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TOPICAL REVIEW

Voltage-gated sodium channels: (Na_V)igating the field to determine their contribution to visceral nociception

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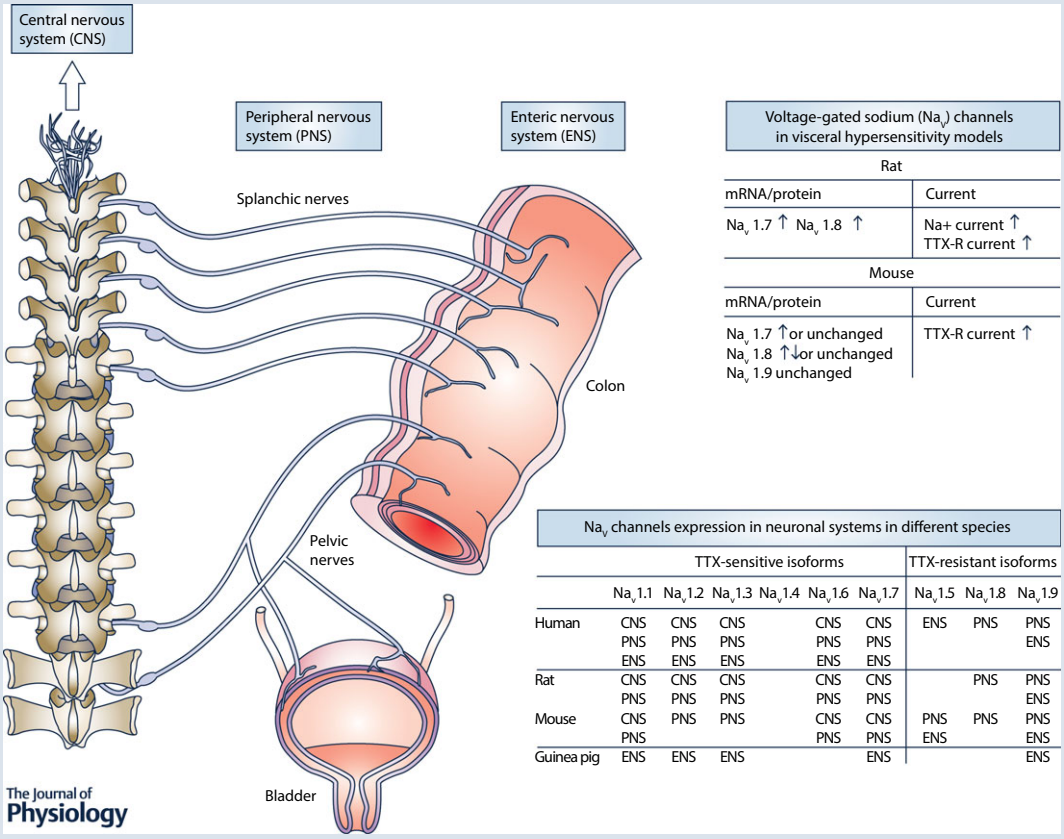

Voltage-gated sodium (Na_V) channels in visceral hypersensitivity models

Rat	
mRNA/protein	Current
Na_V 1.7 ↑ Na_V 1.8 ↑	Na+ current ↑ TTX-R current ↑
Mouse	
mRNA/protein	Current
Na_V 1.7 ↑or unchanged Na_V 1.8 ↑↓or unchanged Na_V 1.9 unchanged	TTX-R current ↑

Na_V channels expression in neuronal systems in different species

	TTX-sensitive isoforms						TTX-resistant isoforms		
	Na_V1.1	Na_V1.2	Na_V1.3	Na_V1.4	Na_V1.6	Na_V1.7	Na_V1.5	Na_V1.8	Na_V1.9
Human	CNS PNS ENS	CNS PNS ENS	CNS PNS ENS		CNS PNS ENS	CNS PNS ENS	ENS	PNS	PNS ENS
Rat	CNS PNS	CNS PNS	CNS PNS		CNS PNS	CNS PNS		PNS	PNS ENS
Mouse	CNS PNS	PNS	PNS		CNS PNS	CNS PNS	PNS ENS	PNS	PNS ENS
Guinea pig	ENS	ENS	ENS			ENS			ENS

Joel Castro, **Andrea Harrington**, **Luke Grundy**, **Annemie Deiteren** and **Sonia Garcia-Caraballo** are postdoctoral research fellows within the Visceral Pain Research Group, whilst **Andelain Erickson** and **Ashlee Caldwell** are PhD students enrolled via the University of Adelaide. Our research comprises pre-clinical and translational science investigating the causes and cures of chronic abdominal and pelvic pain associated with highly prevalent gastrointestinal disorders such as irritable bowel syndrome and inflammatory bowel disease, and bladder disorders such as interstitial cystitis/painful bladder syndrome. **Stuart M. Brierley** is an NHMRC R.D. Wright Fellow and Matthew Flinders Research Fellow in Gastrointestinal Neuroscience. He is Head of the Visceral Pain Research Group located at Flinders University and the South Australian Health and Medical Research Institute (SAHMRI) in Adelaide, Australia.

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Abstract Chronic visceral pain, altered motility and bladder dysfunction are common, yet poorly managed symptoms of functional and inflammatory disorders of the gastrointestinal and urinary tracts. Recently, numerous human channelopathies of the voltage-gated sodium (Na_V) channel family have been identified, which induce either painful neuropathies, an insensitivity to pain, or alterations in smooth muscle function. The identification of these disorders, in addition to the recent utilisation of genetically modified Na_V mice and specific Na_V channel modulators, has shed new light on how Na_V channels contribute to the function of neuronal and non-neuronal tissues within the gastrointestinal tract and bladder. Here we review the current pre-clinical and clinical evidence to reveal how the nine Na_V channel family members ($Na_V1.1$–$Na_V1.9$) contribute to abdominal visceral function in normal and disease states.

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Abstract figure legend Expression of voltage-gated sodium (Na_V) channels in neuronal cells relevant to visceral sensation.

Introduction

Chronic visceral pain, altered intestinal motility and bladder dysfunction remain poorly managed symptoms of functional and inflammatory disorders of the gastrointestinal and urinary tracts. A lack of suitable treatments for these disorders is a major contributing factor to their debilitating nature and the large socio-economic cost accrued by patients, their families and society (NIH, 2009; Gaskin & Richard, 2012; Enck *et al.* 2016). Conventional analgesics, such as opioids and non-steroidal anti-inflammatory drugs (NSAIDs), are unsuitable for treating chronic pain originating in the gastrointestinal and lower urinary tract, as they are associated with severe side effects. This includes tolerance, a lack of efficacy and importantly for some inflammatory gastrointestinal disorders the potential to exacerbate the disease (Sikandar & Dickenson, 2012; Farrell *et al.* 2014). The colon, rectum and bladder are innervated by specialised sensory afferents travelling via the splanchnic and pelvic nerves that terminate within the dorsal horn of the thoracolumbar and lumbosacral spinal cord, respectively (Brierley *et al.* 2004; Harrington *et al.* 2012; Brierley & Linden, 2014). These neurons detect both non-noxious physiological stimuli, including muscle stretch during organ distension, and noxious mechanical and chemical stimuli such as bloating, intense distension/contraction, or the presence of inflammatory mediators (Brierley & Linden, 2014; Brierley, 2016). To encode for such wide-ranging stimuli, visceral organs rely on an array of stimuli-activated primary 'sentinel' transducers, including transient receptor potential (TRP) channels, acid-sensing ion channels (ASIC), mechano-sensitive two-pore domain K (K2P) channels and Piezo channels (Grundy, 2002; Brierley, 2010; Christianson & Davis, 2010; La & Gebhart, 2011; Brierley, 2016; Alcaino *et al.* 2017). Furthermore, primary transducers and ion channels involved in sensory signalling can be modulated and controlled by G-protein coupled receptors (GPCRs) and regulators of GPCR signalling proteins, in response to endogenous mediators (Geppetti *et al.* 2015; Salaga *et al.* 2016).

Voltage-gated sodium (Na_V) channels are secondary in the neuronal response to non-noxious or noxious stimuli. They perform the crucial role of regulating neuronal excitability and the key function of amplifying cation influx generated by the primary transducers to generate and propagate action potentials (Catterall, 2012; King & Vetter, 2014). Voltage-gated potassium (K_V) channels repolarise the membrane potential following Na^+ influx and modulate firing frequency, and have been reported to contribute to visceral hypersensitivity in peripheral neurons in animal models (Hirano *et al.* 2007; Qian *et al.* 2009; Luo *et al.* 2011; Du & Gamper, 2013); however, this family of ion channels is not covered within the scope of this review.

The Na_V channel family contains nine isoforms ($Na_V1.1$–$Na_V1.9$), which are encoded by nine *SCN* genes (*SCN1A*, *SCN2A*, *SCN3A*, *SCN4A*, *SCN5A*, *SCN8A*, *SCN9A*, *SCN10A*, *SCN11A*). Functionally, these channels are historically categorised as either tetrodotoxin-sensitive (TTX-S: $Na_V1.1$-$Na_V1.4$, $Na_V1.6$ and $Na_V1.7$), or tetrodotoxin-resistant (TTX-R: $Na_V1.5$, $Na_V1.8$ and $Na_V1.9$). Anatomically, these channels display wide and diverse expression patterns across neuronal and smooth muscle cells throughout the body (Table 1), as well as cells of the immune system (including macrophages and mast cells) where they are involved in migration and phagocytosis (Bradding *et al.* 2003; Roselli *et al.* 2006; Carrithers *et al.* 2011; Black & Waxman, 2013). $Na_V1.1$, $Na_V1.2$, $Na_V1.3$ and $Na_V1.6$ are traditionally considered to be the predominant isoforms expressed in the brain and spinal cord, whilst $Na_V1.7$, $Na_V1.8$

Table 1. Expression of Na_V isoforms in neuronal and non-neuronal cells in different species relevant for visceral sensation and processing

Na_V isoform	Species	System or tissue	Found in:	Not found in:	Reference
$Na_V1.1$	Human	CNS	Cerebral cortex, cerebellum, hypothalamus, caudate, hippocampus, amygdala, C1 level spinal cord		GTEx Consortium *et al*. 2017)
	Rat	CNS	Hippocampus, cerebellum, spinal cord (dorsal horn, ventral horn, primarily grey matter restricted)	Embryonic brain and spinal cord	(Beckh *et al*. 1989; Westenbroek *et al*. 1989)
	Mouse	CNS	Cerebral cortex, cerebellum, hippocampus, thalamus, central grey, pons, medulla	Fimbria, corpus callosum	(Duflocq *et al*. 2008)
	Human	PNS	L3–L5		(Chang *et al*. 2018)
	Rat	PNS	L4–L5; L5		(Black *et al*. 1996; Fukuoka *et al*. 2008; Wang *et al*. 2011)
	Mouse	PNS	Colonic neurons in T10–L1 and L5–S1; T10–L1	L3–L6 dorsal and ventral roots	(Duflocq *et al*. 2008; Osteen *et al*. 2016; Hockley *et al*. 2017)
	Human	ENS	Colonic myenteric plexus		(Hetz *et al*. 2014)
	Guinea pig	ENS		Duodenal myenteric plexus	(Sage *et al*. 2007)
$Na_V1.2$	Human	CNS	Cerebral cortex, cerebellum, hypothalamus, caudate, hippocampus, amygdala, C1 level spinal cord		(GTEx Consortium *et al*. 2017)
	Rat, cat	CNS	Cortex, hippocampus, cerebellum, hypothalamus, spinal cord grey matter		(Jarnot & Corbett, 2006)
	Rat	CNS	Hippocampus and cerebellum; embryonic brain and spinal cord		(Beckh *et al*. 1989; Westenbroek *et al*. 1989)
	Human	PNS	L3–L5		(Chang *et al*. 2018)
	Rat	PNS	L4–L5; L5		(Black *et al*. 1996; Fukuoka *et al*. 2008)
	Mouse	PNS	Colonic neurons in T10–L1 and L5–S1		(Chang *et al*. 2018; Hockley *et al*. 2017)
	Human	ENS	Colonic myenteric plexus		(Hetz *et al*. 2014)
	Guinea pig	ENS		Duodenal myenteric plexus	(Sage *et al*. 2007)
$Na_V1.3$	Human	CNS	Caudate, cerebellum, cerebral cortex, hippocampus, hypothalamus, amygdala, C1 level spinal cord		(GTEx Consortium *et al*. 2017)
	Rat	CNS	Embryonic brain and spinal cord	Adult brain and spinal cord	(Beckh *et al*. 1989)
	Human	PNS	L3–L5		(Chang *et al*. 2018)
	Rat	PNS	L4–L5	L5	(Black *et al*. 1996; Fukuoka *et al*. 2008)
	Mouse	PNS	Colonic neurons in T10–L1 and L5–S1; DRG		(Chang *et al*. 2018; Hockley *et al*. 2017)
	Human	ENS	Colonic myenteric plexus		(Hetz *et al*. 2014)
	Guinea pig	ENS	Duodenal myenteric plexus		(Sage *et al*. 2007)

(*Continued*)

Table 1. Continued

Na_V isoform	Species	System or tissue	Found in:	Not found in:	Reference
	Human, mouse	Neuroendocrine	Jejunal and colonic enterochromaffin cells		(Bellono *et al*. 2017; Strege *et al*. 2017*a*,*b*)
$Na_V1.4$	Human	CNS		Brain	(GTEx Consortium *et al*. 2017)
	Rat	PNS		L5	(Fukuoka *et al*. 2008)
	Mouse	PNS		Colonic neurons in T10–L1 and L5–S1	(Hockley *et al*. 2017)
	Human	ENS		Colonic myenteric plexus	(Hetz *et al*. 2014)
	Human	Muscle	Oesophageal smooth muscle		(Deshpande *et al*. 2002)
$Na_V1.5$	Human	CNS		Brain	(GTEx Consortium *et al*. 2017)
	Mouse	PNS	Colonic neurons in T10–L1 and L5–S1		(Hockley *et al*. 2017)
	Human	ENS	Colonic myenteric plexus		(Hetz *et al*. 2014)
	Mouse	ENS	Duodenal myenteric plexus		(Osorio *et al*. 2014)
	Human	Interstitial cells	Jejunal interstitial cells of Cajal		(Strege *et al*. 2003)
	Human, dog, rat	Muscle	Jejunal circular smooth muscle		(Holm *et al*. 2002; Ou *et al*. 2002; Strege *et al*. 2007; Beyder *et al*. 2016)
	Human, rat	Muscle	Colonic circular smooth muscle		(Strege *et al*. 2003; Beyder *et al*. 2016)
	Human, mouse	Muscle		Jejunal longitudinal smooth muscle	(Ou *et al*. 2002; Strege *et al*. 2007)
	Pig, guinea pig	Muscle		Jejunal circular smooth muscle	(Strege *et al*. 2007)
	Human	Macrophages	Macrophages		(Carrithers *et al*. 2007, 2011; Black & Waxman, 2013)
$Na_V1.6$	Human	CNS	Cerebral cortex, cerebellum, hypothalamus, caudate, hippocampus		(Whitaker *et al*. 1999; GTEx Consortium *et al*. 2017)
	Rat	CNS	Cerebellum, hippocampus, spinal cord (white and grey matter)		(Tzoumaka *et al*. 2000)
	Mouse	CNS	Spinal cord white and grey matter		(Duflocq *et al*. 2008)
	Human	PNS	L3–L5		(Chang *et al*. 2018)
	Rat	PNS	L4–L5; L5		(Tzoumaka *et al*. 2000; Fukuoka *et al*. 2008)
	Mouse	PNS	L3–L6 dorsal and ventral roots; DRG		(Duflocq *et al*. 2008; Chang *et al*. 2018)
	Mouse	PNS	Colonic neurons in T10–L1 and L5–S1; T10–L1; T9–T13; L6		(King *et al*. 2009; Feng *et al*. 2015; Hockley *et al*. 2017; Inserra *et al*. 2017)
	Human	ENS	Colonic myenteric plexus		(Hetz *et al*. 2014)
	Guinea pig	ENS		Duodenal myenteric plexus	(Sage *et al*. 2007)

(*Continued*)

Table 1. Continued

Na_V isoform	Species	System or tissue	Found in:	Not found in:	Reference
	Human	Macrophages	Macrophages		(Carrithers *et al*. 2007, 2011; Black & Waxman, 2013)
$Na_V1.7$	Human	CNS	Hypothalamus		(GTEx Consortium *et al*. 2017)
	Rat	CNS	Hypothalamus, subfornical organ, intermediolateral cell column	Cerebellum, cerebral cortex, hippocampus, striatum, septum, thalamic nuclei	(Morinville *et al*. 2007)
	Mouse	CNS	Hypothalamus		(Branco *et al*. 2016)
	Human	PNS	L3–L5; DRG		(Flegel *et al*. 2015; Chang *et al*. 2018)
	Rat	PNS	L5		(Fukuoka *et al*. 2008)
	Mouse	PNS	Colonic neurons in T10–L1 and L5–S1; T10–L1; L6		(Feng *et al*. 2015; Hockley *et al*. 2017; Inserra *et al*. 2017)
	Human	ENS	Colonic myenteric plexus		(Hetz *et al*. 2014)
	Guinea pig	ENS	Duodenal myenteric plexus		(Sage *et al*. 2007)
$Na_V1.8$	Human	CNS		Brain	(Flegel *et al*. 2015; GTEx Consortium *et al*. 2017)
	Human	PNS	L3–L5; DRG		(Flegel *et al*. 2015; Chang *et al*. 2018)
	Rat	PNS	Colonic neurons T13–L2		(Hu *et al*. 2013*a*, 2016; Lin *et al*. 2017)
	Mouse	PNS	Colonic neurons in T10–L1 and L5–S1; T10–L1; T9–T13; T9–L1; L6		(Beyak *et al*. 2004; Hillsley *et al*. 2006; King *et al*. 2009; Feng *et al*. 2015; Hockley *et al*. 2017; Inserra *et al*. 2017)
	Human	ENS		Colonic myenteric plexus	(Hetz *et al*. 2014)
	Mouse	ENS		Duodenal myenteric plexus	(Osorio *et al*. 2014)
$Na_V1.9$	Human	CNS		Brain	(GTEx Consortium *et al*. 2017)
	Human	PNS	L3–L5; DRG		(Flegel *et al*. 2015; Chang *et al*. 2018)
	Rat	PNS	L4–L5		(Dib-Hajj *et al*. 1998)
	Mouse	PNS	Colonic neurons T9–T13; trigeminal ganglia		(Beyak *et al*. 2004; Padilla *et al*. 2007; King *et al*. 2009)
	Human	ENS	Colonic submucosal and myenteric plexus		(Hetz *et al*. 2014; O'Donnell *et al*. 2016)
	Rat	ENS	Duodenal myenteric plexus		(Rugiero *et al*. 2003)
	Mouse	ENS	Sensory, Dogiel type II, myenteric and submucosal neurons		(Padilla *et al*. 2007; Osorio *et al*. 2014)
	Guinea pig	ENS	Duodenal intrinsic primary afferent neurons; duodenal myenteric plexus		(Rugiero *et al*. 2003; Copel *et al*. 2009)
	Human	Muscle	Colonic smooth muscle		(O'Donnell *et al*. 2016)
	Human	Mast cells	lung, skin and cord blood-derived mast cells		(Bradding *et al*. 2003)

and $Na_V1.9$ are preferentially expressed in the peripheral nervous system (PNS). $Na_V1.4$ is found predominantly within skeletal muscle and $Na_V1.5$ is the major isoform in cardiac myocytes (Catterall *et al.* 2005). Furthermore, Na_V channels are regulated by a range of enzymes and structural proteins, including auxiliary β-subunits ($\beta1$, $\beta1_B$, $\beta2$, $\beta3$, $\beta4$) (Qin *et al.* 2003; Tseng *et al.* 2007), kinases and ubiquitin-protein ligases (Feng *et al.* 2012; Savio-Galimberti *et al.* 2012; Laedermann *et al.* 2015), which collectively regulate Na_V channel biophysical properties and expression.

Recently, numerous studies have reported Na_V isoform channelopathies, including for $Na_V1.7$ (*SCN9A*), $Na_V1.8$ (*SCN10A*) and $Na_V1.9$ (*SCN11A*) as the primary cause of increased pain or loss of pain phenotypes in humans (Yang *et al.* 2004; Cox *et al.* 2006; Fertleman *et al.* 2006; Klein *et al.* 2013; Leipold *et al.* 2013; Huang *et al.* 2014, 2017; Waxman *et al.* 2014; Dib-Hajj *et al.* 2015; Han *et al.* 2015). Pharmacological modulation of Na_V channels supports these genetic observations, including the finding that activation of all Na_V channels by Pacific ciguatoxin 1 (P-CTX-1) or veratridine due to accidental consumption manifests as acute and severe gastrointestinal disturbances associated with abdominal pain in humans (Schep *et al.* 2006; Stewart *et al.* 2010). Intracolonic administration of purified P-CTX-1 also causes pain behaviour in mice (Inserra *et al.* 2017). On the other hand, TTX (which blocks $Na_V1.1$–$Na_V1.4$, $Na_V1.6$ and $Na_V1.7$) poisoning in humans is associated with paralysis rather than pain (Lago *et al.* 2015). Whilst potentially fatal upon consumption, administration of Na_V-selective agents such as TTX and neosaxitoxin has been shown to decrease pain responses in a range of pain modalities including visceral pain in humans (Hagen *et al.* 2011, 2017; Manriquez *et al.* 2015) and rodents (Marcil *et al.* 2006; Gonzalez-Cano *et al.* 2017). Similarly, intrarectal administration of lidocaine (lignocaine) in irritable bowel syndrome (IBS) patients reduces rectal sensitivity and abdominal pain, suggesting Na_V channels and activation of peripheral afferent endings in the colon play key roles in the pathogenesis of chronic visceral pain in IBS patients (Verne *et al.* 2005).

Human genetic studies have triggered widespread investigation into the therapeutic potential of Na_V channels in the treatment of acute and chronic pain and also prompted studies to identify the wider roles of these channels throughout the body. It is also clear from most studies utilising inflammatory, nematode or bacterial models that gut- and bladder-innervating neurons become hyperexcitable after the initial insult, which involves changes in TTX-R and TTX-S Na_V currents, amongst others. This is apparent in neurons innervating the stomach (Gebhart *et al.* 2002; Bielefeldt *et al.* 2002*a*, *b*; Dang *et al.* 2004), small intestine (Moore *et al.* 2002; Stewart *et al.* 2003; Hillsley *et al.* 2006; Keating *et al.* 2008), the colon (Beyak *et al.* 2004; Ibeakanma *et al.* 2009; King *et al.* 2009) and the bladder (Yoshimura & deGroat, 1997). This review presents recent evidence on the specific roles of $Na_V1.1$–$Na_V1.9$ in transmitting sensation and nociception from the distal gut and bladder in healthy and pathological states.

$Na_V1.1$

$Na_V1.1$ is predominantly expressed in cell bodies, axon initial segments and at the nodes of Ranvier in the central nervous system (CNS) (Westenbroek *et al.* 1989; Duflocq *et al.* 2008; Carithers *et al.* 2015; Uhlen *et al.* 2015; GTEx Consortium *et al.* 2017). It is also expressed in human, rat and mouse PNS (Fukuoka *et al.* 2008; Wang *et al.* 2011; Osteen *et al.* 2016; Chang *et al.* 2018), and in human, but not guinea pig, myenteric plexus (Sage *et al.* 2007; Hetz *et al.* 2014) (Table 1). In thoracolumbar (T10–L1) and lumbar (L5) dorsal root ganglia (DRG) neurons, which contain the cell bodies of sensory neurons innervating the colon, rectum, bladder and skin, $Na_V1.1$ is expressed in 15–35% of all neurons. Expression is predominantly in Tropomyosin-related kinase C (TrkC)- and Tropomyosin-related kinase A (TrkA)-expressing myelinated A-fibres of medium to large diameter and nearly absent in C-fibre small diameter neurons innervating the skin (Fukuoka *et al.* 2008; Wang *et al.* 2011; Osteen *et al.* 2016). However, $Na_V1.1$ mRNA transcript is detected in approximately half of thoracolumbar (T10–L1) and lumbosacral (L5–S1) mouse DRG neurons innervating the colon (Osteen *et al.* 2016; Hockley *et al.* 2017). As colonic afferents are predominantly peptidergic C-fibres, there are clearly key differences in the populations of afferent neurons expressing $Na_V1.1$ when comparing between the colon and the skin. In colon-innervating DRG neurons, $Na_V1.1$ is frequently co-localised with $Na_V1.2$, $Na_V1.3$, $Na_V1.6$, $Na_V1.7$, $Na_V1.8$ and $Na_V1.9$ (Osteen *et al.* 2016; Hockley *et al.* 2017). Functional studies of colonic afferents reveal that $Na_V1.1$ plays a crucial role in the signalling of mechanical pain from the colon (Osteen *et al.* 2016). Application of the selective $Na_V1.1$ agonist, δ-theraphotoxin-Hm1a (Hm1a), enhances mechanically evoked firing in a subpopulation of high-threshold colonic nociceptors. Notably, the mechanical hypersensitivity evoked by Hm1a was blocked by incubation with the $Na_V1.1$/$Na_V1.3$ antagonist ICA-121431 (Table 2) (Osteen *et al.* 2016). Furthermore, Hm1a also induces hyperexcitability of isolated colon-innervating DRG neurons from healthy control mice (Osteen *et al.* 2016). Notably, the percentage of colon-innervating afferents/neurons affected by Hm1a is similar to the percentage of colon-innervating DRG neurons expressing $Na_V1.1$, as determined by single cell PCR (Osteen *et al.* 2016; Hockley *et al.* 2017). Importantly, colon-innervating DRG neurons isolated from mice with chronic visceral hypersensitivity (CVH)

Table 2. Predominant Na_V isoforms contributing functionally to visceral sensation

A. Healthy states

Species	Test	Response	Reference
Human	Appendix distension (*ex vivo* extracellular recordings of mesenteric afferents) before and after exposure to PF-5198007 ($Na_V1.7$ antagonist)	No difference in mesenteric afferent peak firing	(Hockley *et al*. 2017)
Mouse	Colonic mechanical stimulation (*ex vivo* extracellular recordings of colonic afferents in the splanchnic nerve) after Hm1a (highly selective $Na_V1.1$ agonist, mucosal application)	Increase in colonic nociceptor response to mechanical stimuli in a sub-population of afferents.	(Osteen *et al*. 2016)
	Colonic stretch (*ex vivo* extracellular recordings of colorectal afferents in the pelvic nerve) μ-conotoxin GIIIa, and μ-conotoxin PIIIa, serosal/mucosal application)	Reduced action potential firing of stretch-sensitive afferent response	(Feng *et al*. 2015)
	Colonic stretch (*ex vivo* extracellular recordings of colorectal afferents in the pelvic nerve) - ProTxII ($Na_V1.7$ antagonist, serosal/mucosal application)	No difference in stretch-sensitive afferent response	(Feng *et al*. 2015)
	Ciguatoxin (pan-Na_V agonist) (intracolonic)	Increased pain behavioural response	(Inserra *et al*. 2017)
	Colonic incubation with A-803467 ($Na_V1.8$ antagonist) (*ex vivo* extracellular recordings of colorectal nociceptors), followed by ciguatoxin	Inhibited afferent firing induced by ciguatoxin	
	Incubation with supernatant from colitis patients Tumour necrosis factor-α incubation	Increased excitability of colonic DRG neurons associated with enhanced $Na_V1.8$ currents	(Ibeakanma & Vanner, 2010)

B. Knock-out and knock-down models

Model	Species	Test	Response	Reference
$Na_V1.7^{Nav1.8}$	Mouse	Formalin (intraplantar)	Reduction in pain behavioural response in phase I and phase II of formalin response	(Nassar *et al*. 2004)
		Complete Freund's adjuvant (intraplantar)	Reduction in thermal hyperalgesia and mechanical allodynia from day 1 to day 10	
		Carrageenan (intraplantar)	Reduction in thermal hyperalgesia from 1 to 4 h	
		Nerve growth factor (intraplantar)	Absence of phase I thermal hyperalgesia and reduction in phase II	
		Colonic distension (*ex vivo* extracellular recordings of lumbar splanchnic nerve activity)	No difference in afferent firing in physiological range (0–80 mmHg) Reduction in firing in supramaximal range (80–145 mmHg)	(Hockley *et al*. 2017)
		Capsaicin (intracolonic) Mustard oil (intracolonic)	Normal pain behavioural response	

(*Continued*)

Table 2. Continued

B. Knock-out and knock-down models

Model	Species	Test	Response	Reference
		Cyclophosphamide-induced cystitis	Normal level of referred mechanical hyperalgesia responses	
$Na_V1.8^{-/-}$	Mouse	Whole-cell patch clamp	Reduced action potential amplitude in retrogradely labelled neurons projecting to the peritoneal cavity (DRG, T9–T13)	(Hillsley *et al*. 2006)
		Nippostrongylus brasiliensis post-infectious stage, whole-cell patch clamp	Absence of neuronal hyperexcitability 19–25 days post-infection in retrogradely labelled neurons projecting to the peritoneal cavity (DRG, T9–T13)	
		Acetylcholine (intraperitoneal injection)	Normal pain behavioural response	(Laird *et al*. 2002)
		Capsaicin (intracolonic) Mustard oil (intracolonic)	Reduced pain behavioural response	
		Cyclophosphamide-induced cystitis	Normal pain and inflammatory responses	
$Na_V1.8$ knock-down (L6–S1)	Rat	Cystometry (saline)	No change in intercontraction intervals	(Yoshimura *et al*. 2001)
		Acetic acid (intravesical)	Hyper-reflexia attenuated	
$Na_V1.9^{-/-}$	Mouse	Whole-cell patch clamp	Normal excitability and action potential characteristics in colonic neurons (DRG T9–T13)	(Hillsley *et al*. 2006)
		Nippostrongylus brasiliensis post-infectious stage, whole-cell patch clamp	No change in neuronal hyperexcitability 19–25 days post-infection in retrogradely labelled neurons projecting to the peritoneal cavity (DRG, T9–T13)	
		Colorectal distension	Normal pain behavioural response	(Martinez & Melgar, 2008)
		R-848 (toll-like receptor 7 activator)-induced colonic inflammation, colorectal distension	Reduced pain behavioural response	
		Colonic distension (*ex vivo* extracellular recordings of splanchnic nerve activity)	Reduced afferent discharge	(Hockley *et al*. 2014)
		Ex vivo extracellular recordings of lumbar splanchnic nerve activity following inflammatory soup (bradykinin, ATP, histamine, PGE2 and 5-HT), or inflammatory bowel disease patient colonic supernatant application	Reduced afferent fibre responses	

(*Continued*)

Table 2. Continued

Model	Species	Test	Response	Reference
		Ex vivo extracellular recordings of lumbar splanchnic nerve activity following UTP (P2Y2 and P2Y4 agonist) or ADP (P2Y1, P2Y12 and P2Y13 agonist), application	Reduced afferent fibre responses	(Hockley *et al*. 2016*a*)
		Cystometry (saline)	No change in basal urodynamics	(Ritter *et al*. 2009)
		Cyclophosphamide-induced cystitis Bladder distension (*ex vivo* extracellular recordings of bladder nerve activity) following PGE2 bladder infusion application	Reduced afferent excitability	
C. Inflammatory hypersensitivity models				
Model/disease	Species	Test	Response	Reference
Neonatal induced colitis	Rat	Protein expression	Increase in $Na_V1.7$ and $Na_V1.8$ protein in colonic (DRG, T13–L2) neurons post-inflammation	(Qu *et al*. 2013)
		Whole-cell patch clamp	Increase in Na^+ current in colonic neurons (DRG, T13–L2) 6 weeks post-inflammation No change in Na^+ current in colonic neurons (DRG, T13–L2) 10 weeks post-inflammation No change in Na^+ current in non-colonic neurons (DRG, L4–L5) 6 or 10 weeks post inflammation	
Acute TNBS-induced colitis	Mouse	Whole-cell patch clamp	Increased slow TTX-R Na^+ current in colonic neurons (DRG, T9–L1) 7–10 days post- induction No change in persistent TTX-R Na^+ currents in colonic neurons (DRG, T9–L1) 7–10 days post-induction	(Beyak *et al*. 2004)
		Gene and protein expression	No change in $Na_V1.7$ mRNA or protein in retrogradely labelled colonic neurons (DRG, T9–T13) 1 week post-induction	(King *et al*. 2009)
		Gene expression	Tenfold reduction in $Na_V1.8$ mRNA 2–4 days post-induction, no change at day 7, in retrogradely labelled colonic neurons (DRG, T9–T13)	
		Protein expression	No change in $Na_V1.8$ protein 2–4 days post-induction, up-regulation at day 7, in retrogradely labelled colonic neurons (DRG, T9–T13) 1 week post-induction No change in $Na_V1.9$ protein in colonic neurons (DRG, T9–T13) day 7 post-induction	

(*Continued*)

Table 2. Continued

C. Inflammatory hypersensitivity models				
Model/disease	Species	Test	Response	Reference
Post-TNBS-induced colitis	Mouse	Whole-cell patch clamp in the presence of Hm1a ($Na_V1.7$ agonist)	Pronounced increase in excitability of colonic DRG neurons: significant lowering of rheobase and a dramatic increase in the number of action potentials fired at 2× rheobase	(Osteen *et al*. 2016)
		Gene expression	Up-regulation of $Na_V1.7$ mRNA in retrogradely labelled colonic neurons (DRG, L6–S1) 4 weeks post-induction	(Campaniello *et al*. 2016)
Nippostrongylus brasiliensis post-infectious stage	Mouse	Gene expression	No change in $Na_V1.8$ or $Na_V1.9$ mRNA 19–25 days post -infection in retrogradely labelled neurons projecting to the peritoneal cavity (DRG, T9–T13)	(Hillsley *et al*. 2006)
Interstitial cystitis/bladder pain syndrome	Human	Neosaxitoxin (blocker of TTX-S Na_V channels) (bladder infiltration)	Analgesia and reduced frequency lasting up to 90 days	(Manriquez *et al*. 2015)
Cyclophosphamide-induced cystitis	Rat	A-803467 administration (intraperitoneal)	No change in pain behavioural response	(Jarvis *et al*. 2007)
D. Non-inflammatory hypersensitivity models				
Model/disease	Species	Test	Response	Reference
Clinical rectal hypersensitivity	Human	Protein expression (full thickness rectal biopsies)	Increased $Na_V1.7$-immunoreactive nerve fibres in mucosal, submucosal and muscle layers	(Yiangou *et al*. 2007)
Maternal separation model (visceral hypersensitivity)	Rat	Gene expression	No change in $Na_V1.8$ mRNA in colonic neurons (DRG, T13–L2)	(Hu *et al*. 2013*a*)
		Protein expression	Increase in $Na_V1.8$ protein in colonic neurons (DRG, T13–L2)	
		Whole-cell patch clamp	Increased TTX-R Na^+ current in colonic neurons (DRG, T13–L2)	
Streptozotocin-induced diabetes (visceral hypersensitivity)		Protein expression	Increase in $Na_V1.7$ and $Na_V1.8$ protein in colonic neurons (DRG, T13–L2)	(Hu *et al*. 2016)
		Whole-cell patch clamp	Increased TTX-R Na^+ current in colonic neurons (DRG, T13–L2)	
Partial colonic obstruction (visceral hypersensitivity)		Gene expression	Increase in $Na_V1.8$ mRNA in colonic neurons (DRG, T13–L2)	(Lin *et al*. 2017)
		Whole-cell patch clamp	Increased TTX-R Na^+ current in colonic neurons (DRG, T13–L2)	
T8 spinal transection		Whole-cell patch clamp	Reduced TTX-R Na^+ current in bladder neurons	(Yoshimura & deGroat, 1997)

show significantly enhanced responsiveness to Hm1a compared to healthy control mice, suggesting that $Na_V1.1$ may be essential for the development and maintenance of chronic visceral pain conditions (Osteen *et al.* 2016). As such, antagonism of $Na_V1.1$ may be a future target for the treatment of disorders accompanied by chronic visceral pain originating from the colon. There are currently no reports on the expression profile or function of $Na_V1.1$ in the bladder or bladder-innervating sensory neurons.

$Na_V1.2$

$Na_V1.2$ is extensively expressed in the CNS (Jarnot & Corbett, 2006) but has also been detected at low levels in small-diameter DRG neurons (Black *et al.* 1996; Fukuoka *et al.* 2008; Chang *et al.* 2018). Conversely, in colon-innervating DRG neurons of the mouse, $Na_V1.2$ mRNA transcript is present in 69% of thoracolumbar (T10–L1) neurons and at a similar level in lumbosacral (L5–S1) neurons (Hockley *et al.* 2017) (Table 1). Despite this mRNA expression, there is currently no functional data to support a role for $Na_V1.2$ in colonic sensory signalling or pain. Similarly, there are currently no reports on the expression profile or function of $Na_V1.2$ in the bladder or bladder-innervating sensory neurons.

$Na_V1.3$

$Na_V1.3$ is highly expressed in sensory neurons during embryogenesis in rats, but its expression traditionally subsides in fully developed neurons (Beckh *et al.* 1989). The major body of $Na_V1.3$ research in nociception focuses on its role in neuropathic pain, as $Na_V1.3$ is re-expressed following neuropathic injury in large diameter, myelinated A-fibre neurons where it may contribute to ectopic discharge and painful neuropathy (Waxman *et al.* 1994; Zang *et al.* 2010). However, due to the limited expression of this channel in adult tissues and lack of channelopathy-associated pain syndromes, studies investigating the role of $Na_V1.3$ in other pain pathways are few. In relation to the viscera, $Na_V1.3$ mRNA is detected in adult guinea-pig enteric nervous system (ENS) neurons (Sage *et al.* 2007), but its functional role has yet to be determined. Initial experiments indicate that $Na_V1.3$ expression is low in rat lumbar (L5) DRG neurons (Fukuoka *et al.* 2008). However, $Na_V1.3$ mRNA transcripts are detected in approximately half of the colon-innervating thoracolumbar (T10–L1) and lumbosacral (L5–S1) DRG neurons in the mouse (Hockley *et al.* 2017) (Table 1).

More recent studies show a key role for $Na_V1.3$ in non-neuronal tissues, specifically within enterochromaffin cells located within the epithelium from the small and large intestine of humans and mice (Bellono *et al.* 2017; Strege *et al.* 2017*a*,*b*). Voltage-gated sodium currents generated by $Na_V1.3$ likely allow enterochromaffin cells to respond to the detection of mechanical and chemical stimuli within the lumen of the intestine (Bellono *et al.* 2017; Strege *et al.* 2017*b*). In contrast, expression of the other eight Na_V isoforms is very low, or indeed lacking from both intestinal enterochromaffin cells and the wider population of intestinal epithelial cells (Bellono *et al.* 2017). There are currently no reports on the role of $Na_V1.3$ in the bladder or bladder-innervating sensory neurons.

$Na_V1.4$

$Na_V1.4$ is the predominant Na_V isoform in skeletal muscle (Trimmer *et al.* 1990) but is also found in human oesophageal smooth muscle tissue (Deshpande *et al.* 2002). In peripheral neurons, $Na_V1.4$ transcripts are nearly absent in rat lumbar (L5) DRG (Fukuoka *et al.* 2008) and in colon-innervating mouse DRG neurons (Hockley *et al.* 2017) (Table 1). In agreement with tissue distribution, $Na_V1.4$ channelopathies appear to exclusively involve deficits in skeletal muscle function, and to date no involvement in colon or bladder function has been shown.

$Na_V1.5$

$Na_V1.5$ channels have been identified in circular smooth muscle of the jejunum of human, dog, rat and mouse but are absent in pig and guinea pig. $Na_V1.5$ is also absent from human and mouse jejunal longitudinal smooth muscle (Holm *et al.* 2002; Ou *et al.* 2002; Strege *et al.* 2007; Beyder *et al.* 2016). $Na_V1.5$ has been found in colonic circular smooth muscle of human and rat (Strege *et al.* 2003), in jejunal interstitial cells of Cajal in human (Strege *et al.* 2003), and in myenteric plexuses of human and mouse (Hetz *et al.* 2014; Osorio *et al.* 2014).

$Na_V1.5$ in circular smooth muscle may contribute to normal intestinal motility through modulation of slow-wave activity and muscle contractility (Ou *et al.* 2002; Strege *et al.* 2007). These findings are supported by data showing that ranolazine, a treatment for chronic angina, is able to inhibit $Na_V1.5$ currents in human colonic smooth muscle cells (Neshatian *et al.* 2015), which is likely to be responsible for the constipation seen during long-term ranolazine treatment (Nash & Nash, 2008). These data strongly point towards a primary role for $Na_V1.5$ channels in mediating gastrointestinal motility and transit (Beyder & Farrugia, 2016). Similarly, several loss-of-function mutations in *SCN5A*, the gene encoding $Na_V1.5$ channels, are associated with IBS and abdominal pain (Saito *et al.* 2009; Beyder *et al.* 2014; Strege *et al.* 2017*c*). Whether this is purely a consequence of reduced gastrointestinal contractility or whether $Na_V1.5$ channels also play a direct role in visceral sensation remains unclear, as $Na_V1.5$ mRNA transcripts are expressed in 18% of thoracolumbar and 51% of lumbosacral colon-innervating DRG neurons (Hockley *et al.* 2017) (Table 1). Whether this translates into channel

expression and a functional role remains to be determined. There are currently no reports on the expression profile or function of $Na_V1.5$ in the bladder or bladder-innervating sensory neurons.

$Na_V1.6$

$Na_V1.6$ is extensively expressed within the CNS and PNS (Whitaker *et al.* 1999; Tzoumaka *et al.* 2000; Catterall *et al.* 2005; Catterall, 2012; Chang *et al.* 2018), commonly located in clusters at the nodes of Ranvier (Duflocq *et al.* 2008), indicating that $Na_V1.6$ may have a primary role in transmitting rather than initiating action potentials. In rat lumbar (L5) DRG neurons, $Na_V1.6$ transcripts are detected in a third of all neurons and selectively expressed in TrkC- and TrkA-expressing myelinated A-fibre nociceptors (Fukuoka *et al.* 2008). In colon-innervating mouse DRG neurons, $Na_V1.6$ mRNA transcript is present in 63–87% of thoracolumbar (T10–L1) neurons, and in 51% of lumbosacral (L5–S1) neurons (Hockley *et al.* 2017; Inserra *et al.* 2017). Immunohistochemical and western blot analysis show that $Na_V1.6$ protein is present in the cell bodies of sensory neurons and on sensory afferent nerve endings innervating the distal colon and rectum in mice (Feng *et al.* 2015) (Table 1). Antagonism of $Na_V1.6$ reduces action potential firing of stretch-sensitive colorectal afferents *in vitro* (Feng *et al.* 2015) (Table 2). Whether these effects are altered in animal models of inflammatory or chronic visceral pain remains to be investigated. It has, however, been reported that there is no change in $Na_V1.6$ expression in colon-innervating DRG neurons (T9–T13) during the acute inflammatory phase of the mouse model of trinitrobenzenesulphonic acid (TNBS)-induced colitis (King *et al.* 2009). This corresponds with the phase when colorectal afferent hypersensitivity also occurs (Hughes *et al.* 2009). Activation of low-threshold stretch-sensitive afferents is essential for normal physiological function of the colon (Brierley *et al.* 2004; Kyloh *et al.* 2011) and $Na_V1.6$ appears to play a key integrative role in this process. Whether $Na_V1.6$ contributes to aberrant colonic afferent sensory signalling during chronic visceral hypersensitivity remains to be determined. There are currently no reports on the expression profile or function of $Na_V1.6$ in the bladder or bladder-innervating sensory neurons.

$Na_V1.7$

$Na_V1.7$ has become a key target of interest as several human mutations in the *SCN9A* gene, which encodes $Na_V1.7$, lead to either a loss of pain or increased pain perception (Bennett & Woods, 2014). For example, a loss-of-function mutation of *SCN9A* results in a congenital insensitivity to pain (CIP) (Cox *et al.* 2006; Goldberg *et al.* 2007), whereas gain-of-function mutations produce distinct pain syndromes, such as erythromelalgia, small-fibre neuropathy and paroxysmal extreme pain disorder (Fertleman *et al.* 2006). $Na_V1.7$ is extensively expressed in sensory and sympathetic neurons of the PNS, as well as ENS neurons, and is highly restricted in the CNS (Klugbauer *et al.* 1995; Catterall *et al.* 2005; Morinville *et al.* 2007; Sage *et al.* 2007; Branco *et al.* 2016; Chang *et al.* 2018). In rat lumbar (L5) DRG neurons, $Na_V1.7$ transcripts are preferentially expressed in TrkA-expressing C-fibre neurons, and in a subset of A-fibre neurons (Fukuoka *et al.* 2008). Robust immunolabelling of $Na_V1.7$ is present within the peripheral endings of sensory nerves in the skin (Black *et al.* 2012).

From mouse knock-out studies, it appears that $Na_V1.7$ in $Na_V1.8$-expressing cells ($Na_V1.7^{Nav1.8}$) does not contribute in the development of neuropathic pain, nor noxious cold or heat detection (Nassar *et al.* 2004, 2005; Minett *et al.* 2012, 2014; Hockley *et al.* 2017). However, $Na_V1.7^{Nav1.8}$ mice have significantly reduced behavioural responses to inflammatory mediators (formalin, complete Freund's adjuvant, carrageenan and nerve growth factor) when injected into the sole of the hind paw (Nassar *et al.* 2004) and impaired somatic noxious mechanosensation (Minett *et al.* 2012, 2014). Thus far, only the deletion of $Na_V1.7$ in sympathetic and sensory (Wnt1-expressing) neurons and the global $Na_V1.7$ knock-out have been able to significantly reduce pain responses to a range of stimuli and recapitulate the human *SCN9A*-associated CIP phenotype (Gingras *et al.* 2014; Minett *et al.* 2014). Recent studies also show that endogenous opioids contribute to pain insensitivity in both humans and mice lacking $Na_V1.7$, as the opioid antagonist naloxone reverses analgesia associated with the loss of $Na_V1.7$ expression (Minett *et al.* 2015). This suggests that $Na_V1.7$ channel blockers alone may not replicate the analgesic phenotypes of $Na_V1.7$ null mutants, but may be potentiated with exogenous opioids. $Na_V1.7$-selective inhibitors are currently in clinical trial for different types of pain (Pennington *et al.* 2017; Yekkirala *et al.* 2017).

In relation to visceral sensation, $Na_V1.7$ is highly abundant in human lumbar DRG, and is expressed in 100% of mouse colon-innervating thoracolumbar (T10–L1) DRG neurons, and in most colon-innervating lumbosacral (L5–S1) DRG neurons (Chang *et al.* 2018; Hockley *et al.* 2017; Inserra *et al.* 2017) (Table 1). Accordingly, $Na_V1.7$ constitutes the most prevalent TTX-S isoform within colon-innervating DRG neurons. It is of interest to note that 'paroxysmal extreme pain disorder', caused by the human gain of function *SCN9A* mutation, was originally called 'familial rectal pain syndrome'. As the name implies, this disorder is characterised by excruciating rectal and abdominal pain commonly associated with defecation (Fertleman *et al.* 2006), suggesting a key role for $Na_V1.7$ in visceral pain. Moreover, pain perception in a subset of patients with interstitial cystitis/bladder pain syndrome (IC/BPS) is shown to correlate with a

polymorphism in *SCN9A* (Reeder *et al.* 2013). IC/BPS patients treated with a bladder infiltration of neosaxitoxin, a blocker of TTX-S Na_V channels, resulted in significant analgesia and reduced bladder overactivity for 90 days after the treatment (Manriquez *et al.* 2015). Normal physiological function of the bladder, however, appears to be independent of $Na_V1.7$, as *SCN9A*-associated CIP individuals have normal bladder control, and no increased incidence of urinary infections, incontinence, or retention (Cox *et al.* 2006).

Despite these studies, the initial promise of $Na_V1.7$'s contribution to visceral pain is somewhat tempered by experimental studies showing that $Na_V1.7^{Nav1.8}$ mice exhibit normal nocifensive responses to intracolonic administration of capsaicin (TRPV1 agonist) and mustard oil (TRPA1 agonist), indicating that $Na_V1.7$ is not crucial for acute visceral pain signalling (Hockley *et al.* 2017). Low-threshold stretch-sensitive pelvic afferents are unaffected by the $Na_V1.7$ antagonist ProTX-II (Feng *et al.* 2015) (Table 2). Similarly, *ex vivo* extracellular recordings of mesenteric afferents from resected human appendices show that peak firing before and after exposure to a novel $Na_V1.7$-selective antagonist, PF-5198007, is unchanged during repeat noxious ramp distensions (Hockley *et al.* 2017). Afferent responses in mouse *ex vivo* colorectal recordings are attenuated by application of TTX (Feng *et al.* 2015), indicating that TTX-S channels other than $Na_V1.7$ may be important in responding to innocuous and noxious mechanical stimuli. Accordingly, intracolonic co-administration of TTX and P-CTX-1 did not significantly alter the pain response induced by P-CTX-1 (Inserra *et al.* 2017).

Ex vivo extracellular recordings of splanchnic nerve activity from the distal colon of $Na_V1.7^{Nav1.8}$ mice show no difference in peak firing between $Na_V1.7^{Nav1.8}$ and littermate control afferents in the physiological and supra-physiological pressure range (0–80 mmHg) (Hockley *et al.* 2017). However, significantly less action potential firing in afferents from $Na_V1.7^{Nav1.8}$ mice at distension pressures in the supramaximal range (80–145 mmHg) is observed, suggesting that $Na_V1.7$ in $Na_V1.8$-positive colonic afferent neurons may be involved in transducing non-physiological extremes of pressure. This may be important and more relevant to chronic visceral pain states, when splanchnic afferents show mechanical hypersensitivity and decreased activation thresholds to mechanical stimuli (Hughes *et al.* 2009; Castro *et al.* 2013, 2017; de Araujo *et al.* 2014; Osteen *et al.* 2016). In the bladder, $Na_V1.7^{Nav1.8}$ mice have comparable levels of referred hyperalgesia in an acute cyclophosphamide-induced cystitis model compared to littermates (Hockley *et al.* 2017). Overall, these findings suggest that $Na_V1.7$ has a role in mediating acute inflammatory pain in somatic but not visceral pathways. While studies on visceral nociception using $Na_V1.7^{Nav1.8}$ mice have provided valuable insight, replication of these studies in mice with sensory neuron-specific deletion of $Na_V1.7$ (e.g. $Na_V1.7^{Advill}$) will be beneficial to strengthen conclusions concerning $Na_V1.7$ in visceral pain signalling.

Diseases that have a significant visceral pain component are commonly chronic and have unmet needs in terms of clinical treatment. Therefore, further investigations into the role of $Na_V1.7$ in long term and chronic visceral pain models, which are more clinically relevant to pathological chronic visceral pain states, are critical. For example, significant up-regulation of $Na_V1.7$ mRNA occurs 4 weeks after induction of colitis in colon-innervating DRG (L6–S1) neurons (Campaniello *et al.* 2016). Similarly, rats with streptozotocin-induced diabetes show hypersensitivity to colonic distension, which corresponds with the up-regulation of $Na_V1.7$ protein in thoracolumbar (T13–L2) DRG neurons 4 weeks post-induction (Hu *et al.* 2016). In support of these findings, rat neonatal colitis-induced visceral hypersensitivity induces up-regulation of $Na_V1.7$ protein levels in DRG from higher spinal levels (T13–L2), but not lower spinal levels (L4–L5) at 6 weeks post-colitis compared to control animals (Qu *et al.* 2013). Taken together, these findings suggest that $Na_V1.7$ may have an acquired role during chronic visceral pain states. It is well documented that inflammation, tissue damage and healing of visceral organs can induce structural, synaptic or intrinsic neuroplasticity, altering neuronal and gastrointestinal function in the long term (Brierley & Linden, 2014). For example, rectal samples from patients with physiologically characterised rectal hypersensitivity show significantly increased numbers of $Na_V1.7$-immunoreactive nerve fibres in the mucosal, submucosal and muscle layers compared to control tissues (Yiangou *et al.* 2007). In addition to these findings, changes in the ratios of $Na_V1.7$ to a pan-neuronal structural marker, PGP9.5, indicate that increased $Na_V1.7$ expression and nerve sprouting occurs in rectal mucosa, which may contribute to enhanced sensitivity in these patients (Yiangou *et al.* 2007).

Overall, the role of $Na_V1.7$ in pain sensation is complicated, and species differences in expression, assumed translatability of isoform-compound interaction, and effects of Na_V knock-out on other genes may confound overall conclusions. Furthermore, few studies using human tissue have been completed, and healthy tissue is often obtained from patients with colonic or rectal carcinoma (Yiangou *et al.* 2007; Hetz *et al.* 2014; Hockley *et al.* 2017). In addition to species-dependent differences in tissue distributions (Table 1), there are also differences in relative isoform distributions, for example, $Na_V1.7$ is the most abundant isoform in human lumbar DRG, whereas $Na_V1.8$ is more abundant in mouse (Chang *et al.* 2018). $Na_V1.7$ isoforms from different species can also have different compound selectivity in heterologous expression systems that should be carefully considered during experimental design. For example,

human, monkey, dog and mouse $Na_V1.7$ isoforms were found to be largely insensitive to a small molecule inhibitor of $Na_V1.1$/$Na_V1.3$ (ICA-121431) and potently inhibited by a small molecule inhibitor of $Na_V1.7$ (PF-04856264), whereas rat $Na_V1.7$ was potently inhibited by ICA-121431, but largely insensitive to PF-04856264 (McCormack *et al.* 2013). A ProTxII analogue, JNJ63955918, on the other hand was equipotent at human and rat $Na_V1.7$ (Flinspach *et al.* 2017). Single cell studies have shown that Na_V channel expression is heterologous across cells, and there is high co-localisation of $Na_V1.7$ with $Na_V1.6$, $Na_V1.8$ and $Na_V1.9$ in colon-innervating thoracolumbar and lumbosacral neurons in mice (Hockley *et al.* 2017). However, functional relationships of co-expression and investigations of redundancy between Na_V channels are unclear. In knock-out models, deletion of one Na_V gene can lead to a change in expression levels of over 190 genes (Minett *et al.* 2015). Studies investigating Na_V channel contribution to pain signalling using knock-out models or pharmacological modification may benefit from collecting data on regulation of other Na_V family genes and auxiliary β-subunits in parallel, and other key genes where possible. Furthermore, inducible knock-out models offer the advantage of normal development and being able to compare Na_V channel contribution pre- and post-induction of visceral hypersensitivity in the adult, thereby increasing therapeutic potential of these findings.

$Na_V1.8$

$Na_V1.8$ mediates slowly inactivating TTX-R Na^+ currents and carries the majority of the current underlying the upstroke of the action potential in nociceptive neurons. Hence, they are considered to play an important role in action potential electrogenesis (Renganathan *et al.* 2001). $Na_V1.8$-null mice display reduced sensitivity to noxious mechanical stimuli (tail pressure) and noxious thermal stimuli (radiant heat), but normal sensitivity to acute noxious colonic distension by isotonic saline and intraperitoneal acetylcholine (Akopian *et al.* 1999; Laird *et al.* 2002). $Na_V1.8$ is the most abundant isoform expressed in mouse lumbar DRG (Chang *et al.* 2018), and is prevalently expressed in thoracolumbar (96%) and lumbosacral (91%) colonic sensory DRG neurons, with almost complete co-expression with $Na_V1.7$ (Hockley *et al.* 2017). Consistent with this, knock-down of $Na_V1.8$ in DRG neurons results in action potentials with reduced peak amplitude and slower rise times, but similar baseline excitability (Renganathan *et al.* 2001; Hillsley *et al.* 2006). Similarly, A-803467, a selective $Na_V1.8$ antagonist, does not significantly affect the frequency of action potential firing from low-threshold mechanosensitive colonic afferent nerve endings (Feng *et al.* 2015). Together, these data seem to suggest that $Na_V1.8$ channels do play a major role in mediating visceral sensations and pain under physiological conditions.

$Na_V1.8$ channels also have a major role in visceral signalling under pathophysiological conditions. Several studies support increased expression of $Na_V1.8$ protein in colon-innervating sensory DRG neurons in murine models of visceral hypersensitivity (Beyak *et al.* 2004; Hillsley *et al.* 2006; King *et al.* 2009; Qu *et al.* 2013; Hu *et al.* 2013*a*,*b*; Inserra *et al.* 2017; Lin *et al.* 2017) (Table 2). In most studies, increased channel expression correlates with enhanced TTX-R Na^+ current density in colon-innervating DRG neurons *in vitro*, and with visceral hypersensitivity *in vivo*, as the visceromotor response to noxious colonic distension in rats is significantly reduced following intraperitoneal administration of the $Na_V1.8$-specific antagonist A-803467 (Jarvis *et al.* 2007). Similarly, colonic co-administration of A-803467 with P-CTX-1 significantly reduces P-CTX-1-induced nocifensive behaviours in mice (Inserra *et al.* 2017). These findings are consistent with studies in $Na_V1.8$-null mice, which do not develop visceral hypersensitivity after intracolonic administration of sensitising agents such as capsaicin (TRPV1 agonist) and mustard oil (TRPA1 agonist). Furthermore, unlike their wild-type littermates, DRG neurons from $Na_V1.8$-null mice do not display enhanced neuronal hyperexcitability following intestinal infection with *Nippostrongylus brasiliensis* (Laird *et al.* 2002; Hillsley *et al.* 2006).

Inflammatory mediators acting via GPCRs are powerful modulators of $Na_V1.8$ currents, and are believed to underlie increased excitability of nociceptive DRG neurons and associated hyperalgesia (Beyak *et al.* 2004). In this regard, colon-innervating DRG neurons incubated with supernatant from colonic biopsies from patients with active ulcerative colitis (a chronic inflammatory bowel disease) show increased action potential discharge and enhanced $Na_V1.8$ currents (Ibeakanma & Vanner, 2010). These effects were replicated by incubation with tumour necrosis factor α (TNFα), whose levels are enhanced in the ulcerative colitis supernatant. Similar sensitising effects have been reported for prostaglandin E2 (PGE2), adenosine, serotonin (5-HT), ATP, as well as nerve growth factor (NGF), which may persist during and possibly after the inflammation as a result of increased expression of $Na_V1.8$ channels (Gold, 1999; Gold *et al.* 2002; Beyak *et al.* 2004). Recent data, however, indicate that these effects are not limited to inflammatory conditions, but may extend to non-inflammatory chronic pain states. Partial colonic obstruction is associated with an increase in $Na_V1.8$ mRNA expression, as well as enhanced TTX-R Na^+ currents and referred *in vivo* hyperalgesia, effects that were abolished by anti-NGF treatment (King *et al.* 2009; Ibeakanma & Vanner, 2010).

Decreased TTX-R currents occur in bladder-innervating DRG neurons from T8 spinal transected

rats (Yoshimura & deGroat, 1997), which has since been attributed to a down-regulation of $Na_V1.8$ (Black *et al.* 2003). This change is only seen in bladder-innervating DRG neurons, and is accompanied by an up-regulation of TTX-S current, which may also enhance the excitability of these afferent neurons. Knock-down of $Na_V1.8$ in rats at spinal levels L6–S1, known to contain the majority of bladder sensory terminals, does not have an effect on intercontraction intervals following cystometry with saline; however, intravesical acetic acid-induced hyper-reflexia is attenuated in knock-down rats (Yoshimura *et al.* 2001). $Na_V1.8$-null mice develop normal pain and inflammatory responses during cyclophosphamide-induced cystitis compared to littermates (Laird *et al.* 2002), and pain behaviours are sustained in rats with cyclophosphamide-induced cystitis following intraperitoneal administration of A-803467 (Jarvis *et al.* 2007).

Cross-organ sensitisation of the gastrointestinal and lower urinary tract is evident clinically and in animal models (Malykhina *et al.* 2004, 2012; Lei & Malykhina, 2012), highlighting the importance of understanding the mechanisms of viscero-visceral crosstalk. Several studies report increases in TTX-resistant Na^+ current in bladder-innervating DRG neurons following colitis, implicating some involvement of TTX-R channels in bladder pain as a consequence of gastrointestinal tract inflammation. C-fibre bladder-innervating DRG neurons, involved in the transduction of noxious stimuli signalling (Fowler *et al.* 2008), in the majority express TTX-R

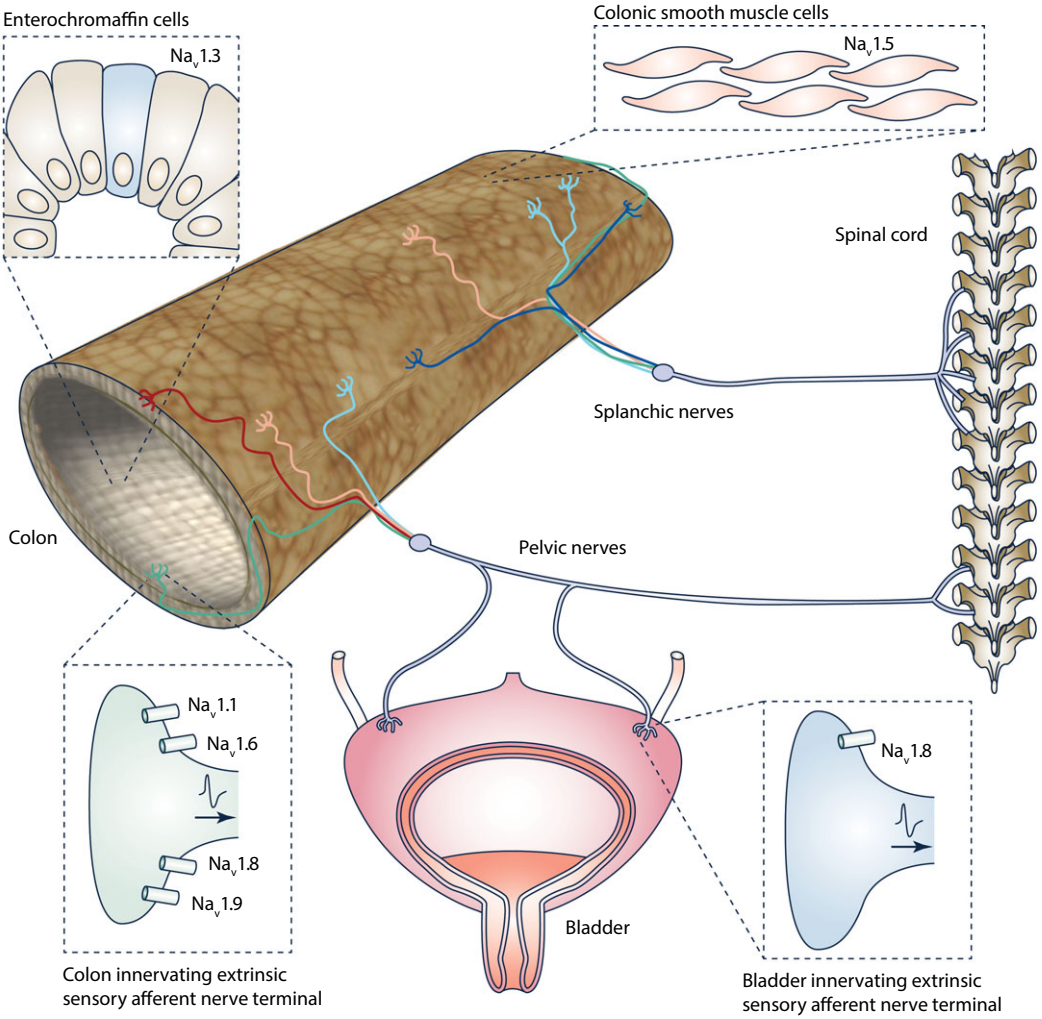

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Figure 1. Current understanding of how specific voltage-gated sodium channels (Na_V) contribute to the functioning of neurons and non-neuronal cells within visceral organs

currents (Yoshimura & deGroat, 1997). Collectively, experimental findings to date indicate that $Na_V1.8$ is not crucial for visceral pain signalling from the bladder in response to several noxious stimuli, but it may have an important role during referred hyperalgesia and in response to certain irritants.

$Na_V1.9$

Several human $Na_V1.9$ channelopathies are associated with congenital episodic pain syndromes, painful neuropathy, and an insensitivity to pain (Huang *et al.* 2014, 2017). $Na_V1.9$ channels are preferentially expressed in small-diameter nociceptors (Dib-Hajj *et al.* 1998; Tate *et al.* 1998), and mediate ultraslow or persistent TTX-R Na^+ currents. Due to their kinetic properties, $Na_V1.9$ channels are unlikely to contribute to action potential generation, but instead regulate neuronal excitability by setting the resting membrane potential closer to threshold (Dib-Hajj *et al.* 1998, 2002; Tate *et al.* 1998). In colonic afferents, action potential firing in response to colonic ramp distension is reduced in $Na_V1.9^{-/-}$ mice, and accompanied by a run-down of responses to repeated phasic distension (Hockley *et al.* 2014). Similar to $Na_V1.8$ channels, several studies indicate that $Na_V1.9$ currents can be enhanced via GPCRs (Maingret *et al.* 2008; Ostman *et al.* 2008; Vanoye *et al.* 2013; Hockley *et al.* 2016*b*). Colonic afferent excitatory responses to the application of multiple inflammatory mediators (applied at once, either in the form of supernatants from chronically inflamed human bowel or as an experimental inflammatory soup containing ATP, PGE2, bradykinin, histamine and 5-HT) are significantly reduced in visceral afferents from $Na_V1.9^{-/-}$ mice (Hockley *et al.* 2014, 2016*a*) (Table 2).

$Na_V1.9^{-/-}$ mice have similar baseline visceromotor responses to colonic distension to wild-type littermates, but reduced visceral hypersensitivity *in vivo* after colonic inflammation induced by activation of toll-like receptor 7 (Martinez & Melgar, 2008). Neuronal hyperexcitability following *Nippostrongylus bransiliensis* infection is unchanged in $Na_V1.9^{-/-}$ mice compared to wild-type littermates, reporting similar action potential characteristics and excitability of colon-innervating DRG neurons (Hillsley *et al.* 2006). Likewise, others do not see changes in $Na_V1.9$ protein expression in colon-innervating DRG neurons, nor differences in either the numbers of neurons expressing persistent TTX-R ($Na_V1.9$) currents or the magnitude of these currents in acute TNBS-induced colitis (Beyak *et al.* 2004; King *et al.* 2009). It is unclear whether these discrepancies in the contribution of $Na_V1.9$ to neuronal (hyper)excitability relates to differences in knock-out constructs and mice strains, or to differences in the inflammatory insult studied. The latter may be of considerable importance as inflammatory mediators such as bradykinin, ATP, histamine, PGE2 and noradrenaline (norepinephrine), potentiate $Na_V1.9$ channel activity when applied conjointly, but fail to modulate $Na_V1.9$ currents when applied separately (Maingret *et al.* 2008).

$Na_V1.9$ channels are also present in myenteric plexus neurons in human, mouse, rat and guinea-pig (Rugiero *et al.* 2003; Padilla *et al.* 2007; Copel *et al.* 2009; Osorio *et al.* 2014) pointing towards an additional role in intestinal motor function. In line with this, colonic migrating motor complex patterns are altered in $Na_V1.9^{-/-}$ mice (Copel *et al.* 2013). Moreover, expression of $Na_V1.9$ channels is decreased in submucosal and myenteric plexus neurons (most likely intrinsic primary afferent neurons) in Hirschsprung's disease (O'Donnell *et al.* 2016). Interestingly, these findings apply not only to aganglionic bowel sections, but in some patients extend to those sections containing normal ganglia numbers, which could explain some of the post-surgery bowel dysmotility issues frequently encountered by these patients (O'Donnell *et al.* 2016). Conversely, a gain-of-function mutation (L811P) in the $Na_V1.9$ gene, *SCN11A*, identified in three unrelated individuals with congenital insensitivity to pain, is associated with severe gastrointestinal dysmotility, including alternating episodes of diarrhoea and constipation (Leipold *et al.* 2013; Woods *et al.* 2015). In contrast, other gain-of-function mutations are predominantly linked to chronic pain syndromes such as autosomal-dominant episodic pain and small fibre neuropathy (Zhang *et al.* 2013; Huang *et al.* 2014; Han *et al.* 2015).

No difference is observed in basal urodynamics between wild-type and $Na_V1.9^{-/-}$ mice; however, the change of urodynamic parameters associated with cyclophosphamide-induced cystitis is absent in $Na_V1.9^{-/-}$ mice, as well as attenuation of PGE2-induced afferent excitability during bladder distension (Ritter *et al.* 2009). It remains to be investigated whether this involvement of $Na_V1.9$ in bladder nociception is due to functional up-regulation of $Na_V1.9$ in bladder afferents, or whether $Na_V1.9$ has a role in central processing of bladder nociceptive pathways.

Conclusion

Recent findings highlight the diversity in expression patterns of Na_V isoforms in abdominal visceral organs. This diversity extends across neurons (enteric, extrinsic sensory DRG innervating the intestine or bladder) and non-neuronal cells (intestinal enterochromaffin cells, intestinal smooth muscle cells, and interstitial cells of Cajal). Na_V channels have a range of functions in health and disease and we are only now, with the development of novel pharmacological and genetic tools, beginning to unpick their complex physiological and pathophysiological interactions. $Na_V1.1$, $Na_V1.6$, $Na_V1.8$ and $Na_V1.9$, contribute to visceral hypersensitivity,

particularly within colonic pathways, and respond to inflammatory mediators in pathophysiological models (Fig. 1).

Whilst $Na_V1.3$ contributes to enterochromaffin cell function and $Na_V1.5$ contributes to intestinal smooth muscle cells and interstitial cells of Cajal function, there is currently no determined function in visceral afferents for $Na_V1.2$, $Na_V1.3$, $Na_V1.4$ or $Na_V1.5$, despite significant mRNA expression of $Na_V1.2$ and $Na_V1.5$ in visceral afferent pathways. $Na_V1.7$ is one of the most extensively expressed and studied Na_V channels, but a role in visceral pain, like that attributed to $Na_V1.7$ in somatic pain studies is currently unclear. Although many of these Na_V channels have been investigated under physiological conditions or in models of acute pain, chronic visceral pain models are necessary for the determination of a precise role in long term pathological visceral pain. Future studies would benefit from the further development of novel, specific agonists and antagonists, as we have seen with recent advances in the role of $Na_V1.1$ in mechanical pain. Likewise, selective Na_V modulators with low systemic uptake for *in vivo* studies will advance our understanding of Na_V channels in visceral pain signalling and the suitability of targeting Na_V channels in the treatment of pain originating in the distal gut and bladder.

References

Akopian AN, Souslova V, England S, Okuse K, Ogata N, Ure J, Smith A, Kerr BJ, McMahon SB, Boyce S, Hill R, Stanfa LC, Dickenson AH & Wood JN (1999). The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways. *Nat Neurosci* **2**, 541–548.

Alcaino C, Farrugia G & Beyder A (2017). Mechanosensitive piezo channels in the gastrointestinal tract. *Curr Top Membr* **79**, 219–244.

Beckh S, Noda M, Lübbert H & Numa S (1989). Differential regulation of three sodium channel messenger RNAs in the rat central nervous system during development. *EMBO J* **8**, 3611–3616.

Bellono NW, Bayrer JR, Leitch DB, Castro J, Zhang C, O'Donnell TA, Brierley SM, Ingraham HA & Julius D (2017). Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. *Cell* **170**, 185–198.e16.

Bennett DL & Woods CG (2014). Painful and painless channelopathies. *Lancet Neurol* **13**, 587–599.

Beyak MJ, Ramji N, Krol KM, Kawaja MD & Vanner SJ (2004). Two TTX-resistant Na^+ currents in mouse colonic dorsal root ganglia neurons and their role in colitis-induced hyperexcitability. *Am J Physiol Gastrointest Liver Physiol* **287**, G845–G855.

Beyder A & Farrugia G (2016). Ion channelopathies in functional GI disorders. *Am J Physiol Gastrointest Liver Physiol* **311**, G581–G586.

Beyder A, Gibbons SJ, Mazzone A, Strege PR, Saravanaperumal SA, Sha L, Higgins S, Eisenman ST, Bernard CE, Geurts A, Kline CF, Mohler PJ & Farrugia G (2016). Expression and function of the *Scn5a*-encoded voltage-gated sodium channel $Na_v1.5$ in the rat jejunum. *Neurogastroenterol Motil* **28**, 64–73.

Beyder A, Mazzone A, Strege PR, Tester DJ, Saito YA, Bernard CE, Enders FT, Ek WE, Schmidt PT, Dlugosz A, Lindberg G, Karling P, Ohlsson B, Gazouli M, Nardone G, Cuomo R, Usai-Satta P, Galeazzi F, Neri M, Portincasa P, Bellini M, Barbara G, Camilleri M, Locke GR III, Talley NJ, D'Amato M, Ackerman MJ & Farrugia G (2014). Loss-of-function of the voltage-gated sodium channel $Na_v1.5$ (channelopathies) in patients with irritable bowel syndrome. *Gastroenterology* **146**, 1659–1668.

Bielefeldt K, Ozaki N & Gebhart GF (2002*a*). Experimental ulcers alter voltage-sensitive sodium currents in rat gastric sensory neurons. *Gastroenterology* **122**, 394–405.

Bielefeldt K, Ozaki N & Gebhart GF (2002*b*). Mild gastritis alters voltage-sensitive sodium currents in gastric sensory neurons in rats. *Gastroenterology* **122**, 752–761.

Black JA, Cummins TR, Yoshimura N, de Groat WC & Waxman SG (2003). Tetrodotoxin-resistant sodium channels $Na_v1.8$/SNS and $Na_v1.9$/NaN in afferent neurons innervating urinary bladder in control and spinal cord injured rats. *Brain Res* **963**, 132–138.

Black JA, Dib-Hajj S, McNabola K, Jeste S, Rizzo MA, Kocsis JD & Waxman SG (1996). Spinal sensory neurons express multiple sodium channel alpha-subunit mRNAs. *Brain Res Mol Brain Res* **43**, 117–131.

Black JA, Frezel N, Dib-Hajj SD & Waxman SG (2012). Expression of Nav1.7 in DRG neurons extends from peripheral terminals in the skin to central preterminal branches and terminals in the dorsal horn. *Mol Pain* **8**, 82.

Black JA & Waxman SG (2013). Noncanonical roles of voltage-gated sodium channels. *Neuron* **80**, 280–291.

Bradding P, Okayama Y, Kambe N & Saito H (2003). Ion channel gene expression in human lung, skin, and cord blood-derived mast cells. *J Leukoc Biol* **73**, 614–620.

Branco T, Tozer A, Magnus CJ, Sugino K, Tanaka S, Lee AK, Wood JN & Sternson SM (2016). Near-perfect synaptic integration by $Na_v1.7$ in hypothalamic neurons regulates body weight. *Cell* **165**, 1749–1761.

Brierley SM (2010). Molecular basis of mechanosensitivity. *Auton Neurosci* **153**, 58–68.

Brierley SM (2016). Altered ion channel/receptor expression and function in extrinsic sensory neurons: the cause of and solution to chronic visceral pain? *Adv Exp Med Biol* **891**, 75–90.

Brierley SM, Jones RCW, Gebhart GF & Blackshaw LA (2004). Splanchnic and pelvic mechanosensory afferents signal different qualities of colonic stimuli in mice. *Gastroenterology* **127**, 166–178.

Brierley SM & Linden DR (2014). Neuroplasticity and dysfunction after gastrointestinal inflammation. *Nat Rev Gastroenterol Hepatol* **11**, 611–627.

Campaniello MA, Harrington AM, Martin CM, Ashley Blackshaw L, Brierley SM & Hughes PA (2016). Activation of colo-rectal high-threshold afferent nerves by interleukin-2 is tetrodotoxin-sensitive and upregulated in a mouse model of chronic visceral hypersensitivity. *Neurogastroenterol Motil* **28**, 54–63.

Carithers LJ, Ardlie K, Barcus M, Branton PA, Britton A, Buia SA, Compton CC, DeLuca DS, Peter-Demchok J, Gelfand ET, Guan P, Korzeniewski GE, Lockhart NC, Rabiner CA, Rao AK, Robinson KL, Roche NV, Sawyer SJ, Segre AV, Shive CE, Smith AM, Sobin LH, Undale AH, Valentino KM, Vaught J, Young TR & Moore HM; GTEx Consortium (2015). A novel approach to high-quality postmortem tissue procurement: The GTEx Project. *Biopreserv Biobank* **13**, 311–319.

Carrithers LM, Hulseberg P, Sandor M & Carrithers MD (2011). The human macrophage sodium channel NaV1.5 regulates mycobacteria processing through organelle polarization and localized calcium oscillations. *FEMS Immunol Med Microbiol* **63**, 319–327.

Carrithers MD, Dib-Hajj S, Carrithers LM, Tokmoulina G, Pypaert M, Jonas EA & Waxman SG (2007). Expression of the voltage-gated sodium channel NaV1.5 in the macrophage late endosome regulates endosomal acidification. *J Immunol* **178**, 7822–7832.

Castro J, Harrington AM, Garcia-Caraballo S, Maddern J, Grundy L, Zhang J, Page G, Miller PE, Craik DJ, Adams DJ & Brierley SM (2017). α-Conotoxin Vc1.1 inhibits human dorsal root ganglion neuroexcitability and mouse colonic nociception via $GABA_B$ receptors. *Gut* **66**, 1083–1094.

Castro J, Harrington AM, Hughes PA, Martin CM, Ge P, Shea CM, Jin H, Jacobson S, Hannig G, Mann E, Cohen MB, MacDougall JE, Lavins BJ, Kurtz CB, Silos-Santiago I, Johnston JM, Currie MG, Blackshaw LA & Brierley SM (2013). Linaclotide inhibits colonic nociceptors and relieves abdominal pain via guanylate cyclase-C and extracellular cyclic guanosine 3′,5′-monophosphate. *Gastroenterology* **145**, 1334–1346.

Catterall WA (2012). Voltage-gated sodium channels at 60: structure, function and pathophysiology. *J Physiol* **590**, 2577–2589.

Catterall WA, Goldin AL & Waxman SG (2005). International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. *Pharmacol Rev* **57**, 397–409.

Chang W, Berta T, Kim YH, Lee S, Lee S-Y & Ji R-R (2018). Expression and role of voltage-gated sodium channels in human dorsal root ganglion neurons with special focus on Nav1.7, species differences, and regulation by paclitaxel. *Neurosci Bull* **34**, 4–12.

Christianson JA & Davis BM (2010). The role of visceral afferents in disease. In *Translational Pain Research: From Mouse to Man*, chap. 3, ed. Kruger L & Light AR. CRC Press/Taylor & Francis, Boca Raton, FL, USA.

Copel C, Clerc N, Osorio N, Delmas P & Mazet B (2013). The $Na_v1.9$ channel regulates colonic motility in mice. *Front Neurosci* **7**, 1–8.

Copel C, Osorio N, Crest M, Gola M, Delmas P & Clerc N (2009). Activation of neurokinin 3 receptor increases $Na_v1.9$ current in enteric neurons. *J Physiol* **587**, 1461–1479.

Cox JJ, Reimann F, Nicholas AK, Thornton G, Roberts E, Springell K, Karbani G, Jafri H, Mannan J, Raashid Y, Al-Gazali L, Hamamy H, Valente EM, Gorman S, Williams R, McHale DP, Wood JN, Gribble FM & Woods CG (2006). An *SCN9A* channelopathy causes congenital inability to experience pain. *Nature* **444**, 894–898.

Dang K, Bielefeldt K & Gebhart GF (2004). Gastric ulcers reduce A-type potassium currents in rat gastric sensory ganglion neurons. *Am J Physiol Gastrointest Liver Physiol* **286**, G573–G579.

de Araujo AD, Mobli M, Castro J, Harrington AM, Vetter I, Dekan Z, Muttenthaler M, Wan J, Lewis RJ, King GF, Brierley SM & Alewood PF (2014). Selenoether oxytocin analogues have analgesic properties in a mouse model of chronic abdominal pain. *Nat Commun* **5**, 1–12.

Deshpande MA, Wang J, Preiksaitis HG, Laurier LG & Sims SM (2002). Characterization of a voltage-dependent Na^+ current in human esophageal smooth muscle. *Am J Physiol Cell Physiol* **283**, C1045–C1055.

Dib-Hajj S, Black JA, Cummins TR & Waxman SG (2002). NaN/$Na_v1.9$: a sodium channel with unique properties. *Trends Neurosci* **25**, 253–259.

Dib-Hajj SD, Black JA & Waxman SG (2015). $Na_V1.9$: a sodium channel linked to human pain. *Nat Rev Neurosci* **16**, 511–519.

Dib-Hajj SD, Tyrrell L, Black JA & Waxman SG (1998). NaN, a novel voltage-gated Na channel, is expressed preferentially in peripheral sensory neurons and down-regulated after axotomy. *Proc Natl Acad Sci USA* **95**, 8963–8968.

Du X & Gamper N (2013). Potassium channels in peripheral pain pathways: expression, function and therapeutic potential. *Curr Neuropharmacol* **11**, 621–640.

Duflocq A, Le Bras B, Bullier E, Couraud F & Davenne M (2008). Nav1.1 is predominantly expressed in nodes of Ranvier and axon initial segments. *Mol Cell Neurosci* **39**, 180–192.

Enck P, Aziz Q, Barbara G, Farmer AD, Fukudo S, Mayer EA, Niesler B, Quigley EM, Rajilic-Stojanovic M, Schemann M, Schwille-Kiuntke J, Simren M, Zipfel S & Spiller RC (2016). Irritable bowel syndrome. *Nat Rev Dis Primers* **2**, 16014.

Farrell KE, Callister RJ & Keely S (2014). Understanding and targeting centrally mediated visceral pain in inflammatory bowel disease. *Front Pharmacol* **5**, 1–4.

Feng B, Zhu Y, La JH, Wills ZP & Gebhart GF (2015). Experimental and computational evidence for an essential role of $Na_v1.6$ in spike initiation at stretch-sensitive colorectal afferent endings. *J Neurophysiol* **113**, 2618–2634.

Feng S, Pflueger M, Lin SX, Groveman BR, Su J & Yu XM (2012). Regulation of voltage-gated sodium current by endogenous Src family kinases in cochlear spiral ganglion neurons in culture. *Pflugers Arch* **463**, 571–584.

Fertleman CR, Baker MD, Parker KA, Moffatt S, Elmslie FV, Abrahamsen B, Ostman J, Klugbauer N, Wood JN, Gardiner RM & Rees M (2006). *SCN9A* mutations in paroxysmal extreme pain disorder: allelic variants underlie distinct channel defects and phenotypes. *Neuron* **52**, 767–774.

Flegel C, Schobel N, Altmuller J, Becker C, Tannapfel A, Hatt H & Gisselmann G (2015). RNA-Seq analysis of human trigeminal and dorsal root ganglia with a focus on chemoreceptors. *PLoS One* **10**, e0128951.

Flinspach M, Xu Q, Piekarz AD, Fellows R, Hagan R, Gibbs A, Liu Y, Neff RA, Freedman J, Eckert WA, Zhou M, Bonesteel R, Pennington MW, Eddinger KA, Yaksh TL, Hunter M, Swanson RV & Wickenden AD (2017). Insensitivity to pain induced by a potent selective closed-state Nav1.7 inhibitor. *Sci Rep* **7**, 39662.

Fowler CJ, Griffiths D & de Groat WC (2008). The neural control of micturition. *Nat Rev Neurosci* **9**, 453–466.

Fukuoka T, Kobayashi K, Yamanaka H, Obata K, Dai Y & Noguchi K (2008). Comparative study of the distribution of the α-subunits of voltage-gated sodium channels in normal and axotomized rat dorsal root ganglion neurons. *J Comp Neurol* **510**, 188–206.

Gaskin DJ & Richard P (2012). The economic costs of pain in the United States. *J Pain* **13**, 715–724.

Gebhart GF, Bielefeldt K & Ozaki N (2002). Gastric hyperalgesia and changes in voltage gated sodium channel function in the rat. *Gut* **51**, i15.

Geppetti P, Veldhuis NA, Lieu T & Bunnett NW (2015). G protein-coupled receptors: dynamic machines for signaling pain and itch. *Neuron* **88**, 635–649.

Gingras J, Smith S, Matson DJ, Johnson D, Nye K, Couture L, Feric E, Yin RY, Moyer BD, Peterson ML, Rottman JB, Beiler RJ, Malmberg AB & McDonough SI (2014). Global Nav1.7 knockout mice recapitulate the phenotype of human congenital indifference to pain. *PLoS One* **9**, e105895.

Gold MS (1999). Tetrodotoxin-resistant Na^+ currents and inflammatory hyperalgesia. *Proc Natl Acad Sci USA* **96**, 7645–7649.

Gold MS, Zhang L, Wrigley DL & Traub RJ (2002). Prostaglandin E_2 modulates TTX-R I_{Na} in rat colonic sensory neurons. *J Neurophysiol* **88**, 1512–1522.

Goldberg YP, MacFarlane J, MacDonald ML, Thompson J, Dube MP, Mattice M, Fraser R, Young C, Hossain S, Pape T, Payne B, Radomski C, Donaldson G, Ives E, Cox J, Younghusband HB, Green R, Duff A, Boltshauser E, Grinspan GA, Dimon JH, Sibley BG, Andria G, Toscano E, Kerdraon J, Bowsher D, Pimstone SN, Samuels ME, Sherrington R & Hayden MR (2007). Loss-of-function mutations in the $Na_v1.7$ gene underlie congenital indifference to pain in multiple human populations. *Clin Genet* **71**, 311–319.

Gonzalez-Cano R, Tejada MA, Artacho-Cordon A, Nieto FR, Entrena JM, Wood JN & Cendan CM (2017). Effects of tetrodotoxin in mouse models of visceral pain. *Mar Drugs* **15**, E188.

Grundy D (2002). Neuroanatomy of visceral nociception: vagal and splanchnic afferent. *Gut* **51** Suppl. 1, i2–5.

GTEx Consortium; Laboratory, Data Analysis &Coordinating Center (LDACC) – Analysis Working Group; Statistical Methods groups – Analysis Working Group; Enhancing GTEx (eGTEx) groups; NIH Common Fund; NIH/NCI; NIH/NHGRI; NIH/NIMH; NIH/NIDA; Biospecimen Collection Source Site – NDRI; Biospecimen Collection Source Site – RPCI; Biospecimen Core Resource – VARI; Brain Bank Repository – University of Miami Brain Endowment Bank; Leidos Biomedical – Project Management; ELSI Study; Genome Browser Data Integration &Visualization – EBI; Genome Browser Data Integration &Visualization – UCSC Genomics Institute, University of California Santa Cruz; Lead analysts:; Laboratory, Data Analysis &Coordinating Center (LDACC):; NIH program management:; Biospecimen collection:; Pathology:; eQTL manuscript working group: Battle A, Brown CD, Engelhardt BE & Montgomery SB (2017). Genetic effects on gene expression across human tissues. *Nature* **550**, 204–213.

Hagen NA, Cantin L, Constant J, Haller T, Blaise G, Ong-Lam M, du Souich P, Korz W & Lapointe B (2017). Tetrodotoxin for moderate to severe cancer-related pain: a multicentre, randomized, double-blind, placebo-controlled, parallel-design trial. *Pain Res Manag* **2017**, 7212713.

Hagen NA, Lapointe B, Ong-Lam M, Dubuc B, Walde D, Gagnon B, Love R, Goel R, Hawley P, Ngoc AH & du Souich P (2011). A multicentre open-label safety and efficacy study of tetrodotoxin for cancer pain. *Curr Oncol* **18**, e109–e116.

Han C, Yang Y, de Greef BT, Hoeijmakers JG, Gerrits MM, Verhamme C, Qu J, Lauria G, Merkies IS, Faber CG, Dib-Hajj SD & Waxman SG (2015). The domain II S4-S5 linker in Nav1.9: a missense mutation enhances activation, impairs fast inactivation, and produces human painful neuropathy. *Neuromolecular Med* **17**, 158–169.

Harrington AM, Brierley SM, Isaacs N, Hughes PA, Castro J & Blackshaw LA (2012). Sprouting of colonic afferent central terminals and increased spinal mitogen-activated protein kinase expression in a mouse model of chronic visceral hypersensitivity. *J Comp Neurol* **520**, 2241–2255.

Hetz S, Acikgoez A, Moll C, Jahnke H-G, Robitzki AA, Metzger R & Metzger M (2014). Age-related gene expression analysis in enteric ganglia of human colon after laser microdissection. *Front Aging Neurosci* **6**, 276.

Hillsley K, Lin JH, Stanisz A, Grundy D, Aerssens J, Peeters PJ, Moechars D, Coulie B & Stead RH (2006). Dissecting the role of sodium currents in visceral sensory neurons in a model of chronic hyperexcitability using Nav1.8 and Nav1.9 null mice. *J Physiol* **576**, 257–267.

Hirano K, Kuratani K, Fujiyoshi M, Tashiro N, Hayashi E & Kinoshita M (2007). Kv7.2–7.5 voltage-gated potassium channel (KCNQ2–5) opener, retigabine, reduces capsaicin-induced visceral pain in mice. *Neurosci Lett* **413**, 159–162.

Hockley JRF, Boundouki G, Cibert-Goton V, McGuire C, Yip PK, Chan C, Tranter M, Wood JN, Nassar MA, Blackshaw LA, Aziz Q, Michael GJ, Baker MD, Winchester WJ, Knowles CH & Bulmer DC (2014). Multiple roles for $Na_V1.9$ in the activation of visceral afferents by noxious inflammatory, mechanical, and human disease-derived stimuli. *Pain* **155**, 1962–1975.

Hockley JRF, González-Cano R, McMurray S, Tejada-Giraldez MA, McGuire C, Torres A, Wilbrey AL, Cibert-Goton V, Nieto FR, Pitcher T, Knowles CH, Baeyens JM, Wood JN, Winchester WJ, Bulmer DC, Cendán CM & McMurray G (2017). Visceral and somatic pain modalities reveal $Na_V1.7$-independent visceral nociceptive pathways. *J Physiol* **595**, 2661–2679.

Hockley JRF, Tranter MM, McGuire C, Boundouki G, Cibert-Goton V, Thaha MA, Blackshaw LA, Michael GJ, Baker MD, Knowles CH, Winchester WJ & Bulmer DC (2016*a*). P2Y receptors sensitize mouse and human colonic nociceptors. *J Neurosci* **36**, 2364–2376.

Hockley JRF, Winchester WJ & Bulmer DC (2016*b*). The voltage-gated sodium channel $Na_V1.9$ in visceral pain. *Neurogastroenterol Motil* **28**, 316–326.

Holm AN, Rich A, Miller SM, Strege P, Ou Y, Gibbons S, Sarr MG, Szurszewski JH, Rae JL & Farrugia G (2002). Sodium current in human jejunal circular smooth muscle cells. *Gastroenterology* **122**, 178–187.

Hu J, Song Z-Y, Zhang H-H, Qin X, Hu S, Jiang X & Xu G-Y (2016). Colonic hypersensitivity and sensitization of voltage-gated sodium channels in primary sensory neurons in rats with diabetes. *J Neurogastroenterol Motil* **22**, 129–140.

Hu S, Xiao Y, Zhu L, Li L, Hu CY, Jiang X & Xu GY (2013*a*). Neonatal maternal deprivation sensitizes voltage-gated sodium channel currents in colon-specific dorsal root ganglion neurons in rats. *Am J Physiol Gastrointest Liver Physiol* **304**, G311–G321.

Hu S, Xu W, Miao X, Gao Y, Zhu L, Zhou Y, Xiao Y & Xu GY (2013*b*). Sensitization of sodium channels by cystathionine β-synthetase activation in colon sensory neurons in adult rats with neonatal maternal deprivation. *Exp Neurol* **248**, 275–285.

Huang J, Han C, Estacion M, Vasylyev D, Hoeijmakers JGJ, Gerrits MM, Tyrrell L, Lauria G, Faber CG, Dib-Hajj SD, Merkies IS, Waxman SG & PROPANE Study Group (2014). Gain-of-function mutations in sodium channel $Na_v1.9$ in painful neuropathy. *Brain* **137**, 1627–1642.

Huang J, Vanoye CG, Cutts A, Goldberg YP, Dib-Hajj SD, Cohen CJ, Waxman SG & George AL Jr (2017). Sodium channel $Na_v1.9$ mutations associated with insensitivity to pain dampen neuronal excitability. *J Clin Invest* **127**, 2805–2814.

Hughes PA, Brierley SM, Martin CM, Brookes SJH, Linden DR & Blackshaw LA (2009). Post-inflammatory colonic afferent sensitisation: different subtypes, different pathways and different time courses. *Gut* **58**, 1333–1341.

Ibeakanma C, Miranda-Morales M, Richards M, Bautista-Cruz F, Martin N, Hurlbut D & Vanner S (2009). *Citrobacter rodentium* colitis evokes post-infectious hyperexcitability of mouse nociceptive colonic dorsal root ganglion neurons. *J Physiol* **587**, 3505–3521.

Ibeakanma C & Vanner S (2010). TNFα is a key mediator of the pronociceptive effects of mucosal supernatant from human ulcerative colitis on colonic DRG neurons. *Gut* **59**, 612–621.

Inserra MC, Israel MR, Caldwell A, Castro J, Deuis JR, Harrington AM, Keramidas A, Garcia-Caraballo S, Maddern J, Erickson A, Grundy L, Rychkov GY, Zimmermann K, Lewis RJ, Brierley SM & Vetter I (2017). Multiple sodium channel isoforms mediate the pathological effects of Pacific ciguatoxin-1. *Sci Rep* **7**, 42810.

Jarnot M & Corbett AM (2006). Immunolocalization of $Na_V1.2$ channel subtypes in rat and cat brain and spinal cord with high affinity antibodies. *Brain Res* **1107**, 1–12.

Jarvis MF, Honore P, Shieh CC, Chapman M, Joshi S, Zhang XF, Kort M, Carroll W, Marron B, Atkinson R, Thomas J, Liu D, Krambis M, Liu Y, McGaraughty S, Chu K, Roeloffs R, Zhong C, Mikusa JP, Hernandez G, Gauvin D, Wade C, Zhu C, Pai M, Scanio M, Shi L, Drizin I, Gregg R, Matulenko M, Hakeem A, Gross M, Johnson M, Marsh K, Wagoner PK, Sullivan JP, Faltynek CR & Krafte DS (2007). A-803467, a potent and selective $Na_v1.8$ sodium channel blocker, attenuates neuropathic and inflammatory pain in the rat. *Proc Natl Acad Sci USA* **104**, 8520–8525.

Keating C, Beyak M, Foley S, Singh G, Marsden C, Spiller R & Grundy D (2008). Afferent hypersensitivity in a mouse model of post-inflammatory gut dysfunction: role of altered serotonin metabolism. *J Physiol* **586**, 4517–4530.

King DE, Macleod RJ & Vanner SJ (2009). Trinitrobenzenesulphonic acid colitis alters $Na_v1.8$ channel expression in mouse dorsal root ganglia neurons. *Neurogastroenterol Motil* **21**, 880–e864.

King GF & Vetter I (2014). No gain, no pain: $Na_v1.7$ as an analgesic target. *ACS Chem Neurosci* **5**, 749–751.

Klein CJ, Wu Y, Kilfoyle DH, Sandroni P, Davis MD, Gavrilova RH, Low PA & Dyck PJ (2013). Infrequent *SCN9A* mutations in congenital insensitivity to pain and erythromelalgia. *J Neurol Neurosurg Psychiatry* **84**, 386–391.

Klugbauer N, Lacinova L, Flockerzi V & Hofmann F (1995). Structure and functional expression of a new member of the tetrodotoxin-sensitive voltage-activated sodium channel family from human neuroendocrine cells. *EMBO J* **14**, 1084–1090.

Kyloh M, Nicholas S, Zagorodnyuk VP, Brookes SJ & Spencer NJ (2011). Identification of the visceral pain pathway activated by noxious colorectal distension in mice. *Front Neurosci* **5**, 16.

La JH & Gebhart GF (2011). Colitis decreases mechanosensitive K_{2P} channel expression and function in mouse colon sensory neurons. *Am J Physiol Gastrointest Liver Physiol* **301**, G165–G174.

Laedermann CJ, Abriel H & Decosterd I (2015). Post-translational modifications of voltage-gated sodium channels in chronic pain syndromes. *Front Pharmacol* **6**, 263.

Lago J, Rodríguez LP, Blanco L, Vieites JM & Cabado AG (2015). Tetrodotoxin, an extremely potent marine neurotoxin: distribution, toxicity, origin and therapeutical uses. *Mar Drugs* **13**, 6384–6406.

Laird JM, Souslova V, Wood JN & Cervero F (2002). Deficits in visceral pain and referred hyperalgesia in Nav1.8 (SNS/PN3)-null mice. *J Neurosci* **22**, 8352–8356.

Lei Q & Malykhina AP (2012). Colonic inflammation up-regulates voltage-gated sodium channels in bladder sensory neurons via activation of peripheral transient potential vanilloid 1 receptors. *Neurogastroenterol Motil* **24**, 575–e257.

Leipold E, Liebmann L, Korenke GC, Heinrich T, Giesselmann S, Baets J, Ebbinghaus M, Goral RO, Stodberg T, Hennings JC, Bergmann M, Altmueller J, Thiele H, Wetzel A, Nuernberg P, Timmerman V, De Jonghe P, Blum R, Schaible H-G, Weis J, Heinemann SH, Huebner CA & Kurth I (2013). A *de novo* gain-of-function mutation in *SCN11A* causes loss of pain perception. *Nat Genet* **45**, 1399–1404.

Lin YM, Fu Y, Winston J, Radhakrishnan R, Sarna SK, Huang LM & Shi XZ (2017). Pathogenesis of abdominal pain in bowel obstruction: Role of mechanical stress-induced upregulation of nerve growth factor in gut smooth muscle cells. *Pain* **158**, 583–592.

Luo JL, Qin HY, Wong CK, Tsang SY, Huang Y & Bian ZX (2011). Enhanced excitability and down-regulated voltage-gated potassium channels in colonic DRG neurons from neonatal maternal separation rats. *J Pain* **12**, 600–609.

McCormack K, Santos S, Chapman ML, Krafte DS, Marron BE, West CW, Krambis MJ, Antonio BM, Zellmer SG, Printzenhoff D, Padilla KM, Lin Z, Wagoner PK, Swain NA, Stupple PA, de Groot M, Butt RP & Castle NA (2013). Voltage sensor interaction site for selective small molecule inhibitors of voltage-gated sodium channels. *Proc Natl Acad Sci USA* **110**, E2724–E2732.

Maingret F, Coste B, Padilla F, Clerc N, Crest M, Korogod SM & Delmas P (2008). Inflammatory mediators increase $Na_v1.9$ current and excitability in nociceptors through a coincident detection mechanism. *J Gen Physiol* **131**, 211–225.

Malykhina AP, Qin C, Foreman RD & Akbarali HI (2004). Colonic inflammation increases Na^+ currents in bladder sensory neurons. *Neuroreport* **15**, 2601–2605.

Malykhina AP, Wyndaele J-J, Andersson K-E, De Wachter S & Dmochowski RR (2012). Do the urinary bladder and large bowel interact, in sickness or in health? *Neurourol Urodyn* **31**, 352–358.

Manriquez V, Caperan DC, Guzman R, Naser M, Iglesia V & Lagos N (2015). First evidence of neosaxitoxin as a long-acting pain blocker in bladder pain syndrome. *Int Urogynecol J* **26**, 853–858.

Marcil J, Walczak JS, Guindon J, Ngoc AH, Lu S & Beaulieu P (2006). Antinociceptive effects of tetrodotoxin (TTX) in rodents. *Br J Anaesth* **96**, 761–768.

Martinez V & Melgar S (2008). Lack of colonic-inflammation-induced acute visceral hypersensitivity to colorectal distension in Nav1.9 knockout mice. *Eur J Pain* **12**, 934–944.

Minett MS, Eijkelkamp N & Wood JN (2014). Significant determinants of mouse pain behaviour. *PLoS One* **9**, e104458.

Minett MS, Nassar MA, Clark AK, Passmore G, Dickenson AH, Wang F, Malcangio M & Wood JN (2012). Distinct Nav1.7-dependent pain sensations require different sets of sensory and sympathetic neurons. *Nat Commun* **3**, 791.

Minett MS, Pereira V, Sikandar S, Matsuyama A, Lolignier S, Kanellopoulos AH, Mancini F, Iannetti GD, Bogdanov YD, Santana-Varela S, Millet Q, Baskozos G, MacAllister R, Cox JJ, Zhao J & Wood JN (2015). Endogenous opioids contribute to insensitivity to pain in humans and mice lacking sodium channel $Na_v1.7$. *Nat Commun* **6**, 8967.

Moore BA, Stewart TM, Hill C & Vanner SJ (2002). TNBS ileitis evokes hyperexcitability and changes in ionic membrane properties of nociceptive DRG neurons. *Am J Physiol Gastrointest Liver Physiol* **282**, G1045–G1051.

Morinville A, Fundin B, Meury L, Juréus A, Sandberg K, Krupp J, Ahmad S & O'Donnell D (2007). Distribution of the voltage-gated sodium channel $Na_v1.7$ in the rat: Expression in the autonomic and endocrine systems. *J Comp Neurol* **504**, 680–689.

Nash DT & Nash SD (2008). Ranolazine for chronic stable angina. *Lancet* **372**, 1335–1341.

Nassar MA, Levato A, Stirling LC & Wood JN (2005). Neuropathic pain develops normally in mice lacking both $Na_v1.7$ and $Na_v1.8$. *Mol Pain* **1**, 1–9.

Nassar MA, Stirling LC, Forlani G, Baker MD, Matthews EA, Dickenson AH & Wood JN (2004). Nociceptor-specific gene deletion reveals a major role for $Na_v1.7$ (PN1) in acute and inflammatory pain. *Proc Natl Acad Sci USA* **101**, 12706–12711.

Neshatian L, Strege PR, Rhee PL, Kraichely RE, Mazzone A, Bernard CE, Cima RR, Larson DW, Dozois EJ, Kline CF, Mohler PJ, Beyder A & Farrugia G (2015). Ranolazine inhibits voltage-gated mechanosensitive sodium channels in human colon circular smooth muscle cells. *Am J Physiol Gastrointest Liver Physiol* **309**, G506–G512.

NIH (2009). Opportunities & Challenges in Digestive Diseases Research: Recommendations of the National Commission on Digestive Diseases. United States Department of Health & Human Services, National Institute of Diabetes and Digestive and Kidney Diseases, NIH Publication No. 08–6514, Bethesda, MD, USA.

O'Donnell AM, Coyle D & Puri P (2016). Decreased $Na_v1.9$ channel expression in Hirschsprung's disease. *J Pediatr Surg* **51**, 1458–1461.

Osorio N, Korogod S & Delmas P (2014). Specialized functions of Nav1.5 and Nav1.9 channels in electrogenesis of myenteric neurons in intact mouse ganglia. *J Neurosci* **34**, 5233–5244.

Osteen JD, Herzig V, Gilchrist J, Emrick JJ, Zhang C, Wang X, Castro J, Garcia-Caraballo S, Grundy L, Rychkov GY, Weyer AD, Dekan Z, Undheim EAB, Alewood P, Stucky CL, Brierley SM, Basbaum AI, Bosmans F, King GF & Julius D (2016). Selective spider toxins reveal a role for the $Na_v1.1$ channel in mechanical pain. *Nature* **534**, 494–499.

Ostman JA, Nassar MA, Wood JN & Baker MD (2008). GTP up-regulated persistent Na^+ current and enhanced nociceptor excitability require $Na_V1.9$. *J Physiol* **586**, 1077–1087.

Ou Y, Gibbons SJ, Miller SM, Strege PR, Rich A, Distad MA, Ackerman MJ, Rae JL, Szurszewski JH & Farrugia G (2002). *SCN5A* is expressed in human jejunal circular smooth muscle cells. *Neurogastroenterol Motil* **14**, 477–486.

Padilla F, Couble M-L, Coste B, Maingret F, Clerc N, Crest M, Ritter AM, Magloire H & Delmas P (2007). Expression and localization of the Nav1.9 sodium channel in enteric neurons and in trigeminal sensory endings: Implication for intestinal reflex function and orofacial pain. *Mol Cell Neurosci* **35**, 138–152.

Pennington MW, Czerwinski A & Norton RS (2017). Peptide therapeutics from venom: Current status and potential. *Bioorg Med Chem*; https://doi.org/10.1016/j.bmc.2017.1009.1029.

Qian AH, Liu XQ, Yao WY, Wang HY, Sun J, Zhou L & Yuan YZ (2009). Voltage-gated potassium channels in IB4-positive colonic sensory neurons mediate visceral hypersensitivity in the rat. *Am J Gastroenterol* **104**, 2014–2027.

Qin N, D'Andrea MR, Lubin ML, Shafaee N, Codd EE & Correa AM (2003). Molecular cloning and functional expression of the human sodium channel β_{1B} subunit, a novel splicing variant of the β_1 subunit. *Eur J Biochem* **270**, 4762–4770.

Qu R, Tao J, Wang Y, Zhou Y, Wu G, Xiao Y, Hu C-Y, Jiang X & Xu G-Y (2013). Neonatal colonic inflammation sensitizes voltage-gated Na^+ channels via upregulation of cystathionine β-synthetase expression in rat primary sensory neurons. *Am J Physiol Gastrointest Liver Physiol* **304**, G763–G772.

Reeder JE, Byler TK, Foster DC, Landas SK, Okafor H, Stearns G, Wood RW, Zhang Y & Mayer RD (2013). Polymorphism in the SCN9A voltage-gated sodium channel gene associated with interstitial cystitis/bladder pain syndrome. *Urology* **81**, 210e1–210e4.

Renganathan M, Cummins TR & Waxman SG (2001). Contribution of $Na_v1.8$ sodium channels to action potential electrogenesis in DRG neurons. *J Neurophysiol* **86**, 629–640.

Ritter AM, Martin WJ & Thorneloe KS (2009). The voltage-gated sodium channel Nav1.9 is required for inflammation-based urinary bladder dysfunction. *Neurosci Lett* **452**, 28–32.

Roselli F, Livrea P & Jirillo E (2006). Voltage-gated sodium channel blockers as immunomodulators. *Recent Pat CNS Drug Discov* **1**, 83–91.

Rugiero F, Mistry M, Sage D, Black JA, Waxman SG, Crest M, Clerc N, Delmas P & Gola M (2003). Selective expression of a persistent tetrodotoxin-resistant Na^+ current and $Na_V1.9$ subunit in myenteric sensory neurons. *J Neurosci* **23**, 2715–2725.

Sage D, Salin P, Alcaraz G, Castets F, Giraud P, Crest M, Mazet B & Clerc N (2007). $Na_v1.7$ and $Na_v1.3$ are the only tetrodotoxin-sensitive sodium channels expressed by the adult guinea pig enteric nervous system. *J Comp Neurol* **504**, 363–378.

Saito YA, Strege PR, Tester DJ, Locke GR, Talley NJ, Bernard CE, Rae JL, Makielski JC, Ackerman MJ & Farrugia G (2009). Sodium channel mutation in irritable bowel syndrome: evidence for an ion channelopathy. *Am J Physiol Gastrointest Liver Physiol* **296**, G211–G218.

Salaga M, Storr M, Martemyanov KA & Fichna J (2016). RGS proteins as targets in the treatment of intestinal inflammation and visceral pain: New insights and future perspectives. *Bioessays* **38**, 344–354.

Savio-Galimberti E, Gollob MH & Darbar D (2012). Voltage-gated sodium channels: biophysics, pharmacology, and related channelopathies. *Front Pharmacol* **3**, 124.

Schep LJ, Schmierer DM & Fountain JS (2006). Veratrum poisoning. *Toxicol Rev* **25**, 73–78.

Sikandar S & Dickenson AH (2012). Visceral pain – the Ins and Outs, the Ups and Downs. *Curr Opin Support Palliat Care* **6**, 17–26.

Stewart I, Lewis RJ, Eaglesham GK, Graham GC, Poole S & Craig SB (2010). Emerging tropical diseases in Australia. Part 2. Ciguatera fish poisoning. *Ann Trop Med Parasitol* **104**, 557–571.

Stewart T, Beyak MJ & Vanner S (2003). Ileitis modulates potassium and sodium currents in guinea pig dorsal root ganglia sensory neurons. *J Physiol* **552**, 797–807.

Strege PR, Knutson K, Eggers SJ, Li JH, Wang F, Linden D, Szurszewski JH, Milescu L, Leiter AB, Farrugia G & Beyder A (2017*a*). Sodium channel $Na_V1.3$ is important for enterochromaffin cell excitability and serotonin release. *Sci Rep* **7**, 15650.

Strege PR, Knutson KR, Eggers SJ, Wang F, Li J, Leiter A, Farrugia G & Beyder A (2017*b*). *SCN3A*-encoded voltage-gated sodium channel $Na_V1.3$ is specifically expressed in human and mouse gastrointestinal enterochromaffin cells and is important for enterochromaffin cell excitability. *FASEB J* **31**, 1007–1040.

Strege PR, Mazzone A, Bernard CE, Neshatian L, Gibbons SJ, Saito YA, Tester DJ, Calvert ML, Mayer EA, Chang L, Ackerman MJ, Beyder A & Farrugia G (2017*c*). Irritable bowel syndrome (IBS) patients have *SCN5A* channelopathies that lead to decreased $Na_V1.5$ current and mechanosensitivity. *Am J Physiol Gastrointest Liver Physiol*; https://doi.org/10.1152/ajpgi.00016.2017.

Strege PR, Mazzone A, Kraichely RE, Sha L, Holm AN, Ou Y, Lim I, Gibbons SJ, Sarr MG & Farrugia G (2007). Species dependent expression of intestinal smooth muscle mechanosensitive sodium channels. *Neurogastroenterol Motil* **19**, 135–143.

Strege PR, Ou YJ, Sha L, Rich A, Gibbons SJ, Szurszewski JH, Sarr MG & Farrugia G (2003). Sodium current in human intestinal interstitial cells of Cajal. *Am J Physiol Gastrointest Liver Physiol* **285**, G1111–G1121.

Tate S, Benn S, Hick C, Trezise D, John V, Mannion RJ, Costigan M, Plumpton C, Grose D, Gladwell Z, Kendall G, Dale K, Bountra C & Woolf CJ (1998). Two sodium channels contribute to the TTX-R sodium current in primary sensory neurons. *Nat Neurosci* **1**, 653–655.

Trimmer JS, Cooperman SS, Agnew WS & Mandel G (1990). Regulation of muscle sodium channel transcripts during development and in response to denervation. *Dev Biol* **142**, 360–367.

Tseng TT, McMahon AM, Johnson VT, Mangubat EZ, Zahm RJ, Pacold ME & Jakobsson E (2007). Sodium channel auxiliary subunits. *J Mol Microbiol Biotechnol* **12**, 249–262.

Tzoumaka E, Tischler AC, Sangameswaran L, Eglen RM, Hunter JC & Novakovic SD (2000). Differential distribution of the tetrodotoxin-sensitive rPN4/NaCh6/Scn8a sodium channel in the nervous system. *J Neurosci Res* **60**, 37–44.

Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J & Ponten F (2015). Proteomics. Tissue-based map of the human proteome. *Science* **347**, 1260419.

Vanoye CG, Kunic JD, Ehring GR & George AL Jr (2013). Mechanism of sodium channel $Na_v1.9$ potentiation by G-protein signaling. *J Gen Physiol* **141**, 193–202.

Verne GN, Sen A & Price DD (2005). Intrarectal lidocaine is an effective treatment for abdominal pain associated with diarrhea-predominant irritable bowel syndrome. *J Pain* **6**, 493–496.

Wang W, Gu J, Li Y-Q & Tao Y-X (2011). Are voltage-gated sodium channels on the dorsal root ganglion involved in the development of neuropathic pain? *Mol Pain* **7**, 1–9.

Waxman SG, Kocsis JD & Black JA (1994). Type III sodium channel mRNA is expressed in embryonic but not adult spinal sensory neurons, and is reexpressed following axotomy. *J Neurophysiol* **72**, 466–470.

Waxman SG, Merkies IS, Gerrits MM, Dib-Hajj SD, Lauria G, Cox JJ, Wood JN, Woods CG, Drenth JP & Faber CG (2014). Sodium channel genes in pain-related disorders: phenotype-genotype associations and recommendations for clinical use. *Lancet Neurol* **13**, 1152–1160.

Westenbroek RE, Merrick DK & Catterall WA (1989). Differential subcellular localization of the RI and RII Na^+ channel subtypes in central neurons. *Neuron* **3**, 695–704.

Whitaker W, Faull R, Waldvogel H, Plumpton C, Burbidge S, Emson P & Clare J (1999). Localization of the type VI voltage-gated sodium channel protein in human CNS. *Neuroreport* **10**, 3703–3709.

Woods CG, Babiker MOE, Horrocks I, Tolmie J & Kurth I (2015). The phenotype of congenital insensitivity to pain due to the $Na_V1.9$ variant p.L811P. *Eur J Hum Genet* **23**, 561–563.

Yang Y, Wang Y, Li S, Xu Z, Li H, Ma L, Fan J, Bu D, Liu B, Fan Z, Wu G, Jin J, Ding B, Zhu X & Shen Y (2004). Mutations in *SCN9A*, encoding a sodium channel alpha subunit, in patients with primary erythermalgia. *J Med Genet* **41**, 171–174.

Yekkirala AS, Roberson DP, Bean BP & Woolf CJ (2017). Breaking barriers to novel analgesic drug development. *Nat Rev Drug Discov* **16**, 545–564.

Yiangou Y, Facer P, Chessell IP, Bountra C, Chan C, Fertleman C, Smith V & Anand P (2007). Voltage-gated ion channel $Na_v1.7$ innervation in patients with idiopathic rectal hypersensitivity and paroxysmal extreme pain disorder (familial rectal pain). *Neurosci Lett* **427**, 77–82.

Yoshimura N & deGroat WC (1997). Plasticity of Na^+ channels in afferent neurones innervating rat urinary bladder following spinal cord injury. *J Physiol* **503**, 269–276.

Yoshimura N, Seki S, Novakovic SD, Tzoumaka E, Erickson VL, Erickson KA, Chancellor MB & de Groat WC (2001). The involvement of the tetrodotoxin-resistant sodium channel $Na_v1.8$ (PN3/SNS) in a rat model of visceral pain. *J Neurosci* **21**, 8690–8696.

Zang Y, He XH, Xin WJ, Pang RP, Wei XH, Zhou LJ, Li YY & Liu XG (2010). Inhibition of NF-kappaB prevents mechanical allodynia induced by spinal ventral root transection and suppresses the re-expression of Nav1.3 in DRG neurons *in vivo* and *in vitro*. *Brain Res* **1363**, 151–158.

Zhang XY, Wen J, Yang W, Wang C, Gao L, Zheng LH, Wang T, Ran K, Li Y, Li X, Xu M, Luo J, Feng S, Ma X, Ma H, Chai Z, Zhou Z, Yao J, Zhang X & Liu JY (2013). Gain-of-function mutations in *SCN11A* cause familial episodic pain. *Am J Hum Genet* **93**, 957–966.

Additional information

Competing interests

In relation to the content covered within this review, the authors have nothing to declare.

Author contributions

All authors contributed to searching the published literature and to writing the review. All authors approved the final version and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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