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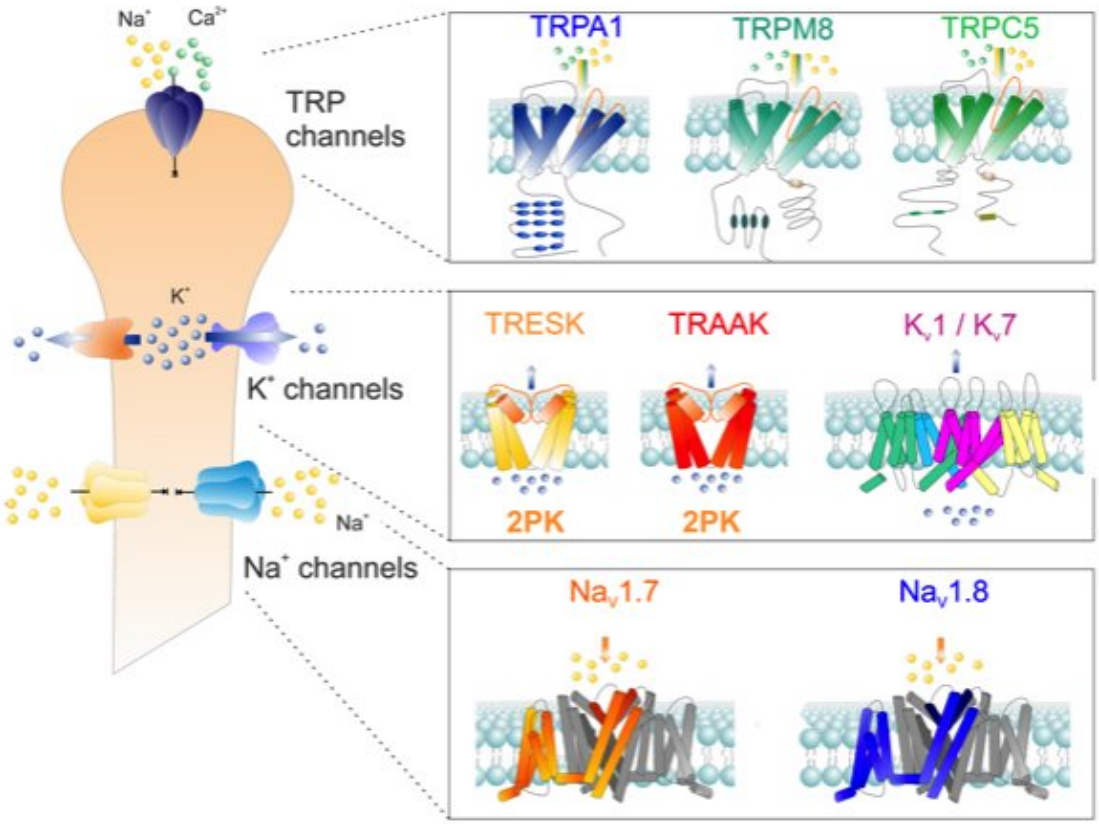
1 **Graphical**

2 **abstract**



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Therapeutic opportunities for targeting cold pain pathways

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Abstract: Cold pain is a frequent symptom in neuropathic pain. Compared to other pain modalities, such as heat pain, the mechanisms behind physiological and pathological cold pain remain elusive. Moreover, it is becoming increasingly evident that cold pain pharmacology differs between various neuropathic pain conditions, making mechanism-directed treatment based on an understanding of the underlying pathophysiological mechanisms imperative to achieving clinical success. Here we review the processes of physiological and abnormal cold sensing, the pharmacology of cold nociception, cold hyperalgesia and cold allodynia, and provide an overview of cold pain syndromes and their current and potential treatments.

Keywords: cold pain signalling, ion channels, TRP channels, pain pathways, treatment, cold pain sensitisation

The authors declare no conflict of interest.

Introduction

The perception of temperature is vital for human survival. As homoeothermic animals, humans rely on temperature regulation mechanisms to maintain a constant body temperature of ~ 37 °C irrespective of activity or the external environment. However, while innocuous hot or cold is considered harmless and often pleasurable, temperature extremes are painful and interpreted as signs of impeding tissue damage. The sensation of pleasant cool is usually elicited at temperatures just below normal skin temperature (32 °C), with temperatures approaching 10–15 °C and below gradually eliciting burning, aching and pricking pain [1-3]. Indeed, many of us are familiar with the excruciating pain that follows consumption of cold beverages or food, resulting in a cold-induced headache also known as "brain freeze". Similarly, immersion in ice-cold water is a commonly used nociceptive assessment in sensory testing to quantify an individual's pain threshold [4]. In this review, we describe the different cold pain pathways, ion channels involved in cold pain signalling, their involvement in pathological cold pain, and discuss mechanisms of current and potential new treatments for cold pain that might alleviate chronic cold pain sensitisation.

Cold pain pathways

Specific peripheral nerves activated by innocuous cold were discovered almost a century ago, first in the cat [5] and later in primates, where *in vivo* studies in anesthetised animal identified peripheral nerve fibres that discharged action potentials in response to cold stimulation of their receptive fields [6, 7]. Initially, these studies identified afferent fibres that only responded to innocuous temperatures (below 27 °C) and not to heat or mechanical stimuli. The firing frequency of these cold-sensitive neurons, later regarded as 'classic'

innocuous cold thermosensors, is inversely proportional to temperature, with peak discharge rates occurring on cooling between 30 and 25 °C [7]. Since this early discovery, other cold-sensitive sensory fibre subtypes were characterised in primates and in other species, as shown in **Table 1** [6-13]. Subsets of slow-conducting (typically < 1 m/s) C-fibres are monomodal and sense innocuous and noxious cold or are multimodal mechanothermal nociceptors, with the remaining cold-sensitive neurons classified as fast-conducting (up to 16 m/s) Aδ-fibres [3, 13-15]. Given that C-fibres sensitive to noxious cold are mostly polymodal nociceptors that respond to other stimuli, including mechanical stimulation, it appears likely that C-fibres conduct pain signals without discriminating the nature of the stimuli, while Aδ-fibres recognise the discomfort is caused specifically by cold [7, 16]. It is thus very likely that the full sensation of cold pain requires the presence of both C- and Aδ-fibres.

In humans, the physiological cold pain threshold is significantly more variable than the heat pain threshold, with temperatures below approximately 15 °C eliciting pain. Broadly, the intensity of cold pain increases rapidly between approximately 20 and 0 °C [1, 2, 17]. In rodents, frank nocifensive behaviour on contact exposure to cold surfaces is usually only elicited at temperatures below 5 °C [18, 19], which may reflect the relative insensitivity of these behavioural assays to detect mild to moderate pain-related behaviours in rodents.

Centrally, temperature signals reach the brain via the lateral spinothalamic tract, whereas pain signals are additionally carried via the spinoreticular and spinothalamic tracts, which terminate in the thalamus and the frontal cortices, respectively [20-23]. In healthy humans, stimulation of the forearm skin with innocuous cold leads to activation of the contra- and ipsilateral- posterior insular cortices as the primary somatosensory area [24]. In contrast, noxious cold stimuli activate the contra- and ipsilateral- insular cortices and secondary somatosensory cortices and the cingulate cortex, as revealed by magnetoencephalography [24]. Functional magnetic resonance imaging has been employed to investigate brain areas

activated during menthol-induced cold allodynia, identifying increased activation within the ipsilateral dorsolateral prefrontal cortices and the brainstem (ipsilateral parabrachial nucleus) [25]. These observations illustrate some of the differences between the brain regions responsible for innocuous cold, cold pain, and pathological cold pain. In contrast, the molecular mechanisms underlying central cold pain processing are less clear. Studies to date have revealed that the beneficial effects of spinal cord stimulation were abolished by serotonin receptor antagonists in a murine spinal nerve ligation model [26], and that ablating neuropeptide Y receptors in the superficial spinal dorsal horn reduced cold allodynia in CFA-induced inflammation in rats [27]. Interestingly, μ-opioid receptor binding potential in the striatum predicted cold pressor test threshold in humans, but not cold tolerance, possibly correlating peripheral cold pain threshold with central opioid-dependent inhibition [28].

Pathological Cold Pain Conditions

Pathological cold pain is a frequent symptom in a range of neuropathic pain syndromes, including those of peripheral and central origin, and usually presents as cold allodynia or hyperalgesia [29]. While many conditions, including diabetic neuropathy, peripheral nerve injury, chemotherapy-induced neuropathy, post-stroke central pain and ciguatera (see **Table 2**) can present with the symptom of pathological cold pain, the mechanisms by which cold pain arise are still poorly understood and can vary significantly between diseases [30-35].

A condition that is defined by cold allodynia is ciguatera, a form of marine food poisoning arising from the consumption of tropical and subtropical fish contaminated with ciguatoxins. Ciguatoxins originate from dinoflagellates of the *Gambierdiscus* family and accumulate in reef fish through the marine food chain [36]. Symptoms of ciguatera include dysaesthesias, headache, dental pain, and myalgia, with cold allodynia occurring in almost all ciguatera patients (for review see [36]). Experiments with human volunteers showed that intracutaneous

injection of ciguatoxin induced local cold allodynia which faded after a few hours, suggesting ciguatoxin acts acutely and peripherally to cause cold pain [37]. Similarly, mice of the C57BL/6 strain develop cold, but not mechanical or heat allodynia, within one hour after intraplantar ciguatoxin injection, an effect that is mediated predominantly through peripheral sensory neurons expressing the transient receptor potential (TRP) cold sensor TRPA1 [38].

In contrast, cold allodynia elicited after intraplantar injection of the chemotherapeutic agent oxaliplatin involved sensory neurons expressing voltage-gated sodium channel (Na_v) subtype 1.6 and voltage-gated potassium channel (K_v) channels, and developed independent of cold-sensitive TRP channels [39]. While oxaliplatin is administered intravenously in humans, intraplantar injection of oxaliplatin in mice recapitulated the immediate-onset cold-induced dysaesthesias that often occur in humans undergoing therapy with the platinum-derived anti-tumour agent. The immediate occurrence of painful symptoms after subcutaneous oxaliplatin exposure in mice implies that primary sensory effects are sufficient to elicit cold allodynia. However, the long-lasting progression of the disease in humans after intravenous infusion does not exclude an altered expression of cold-sensing TRP channels contributing or aggravating the disease at a later stage, as found for TRPA1 or TRPM8 in mouse models of chronic oxaliplatin-induced neuropathy [40-43].

Treatment with paclitaxel, a chemotherapy agent used in the management of solid tumours such as breast cancer, is also associated with a high incidence of cold allodynia. Paclitaxel-induced cold allodynia commences in the hands and feet and gradually progresses centrally. Cold allodynia appears to parallel the development of paclitaxel-induced neuropathy with signs such as demyelination, accumulation of abnormal mitochondria in sensory nerves, and fibre loss in severe cases [44, 45]. Subcutaneous injection of tetrodotoxin (TTX) reduced paclitaxel-induced mechanical, heat and cold allodynia [46], suggesting the involvement of TTX-sensitive Na_v isoforms as observed for oxaliplatin-induced cold allodynia.

Interestingly, diabetic animals with pre-existing peripheral neuropathy are more susceptible to develop cold allodynia when treated with paclitaxel [45]. This is perhaps not surprising as diabetic neuropathy, a late symptom of chronic diabetes, is in itself a complex neuropathic pain syndrome associated with cold allodynia and increased TRPA1 gene expression. More than 50% of patients diagnosed with diabetes will eventually develop diabetic neuropathy, which is characterised by distal paraesthesias and dysaesthesias, including cold-induced allodynia and hyperalgesia [47, 48]. The causes of diabetic neuropathy are multifactorial, revolving around the cellular results of chronic hyperglycaemia which in turn cause oxidative stress and cellular damage [49]. While the precise pathophysiological mechanisms leading to cold pain in diabetic neuropathy remain to be elucidated, abnormal peripheral sensory nerve function, including functional changes of the molecular players implicated in cold sensing, are likely to be involved. For example, methylglyoxal, an endogenous reactive metabolite, contributes to gain-of-function dysregulation of peripheral sensory neurons by increasing signalling through voltage-gated sodium channels and the cold-sensitive receptor TRPA1 [50, 51].

Cold intolerance also occurs in over 40% of patients with upper-extremity nerve trauma or hand/finger amputation and in generalised pain states such as complex regional pain syndrome, and may involve both peripheral sensory neuron abnormalities as well as altered central processing [52-55]. In addition to dysfunction of peripheral nerves, changes in central signalling can also lead to cold allodynia. For example, central post-stroke pain (CPSP) is a chronic pain condition [56, 57] that features painful symptoms in the body parts related to the brain territory affected by the cerebrovascular lesion. Estimations about cold pain prevalence are difficult because pre-existing chronic pain disorders are frequent [56]. Nevertheless, thermal allodynia is a frequent sensory symptom of CPSP and the allodynia presumably arises when CNS structures that form parts of somatosensory pathways, such as the medulla,

thalamus, and cortex, are among damaged areas [56]. Interestingly, brain lesions alone are insufficient to cause cold allodynia, suggesting additional secondary pathophysiological changes contribute to the development of cold allodynia following a stroke [56].

Ion Channels Involved in Cold Pain

Cold temperature detection involves the process of sensory transduction in the cutaneous primary sensory nerve terminals, which converts a thermal stimulus into depolarisation of the membrane. This transformation into an electrical signal is followed by the subsequent propagation of the action potentials in the cold-sensitive afferent nerves. A large array of ion channels, including TRPs and sodium and potassium channels, shape this process as outlined below **(Fig. 1).**

TRP Channels

Cold-sensitive TRPM8: The discovery of the transient receptor potential melastatin 8 channel (TRPM8) [58, 59] has significantly advanced our understanding of the processes underlying transduction and transformation of external cold into electrical signals. The channel is essential for the detection of environmental cold, and a series of potassium channels contribute to threshold adjustment and amplification of TRPM8-dependent cold transduction [60-62]. TRPM8$^{-/-}$ mice show drastically reduced responses to innocuous cold [63-65]. Cultured trigeminal ganglia from TRPM8$^{-/-}$ mice are also insensitive to menthol or innocuous cold (22 °C), while the number of cold-responsive cutaneous myelinated and unmyelinated nerve endings is decreased significantly [63]. However, the extent to which TRPM8 is essential for pathological forms of cold pain is controversial, and conflicting evidence exists, as outlined in **Table 3**.

TRPM8$^{-/-}$ mice, mice treated with intrathecal TRPM8 antisense oligonucleotide, and mice with diphtheria toxin-mediated ablation of TRPM8-positive neurons all had reduced physiological cold sensitivity, but also reduced cold hypersensitivity following Complete Freund's Adjuvant (CFA)-induced inflammation and chronic constriction injury (CCI), as examined by the acetone test [64, 66, 67]. At the same time, TRPM8$^{-/-}$ mice retain noxious cold sensitivity and exhibit similar nocifensive behaviours to control mice at temperatures below 10 °C [63, 65]. A further confounding factor in studies assessing the contribution of TRPM8 to cold sensing and cold pain is the specificity and selectivity of pharmacological "tool" compounds such as menthol. Many studies equate menthol-induced responses or effects with selective TRPM8 activation. However, the prototypical TRPM8 agonist menthol, as discussed below, is a promiscuous compound and elicits responses in TRPM8-negative sensory neurons. Menthol inhibits nicotinic acetylcholine receptors [68], inactivates voltage-gated calcium currents [69], inhibits K_v7.2/7.3 channels [60], and activates and/or desensitises the cold-sensitive TRPA1 channel [70].

Cold-sensitive TRPA1: TRPA1 is considered a noxious cold and irritant sensor. The channel mediates, among others, the reaction to the pungent components of mustard, horseradish, and wasabi. TRPA1 is also activated by formaldehyde, bacterial endotoxins and pro-inflammatory mediators such as bradykinin, methylglyoxal, and prostaglandin E2 [50, 71-79]. TRPA1 expression in sensory neuronal populations overlaps little with TRPM8 expression, suggesting distinct physiological functions for the two channels. Moreover, TRPA1 is not only highly co-expressed with the noxious heat sensor TRPV1, but can also form functional heteromultimers [80], which adds to the functional complexity of this channel.

Activation of TRPA1 by cold in cellular models varies between species [81] and has been investigated extensively, as summarised in **Table 4**. The overwhelming majority of studies

assessed TRPA1 activation by cold using cultured rodent dorsal root ganglion (DRG) neurons, or rodent TRPA1 overexpressed in mammalian HEK293 cells, CHO cells, or *Xenopus* oocytes. Many such studies demonstrated increased intracellular Ca^{2+} or TRPA1-mediated inward current upon exposure to cool temperatures (5-18 °C ; see **Table 4**) [59, 77, 81-86]. However, while questions about the cold-sensitivity of TRPA1 have been raised [61, 74, 81, 87, 88], species- and tissue-specific differences in the cold sensitivity of TRPA1 are becoming apparent. A number of studies also based their conclusions regarding the cold sensitivity of TRP channels on the perhaps erroneous assumption of the pharmacological specificity of isothioscyanates, icilin and menthol [59, 70, 74, 89-92]. In fact, while rodent TRPA1 is cold-sensitive, snake and drosophila TRPA1 are heat-sensitive [87, 93, 94]. In TRPA1-deficient mice, altered nocifensive behaviour in various noxious cold tests has been described and is summarised in **Table 5**. In addition, a recent functional MRI study unveiled altered central processing of cold stimuli at temperatures above the noxious cold range (15 °C) where no measurable aversive behaviour is observed, suggesting that standard cold pain tests in mice are less sensitive than commonly believed [38]. In contrast to mice TRPA1, primate TRPA1 does not respond to cooling in *in vitro* systems [81]. Nevertheless, a distinct role for TRPA1 in cold sensing and cold pain in humans cannot be denied, as a monogenic TRPA1 gain-of-function mutation is linked to a hereditary disease of episodic debilitating pain, which is triggered by cold [95]. Turning to ciguatoxin as a model of cold allodynia, *de novo* TRPA1-mediated cold responses in previously cold-insensitive mouse DRG neurons emerged after treatment with ciguatoxin and correlate well with a key contribution of TRPA1 to ciguatoxin-induced cold allodynia in mice [38]. Comparable mechanisms of TRPA1 sensitisation to cold may apply for cold hypersensitivity in inflammatory conditions and diabetic neuropathy [50, 71, 83, 96]. In addition, evidence for TRPA1 contribution to neuropathic cold allodynia of various origins is brought together in **Table 5**.

Insight into the biophysical mechanisms underlying temperature-sensitivity of TRP channels comes from a range of elegant studies assessing gating behavior of TRP channel chimeras and orthologs. Although temperature can have a profound effect on general phospholipid phase transitions, conformational changes, and protein denaturation, the remarkable effect of temperature on thermo-TRP function likely arises from direct effects on structural components which alter channel open probability [97]. In the case of TRPA1, residues contributing to thermal sensitivity appear to be located in the region of the N-terminal ankyrin domains which converted the cold-sensitive mouse TRPA1 into a heat-gated channel akin to the rattlesnake TRPA1 ortholog [87, 98], while point mutations within the S5 and S6 transmembrane domain rendered rodent and drosophila TRPA1, respectively, temperature-insensitive [81, 99]. Similarly, replacement of the TRPM8 C-terminus with the corresponding TRPV1 sequence reversed the temperature-dependence of the channel [100]. In addition, temperature and voltage exert synergistic, or allosteric, effects on channel gating, with cooling shifting the voltage-dependence of TRPM8 activation closer to physiological membrane potential [101].

Cold-sensitive TRPC5: The recent discovery of the involvement of the TRPC5 channel in peripheral cold sensation has recently expanded the list of cold-sensitive channels [102] and may provide an explanation for the TRPM8- and TRPA1-independent cold sensitivity observed in peripheral sensory neurons [90]. TRPC5 homomers, but not TRPC5-TRPC1 heteromultimers, are most active between 37 and 25 °C [102]. In mice and humans, TRPC5 is expressed at all levels of the nociceptive system, including sensory nerve endings, axons, DRG neurons and the superficial nociceptive laminae in the spinal cord [102]. TRPC5$^{-/-}$ mice, based on the 129S1/SvImJ strain, had fewer TRPM8-expressing DRG neurons but significantly higher numbers of cutaneous CMC-fibres with increased sensitivity to cold and

menthol. However, at the behavioural level, no differences appeared in the cold plate and the two-plate temperature preference assays. Rather than being a noxious cold sensor, TRPC5 probably mediates changes in detection and regional adaption to cold. Cold-induced gating of TRPC5 is potentiated by activation of Gq-coupled receptors, which modulates cold sensing by TRPC5 [102]. Neither oxaliplatin nor ciguatoxin-activated models of cold allodynia involve TRPC5 [38, 39] .

Voltage-gated sodium channels

$Na_v1.1$, $Na_v1.2$, $Na_v1.6$, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ are the Na_v isoforms expressed in adult DRG neurons and co-localise to a variable degree with peripherin, a marker of non-myelinated C-fibre neurons and with neurofilament, which stains medium and large diameter neurons associated with A-fibres. While $Na_v1.6$ localises to all sizes of neurons, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ predominate the small neurons. In contrast, $Na_v1.1$ is exclusively confined to large neurons [103]. $Na_v1.3$ is the major Na_v subtype in embryonic neurons and is present in sympathetic neurons [104], but undetectable in adult DRGs, where it re-emerges after injuries such as axotomy [105]. Given their diverse roles in cold pain pathways, it is likely that these subtypes contribute to modality- and pathway-specific pain, including nociceptive cold pain, cold allodynia and cold hyperalgesia as discussed below.

$Na_v1.7$: $Na_v1.7$ has gained prominence as a putative pain target, with rare genetic mutations rendering $Na_v1.7$ non-functional in humans causing cases of congenital insensitivity to pain (CIP), without affecting the ability to discriminate hot from cold [106-109]. Its contribution to physiological and pathological cold pain was assessed using three different $Na_v1.7$ knockout mouse strains (see **Table 6**): $Na_v1.7^{Nav1.8}$, with $Na_v1.7$ deleted from $Na_v1.8$-expressing neurons; $Na_v1.7^{Advill}$, with $Na_v1.7$ deleted from all sensory neurons; and $Na_v1.7^{Wnt1}$, with

$Na_v1.7$ deleted from sensory and sympathetic neurons [110]. Deletion of $Na_v1.7$ from $Na_v1.8$-expressing nociceptors reduced but did not abolish withdrawal responses to an acetone drop applied to the hind paw, while cold nociception was virtually abolished in $Na_v1.7^{Advill}$ and $Na_v1.7^{Wnt1}$ animals [110]. Although this suggests a crucial role for $Na_v1.7$ in $Na_v1.8$-negative neurons during cold nociception, all three $Na_v1.7$ knockout mouse lines displayed normal temperature preference behaviour between 30 °C and 5 °C [110]. Additionally, $Na_v1.7$ in DRG neurons is essential for cold allodynia in the CCI-model of neuropathic pain, but not in the sympathetically mediated spinal nerve transection (SNT) model of neuropathic pain, which only requires $Na_v1.7$ in sympathetic neurons. Consistent with these roles, $Na_v1.7$ does not contribute to oxaliplatin-induced cold allodynia [39, 111].

$Na_v1.8$: Low-threshold TTX-sensitive ion channels such as $Na_v1.7$ enter a state of slow inactivation upon cooling at temperatures lower than 26 °C [112]. In contrast, the high-threshold, slow TTX-resistant $Na_v1.8$ is resistant to cold-induced slow inactivation and remains available to generate action potentials at noxious cold temperatures. Consequentially, $Na_v1.8$ expressing C-fibres predominate our perceptions in cold temperatures, while Aβ-fibre mediated mechanosensitivity and manual dexterity is lost [112]. Thus, $Na_v1.8$ is not only responsible for pain caused by noxious cold, but also for any other pain modality at low temperatures. Accordingly, cold pain thresholds are significantly increased in $Na_v1.8$ global knockout animals and after diphtheria toxin-mediated ablation of $Na_v1.8$-expressing DRG neurons [110, 112, 113] (see **Table 7)**. Similar to $Na_v1.7$, the contribution of $Na_v1.8$ to pathological cold pain appears to be modality- and disease-specific. In the post-CCI cold allodynia model, $Na_v1.8$ knock-out mice showed attenuated flinching responses to acetone, while in the post-SNT cold allodynia model the withdrawal responses of $Na_v1.8$ knock-out animals were similar to wild-type litter mates [111].

$Na_v1.8$ appears less important in conditions where cold allodynia occurs at more moderate temperatures, potentially because TTX-sensitive Na_v isoforms remain functional. This is likely the case for ciguatoxin-induced cold allodynia, where $Na_v1.8$-expressing C-fibre nociceptive pathways contribute partially to cold pain behaviour in concert with TTX-sensitive ion channels on A-fibre pathways [38]. In contrast to ciguatoxin, cold allodynia elicited by intraplantar injection of the chemotherapeutic agent oxaliplatin is mediated entirely by TTX-sensitive Na_v isoforms, and cold pain behaviour at 10 °C was not affected in $Na_v1.8$ knockout animals, or after treatment with the $Na_v1.8$ inhibitor A803467 [39]. These findings were also confirmed in a more conventional model where cold allodynia develops slowly after repeated intravenous administration of oxaliplatin [111] and which was independent of $Na_v1.7$, $Na_v1.8$, and TRP channels.

$Na_v1.6$ and $Na_v1.3$: Involvement of $Na_v1.3$ in pain behaviour seems minor, despite being upregulated in various inflammatory and neuropathic pain conditions [114-116]. Some contribution of $Na_v1.3$ was shown in CCI, but not in the sciatic nerve ligation (SNL) or oxaliplatin-induced models of cold allodynia [39, 111]. Indeed, oxaliplatin-induced cold allodynia was completely abolished by TTX and the $Na_v1.6$ inhibitor GIIIA, while inhibition by subtype-selective modulators or genetic deletion of other Na_v isoforms, including $Na_v1.7$ and $Na_v1.3$, had no effect [39]. Although activation of $Na_v1.6$ by the scorpion toxin Cn2 was not sufficient to cause cold allodynia, it amplified cold pain caused by potassium channel inhibition [39]. Indeed, oxaliplatin is likely to elicit direct effects on $Na_v1.6$-expressing sensory neurons independent of changes in neuronal viability or frank neuronal toxicity. When exposed to oxaliplatin, compound after-potentials were triggered in A-fibres from human nerve fascicles upon cooling, but not in C-fibres [117]. This effect was abolished in sural nerve fascicles from $SCN8A^{med/med}$ mice that lack functional $Na_v1.6$ [117]. Oxaliplatin also

induced $Na_v1.6$-mediated resurgent and persistent currents in large (but not small) diameter DRG neurons in response to cooling, and significantly slowed inactivation of heterologously expressed $Na_v1.6$ [117]. These effects corroborate a crucial role for $Na_v1.6$ in cold pain pathways. Indeed, the contribution of $Na_v1.6$ to cold pain may extend to other conditions, as ciguatoxin-induced cold allodynia was also partially inhibited by the $Na_v1.6$ inhibitor GIIIA [37].

Potassium channels

A range of potassium channels, including the two-pore domain (2PK) background channels TRAAK, TASK-3, TREK-1, TRESK [118, 119] as well as voltage-gated potassium channels K_v1 and $K_v7.2/7.3$, have been implicated in the physiology of cold sensing and the pathophysiology of cold pain. Closure of background 2PK channels underpins cooling-induced increase in excitability of cultured DRG neurons, leading to membrane depolarisation and increased firing [120, 121].

TREK-1 and TRAAK are temperature- and mechano-sensitive background potassium channels of the 2PK family [122, 123] that stabilise the resting membrane potential at normal skin and body temperature. Their closure with cooling and heating facilitates temperature transduction-induced membrane depolarisation in C-fibres [120, 121, 123, 124]. However, while C-fibres from TREK-1 knock-out mice exhibit a reduced heat threshold, these mice did not display any difference in cold sensitivity compared to control during the acetone test and cold water tail immersion [124]. A more detailed analysis using temperature-controlled cold plates revealed that TREK-1 knock-out mice had reduced cold avoidance at 18°C but not at other temperatures [62], although cold allodynia (as evidenced by decreased paw withdrawal responses in the acetone test) was decreased in TREK-$1^{-/-}$ mice after SNL [124].

TRAAK knockout mice, like TREK-1 knockouts, had normal cold-sensitive C-fibres and no cold sensing deficits in the cold plate assay, the temperature-preference assay from 27 °C to 12 °C, or in cold allodynia following CCI [62]. A cold sensing phenotype became apparent only when both TREK1 and TRAAK were removed. The double knockout mice showed cold hypersensitivity in the 30°C thermal preference test, increased cold avoidance in the cold plate test, and increased cold allodynia post-CCI [62]. Thu,s during cold allodynia, where normally silent sensory neurons increasingly respond to mild cooling, reduced background potassium conductance may contribute to altered thermal thresholds and heightened cold sensitivity.

The presence of 4-aminopyridine-sensitive K_v channels appears to define cold-insensitive neurons [61, 121]. Activity of these channels prevents firing of cold-insensitive neurons during cooling by acting as excitability brakes. In addition, the setting of the temperature threshold in cultured trigeminal neurons is conferred by equilibrium between TRPM8 and K_v1 channels. Indeed, the temperature threshold of trigeminal neurons is defined by balanced expression of the cold-sensitive thermosensor TRPM8 and K_v1 channels, with cold-sensitive neurons defined by large TRPM8-mediated responses and low K_v1 current density, while the inverse relationship is true for cold-insensitive neurons [61].

Investigating ion channel constellations in low- and high-threshold cold-sensitive DRG neurons revealed that $K_v1.1$ and $K_v1.2$ selective blockers increased baseline calcium levels of high-threshold cold sensors but not low-threshold sensors, confirming differential expression of K_v1 channels in cold-sensitive neurons responsive to different temperature ranges [85]. Selective $K_v1.2$ block converted high-threshold cold sensors into low-threshold cold sensors that respond to minor fluctuations in temperature (± 1 °C) [85]. This effect translates to increased nocifensive behaviour of mice on a 0 °C cold plate and increased flinching in the acetone test after intraplantar injection of a K_v1 channel blocker [61]. These results support a pivotal role of K_v1 channels in setting the temperature threshold of cultured DRGs with high,

but not low threshold, cold-sensitivity. Whether K_v1 channels also contribute to the setting of the temperature thresholds, and thereby shape the broad dynamic range of monomodal cold-sensitive fibres in the murine skin, has not been investigated so far.

A similar inverse relationship exists for K_v7 availability and temperature threshold of activation in cutaneous cold nociceptive nerve fibre terminals [60]. While K_v7 expression levels or current density cannot be determined in terminal nerve endings, the cold sensitising effect of the K_v7 inhibitors XE991 and camphor were directly correlated to the fibre's temperature threshold, with low threshold cold-sensitive fibres showing little cold sensitisation in response to K_v7 inhibition [60]. Instead, relatively cold-insensitive fibres with high temperature thresholds showed pronounced cold sensitisation in response to both camphor and XE991 [60]. However, treatment with the K_v7 specific inhibitor XE991 or camphor, an M-channel inhibitor and weak TRPM8 agonist, were unable to trigger a cold response in the absence of TRPM8, suggesting that K_v7 channels act as suprathreshold amplifiers of the cold-activated generator potential provided by TRPM8 [60]. In addition, menthol appears to be an M-channel inhibitor and thus combines dual cold-activating and cold-sensitising pharmacology in one molecule [60].

Animal Models of Cold Pain

Rodent models are widely used to assess the contribution of various ion channels to cold sensing and nociception as well as cooling-induced allodynia and hyperalgesia, largely due to the accessibility of rodents in a laboratory setting and the ease of genetic manipulation in mice in particular. However, results from rodent studies, especially mice, are limited by difficulties relating to behavioural responses to pain sensation in animals, with poor distinction often made between innocuous cool, cold and noxious cold. Overt aversive behaviours upon exposure to temperature-controlled surfaces such as paw lifting, licking, flinching, shaking or

jumping occur at temperatures below 5 °C, suggesting this should be considered the threshold for noxious cold in rodents [19]. Moreover, large variation in response latency occurs in the traditional measure of hind paw withdrawal upon contact with a 0 °C cold plate, which can range from 5-200 seconds in several independent reports [63-65, 78]. Attempts to correlate more subtle changes in exploratory behaviour, such as walking backwards, to cold-induced pain responses have been reported [125]. Nevertheless, the validity and reproducibility of such approaches are unclear. An alternative approach to quantification of cold-induced pain behaviour is to assess latency to forepaw rather than hind paw withdrawal or shaking, an approach used successfully to assess the contribution of TRPM8 to noxious cold sensing at 0 °C [66, 82].

Another measure of cold avoidance is the temperature preference assay. The two-plate temperature preference assay allows animals to choose between two adjacent surfaces maintained at different temperatures, while the temperature gradient assay determines a preference temperature directly by using a continuous gradient. In these assays, choosing the appropriate temperature range is crucial as differences in phenotype or behaviour can only be detected if the thermal environment lies in the appropriate range of being mildly aversive without being overtly noxious. However, although clear differences in time spent at cool temperatures are apparent in models such as TRPM8$^{-/-}$ animals, it is not entirely clear to what degree these assays reflect physiological warmth preference rather than a sensation of pain, or aversion to cool.

The acetone test consists of applying a drop or a small spray of acetone to one hind paw, which then leads to evaporation-induced cooling and aversive behaviour. Application of acetone to the hind paw is often considered an innocuous cold stimulus as a decrease of approximately ~8-12 °C in skin temperature can be achieved with this technique [64, 126]. An additional advantage of the acetone test is the ability to stimulate unilateral aversive

behaviour, which may be easier to quantify. However, application of acetone to the hind paw of mice, and to a lesser degree of rats, elicits withdrawal responses in some naïve animals [64], suggesting the use of the acetone evaporation test is not suitable for quantification of cold allodynia and may more accurately represent general noxious withdrawal responses. Acetone-evoked withdrawal responses may also incorporate elements of mechanical stimulation or aversive responses to the odour of acetone, and control over the rate and degree of cooling is not possible. In addition, acetone has high vapour pressure and a small surface tension, and forming a uniform "drop" on a syringe that can be applied to the animal's hind paw can rarely be achieved.

An additional factor to be considered in observer-based behavioural studies is the need for appropriate blinding and randomisation to eliminate conscious, as well as unconscious, bias. This is illustrated by our observation that cold allodynia was erroneously observed when a blinded observer quantified paw withdrawal responses to the application of acetone between two distinct groups of mice while knowing one group was an experimental cohort and the other a control cohort. This effect completely disappeared when individual mice from the two cohorts were observed at random (unpublished observation).

Despite the wide availability of animal models of cold allodynia, it is difficult to evaluate how well the pathophysiological mechanisms of animal models translate to human conditions. This difficulty arises in part from differences in the time course of disease and methods used to induce and measure pain behaviour. Currently, animal models are considered adequate as long as the animals exhibit similar neuropathic pain symptoms to human patients after exposure to the same chemicals or injuries. The extent to which such animal models are based on similar disease mechanism as their human equivalents is not always clear and needs to be assessed using well-designed clinical studies.

Treatment of Pathological Cold Pain

Despite our growing understanding of the pathophysiology of cold pain, treatment of neuropathic cold pain remains largely symptomatic, with standard analgesics and adjuvants used in the majority of conditions associated with cold allodynia or hyperalgesia.

Neuropathic pain patients often present with a multitude of sensory abnormalities that are based on diverse pathophysiological mechanisms. Most clinical trials assessing analgesic efficacy for specific diseases rely on visual analogue scales or equivalent self-reporting of pain intensity rather than quantitative sensory testing. In addition, co-morbidities are common in diseases that present with cold allodynia, adding to heterogeneity in the clinical presentation. Accordingly, delineating clinical efficacy for treatment of cold pain specifically is often difficult, although inclusion of quantitative sensory profiling in clinical trials is likely to improve our success in mechanism-based treatment approaches [127].

As outlined in the preceding sections, it is becoming increasingly clear that cold pain and cold sensing are complex phenomena involving diverse modality- and disease-specific mechanisms. This complexity is illustrated by the diverse molecular mechanisms underlying cold pain induced by intraplantar injection of ciguatoxin and oxaliplatin. While ciguatoxin-induced cold allodynia requires TRPA1 and is decreased significantly without functional $Na_v1.8$, acute oxaliplatin-induced cold allodynia develops in sensory neurons expressing TTX-sensitive $Na_v1.6$ independent of thermosensitive TRP channels. It is thus likely there will be no single molecular target or drug that can treat the wide pathophysiological spectrum of cold allodynia and hyperalgesia. Nonetheless, mechanism-directed drug therapy for neuropathic pain is on the rise, with drugs targeting TRPA1 and TRPM8 as well as $Na_v1.8$ and $Na_v1.7$ considered particularly attractive for treatment of cold pain, albeit the responding patient populations will need to be chosen carefully based on the underlying pathophysiological mechanisms. Based on the role of K^+ channels in setting the temperature threshold in cold-

sensitive peripheral sensory neurons, K^+ channel agonists may also prove useful in the clinical management of neuropathic cold pain. Indeed, the K^+ channel agonists flupirtine and retigabine were effective at decreasing neuronal excitability in an *in vitro* model of oxaliplatin-induced cold hypersensitivity and also decreased oxaliplatin-induced cold allodynia *in vivo*, as did analgesics with mixed activity at Na_v and K_v channels such as lamotrigine and phenytoin (See **Table 12**) [128, 129].

Although selective channel modulators may be a feasible strategy for reducing cold allodynia due to the defined role of many channels such as TTX-sensitive Na_v isoforms, $Na_v1.8$, TRPM8 and TRPA1, limited research on the clinical applications of these findings is available and accurate analgesic efficacy prediction based on cell or rodent models may be difficult. For example, while amitriptyline reduced ciguatoxin-induced calcium influx in *in vitro* models, it was not effective at treating ciguatoxin-induced cold allodynia in a rodent model [37]. In the absence of systematic clinical trials in human ciguatera sufferers, this result is consistent with an anecdotal report of lack of efficacy of amitriptyline for treatment of cold hypersensitivity, although the tricyclic antidepressant may be efficacious at treating other symptoms associated with ciguatera [130]. Conversely, the antiepileptic lamotrigine and the analgesic flupirtine were analgesic *in vivo* but had little effect on ciguatoxin-induced Ca^{2+} responses *in vitro*, being 100- and 10-times less potent than amitriptyline, respectively [37].

A similar analgesic efficacy profile was observed for oxaliplatin-induced cold allodynia, with lamotrigine, retigabine, and phenytoin being most effective while amitriptyline was not analgesic [128, 131]. Gabapentin as well as Ca^{2+}/Mg^{2+} also partially reduced oxaliplatin-induced cold allodynia [131]. However, while efficacy of Ca^{2+}/Mg^{2+} is in accordance with several clinical studies [132-134], gabapentin was not effective in a randomised controlled trial on oxaliplatin-induced neuropathic pain [135]. In fact, more patients in the gabapentin treatment group developed neuropathy than those in the control group [135]. This apparent

discrepancy highlights the difficulty in translating findings from cellular models to murine models and human disease. Similarly, venlafaxine displayed effective analgesia in a murine model of oxaliplatin-induced cold allodynia [136], yet did not significantly improve cold allodynia in human patients despite efficacy towards other symptoms of oxaliplatin-induced neuropathy [137]. Curiously, morphine treatment displayed significant benefit in murine models of acute and chronic oxaliplatin-induced cold allodynia [136, 138], while the opioid receptor agonist fentanyl was ineffective at reversing cold allodynia induced by intraplantar injection of oxaliplatin [128].

Few human studies have specifically examined analgesic efficacy in paclitaxel-induced cold allodynia. Randomised controlled trials assessing the effect of gabapentin, lamotrigine and glutathione found a lack of efficacy in chemotherapy-induced peripheral neuropathy (CIPN) [139-141]. However, these studies did not specifically investigate cooling-related symptoms. Currently, the most promising treatment targeting paclitaxel-induced cold allodynia that has undergone human trials appears to be duloxetine. In one case study, a patient complaining of persistent and uncontrolled paclitaxel-induced neuropathy was treated unsuccessfully with pregabalin and trazodone. The addition of duloxetine reversed the symptoms and the patient experienced almost no pain two and half months later [142]. While this case study did not specify what type of allodynia the patient suffered from, evidence from a murine model supported the lack of efficacy of pregabalin in cold allodynia after paclitaxel administration [143], while a clinical trial showed duloxetine to be efficacious in CIPN [144]. This suggests that duloxetine, but not pregabalin, may be a potential treatment for paclitaxel-induced cold allodynia, although further studies are warranted.

In comparison to studies on CIPN, a much larger body of literature is devoted to the current and prospective treatments for diabetic neuropathy. A vast array of drugs, ranging from tight control of blood glucose levels to topical capsaicin applications, are used clinically to treat

pain and allodynia associated with diabetic neuropathy. Cold allodynia, however, does not present in every case of diabetic neuropathy. Moreover, human clinical trials on diabetic neuropathy frequently use pain scores and visual analogue scales as measurements of analgesia and do not necessarily assess the extent of stimulus-evoked pain or cold allodynia. Trials that include cold pain patients also often do not report separate analysis of modality-specific pain [145, 146]. Therefore, it is often difficult to gauge the specific impact of treatments on cold pain. Notably, clinical studies on lamotrigine, gabapentin, and amitriptyline specified the inclusion of cold pain patients, and the overall results were positive [147, 148]. A rat study also verified that amitriptyline is effective in diabetic animals with associated cold allodynia [149]. However, while venlafaxine and pregabalin were effective for the treatment of diabetic neuropathy, it is unclear if these human trials included patients with cold pain [146, 150].

Unlike the peripheral neuropathies that are associated with cold pain, central post-stroke pain (CPSP) triggers cold pain through damage to central pain tracts. Its treatment broadly follows that of other neuropathies, with drugs such as pregabalin and amitriptyline effectively alleviating neuropathic pain [151, 152]. While systematic clinical trials assessing analgesic efficacy in CPSP-associated cold pain are largely lacking, treatment with duloxetine for eight weeks significantly decreased cold allodynia evoked by the acetone test in humans [153]. Therefore, while CPSP in general can be effectively treated by a variety of drugs commonly used in neuropathic pain, duloxetine appears as a promising new treatment of cold allodynia associated with central nerve injury.

With increasing understanding of the pathophysiological mechanisms underlying cold pain, new mechanism-based treatments are being developed. Extensive drug discovery efforts directed at thermosensitive ion channels, most notably TRPA1 and TRPM8, have led to the discovery of a number of molecules with putative analgesic efficacy in cold allodynia. HC-

030031, a purine acetamide and selective TRPA1 antagonist, has been used extensively in a range of models associated with cold pain (see **Table 5**), with variable effects. Notably, HC-030031 was effective in rodent models of nerve injury and inflammation [83, 154], as was another small molecule TRPA1 inhibitor, A-967079 [155].

Presently, a TRPA1 selective antagonists (GRC 17536) is being evaluated in human studies for the treatment of diabetic neuropathy and reached Phase II trials (http://clinicaltrials.gov/ct2/show/NCT01726413), and further Phase I clinical trials have been initiated to assess safety and efficacy of the TRPA1 antagonist CB-625 [156]. In addition, a range of TRPM8 antagonists are being developed currently, including compounds that showed efficacy in rodent models of chronic constriction injury- and oxaliplatin-induced cold allodynia [157]. Other selective TRP as well as Na^+ or K^+ channel modulators remain in the pre-clinical development stage where their efficacy, selectivity, and dose *in vitro* and *in vivo* remain a topic of investigation. Thus, promising new approaches to the treatment of cold pain are on the horizon.

Conclusion

This review has highlighted that cold pain and cold sensing are complex phenomena, with clear differences in molecular pathophysiology between conditions associated with cold pain. Peripheral sensory neuron ion channels, such as voltage-gated sodium channels, potassium channels, and TRP channels, are involved to varying degrees in cold nociception, hyperalgesia and allodynia and may be suitable targets for improved treatment of these pain modalities. Notably, in addition to $Na_v1.7$, $Na_v1.8$, TRPA1, TRPM8, K_v1, TRESK, and TRAAK, more recently $K_v7.2/7.3$, TRPC5, and $Na_v1.6$ have also been implicated in cold sensing and pain. In light of the diverse molecular players and mechanisms involved in pathological cold pain, it is not surprising that there is currently no universally effective

treatment. In addition, the cold pain symptoms present in each neuropathic syndrome are likely to result from specific sets of interactions between peripheral fibres, ion channels, and central modulation. Given this complexity, extensive research is required to unravel the processes underlying neuropathic cold pain and to allow the rational development of new drugs that improve the clinical management of this often-neglected syndrome.

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Table 1. Peripheral nerve subtypes involved in sensing innocuous and noxious cold

Fibre type	Fibre subtype	Sensitivity	References
C-fibres	CC (C-cold)	Only respond to cold, menthol-sensitive	[3, 6, 15, 102, 158]
	CMHC (C-mechano-heat-cold)	Polymodal nociceptors, respond to noxious mechanical, heat and cold	[14, 60, 159]
	CMC (C-mechano-cold)	Polymodal nociceptors, respond to noxious mechanical and cold	[13, 60, 158, 159]
	High-threshold cold-sensitive fibres	Only respond to noxious cold (< 20 °C)	[7]
Aδ-fibres	Low-threshold cold-sensitive fibres	Respond to innocuous cold	[160]
	AδMHC (Aδ-mechano-heat-cold)	Polymodal nociceptors, respondto noxious mechanical, heat and cold	[13, 159]
	AδMC (Aδ-mechano-cold) fibres	Polymodal nociceptors, respondg to noxious mechanical and cold	[159]
	Mechano-insensitive cold-sensitive fibres	Respond to innocuous or noxious cold	[6, 160]

Table 2. Common neuropathic pain conditions exhibiting symptoms of cold allodynia

Conditions associated with Pathological Cold Pain	**Cold Pain Prevalence**
Ciguatera	71-88% [161-163]
Oxaliplatin-induced neuropathy	81-98% [164]
Paclitaxel-induced neuropathy	84% [165]
Diabetic neuropathy	Uncertain
Central post-stroke pain (CPSP)	17-70% [166-168]
Post-traumatic cold intolerance following upper limb injury	38-82% [52, 54, 55]

Table 3. TRPM8 in *in vivo* behavioural models of cold pain

Model	Pain test	Inhibition	Cold sensitivity	Species	Ref
Nociception					
Paw	Acetone test	KO	Reduced	Mouse	[63]
	Temperature preference test (30 °C vs 25-5 °C)	KO	Reduced	Mouse	
	10 °C, 0 °C, -5 °C and -10 °C cold plates	KO	Reduced	Mouse	
	0 °C cold plate. Temperature preference test (room temperature vs 25/5 °C)	KO	Reduced	Mouse	[64]
	15 – 53.5 °C multi-zone plate	KO	Reduced	Mouse	[65]
	Acetone test	KO	Reduced	Mouse	
	-1°C cold plate	KO	Unchanged	Mouse	
	Icilin (intraperitoneal)	KO	Reduced	Mouse	
	Acetone test	KO	Reduced	Mouse	[66]
	Icilin (intraperitoneal)				
	0 °C cold plate				
	Temperature preference test (5-50 °C)				
	Acetone test	TRPM8-positive DRG neurons ablated by diphtheria toxin		Mouse	
	Icilin (intraperitoneal)				
	0 °C cold plate				
	Temperature preference test (5-50 °C)				
Inflammation					
Complete Freund's Adjuvant injection (CFA)	Acetone test	KO	Reduced	Mouse	[64]
	Acetone test	KO	Reduced	Mouse	[66]
	Acetone test	TRPM8-positive DRG neurons ablated by diphtheria toxin	Reduced	Mouse	
Nerve injury					
CCI	Acetone test	KO	Reduced	Mouse	[64]
	Acetone test	KO	Reduced	Mouse	[66]
	Acetone test	TRPM8-positive DRG neurons ablated by diphtheria toxin	Reduced	Mouse	
	4 °C cold plate	Local KO with oligonucleotides	Reduced	Rat	[67]
	4 °C cold plate	Menthol (intrathecal)	Increased	Rat	
	1-cm-deep 4 °C water on cage floor	Local KO with oligonucleotides	Reduced	Rat	[169]

Neuropathies					
Ciguatoxin	15 °C cold plate	KO	Unchanged	Mouse	[38]
	15 °C cold plate	AMTB (intraplantar)	Unchanged	Mouse	
Oxaliplatin	Temperature preference test (25 °C vs 21/23 °C)	KO	Reduced	Mouse	[42]
	10 °C cold plate	KO	Unchanged	Mouse	[39]
	10 °C cold plate	AMTB (intraplantar)	Unchanged	Mouse	
	10 °C cold plate	M8-B (intraplantar)	Unchanged	Mouse	
	Acetone test	Capsazepine (intraperitoneal)	Reduced	Mouse	[43]
	Acetone test	Capsazepine (intraperitoneal)	Unchanged	Mouse	[40]

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Table 4. TRPA1 in *in vitro* models of cold pain

Model	Species	Temp	Respond to cold	Comment	Ref
CHO cells	Mouse	9 °C	Yes	Increased inward current	[77]
	Mouse	10 °C	Yes	Inward current increase	[82]
	Mouse	17 –11 °C	Yes	Calcium influx and inward current increase	[59]
Xenopus oocytes	Human	5 °C	Yes	Increased inward current	[77]
	Mouse	5 °C	Yes	Increased inward current	
	Rattlesnake	15 – 45 °C	No	Rattlesnake, rat snake, *Drosophila* TRPA1 responded to >38°C. Human TRPA1 responded to neither heat or cold	[87]
	Rat snake	15 – 45 °C	No		
	Drosophila	15 – 45 °C	No		
	Human	15 – 45 °C	No		
	Mouse	5 °C	Yes	Inward current increase	[59]
HEK cells	Rat	10 °C	Yes	Increased inward current	[83]
	Rat	24 – 8 °C	Yes	Calcium influx and inward current increased for rat and mouse TRPA1, but not for human or Rhesus monkey TRPA1	[81]
	Mouse	24 – 8 °C	Yes		
	Human	24 – 8 °C	No		
	Rhesus monkey	24 – 8 °C	No		
	Mouse	16 – 5 °C	Yes	Calcium influx and inward current increase	[84]
	Human	10 °C	No	Calcium influx increased for both TRPA1-positive and control cells upon cold stimulation	[88]
	Human	5 °C	No	No calcium influx	[74]
Cultured DRG neurons	Rat	10 °C	Yes	Increased inward current	[83]
	Mouse	16 – 5 °C	Yes	Calcium influx increase	[84]
	Rat	17/5 °C	Yes	Many rat cold-sensitive neurons express TRPA1, very few mouse cold sensors express the channel	[85]
	Mouse	17/5 °C	No		
Cultured trigeminal neurons	Mouse	16 – 8 °C	No	No increase in calcium influx	[61]
	Mouse	25 – 10 °C	Yes	Calcium influx increase	[82]
	Rat	5 °C	No	No cold response in neurons responsive to mustard oil	[74]
Nodose ganglion neurons	Rat	12 °C	Yes	Ca^{2+} responses and currents in vagal neurons	[86]

1 **Table 5**. TRPA1 in *in vivo* behavioural models of cold pain

Model	Pain test	Inhibition	Cold sensitivity	Species	Ref
Nociception					
Paw	0 °C cold plate	KO	Reduced	Mouse	[83]
	-5 °C cold plate	HC-030031 (intraperitoneal)	Unchanged	Rat	
	0 °C cold plate	KO	Reduced	Mouse	[82]
	10 °C cold plate	KO	Reduced	Mouse	[170]
	Acetone test	KO	Reduced	Mouse	[170]
	5 – 0 °C cold plate	A-967079 (oral)	No effect	Mouse	[155]
Tail	-10 °C tail immersion	KO	Reduced	Mouse	[82]
Inflammation					
CFA	5 °C cold plate	HC-030031 (intraperitoneal)	Reduced	Rat	[83]
	5 °C cold plate. Hind paw immersion in water at 28 °C, 16 °C, and 4 °C	Local KO with oligonucleotides	Reduced	Rat	[171]
Nerve injury					
CCI	Acetone test	A-967079 (oral)	Reduced	Rat	[155]
SNL	10 °C cold plate	HC-030031 (intraperitoneal)	Reduced	Rat	[83]
	5 °C cold plate	Local KO with oligonucleotides	Reduced	Rat	[172]
	5 °C cold plate. Hind paw immersion in water at 28 °C, 16 °C, and 4 °C	Local KO with oligonucleotides	Reduced	Rat	[171]
Neuropathies					
Ciguatoxin	15 °C cold plate	KO	Reduced	Mouse	[38]
Oxaliplatin	10 °C tail immersion	HC-030031 (intragastric)	Reduced	Rat	[40]
	Acetone test	KO	Reduced	Mouse	
	10 °C cold plate	HC-030031 (intraplantar)	Unchanged	Mouse	[39]
	10 °C cold plate	KO	Unchanged	Mouse	
	30–1 °C dynamic cold plate	HC-030031 (intraperitoneal)	Unchanged	Mouse	[42]

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Table 6. $Na_v1.7$ in *in vivo* behavioural models of cold pain

Model	Pain test	Inhibition	Cold sensitivity	Species	Reference
Nociception					
Paw	Acetone test	$Na_v1.7^{AdvIII}$	Reduced	Mouse	[110]
	Temperature preference test (30 °C and 5 °C cold plates)	$Na_v1.7^{AdvIII}$	Unchanged	Mouse	
	Acetone test	$Na_v1.7^{Nav1.8}$	Unchanged	Mouse	
	Temperature preference test (30 °C and 5 °C cold plates)	$Na_v1.7^{Nav1.8}$	Unchanged	Mouse	
	Acetone test	$Na_v1.7^{Wnt1}$	Reduced	Mouse	
	Temperature preference test (30 °C and 5 °C cold plates)	$Na_v1.7^{Wnt1}$	Unchanged	Mouse	
Nerve injury					
Spare nerve injury (SNI)	Acetone test	$Na_v1.7^{AdvIII}$	Unchanged	Mouse	[111]
	Acetone test	$Na_v1.7^{Nav1.8}$	Unchanged	Mouse	
	Acetone test	$Na_v1.7^{Wnt1}$	Reduced	Mouse	
CCI	Acetone test	$Na_v1.7^{AdvIII}$	Reduced	Mouse	
	Acetone test	$Na_v1.7^{Nav1.8}$	Reduced	Mouse	
	Acetone test	$Na_v1.7^{Wnt1}$	Reduced	Mouse	
Neuropathies					
Ciguatoxin	15 °C cold plate	ProTxII (intraplantar)	Unchanged	Mouse	[37]
Oxaliplatin	Acetone test	$Na_v1.7^{Wnt1}$	Unchanged	Mouse	[111]
	10 °C cold plate	ProTxII (intraplantar)	Unchanged	Mouse	[39]

Table 7. $Na_v1.8$ in *in vivo* behavioural models of cold pain

Model	Pain test	Inhibition	Cold sensitivity	Species	Reference
Nociception					
Paw	Temperature preference test (30 °C and 5 °C cold plates)	KO	Reduced	Mouse	[110]
	Acetone test	KO	Unchanged	Mouse	
	0 °C cold plate	KO	Reduced	Mouse	[112]
	0 °C cold plate	$Na_v1.8$-positive DRG neurons ablated by diphtheria toxin	Reduced	Mouse	[113]
Nerve injury					
SNI	Acetone test	KO	Unchanged	Mouse	[111]
	Acetone test	KO	Reduced	Mouse	[173]
CCI	Acetone test	KO	Reduced	Mouse	[111]
	Acetone test	KO	Unchanged	Mouse	[173]
Neuropathies					
Ciguatoxin	15 °C cold plate	A803467 (intraplantar)	Reduced	Mouse	[38]
	15 °C cold plate	$Na_v1.8$-positive DRG neurons ablated by diphtheria toxin	Reduced	Mouse	
	15 °C cold plate	KO	Reduced	Mouse	
Oxaliplatin	15 °C cold plate	A803467 (intraplantar)	Unchanged	Mouse	[37]

Table 8. $Na_v1.3$ in *in vivo* behavioural models of cold pain

Model	Pain test	Inhibition	Cold sensitivity	Species	Reference
Nerve injury					
SNI	Acetone test	KO	Unchanged	Mouse	[111]
	Acetone test	Intrathecal injection of oligonucleotides	Unchanged	Rat	[174]
CCI	Acetone test	KO	Reduced	Mouse	[111]
Neuropathies					
Ciguatoxin	15 °C cold plate	KO	Unchanged	Mouse	[37]
Oxaliplatin	10 °C cold plate	KO	Unchanged	Mouse	[39]

Table 9. $Na_v1.6$ in *in vivo* behavioural models of cold pain

Model	Pain test	Inhibition	Cold sensitivity	Species	Reference
Neuropathies					
Ciguatoxin	15 °C cold plate	GIIIA (intraplantar)	Reduced	Mouse	[37]
Oxaliplatin	10 °C cold plate	GIIIA (intraplantar)	Reduced	Mouse	[39]

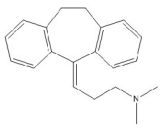
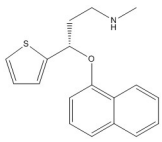
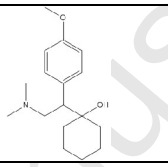
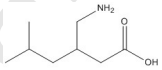
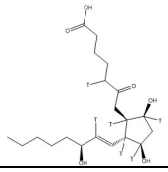
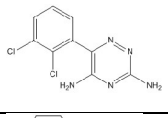
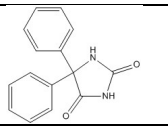
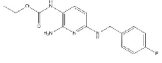
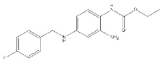
Table 10. $Na_v1.9$ in *in vivo* behavioural models of cold pain

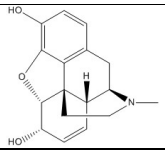
Model	Pain test	Inhibition	Cold sensitivity	Species	Reference
Nociception					
Paw	0 °C cold plate	KO	Unchanged	Mouse	[175]
Nerve injury					
SNI	Acetone test	KO	Unchanged	Mouse	[111]
	Acetone test	KO	Reduced	Mouse	[173]
CCI	Acetone test	KO	Reduced	Mouse	[111]
	Acetone test	KO	Reduced	Mouse	[173]
Neuropathies					
Ciguatoxin	15 °C cold plate	KO	Unchanged	Mouse	[38]
Oxaliplatin	10 °C cold plate	KO	Unchanged	Mouse	[39]

Table 11: Comparison of animal cold allodynia models with human disease

	Causes of disease	Disease time course	Acute Symptoms	Chronic symptoms
Diabetic neuropathy-induce cold allodynia				
Animal model	Streptozotocin-induced hyperglycaemia	21 days from streptozotocin injection to hyperglycaemia [45, 176]	N/A	Cold allodynia, heat allodynia, heat hyperalgesia, mechanical hyperalgesia [176]
Animal model	Zucker Fatty rats	Hyperglycaemia established from ~8 weeks [177]	N/A	Reduced nerve conduction speed, mechanical allodynia [177]
Human patients	Chronic diabetes mellitus type 1 or 2	A minimum of 6 months to 2 years from diabetes diagnosis to established neuropathy, dependent on hyperglycaemia [147, 150, 178]	N/A	Peripheral pain, tingling, paraesthesia, numbness, reduced vibration perception, nerve conduction abnormalities [147, 178]
Ciguatera				
Animal model	Ciguatoxin (intraplantar)	Immediate [38]	Spontaneous pain, cold allodynia [38]	N/A
Animal model	Ciguatoxin (intraperitoneal)	Immediate [179]	Hypothermia, reduced locomotor activity, hyporeflexia, lachrymation, salivation, diarrhoea	N/A
Human patients	Ingestion of ciguatoxin-contaminated fish	Immediate [161]	Paraesthesia, diarrhoea, abdominal pain, vomiting, headache [161]	Paraesthesia, myalgia, muscle weakness, vertigo, ataxia [161]
Oxaliplatin-induced cold allodynia				
Animal model	Injected oxaliplatin (acute or chronic)	Immediate [39]	Cold allodynia, mechanical allodynia [39, 180]	Mechanical allodynia, cold allodynia [136]
Human patients	Chronic oxaliplatin therapy	Immediately after injection [181] and chronic	Peripheral paraesthesia and cold allodynia [181]	Cold and heat allodynia, impaired vibration detection [181]

1 **Table 12.** Common drugs used in the treatment of neuropathic cold allodynia

Drug name	Drug Target	Condition	Effect	Structure	References
Amitriptyline	Serotonin-noradrenaline reuptake inhibitor and Na_v inhibitor [182]	Ciguatera	Uncertain		[37, 163]
		Oxaliplatin-induced	No		[128, 131]
		Diabetic neuropathy	Yes		[148, 149]
Duloxetine	Serotonin-noradrenaline reuptake inhibitor	Paclitaxel-induced	Yes		[142, 144]
		Post-stroke central pain	Yes		[153]
Venlafaxine	Serotonin-noradrenaline reuptake inhibitor	Oxaliplatin-induced	Uncertain		[136, 137]
Pregabalin	Ca_v in the CNS	Oxaliplatin-induced	Uncertain		[138, 143]
		Paclitaxel-induced	No		[142, 143]
Gabapentin	Ca_v inhibitor [183]	Oxaliplatin-induced	Uncertain		[129, 131, 135, 136, 139]
		Diabetic neuropathy	Yes		[148]
Lamotrigine	Na_v, Ca_v inhibitor, K_v activator [184]	Ciguatera	Yes		[37]
		Oxaliplatin-induced	Yes		[128]
		Diabetic neuropathy	Yes		[147]
Phenytoin	Na_v inhibitor	Ciguatera	Yes		[37]
		Oxaliplatin-induced	Yes		[128]
Flupirtine	K_v activator	Ciguatera	Yes		[37]
		Oxaliplatin-induced	Yes		[129]
Retigabine	K_v7 activator	Oxaliplatin-induced	Yes		[128]
Ca^{2+}/Mg^{2+} infusion	Chelation to oxaliplatin metabolite, or Na_v inhibition	Oxaliplatin-induced	Yes		[131-134]

Morphine	μ-opioid receptor agonist	Oxaliplatin-induced	Yes		[136, 138]

1

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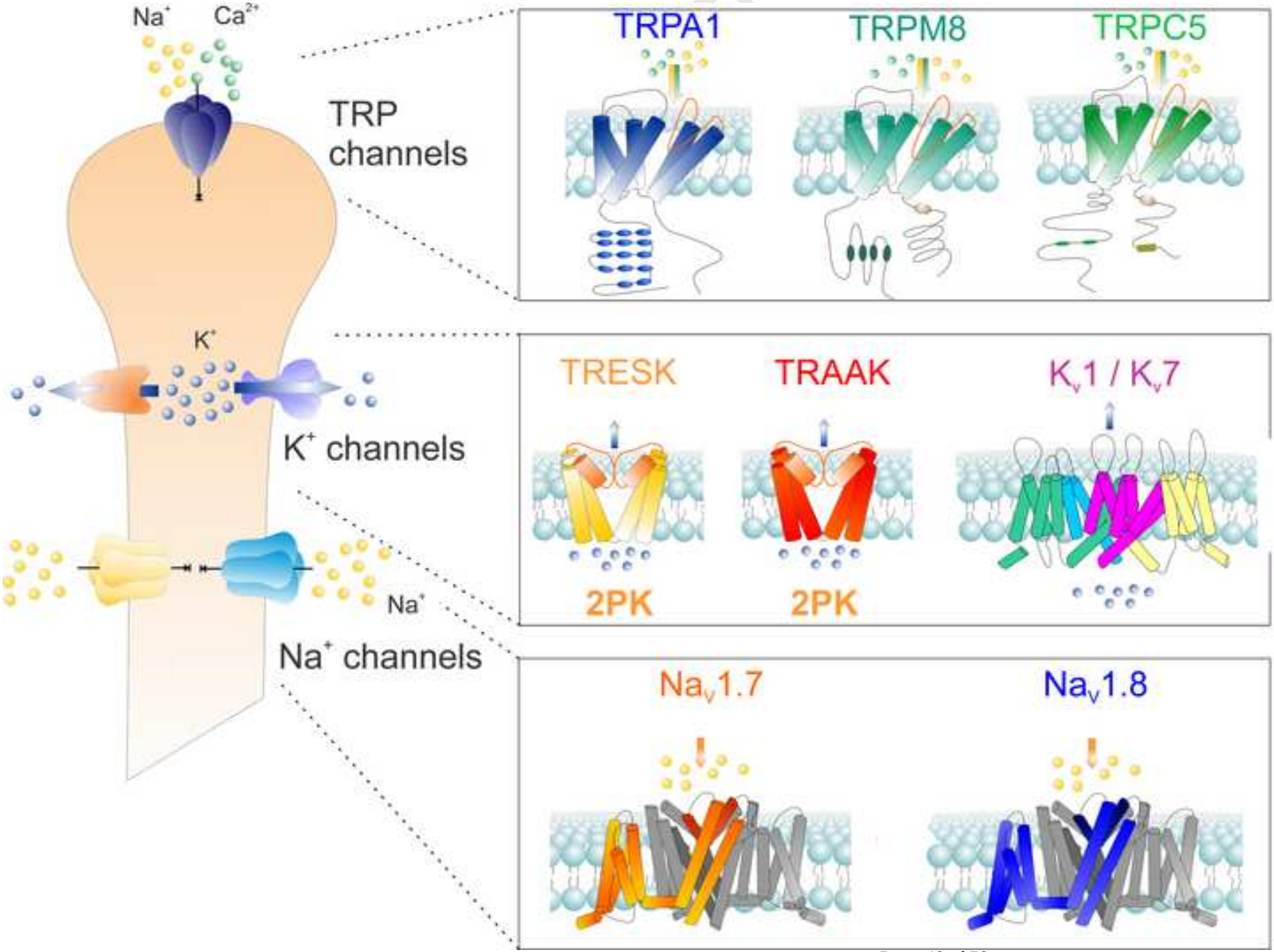
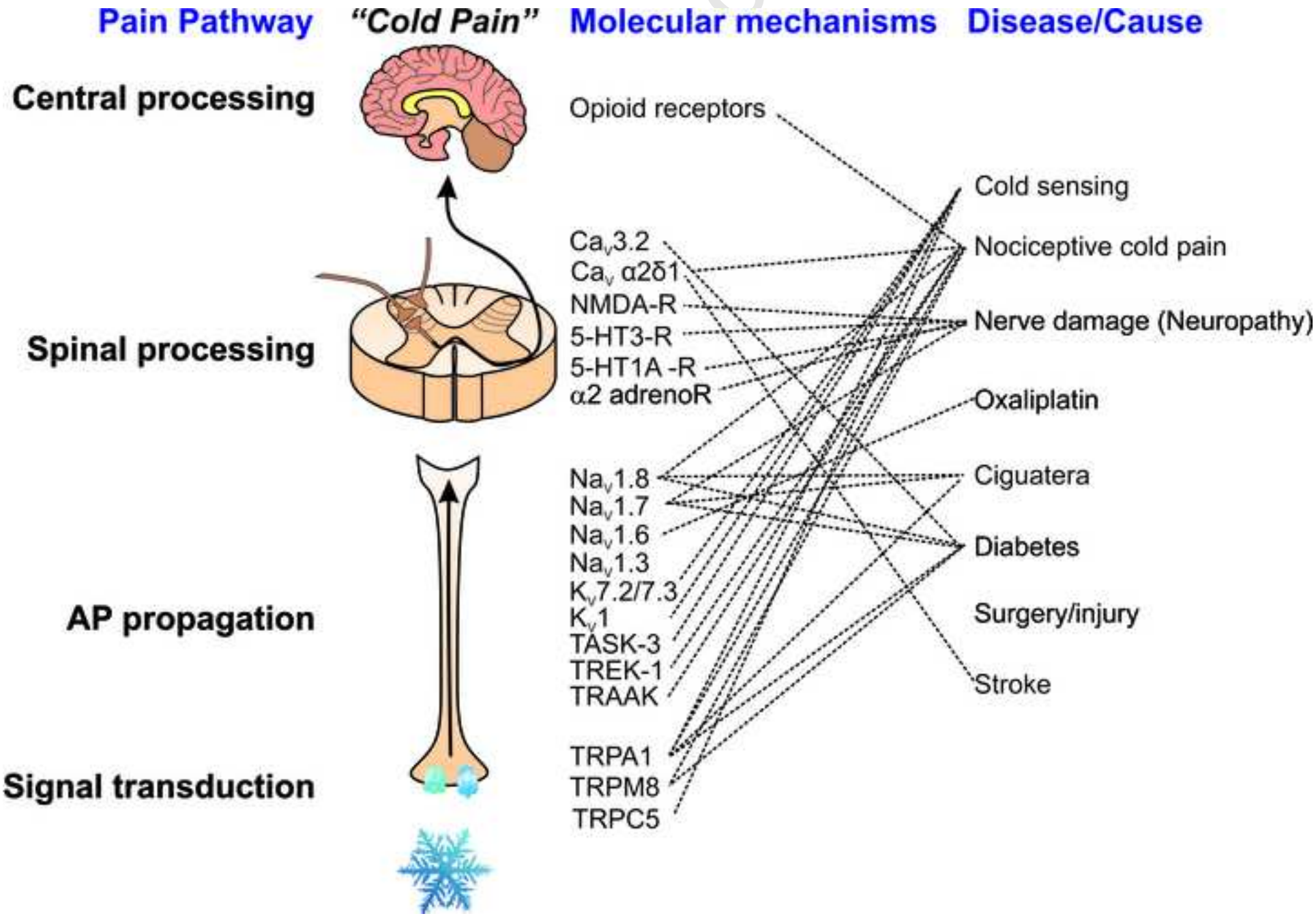
Fig. 1. Ion channels involved in cold sensing and cold pain. A range of neuronal ion channels have been identified that differentially contribute to physiological cold sensing, cold nociception, as well as pathological cold pain states such as cold allodynia and cold hyperalgesia. Transient receptor potential channels TRPA1, TRPM8 and TRPC5 are gated by cold and are involved in transformation of an external thermal stimulus into a neuronal signal. Voltage-gated potassium channels, most notably K_v1 and $K_v7.2/7.3$, act as excitability brakes in cold-insensitive neurons and contribute to setting the temperature threshold. In addition, background potassium channels such as TRAAK and TREK-1, which regulate the resting membrane potential of sensory neurons, are instrumental in reducing neuronal excitability and thus contribute crucially to thermal pain. Voltage-gated sodium channels, including Nav1.7 and Nav1.8 are expressed in unmyelinated nociceptive fibres and contribute to neuronal depolarisation and hyper-excitability.

Fig. 2. Pharmacology of cold pain pathways. The molecular mechanisms that integrate to produce cold pain in different disease states are diverse. These include altered signal transduction in myelinated and unmyelinated peripheral sensory nerve endings, altered action potential (AP) propagation, and altered spinal transmission and central processing. Key channels and receptors contributing to physiological and pathological cold pain pathways are illustrated.

Figure


Na+
Ca2+
TRP channels
TRPA1
TRPM8
TRPC5
K+
K+ channels
TRESK
TRAAK
Kv1 / Kv7
2PK
2PK
Na+
Na+ channels
Nav1.7
Nav1.8

Figure


Pain Pathway
"Cold Pain"
Molecular mechanisms
Disease/Cause
Central processing
Spinal processing
AP propagation
Signal transduction
Opioid receptors
Cav3.2
Cav α2δ1
NMDA-R
5-HT3-R
5-HT1A -R
α2 adrenoR
Nav1.8
Nav1.7
Nav1.6
Nav1.3
Kv7.2/7.3
Kv1
TASK-3
TREK-1
TRAAK
TRPA1
TRPM8
TRPC5
Cold sensing
Nociceptive cold pain
Nerve damage (Neuropathy)
Oxaliplatin
Ciguatera
Diabetes
Surgery/injury
Stroke