

1 **The Voltage-gated Sodium Channel Nav1.9 in Visceral Pain**

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15 **Short title:** Nav1.9 and Visceral Pain

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24 ABSTRACT

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26 **Background** Visceral pain is a common symptom for patients with gastrointestinal (GI)
27 disease. It is unpleasant, debilitating and represents a large unmet medical need for
28 effective clinical treatments. Recent studies have identified Nav1.9 as an important
29 regulator of afferent sensitivity in visceral pain pathways to mechanical and
30 inflammatory stimuli, suggesting that Nav1.9 could represent an important therapeutic
31 target for the treatment of visceral pain. This potential has been highlighted by the
32 identification of patients who have an insensitivity to pain or painful neuropathies
33 associated with mutations in *SCN11A*, the gene encoding voltage-gated sodium channel
34 subtype 1.9 (Nav1.9).

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36 **Purpose** Here we address the role of Nav1.9 in visceral pain and what known human
37 Nav1.9 mutants can tell us about Nav1.9 function in gut physiology and pathophysiology.

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39 **Key words**

40 Irritable bowel syndrome; inflammatory bowel disease; visceral pain; voltage-gated
41 sodium channel; Nav1.9; Nociceptor sensitivity; visceral afferent; enteric nervous
42 system

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44 KEY MESSAGES

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

49 • Recent evidence implicates the voltage-gated sodium channel subtype 1.9
50 (Nav1.9) as a regulator of primary visceral nociceptor sensitivity and as a
51 contributor to sodium current conductance in neurones of the enteric nervous
52 system.

53 • Patients with painful and painless phenotypes associated with mutations in
54 Nav1.9 have been identified, which, in some cases, possess complex
55 gastrointestinal disorders.

56 • These studies have served to confirm a key role for Nav1.9 in visceral pain and
57 suggest that Nav1.9 may contribute to gastrointestinal disorders.

58

59 INTRODUCTION

60

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

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

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

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

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

110 Voltage-gated sodium channels (Nav) have the potential to act as one such point of
111 convergence in nociceptor activation, being critical to the electrogenesis of excitable
112 cells. In particular, three (Nav1.7, Nav1.8 and Nav1.9) subtypes of the nine Nav1.1-
113 Nav1.9 α -subunits encoded by the genes (*SCN1A-SCN5A* and *SCN8A-SCN11A*) are
114 strongly expressed in sensory neurones and have been associated with human pain
115 disorders.(19-24) Of these, the recent association of mutations in Nav1.9 with human
116 pain phenotypes (23-25) has led to renewed interest in this channel as a therapeutic
117 approach to the treatment of pain. Alongside Nav1.9 channelopathies, expression in
118 nociceptive sensory neurones and attenuated pain behaviours in Nav1.9 knock-out mice
119 have linked the channel to the transduction of noxious stimuli. This review addresses
120 the role of Nav1.9 in visceral pain and the effect of Nav1.9 channelopathies on
121 gastrointestinal phenotype.

122 BIOPHYSICAL CHARACTERISTICS OF NAV1.9

123

124 The tetrodotoxin (TTX)-resistant Nav1.9 isoform is expressed primarily by small
125 diameter neurones of the peripheral nervous system and is implicated in
126 nociception.(26) The channel possesses unique biophysical characteristics producing a
127 persistent sodium current, with slow activation and inactivation kinetics, and a
128 hyperpolarised voltage-dependent activation, activating close to the resting membrane
129 potential (threshold of activation $\sim -65\text{mV}$). (27-30) The slow kinetics of Nav1.9
130 activation likely precludes the channel from contributing to the action potential up-
131 stroke.(27) Indeed, the sub-threshold membrane potential activation of Nav1.9 and

132 significant overlap in activation/inactivation gating at around the resting membrane
133 potential mean that Nav1.9 acts as a contributor to resting membrane conductance.(31-
134 33) It is in this capacity that Nav1.9 is likely capable of regulating resting neuronal
135 excitability and the development of generator potentials in nerve terminals to
136 depolarising stimuli, such as mechanical force, acidity or temperature. In addition to
137 these voltage-dependent roles of Nav1.9, we and others have shown that Nav1.9-
138 mediated persistent sodium currents are greatly enhanced in the presence of
139 inflammatory mediators, leading to more depolarised resting membrane potentials,
140 which may be sufficient to trigger action potential electrogenesis. Therefore in the
141 presence of inflammation, Nav1.9 has the potential to be both a key mechanism by
142 which inflammatory mediators trigger spontaneous action potential firing and an
143 important regulator of afferent sensitivity to external stimuli (see Figure 1).

144 **NAV1.9 IN VISCERAL NEURONES AND BEHAVIOURAL PAIN PHENOTYPES**

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146 In line with these proposed roles for Nav1.9 in the modulation of sensory neuronal
147 excitability, several studies have provided evidence for the involvement of Nav1.9 in
148 visceral pain and the development of inflammatory mechanical hypersensitivity.(34-38)
149 Most recently, Hockley *et al.* has shown using a combination of *in situ* hybridisation and
150 immunohistochemistry that approximately half of thoracolumbar DRG neurones back-
151 labelled by tracer injection into the wall of the distal colon express Nav1.9,(36) building
152 on previous qualitative studies.(39-41) Consistent with the expression of Nav1.9 in gut-
153 projecting DRG neurones, the colonic afferent fibre response to a broad range of
154 algogenic inflammatory mediators, such as ATP, PGE₂, adenosine, and bradykinin are
155 greatly attenuated in Nav1.9 -/- mice.(36, 42, 43) Importantly, from a translational

156 perspective, responses to the application of multiple inflammatory mediators applied at
157 once, either in the form of supernatants from both chronically and acutely inflamed
158 human bowel (e.g. resected tissue from patients with inflammatory bowel disease or
159 appendicitis), or as an experimental inflammatory soup (ATP, PGE₂, bradykinin,
160 histamine & 5-hydroxytryptamine) were also significantly reduced in visceral afferents
161 of Nav1.9 -/- mice.(36, 44) This illustrates Nav1.9 as a down-stream effector of afferent
162 excitability to multiple inflammatory mediators, including those present in human
163 disease, and highlights its value as a target for the treatment of inflammatory visceral
164 pain. Afferent responses to direct nerve activators such as capsaicin or mechanical
165 stimuli, which stimulate pain-sensing nerves through the activation of channels such as
166 TRPV1, TRPV4, TRPA1 or ASIC3 are also significantly reduced in Nav1.9 -/- mice, as was
167 the mechanical hypersensitivity observed following application of inflammatory
168 soup.(36) The translation of these observations into a loss of the perception of pain
169 centrally is supported by behavioural studies that have demonstrated robust visceral
170 phenotypes in Nav1.9 -/- mice to colorectal distension after intracolonic instillation of
171 an inflammatory agent.(45) A reduction in bladder afferent activity to PGE₂ has also
172 been reported in Nav1.9 -/- mice, and thermal and mechanical hypersensitivity
173 following intraplantar injections of inflammatory agents (including carrageenan and
174 complete Freud's adjuvant) is greatly attenuated in Nav1.9-/- mice implicating Nav1.9 in
175 the broader regulation of nociceptor activation by inflammatory mediators.(33-35, 46,
176 47)

177 GUT PHENOTYPES ASSOCIATED WITH HUMAN NAV1.9 CHANNELOPATHIES
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179 The recent identification of human Nav1.9 channelopathies associated with congenital
180 insensitivity to pain, episodic pain syndrome and painful neuropathy has led to
181 functional studies of this channel. Familial episodic pain has been associated with point
182 mutations in *SCN11A*, the gene encoding Nav1.9, in two Chinese families.(24) These two
183 separate mutations at Ala808Gly and Arg225Cys cause episodic pain predominantly in
184 the lower distal extremities and was worsened by fatigue (see Figure 2A). Specifically,
185 these mutations possess significantly increased current density and unaltered voltage
186 dependence of activation and inactivation.(24) This leads to increased neuronal
187 excitability, which was not attributed to changes in resting membrane potential or
188 action potential threshold. Instead, increased action potential firing following current
189 injections is likely causal to the observed hyperexcitability of sensory neurones.

190 Recently eight heterozygous variants of *SCN11A* were identified in 12 patients from a
191 cohort of 393 patients with painful neuropathy.(25) Mutations in *SCN9A* and *SCN10A*
192 were not found in these patients and a detailed functional electrophysiological analysis
193 was carried out on two Nav1.9 mutations (Ile381Thr and Leu1158Pro) possessed by
194 four patients, which were shown to confer gain-of-function characteristics to the Nav1.9
195 channel (see Figure 2A). Symptoms presented late in life (>50 years of age) and
196 consisted of numbness, tingling and typically dull burning pain in the lower limbs. These
197 symptoms were associated with autonomic changes including diarrhoea, hyperhidrosis,
198 dry mouth/eyes and altered blood flow. Ile381Thr and Leu1158Pro mutations were
199 located in membrane-spanning segments lining the pore (DI/S6) and in the voltage-
200 sensor (DIII/S3) of the channel, respectively (see Figure 2A). Both mutations lead to
201 hyperpolarising shifts in the voltage dependence of activation and, in the case of
202 Ile381Thr, a depolarising shift in the voltage dependence of inactivation, whilst this

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

206 Finally, a single *de novo* Leu811Pro mutation located at the distal end of the S6
207 transmembrane helix in domain II of Nav1.9 has been linked to the inability to
208 experience pain in humans (see Figure 2A).(23) Clinically, this results in multiple
209 painless fractures and slow wound healing. Further, gastrointestinal function is also
210 impaired with patients requiring temporary parenteral nutrition and possessing
211 morphologically abnormal small intestine and enlarged colon.(23) This phenotype is
212 driven by changes in Nav1.9 voltage-dependant gate closure and channel inactivation
213 caused by the mutation. As such, Nav1.9 Leu811Pro possesses a leftward shift in
214 activation and deactivation kinetics ($\sim -29\text{mV}$), and results in increased Nav1.9 Na⁺
215 current flux at rest, and a subsequent $\sim 7\text{mV}$ depolarisation of the resting membrane
216 potential. Leipold *et al.* hypothesise that other voltage-gated sodium channels and
217 voltage-gated calcium channels are therefore progressively inactivated and the sensory
218 neurone experiences conduction block.(23, 31) Given the extensive expression of
219 Nav1.9 within small DRG neurones, this could result in a selective blockade of primary
220 nociceptive pathways and an inability to sense pain. This hypothesis had been
221 challenged by recent studies linking mutations in Nav1.9 with hyperexcitability of
222 sensory neurones. Whilst the change in the voltage dependence of activation and
223 deactivation associated with the Leu811Pro mutation are far greater than those seen in
224 the Huang *et al.* and Zhang *et al.* mutations, the subsequent depolarisation in resting
225 membrane potential (RMP) observed by Leipold *et al.* of $\sim 7\text{mV}$ is comparable to that
226 seen by Huang *et al.*(23-25) As such it is difficult to reconcile these findings with current

227 hypotheses correlating point mutations in both Nav1.7 and Nav1.9 to depolarising
228 changes in resting membrane potential and subsequent *hyperexcitability* of DRG
229 neurones.(25, 48) Indeed, Huang *et al.* go on to state that ‘hypoexcitability of mouse
230 DRG neurones that express the Nav1.9 L811P mutation cannot be explained by the shift
231 in RMP of these neurones’.(25) This is likely due to the expression of Nav1.8 in the vast
232 majority of small sensory neurones, which unlike other voltage-gated sodium channels,
233 has voltage-dependencies of activation and inactivation 20-40mV more
234 depolarised.(49) As such a 5-7mV depolarising shift in resting membrane potential is
235 unlikely to drive significant proportions of Nav1.8 channels into inactivated states and
236 will result in hyperexcitability of these neurones,(25, 50) however in those cells not
237 expressing Nav1.8, hypoexcitability is the likely phenotype.(51) Further, Huang *et al.*
238 suggest that the hypoexcitability observed in sensory neurones expressing the Nav1.9
239 Leu811Pro mutation may potentially be explained by a sampling bias towards these
240 cells, with cells expressing Nav1.8 significantly fatigued or subnormal.(25)

241 Nav1.9, in conjunction with Nav1.5, have been shown to be the two sodium channels
242 responsible for carrying the TTX-R Na⁺ currents observed in myenteric neurones of the
243 enteric nervous system, and responsible for regulating tonic firing and the amplification
244 of incoming signals.(52-54) The enteric nervous system regulates digestive functions
245 including secretomotor reflexes and the detection of luminal contents; and is organised
246 into two plexuses: the myenteric plexus, located between the circular and longitudinal
247 muscle layers, the submucosal plexus, located between the mucosa and the circular
248 muscle.(55) The contribution of Nav1.9 to secretomotor function is supported by mice
249 lacking Nav1.9 possessing altered gut motility(56) and the complex gut phenotypes
250 observed in patients with Nav1.9 Leu811Pro variants.(23) These GI phenotypes may be

251 a manifestation of myenteric dysfunction, possibly driven by the same aberrant
252 mechanism speculated for sensory neurones possessing Nav1.9 mutations.(23, 25) This
253 is especially pertinent given the lack of Nav1.8 in myenteric neurones.(52) Recent
254 successes modelling *in silico* the role of Nav1.5 and Nav1.9 in the control of myenteric
255 neuronal excitability provide an opportunity to explore how altered channel kinetics,
256 such as those seen in Nav1.9 mutants, may impact myenteric neuronal function.(52)

257 The association of multiple variants in *SCN5A*, the gene encoding Nav1.5, with a subset
258 of diarrhoea-predominant irritable bowel syndrome (IBS) patients suggests that altered
259 sodium channel function may be clinically relevant to functional GI disorders.(57)

260 Whilst the mechanism underpinning altered GI function in Nav1.5 mutants is yet to be
261 fully resolved, expression of Nav1.5 in pace-maker interstitial cells of Cajal (ICC),(58)
262 smooth muscle(59) and myenteric neurones,(52) implicates a significant contribution
263 to normal secretomotor function in tissues other than those of primary expression, i.e.
264 cardiac myocytes. Importantly, such Nav1.5 mutants may not necessarily possess a
265 cardiac phenotype, suggesting that phenotypic presentation of specific sodium channel
266 variants is dependent on the tissue or cell-type of expression.

267 Given this, and the role of Nav1.9 in both visceral extrinsic afferent pathways and the
268 enteric nervous system, it suggests that potentially novel disease-causing Nav1.9
269 mutations may exist for gastrointestinal disorders. Specifically, that Nav1.5 is co-
270 expressed alongside Nav1.9 in myenteric neurones, novel therapeutics impacting Nav1.9
271 channel function may also possess beneficial disease-modifying characteristics in IBS, in
272 addition to any putative analgesic properties. As such, further research is warranted
273 into the effects of altered Nav1.9 function in the enteric nervous system, in addition to a
274 more comprehensive phenotyping of visceral sensation in Nav1.9 mutants. These

275 findings also suggest that the interrogation of existing genome-wide association studies
276 (GWAS) for IBS and other function GI disorders may be beneficial in defining any
277 pathogenic role for mutations in Nav1.9.

278 What do human channelopathies tell us about Nav1.9 as an analgesic target for
279 inflammatory pain? The GI phenotype presented by Leipold *et al.*, alongside rodent
280 models of enteric neuronal function, suggests that Nav1.9 contributes to the effective
281 function of the GI tract, including peristaltic propulsion.(23, 53, 54, 56) Mouse knock-
282 out shows that Nav1.9 likely acts to regulate the site of origin and frequency of
283 migrating motor complexes along the GI tract.(56) By contrast, in patients with familial
284 episodic pain as a result of alterations in Nav1.9, no GI dysmotility was reported.(24)
285 Collectively this data would suggest that complete 'conduction block' of myenteric nerve
286 action potential firing, as may be occurring in Leu811Pro Nav1.9 mutants, is
287 significantly more detrimental to GI function than either the loss of Nav1.9 or
288 modulation of current amplitude through genetic alteration. As such, it is hard to
289 predict whether pharmacological inhibition of Nav1.9 will significantly alter
290 gastrointestinal function; however as observed in Nav1.9 -/- mice complete loss of
291 Nav1.9 current may not induce significant GI dysmotility.(56) The ability to test this
292 hypothesis in human bowel tissues is critical in the understanding of this pathway
293 before commencing costly clinical studies.(60, 61)

294 Importantly, cognitive function and brain development appears normal in patients
295 possessing these Nav1.9 mutations.(23-25) This may have been unexpected given the
296 purported requirement of Nav1.9 in neurotrophin-evoked depolarisations in
297 mammalian brain.(62) Interestingly, patients with Nav1.9 Leu811Pro presented with
298 delayed motor development and mild muscular weakness, although biopsies and

299 electromyography were normal.(23) Such motor phenotypes were not explicitly
300 mentioned for Ala808Gly and Arg225Cys mutations(24), or Ile381Thr and Leu1158Pro
301 mutations.(25) Indeed, one patient is reported to have been a soldier previously, at least
302 suggesting that motor or muscular deficits, if present, were not incapacitating.(24)
303 Nav1.9 has been implicated in the development of motoneurone axons, with Nav1.9 -/-
304 mice showing marked reductions in axon growth.(63) This axon growth is dependent
305 upon voltage-gated calcium channel activation and suggests that aberrant Leu811Pro
306 Nav1.9 Na⁺ flux may impair motoneurone development in patients with this
307 mutation.(23) However, it is clear that these patients ultimately exhibit normal motor
308 control, suggesting that compensation at least within motoneurons for Nav1.9 deficits
309 can occur.

310 The recent reporting of Nav1.9 possessing a specialised role in cold pain sensation and
311 cold allodynia is consistent with patients with episodic pain syndrome reporting the
312 pain region as feeling extremely cold.(24) This is in stark contrast to the presentation of
313 Nav1.7-dependent erythromelalgia where severe burning pain in the extremities may be
314 relieved by ice bath or cold compress.(64) Whether there are consequences to visceral
315 sensation of the involvement of Nav1.9 in detecting noxious cold, it remains to be seen.

316 Our current understanding of the function of Nav1.9 in conjunction with pain
317 phenotypes of human mutants suggests a significant role for the channel in the
318 development of visceral inflammatory pain. Together these findings indicate that
319 pharmacological blockade of Nav1.9 may prove an effective analgesic strategy in
320 pathologies where the predominant pain is caused by acute or on-going inflammation.
321 This may be particularly relevant for gastrointestinal disorders, where there is an
322 unmet medical need for mechanistically novel analgesics. As such, Nav1.9 represents a

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

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333

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336

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488

489 FIGURE LEGENDS

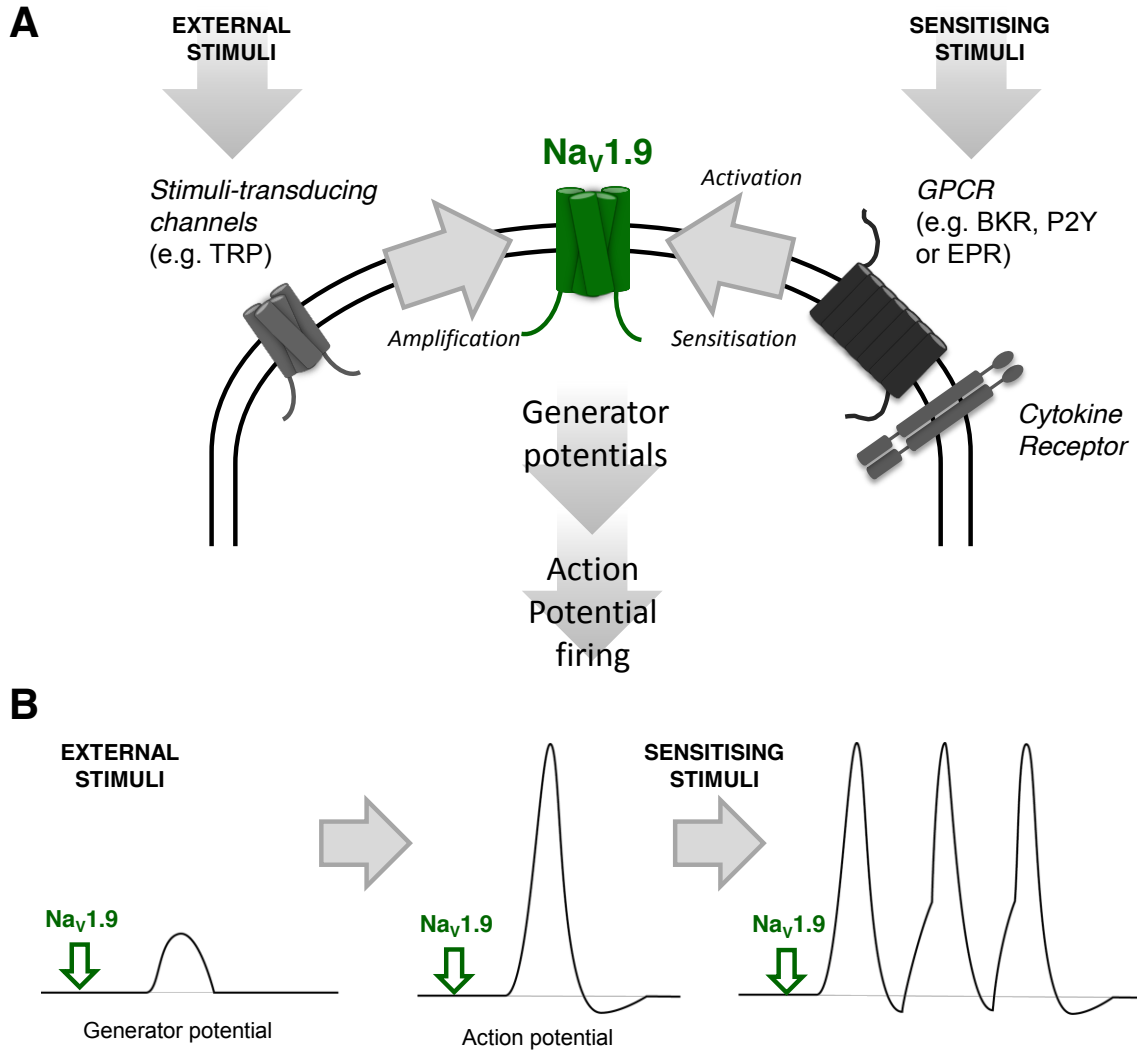
490

491 Figure 1. Contribution of Nav1.9 to visceral afferent action potential generation. A
492 Schematic of visceral afferent ending in the gastrointestinal tract. When expressed,
493 Nav1.9 contributes to setting resting membrane potential, acts to amplify generator
494 potentials evoked by external stimuli, such as a mechanical force and functions to
495 transduce sensitising stimuli such as inflammatory mediators. B Nav1.9's contribution
496 to the resting membrane potential and amplification of depolarising stimuli means that
497 in a sensitised state where a greater Nav1.9 Na⁺ current is present, a smaller stimuli and
498 generator potential is required to evoke action potential firing. TRP, transient receptor
499 potential; BKR, bradykinin receptor; EPR, prostaglandin receptor.

500

501 Figure 2. Nav1.9 variants associated with clinical pain or painless phenotypes and
502 expression of Nav1.9 in gastrointestinal tissues. A In purple, two mutations (R225C and
503 A808G) linked to episodic pain syndrome (24). In red, seven mutations (I381T, K419N,
504 A582T, A681D, A842P, L1158P and F1689L) associated with painful neuropathy. An
505 eighth mutation was also identified at the 3' acceptor splice site of intron 24 (25). In
506 purple, gain-of-function mutation (L811P) linked to congenital insensitivity to pain
507 (23). B Nav1.9 has been identified in extrinsic afferents innervating the mesentery and
508 gastrointestinal wall and contributes to the development of inflammatory
509 hypersensitivity and visceral pain. The expression of Nav1.9 by enteric neurones
510 present in both the submucosal and myenteric plexi suggest that Nav1.9 influences
511 secretomotor function and may have a role in the development of conditions involving
512 GI dysmotility such as irritable bowel syndrome (IBS).

513 Figure 1



514

515

516 Figure 2

