated with autonomic changes including diarrhoea, hyperhidrosis, dry mouth/eyes and altered blood flow. Ile381Thr and Leu1158Pro mutations were located in membrane-spanning segments lining the pore (DI/S6) and in the voltage-sensor (DIII/S3) of the channel, respectively (see Figure 2A). Both mutations lead to hyperpolarising shifts in the voltage dependence of activation and, in the case of Ile381Thr, a depolarising shift in the voltage dependence of inactivation, whilst this

remained unchanged for Leu1158Pro. Resting membrane potential was depolarised and action potential current threshold was reduced by both mutations, resulting in significant increases in action potential firing during depolarising current steps.

Finally, a single *de novo* Leu811Pro mutation located at the distal end of the S6 transmembrane helix in domain II of $Na_V1.9$ has been linked to the inability to experience pain in humans (see Figure 2A).(23) Clinically, this results in multiple painless fractures and slow wound healing. Further, gastrointestinal function is also impaired with patients requiring temporary parenteral nutrition and possessing morphologically abnormal small intestine and enlarged colon.(23) This phenotype is driven by changes in $Na_V1.9$ voltage-dependant gate closure and channel inactivation caused by the mutation. As such, $Na_V1.9$ Leu811Pro possesses a leftward shift in activation and deactivation kinetics (~-29mV), and results in increased $Na_V1.9$ Na^+ current flux at rest, and a subsequent ~7mV depolarisation of the resting membrane potential. Leipold *et al.* hypothesise that other voltage-gated sodium channels and voltage-gated calcium channels are therefore progressively inactivated and the sensory neurone experiences conduction block.(23, 31) Given the extensive expression of $Na_V1.9$ within small DRG neurones, this could result in a selective blockade of primary nociceptive pathways and an inability to sense pain. This hypothesis had been challenged by recent studies linking mutations in $Na_V1.9$ with hyperexcitability of sensory neurones. Whilst the change in the voltage dependence of activation and deactivation associated with the Leu811Pro mutation are far greater than those seen in the Huang *et al*. and Zhang *et al*. mutations, the subsequent depolarisation in resting membrane potential (RMP) observed by Leipold *et al*. of ~7mV is comparable to that seen by Huang *et al.*(23-25) As such it is difficult to reconcile these findings with current

hypotheses correlating point mutations in both $Na_V1.7$ and $Na_V1.9$ to depolarising changes in resting membrane potential and subsequent *hyperexcitability* of DRG neurones.(25, 48) Indeed, Huang *et al*. go on to state that 'hypoexcitability of mouse DRG neurones that express the $Na_V1.9$ L811P mutation cannot be explained by the shift in RMP of these neurones'.(25) This is likely due to the expression of $Na_V1.8$ in the vast majority of small sensory neurones, which unlike other voltage-gated sodium channels, has voltage-dependencies of activation and inactivation 20-40mV more depolarised.(49) As such a 5-7mV depolarising shift in resting membrane potential is unlikely to drive significant proportions of $Na_V1.8$ channels into inactivated states and will result in hyperexcitability of these neurones,(25, 50) however in those cells not expressing $Na_V1.8$, hypoexcitability is the likely phenotype.(51) Further, Huang *et al*. suggest that the hypoexcitability observed in sensory neurones expressing the $Na_V1.9$ Leu811Pro mutation may potentially be explained by a sampling bias towards these cells, with cells expressing $Na_V1.8$ significantly fatigued or subnormal.(25)

$Na_V1.9$, in conjunction with $Na_V1.5$, have been shown to be the two sodium channels responsible for carrying the TTX-R Na^+ currents observed in myenteric neurones of the enteric nervous system, and responsible for regulating tonic firing and the amplification of incoming signals.(52-54) The enteric nervous system regulates digestive functions including secretomotor reflexes and the detection of luminal contents; and is organised into two plexuses: the myenteric plexus, located between the circular and longitudinal muscle layers, the submucosal plexus, located between the mucosa and the circular muscle.(55) The contribution of $Na_V1.9$ to secretomotor function is supported by mice lacking $Na_V1.9$ possessing altered gut motility(56) and the complex gut phenotypes observed in patients with $Na_V1.9$ Leu811Pro variants.(23) These GI phenotypes may be

a manifestation of myenteric dysfunction, possibly driven by the same aberrant mechanism speculated for sensory neurones possessing $Na_V1.9$ mutations.(23, 25) This is especially pertinent given the lack of $Na_V1.8$ in myenteric neurones.(52) Recent successes modelling *in silico* the role of $Na_V1.5$ and $Na_V1.9$ in the control of myenteric neuronal excitability provide an opportunity to explore how altered channel kinetics, such as those seen in $Na_V1.9$ mutants, may impact myenteric neuronal function.(52)

The association of multiple variants in *SCN5A*, the gene encoding $Na_V1.5$, with a subset of diarrhoea-predominant irritable bowel syndrome (IBS) patients suggests that altered sodium channel function may be clinically relevant to functional GI disorders.(57) Whilst the mechanism underpinning altered GI function in $Na_V1.5$ mutants is yet to be fully resolved, expression of $Na_V1.5$ in pace-maker interstitial cells of Cajal (ICC),(58) smooth muscle(59) and myenteric neurones,(52) implicates a significant contribution to normal secretomotor function in tissues other than those of primary expression, i.e. cardiac myocytes. Importantly, such $Na_V1.5$ mutants may not necessarily possess a cardiac phenotype, suggesting that phenotypic presentation of specific sodium channel variants is dependent on the tissue or cell-type of expression.

Given this, and the role of $Na_V1.9$ in both visceral extrinsic afferent pathways and the enteric nervous system, it suggests that potentially novel disease-causing $Na_V1.9$ mutations may exist for gastrointestinal disorders. Specifically, that $Na_V1.5$ is co-expressed alongside $Na_V1.9$ in myenteric neurones, novel therapeutics impacting $Na_V1.9$ channel function may also possess beneficial disease-modifying characteristics in IBS, in addition to any putative analgesic properties. As such, further research is warranted into the effects of altered $Na_V1.9$ function in the enteric nervous system, in addition to a more comprehensive phenotyping of visceral sensation in $Na_V1.9$ mutants. These

findings also suggest that the interrogation of existing genome-wide association studies (GWAS) for IBS and other function GI disorders may be beneficial in defining any pathogenic role for mutations in $Na_V1.9$.

What do human channelopathies tell us about $Na_V1.9$ as an analgesic target for inflammatory pain? The GI phenotype presented by Leipold *et al.*, alongside rodent models of enteric neuronal function, suggests that $Na_V1.9$ contributes to the effective function of the GI tract, including peristaltic propulsion.(23, 53, 54, 56) Mouse knock-out shows that $Na_V1.9$ likely acts to regulate the site of origin and frequency of migrating motor complexes along the GI tract.(56) By contrast, in patients with familial episodic pain as a result of alterations in $Na_V1.9$, no GI dysmotility was reported.(24) Collectively this data would suggest that complete 'conduction block' of myenteric nerve action potential firing, as may be occurring in Leu811Pro $Na_V1.9$ mutants, is significantly more detrimental to GI function than either the loss of $Na_V1.9$ or modulation of current amplitude through genetic alteration. As such, it is hard to predict whether pharmacological inhibition of $Na_V1.9$ will significantly alter gastrointestinal function; however as observed in $Na_V1.9$ -/- mice complete loss of $Na_V1.9$ current may not induce significant GI dysmotility.(56) The ability to test this hypothesis in human bowel tissues is critical in the understanding of this pathway before commencing costly clinical studies.(60, 61)

Importantly, cognitive function and brain development appears normal in patients possessing these $Na_V1.9$ mutations.(23-25) This may have been unexpected given the purported requirement of $Na_V1.9$ in neurotrophin-evoked depolarisations in mammalian brain.(62) Interestingly, patients with $Na_V1.9$ Leu811Pro presented with delayed motor development and mild muscular weakness, although biopsies and

electromyography were normal.(23) Such motor phenotypes were not explicitly mentioned for Ala808Gly and Arg225Cys mutations(24), or Ile381Thr and Leu1158Pro mutations.(25) Indeed, one patient is reported to have been a soldier previously, at least suggesting that motor or muscular deficits, if present, were not incapacitating.(24) $Na_V1.9$ has been implicated in the development of motoneurone axons, with $Na_V1.9$ -/- mice showing marked reductions in axon growth.(63) This axon growth is dependent upon voltage-gated calcium channel activation and suggests that aberrant Leu811Pro $Na_V1.9$ Na^+ flux may impair motoneurone development in patients with this mutation.(23) However, it is clear that these patients ultimately exhibit normal motor control, suggesting that compensation at least within motoneurones for $Na_V1.9$ deficits can occur.

The recent reporting of $Na_V1.9$ possessing a specialised role in cold pain sensation and cold allodynia is consistent with patients with episodic pain syndrome reporting the pain region as feeling extremely cold.(24) This is in stark contrast to the presentation of $Na_V1.7$-dependent erythromelalgia where severe burning pain in the extremities may be relieved by ice bath or cold compress.(64) Whether there are consequences to visceral sensation of the involvement of $Na_V1.9$ in detecting noxious cold, it remains to be seen.

Our current understanding of the function of $Na_V1.9$ in conjunction with pain phenotypes of human mutants suggests a significant role for the channel in the development of visceral inflammatory pain. Together these findings indicate that pharmacological blockade of $Na_V1.9$ may prove an effective analgesic strategy in pathologies where the predominant pain is caused by acute or on-going inflammation. This may be particularly relevant for gastrointestinal disorders, where there is an unmet medical need for mechanistically novel analgesics. As such, $Na_V1.9$ represents a

unique modulator of visceral afferent excitability capable of significantly impacting the development of visceral pain.

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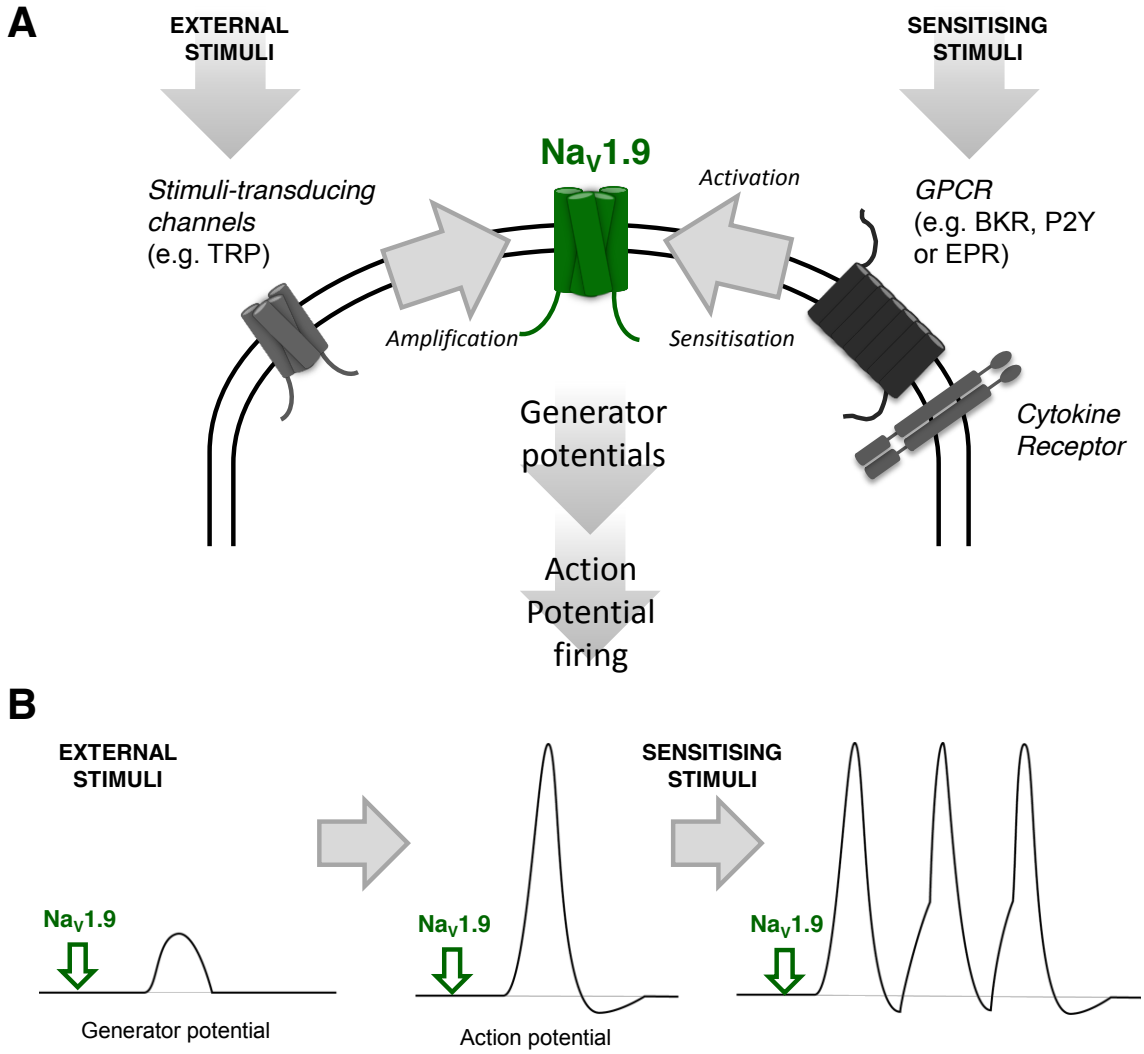
FIGURE LEGENDS

Figure 1. Contribution of $Na_V1.9$ to visceral afferent action potential generation. A Schematic of visceral afferent ending in the gastrointestinal tract. When expressed, $Na_V1.9$ contributes to setting resting membrane potential, acts to amplify generator potentials evoked by external stimuli, such as a mechanical force and functions to transduce sensitising stimuli such as inflammatory mediators. B $Na_V1.9$'s contribution to the resting membrane potential and amplification of depolarising stimuli means that in a sensitised state where a greater $Na_V1.9$ Na^+ current is present, a smaller stimuli and generator potential is required to evoke action potential firing. TRP, transient receptor potential; BKR, bradykinin receptor; EPR, prostaglandin receptor.

Figure 2. $Na_V1.9$ variants associated with clinical pain or painless phenotypes and expression of $Na_V1.9$ in gastrointestinal tissues. A In purple, two mutations (R225C and A808G) linked to episodic pain syndrome (24). In red, seven mutations (I381T, K419N, A582T, A681D, A842P, L1158P and F1689L) associated with painful neuropathy. An eighth mutation was also identified at the 3' acceptor splice site of intron 24 (25). In purple, gain-of-function mutation (L811P) linked to congenital insensitivity to pain (23). B $Na_V1.9$ has been identified in extrinsic afferents innervating the mesentery and gastrointestinal wall and contributes to the development of inflammatory hypersensitivity and visceral pain. The expression of $Na_V1.9$ by enteric neurones present in both the submucosal and myenteric plexi suggest that $Na_V1.9$ influences secretomotor function and may have a role in the development of conditions involving GI dysmotility such as irritable bowel syndrome (IBS).

513 Figure 1



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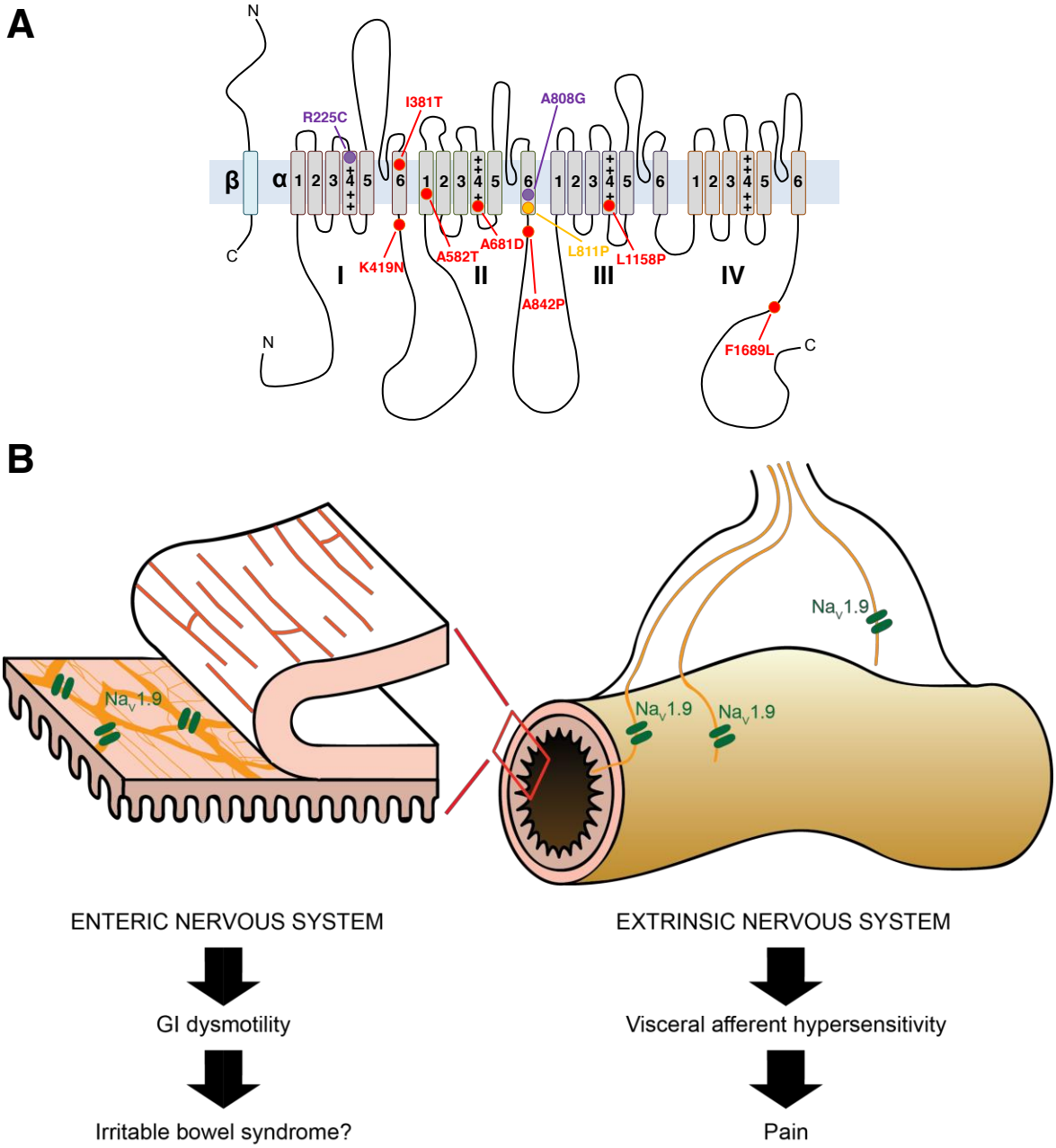
516 Figure 2



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