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Sodium Channels in Pain Disorders: Pathophysiology and Prospects for Treatment

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Introduction

Pain is deemed chronic if it lasts more than 12 weeks and can sometimes last a lifetime. While some clinical pain syndromes are traced back to specific causes such as traumatic or metabolic nerve injuries the majority of cases have no clear causative event/injury [11; 106]. Pain can even be perceived to arise from an amputated, and hence an absent, limb [93; 121]. Genetic and functional findings have linked voltage-gated sodium channels that are expressed in peripheral sensory neurons to human pain disorders, and support targeting these channels for development of new analgesics.

Nine genes (*SCN1A*-*SCN5A* and *SCN8A*-*SCN11A*) encode diverse pore-forming sodium channel α-subunits ($Na_v1.1$-$Na_v1.9$) which manifest distinct expression patterns and biophysical and pharmacological properties [24]. Sodium channels are composed of 1700–2000 amino acids which fold into four domains (DI-DIV) with each domain consisting of six transmembrane segments, linked by three intracellular loops, and cytoplasmic N- and C-termini [23]. Adult peripheral sensory neurons can express channels $Na_v1.1$, $Na_v1.6$ and $Na_v1.7$, which are blocked by nanomolar concentrations of the neurotoxin tetrodotoxin (TTX-S), and channels $Na_v1.8$ and $Na_v1.9$, which are resistant to micromolar concentrations of TTX (TTX-R). The TTX-S channel $Na_v1.3$ is predominantly expressed in embryonic sensory neurons, [116] but is up-regulated following traumatic or metabolic nerve injury in rodent DRG neurons [35; 116]. Functional studies of these channels within neurons in which they are normally expressed [38] as well as studies in knock-out mice have yielded

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important information about the contribution of individual sodium channels to electrogenesis within these neurons [42; 47; 97](Figure 1).

Three of the channels that are expressed in sensory neurons — $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ — are preferentially expressed in peripheral neurons, whereas $Na_v1.1$ and $Na_v1.6$ are also widely expressed in the CNS. $Na_v1.7$ and $Na_v1.8$ channels have garnered intense interest because of the accumulating evidence for their role in human pain disorders and relative ease of studying these channels in isolation in heterologous expression systems or in native neurons [43; 57]. Evidence for mutations in $Na_v1.9$ [60; 61; 64; 72; 73; 87; 118; 127] and $Na_v1.6$ [108] in human pain conditions has started to appear in the past few years. Recent comprehensive reviews have covered this topic in detail [15; 36; 37; 40; 43; 57]. We provide in this article an overview of the more recent developments that further our understanding of the contribution of peripheral sodium channels to a spectrum of human pain disorders, and the clinical testing of both new and existing sodium channel blockers for treatment of patients carrying mutations in $Na_v1.7$.

$Na_v1.6$ in trigeminal neuralgia

$Na_v1.6$ is the major sodium channel at axon initial segments (AIS) and mature nodes of Ranvier in myelinated fibers of the central and peripheral nervous systems [21; 100], and contributes to the C-wave of compound action potential of the sciatic nerve, consistent with its expression in small DRG neurons [17]. $Na_v1.6$ produces a TTX-S current characterized by fast activation, inactivation and repriming, and produces persistent current –incomplete inactivation of the channel– and resurgent current – channel reopening upon hyperpolarization following strong depolarization – in DRG neurons [32; 98]. These properties poise $Na_v1.6$ to contribute to neuronal repetitive firing.

A recent study reported a $Na_v1.6$ mutation in a patient with trigeminal neuralgia [108], adding to evidence from animal studies [34; 105; 122; 123] supporting a role for this channel in pain. Trigeminal neuralgia (TN) is characterized by paroxysmal facial neuropathic pain along one or more branches of the trigeminal nerve, often triggered by innocuous stimuli, such as light touch, eating, shaving, or applying makeup [30]. Although neurovascular compression of the trigeminal nerve is prevalent in patients with TN, symptoms can occur in the absence of nerve compression or only in a subpopulation of patients with nerve compression [5; 68; 69; 77], which suggests the contribution of other factors in disease manifestation. The sodium channel blockers carbamazepine (CBZ) and oxcarbazepine are the first-line treatment for classical TN [30], which supports involvement of sodium channels in the pathophysiology of TN. We have recently reported the first mutation located in transmembrane segment 1 of domain I in $Na_v1.6$ in a patient with trigeminal neuralgia and no other comorbidity The Met136Val mutation in $Na_v1.6$, which was not previously reported in any of the sequence databases, confers gain-of-function attributes on the channel – increased transient and resurgent current density – and enhances excitability of trigeminal neurons –reduced threshold for action potential and increased repetitive firing– that express this mutant channel [108]. Although this mutation by itself is not enough to cause TN, it may act to predispose the carrier to the manifestation of TN

symptoms. This study provides the first link of voltage-gated sodium channel $Na_V1.6$ to a human pain disorder.

$Na_v1.8$ in human peripheral painful neuropathy

$Na_v1.8$ is preferentially expressed in peripheral sensory neurons, and is present in >90% of small DRG neurons and in about 40% of cutaneous afferents [103]. $Na_v1.8$ is characterized by depolarized voltage dependence of activation and inactivation, compared to other sodium channels, and a slow rate of inactivation and rapid recovery from inactivation [2; 3], and a slow resurgent current [107]. $Na_v1.8$ contributes most the current underlying the upstroke of the action potential in small DRG neurons [18; 96]. As a result of these properties, $Na_v1.8$ supports repetitive firing in response to sustained depolarization. Recently, we have shown species-specific differences in $Na_v1.8$ (Figure 2), for example a larger persistent current is produced by human $Na_v1.8$, compared to rodent $Na_v1.8$ channels, which is correlated by wider action potentials in both human DRG neurons or when the human $Na_v1.8$ channel is expressed in rodent DRG neurons [57]. The presence or absence of $Na_v1.8$ in a particular cell is a major contributor to the cell background effect, which is manifested when the same mutations that cause depolarization of resting membrane potential will lead to hypoexcitabile neurons if $Na_v1.8$ is absent and hyperexcitable neurons if $Na_v1.8$ is present [58; 97].

De novo gain-of-function mutations of $Na_v1.8$ that produce DRG neuron hyperexcitability have been found in 4% of a group of 393 consecutive patients diagnosed with small fiber neuropathy (SFN) at one medical center, including familial episodic pain syndrome (FEPS2, OMIM#61551), establishing a role for this channel in pathogenesis [19; 59], and a single nucleotide polymorphism biases experimental pain in healthy human subjects [46]. The gain-of-function attributes that these mutations confer on $Na_v1.8$ include hyperpolarized voltage-dependence of activation, accelerated recovery from inactivation, enhanced ramp current and impaired inactivation. $Na_v1.8$ selective blockers have been developed and showed efficacy in animal models [65; 88], providing proof-of-principle that it is possible to target $Na_v1.8$ *in vivo*. The new links of $Na_v1.8$ in human pain disorders and success in pre-clinical studies using $Na_v1.8$ selective blockers suggest new therapeutic strategies when clinically-relevant blockers become available.

$Na_v1.9$ in human painful and painless disorders

$Na_v1.9$ is preferentially expressed in small-diameter (<30 μm diameter) DRG neurons, trigeminal ganglion neurons, including functionally-identified nociceptors, and intrinsic myenteric neurons [37]. $Na_v1.9$ channels produce a TTX-R current which characteristically activates at hyperpolarized voltages, compared to other neuronal sodium channels, and inactivates with unusually ‘ultra-slow’ kinetics causing the persistence of the sodium current after activation [31] [41]. The large overlap between activation and inactivation of $Na_v1.9$ results in a big window current within the physiological voltage domain close to resting membrane potential of DRG neurons (-70 mV to -40 mV), permitting these channels to depolarize the membrane potential to reach threshold for firing the all-or-none action potential. These features have led to the designation of $Na_v1.9$ as a threshold channel.

Although studies on $Na_v1.9$ have been hampered by poor expression in heterologous expression systems, recently, expression of $Na_v1.9$ in the DRG-derived cell line ND7/23 [111] and HEK293 cells [75] have been reported which may accelerate studies of this channel and development of isoform-selective blockers.

A spectrum of human pain disorders has been linked to dominant gain-of-function mutations in $Na_v1.9$, including painful (FEPS3, OMIM#615552) and painless channelopathies (HSAN VII, OMIM#615548) [37]. Mutations in $Na_v1.9$ have been identified in several families with pain in distal extremities [61; 87; 127] and in one family with cold-aggravated pain [72]. Sporadic mutations of $Na_v1.9$ have been identified in patients with painful small fiber neuropathy [60; 64]. Unexpectedly a gain-of-function mutation in $Na_v1.9$ has been reported in patients with insensitivity to pain [73; 118], and another mutation was identified in a family with early-onset insensitivity to pain associated with chronic diarrhea, but functional assessment of this mutation has not been reported thus far [90]. The gain-of-function attributes that these mutations confer on $Na_v1.9$ include massive hyperpolarizing shift in activation, increased amplitude of ramp current and slower deactivation. The expression of $Na_v1.9$ in nociceptors and myenteric neurons is consistent with the symptoms of distal extremity pain and gastrointestinal disturbances reported by patients carrying mutations in this channel [61; 64; 72; 87; 127]. However, a mechanistic basis for the loss of pain sensation in patients with gain-of-function mutations in $Na_v1.9$ remains to be determined [73; 118].

$Na_v1.7$ in human pain disorders

Arguably, we know more about the contribution of $Na_v1.7$ to pain in humans than any other sodium channel. $Na_v1.7$ is preferentially expressed in peripheral somatic and visceral sensory neurons – including the vast majority of functionally-identified nociceptors – olfactory sensory neurons, and sympathetic ganglion neurons [43]. $Na_v1.7$ accumulates both at nerve fiber endings in the periphery and presynaptic terminals in the dorsal horn of the spinal cord [43]. $Na_v1.7$ produces a TTX-S current that rapidly activates and inactivates but slowly recovers from inactivation (reprimes) [67], and is also characterized by slow closed-state inactivation, allowing the channel to produce a substantial current in response to small, slow “ramp” depolarizations [33; 63]. This ability of $Na_v1.7$ to boost subthreshold stimuli increases the probability of neurons reaching their threshold for action potential firing [42; 47; 97]. The implementation of dynamic-clamp recordings in native rodent DRG neurons using physiologically-relevant levels of $Na_v1.7$ currents with native biophysical properties, showed a linear correlation between the level of $Na_v1.7$ conductance and current threshold in these cells [112]. Pharmacological block of $Na_v1.7$ increases threshold and reduces amplitude of action potential in DRG neurons and reduces neurotransmitter release from both peripheral and central terminals of primary afferents [4]. Thus, both anatomical and functional evidence supports a role for $Na_v1.7$ as a threshold channel.

Many mutations in $Na_v1.7$ have been found in patients with heritable pain disorders that follow a dominant Mendelian inheritance pattern and confer gain-of-function attributes on the channel, supporting the conclusion that these mutations are pathogenic [117] and confirming a definitive role for $Na_v1.7$ in human pain signaling. Dominantly inherited gain-

of-function missense mutations in *SCN9A*, the gene encoding $Na_v1.7$, are found in patients with inherited erythromelalgia (IEM, also known as primary EM, OMIM #133020)[125] and paroxysmal extreme pain disorder (PEPD, previously known as familial rectal pain, OMIM #167400)[49]. By contrast, recessively inherited loss-of-function mutations in *SCN9A* are linked to congenital insensitivity (indifference) to pain (CIP, OMIM #243000) characterized by sensory loss limited to pain sensation and anosmia, and without autonomic deficits [29], and hereditary sensory and autonomic neuropathy IID (HSANIID, OMIM#243000) which is characterized by adolescent or congenital onset with loss of pain and temperature sensation, autonomic nervous dysfunctions, hearing loss, and hyposmia [126]. These data provided the biological basis to investigate the contribution of this channel to more common pain disorders and led to the identification of gain-of-function variants of $Na_v1.7$ in roughly 30% of patients with idiopathic small fiber neuropathy and biopsy-confirmed loss of intra-epidermal nerve fibers [48]. These studies have solidified the status of $Na_v1.7$ as a major contributor to human pain disorders.

Although patients with IEM and PEPD present with distinct clinical symptoms, they both carry gain-of-function mutations in $Na_v1.7$. Patients with PEPD report severe perirectal pain starting typically in infancy and is normally triggered by bowel movement or probing of the rectal or perineal areas, which is accompanied by tonic posturing and immediately followed by flushing in a uni- or bi-lateral fashion [50], and sometimes in a harlequin pattern which can alternate between the left and right sides of the body during different pain episodes [26]. Ocular and mandibular pain, sometimes triggered by cold or irritants, becomes the more prominent complaints in older patients. Patients with IEM start to exhibit symptoms as early as 1 year of age and as late as late-teens, and episodes of burning pain are triggered by mild warmth or exercise together with erythema and mild swelling in the hands and feet, with partial relief of symptoms by cooling affected extremities [45]. Despite the apparent uniformity of these symptoms, one recent study that looked in more details into the natural history of IEM in a cohort of 13 patients from four families demonstrated substantial variability in symptom presentation and triggers among members of the same family, including, for example, patients who reported that cooling evokes pain rather than relieves pain [82], while another study on two patients (parent/child) showed large variability in the severity of ongoing pain and in the number of nightly awakening due to pain [55]. The distinct patterns of affected body regions in IEM and PEPD, and the substantial variability of pain symptoms among members of the same family with the same mutation in IEM, suggest additional factors that regulate the severity of the symptoms and which parts of the body are affected.

Functional characterization of the mutations in $Na_v1.7$ has shed light on the pathophysiological basis for nociceptor excitability in these disorders, establishing a mechanistic link to pain. At the channel level, these mutations confer multiple gain-of-function attributes on mutant channels. These include hyperpolarizing shift in activation, increased amplitude of ramp current and slower deactivation, impaired inactivation and increased persistent current, and enhancement of resurgent current. At the cellular levels, expression of these mutant channels in sensory neurons lead to increased excitability of DRG neurons [43], which reflects a cellular correlate of pain that these patients experience.

Studies of mutant $Na_v1.7$ channels have also unmasked the important observation that the neuronal background governs the cellular response to the expression of a mutant $Na_v1.7$ channel. Experimental evidence shows that the same mutation can induce hyperexcitability of sensory neurons while causing hypoexcitability of sympathetic neurons, and that this dichotomy is caused by the presence of $Na_v1.8$ in sensory neurons and its absence in sympathetic neurons [58; 97]. This divergent effect of the same mutation in different neuronal backgrounds has been linked to the mutation-induced depolarization of the resting potential of the neurons. The depolarization of resting membrane potential of sensory neurons facilitates the activation of the $Na_v1.8$ channels that have a depolarized voltage-dependence of activation and inactivation compared to the other sodium channels and can support firing action potentials. The depolarization of resting membrane potential of sympathetic neurons leads to resting inactivation of the sodium channels leading to reduced probability of firing an action potential. While hyperexcitability of sensory neurons is intuitively linked to pain symptoms, hypoexcitability of sympathetic neurons may lead to reduced sympathetic vasoconstriction tone, contributing to skin flushing that is observed in these patients.

The majority of the functional studies discussed above have been carried out in an overexpression system in which the wild-type or mutant channels are expressed at supraphysiological levels in rodent DRG neurons; however, additional lines of evidence from assays under more physiological settings produced results that are in agreement with these data. Implementation of dynamic-clamp recordings in which physiologically-relevant levels of a wild-type or mutant $Na_v1.7$ channel could be injected into DRG neurons using a computer-controlled amplifier, showed that the $Na_v1.7$ Leu858His mutant current caused a 27-fold amplification of net sodium influx during subthreshold depolarizations and even greater amplification during interspike intervals [112]. These findings contribute to the mechanistic basis for reduced current threshold and enhanced action potential firing probability in sensory neurons. Another independent line of evidence is provided by studies using sensory neurons that are differentiated from induced pleuripotent stem cells from patients with IEM. These patient-derived neurons have a lower threshold and increased firing frequency [22]. Taken together, the genetic and functional studies show that $Na_v1.7$ channels play as a major role in pain signaling in humans.

There are no published animal models for a gain-of-function mutation in $Na_v1.7$, thus it has not been possible to assess the effect of mutant channels *in vivo*. By contrast, global, or conditional knockout of $Na_v1.7$ in sensory and sympathetic ganglia, have been reported to recapitulate CIP and anosmia in the mouse [56; 85]. Importantly, these animal studies have shown no haploinsufficiency due to the loss of one Scn9a allele, just as is the case in humans [29]. Whether a mouse with a knock-in mutation in $Na_v1.7$ recapitulates the IEM or PEPD phenotypes remains to be seen.

$Na_v1.7$ as a validated target for development of pain therapeutics

Despite the clear role of sodium channels in the pathogenesis of pain in individuals carrying mutations in $Na_v1.7$, treatment with sodium channel inhibitors which bind to the local anesthetics (LA) site of sodium channels has produced mixed outcomes [43]. However, the

genetic and functional validation of $Na_v1.7$ as a major contributor to human pain spurred the development of isoform-specific blockers. Several small-molecule inhibitors of $Na_v1.7$ have been recently described [10]. A new class of sulfonamide-based molecules with selectivity for $Na_v1.7$ have been developed which bind to a novel site in the voltage-sensing domain of domain IV of $Na_v1.7$ [1; 81]. An orally-bioavailable arylsulfonamide blocker, PF-05089771, with notable selectivity for $Na_v1.7$ over other voltage-gated sodium channel isoforms (by 10–900-fold), and 1000-fold selectivity over potassium and calcium channels, binds preferentially to the slow-inactivated state of the channel in a use-dependent manner [4]. Other sulfonamide compounds have also been reported with comparable selectivity for $Na_v1.7$ over other sodium channels [1; 44]. These molecules combine molecular selectivity and functional selectivity for hyperactive channels, and hold the promise for improved therapeutic potential.

Patients with well-defined gain-of-function mutations in $Na_v1.7$ present an opportunity to validate therapeutic efficacy of existing and novel small molecule sodium channel blockers because of the near certainty that the target — Nav1.7 — plays a major role in pain. Capitalizing on the availability of a cohort of patients carrying mutations in Nav1.7 that have been functionally tested and shown to produce DRG neuron hypersensitivity, and the development of an orally bioavailable Nav1.7 selective inhibitor, a phase II proof-of-principle randomized, double-blind placebo-controlled cross-over clinical trial (NCT01769274) was conducted to evaluate safety and efficacy of a single dose PF-05089771 (1600 mg) in these patients following a thermal challenge to the foot that was previously shown to evoke a pain episode [82]. There were 5 individuals who met inclusion/exclusion criteria and carried the following mutations: Ser241Thr (1 subject), Val400Meth (2 subjects), Ile848Thr (I subject) and Phe1449Val (1 subject). The clinical data show variability in the response of the subjects with different mutations, differential responses of subjects carrying the same mutation, and the differential response in the two testing sessions. The primary endpoint for the trial, the average pain score on the numeric rating scale (NRS) at 0–4 hrs postdose, was not met, possibly due to lower than expected plasma concentrations of the PF05089771 which reached T_{Max} at 4–6 hours post dosing. However, compared to placebo, four of the patients showed a reduction in the maximum pain score of at least 2 points on the 10-point NRS in at least one session and three patients responded in both sessions; improvement in pain scores occurred more than 4 hrs post-dose [22]. Interestingly, the subject with the Ser241Thr mutation was the only subject who did not respond to treatment in either session. Despite the limitations of this study especially the small number of subjects and the single dose design, it is encouraging that individual subjects achieved a substantial level of pain relief, which supports further evaluation of $Na_v1.7$ blockers in larger cohorts with multiple dosing that might produce more favorable pharmacokinetics, or with an improved generation of $Na_v1.7$ selective compounds.

Subjects who were enrolled in the clinical trial gave additional consent to generate induced pluripotent cells, which were used to differentiate patient-specific sensory neurons that were evaluated for firing properties in response to a physiologically-relevant thermal stimulus, and for their response to incubation with clinically-relevant concentration of PF-05089771. The data show that the patient-specific sensory neurons with the mutations Val400Meth, Ile848Thr and Phe1449Val recapitulated the clinical phenotype in that they fired more action

potentials compared to sensory neurons that were differentiated from control subjects; there was attenuation of excitability of these neurons upon incubation with PF-05089771. Sensory neurons from the subject with Ser241Thr showed a rheobase within the range of control neurons, and unlike the neurons from the other IEM subjects, did not manifest a reduction in the rheobase upon exposure to a thermal stimulus of 40°C. Moreover, the reversal of elevated heat sensitivity seen with the other IEM-derived sensory neurons following exposure to an analogue of PF-05089771 was not seen with the Ser241Thr sensory neurons [22]. This *in vitro* pharmacological data parallels that clinical data showing the subject with the Ser241Thr mutation did not respond to the single dose treatment with PF-05089771. The reason for the lack of efficacy of the selective $Na_v1.7$ blocker on cells with the Ser241Thr mutation and in the subject carrying this mutation is not known at this time.

Although the majority of patients with IEM due to mutations in $Na_v1.7$ do not respond to treatment with sodium channel blockers in clinical use, a few cases of IEM patients and the PEPD patients respond to treatment with these drugs, indicating that monotherapy could be effective when there is adequate target engagement. Pharmacological studies have shown that carbamazepine inhibits gain-of-function attributes of PEPD $Na_v1.7$ mutations, namely reducing the persistent current and shifting fast-inactivation in a hyperpolarizing direction, consistent with the effective response of these patients to treatment with carbamazepine [14; 49]. The lack of efficacy of sodium channel blockers in patients with $Na_v1.7$ IEM mutations is not well understood, albeit in one case it has been linked to a reduced affinity of the mutant channel, Asn395Lys, to sodium channel blockers that bind at the local anesthetic binding site [102]. However, there are case reports of effective treatment with mexiletine in a patient carrying the Val872Gly [27], and CBZ in patients carrying the mutations Val400Met [52] and Ser241Thr [55]. The efficacy of mexiletine in the patient carrying the Val872Gly mutation has been linked to a stronger use-dependent fall-off in the current [27]. By contrast, CBZ was shown to shift the Val400Met mutant channel activation in a depolarizing direction, bringing it closer to that of wild-type channels [52].

In the era of affordable individualized genomic screening, we implemented a multi-disciplinary approach to predict the effect of treatment of a novel channel variant based on the response of a “seed” variant, thus potentially moving away from a trial and error approach to a more predictive approach for choice of pain therapeutic. Using atomic-level modeling and thermodynamic analysis, we were able to show that clinically relevant concentration of CBZ attenuates firing of DRG neurons expressing the Ser241Thr mutant channels (Figure 3), thus predicting CBZ-responsiveness of the Ser241Thr mutation and shown that clinically-relevant concentrations [124], and this pharmacogenomic guided therapy was successfully implemented in a placebo-controlled double-blind clinical trial in two patients carrying this mutation [55]. This represents a case study that illustrates the power of structural modeling and pharmacogenomic approaches for the treatment of pain, moving us closer to the promise of personalized medicine.

Functional brain imaging in pain studies

Given that pain reports are subjective and rely on patients’ ratings it follows therefore that consciousness, and hence activity within cortical circuitry, are a pre-requisite for the

experience of pain [12; 53; 93; 94]. More than half-a-century of animal research in the setting of a peripheral injury has now extensively described the neural nociceptive machinery that transmits information about potentially painful stimuli cephalad and demonstrates its plasticity in animal models of pain [119; 120]. However, conditions such as fibromyalgia can defy modeling with animal research because the causative injury, if any, is unclear [106]. The advent of functional brain imaging (fMRI) has opened a window into studying the subjective experience of pain by allowing the simultaneous measurement of subjective pain ratings and brain activity.

In the past 25 years hundreds of positron emission tomography and functional brain imaging studies have reported brain responses to acute painful thermal, mechanical, chemical and visceral stimuli [7; 20; 79]. These studies have described activations in the thalamus, primary (SI) and secondary somatosensory area (SII), insula, and anterior cingulate cortex (ACC), areas collectively referred to as the pain matrix [109], with variable reports of activation in the cerebellum, striatum, amygdala, medial and dorso-lateral prefrontal cortices. Pain intensity ratings collected during some of these experiments covaried with activity in the insula and ACC [28]. Such findings were consistent across hundreds of studies in healthy participants, providing support for the suggestion that the pain matrix might be a mediator of the conscious perception of pain or provide a brain signature of pain [109; 114]. Nevertheless, brain activity associated with chronic clinical pain such as low-back pain (CBP), fibromyalgia, migraine or neuropathic pain demonstrated a very different picture, engaging mainly limbic brain areas such as amygdala, striatum (dorsal and ventral), and medial prefrontal cortex, mPFC [6; 7; 12; 78; 104]. These studies provided evidence that areas mediating emotional decision-making, learning and valuation [62; 66; 92] correlate more strongly with chronic pain than areas mediating accurate sensations [84] and attention or salience [83]. These findings also challenged the concept of the pain matrix [7]. Behavioral studies describing deficits in hedonic perception in CBP [54] and emotional decision making tasks [9; 16; 115] in CBP and fibromyalgia patients are consistent with the idea that chronic pain taxes the limbic brain.

Recent findings provide evidence that altered structure and function of areas in the limbic brain are critical factors in determining the risk of transition from acute clinical pain to the chronic condition [13; 110]. Baliki et al., [13] showed that the magnitude of cortico-striatal functional connectivity between the nucleus accumbens (NAc, part of the ventral striatum) and the mPFC predicts the odds that an episode of sub-acute back pain (period between 6 to 12 weeks) would persist or remit one year later. The same group provided evidence that the volume of amygdala and hippocampus and their functional and structural connectivity to the medio-dorsal prefrontal cortex predict the magnitude of the risk of transitioning to chronic low-back pain at 1 and 3 years follow-up, and mediate the effect of genetic risk factors for that transition [110]. Both the amygdala and hippocampus are crucial brain areas for learning and memory formation [91; 92] implying that pain persistence presupposes new learning, most likely driven by peripheral nociceptive input. In agreement with these conclusions, a recent study demonstrated that adult hippocampal neurogenesis, which is a hallmark of new learning, is necessary for the development of neuropathic pain in rodents [8]. Collectively, these results show that the brain correlates of adaptive (i.e. protective) acute pain are different from those of chronic (i.e. maladaptive) pain and point to a causal

role of the limbic circuitry and limbic plasticity in clinical pain perception and the transition to the chronic state.

Functional brain imaging in pain studies: Lessons from IEM

As discussed in previous sections, IEM is a rare chronic pain condition caused by gain-of-function mutations in the $Na_v1.7$ channel present on peripheral nociceptors. Patients with IEM suffer from severe pain attacks when they are exposed to thermal stimulus that would not otherwise elicit pain in individuals with normal $Na_v1.7$ channels. These attacks can be relieved by cooling of the affected body part [39]. Therefore, if chronic pain is conceived, at least in part, as a persistent peripheral barrage from nociceptors [113], IEM offers a unique opportunity to examine this model of hyperactive nociceptors in the context of brain imaging because peripheral input can be manipulated via the control of skin temperature (Figure 3). Seghredahl et al. [101] measured cerebral blood flow (CBF) in a patient with IEM to assess differences between acute thermal heating and cooling using arterial spin labeling. Acute thermal heating triggered a pain attack while cooling induced analgesia. Compared to cooling, the pain attacks were accompanied by increased CBF in the thalamus, striatum, SI, insula, inferior frontal gyrus, posterior, mid- and anterior cingulate cortex. Activation in these areas has been shown to predict thermal heat pain intensity [114], and is reminiscent of activity in the areas of the pain matrix [109]. One disadvantage of presenting an outside painful stimulation during brain imaging, however, is the difficulty of controlling for the activation elicited by the saliency of the stimulus itself [71]. Interestingly, activation of the pain matrix can be elicited by stimuli from other modalities (i.e. visual, auditory, tactile) [86], and occurs in the absence of pain in patients with a loss-of-function mutation of the $Na_v1.7$ channel [99], raising issues about the specificity of activation in these brain areas to pain perception.

In keeping with the learning model of chronic pain described above, we therefore hypothesized that examining IEM patients' stimulus-free pain, an approach used with multiple other chronic pain conditions [6], would uncover the shift of neural correlate of this chronic pain syndrome towards the limbic brain. We studied two patients suffering from IEM due to the same gain-of-function mutation Nav1.7-S241T using fMRI blood oxygen level dependent (BOLD) signal. BOLD signal was collected simultaneously with continuous pain intensity ratings under two different conditions: (1) during an attack of IEM pain elicited by a thermal heating boot wrapped around the foot; and (2) after the termination of the stimulus, while patients reported their ongoing stimulus-free pain during attacks that persisted for more than an hour. The neural correlates of stimulus-free pain engaged mostly the limbic brain including the prefrontal cortex, dorsal and ventral striatum and showed a minimal overlap (~ 2%) with areas of the pain matrix in the insula. In contrast, the neural correlates of the acute thermal pain stimulation showed a larger overlap with areas of the pain matrix (~11%) and resembled more the results reported by Segherdahl et al [101]. More importantly, we assessed the treatment effects of CBZ vs. placebo on IEM pain and brain activity. Our choice of CBZ was genomically guided by *in vitro* work pointing to its specific effect on the $Na_v1.7$-S241T mutation carried by both patients [55; 124]. Clinical pain improvement was observed after two weeks of treatment with CBZ but not placebo and was accompanied by a shift in brain activity from ventral striatum, rostral ACC and posterior

cingulate cortex towards primary sensori-motor and attention areas [55]. This shift in brain activity suggests that chronic IEM pain treatment is associated with increased activity in areas mediating accurate sensation and attention and decreased activity in areas mediating emotional learning (Figure 3).

Half a century ago Wilder Penfield described the brain as a “passive observer” of peripheral nociceptive input based on the absence of evidence for specific cortical neuronal responses supporting the presence of a “pain cortex” [89]. More recent cortical stimulation studies confirmed that painful responses to cortical stimulation are very rare and confined to the posterior insula and adjacent parietal operculum only [80]. However, brain imaging findings described above as well as a large literature on the psychological modulation of pain [20; 51] support the role of the limbic brain as mechanistically involved in the perception of pain and the “chronification” of clinical conditions. Two different rodent studies of nerve injury induced pain [70; 95] to-date have been able to control pain behavior via the modulation of activity in neuronal projections from the pre-limbic PFC (the rodent equivalent of the human mPFC) into the ventral striatum. In agreement with these observations, imaging of IEM patients suggests that although the peripheral source of afferent barrage is well defined and can be readily manipulated, pain perception and pain relief involve the limbic brain, in particular the valuation circuitry [55; 66; 74]. Taken together, evidence from animal and human research, support the hypothesis therefore that this circuitry, particularly the ventral striatum-medial prefrontal axis, may function as a gate-keeper controlling which nociceptive input emerges into consciousness as pain [12].

However promising the findings of brain imaging in clinical pain conditions including IEM are, many limitations persist. For example, it is still unclear how conscious perception of pain arises from the activation of nociceptors. Some advance in predicting pain intensity using fMRI data has been recently demonstrated [25; 114]. While IEM pain can be reproducibly triggered in study subjects and imaging data can thus be correlated with well-defined pain trigger, the causative chain of events in the overwhelming majority of clinical pain conditions that are studied by fMRI remain unknown. Nevertheless, studies using fMRI data within the framework of a longitudinal design have proven crucial in understanding the mechanism of, and predicting the transition from early to chronic pain syndromes [13; 110]. Finally, fMRI measures brain activity based on the BOLD signal. BOLD is a slow signal relative to the frequency of neuronal firing [76]. Combined electroencephalogram-fMRI studies may help on this front.

Conclusions

Elucidation of the role of individual sodium channels in action potential firing has informed our understanding of pathophysiological mechanisms underlying painful channelopathies. The parallel outcome of attenuation of firing of DRG neurons expressing the Ser241Thr mutation and pain relief of the subjects carrying this mutation, and the lack of efficacy of treatment with PF-05089771 in the in vitro pharmacological assay and the clinical outcome in the subject with this mutation, suggests that *in vitro* pharmacological assays using patient-specific differentiated neurons might be predictive of clinical outcomes of subjects receiving this treatment. These are examples of a complicated pharmacogenomic picture that may

underlie the diverse response that is seen in the clinic. The variability in the response of individual patient to existing drugs or to new ones like PF-05089771 is to be expected, but we'll have to wait for additional clinical trials with larger cohorts of subjects to make more definitive conclusions. The promise of the new generation of sodium channel blockers is that their isoform selectivity will reduce the potential adverse effects that limit their effective clinical utility.

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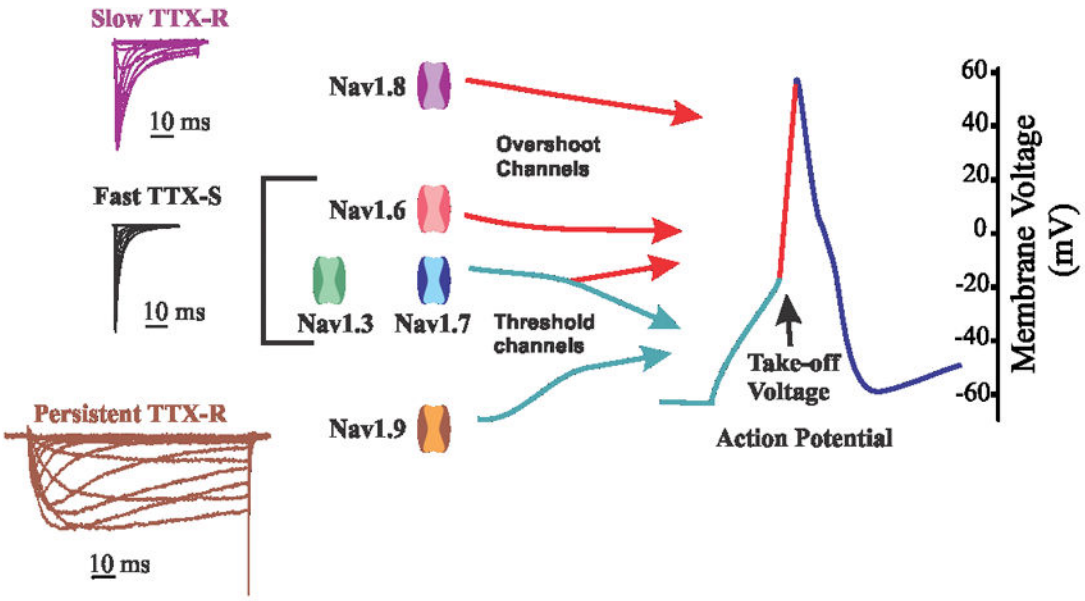

Figure 1. Contribution of sodium channel isoforms to action potential

Based on their ability to boost subthreshold stimuli -- TTX-S channels $Na_v1.3$ and $Na_v1.7$ -- or hyperpolarized and persistent current -- TTX-R channel $Na_v1.9$ -- these channels have been considered threshold channels for action potential firing. TTX-S channels $Na_v1.3$, $Na_v1.6$ (especially at nodes of Ranvier), and TTX-R channel $Na_v1.8$ (in the neurons in which it is expressed), contribute most of the current for the action potential. Adapted with permission from [42]

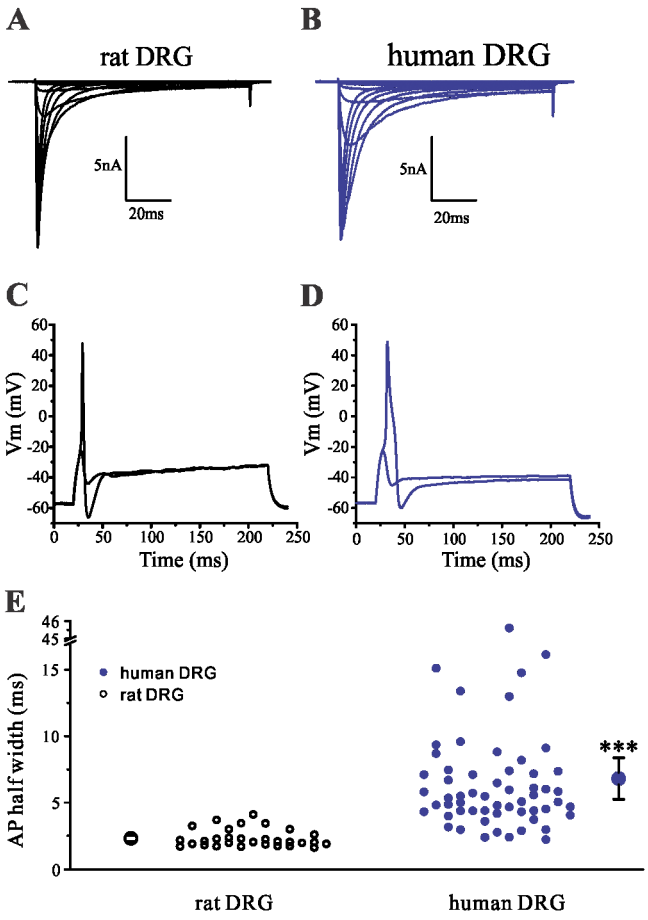

Figure 2. Species-specific differences in $Na_V1.8$ and action potential properties

(**A, B**) Representative $Na_V1.8$ current family traces recorded from rat (**A**) or human DRG neuron (**B**). Human DRG neurons produce a Nav1.8 current with slower kinetics of inactivation, and larger persistent current compared to the Nav1.8 current in rat DRG neurons. (**C, D**) Representative action potential traces recorded from rat (**C**) or human (**D**) DRG neuron. Human DRG neurons produce broader action potentials than rat neurons. (**E**) Comparison of the half-width of action potential between rat and human DRG neurons. The mean ± standard error of the half-width is represented by the two larger symbols. Adapted with permission from [57].

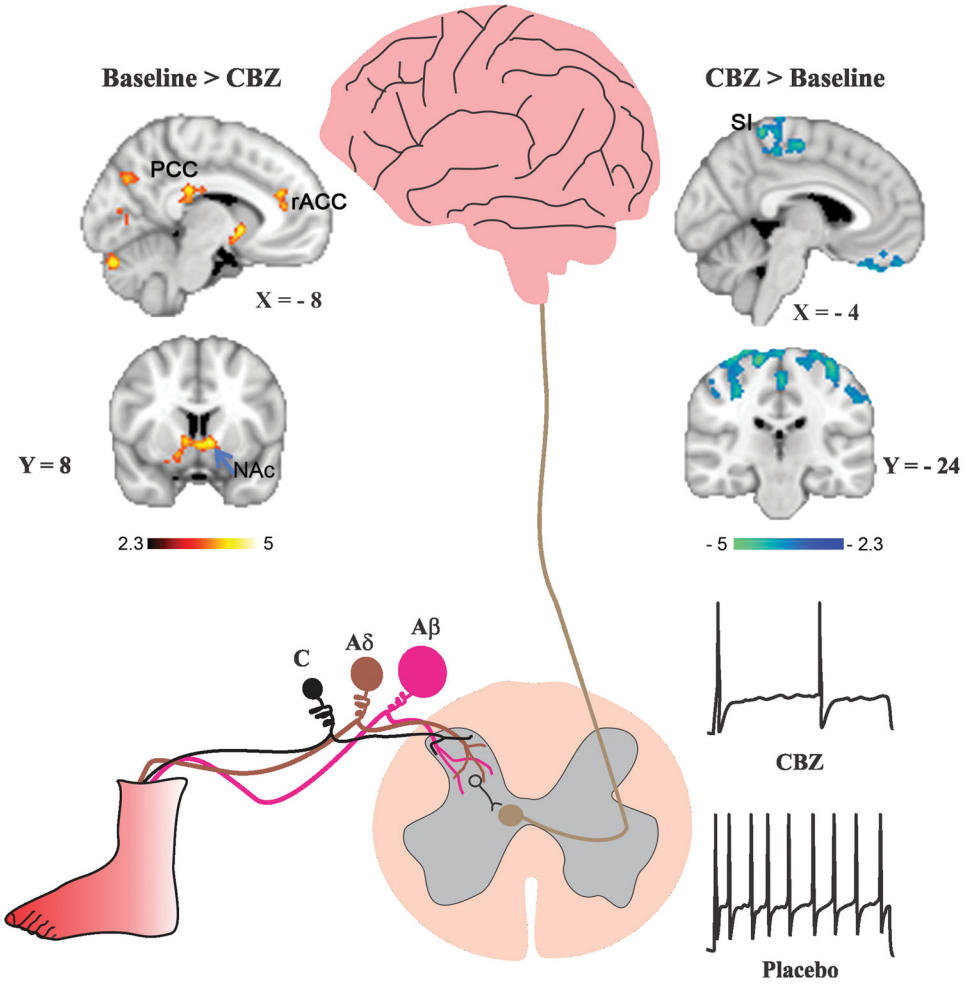

Figure 3. Carbamazepine reduces excitability of DRG neurons expressing the $Na_V1.7$-S241T mutation and is accompanied by a shift in brain activity toward a pattern associated with decreased pain

Schematic represents burning pain pathway in patients with inherited erythromelalgia. Typically, pain attacks which can be reproducibly and predictably triggered by warming distal limbs, are accompanied by skin flushing and swelling of affected limb which also becomes hot. Primary afferents innervating the foot consist of unmyelinated C fibers (black), lightly myelinated Aδ fibers (brown) and heavily myelinated Aβ fibers (magenta). Primary afferents form their first synapse in the spinal cord, and the signal is transmitted to higher brain centers. DRG neurons that express the $Na_V1.7$-S241T mutation fire repetitively, and the firing frequency is markedly attenuated when neurons are treated with a clinically-relevant concentration of carbamazepine (CBZ). Functional brain imaging in a patient treated with placebo shows that pain, after termination of the thermal stimulus, is accompanied by increased activity in valuation areas posterior cingulate cortex (PCC) and anterior cingulate cortex (ACC), and nucleus accumbans (NAc). Treatment with CBZ is accompanied by a shift in brain activity to the primary somatosensory cortex (SI) and parietal attention areas. Adapted with permission from [55].