Understanding the physiological role of $Na_V1.9$: challenges and opportunities for pain modulation

Wayra Brackx[*], Rita de Cássia Collaço[*], Margaux Theys[*], Jolien Vander Cruyssen[*], Frank Bosmans[#]

Molecular Physiology and Neurophysics Group, Department of Basic and Applied Medical Sciences, University of Ghent, Ghent, Belgium.

[*] Co-first authors

[#] Corresponding author: Corneel Heymanslaan 10, building B, entrance 36, Ghent 9000, Belgium; Tel. +32(0)93323938; email: frank.bosmans@ugent.be

Abstract

Voltage-activated Na^+ (Na_V) channels are crucial contributors to rapid electrical signaling in the human body. As such, they are among the most targeted membrane proteins by clinical therapeutics and natural toxins. Several of the nine mammalian Na_V channel subtypes play a documented role in pain or other sensory processes such as itch, touch, and smell. While causal relationships between these subtypes and biological function have been extensively described, the physiological role of $Na_V1.9$ is less understood. Yet, mutations in $Na_V1.9$ can cause striking disease phenotypes related to sensory perception such as loss or gain of pain and chronic itch. Here, we explore our current knowledge of the mechanisms by which $Na_V1.9$ may contribute to pain and elaborate on the challenges associated with establishing links between experimental conditions and human disease. This review also discusses the lack of comprehensive insights into $Na_V1.9$ pharmacology, an unfortunate situation since modulatory compounds may have tremendous potential in the clinic to treat pain or as precision tools to examine the extent of $Na_V1.9$ participation in sensory perception processes.

Keywords

Pain, voltage-activated sodium ion channel, $Na_V1.9$, sensory perception

Abbreviations

AGAP - Antitumor-analgesic peptide;

AP – Action Potential;

ATP - adenosine triphosphate;

BAM8-22 - Bovine Adrenal Medulla 8-22;

BDNF – Brain-Derived Neurotrophic Factor;

CFA – Complete Freund's adjuvant;

CGRP - Calcitonin-Gene Related Peptide;

CHO cells - Chinese Hamster Ovary cells;

CIP – Congenital Insensitivity to Pain;

CXCR4 - C-X-C chemokine Receptor type 4;

DAG – Diacylglycerol;

DRG – Dorsal Root Ganglion;

ERK1/2 - Extracellular signal-Regulated Kinase ½;

ERK5/CREB - Extracellular signal-Regulated Kinase 5/cAMP Response Element Binding protein;

FEP – Familial Episodic Pain;

GPCR – G protein-coupled receptor;

GPI – GlycosylPhosphatidylInositol;

Gr4b - δ-theraphotoxin-Gr4b;

GrTx-SIA - omega-Grammotoxin SIA;

GTP – Guanosine Triphosphate;

HaTx – Hanatoxin;

HEK293 – Human Embryonic Kidney-293;

HpTx1 – Heteropodatoxin 1;

IB4 – Isolectin B4

IP_3 – inositol triphosphate;

JAK2-STAT3 - Janus Kinase 2/Signal Transducer and Activator of Transcription 3;

JNK - c-Jun N-terminal Kinase;

K_V channels - voltage-activated K^+ channels;

MAPK - Mitogen-Activated Protein Kinase;

MOH - Medication Overuse Headache;

MrgprC11/X1 - Mas-related G-protein Coupled Receptor Member X1;

Na_V channels – voltage-activated Na^+ channels;

NGF – Nerve Growth Factor;

P-CTX-1 - Pacific Ciguatoxin-1;

PGE_2 – Prostaglandine E2;

PIP_2 – Phosphatidylnositol 4,5-bisphosphate;

PK(A)(C) – Protein Kinase (A)(C);

PLC – Phospholipase C; PMA - Phorbol 12-myristate 13-acetate;

PPN – Painful Peripheral Neuropathy;

ProTx-I - Protoxin I;

PTX - Pertussis Toxin;

SDF-1 - Stromal cell-derived factor 1;

SFN – Small Fiber Neuropathy;

TRK(A)(B) – Tropomyosin Receptor Kinase (A)(B);

TTX – Tetrodotoxin;

VSD – Voltage-Sensing Domain

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1. Mutations in Na_V channels and subsequent pain phenotypes

Pain is an unpleasant sensory and emotional experience associated with, or resembling, actual or potential tissue harm (Raja, et al., 2020). When pain persists beyond typical remedial efforts, it becomes chronic and loses its protective value. Chronic pain can be caused by a wide variety of afflictions and is estimated to affect about 30% of people worldwide, of which 5% experience limitations in their daily activities (source: Centers for Disease Control and Prevention and the International Association for the Study of Pain). Additionally, patients may suffer from depression and opioid dependency when standard pain management strategies fail, leading it to be an enormous personal and economic burden (Cohen, Vase, & Hooten, 2021). As such, uncovering new pathways to combat chronic pain is a key challenge in today's medicine.

In general, the neuroanatomy of pain pathways involves the sensory input from mechanical, thermal or chemical stimuli that is provided to primary afferent axons that innervate peripheral tissues and organs (Figure 1) (Willis & Westlund, 1997). Large myelinated (Aβ) afferents are proprioceptors that convey mechanical and tactile information whereas most myelinated (Aδ) and unmyelinated (C) fibers function as thermoreceptors or nociceptors, taking charge of heat/cold and pain signaling. The cell bodies of these afferents reside in the dorsal root ganglion (DRG), from which the axons enter the spinal cord through the dorsal root. Finally, they form excitatory synapses with neurons within the dorsal horn, where interneurons arborize locally. In contrast, the axons of projection neurons advance via the spinothalamic, spinomesencephalic, and spinoreticular tracts to the brain. Descending pain modulatory pathways originate in the periaqueductal gray, form synapses in the rostral ventromedial medulla and terminate diffusely within the dorsal horn (Todd, 2010). The involvement of numerous components in the peripheral and central nervous system illustrate the complexities in mapping the pain landscape but also provides opportunities for drug discovery.

Understanding the mechanisms of pain signal transduction has been greatly promoted by investigating Mendelian disorders of pain, such as hereditary sensory neuropathies, Congenital Indifference to Pain (CIP), primary erythromelalgia and familial hemiplegic migraine (Dib-Hajj & Waxman, 2019; Huang, et al., 2017; Kahlig,

et al., 2008; King, Leipold, Goehringer, Kurth, & Challman, 2017). Key players and pathways have been unveiled by identifying the underlying genetic mutations to these afflictions. Na_V channels occupy a distinctly important place among contributors to pain, mainly because of their fundamental role in generating action potentials (APs) and driving neuronal transmission (Ahern, Payandeh, Bosmans, & Chanda, 2016). Of the nine different subtypes ($Na_V1.1$–$Na_V1.9$), $Na_V1.7$, $Na_V1.8$ and $Na_V1.9$ (Figure 1) are primarily expressed in nociceptive sensory neurons and are documented regulators of pain processing (Dib-Hajj & Waxman, 2019; Habib, Wood, & Cox, 2015; Lischka, et al., 2022). Multiple case studies identified mutations in the respectively encoding genes *SCN9A, SCN10A,* and *SCN11A* in patients with a pain phenotype. $Na_V1.9$ in particular, has long been a puzzling channel to study in relation to sensory perception. With its prominent expression in DRG (Figure 1) and the superficial layers of the dorsal horn – hot spots in pain pathways – $Na_V1.9$ has the potential to be a robust drug target (Bennett, Clark, Huang, Waxman, & Dib-Hajj, 2019; Black, Liu, Tanaka, Cummins, & Waxman, 2004; Cummins, et al., 1999; Fjell, et al., 2000; Lischka, et al., 2022; Salvatierra, et al., 2018). This has been emphasized multiple times as *SCN11A* gain-of-function mutations have been implicated in CIP, familial episodic pain (FEP), small fiber sensory neuropathy (SFN) and painful peripheral neuropathy (PPN) (Figure 2, see Table 1 for references). For example, the *de novo* heterozygous missense mutation $Na_V1.9$ p.L811P was identified in individuals with a congenital inability to experience pain (Leipold, et al., 2013; Salvatierra, et al., 2018; Woods, Babiker, Horrocks, Tolmie, & Kurth, 2015). Knock-in mice carrying the orthologous p.L799P mutation present a reduced sensitivity to pain, thereby partly recapitulating the human phenotype. The mutant channel is considered gain-of-function and exhibits excessive activity at membrane resting voltages leading to tonic depolarization and Na_V channel inactivation, and impaired generation of APs. Markedly, gain-of-function mutations in $Na_V1.9$ have also been linked to gain-of-pain phenotypes. For example, $Na_V1.9$ p.R225C and p.A808G showed a co-segregation in two families with episodic pain (Kabata, et al., 2018). Biophysical measurements in DRG from transgenic mice indicated increases in current density and both mutants were therefore labeled gain-of-function. The p.N816K variant also causes episodic pain, possibly due to an increase in $Na_V1.9$ current density and hyperpolarization of the voltage-dependence of channel

activation (Huang, et al., 2019). Subsequent recordings in $Na_V1.9$-null mouse DRG electroporated with the mutant channel uncovered that the p.N816K substitution reduces current threshold to fire APs and renders DRG hyperexcitable. To help explain the spectrum of clinical phenotypes produced by gain-of-function mutations in $Na_V1.9$, a unifying mechanism was proposed (Huang, et al., 2017). This entails that a U-shaped relationship exists between the membrane resting potential and the AP threshold. $Na_V1.9$ mutations that evoke small changes in membrane depolarization cause neuronal hyperexcitability and pain, whereas substitutions that lead to large membrane depolarizations cause hypoexcitability and insensitivity to pain. A further validation of this theory requires additional studies on a genetic and pharmacological level. The next sections in this review provide a condensed overview of mechanistic research into the role of $Na_V1.9$ in nociception using a range of rodent models and biophysical studies. Pain-related modifiers of $Na_V1.9$ function and pharmacological agents that target this enigmatic Na_V channel subtype are also discussed.

2. Studies on $Na_V1.9$-related phenotypes using rodents

The use of genetically modified rodents remains a staple in modern-day pain research (Mogil, 2019). Regarding $Na_V1.9$, ambitious genetic and functional rodent studies led to remarkable insights but also generated additional questions. We clustered available data into a neuropathic, inflammatory, and visceral pain section. A first group of experiments involves $Na_V1.9$ knockout mice or rats. It is worth noting that a knockout background has yet to be identified in humans. With respect to neuropathic pain, partial ligation of the sciatic nerve in $Na_V1.9^{-/-}$ mice led to a reduction in cold sensitivity but not mechanical stimuli (Leo, D'Hooge, & Meert, 2010; Minett, Falk, et al., 2014; Priest, et al., 2005). Trigeminal neuralgia, another neuropathic pain condition, causes sudden and severe facial pain mediated by the trigeminal nerve (Maarbjerg, Di Stefano, Bendtsen, & Cruccu, 2017). Indeed, $Na_V1.9^{-/-}$ mice failed to develop thermal and mechanical hypersensitivity after infraorbital nerve constriction, a model of trigeminal neuralgia (Luiz, Kopach, Santana-Varela, & Wood, 2015). However, spinal nerve ligation injury and spinal nerve transections in $Na_V1.9$ knockdown rats and knockout mice respectively, did not alleviate mechanical

or thermal hypersensitivity to nociceptive stimuli (Minett, Falk, et al., 2014; Porreca, et al., 1999). Moreover, spared nerve injury experiments in $Na_V1.9^{-/-}$ mice with an intact sural nerve, showed no reduction in cold or mechanical hypersensitivity whereas the development of cold sensitivity was prevented when leaving the tibial nerve intact (Amaya, et al., 2006; Leo, et al., 2010). To help address the conflicting results with respect to $Na_V1.9$ involvement in mechanical and heat responsiveness, refined behavioural tests were conducted and uncovered different responses to ramp-shaped noxious pressure and slowly increasing radiant heat in WT and $Na_V1.9^{-/-}$ mice (Minett, Eijkelkamp, & Wood, 2014). Furthermore, a detailed study from Hoffmann and colleagues (Hoffmann, et al., 2017) demonstrated that $Na_V1.9$ knockout mice exhibit impaired mechanical and thermal sensory capacities and reduced electrical excitability of nociceptors. Using an array of techniques, they concluded that $Na_V1.9$ likely contributes to thermal and mechanical nociception, presumably through augmenting neuronal excitability but also by amplifying receptor potentials irrespective of stimulus modality.

In contrast to neuropathic pain, a role for $Na_V1.9$ in inflammatory sensitization and hyperalgesia has been more consistently reported (Amaya, et al., 2006; Maingret, et al., 2008; Ostman, Nassar, Wood, & Baker, 2008; Priest, et al., 2005). Discrepancies in test results do exist and may relate to the rodent line or behavioural paradigm used to generate data. Inflammatory pain is typically evoked by administrating agents that activate an immune response, such as Complete Freund's Adjuvant (CFA), carrageenan, or by inflammatory mediators such as prostaglandin E2 (PGE_2) (Barrot, 2012). Peripheral inflammation induced by intraplantar injection of CFA typically results in mechanical hypersensitivity (Amaya, et al., 2006; Leo, et al., 2010). In $Na_V1.9^{-/-}$ animals, several studies uncovered a significant reduction or lack in thermal hypersensitivity after CFA injection illustrating a contribution of $Na_V1.9$ to inflammatory pain (Amaya, et al., 2006; Priest, et al., 2005). However, Leo *et al.* noted an unaltered paw withdrawal latency in response to high temperatures in $Na_V1.9^{-/-}$ mice after CFA treatment (Leo, et al., 2010). In addition, rats treated with $Na_V1.9$ antisense oligodeoxynucleotides did not show an alleviation of thermal or mechanical hypersensitivity induced by CFA injection despite an increase in $Na_V1.9$ expression (Yu, Zhao, Guan, & Chen, 2011). Leo *et al.* also described that thermal hypersensitivity was still present after carrageenan injection in

paws of $Na_V1.9^{-/-}$ mice (Leo, et al., 2010). In contrast, Priest *et al.* observed a reduced duration of thermal hypersensitivity under similar conditions (Priest, et al., 2005). Similarly, administration of $Na_V1.9$ antisense oligodeoxynucleotides in rats and deletion of $Na_V1.9$ in mice was accompanied by a reduction in mechanical and thermal hypersensitivity (Lolignier, et al., 2011). Contrary to these few conflicting reports, virtually all studies in which experiments were carried out with PGE_2 and formalin conclude that $Na_V1.9$ does play a vital role in inflammatory pain (Amaya, et al., 2006; Leo, et al., 2010; Lolignier, et al., 2011; Priest, et al., 2005). It is worth noting here that the formalin test may not be ideally suited to investigate inflammation (Hoffmann, et al., 2022).

Humans harbouring a $Na_V1.9$ mutation also report a range of gastrointestinal discomforts (Cibert-Goton, et al., 2021; Hockley, et al., 2014; Hockley, Winchester, & Bulmer, 2016). Combined with accumulating evidence of functional expression in the enteric nervous system (Coste, Osorio, Padilla, Crest, & Delmas, 2004; Osorio, Korogod, & Delmas, 2014; Rugiero, et al., 2003), researchers have been investigating the extent of $Na_V1.9$ involvement in gut function and a possible contribution to visceral pain using $Na_V1.9$ knockout models. For example, injection of acetic acid into the peritoneal cavity increases the nocifensive response in $Na_V1.9^{-/-}$ mice (Leo, et al., 2010). $Na_V1.9$ does not seem to play a role in the development of visceral hypersensitivity after colorectal distension in basal conditions. Yet, upon induction of colonic acute inflammation, a reduction in mechanical visceral hyperalgesia became apparent in $Na_V1.9^{-/-}$ mice (Martinez & Melgar, 2008). Moreover, $Na_V1.9$ may also play a role in colonic motility, since deletion of this gene resulted in changes to the colonic migrating motor complex (Copel, Clerc, Osorio, Delmas, & Mazet, 2013). Although more studies are needed, accruing data suggest a potential role for $Na_V1.9$ in regulating visceral functions.

In addition to knockout studies, mouse models of human diseases caused by $Na_V1.9$ mutations (Table 1) have also been created (Table 2). For example, patients with the heterozygous p.A808G mutation experience episodic pain, which can be triggered or aggravated by cold or alcohol intake (Yang, et al., 2020). Transgenic mice homozygous for the orthologous p.A796G mutation exhibited an apparent hypersensitivity to cold, mechanical, and heat stimulation, and presented hypersensitivity to acetaldehyde, which could relate to the alcohol intake-

induced pain phenotype in patients. Interestingly, acetaldehyde increased current levels of the mutant mouse $Na_V1.9$ channel. Another example is the $Na_V1.9$ p.R222S mutation, which is linked to familial episodic pain in humans with symptoms including episodic pain and cold hypersensitivity (Okuda, et al., 2016; Zhao, Jin, Hu, Zhou, & Shi, 2022). Interestingly, Zhao *et al.* reported heat hypersensitivity to be exclusively present in homozygous mutant mice (Zhao, et al., 2022), whereas Okuda *et al.* recognized this phenotype also in heterozygous mutant mice (Okuda, et al., 2016). $Na_V1.9^{p.R222S/WT}$ mice did exhibit amplified nociceptive behaviour when exposed to cold temperatures, an observation that corresponds to patient experiences. An increased nociceptive response was observed in homozygous mutant mice after intraplantar formalin injection (Okuda, et al., 2016). Markedly, patients with this mutation also report visceral dysfunction. $Na_V1.9^{p.R222S/p.R222S}$ mice indeed showed reduced intestinal motility, visceral inflammation, and an increased sensitivity to mechanical colorectal distension (Zhao, et al., 2022). A final example is that of the human $Na_V1.9$ p.L811P orthologous mutation which was introduced in mouse *SCN11a* (p.L799P) by multiple groups (Ebbinghaus, et al., 2020; Leipold, et al., 2013; Salvatierra, et al., 2018). In addition to CIP, patients heterozygous to this mutation report severe itch, self-inflicted lesions, and gastrointestinal dysfunction such as constipation and intermitted diarrhoea (Woods, et al., 2015). While patients display an extremely elevated pain threshold, *SCN11a* p.L799P/WT mice still can experience pain sensations, albeit to a lesser extent (Leipold, et al., 2013). For example, mutant mice present a higher threshold for noxious heat stimuli when compared to WT littermates. Although the withdrawal threshold for plantar mechanical and thermal stimulation did not differ under basal conditions, *SCN11a* p.L799P/WT mice displayed a smaller reduction in withdrawal threshold after zymosan-induced inflammation, indicating reduced thermal and mechanical hyperalgesia. Markedly, Salvatierra *et al.* reported an increase in spontaneous scratching in heterozygous knock-in mice (Salvatierra, et al., 2018). Moreover, 11% of *SCN11a* p.L799P/WT mice exhibited skin lesions, as seen in patients (Leipold, et al., 2013). Interestingly, Ebbinghaus *et al.* stated that this increase in spontaneous scratching in mice only surfaces at a late age, unlike in human patients (Ebbinghaus, et al., 2020).

Even with the noted discrepancies that can easily emerge from comparing complex rodent studies to each other or to human physiology, these and other explorations on rats provide intriguing evidence of an important role for $Na_V1.9$ in pain (Black, et al., 2004; Coskun, Ocal, & Gunay, 2021; Craner, Klein, Renganathan, Black, & Waxman, 2002; Hirofuji, Yokota, Ohno, Kinoshita, & Neo, 2014; Ito, et al., 2012; M. Liu, Zhong, Xia, Dou, & Li, 2019; Ohno, et al., 2010; Ri-Ge-le, et al., 2018; Sleeper, et al., 2000; Strickland, et al., 2008; Xu, Zhang, Wang, Wang, & Wang, 2016; Yu, et al., 2013). The next sections will discuss potential underlying mechanisms as well as challenges in studying this channel in detail on a biophysical and systemic level.

3. Distinctive biophysical properties of $Na_V1.9$

$Na_V1.9$ is one of nine Na_V channel subtypes identified in mammals (Ahern, et al., 2016; Catterall, Goldin, & Waxman, 2005; Hille, 2001), all with an amino acid sequence homology likely to result in a similar three-dimensional architecture (Noreng, Li, & Payandeh, 2021) (Figure 3A). The pore-forming subunit consists of four connected domains (DI–IV), each having six transmembrane segments (S1–S6). These homologous regions each contain a voltage-sensing domain (VSD; S1–S4) encompassing positively charged residues along S4, and one quarter of the structure that forms the Na^+-selective pore (S5–S6). The pore can open after the VSDs in DI–III have moved in response to changes in membrane voltage. Subsequent activation of the DIV VSD will initiate channel inactivation resulting in a non-conductive state (Ahern, et al., 2016) (Figure 2 and Figure 3A).

Of all Na_V channel subtypes, $Na_V1.9$ gating remains the most perplexing. More than two decades ago, the slowly activating and inactivating Na^+ current generated by $Na_V1.9$ (Figure 3B, C) was discovered in a subset of DRG neurons where it appears alongside the much faster currents produced by other Na_V channel subtypes (Cummins, et al., 1999). To isolate $Na_V1.9$-mediated ionic currents, researchers came up with creative solutions. For example, early channel gating data was obtained by recordings from DRG neurons in $Na_V1.8^{-/-}$ mice in which only $Na_V1.9$ currents are present when measured in the presence of tetrodotoxin (TTX), a biological compound that potently inhibits $Na_V1.1$-$Na_V1.4$ and $Na_V1.6$-$Na_V1.7$ (Maruyama, et al., 2004). Alternatively, researchers can

add fluoride ions to the intracellular solution, thereby shifting the activation voltage of $Na_V1.9$ to more hyperpolarized voltages, possibly through a G protein-coupled receptor (GPCR)-mediated mechanism (Amaya, et al., 2006; Blair & Bean, 2002; Coste, Crest, & Delmas, 2007; Coste, et al., 2004; Maingret, et al., 2008; Maruyama, et al., 2004; Vanoye, Ehring, & George, 2012). $Na_V1.8$ gating is virtually unaltered under these conditions, thus resulting in a membrane voltage window in which $Na_V1.9$ function can be examined in native tissues. These techniques are labor intensive and typically unsuitable for setting up high-throughput screening platforms that could allow the identification of $Na_V1.9$ modulators. $Na_V1.9$ research and drug discovery would therefore greatly benefit from the establishment of a robust and reproducible heterologous expression system. However, contrary to other Na_V channel subtypes this has proven to be quite the challenge. Some tools have been described to boost channel expression, but most of these yield relatively small improvements. Measurable currents have been recorded after transfection of $Na_V1.9$ in the DRG-derived ND7/23 hybridoma cell line as well as in HEK293 cells stably transfected with β_1 and β_2 auxiliary subunit DNA (Lin, Santos, Padilla, Printzenhoff, & Castle, 2016; Vanoye, et al., 2012), but additional interventions are usually required to achieve $Na_V1.9$ current densities that are sufficient for biophysical characterization. These include extended low temperature incubation (28-30°C), the use of $Na_V1.9$ chimeras containing C-terminal enhanced Green Fluorescent Protein (Zhou, et al., 2017), or C-terminal fragments of $Na_V1.4$ (Goral, Leipold, Nematian-Ardestani, & Heinemann, 2015) or $Na_V1.7$ (Sizova, et al., 2020) as well as the inclusion of GTPγS, a non-hydrolysable form of guanosine triphosphate (GTP), in the pipette solution (Vanoye, et al., 2012). With the exception of adjustments to the formulation of the electrophysiological recording solutions, most of these interventions are believed to promote trafficking. It should however be noted that the resulting currents typically are still not equivalent to those observed in primary DRGs.

Unlike other subtypes, $Na_V1.9$ currents in native tissues consistently display ultraslow activation and inactivation kinetics when measured using conventional electrophysiological techniques on tissues or cells dissociated from various mammals (Baker, Chandra, Ding, Waxman, & Wood, 2003; Cummins, et al., 1999; Herzog, Cummins, & Waxman, 2001) (Figure 3B, C). This unique feature may result, in part, from the markedly different

amino acid sequence, particularly in the VSDs. Indeed, voltage-activated K^+ (K_V) channels containing defined $Na_V1.9$ voltage-sensing regions from DII-IV exhibit slow kinetics compared to those from other Na_V channel subtypes (Bosmans, Puopolo, Martin-Eauclaire, Bean, & Swartz, 2011). Because of its slow gating characteristics *in vitro*, it was initially thought that $Na_V1.9$ is not directly responsible for action potential generation but rather helps tune the resting membrane voltage (Cummins, et al., 1999). In turn, this process could influence the gating properties of other Na_V channel subtypes that are expressed in the same cell and thus help shape overall neuronal excitability. However, typical electrophysiology experiments are often performed at room temperature (~20°C) (Lin, et al., 2016; Vanoye, et al., 2012). Yet, $Na_V1.9$ undergoes a considerable increase in conductance (~4-fold) and a remarkable speeding of kinetics with heating to physiological temperatures (Touska, et al., 2018) (Figure 3C). Therefore, it is likely that *in vivo*, $Na_V1.9$ plays an important role in bringing particular neurons to firing threshold and does contribute to the upstroke of the action potential. Although differences in $Na_V1.9$ gating have been noted between species, data from human DRG indeed demonstrate that ramp currents are mostly driven by TTX-resistant channels, including $Na_V1.9$, suggesting that these currents underlie spike initiation and action potential overshoot (X. Zhang, Priest, Belfer, & Gold, 2017). In addition, the observed absence of use-dependent pharmacological block of TTX-resistant currents in human DRG would enable these channels to underlie sustained neural activity such as that associated with enduring pain (Bennett, et al., 2019). Combined, a growing body of work illustrates the distinctive biophysical characteristics of $Na_V1.9$ but also highlights species differences that may complicate drug trials.

4. Pain-related modifiers of $Na_V1.9$ function

Consistent with behavioral tests, $Na_V1.9$ ionic currents are enhanced by a plethora of pro-inflammatory mediators, including PGE_2, norepinephrine, adenosine triphosphate (ATP), bradykinin and histamine (Amaya, et al., 2006; Hockley, Tranter, et al., 2016; Maingret, et al., 2008; Rush & Waxman, 2004; Sukhanova, Koirala, & Elmslie, 2022). Interestingly, the effects of these compounds are synergistic, meaning that a mixture of some of these compounds

at low concentrations can produce a significant functional upregulation (Figure 4). Considering that potentiation occurs within minutes, a likely mechanism involves second messengers. The ability of GTPγS and fluoride ions to mimic these effects suggests a participation of G-proteins, and more specifically $G_{i/o}$ proteins (Coste, et al., 2004; Maingret, et al., 2008; Ostman, et al., 2008). Involvement of these proteins can be demonstrated by the use of pertussis toxin (PTX), a compound produced by *Bordetella pertussis* that prevents the interaction $G_{i/o}$ subunit signaling by precluding their interaction with corresponding GPCRs (Campbell & Smrcka, 2018). PTX abolishes $Na_V1.9$ potentiation by PGE_2 as well as stromal cell-derived factor 1 (SDF-1), but is insensitive to cholera toxin, a similar compound specific for G_s subunits (Qiu, et al., 2016). SDF-1 is the endogenous ligand of CXCR4, a GPCR involved in neuropathic pain. PTX also diminishes the stimulating effect of BAM8-22, a pruritogen acting via MrgprC11/X1, on total TTX-resistant currents from small-diameter DRGs (Tseng, Zheng, Li, & Dong, 2019). There is also evidence to suggest the involvement of $G_{q/11}$ subunits, considering that stimulation of both P2Y and NK3 receptors in visceral afferents and M1 muscarinic receptors in medial prefrontal cortex neurons enhance $Na_V1.9$ channel activity (Copel, et al., 2009; Hockley, Tranter, et al., 2016; Kurowski, Gawlak, & Szulczyk, 2015).

Downstream, these GPCRs are capable of activating protein kinase C (PKC) that can phosphorylate the channel to modulate its activity. In particular, the PKCε and PKCγ isoforms have been demonstrated to play a pivotal role in peripheral and central sensitization, respectively (Velazquez, Mohammad, & Sweitzer, 2007). Other Na_V channel subtypes involved in pain have also shown susceptibility to PKC modulation. $Na_V1.8$ phosphorylation by PKCε increases its activity and causes mechanical hyperalgesia, and several studies support $Na_V1.7$ modulation by PKC as well (Cang, Zhang, Zhang, & Zhao, 2009; Vijayaragavan, Boutjdir, & Chahine, 2004; Wu, et al., 2012). In case of $Na_V1.9$, PKC activation with phorbol 12-myristate 13-acetate (PMA) produces channel upregulation in cultured DRGs, and PMA injection increases $Na_V1.9$ expression in naïve mice while a PKC inhibitor lowers elevated $Na_V1.9$ levels in rats injected with CFA to establish inflammatory arthritis (Bai, et al., 2020). When combined with a non-selective protein kinase inhibitor, the effects of GTPγS on $Na_V1.9$ currents diminish, indicating that potentiating effects of GPCRs may indeed involve phosphorylation of $Na_V1.9$ and/or associated proteins (Ostman,

et al., 2008). PMA stimulation also mimics the effects of NK3 receptor stimulation on $Na_V1.9$ currents (Copel, et al., 2009). Another effector known to influence pain-related Na_V channel subtypes is protein kinase A (PKA), but reports on its modulatory effects on $Na_V1.9$ activity are currently inconsistent (Kakimura, Zheng, Uryu, & Ogata, 2010; Qiu, et al., 2016; Scroggs, 2012; X. Y. Zhang, Wu, Zhang, & Gan, 2022). One study investigating the role of $Na_V1.9$ in medication overuse headache (MOH) demonstrated that activation of the NO-cGMP pathway increases $Na_V1.9$ currents in dural afferents from MOH but not WT mice (Bonnet, et al., 2019). This effect may originate in a relief from PKA inhibition on NO-$Na_V1.9$ coupling. Several reports have also demonstrated the involvement of MAPK and JAK2-STAT3 pathways in the regulation of $Na_V1.8$ and $Na_V1.9$ expression *in vivo*, which is interesting considering the established role of these pathways in neuropathic and inflammatory pain (de Lima, et al., 2022; Li, Yang, Sun, Feng, & Song, 2021; Ma, et al., 2022; F. Zhang, et al., 2019).

In addition to mediators, $Na_V1.9$ channel activity also depends on its location in the neuronal membrane. Under unstimulated conditions, $Na_V1.9$ tends to be localized in cholesterol-rich lipid raft domains, possibly through an interaction with glycosylphoshphatidylinositol (GPI)-anchored proteins such as contactin, an adhesion molecule that promotes $Na_V1.9$ surface expression in transfected CHO cells and $IB4^+$ DRG neurons (C. J. Liu, et al., 2001; Rush, et al., 2005). In myelinated neurons, contactin targets Na_V channels to the nodes of Ranvier in between the myelin sheaths (Poliak & Peles, 2003); however, $IB4^+$ neurons are unmyelinated and ion channels are spread along the axon at much lower densities (Rush, et al., 2005). Here, lipid rafts can acts as hubs on the membrane where voltage-activated ion channels, including $Na_V1.8$, cluster to allow micro-saltatory conduction of APs along unmyelinated axons (Neishabouri & Faisal, 2014; Pristera, Baker, & Okuse, 2012). Indeed, several studies have shown that disruption of lipid rafts can impair neuronal excitability (Pristera, et al., 2012). Inflammatory stimuli can reduce cholesterol levels in affected tissues, and this may be sufficient to increase neuronal excitability and cause pain. Surprisingly however, pain induced by cholesterol depletion depends on $Na_V1.9$ but not $Na_V1.8$ (Amsalem, Poilbout, Ferracci, Delmas, & Padilla, 2018). Treatment of DRGs with methyl-β-cyclodextrin increases $Na_V1.9$ current amplitude and hyperpolarizes voltage-dependence of activation. A similar

effect can be achieved by stimulation with inflammatory mediators, which translocate $Na_V1.9$ to non-raft cholesterol-poor regions through a mechanism that relies on the production of reactive oxygen species (ROS) (Amsalem, et al., 2018). These regions may be more permissive to conformational changes and therefore facilitate activation. It is worth noting that the GPI-anchored tyrosine kinase receptor c-Ret is also expressed in $IB4^+$ neurons and translocated to lipid rafts in response to stimulation with its ligand, glial cell-line derived neurotrophic factor (GDNF) (Tansey, Baloh, Milbrandt, & Johnson, 2000). GDNF upregulates $Na_V1.9$ expression and is therefore often added to DRG culture media (Cummins, Black, Dib-Hajj, & Waxman, 2000). Similar mechanisms have been described for other neurotrophic factor receptors, including TrkA stimulation by nerve growth factor (NGF) and TrkB stimulation by brain-derived neurotrophic factor (BDNF) in hippocampal neurons (Suzuki, et al., 2004; Tsui-Pierchala, Encinas, Milbrandt, & Johnson, 2002). Altogether, these insights provide a multitude of potential pathways to influence $Na_V1.9$ function and investigate or address pain perception. In the next section, we will focus on compounds that can directly affect the channel itself.

5. Pharmacological susceptibility of $Na_V1.9$

$Na_V1.9$ is an emerging drug target with promising clinical applications. Moreover, the availability of specific compounds can complement genetic studies and help generate new insights into sensory perception mechanisms. However, for reasons outlined above, the search for subtype-specific ligands is progressing slowly. The two cell lines with a reported stable expression of $Na_V1.9$ were used to identify a series of modulators including well-established anesthetic and antiepileptic drugs (Lin, et al., 2016; Vanoye, et al., 2012). However, these compounds affect virtually all Na_V channel subtypes resulting in a limited use for research purposes and potential side effects when used in the clinic. More molecules were found to affect $Na_V1.9$ function, sometimes fortuitously and often in a non-subtype-specific manner (Figure 4). Mepyramine, a first-generation antihistamine prescribed to patients suffering from urticaria, inhibits a variety of Na_V channel variants, including $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$ (Hao, et al., 2021). Orphenadrine is an anticholinergic agent used mainly to treat Parkinson's disease. However, it is

sometimes employed as an analgesic by itself or in combination with non-steroidal anti-inflammatory drugs. Clinically relevant concentrations of orphenadrine appear to block $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$ currents (Desaphy, et al., 2009). Amitriptyline is a tricyclic antidepressant used to manage various types of chronic pain. Its efficacy has been attributed to inhibiting Na_V channel function. In acutely dissociated trigeminal ganglia and DRG, amitriptyline reduces $Na_V1.9$-mediated currents in a concentration-dependent manner. This compound was also shown to hyperpolarize $Na_V1.9$ steady-state inactivation without affecting voltage-dependent activation (Genevois, et al., 2021; Hur, et al., 2008; Liang, Liu, Zheng, & Yu, 2013). Combined, these reports illustrate the need to continue investigations geared towards uncovering $Na_V1.9$ pharmacology.

A promising approach to identify $Na_V1.9$ modulators may lie in the use of natural compounds such as peptide toxins. Animal venoms are a documented source of subtype-specific Na_V channel modulators and potential lead compounds for therapeutics (Gilchrist, Olivera, & Bosmans, 2014; Herzig, et al., 2020). For example, δ-theraphotoxin-Gr4b (Gr4b), a peptide isolated from the venom of the *Grammostola rosea* spider, slows $Na_V1.9$ fast inactivation without affecting its close relative $Na_V1.8$. Gr4b also alters channel gating to augment window current. Analysis of $Na_V1.8$/$Na_V1.9$ chimeric channels revealed that Gr4b preferentially binds to VSDIII and has additional interactions with VSDIV (Peng, et al., 2021). Another peptide derived from spider venom, HpTx1 from *Heteropoda venatoria*, increases $Na_V1.9$ current density by hampering fast inactivation and shifts channel availability to more depolarized voltages (Zhou, et al., 2020). At similar concentrations, HpTx1 inhibits $Na_V1.7$ currents seemingly without influencing other channel gating parameters. Subsequent to this dual action, HpTx1 induces nocifensive behavior and hypersensitivity to mechanical and thermal stimuli in $Na_V1.7$ knockout mice. Conversely, pain sensations were attenuated in $Na_V1.9$ knockout animals, presumably by inhibiting $Na_V1.7$ activity. Scorpion toxins have also been reported to act on $Na_V1.9$, albeit in a non-specific manner so far. Tf2 from *Tityus fasciolatus* primarily activates $Na_V1.3$ but residual effects were noted on a chimeric construct in which the C-terminal region of $Na_V1.9$ was replaced with that of $Na_V1.4$ to improve expression ($Na_V1.9\_C4$) (Goral, et al., 2015; Israel, et al., 2020). Tf2 marginally hyperpolarizes the activation voltage of this $Na_V1.9$ mutant. The analgesic β-

type scorpion neurotoxin N58A alters gating and reduces current densities of $Na_V1.8$ and $Na_V1.9$ in a rat model of chronic constriction injury. N58A impedes phosphorylation levels of ERK1/2, P38, JNK, and ERK5/CREB pathways in a dose-dependent manner as well as $Na_V1.8$ and $Na_V1.9$ expression (Li, et al., 2021). Antitumor-analgesic peptide (AGAP) isolated from *Buthus martensii* venom exhibits analgesic effects, possibly by reducing $Na_V1.8$ and $Na_V1.9$ current amplitudes (Li, et al., 2016). Six other toxins from scorpion (TsVII, BomIV, AaHII) and spider venom (ProTx-I, HaTx, GrTx-SIA) were shown to interact with chimeric $K_V2.1$ channels that contained toxin-binding motifs from $Na_V1.9$. Of these, TsVII from *Tityus serrulatus* and ProTx-I from *Thrixopelma pruriens* were tested on $Na_V1.9$-mediated currents in DRG and indeed presented a substantial potentiating effect on channel gating (Bosmans, et al., 2011; Gilchrist & Bosmans, 2012).

Other organisms may also produce molecules that act on $Na_V1.9$. For example, melittin, a polypeptide that constitutes ~50% of dry honeybee (*Apis mellifera*) venom, was found to induce tonic AP firing in isolated DRG in the presence of TTX (Figure 4). Markedly, subcutaneous injection of melittin increased $Na_V1.8$ and $Na_V1.9$ mRNA, protein, and current densities. Antisense-mediated knockdown in DRG of $Na_V1.9$, but not $Na_V1.8$, resulted in the inhibition of melittin-induced pain-related behaviors (Yu, et al., 2013). Another example of potential $Na_V1.9$ modulators are ciguatoxins, marine polycyclic polyethers found in certain strains of dinoflagellates that can cause ciguatera disease, a debilitating fish poisoning illness dominated by sensory and neurological disturbances that include cold allodynia as well as pruritus. Local application of Pacific Ciguatoxin-1 (P-CTX-1) onto the skin of human subjects induces a long-lasting, painful axon reflex flare, in part due to the toxin-induced release of calcitonin-gene related peptide (CGRP) from nerve terminals. Interestingly, the absence of $Na_V1.9$ reduces toxin-induced CGRP release by ~40% and may therefore suggest a link to P-CTX-1 efficacy (Touska, et al., 2017). Although the mechanism remains unclear and unsubstantiated, saxitoxin, an alkaloid also found in dinoflagellates and known to cause paralytic shellfish poisoning, was reported to inhibit BDNF-evoked Na^+ currents, which were presumed to be $Na_V1.9$-mediated, in non-neuronal HEK-293 cells co-expressing $Na_V1.9$ and TrkB (Blum, Kafitz, & Konnerth, 2002). Finally, it is worth noting that potential $Na_V1.9$ ligands or lead compounds may also be deduced from

poisonous events. Pyrethroids are a commonly used family of insecticides that can induce neurotoxic effects when humans are chronically exposed to high concentrations. Indeed, a 60-day exposure of rodents to an insecticide cocktail that included permethrin, a type 1 pyrethroid, induced upregulation of $Na_V1.9$ expression but not $Na_V1.8$. Acute application of permethrin also has the ability to increase current amplitudes of $Na_V1.9$ in skin, muscle, and vascular nociceptors (Nutter & Cooper, 2014). Deltamethrin, another pyrethroid, enhances slow inactivation of $Na_V1.7$, $Na_V1.8$, and $Na_V1.9\_C4$ with comparable sensitivities (Bothe & Lampert, 2021).

6. Conclusions and challenges

While relationships between most Na_V channel subtypes and their biological function have been extensively described, the physiological role of $Na_V1.9$ in pain is less understood. Yet, accumulating evidence for a role of $Na_V1.9$ in nociception is being provided by the discovery of human mutations that cause an array of symptoms including gain- or loss-of-pain. Elegant and extensive research using genetically modified rodents substantiated a key role for $Na_V1.9$ in nociceptive pathways, albeit with occasionally conflicting results. However, the use of more refined and standardized rodent models is overcoming hurdles such as discrepancies between experimental conditions. Equally important knowledge can be gained by investigating the mechanistic details that underlie behavioral phenotypes. Compared to other Na_V channel subtypes, $Na_V1.9$ presents distinct biophysical peculiarities, chief among which are its apparent slow gating kinetics. However, $Na_V1.9$ undergoes a remarkable increase in conductance and speeding of kinetics when heating to temperatures that mimic physiological or pathological conditions such as inflammation. Therefore, it is likely that in contrast to earlier notions, $Na_V1.9$ does contribute to the action potential upstroke. Since most electrophysiological experiments are typically executed at room temperature, this opens up the possibility of mechanistic mismatches between observed biophysical and behavioral phenotypes.

Despite these complications and notwithstanding the sometimes limited clinical efficacy of subtype-specific modulators, such as $Na_V1.7$ inhibitors (Mulcahy, et al., 2019), $Na_V1.9$ still constitutes a compelling target

for analgesic drug development. Even with a $Na_V1.9$-specific compound in hand, a series of challenges will need to be addressed. These include, among others, (1) to precisely delineate expression patterns and associated physiological roles to grasp the extent of potential on-target effects; (2) to define the level of selectivity required to avoid off-target effects; (3) to determine the level of $Na_V1.9$ inhibition (or activation) required to produce robust analgesia; and (4) to assess the influence of tissue barriers, non-selective protein or lipid binding, or state-dependent limitations in compound efficacy. Although seemingly a daunting task, there is reason to believe that developing an effective $Na_V1.9$-targeting compound is achievable with the emergence of refined screening approaches and structure-based drug design (Bagal, Marron, Owen, Storer, & Swain, 2015; Johnson, et al., 2022; Lin, et al., 2016; Mulcahy, et al., 2019; Nguyen & Yarov-Yarovoy, 2022; Noreng, et al., 2021; Vanoye, et al., 2012).

7. Conflict of interest statement

The authors declare that there are no conflicts of interest.

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9. Reference list

Ahern, C. A., Payandeh, J., Bosmans, F., & Chanda, B. (2016). The hitchhiker's guide to the voltage-gated sodium channel galaxy. *J Gen Physiol, 147*, 1-24.

Amaya, F., Wang, H., Costigan, M., Allchorne, A. J., Hatcher, J. P., Egerton, J., Stean, T., Morisset, V., Grose, D., Gunthorpe, M. J., Chessell, I. P., Tate, S., Green, P. J., & Woolf, C. J. (2006). The voltage-gated sodium channel Nav1.9 is an effector of peripheral inflammatory pain hypersensitivity. *J Neurosci, 26*, 12852-12860.

Amsalem, M., Poilbout, C., Ferracci, G., Delmas, P., & Padilla, F. (2018). Membrane cholesterol depletion as a trigger of Nav1.9 channel-mediated inflammatory pain. *Embo Journal, 37*.

Bagal, S. K., Marron, B. E., Owen, R. M., Storer, R. I., & Swain, N. A. (2015). Voltage gated sodium channels as drug discovery targets. *Channels, 9*, 360-366.

Bai, Q., Shao, J., Cao, J., Ren, X., Cai, W., Su, S., George, S., Tan, Z., Zang, W., & Dong, T. (2020). Protein kinase C-alpha upregulates sodium channel Nav1.9 in nociceptive dorsal root ganglion neurons in an inflammatory arthritis pain model of rat. *J Cell Biochem, 121*, 768-778.

Baker, M. D., Chandra, S. Y., Ding, Y., Waxman, S. G., & Wood, J. N. (2003). GTP-induced tetrodotoxin-resistant Na+ current regulates excitability in mouse and rat small diameter sensory neurones. *J Physiol, 548*, 373-382.

Barrot, M. (2012). Tests and models of nociception and pain in rodents. *Neuroscience, 211*, 39-50.

Bennett, D. L., Clark, A. J., Huang, J., Waxman, S. G., & Dib-Hajj, S. D. (2019). The Role of Voltage-Gated Sodium Channels in Pain Signaling. *Physiol Rev, 99*, 1079-1151.

Black, J. A., Liu, S., Tanaka, M., Cummins, T. R., & Waxman, S. G. (2004). Changes in the expression of tetrodotoxin-sensitive sodium channels within dorsal root ganglia neurons in inflammatory pain. *Pain, 108*, 237-247.

Blair, N. T., & Bean, B. P. (2002). Roles of tetrodotoxin (TTX)-sensitive Na+ current, TTX-resistant Na+ current, and Ca2+ current in the action potentials of nociceptive sensory neurons. *J Neurosci, 22*, 10277-10290.

Blum, R., Kafitz, K. W., & Konnerth, A. (2002). Neurotrophin-evoked depolarization requires the sodium channel Na(V)1.9. *Nature, 419*, 687-693.

Bonnet, C., Hao, J., Osorio, N., Donnet, A., Penalba, V., Ruel, J., & Delmas, P. (2019). Maladaptive activation of Nav1.9 channels by nitric oxide causes triptan-induced medication overuse headache. *Nat Commun, 10*, 4253.

Bosmans, F., Puopolo, M., Martin-Eauclaire, M. F., Bean, B. P., & Swartz, K. J. (2011). Functional properties and toxin pharmacology of a dorsal root ganglion sodium channel viewed through its voltage sensors. *J Gen Physiol, 138*, 59-72.

Bothe, S. N., & Lampert, A. (2021). The insecticide deltamethrin enhances sodium channel slow inactivation of human Nav1.9, Nav1.8 and Nav1.7. *Toxicol Appl Pharmacol, 428*, 115676.

Campbell, A. P., & Smrcka, A. V. (2018). Targeting G protein-coupled receptor signalling by blocking G proteins. *Nature reviews. Drug discovery, 17*, 789-803.

Cang, C. L., Zhang, H., Zhang, Y. Q., & Zhao, Z. Q. (2009). PKCepsilon-dependent potentiation of TTX-resistant Nav1.8 current by neurokinin-1 receptor activation in rat dorsal root ganglion neurons. *Mol Pain, 5*, 33.

Catterall, W. A., Goldin, A. L., & Waxman, S. G. (2005). International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. *Pharmacol Rev, 57*, 397-409.

Cibert-Goton, V., Lam, C., Lingaya, M., Falcone, Y., Wood, J. N., Bulmer, D. C., & Spiller, R. (2021). Pain Severity Correlates With Biopsy-Mediated Colonic Afferent Activation But Not Psychological Scores in Patients With IBS-D. *Clin Transl Gastroenterol, 12*, e00313.

Cohen, S. P., Vase, L., & Hooten, W. M. (2021). Chronic pain: an update on burden, best practices, and new advances. *Lancet, 397*, 2082-2097.

Coll, M., Allegue, C., Partemi, S., Mates, J., Del Olmo, B., Campuzano, O., Pascali, V., Iglesias, A., Striano, P., Oliva, A., & Brugada, R. (2016). Genetic investigation of sudden unexpected death in epilepsy cohort by panel target resequencing. *Int J Legal Med, 130*, 331-339.

Copel, C., Clerc, N., Osorio, N., Delmas, P., & Mazet, B. (2013). The Nav1.9 channel regulates colonic motility in mice. *Front Neurosci, 7*, 58.

Copel, C., Osorio, N., Crest, M., Gola, M., Delmas, P., & Clerc, N. (2009). Activation of neurokinin 3 receptor increases Na(v)1.9 current in enteric neurons. *J Physiol, 587*, 1461-1479.

Coskun, C., Ocal, I., & Gunay, I. (2021). A Low-Frequency Pulsed Magnetic Field Reduces Neuropathic Pain by Regulating NaV(1.8) and NaV(1.9) Sodium Channels at the Transcriptional Level in Diabetic Rats. *Bioelectromagnetics, 42*, 357-370.

Coste, B., Crest, M., & Delmas, P. (2007). Pharmacological dissection and distribution of NaN/Nav1.9, T-type Ca2+ currents, and mechanically activated cation currents in different populations of DRG neurons. *J Gen Physiol, 129*, 57-77.

Coste, B., Osorio, N., Padilla, F., Crest, M., & Delmas, P. (2004). Gating and modulation of presumptive NaV1.9 channels in enteric and spinal sensory neurons. *Molecular and cellular neurosciences, 26*, 123-134.

Craner, M. J., Klein, J. P., Renganathan, M., Black, J. A., & Waxman, S. G. (2002). Changes of sodium channel expression in experimental painful diabetic neuropathy. *Ann Neurol, 52*, 786-792.

Cummins, T. R., Black, J. A., Dib-Hajj, S. D., & Waxman, S. G. (2000). Glial-derived neurotrophic factor upregulates expression of functional SNS and NaN sodium channels and their currents in axotomized dorsal root ganglion neurons. *J Neurosci, 20*, 8754-8761.

Cummins, T. R., Dib-Hajj, S. D., Black, J. A., Akopian, A. N., Wood, J. N., & Waxman, S. G. (1999). A novel persistent tetrodotoxin-resistant sodium current in SNS-null and wild-type small primary sensory neurons. *J Neurosci, 19*, RC43.

de Lima, F. O., Lauria, P. S. S., do Espirito-Santo, R. F., Evangelista, A. F., Nogueira, T. M. O., Araldi, D., Soares, M. B. P., & Villarreal, C. F. (2022). Unveiling Targets for Treating Postoperative Pain: The Role of the TNF-alpha/p38 MAPK/NF-kappaB/Nav1.8 and Nav1.9 Pathways in the Mouse Model of Incisional Pain. *International Journal of Molecular Sciences, 23*.

Desaphy, J. F., Dipalma, A., De Bellis, M., Costanza, T., Gaudioso, C., Delmas, P., George, A. L., Jr., & Camerino, D. C. (2009). Involvement of voltage-gated sodium channels blockade in the analgesic effects of orphenadrine. *Pain, 142*, 225-235.

Dib-Hajj, S. D., & Waxman, S. G. (2019). Sodium Channels in Human Pain Disorders: Genetics and Pharmacogenomics. *Annu Rev Neurosci, 42*, 87-106.

Ebbinghaus, M., Tuchscherr, L., Segond von Banchet, G., Liebmann, L., Adams, V., Gajda, M., Hubner, C. A., Kurth, I., & Schaible, H. G. (2020). Gain-of-function mutation in SCN11A causes itch and affects neurogenic inflammation and muscle function in Scn11a+/L799P mice. *PLoS One, 15*, e0237101.

Eijkenboom, I., Sopacua, M., Hoeijmakers, J. G. J., de Greef, B. T. A., Lindsey, P., Almomani, R., Marchi, M., Vanoevelen, J., Smeets, H. J. M., Waxman, S. G., Lauria, G., Merkies, I. S. J., Faber, C. G., & Gerrits, M. M. (2019). Yield of peripheral sodium channels gene screening in pure small fibre neuropathy. *J Neurol Neurosurg Psychiatry, 90*, 342-352.

Fjell, J., Hjelmstrom, P., Hormuzdiar, W., Milenkovic, M., Aglieco, F., Tyrrell, L., Dib-Hajj, S., Waxman, S. G., & Black, J. A. (2000). Localization of the tetrodotoxin-resistant sodium channel NaN in nociceptors. *Neuroreport, 11*, 199-202.

Genevois, A. L., Ruel, J., Penalba, V., Hatton, S., Petitfils, C., Ducrocq, M., Principe, P., Dietrich, G., Greco, C., & Delmas, P. (2021). Analgesic Effects of Topical Amitriptyline in Patients With Chemotherapy-Induced Peripheral Neuropathy: Mechanistic Insights From Studies in Mice. *J Pain, 22*, 440-453.

Gilchrist, J., & Bosmans, F. (2012). Animal toxins can alter the function of Nav1.8 and Nav1.9. *Toxins (Basel), 4*, 620-632.

Gilchrist, J., Olivera, B. M., & Bosmans, F. (2014). Animal toxins influence voltage-gated sodium channel function. *Handb Exp Pharmacol, 221*, 203-229.

Ginanneschi, F., Rubegni, A., Moro, F., Volpi, N., Santorelli, F. M., & Rossi, A. (2019). SCN11A variant as possible pain generator in sensory axonal neuropathy. *Neurological Sciences, 40*, 1295-1297.

Goral, R. O., Leipold, E., Nematian-Ardestani, E., & Heinemann, S. H. (2015). Heterologous expression of NaV1.9 chimeras in various cell systems. *Pflugers Arch, 467*, 2423-2435.

Habib, A. M., Wood, J. N., & Cox, J. J. (2015). Sodium channels and pain. *Handb Exp Pharmacol, 227*, 39-56.

Han, C., Yang, Y., de Greef, B. T., Hoeijmakers, J. G., Gerrits, M. M., Verhamme, C., Qu, J., Lauria, G., Merkies, I. S., Faber, C. G., Dib-Hajj, S. D., & Waxman, S. G. (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.

Han, C., Yang, Y., Te Morsche, R. H., Drenth, J. P., Politei, J. M., Waxman, S. G., & Dib-Hajj, S. D. (2017). Familial gain-of-function Na(v)1.9 mutation in a painful channelopathy. *J Neurol Neurosurg Psychiatry, 88*, 233-240.

Hao, J., Brosse, L., Bonnet, C., Ducrocq, M., Padilla, F., Penalba, V., Desplat, A., Ruel, J., & Delmas, P. (2021). The widely used antihistamine mepyramine causes topical pain relief through direct blockade of nociceptor sodium channels. *Faseb Journal, 35*, e22025.

Herzig, V., Cristofori-Armstrong, B., Israel, M. R., Nixon, S. A., Vetter, I., & King, G. F. (2020). Animal toxins - Nature's evolutionary-refined toolkit for basic research and drug discovery. *Biochem Pharmacol, 181*, 114096.

Herzog, R. I., Cummins, T. R., & Waxman, S. G. (2001). Persistent TTX-resistant Na+ current affects resting potential and response to depolarization in simulated spinal sensory neurons. *J Neurophysiol, 86*, 1351-1364.

Hille, B. (2001). *Ion channels of excitable membranes* (3 ed. Vol. 1). MA, USA: Sinauer Associates, Inc.

Hirofuji, S., Yokota, A., Ohno, K., Kinoshita, M., & Neo, M. (2014). Role of sodium channels in recovery of sciatic nerve-stretch injury in rats. *Muscle Nerve, 50*, 425-430.

Hockley, J. R., Boundouki, G., Cibert-Goton, V., McGuire, C., Yip, P. K., Chan, C., Tranter, M., Wood, J. N., Nassar, M. A., Blackshaw, L. A., Aziz, Q., Michael, G. J., Baker, M. D., Winchester, W. J., Knowles, C. H., & Bulmer, D. C. (2014). Multiple roles for NaV1.9 in the activation of visceral afferents by noxious inflammatory, mechanical, and human disease-derived stimuli. *Pain, 155*, 1962-1975.

Hockley, J. R., Tranter, M. M., McGuire, C., Boundouki, G., Cibert-Goton, V., Thaha, M. A., Blackshaw, L. A., Michael, G. J., Baker, M. D., Knowles, C. H., Winchester, W. J., & Bulmer, D. C. (2016). P2Y Receptors Sensitize Mouse and Human Colonic Nociceptors. *J Neurosci, 36*, 2364-2376.

Hockley, J. R., Winchester, W. J., & Bulmer, D. C. (2016). The voltage-gated sodium channel NaV 1.9 in visceral pain. *Neurogastroenterol Motil, 28*, 316-326.

Hoffmann, T., Kistner, K., Carr, R. W., Nassar, M. A., Reeh, P. W., & Weidner, C. (2017). Reduced excitability and impaired nociception in peripheral unmyelinated fibers from Nav1.9-null mice. *Pain, 158*, 58-67.

Hoffmann, T., Klemm, F., T, I. K., Sauer, S. K., Kistner, K., Riedl, B., Raboisson, P., Luo, L., Babes, A., Kocher, L., Carli, G., Fischer, M. J. M., & Reeh, P. W. (2022). The formalin test does not probe inflammatory pain but excitotoxicity in rodent skin. *Physiol Rep, 10*, e15194.

Huang, J., Estacion, M., Zhao, P., Dib-Hajj, F. B., Schulman, B., Abicht, A., Kurth, I., Brockmann, K., Waxman, S. G., & Dib-Hajj, S. D. (2019). A Novel Gain-of-Function Nav1.9 Mutation in a Child With Episodic Pain. *Front Neurosci, 13*, 918.

Huang, J., Han, C., Estacion, M., Vasylyev, D., Hoeijmakers, J. G., Gerrits, M. M., Tyrrell, L., Lauria, G., Faber, C. G., Dib-Hajj, S. D., Merkies, I. S., Waxman, S. G., & Group, P. S. (2014). Gain-of-function mutations in sodium channel Na(v)1.9 in painful neuropathy. *Brain, 137*, 1627-1642.

Huang, J., Vanoye, C. G., Cutts, A., Goldberg, Y. P., Dib-Hajj, S. D., Cohen, C. J., Waxman, S. G., & George, A. L., Jr. (2017). Sodium channel NaV1.9 mutations associated with insensitivity to pain dampen neuronal excitability. *J Clin Invest, 127*, 2805-2814.

Hur, Y. K., Choi, I. S., Cho, J. H., Park, E. J., Choi, J. K., Choi, B. J., & Jang, I. S. (2008). Effects of carbamazepine and amitriptyline on tetrodotoxinresistant Na+ channels in immature rat trigeminal ganglion neurons. *Archives of Pharmacal Research, 31*, 178-182.

Israel, M. R., Dash, T. S., Bothe, S. N., Robinson, S. D., Deuis, J. R., Craik, D. J., Lampert, A., Vetter, I., & Durek, T. (2020). Characterization of Synthetic Tf2 as a Na(V)1.3 Selective Pharmacological Probe. *Biomedicines, 8*.

Ito, A., Takeda, M., Yoshimura, T., Komatsu, T., Ohno, T., Kuriyama, H., Matsuda, A., & Yoshimura, M. (2012). Anti-hyperalgesic effects of calcitonin on neuropathic pain interacting with its peripheral receptors. *Mol Pain, 8*, 42.

Johnson, J. P., Focken, T., Khakh, K., Tari, P. K., Dube, C., Goodchild, S. J., Andrez, J. C., Bankar, G., Bogucki, D., Burford, K., Chang, E. E., Chowdhury, S., Dean, R., de Boer, G., Decker, S., Dehnhardt, C., Feng, M., Gong, W., Grimwood, M., Hasan, A., Hussainkhel, A., Jia, Q., Lee, S. I., Li, J., Lin, S. P., Lindgren, A., Lofstrand, V., Mezeyova, J., Namdari, R., Nelkenbrecher, K., Shuart, N. G., Sojo, L., Sun, S. Y., Taron, M., Waldbrook, M., Weeratunge, D., Wesolowski, S., Williams, A., Wilson, M., Xie, Z. W., Yoo, R., Young, C., Zenova, A., Zhang, W., Cutts, A. J., Sherrington, R. P., Pimstone, S. N., Winquist, R., Cohen, C. J., Empfield, J. R., & Chanda, B. (2022). NBI-921352, a first-in-class, Na(V)1.6 selective, sodium channel inhibitor that prevents seizures in Scn8a gain-of-function mice, and wild-type mice and rats. *Elife, 11*.

Kabata, R., Okuda, H., Noguchi, A., Kondo, D., Fujiwara, M., Hata, K., Kato, Y., Ishikawa, K., Tanaka, M., Sekine, Y., Hishikawa, N., Mizukami, T., Ito, J., Akasaka, M., Sakurai, K., Yoshida, T., Minoura, H., Hayashi, T., Inoshita, K., Matsuyama, M., Kinjo, N., Cao, Y., Inoue, S., Kobayashi, H., Harada, K. H., Youssefian, S., Takahashi, T., &

Koizumi, A. (2018). Familial episodic limb pain in kindreds with novel Nav1.9 mutations. *PLoS One, 13*, e0208516.

Kahlig, K. M., Rhodes, T. H., Pusch, M., Freilinger, T., Pereira-Monteiro, J. M., Ferrari, M. D., van den Maagdenberg, A. M., Dichgans, M., & George, A. L., Jr. (2008). Divergent sodium channel defects in familial hemiplegic migraine. *Proc Natl Acad Sci U S A, 105*, 9799-9804.

Kakimura, J., Zheng, T., Uryu, N., & Ogata, N. (2010). Regulation of the spontaneous augmentation of Na(V)1.9 in mouse dorsal root ganglion neurons: effect of PKA and PKC pathways. *Mar Drugs, 8*, 728-740.

King, M. K., Leipold, E., Goehringer, J. M., Kurth, I., & Challman, T. D. (2017). Pain insensitivity: distal S6-segment mutations in Na(V)1.9 emerge as critical hotspot. *Neurogenetics, 18*, 179-181.

Kleggetveit, I. P., Schmidt, R., Namer, B., Salter, H., Helas, T., Schmelz, M., & Jorum, E. (2016). Pathological nociceptors in two patients with erythromelalgia-like symptoms and rare genetic Nav 1.9 variants. *Brain Behav, 6*, e00528.

Kurowski, P., Gawlak, M., & Szulczyk, P. (2015). Muscarinic receptor control of pyramidal neuron membrane potential in the medial prefrontal cortex (mPFC) in rats. *Neuroscience, 303*, 474-488.

Leipold, E., Hanson-Kahn, A., Frick, M., Gong, P., Bernstein, J. A., Voigt, M., Katona, I., Oliver Goral, R., Altmuller, J., Nurnberg, P., Weis, J., Hubner, C. A., Heinemann, S. H., & Kurth, I. (2015). Cold-aggravated pain in humans caused by a hyperactive NaV1.9 channel mutant. *Nat Commun, 6*, 10049.

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

Leng, X. R., Qi, X. H., Zhou, Y. T., & Wang, Y. P. (2017). Gain-of-function mutation p.Arg225Cys in SCN11A causes familial episodic pain and contributes to essential tremor. *Journal of Human Genetics, 62*, 641-646.

Leo, S., D'Hooge, R., & Meert, T. (2010). Exploring the role of nociceptor-specific sodium channels in pain transmission using Nav1.8 and Nav1.9 knockout mice. *Behav Brain Res, 208*, 149-157.

Li, C. L., Liu, X. F., Li, G. X., Ban, M. Q., Chen, J. Z., Cui, Y., Zhang, J. H., & Wu, C. F. (2016). Antinociceptive Effects of AGAP, a Recombinant Neurotoxic Polypeptide: Possible Involvement of the Tetrodotoxin-Resistant Sodium Channels in Small Dorsal Root Ganglia Neurons. *Front Pharmacol, 7*, 496.

Li, C. L., Yang, R., Sun, Y., Feng, Y., & Song, Y. B. (2021). N58A Exerts Analgesic Effect on Trigeminal Neuralgia by Regulating the MAPK Pathway and Tetrodotoxin-Resistant Sodium Channel. *Toxins (Basel), 13*.

Liang, J., Liu, X., Zheng, J., & Yu, S. (2013). Effect of amitriptyline on tetrodotoxin-resistant Nav1.9 currents in nociceptive trigeminal neurons. *Mol Pain, 9*, 31.

Lin, Z., Santos, S., Padilla, K., Printzenhoff, D., & Castle, N. A. (2016). Biophysical and Pharmacological Characterization of Nav1.9 Voltage Dependent Sodium Channels Stably Expressed in HEK-293 Cells. *PLoS One, 11*, e0161450.

Lischka, A., Lassuthova, P., Cakar, A., Record, C. J., Van Lent, J., Baets, J., Dohrn, M. F., Senderek, J., Lampert, A., Bennett, D. L., Wood, J. N., Timmerman, V., Hornemann, T., Auer-Grumbach, M., Parman, Y., Hubner, C. A., Elbracht, M., Eggermann, K., Geoffrey Woods, C., Cox, J. J., Reilly, M. M., & Kurth, I. (2022). Genetic pain loss disorders. *Nat Rev Dis Primers, 8*, 41.

Liu, C. J., Dib-Hajj, S. D., Black, J. A., Greenwood, J., Lian, Z., & Waxman, S. G. (2001). Direct interaction with contactin targets voltage-gated sodium channel Na(v)1.9/NaN to the cell membrane. *J Biol Chem, 276*, 46553-46561.

Liu, M., Zhong, J., Xia, L., Dou, N., & Li, S. (2019). The expression of voltage-gated sodium channels in trigeminal nerve following chronic constriction injury in rats. *Int J Neurosci, 129*, 955-962.

Lolignier, S., Amsalem, M., Maingret, F., Padilla, F., Gabriac, M., Chapuy, E., Eschalier, A., Delmas, P., & Busserolles, J. (2011). Nav1.9 channel contributes to mechanical and heat pain hypersensitivity induced by subacute and chronic inflammation. *PLoS One, 6*, e23083.

Luiz, A. P., Kopach, O., Santana-Varela, S., & Wood, J. N. (2015). The role of Nav1.9 channel in the development of neuropathic orofacial pain associated with trigeminal neuralgia. *Mol Pain, 11*, 72.

Ma, T., Li, L., Chen, R., Yang, L., Sun, H., Du, S., Xu, X., Cao, Z., Zhang, X., Zhang, L., Shi, X., & Liu, J. Y. (2022). Protein arginine methyltransferase 7 modulates neuronal excitability by interacting with NaV1.9. *Pain, 163*, 753-764.

Maarbjerg, S., Di Stefano, G., Bendtsen, L., & Cruccu, G. (2017). Trigeminal neuralgia - diagnosis and treatment. *Cephalalgia, 37*, 648-657.

Maingret, F., Coste, B., Padilla, F., Clerc, N., Crest, M., Korogod, S. M., & Delmas, P. (2008). Inflammatory mediators increase Nav1.9 current and excitability in nociceptors through a coincident detection mechanism. *J Gen Physiol, 131*, 211-225.

Martinez, V., & Melgar, S. (2008). Lack of colonic-inflammation-induced acute visceral hypersensitivity to colorectal distension in Na(v)1.9 knockout mice. *Eur J Pain, 12*, 934-944.

Maruyama, H., Yamamoto, M., Matsutomi, T., Zheng, T., Nakata, Y., Wood, J. N., & Ogata, N. (2004). Electrophysiological characterization of the tetrodotoxin-resistant Na+ channel, Na(v)1.9, in mouse dorsal root ganglion neurons. *Pflugers Arch, 449*, 76-87.

Matsubara, Y., Okuda, H., Harada, K. H., Youssefian, S., & Koizumi, A. (2021). Mechanical allodynia triggered by cold exposure in mice with the Scn11a p.R222S mutation: a novel model of drug therapy for neuropathic pain related to Na(V)1.9. *Naunyn Schmiedebergs Arch Pharmacol, 394*, 299-306.

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

Minett, M. S., Falk, S., Santana-Varela, S., Bogdanov, Y. D., Nassar, M. A., Heegaard, A. M., & Wood, J. N. (2014). Pain without nociceptors? Nav1.7-independent pain mechanisms. *Cell Rep, 6*, 301-312.

Mogil, J. S. (2019). The translatability of pain across species. *Philos Trans R Soc Lond B Biol Sci, 374*, 20190286.

Mulcahy, J. V., Pajouhesh, H., Beckley, J. T., Delwig, A., Du Bois, J., & Hunter, J. C. (2019). Challenges and Opportunities for Therapeutics Targeting the Voltage-Gated Sodium Channel Isoform Na(V)1.7. *Journal of Medicinal Chemistry, 62*, 8695-8710.

Neishabouri, A., & Faisal, A. A. (2014). Saltatory conduction in unmyelinated axons: clustering of Na(+) channels on lipid rafts enables micro-saltatory conduction in C-fibers. *Front Neuroanat, 8*, 109.

Nguyen, P. T., & Yarov-Yarovoy, V. (2022). Towards Structure-Guided Development of Pain Therapeutics Targeting Voltage-Gated Sodium Channels. *Frontiers in Pharmacology, 13*.

Noreng, S., Li, T., & Payandeh, J. (2021). Structural Pharmacology of Voltage-Gated Sodium Channels. *J Mol Biol, 433*, 166967.

Nutter, T. J., & Cooper, B. Y. (2014). Persistent modification of Nav1.9 following chronic exposure to insecticides and pyridostigmine bromide. *Toxicol Appl Pharmacol, 277*, 298-309.

Ohno, K., Yokota, A., Hirofuji, S., Kanbara, K., Ohtsuka, H., & Kinoshita, M. (2010). Altered expression of sodium channel distribution in the dorsal root ganglion after gradual elongation of rat sciatic nerves. *J Orthop Res, 28*, 481-486.

Okuda, H., Noguchi, A., Kobayashi, H., Kondo, D., Harada, K. H., Youssefian, S., Shioi, H., Kabata, R., Domon, Y., Kubota, K., Kitano, Y., Takayama, Y., Hitomi, T., Ohno, K., Saito, Y., Asano, T., Tominaga, M., Takahashi, T., & Koizumi, A. (2016). Infantile Pain Episodes Associated with Novel Nav1.9 Mutations in Familial Episodic Pain Syndrome in Japanese Families. *PLoS One, 11*, e0154827.

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.

Ostman, J. A., Nassar, M. A., Wood, J. N., & Baker, M. D. (2008). GTP up-regulated persistent Na+ current and enhanced nociceptor excitability require NaV1.9. *J Physiol, 586*, 1077-1087.

Peng, S., Chen, M., Xiao, Z., Xiao, X., Luo, S., Liang, S., Zhou, X., & Liu, Z. (2021). A Novel Spider Toxin Inhibits Fast Inactivation of the Na(v)1.9 Channel by Binding to Domain III and Domain IV Voltage Sensors. *Front Pharmacol, 12*, 778534.

Phatarakijnirund, V., Mumm, S., McAlister, W. H., Novack, D. V., Wenkert, D., Clements, K. L., & Whyte, M. P. (2016). Congenital insensitivity to pain: Fracturing without apparent skeletal pathobiology caused by an

autosomal dominant, second mutation in SCN11A encoding voltage-gated sodium channel 1.9. *Bone, 84*, 289-298.

Poliak, S., & Peles, E. (2003). The local differentiation of myelinated axons at nodes of Ranvier. *Nature Reviews Neuroscience, 4*, 968-980.

Poojary, S., Jaiswal, S., Shah, K. S., & Bhalala, K. B. (2020). Sisters with No Pain, No Tears: A Report of a New Variant of Hereditary Sensory and Autonomic Neuropathy (Type IX) Caused by a Novel SCN11A Mutation. *Indian Journal of Dermatology, 65*, 299-303.

Porreca, F., Lai, J., Bian, D., Wegert, S., Ossipov, M. H., Eglen, R. M., Kassotakis, L., Novakovic, S., Rabert, D. K., Sangameswaran, L., & Hunter, J. C. (1999). A comparison of the potential role of the tetrodotoxin-insensitive sodium channels, PN3/SNS and NaN/SNS2, in rat models of chronic pain. *Proc Natl Acad Sci U S A, 96*, 7640-7644.

Priest, B. T., Murphy, B. A., Lindia, J. A., Diaz, C., Abbadie, C., Ritter, A. M., Liberator, P., Iyer, L. M., Kash, S. F., Kohler, M. G., Kaczorowski, G. J., MacIntyre, D. E., & Martin, W. J. (2005). Contribution of the tetrodotoxin-resistant voltage-gated sodium channel NaV1.9 to sensory transmission and nociceptive behavior. *Proc Natl Acad Sci U S A, 102*, 9382-9387.

Pristera, A., Baker, M. D., & Okuse, K. (2012). Association between tetrodotoxin resistant channels and lipid rafts regulates sensory neuron excitability. *PLoS One, 7*, e40079.

Qiu, F., Li, Y., Fu, Q., Fan, Y. Y., Zhu, C., Liu, Y. H., & Mi, W. D. (2016). Stromal Cell-Derived Factor 1 Increases Tetrodotoxin-Resistant Sodium Currents Nav1.8 and Nav1.9 in Rat Dorsal Root Ganglion Neurons via Different Mechanisms. *Neurochem Res, 41*, 1587-1603.

Raja, S. N., Carr, D. B., Cohen, M., Finnerup, N. B., Flor, H., Gibson, S., Keefe, F. J., Mogil, J. S., Ringkamp, M., Sluka, K. A., Song, X. J., Stevens, B., Sullivan, M. D., Tutelman, P. R., Ushida, T., & Vader, K. (2020). The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. *Pain, 161*, 1976-1982.

Ri-Ge-le, A., Guo, Z. L., Wang, Q., Zhang, B. J., Kong, D. W., Yang, W. Q., Yu, Y. B., & Zhang, L. (2018). Tanshinone IIA Improves Painful Diabetic Neuropathy by Suppressing the Expression and Activity of Voltage-Gated Sodium Channel in Rat Dorsal Root Ganglia. *Exp Clin Endocrinol Diabetes, 126*, 632-639.

Rugiero, F., Mistry, M., Sage, D., Black, J. A., Waxman, S. G., Crest, M., Clerc, N., Delmas, P., & Gola, M. (2003). Selective expression of a persistent tetrodotoxin-resistant Na+ current and NaV1.9 subunit in myenteric sensory neurons. *J Neurosci, 23*, 2715-2725.

Rush, A. M., Craner, M. J., Kageyama, T., Dib-Hajj, S. D., Waxman, S. G., & Ranscht, B. (2005). Contactin regulates the current density and axonal expression of tetrodotoxin-resistant but not tetrodotoxin-sensitive sodium channels in DRG neurons. *Eur J Neurosci, 22*, 39-49.

Rush, A. M., & Waxman, S. G. (2004). PGE2 increases the tetrodotoxin-resistant Nav1.9 sodium current in mouse DRG neurons via G-proteins. *Brain Res, 1023*, 264-271.

Salvatierra, J., Diaz-Bustamante, M., Meixiong, J., Tierney, E., Dong, X., & Bosmans, F. (2018). A disease mutation reveals a role for NaV1.9 in acute itch. *J Clin Invest, 128*, 5434-5447.

Sambuughin, N., Ren, M., Capacchione, J. F., Mungunsukh, O., Chuang, K., Horkayne-Szakaly, I., O'Connor, F. G., & Deuster, P. A. (2018). Multifactorial Origin of Exertional Rhabdomyolysis, Recurrent Hematuria, and Episodic Pain in a Service Member with Sickle Cell Trait. *Case Rep Genet, 2018*, 6898546.

Scroggs, R. S. (2012). The distribution of low-threshold TTX-resistant Na(+) currents in rat trigeminal ganglion cells. *Neuroscience, 222*, 205-214.

Sizova, D. V., Huang, J., Akin, E. J., Estacion, M., Gomis-Perez, C., Waxman, S. G., & Dib-Hajj, S. D. (2020). A 49-residue sequence motif in the C terminus of Nav1.9 regulates trafficking of the channel to the plasma membrane. *J Biol Chem, 295*, 1077-1090.

Sleeper, A. A., Cummins, T. R., Dib-Hajj, S. D., Hormuzdiar, W., Tyrrell, L., Waxman, S. G., & Black, J. A. (2000). Changes in expression of two tetrodotoxin-resistant sodium channels and their currents in dorsal root ganglion neurons after sciatic nerve injury but not rhizotomy. *J Neurosci, 20*, 7279-7289.

Strickland, I. T., Martindale, J. C., Woodhams, P. L., Reeve, A. J., Chessell, I. P., & McQueen, D. S. (2008). Changes in the expression of NaV1.7, NaV1.8 and NaV1.9 in a distinct population of dorsal root ganglia innervating the rat knee joint in a model of chronic inflammatory joint pain. *Eur J Pain, 12*, 564-572.

Sukhanova, K. Y., Koirala, A., & Elmslie, K. S. (2022). Na(V)1.9 current in muscle afferent neurons is enhanced by substances released during muscle activity. *J Neurophysiol, 128*, 739-750.

Sun, J., Duan, G., Li, N., Guo, S., Zhang, Y., Ying, Y., Zhang, M., Wang, Q., Liu, J. Y., & Zhang, X. (2017). SCN11A variants may influence postoperative pain sensitivity after gynecological surgery in Chinese Han female patients. *Medicine (Baltimore), 96*, e8149.

Suzuki, S., Numakawa, T., Shimazu, K., Koshimizu, H., Hara, T., Hatanaka, H., Mei, L., Lu, B., & Kojima, M. (2004). BDNF-induced recruitment of TrkB receptor into neuronal lipid rafts: roles in synaptic modulation. *J Cell Biol, 167*, 1205-1215.

Tansey, M. G., Baloh, R. H., Milbrandt, J., & Johnson, E. M., Jr. (2000). GFRalpha-mediated localization of RET to lipid rafts is required for effective downstream signaling, differentiation, and neuronal survival. *Neuron, 25*, 611-623.

Todd, A. J. (2010). Neuronal circuitry for pain processing in the dorsal horn. *Nature Reviews Neuroscience, 11*, 823-836.

Touska, F., Sattler, S., Malsch, P., Lewis, R. J., Reeh, P. W., & Zimmermann, K. (2017). Ciguatoxins Evoke Potent CGRP Release by Activation of Voltage-Gated Sodium Channel Subtypes Na(V)1.9, Na(V)1.7 and Na(V)1.1. *Mar Drugs, 15*.

Touska, F., Turnquist, B., Vlachova, V., Reeh, P. W., Leffler, A., & Zimmermann, K. (2018). Heat-resistant action potentials require TTX-resistant sodium channels Na(V)1.8 and Na(V)1.9. *J Gen Physiol, 150*, 1125-1144.

Tseng, P. Y., Zheng, Q., Li, Z., & Dong, X. (2019). MrgprX1 Mediates Neuronal Excitability and Itch Through Tetrodotoxin-Resistant Sodium Channels. *Itch (Philadelphia, Pa.), 4*, e28.

Tsui-Pierchala, B. A., Encinas, M., Milbrandt, J., & Johnson, E. M., Jr. (2002). Lipid rafts in neuronal signaling and function. *Trends Neurosci, 25*, 412-417.

Vanoye, C. G., Ehring, G. R., & George, A. L. (2012). Functional Analysis of Stably Expressed Human Nav1.9. *Biophysical Journal, 102*, 527a-527a.

Velazquez, K. T., Mohammad, H., & Sweitzer, S. M. (2007). Protein kinase C in pain: involvement of multiple isoforms. *Pharmacol Res, 55*, 578-589.

Vijayaragavan, K., Boutjdir, M., & Chahine, M. (2004). Modulation of Nav1.7 and Nav1.8 peripheral nerve sodium channels by protein kinase A and protein kinase C. *J Neurophysiol, 91*, 1556-1569.

Willis, W. D., & Westlund, K. N. (1997). Neuroanatomy of the pain system and of the pathways that modulate pain. *Journal of Clinical Neurophysiology, 14*, 2-31.

Woods, C. G., Babiker, M. O., Horrocks, I., Tolmie, J., & Kurth, I. (2015). The phenotype of congenital insensitivity to pain due to the NaV1.9 variant p.L811P. *Eur J Hum Genet, 23*, 561-563.

Wu, D. F., Chandra, D., McMahon, T., Wang, D., Dadgar, J., Kharazia, V. N., Liang, Y. J., Waxman, S. G., Dib-Hajj, S. D., & Messing, R. O. (2012). PKCepsilon phosphorylation of the sodium channel NaV1.8 increases channel function and produces mechanical hyperalgesia in mice. *J Clin Invest, 122*, 1306-1315.

Xu, W., Zhang, J., Wang, Y., Wang, L., & Wang, X. (2016). Changes in the expression of voltage-gated sodium channels Nav1.3, Nav1.7, Nav1.8, and Nav1.9 in rat trigeminal ganglia following chronic constriction injury. *Neuroreport, 27*, 929-934.

Yang, L., Li, L., Tang, H., Ma, T., Li, Y., Zhang, X., Shi, X., & Liu, J. Y. (2020). Alcohol-aggravated episodic pain in humans with SCN11A mutation and ALDH2 polymorphism. *Pain, 161*, 1470-1482.

Yu, Y. Q., Zhao, F., Guan, S. M., & Chen, J. (2011). Antisense-mediated knockdown of Na(V)1.8, but not Na(V)1.9, generates inhibitory effects on complete Freund's adjuvant-induced inflammatory pain in rat. *PLoS One, 6*, e19865.

Yu, Y. Q., Zhao, Z. Y., Chen, X. F., Xie, F., Yang, Y., & Chen, J. (2013). Activation of tetrodotoxin-resistant sodium channel NaV1.9 in rat primary sensory neurons contributes to melittin-induced pain behavior. *Neuromolecular Med, 15*, 209-217.

Zhang, F., Wang, Y., Liu, Y., Han, H., Zhang, D., Fan, X., Du, X., Gamper, N., & Zhang, H. (2019). Transcriptional Regulation of Voltage-Gated Sodium Channels Contributes to GM-CSF-Induced Pain. *J Neurosci, 39*, 5222-5233.

Zhang, X., Priest, B. T., Belfer, I., & Gold, M. S. (2017). Voltage-gated Na(+) currents in human dorsal root ganglion neurons. *Elife, 6*.

Zhang, X. Y., Wen, J., Yang, W., Wang, C., Gao, L., Zheng, L. H., 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, J. Y. (2013). Gain-of-function mutations in SCN11A cause familial episodic pain. *American Journal of Human Genetics, 93*, 957-966.

Zhang, X. Y., Wu, X., Zhang, P., & Gan, Y. H. (2022). Prolonged PGE(2) treatment increased TTX-sensitive but not TTX-resistant sodium current in trigeminal ganglionic neurons. *Neuropharmacology, 215*, 109156.

Zhao, C., Jin, J., Hu, H., Zhou, X., & Shi, X. (2022). The Gain-of-Function R222S Variant in Scn11a Contributes to Visceral Hyperalgesia and Intestinal Dysmotility in Scn11a (R222S/R222S) Mice. *Front Neurol, 13*, 856459.

Zhou, X., Ma, T., Yang, L., Peng, S., Li, L., Wang, Z., Xiao, Z., Zhang, Q., Wang, L., Huang, Y., Chen, M., Liang, S., Zhang, X., Liu, J. Y., & Liu, Z. (2020). Spider venom-derived peptide induces hyperalgesia in Na(v)1.7 knockout mice by activating Na(v)1.9 channels. *Nat Commun, 11*, 2293.

Zhou, X., Xiao, Z., Xu, Y., Zhang, Y., Tang, D., Wu, X., Tang, C., Chen, M., Shi, X., Chen, P., Liang, S., & Liu, Z. (2017). Electrophysiological and Pharmacological Analyses of Na(v)1.9 Voltage-Gated Sodium Channel by Establishing a Heterologous Expression System. *Front Pharmacol, 8*, 852.

10. Figures and legends

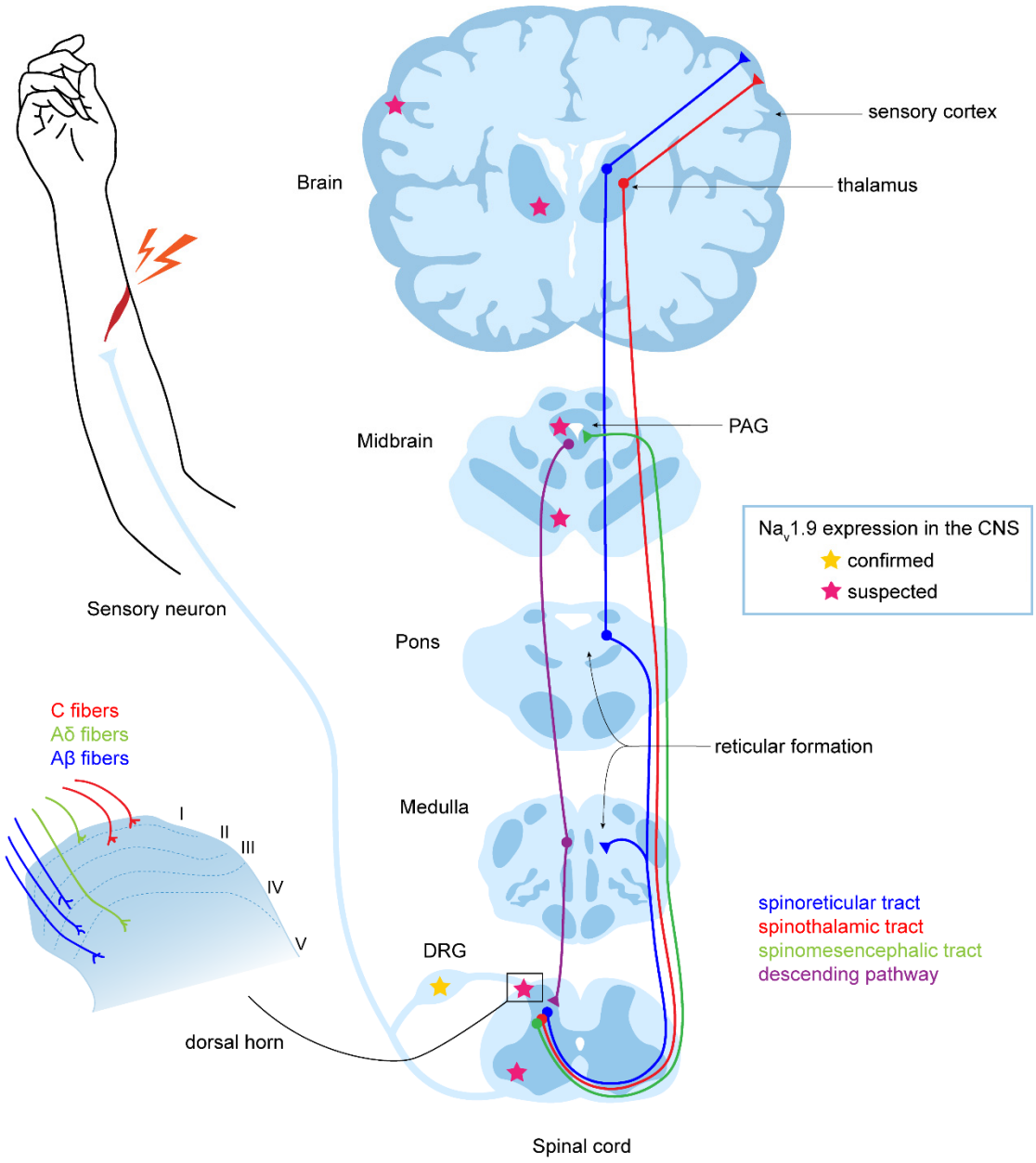

Figure 1: Overview of pain pathways

Sensory neurons transmit pain signals - which are detected at the site of injury - through unmyelinated C-fibers (red) and myelinated Aδ- (green) and Aβ- (blue) fibers. The cell bodies of these neurons cluster in the dorsal root ganglion (DRG) and the axons arborize in laminae I-V of the dorsal root of the spinal cord, where they form synapses with cell bodies of the spinoreticular (blue), spinothalamic (red), and spinomesencephalic (green) tract.

Pain modulation starts in the periaqueductal gray (PAG) and follows a descending pathway (purple) which projects back to the dorsal horn. $Na_V1.9$ is functionally expressed in DRGs. In addition, RNA sequencing and mouse studies allude to expression in various regions of the central nervous system (indicated in the figure).

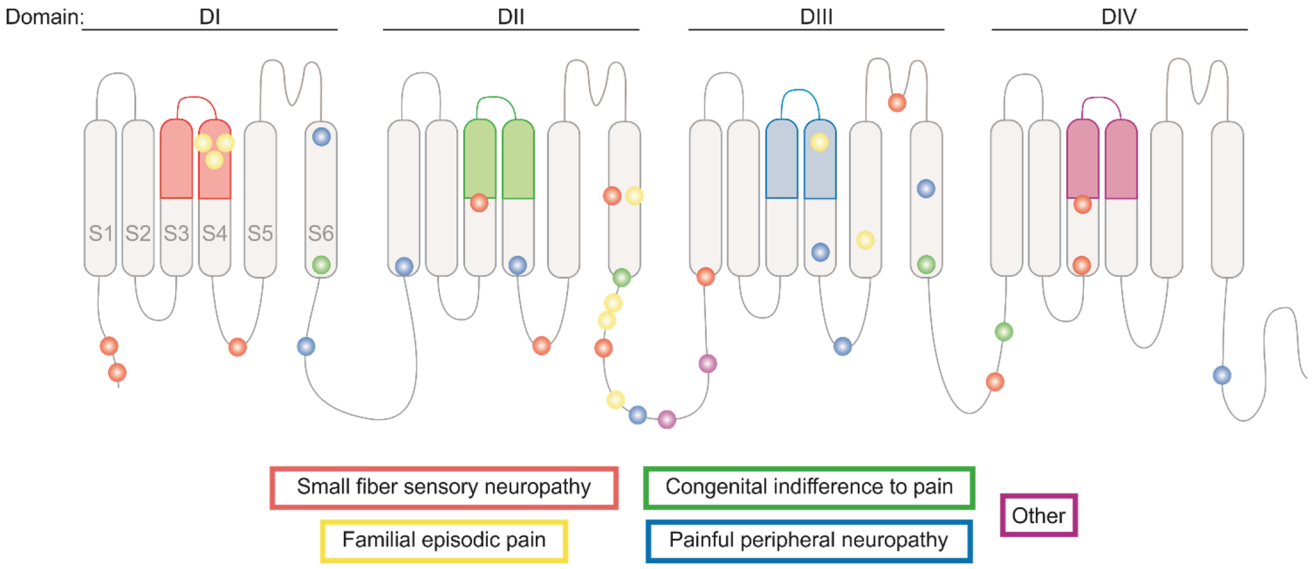

Figure 2: Synopsis of *SCN11A* mutations identified in humans

Na_V channels consist of four domains (DI-DIV), each of these contain six transmembrane segments (S1-S6) of which S1-S4 constitute the voltage-sensing domain (VSD). Mutations are divided in categories based on their phenotype: small fiber neuropathy (red), congenital indifference to pain (green), familial episodic pain (yellow), painful peripheral neuropathy (blue) and other phenotypes (purple). See Table 1 for more information about individual mutations. Interesting to note is the apparent hot spot of disease-causing mutations in the intracellular loop between DII and DIII.

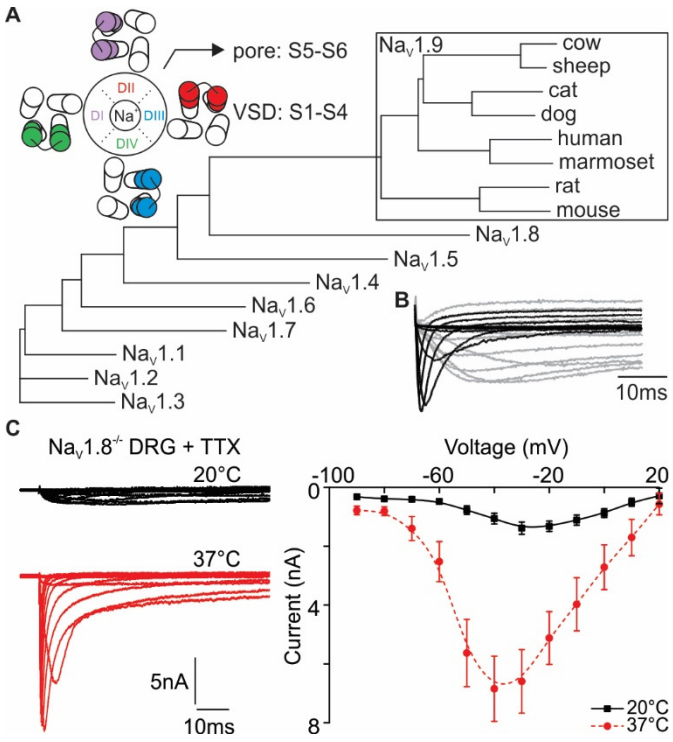

Figure 3: Biophysical properties of Na$_V$1.9

A, Illustration of the global organization of a pseudo-tetrameric Na$_V$ channel showing DI-DIV organized in a pore region surrounded by a clockwise arrangement of the corresponding voltage-sensing domains (VSDs). The phylogenetic relationship between the nine human Na$_V$ channel subtypes is also shown. Square highlights the amino acid similarity between Na$_V$1.9 orthologues. **B,** TTX-resistant Na^+ currents present in a DRG from a wild type C57BL/6J mouse with Na$_V$1.8 traces shown in black and the much slower Na$_V$1.9 traces in grey. Currents were evoked by 10mV step depolarizations from a holding potential of -100mV. **C**, Na$_V$1.9 currents recorded from small-diameter DRG from Na$_V$1.8$^{-/-}$ C57BL/6J mice, acquired with a fluoride-based pipette solution at 20°C (black) and 37°C (red) and in the presence of 1500nM TTX (left). The corresponding current-voltage relationship is shown on the right. Data are represented as means ± SEM.

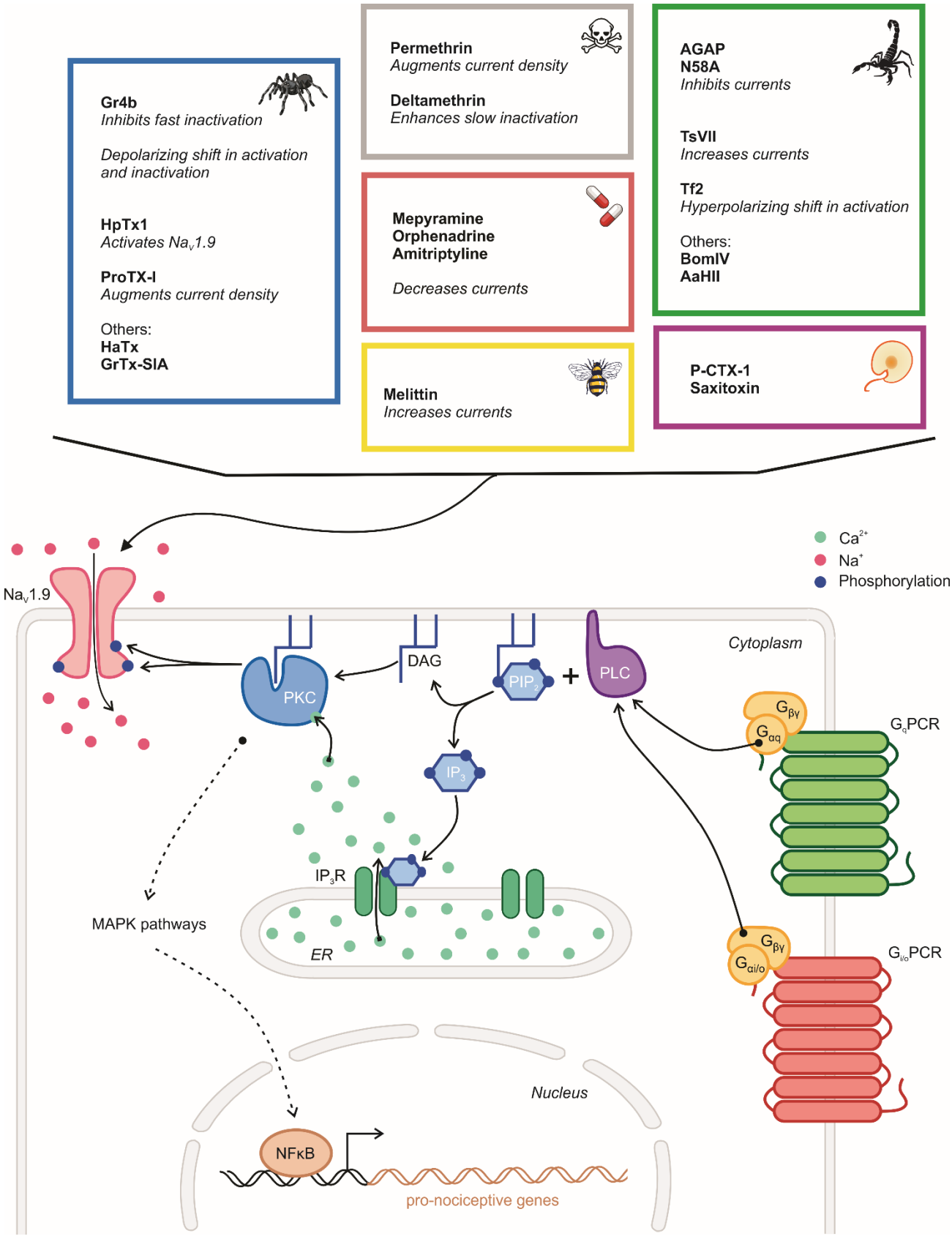
Gr4b
Inhibits fast inactivation
Depolarizing shift in activation and inactivation
HpTx1
Activates Nav1.9
ProTX-I
Augments current density
Others:
HaTx
GrTx-SIA
Permethrin
Augments current density
Deltamethrin
Enhances slow inactivation
Mepyramine
Orphenadrine
Amitriptyline
Decreases currents
Melittin
Increases currents
AGAP
N58A
Inhibits currents
TsVII
Increases currents
Tf2
Hyperpolarizing shift in activation
Others:
BomIV
AaHII
P-CTX-1
Saxitoxin
Ca2+
Na+
Phosphorylation
NaV1.9
PKC
DAG
PIP2
PLC
Cytoplasm
Gβγ
Gαq
GqPCR
IP3
IP3R
ER
MAPK pathways
Gβγ
Gαi/o
Gi/oPCR
Nucleus
NFκB
pro-nociceptive genes

Figure 4: Mediators of $Na_V1.9$ function

Top: Exogenous substances that interact with $Na_V1.9$. Natural toxins, therapeutics, and insecticides can interact with $Na_V1.9$ to influence function. Spider venom toxins can have major (Gr4b, HpTx1 and ProTX-I) or subtle (HaTX and GrTX-SIA) effects on $Na_V1.9$. A similar pattern has been observed for scorpion venom toxins (major: AGAP, N58A, TsVII and Tf2, minor: BomVI and AaHII). Assays on $Na_V1.9$ knockout mice and cells expressing antisense *SCN11A* mRNA suggest that toxins produced by dinoflagellates can also interact with $Na_V1.9$. **Bottom:** $Na_V1.9$ modulation by GPCRs. Stimulation of both Gi/o and Gq subunits activate phospholipase C (PLC), which in turn splits phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol triphosphate (IP3). DAG activates PKC, and this effect is enhanced by the release of Ca^{2+} from intracellular stores when IP3 binds to its receptor. PKC enhances $Na_V1.9$ activity, most likely through phosphorylation of the channel and/or associated subunits. Downstream, PKC also affects the MAPK pathway, which ultimately leads to the activation of transcription factors such as NFκB.

11. Tables

Disorder	*SCN11A* mutation	References
Familial episodic pain (FEP)	p.R222S	Okuda (2016), Yang (2020)
	p.R225C	Zhang (2013), Leng (2017), Kabata (2018)
	p.A808G	Zhang (2013), Yang (2020)
	p.F814C	Kabata (2018)
	p.N816K	Huang (2019)
	p.R838Q	Sambuughin (2018), Eijkenboom (2019)
	p.F1146S	Kabata (2018)
Small fiber sensory neuropathy (SFSN)	p.A32V	Eijkenboom (2019)
	p.R51V	Eijkenboom (2019)
	p.R354V	Eijkenboom (2019)
	p.I643V	Eijkenboom (2019)
	p.G699R	Han (2015)
	p.I807V	Eijkenboom (2019)
	p.N820W	Ginanneschi (2019)
	p.I1074M	Eijkenboom (2019)
	p.A1249Y	Eijkenboom (2019)
	p.R1350Q	Eijkenboom (2019)
	p.G1428S	Eijkenboom (2019)
	p.V1437M	Eijkenboom (2019)
FEP, SFSN	p.R222H	Okuda (2016), Kabata (2018), Han (2017)
FEP, cold-aggravated peripheral pain	p.V1184A	Kabata (2018), Leipold (2015)
Painful peripheral neuropathy (PPN)	p.I381T	Huang (2014)
	p.K419N	Huang (2014)
	p.A582T	Huang (2014)
	p.A681D	Huang (2014)
	p.A842P	Huang (2014)
	p.L1158P	Huang (2014)
	p.N1169S	Kleggetveit (2016)
	p.I1293V	Kleggetveit (2016)
	p.F1689L	Huang (2014)
Congenital indifference to pain (CIP)	p.L396P	King (2017)
	p.L811P	Huang (2017), Woods (2015), Leipold (2013), Salvatierra (2018), Ebbinghaus (2020)
	p.L1302F	Huang (2017), Phatarakijnirund (2016)
	p.C1355F	Poojary (2020)
SUDEP	p.C844Y	Coll (2016)
Post-operative pain	p.V909I	Sun (2017)

Table 1: Overview of identified human *SCN11A* mutations and associated clinical phenotypes. References include (Coll, et al., 2016; Ebbinghaus, et al., 2020; Eijkenboom, et al., 2019; Ginanneschi, et al., 2019; Han, et al., 2015; Han, et al., 2017; Huang, et al., 2019; Huang, et al., 2014; Huang, et al., 2017; Kabata, et al., 2018; King, et al., 2017; Kleggetveit, et al., 2016; Leipold, et al., 2015; Leng, Qi, Zhou, & Wang, 2017; Okuda, et al., 2016; Phatarakijnirund, et al., 2016; Poojary, Jaiswal, Shah, & Bhalala, 2020; Salvatierra, et al., 2018; Sambuughin, et al., 2018; Sun, et al., 2017; Woods, et al., 2015; Yang, et al., 2020; X. Y. Zhang, et al., 2013).

Human mutation	Mouse mutation		Mouse phenotypes	Biophysics	References
p.R222S	p.R222S	Nav1.9$^{R222S/R222S}$	Heat hypersensitivity; Inflammatory hypersensitivity; Visceral mechanical hypersensitivity; Inflammatory visceral pain; Intestinal dysmotility	Increased AP frequency	Zhao 2022
		Nav1.9$^{+/R222S}$	Cold and heat hypersensitivity	No change in RMP; Increased AP frequency	Okuda 2016
			Mechanical and heat hypersensitivity after exposure to cold		Matsurbara 2021
p.L811P	p.L799P	Nav1.9$^{+/L799P}$	Increased spontaneous scratching	Hyperpolarized activation voltage; Depolarized RMP; Increased and decreased AP firing threshold	Salvatierra 2018
			Tissue lesions ; Reduced heat hypersensitivity; Reduced inflammatory hypersensitivity	Hyperpolarized activation voltage; Decreased peak current density; Depolarized RMP; Reduced AP duration	Leipold 2013
			Spontaneous scratching; Reduced grip strength		Ebbinghaus 2020
p.A808G	p.A796G	Nav1.9$^{A796G/A796G}$	Mechanical, cold and heat hypersensitivity	Hyperpolarized activation voltage; Increased window current; No change in RMP; Increased AP frequency	Yang 2020
		Nav1.9$^{+/A796G}$	Heat hypersensitivity	Hyperpolarized activation voltage	
p.F814C p.F1146S	p.F802C, p.F1125S	Nav1.9$^{+/F802C}$ Nav1.9$^{+/F1125S}$	Not done	Depolarized RMP; Increased AP firing frequency	Kabata 2018

Table 2: Overview of mouse studies based on human *SCN11A* mutations. References include (Ebbinghaus, et al., 2020; Kabata, et al., 2018; Leipold, et al., 2013; Matsubara, Okuda, Harada, Youssefian, & Koizumi, 2021; Okuda,

et al., 2016; Salvatierra, et al., 2018; Yang, et al., 2020; Zhao, et al., 2022). Biophysical experiments were typically done in DRG (Dorsal Root Ganglion). AP: Action Potential; RMP: Resting Membrane Potential.