Neurosci. Bull. December, 2019, 35(6):1073–1084
https://doi.org/10.1007/s12264-019-00378-5

www.neurosci.cn
www.springer.com/12264

REVIEW

High-Voltage-Activated Calcium Channel in the Afferent Pain Pathway: An Important Target of Pain Therapies

Qi Li[1] · Jian Lu[1,2] · Xiaoxin Zhou[1] · Xuemei Chen[1] · Diansan Su[1] · Xiyao Gu[1] · Weifeng Yu[1]

Received: 7 August 2018 / Accepted: 24 December 2018 / Published online: 7 May 2019

Abstract High-voltage-activated (HVA) Ca^{2+} channels are widely expressed in the nervous system. They play an important role in pain conduction by participating in various physiological processes such as synaptic transmission, changes in synaptic plasticity, and neuronal excitability. Available evidence suggests that the HVA channel is an important therapeutic target for pain management. In this review, we summarize the changes in different subtypes of HVA channel during pain and present the currently available evidence from the clinical application of HVA channel blockers. We also review novel drugs in various phases of development. Moreover, we discuss the future prospects of HVA channel blockers in order to promote "bench-to-bedside" translation.

Keyword Pain · High-voltage-activated calcium channel · Dorsal root ganglion

Introduction

Pain is an unpleasant feeling induced by noxious stimuli. Pain is often accompanied by emotional changes and protective defense responses. At the same time, pain is one of the most common clinical symptoms that facilitate the diagnosis of disease. However, pain brings great suffering to patients and seriously affects their daily work and quality. In a community-based epidemiological survey, 45%–80% of elderly participants were found to suffer from chronic pain [1]. In a study conducted in Europe, chronic pain was found to affect ~20% of the population; in addition, chronic pain was more common among women, the elderly, and the relatively poor [2]. Rapid advances in medicine have created an impetus for the development of better pain therapies. Towards this end, a better understanding of the mechanisms underlying pain generation and signaling is a key imperative in order to identify novel therapeutic targets for pain management. A number of ion channels play important roles in the process of pain generation and transmission [3], including voltage-gated Ca^{2+} channels, especially high-voltage-activated (HVA) Ca^{2+} channels [4]. Various subtypes of HVA channel regulate the conduction of pain by changing their expression and currents. Pharmacological modulation of HVA channels is a viable therapeutic strategy for pain management. In this work, we review the pain conduction process and describe the role of HVA channels. In addition, we review the available evidence from experimental and clinical studies of HVA channel blockers. Lastly, we provide an overview of novel drugs that are currently in various phases of development and discuss their prospects in order to promote the "bench-to-bedside" translation of HVA channel blockers.

Qi Li and Jian Lu have contributed equally to this work.

✉ Xiyao Gu
gxykevin@126.com

✉ Weifeng Yu
yuweifeng@renji.com

[1] Department of Anesthesiology, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200127, China

[2] Department of Anesthesiology, The Second Affiliated Hospital of Jiaxing University, Jiaxing 314000, China

HVA Channels in the Afferent Pain Pathway

There are several types of HVA channels in neural tissue, including N-type, L-type, P/Q-type, and R-type channels. HVA channels are heteromultimeric proteins that essentially consist of $Ca_v\alpha1$, $Ca_v\beta$, and $Ca_v\alpha2\delta$ subunits in a 1:1:1 ratio (Fig. 1) [5]. In addition, some of the HVA channels, such as the L-type channels in skeletal muscle, also have the $Ca_v\gamma$ subunit [6]. The $Ca_v\alpha1$ subunit is a transmembrane protein that determines the subtype of the HVA channel. The coding genes can be divided into two major families, Ca_v1 and Ca_v2. The Ca_v1 family includes $Ca_v1.1$–1.4, all of which encode L-type channels. The Ca_v2 family includes $Ca_v2.1$–2.3; $Ca_v2.1$ encodes P/Q-type channels, and its selective splicing may determine the expression as P- or Q-type channels, while $Ca_v2.2$ encodes the N-type channel, and $Ca_v2.3$ encodes the R-type channel [7, 8]. The different subtypes of HVA channel have their own diverse functions and are amenable to selective blockade (Table 1) [9, 10]. This implies that HVA channels represent a viable therapeutic target that may be modulated for more effective treatment with fewer side-effects. The $Ca_v\beta$ subunit is an intracellular protein that is linked to $Ca_v\alpha1$ *via* domains I-II [11]; it may be involved in increasing membrane channel expression and regulating gene transcription by interfering with ubiquitination [12]. The $Ca_v\alpha2\delta$ subunit can be divided into α2 and δ, each of which is expressed by independent genes and then joined by disulfide bonds [13]. δ is present outside the cell membrane, where it acts as a glycosylated phosphatidylinositol anchored to the monolayer of the outer membrane of the cell membrane [14], promoting transmembrane expression of the α1 subunit [10]. Similar to $Ca_v\beta$, the δ subunit is also involved in regulating the expression of HVA channels by interfering with ubiquitination. While $Ca_v\gamma$ includes four helices, it is unclear whether it is distributed in neurons. A new γ subunit, which is the target of the stargazer mutation in mice, has recently been reported. A group of seven γ subunits exists in brain and other tissues. However, these novel stargazer-like γ subunits mainly modulate glutamate receptors in the postsynaptic membranes of neurons. It is still not clear whether these are associated with the HVA channels in neurons [15].

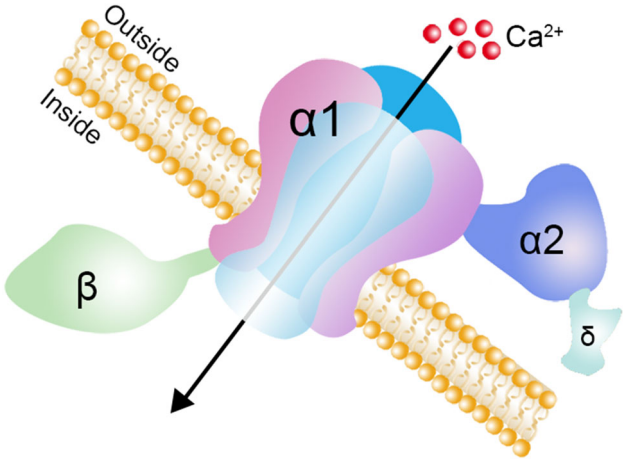

Fig. 1 Structure of HVA channels. Each HVA channel consists of Caα1, $Ca_v\beta$, and Caα2δ subunits [6]. The $Ca_v\alpha1$ subunit is a transmembrane protein that determines the subtype of the HVA channel [8]. The $Ca_v\beta$ subunit is present inside cells, and is involved in increasing membrane channel expression and in regulating gene transcription [11, 12]. The $Ca_v\alpha2\delta$ subunit is present outside cells and is linked by disulfide bonds [13].

Changes in the expression and function of HVA channels are closely associated with the occurrence, development, and persistence of pain [16]. In Table 2, we summarize the contemporary research pertaining to various changes in HVA channels of different subtypes in various pain models, including changes in expression and currents. In the next section, we focus on the role of these different types of HVA channel in pain conduction.

N-type HVA Channels

N-type HVA channels are substantially expressed in neural tissue. They are abundantly expressed in the presynaptic membrane of axons, regulate the release of neurotransmitters [17], and are abundant in DRG neurons [18]. With the influx of a large amounts of Ca^{2+} into a neuron, the presynaptic membrane releases many neurotransmitters, such as glutamic acid and substance P [19]. Sensitivity to pain is significantly reduced in mice with N-type channel deletion [20], while anxiety level and alcohol withdrawal symptoms are only slightly improved [21]. These findings indicate that N-type channels are closely associated with pain transmission.

In the partial sciatic nerve ligation model, the expression of N-type channels in DRG neurons is upregulated, and the proportion of N-type current is elevated [18]; in addition, in formalin-induced inflammatory pain model the N-type channels lack the domain II-III linker regions, which are encoded by $Ca_v2.2$ exon 18a [22]. RT-PCR analysis revealed no significant changes in the mRNA of N-type channels in a mouse model of diabetic neuropathic pain [23], suggesting that the structural changes in N-type channels caused by chronic pain occur at the protein level. In addition, the upregulation of N-type channels may be related to regulation of the ubiquitin-proteasome pathway [24]. There is also evidence that the expression of $Ca_v\alpha2\delta1$ is increased in the dorsal spinal cord in the spinal cord injury model [25], as described above; this increases the level of the transmembrane $Ca_v\alpha1$ subunit of HVA channels. This change is particularly associated with

Table 1 HVA subtypes and their characteristics.

HVA subtype	Encoding gene	Specific blocker	Main distribution	Function
L	$Ca_v1.1$	Dihydropyridines	Skeletal muscle	Excitation-contraction coupling
	$Ca_v1.2$	Dihydropyridines	Cardiomyocytes, neurons, smooth muscle cells	Excitation-contraction coupling, neural conduction
	$Ca_v1.3$	Dihydropyridines	Cardiomyocytes, neurons, endocrine cells	Cardiac rhythm, neuronal conduction, endocrine secretion
	$Ca_v1.4$	Dihydropyridines	Retina	Visual conduction
P/Q	$Ca_v2.1$	ω-Agatoxin	Neurons	Neuronal conduction
N	$Ca_v2.2$	ω-Conotoxin GIVA and MVIIA	Neurons, endocrine cells	Neurotransmitter release
R	$Ca_v2.3$	SNX-482	Neurons	Neuronal conduction

abnormal tactile pain in spinal nerve-ligated (SNL) rats [26]. The above conclusions have also been confirmed in transgenic mice overexpressing $Ca_v\alpha2\delta1$ in DRGs and the spinal cord. $Ca_v\alpha2\delta1$ overexpression has been shown to result in enhanced currents, which exaggerate and prolong the dorsal horn neuronal responses to mechanical and thermal stimuli [27]. In addition, pregabalin, which acts on $Ca_v\alpha2\delta1$ to inhibit N-type channels, has been shown to inhibit the accumulation of $Ca_v\alpha2\delta1$ in the SNL model. Moreover, it reduces the increase of $\alpha2\delta1$ in the presynaptic terminals of DRG neurons in the spinal dorsal horn [28]. Owing to the unique role of $Ca_v\alpha2\delta1$, it has been fully utilized as an important therapeutic target in clinical settings. For example, gabapentin acts on this subunit and has a powerful analgesic effect in patients with neuropathic pain [29].

In general, N-type channels are up-regulated during pain generation, including increased currents and expression. At the same time, inhibition of N-type channels reduces nerve conduction to induce analgesia. These findings suggest that N-type channels play an important role in the process of pain generation. Moreover, N-type channels are viable potential targets for the development of novel analgesics.

L-type HVA Channels

Currently, there is considerable ambiguity with respect to the specific role of L-type HVA channels in the pain afferent pathway. As noted earlier, the Ca_v1 family can be divided into four groups, $Ca_v1.1$–1.4. $Ca_v1.1$ is mainly expressed in skeletal muscle and plays a key role in excitation-contraction coupling. $Ca_v1.4$ is mostly expressed in the retina and plays an important role in normal visual function. However, no evident expression of $Ca_v1.1$ and $Ca_v1.4$ has been found in the brain. Radioreceptor assays and quantitative polymerase chain reaction (qPCR) revealed that $Ca_v1.1$ and $Ca_v1.4$ $\alpha1$ subunits account for 0.08% of the L-type channel transcripts in mouse whole brain; owing to their very low expression, $Ca_v1.1$ and $Ca_v1.4$ $\alpha1$ are unlikely to serve as drug targets for pain treatment [30]. In contrast, $Ca_v1.2$ and $Ca_v1.3$ are expressed in most excitable cells, including neurons, and both genes can even be expressed in the same cell [31]. In the brain, almost 90% of L-type channels are encoded by $Ca_v1.2$ while $Ca_v1.3$-encoded L-type channels account for 10% [30]. Unlike the presence of HVA channels expressed by the Ca_v2 family in the presynaptic membrane, the L-type channels encoded by $Ca_v1.2$ and $Ca_v1.3$ are mainly found in the postsynaptic membrane and are distributed in the spinal cord and dendrites [32]. Here, they generate neuronal discharges and activate Ca^{2+}-dependent pathways to regulate gene expression (termed excitation-transcription coupling) [33]. By promoting synaptic plasticity, they are involved in different forms of learning and memory, drug addiction, and neurodevelopment. For example, deficiency of $Ca_v1.2$ promotes anxiety-like behaviors [34], while deficiency of $Ca_v1.3$ enhances antidepressant behavior which includes reduced immobility time in the forced swim test as well as tail suspension test [35]. Conversely, use of BayK8644 to selectively activate $Ca_v1.3$ *in vivo* has been shown to make depression worse [36].

In neurons, the current of the L-type channels is difficult to quantify because the current produced by HVA channels encoded by the Ca_v2 family has a major advantage. It has been reported that in medium-sized DRG neurons (diameter 30–40 μm), the current of the L-type channels is reduced after SNL [37]. Another study showed upregulation of the expression of L-type channels in mice exposed to chronic pain. In contrast, $Ca_v1.3$-knockout mice exhibit normal pain phenotypes [38], suggesting that L-type channels encoded by Cav1.3 do not play a major role in pain conduction. This result is consistent with the percentage expression of the L-type channels encoded by Cav1.2

Table 2 Role of HVA channels in different models of pathological pain.

HVA subtype	Type of pathological pain	Model of pathological pain	Changes in channels
N	Inflammatory pain	Formalin-induced inflammation	$Ca_v2.2$ mRNA levels lacking exon 18a↑ and amount of full-length variants↓ [22]
		Carrageenan-induced inflammation	Expression↑ [86]
		CFA-induced inflammation	Expression↑ [87]
			(in ipsilateral L4 and L5 ganglia)
	Neuropathic pain	CCI	Expression↑ [88]
		SNL	Expression↑ [89]
		PSL	Current↑ and expression↑ [18]
			(medium/large cells in DRG)
		SCI	Expression↑ [25]
	Cancer pain	CINP	Current↑ and expression↑ [90]
L[#]	Inflammatory pain	TNBS-induced inflammation	$Ca_v1.2$ expression↑ and current↑ [91]
		Propofol-induced inflammation	$Ca_v1.2$ expression↑ [92]
	Neuropathic pain	CCI	$Ca_v1.2$ current↑ [93]
		SNL	$Ca_v1.2$ expression↑ [39]
		PSL	$Ca_v1.2$ expression↑ while $Ca_v1.3$ expression↓ [94]
	Cancer pain	CINP	$Ca_v1.2$ current↓ [90]
		OINP	$Ca_v1.2$ current↑ [95]
R	Inflammatory pain	Formalin-induced inflammation	Current↑ [77]
		TNBS-induced inflammation	Expression↑ and current↑ [91]
	Neuropathic pain	SNL	Expression↑ [78]
		PSL	Expression↑ [45]
	Cancer pain	CINP	Current↓ [90]
		Bone metastasis–associated pain	Expression↓ [96]
		(cultured 2472 fibrosarcoma cells injected into and around the calcaneus)	
P/Q	Inflammatory pain	Formalin-induced inflammation	Current↑ [97]
		CFA-induced inflammation	Current↓ [87]
	Neuropathic pain	PSL	Current↓
			(medium/large cells in DRG) [18]
		Streptozocin-induced neuropathy	Expression↑ [98]
	Cancer pain	CINP	Current↓ [90]

CFA, complete Freund's adjuvant; CCI, chronic constriction injury; SNL, spinal nerve ligation; PSL, partial sciatic nerve ligation; SCI, spinal cord injury; CINP, cisplatin-induced neuropathic pain; TNBS, 2,4,6-trinitrobenzenesulfonic acid; OINP, oxaliplatin-induced neuropathic pain.
[#]$Ca_v1.1$ and $Ca_v1.4$ are not evident in nervous tissue [30].

and Cav1.3 in nervous tissue. Interestingly, knockdown of mir-103 encoding a microRNA in rat leads to increased sensitivity to pain and decreased expression of $Ca_v1.2$ [39]. Similarly, the severe cardiovascular side-effects of the L-type channel-specific blockers dihydropyridines are a key constraint for further studies of L-type channels. In a clinical study, subtle changes in the central nervous system have been detected in healthy volunteers after treatment with blockers of L-type channels [40].

The role of the L-type channels in the central nervous system may be more conspicuous than in the peripheral nervous system. The absence of expression of L-type channels in the anterior cingulate cortex in mice is associated with reduced pain and learning disabilities [41].

Altogether, the role of L-type channels in pain is still unclear. The inconsistency among findings may be related to differences in the experimental models, the tissues collected from various regions of laboratory animals, and the method of detection. In addition, the difficulty in quantifying the current of L-type channels and the cardiovascular side-effects of their antagonists make it more difficult to study these channels. At least, these findings

suggest a postsynaptic effect of L-type Ca^{2+} channels in afferent pain signaling, although it is unclear whether these channels can be used as targets for pain management. The development of Ca^{2+} imaging and optogenetic techniques may provide new ideas for the study of L-type channels [42].

R-type HVA Channels

R-type HVA channels exist in various types of neurons and are closely associated with the release of neurotransmitters and the regulation of neuronal excitability. They are also expressed in DRG neurons and are involved in the regulation of pain transmission. The $Ca_v2.3$ gene encoding the R-type channel can be divided into six groups, $Ca_v2.3a$–$2.3f$. The results of RT-PCR have shown that the R-type channels in DRG tissues are mostly encoded by $Ca_v2.3a$ and $Ca_v2.3e$. By single cell RT-PCR, it has been shown that DRG neurons expressing $Ca_v2.3e$ account for 20%, while those expressing $Ca_v2.3a$ account for 2.8%. In addition, the mRNA of $Ca_v2.3e$ is mainly present in small DRG neurons. In contrast, $Ca_v2.3e$ mRNA is less abundantly expressed in medium DRG neurons, but almost none in large DRG neurons. This suggests that the R-type channels expressed by $Ca_v2.3e$ predominate in noxious DRG neurons. Furthermore, DRG neurons with Cav2.3e expression are positive for TrkA and transient receptor potential vanilloid 1, and negative for isolectin B4 [43]. In general, the R-type channels present in nociceptive DRG neurons are primarily encoded by $Ca_v2.3e$ and are closely associated with pain transmission. Partial ligation of the spinal nerve has been shown to induce upregulation of R-type channels in DRG neurons [44]; exposure of $Ca_v2.3$-knockdown mice to inflammatory conditions reveals a significant reduction in sensitivity to pain [45]. Overall, in the DRG, the expression of R-type channels is typically upregulated during pain and the current is increased, especially in neuropathic pain. Furthermore, blockade of R-type channel can have a significant analgesic effect. Therefore, R-type channels play an important role in the pain afferent pathway and may be an important therapeutic target for pain management.

P/Q-type HVA Channels

As with L-type HVA channels, the role of P/Q HVA channels in the process of pain transmission is not clear. Mice lacking $Ca_v2.1$ have been shown to exhibit severe ataxia and an absence of seizures, and die in $\sim$4 weeks. Interestingly, mice with postnatal deletion of the $Ca_v2.1$ gene have a slower onset of neurological deficit [46]. SNL has been shown to induce upregulation of N-type channel expression and downregulation of P/Q-type channel expression in medium-sized DRG neurons, leading to changes in the relative proportion of current [18]. Young mice with $Ca_v2.1$ knockdown exhibit hyperalgesia to acute thermal pain but reduced sensitivity to chronic pain [47]. There is less evidence to support a role of P/Q-type channels in pain transmission; however, it is undeniable that they play a certain role in this process.

As noted earlier, various studies have demonstrated that each subtype of HVA channel undergoes changes in expression and/or current in various pain models. Changes in HVA channel activity regulate other important physiological processes that play important roles in pain conduction, although the changes and effects of HVA channels in some subtypes remain unclear (Fig. 2). Many researchers are currently working on this, which may facilitate a better understanding of pain mechanisms.

Current Applications and New Drug Development of HVA Channel Blockers

So far, the most commonly-used Ca^{2+} channel blockers for the treatment of pain are gabapentin and pregabalin. Gabapentin has been shown to alleviate postoperative pain [48]. Gabapentin and its derivative pregabalin were originally analogs designed to mimic GABA. However, the site of action of gabapentin is at $Ca_v\alpha2\delta$-1 subunits rather than GABA receptors. Although the structure of gabapentin is similar to that of GABA, it neither acts on $GABA_A$ nor $GABA_B$ receptors [49]. Moreover, gabapentin does not affect the synthesis and metabolism of GABA. Under neuropathological conditions, the anti-nociceptive effect of gabapentin is attenuated in rats with deletion of the $Ca_v\alpha2\delta$-1 gene [50]. This also confirmed that $Ca_v\alpha2\delta$-1 is the site of action of gabapentin. Gabapentin binds to the $Ca_v\alpha2\delta$-1 subunit and enters the neuronal cytoplasm through the system-L-neutral amino-acid transporter, and then acts in the cell [51]. In the case of neuropathic pain, intraperitoneal injection of gabapentin blocks the rapid transport of $Ca_v\alpha2\delta$-1 subunits from the endoplasmic reticulum to the cell membrane in afferent nerve terminals within 30 min, impeding an increase in expression levels. Under non-neuropathic conditions, gabapentin blocks the transport of $Ca_v\alpha2\delta$ subunits from the cell body to nerve terminals [52]. In laboratory models, gabapentin acts on almost every rat; however, in clinical trials, only 50% of patients experience pain relief [53]. This may be related to the downregulation of $\alpha2\delta$-1 subunits [54] as well as time and the down-regulation of glutamate transporter 1 expression [53, 55]. Despite the limited persistence of gabapentin, it is still the first line of pain treatment due to its broad prospects. In addition, although large numbers of Ca^{2+} channel blockers have been discovered, they are rarely

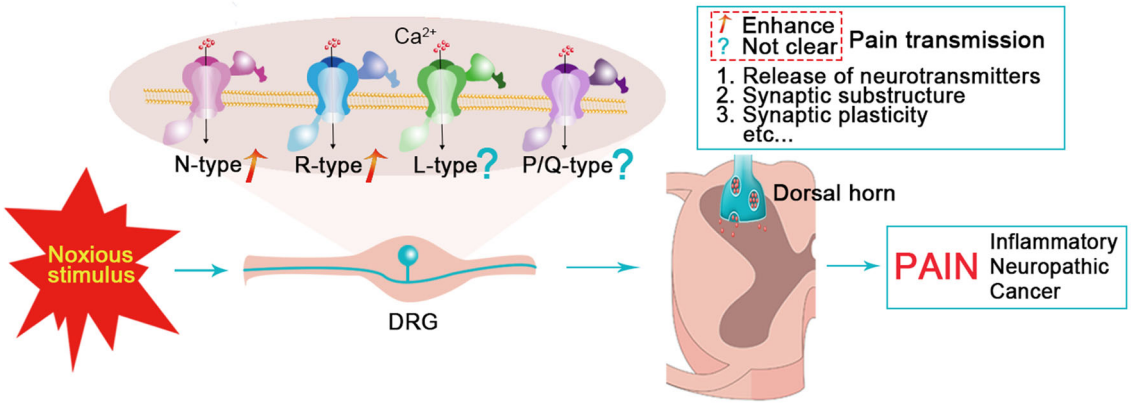

Fig. 2 Changes in HVA channels during pain conduction. Upon exposure to noxious stimuli, different subtypes of HVA channels in the DRG or spinal cord produce different degrees of change in current and expression, thereby triggering a series of physiological processes (e.g. release of neurotransmitters, synaptic substructure, and plasticity) that eventually produce pain sensation. The expression and current of N-type and R-type channels are upregulated in various pain models. The changes in P/Q-type and L-type channels are still not clear.

used in clinical practice. This thorny problem can be due to various factors, such as patient heterogeneity and the choice of appropriate test endpoints. Among these, the lack of understanding of pain mechanism is a key factor [53]. Therefore, the exploration of the pain mechanism still has a long way to go.

A-1048400 (1-[2-(4-benzhydryl-piperazin-1-yl)-2-oxo-ethyl]-3,3-diphenyl-piperidin-2-one) is a novel, oral, state-dependent Ca^{2+} channel blocker. In an electrophysiological study, A-1048400 has been shown to bind to the N-type, L-type, and P/Q-type channels of human and rat neurons and act as a blocking agent with comparable potency. A-1048400 has exhibited an analgesic effect in animal models of inflammatory and neuropathic pain; in addition, the analgesic effect is dose-dependent. At the same time, A-1048400 plays a more powerful role in the SNL model than in the chronic constriction injury model, but the mechanism is still unclear. Moreover, A-1048400 is also a state-dependent blocker to which channels are more sensitive in the inactive state. A-1048400 has fewer cardiovascular side-effects than traditional Ca^{2+} channel blockers. A-1048400 blocks neuronal Ca^{2+} channels in a voltage-dependent (state-dependent) manner; in addition, it does not significantly alter hemodynamic functions in rats at plasma concentrations much higher than the desired concentration for antinociception [56]. Voltage-dependent blockers act on neuronal ion channels in specific states; they may preferentially regulate overactive neurons and have relatively little effect on normal neuronal activity. A-1048400 has been shown to have potent analgesic effects in models of inflammation and osteoarthritis pain with fewer cardiovascular side-effects; this property extends the potential therapeutic utility of Ca^{2+} channel blockers in chronic pain states.

As already noted, different types of HVA channel can be specifically blocked by different blockers. Next, we elaborate on these specific HVA blockers.

Blockers of N-type HVA Channels

The ω-conotoxins GIVA and MVIIA are selective inhibitors of N-type channels, composed of 27 and 25 peptides, respectively, and containing disulfide bonds. They inhibit channels by occupying the conduction pore of the α1 subunit [57]; their analgesic effect is irreversible and is exerted by strong hyperpolarization of cells [58]. Intrathecal injection of GIVA and MVIIA into rats has been shown to significantly inhibit algesia [59]. Of these, MVIIA can be synthesized and its ability to block N-type channels is retained *in vitro*. It is currently used as the main component of Ziconotide for treatment of severe chronic pain, such as cancer pain [58]. A systematic review of randomized controlled trials of Ziconotide has shown its therapeutic efficacy against chronic neuropathic pain. Unlike morphine, it rarely causes serious adverse reactions such as hyperalgesia, drug resistance, and respiratory depression. In patients of childbearing age, the endocrine side-effects of morphine such as loss of libido, decreased testosterone levels in men, and osteoporosis of the spine may induce severe pain. However, such side-effects have rarely been reported with Ziconotide [60]. Some experts believe that Ziconotide is the only analgesic drug in this group that is suitable for clinical use [61]. At the same time, Ziconotide's withdrawal response is weak, which solves a major problem in clinical pain management [60].

However, this drug does not easily pass through the blood-brain barrier and requires intrathecal instillation through a micropump [62]. In addition, it has some severe

side-effects such as dizziness, blurred vision, memory defects, and abnormalities of the urinary and digestive systems; therefore, its therapeutic window is narrow [58]. Therefore, the principle of "start low, go slow" should be followed to minimize the side-effects of Ziconotide. According to current recommendations, the dose should not be increased more than once a week [63]. Among the described side-effects, the most worrying are the neuropsychiatric side-effects, such as suicide and schizophrenia, which are believed to be associated with rapid titration and high doses. Hence, considering that a patient's previous mental disorder may be a risk factor for adverse neuropsychiatric reactions, the psychological state of patients should be carefully evaluated before Ziconotide treatment. A history of mental illness should be considered a contraindication [64].

At present, a series of the blockers of N-type channels extracted from *Conus striatus* such as the ω-conotoxins SO-3, FVIA, CVID, and CVIE are undergoing research and trials. CVID has entered clinical trials and may have a wider therapeutic window than Ziconotide [58]. Similar to other ω-conotoxins, SO-3 contains 25 amino-acid residues and 3 disulfide bonds. Among these, Lys6, Ile11, and Asn14 are important functional residues in SO-3 [65]. SO-3 produces a potent, long-lasting spinal analgesic effect in the mouse acetic acid writhing test and the rat formalin test, enhances the analgesic effect of morphine on co-administration. After repeated administration, no tolerance to SO-3 or cross-tolerance to morphine occurs. In addition, the analgesic effects of SO-3 are similar to those of Ziconotide, while the adverse reactions are fewer. Studies conducted on cultured hippocampal neurons have shown that SO-3 has similar efficacy to Ziconotide in inhibiting N-type channels, but has less effect on non-N-type HVA currents at higher concentrations At the same time, SO-3 has no effect on voltage-sensitive Na^+ and K^+ currents, which may explain its fewer side-effects. Within the therapeutic range, SO-3 has no significant adverse effects on exercise. Therefore, SO-3 as an N-type channel blocker has a broader application prospect, either alone or in combination with opioids [66]. Nonetheless, since Ziconotide can only be administered by intrathecal instillation, not only is the risk of administration high, but also the quality of life of the patient is greatly affected. Therefore, other drugs that do not require intrathecal administration are also actively being developed, such as ZC88 [67] and TROX-1 [68].

ZC88 is a non-peptide N-type channel-specific inhibitor that has no effect on L-type, R-type, or P/Q-type channels [67]. In a model of chronic constriction injury, oral administration of ZC88 leads to a dose-dependent reduction in mechanical hyperalgesia, while having no effect on normal mice in the acute thermal pain model. However, ZC88 combined with morphine reduces the tolerance, physical dependence, and withdrawal symptoms of morphine with no effect on morphine-induced psychological dependence in a conditioned place preference model [69]. Therefore, ZC88 is expected to be used as a drug for neuropathic pain, either alone or in combination with morphine.

TROX-1 has similar potency on the channels of the Ca_v2 family, including $Ca_v2.1$, $Ca_v2.2$, and $Ca_v2.3$, under depolarization conditions. However, under inflammatory conditions, TROX-1 has no effect on mice with $Ca_v2.2$ gene deletion [68]. This indicates that TROX-1 plays a major role in the inhibition of $Ca_v2.2$. Moreover, oral administration of TROX-1 has been shown to significantly increase the threshold of the paw withdrawal of rats with SNL [70].

And by far, a series of designed piperazines has been shown to have a significant inhibitory effect on N-type channels. Z160, which can be administered orally, mediates analgesia in several animal models of pain [71]. However, it failed Phase II clinical trials. As an important target for pain therapy, N-type channels play an important role in clinical applications and there is much work to do.

Blockers of L-type HVA Channels

Dihydropyridines are known to mainly act on L-type channels. These drugs bind to the pore of the α1 subunit, which is S5 or S6 in domains III and IV [72]. Nicardipine has been shown to reduce pain behavior in an SNL model [73]. However, the dihydropyridine Ca^{2+} blockers have considerable cardiovascular side-effects, so identification of novel specific blockers of L-type channels to minimize the cardiovascular side-effects of dihydropyridines and maximize their effects in neurons is a key imperative. A new structure-activity relationship has been reported for the new pyrimidine-2,4,6-triones. The most selective candidate is BPN-4689 (also referred to as Cp8). Based on the results of fluorescent imaging plate reader assays, the selectivity of Cp8 for $Ca_v1.3$ is 600 times greater than that for $Ca_v1.2$ [74]. However, in whole-cell patch clamp recordings, Cp8 has been shown to cause a significant time-dependent change in the transient expression of L-type channels in tsA201 cells, characterized by a decrease in the time-course of activation, inactivation, and inactivation, very similar to the activity of activators in L-type channels [75]. Therefore, the effect of Cp8 is related to the experimental conditions, and under some conditions, it may even activate L-type channels.

In addition to these small molecules, the conotoxin TxVII has been shown to inhibit L-type channels. Interestingly, TxVII inhibits dihydropyridine-sensitive currents in lymphocyte RPeD1 neurons, but does not inhibit these currents in PC12 cells, suggesting that TxVII has cell-

subtype selectivity. However, there is currently no research on which cell subtype that may be involved in the regulation of pain signaling is specifically affected by TxVII [76].

In general, L-type channels may play an important role in the post-synaptic process during pain transmission, but there is still a long way to go for the clinical use of L-type channel blockers in pain management.

Blockers of R-type and P/Q-type HVA Channels

When R-type channels are specifically blocked by SNX-482, neuralgia is significantly inhibited. At the same time, SNX-482 has been shown to attenuate formalin-induced pain behavior, neurokinin 1 receptor (NK1R) internalization, and c-Fos expression in the posterior horn of the spinal cord. NK1R is an indicator of substance P and c-Fos is a proto-oncogene that is expressed when neurons are subjected to noxious stimulation [77]. In addition, electrophysiological studies in the SNL model have shown that SNX-482 mediates antinociceptive effects by regulating neuronal activity in the dorsal horn of the spinal cord. At the same time, SNX-482 has a weak effect on sham-operated rats. This phenomenon also supports the results of behavioral testing. SNX-482 has a significant inhibitory effect on nociceptive C-fibers and Aδ-fibers, in contrast to non-noxious Aβ-fibers [78].

P/Q-type channels can be blocked by ω-agatoxin IVA, which suggests that these channels may be another target for pain treatment. The ω-agatoxin IVA is effective in the treatment of pain in knee arthritis [79]. It combines with the channel to change its activation voltage to a positive potential, which is not achievable under normal physiological conditions, and it suppresses P/Q-type channels by raising the voltage-gated energy barrier rather than physically occupying the conduction pore. Therefore, IVA does not change the permeability of the channels, but only works by raising the voltage level required for channel activation [80]. In the tail-flick and tail-pressure tests, intrathecal injection of IVA shows dose-dependent inhibition in the range 0.33 pmol–33 pmol/mouse. At the same time, IVA administered in combination with morphine enhances the effect of morphine at subthreshold doses. This suggests that co-administration of P/Q-type channel inhibitors with morphine can help reduce the dose of morphine and minimize the side-effects [81]. P/Q-type channels play an important part in the pain afferent pathway, so their clinical significance cannot be underestimated.

Tx3-3 is a novel peptide toxin extracted from the venom of the Brazilian armed spider, *Phoneutria nigriventer*. It blocks the action of P/Q-type and R-type channels, and is a state-dependent blocker. Tx3-3 has been shown to have transient anti-nociceptive effects in noxious pain tests (such as the tail-flick test) and long-lasting anti-nociceptive effects in neuropathic pain models. However, Tx3-3 has no effect on inflammatory pain. It is worth noting that the anti-nociceptive effects of Tx3-3 only occur in the case of noxious stimuli such as thermal nociception and mechanical pain. At the same time, it does not alter mechanical sensitivity unless there is a secondary injury. No side-effects have been reported, which may be related to its state-dependent properties. This implies a better safety profile of Tx3-3. It holds promise as a novel therapeutic agent for the control of neuropathic pain [82].

Future Prospects

HVA channels are widely present in the pain afferent pathway, including the DRG, spinal cord, and even the brain. In various pain models, the activity of HVA channels of different subtypes increases to varying degrees on exposure to noxious stimuli. Owing to their important role in the release of neurotransmitters and the structure and plasticity of synapses, HVA channels are undoubtedly an indispensable part of the pain transmission. Therefore, HVA channels are also considered to be important therapeutic targets in pain management. However, since these channels are also present in other tissues (such as the cardiovascular system and skeletal muscle) and participate in many other physiological events, HVA channel blockers inevitably have side-effects. To date, only pregabalin and gabapentin have been used as first-line medications for pain management. Among the specific HVA channel blockers, Ziconotide has also been used in clinical settings. However, its side-effects and intrathecal mode of administration are significant limitations to its wider use. Teams of researchers are currently working to reduce the side-effects of HVA channel blockers. The strategies are broadly divided into increasing the specificity of the blocker and exploring state-dependent blockers. In theory, state-dependent blockers can prevent the blockade of Ca^{2+} ion channels in cells outside of the pain pathway. At the same time, in order to increase the specificity of the blockers, researchers are working to investigate those that target specific splices of HVA channels, but is a huge challenge. Based on this, a substantial number of drugs have been developed. Peptide toxins isolated from predatory species such as cone snails are examples of a large class.

The venoms from various predatory species such as fishing mollusks, scorpions, snakes, and arachnids contain large amounts of toxins, including HVA channel blockers. These blockers act primarily by blocking the physical occupancy of the pores and preventing activation gating.

The ω-conotoxins GIVA, MVIIA, and SNX-482 belong to this class of peptide toxins, which have a high specificity for different subtypes of HVA channels. However, since they do not easily cross the blood-brain barrier, most are delivered by intrathecal injection and cannot be administered systemically. This also limits their clinical application. In response to their shortcomings, numerous researchers have improved their structure to allow for oral administration and to minimize side-effects. Potentially, tethered MVIIA can target specific cells through viral targeting [83]. Although safety concerns continue to persist, the use of peptide toxins may be a promising approach to pain therapies.

Due to the low efficacy or side-effects, the current treatment effects of pain therapies are still not satisfactory. How to use opioids is a problem for every pain physician. At present, opioid abuse in the United States and its catastrophic consequences, as well as the weak evidence base for their use in chronic pain, demonstrates how difficult it is to "hijack" endogenous opioids without harm [84]. With regard to cannabis-based analgesia, there seems to be a gap between public beliefs and existing medical evidence [85]. Although current treatments are not satisfactory, considering the important role of HVA channel subtypes in neurotransmitter release, neuronal excitability, and neurogenic inflammation, HVA channels are still considered to be important targets for pain management. The development of HVA subtype blockers contributes to the development of pain treatment and understanding the role of HVA subtypes in order to achieve the goal of improving the efficacy and safety of drugs for the treatment of pain.

Conclusions

HVA channels are closely associated with pain. Upon exposure to noxious stimuli, different subtypes of HVA channel produce different degrees of change in current and expression, thereby triggering a series of physiological processes that eventually produce pain sensation. Among these, the changes in N-type and R-type channels in the process of pain transmission are more clear, while those in the P/Q-type and L-type channels are still not clear. Given its important role in pain transmission, the HVA channel is considered to be potential therapeutic target for pain management. A number of blockers of HVA channels have been developed or are undergoing clinical trials. Unfortunately, progress in the development of novel HVA channel inhibitors has been slow due to low efficacy and/or side-effects. However, it is still too early to abandon HVA channels as a target for pain management. Increasing the selectivity of HVA channel blockers and the development of state-dependent drugs are expected to enhance their efficacy and reduce side-effects. In conclusion, it is indisputable that the HVA channel is an important target for pain therapies. Through the unremitting efforts of researchers, HVA channel blockers may become an important means of clinical pain treatment.

Acknowledgements This review was supported by the National Natural Science Foundation of China (81701092), Shanghai Municipal Education Commission-Gaofeng Clinical Medicine Support (20171916), and Shanghai Municipal Commission of Health and Family Planning Funding for Key Developing Disciplines (2015ZB0101).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Thakral JM, Shi L, Foust JB, Patel KV, Shmerling RH, Bean JF, *et al.* Pain quality descriptors in community-dwelling older adults with nonmalignant pain. Pain 2016, 157: 2834–2842.
2. van Hecke O, Torrance N, Smith BH. Chronic pain epidemiology and its clinical relevance. Br J Anaesth 2013, 111: 13–18.
3. von Hehn CA, Baron R, Woolf CJ. Deconstructing the neuropathic pain phenotype to reveal neural mechanisms. Neuron 2012, 73: 638–652.
4. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain 2011, 152: S2–15.
5. Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J. International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltagegated calcium channels. Pharmacol Rev 2005, 57: 411–425.
6. Arikkath J, Chen CC, Ahern C, Allamand V, Flanagan JD, Coronado R, *et al.* Gamma 1 subunit interactions within the skeletal muscle L-type voltage-gated calcium channels. J Biol Chem 2003, 278: 1212–1219.
7. Samuel H, Pratt WS, Rees E, Dahimene S, Ferron L, Owen MJ, *et al.* Genetic disruption of voltage-gated calcium channels in psychiatric and neurological disorders. Prog Neurobiol 2015, 134: 36–54.
8. Catterall WA. Voltage-Gated Calcium Channels. Cold Spring Harb Perspect Biol 2011, 3: a003947.
9. Triggle DJ. 1,4-Dihydropyridines as calcium channel ligands and privileged structures. Cell Mol Neurobiol 2003, 23: 293–303.
10. Rajakulendran S, Hanna MG. The Role of Calcium Channels in Epilepsy. Cold Spring Harb Perspect Med 2016, 6: a022723.
11. Van Petegem F, Clark KA, Chatelain FC, Minor DL Jr. Structure of a complex between a voltage-gated calcium channel β-subunit and an α-subunit domain. Nature 2004, 429: 671–675.
12. Altier C, Garcia-Caballero A, Simms B, You H, Chen L, Walcher J, *et al.* The $Ca_v\beta$ subunit prevents RFP2-mediated ubiquitination and proteasomal degradation of L-type channels. Nature Neurosci 2011, 14: 173–180.
13. Dolphin AC. The α2δ subunits of voltage-gated calcium channels, Biochim Biophys Acta 2013, 1828: 1541–1549.
14. Davies A, Kadurin I, Alvarez-Laviada A, Douglas L, Nieto-Rostro M, Bauer CS, *et al.* The alpha2delta subunits of voltage-gated calcium channels form GPI-anchored proteins, a posttranslational modification essential for function. Proc Natl Acad Sci U S A 2010, 107: 1654–1659.

15. Nicoll RA, Tomita S, Bredt DS. Auxiliary subunits assist AMPA-type glutamate receptors. Science 2006, 311: 1253–1256.
16. Yusaf SP, Goodman J, Pinnock RD, Dixon AL, Lee K. Expression of voltage-gated calcium channel subunits in rat dorsal root ganglion neurons. Neurosci Lett 2001, 311: 137–141.
17. Silva FR, Miranda AS, Santos RPM, Olmo IG, Zamponi GW, Dobransky T, *et al.* N-type Ca^{2+} channels are affected by full-length mutant huntingtin expression in a mouse model of Huntington's disease, Neurobiol Aging 2017, 55: 1–10.
18. Murali SS, Napier AI, Mohammadi SA, Alewood PF, Lewis RJ, MacDonald JC. High-voltage-activated calcium current subtypes in mouse DRG neurons adapt in a subpopulation-specific manner after nerve injury. J Neurophysiol 2015, 113: 1511–1519.
19. Smith MT, Cabot PJ, Ross FB, Robertson AD, Lewis RJ. The novel N-type calcium channel blocker, AM336, produces potent dose-dependent antinociception after intrathecal dosing in rats and inhibits substance P release in rat spinal cord slices. Pain 2002, 96: 119–127.
20. Saegusa H, Matsuda Y, Tanabe T. Effects of ablation of N- and R-type Ca^{2+} channels on pain transmission. Neurosci Res. 2002, 43: 1–7.
21. Newton PM, Orr CJ, Wallace MJ, Kim C, Shin HS, Messing RO. Deletion of N-type calcium channels alters ethanol reward and reduces ethanol consumption in mice. J Neurosci 2004, 24: 9862–9869.
22. Asadi S, Javan M, Ahmadiani A, Sanati MH. Alternative splicing in the synaptic protein interaction site of rat Ca(v)2.2 [alpha (1B)] calcium channels: changes induced by chronic inflammatory pain. J Mol Neurosci 2009, 39: 40–48.
23. Umeda M, Ohkubo T, Ono J, Fukuizumi T, Kitamura K. Molecular and immunohistochemical studies in expression of voltage-dependent Ca^{2+} channels in dorsal root ganglia from streptozotocin-induced diabetic mice. Life Sci 2006, 79: 1995–2000.
24. Waithe D, Ferron L, Page KM, Chaggar K, Dolphin AC. Beta-subunits promote the expression of Ca(V)2.2 channels by reducing their proteasomal degradation. J Biol Chem 2011, 286: 9598–9611.
25. Boroujerdi A, Zeng J, Sharp K, Kim D, Steward O, Luo ZD. Calcium channel alpha-2-delta-1 protein upregulation in dorsal spinal cord mediates spinal cord injury-induced neuropathic pain states. Pain 2011,152: 649–655.
26. Li CY, Song YH, Higuera ES, Luo ZD. Spinal dorsal horn calcium channel alpha2delta-1 subunit upregulation contributes to peripheral nerve injury-induced tactile allodynia. J Neurosci 2004, 24: 8494–8499.
27. Li CY, Zhang XL, Matthews EA, Li KW, Kurwa A, Boroujerdi A, *et al.* Calcium channel alpha2delta1 subunit mediates spinal hyperexcitability in pain modulation. Pain 2006, 125: 20–34.
28. Bauer CS, Rahman W, Tran-van-Minh A, Lujan R, Dickenson AH, Dolphin AC. The anti-allodynic alpha(2)delta ligand pregabalin inhibits the trafficking of the calcium channel alpha(2)delta-1 subunit to presynaptic terminals in vivo. Biochem Soc Trans 2010, 38: 525–528.
29. Alles SRA, Smith PA. Etiology and pharmacology of neuropathic pain. Pharmacol Rev 2018, 70: 315–347.
30. Sinnegger-Brauns MJ, Huber IG, Koschak A, Wild C, Obermair GJ, Einzinger U, *et al.* Expression and 1,4-dihydropyridine-binding properties of brain L-type calcium channel isoforms. Mol Pharmacol 2009, 75: 407–414.
31. Dragicevic E, Poetschke C, Duda J, Schlaudraff F, Lammel S, Schiemann J, *et al.* $Ca_v1.3$ channels control D2-autoreceptor responses via NCS-1 in substantia nigra dopamine neurons. Brain 2014, 137: 2287–2302.
32. Jenkins MA, Christel CJ, Jiao Y, Abiria S, Kim KY, Usachev YM, *et al.* Ca^{2+}-dependent facilitation of $Ca_v1.3$ Ca^{2+} channels by densin and Ca^{2+}/calmodulin-dependent protein kinase II. J Neurosci 2010, 30: 5125–5135.
33. Ma H, Cohen S, Li B, Tsien RW. Exploring the dominant role of Ca_v1 channels in signalling to the nucleus. Biosci Rep 2013, 33: 97–101.
34. Lee AS, Ra S, Rajadhyaksha AM, Britt JK, De Jesus-Cortes H, Gonzales KL, *et al.* Forebrain elimination of cacna1c mediates anxiety-like behavior in mice. Mol Psychiatry 2012, 17: 1054–1055.
35. Busquet P, Nguyen NK, Schmid E, Tanimoto N, Seeliger MW, Ben-Yosef T, *et al.* $Ca_v1.3$ L-type Ca^{2+} channels modulate depression-like behaviour in mice independent of deaf phenotype. Int J Neuropsychopharmacol 2010, 13: 499–513.
36. Sinnegger-Brauns MJ, Hetzenauer A, Huber IG, Renström E, Wietzorrek G, Berjukov S, *et al.* isoform-specific regulation of mood behavior and pancreatic beta cell and cardiovascular function by L-type Ca^{2+} channels. J Clin Invest 2004, 113: 1430–1439.
37. McCallum JB, Wu HE, Tang Q, Kwok WM, Hogan QH. Subtype-specific reduction of voltage-gated calcium current in medium-sized dorsal root ganglion neurons after painful peripheral nerve injury. Neuroscience 2011, 179: 244–255.
38. Clark NC, Nagano N, Kuenzi FM, Jarolimek W, Huber I, Walter D, *et al.* Neurological phenotype and synaptic function in mice lacking the CaV1.3 alpha subunit of neuronal L-type voltage-dependent Ca^{2+} channels. Neuroscience 2003, 120: 435–442.
39. Favereaux A, Thoumine O, Bouali-Benazzouz R, Roques V, Papon MA, Salam SA, *et al.* Bidirectional integrative regulation of $Ca_v1.2$ calcium channel by microRNA miR-103: role in pain. EMBO J 2011, 30: 3830–3841.
40. Wankerl K, Weise D, Gentner R, Rumpf JJ, Classen J. L-type voltage-gated Ca^{2+} channels: a single molecular switch for long-term potentiation/long-term depression-like plasticity and activity-dependent metaplasticity in humans. J Neurosci 2010, 30: 6197–6204.
41. Jeon D, Kim S, Chetana M, Jo D, Ruley HE, Lin SY, *et al.* Observational fear learning involves affective pain system and $Ca_v1.2$ Ca^{2+} channels in ACC. Nature Neurosci 2010, 13: 482–488.
42. Anderson M, Zheng Q, Dong X. Investigation of pain mechanisms by calcium imaging approaches. Neurosci Bull 2018, 34: 194–199.
43. Zhi F, Hwang JH, Kim JS, Jung SJ, Oh SB. R-type calcium channel isoform in rat dorsal root ganglion neurons. Korean J Physiol Pharmacol 2010, 14: 45–49.
44. Yang L, Stephens GJ. Effects of neuropathy on high-voltage-activated Ca^{2+} current in sensory neurons. Cell Calcium 2009, 46: 248–256.
45. Saegusa H, Kurihara T, Zong S, Minowa O, Kazuno A, Han W, *et al.* Altered pain responses in mice lacking alpha 1E subunit of the voltage-dependent Ca^{2+} channel. Proc Natl Acad Sci U S A 2000, 97: 6132–6137.
46. Mark MD, Maejima T, Kuckelsberg D, Yoo JW, Hyde RA, Shah V, *et al.* Delayed postnatal loss of P/Q-type calcium channels recapitulates the absence epilepsy, dyskinesia, and ataxia phenotypes of genomic Cacna1a mutations. J Neurosci 2011, 31: 4311–4326.
47. Luvisetto S, Marinelli S, Panasiti MS, D'Amato FR, Fletcher CF, Pavone F, *et al.* Pain sensitivity in mice lacking the Ca(v)2.1alpha1 subunit of P/Q-type Ca^{2+} channels. Neuroscience 2006, 142: 823–832.
48. Clarke H, Bonin RP, Orser BA, Englesakis M, Wijeysundera DN, Katz J. The prevention of chronic postsurgical pain using gabapentin and pregabalin: a combined systematic review and meta-analysis. Anesth Analg 2012, 115: 428–442.

49. Li Z, Taylor CP, Weber M, Piechan J, Prior F, Bian F, *et al.* Pregabalin is a potent and selective ligand for α2δ-1 and α2δ-2 calcium channel subunits. Eur J Pharmacol 2011, 667 : 80–90.
50. Patel R, Bauer CS, Nieto-Rostro M, Margas W, Ferron L, Chaggar K, *et al.* alpha2delta-1 gene deletion affects somatosensory neuron function and delays mechanical hypersensitivity in response to peripheral nerve damage. J Neurosci 2013, 33: 16412–16426.
51. Biggs JE, Stemkowski PL, Knaus EE, Chowdhury MA, Ballanyi K, Smith PA. Suppression of network activity in dorsal horn by gabapentin permeation of TRPV1 channels: implications for drug access to cytoplasmic targets. Neurosci Lett 2015, 584: 397–402.
52. Alles SRA, Smith PA. The anti-allodynic gabapentinoids: myths, paradoxes, and acute effects. Neuroscientist 2016, 23: 40–55.
53. Wiffen PJ, Derry S, Bell RF, Rice AS, Tölle TR, Phillips T, *et al.* Gabapentin for chronic neuropathic pain and fibromyalgia in adults. Cochrane Database Syst Rev 2017, 6: CD007938.
54. Yang Y, Yang F, Yang F, Li CL, Wang Y, Li Z, *et al.* Gabapentinoid insensitivity after repeated administration is associated with downregulation of the α2δ-1 subunit in rats with central post-stroke pain hypersensitivity. Neurosci Bull 2016, 32: 41–50.
55. Kimura M, Eisenach JC, Hayashida K. Gabapentin loses efficacy over time after nerve injury in rats: role of glutamate transporter-1 in the locus coeruleus. Pain 2016, 157: 2024–2032.
56. Scott VE, Vortherms TA, Niforatos W, Swensen AM, Neelands T, Milicic I, *et al.* A-1048400 is a novel, orally active, state-dependent neuronal calcium channel blocker that produces dose-dependent antinociception without altering hemodynamic function in rats. Biochem Pharmacol 2012, 83: 406–418.
57. Bourinet E, Zamponi GW. Block of voltage-gated calcium channels by peptide toxins. Neuropharmacology 2017, 127: 109–115.
58. Ramírez D, Gonzalez W, Fissore RA, Carvacho I. Conotoxins as Tools to Understand the Physiological Function of Voltage-Gated Calcium (Ca_v) Channels. Mar Drugs 2017, 15: 313.
59. Scott DA, Wright CE, Angus JA. Actions of intrathecal omega-conotoxins CVID, GVIA, MVIIA, and morphine in acute and neuropathic pain in the rat. Eur J Pharmacol 2002, 451: 279–286.
60. Brookes ME, Eldabe S, Batterham A. Ziconotide Monotherapy: A systematic review of randomised controlled trials. Curr Neuropharmacol 2017, 15: 217–231.
61. Vitale V, Battelli D, Gasperoni E, Monachese N. Intrathecal therapy with ziconotide: clinical experience and considerations on its use. Minerva Anestesiol. 2008, 74: 727–733.
62. Ver Donck A, Collins R, Rauck RL, Nitescu P. An open-label, multicenter study of the safety and efficacy of intrathecal ziconotide for severe chronic pain when delivered via an external pump. Neuromodulation 2008, 11: 103–111.
63. Fisher R, Hassenbusch S, Krames E, Leong M, Minehart M, Prager J, *et al.* A consensus statement regarding the present suggested titration for prialt (ziconotide). Neuromodulation 2005, 8: 153–154.
64. Deer TR, Prager J, Levy R, Rathmell J, Buchser E, Burton A, *et al.* Polyanalgesic Consensus Conference 2012: recommendations for the management of pain by intrathecal (intraspinal) drug delivery: report of an interdisciplinary expert panel. Neuromodulation 2012, 15: 436–466.
65. Dong M, Wang F, Yan Z, Yu S, Wei J, Wu Q, *et al.* Structure-activity analysis of N-type calcium channel inhibitor SO-3. Biochemistry 2018, 57: 6349–6355.
66. Yan LD, Liu YL, Zhang L, Dong HJ, Zhou PL, Su RB, *et al.* Spinal antinociception of synthetic omega-conotoxin SO-3, a selective N-type neuronal voltage-sensitive calcium channel blocker, and its effects on morphine analgesia in chemical stimulus tests in rodent. Eur J Pharmacol 2010, 636: 73–81.
67. Zhang S, Yang L, Zhang K, Liu X, Dai W, Zhang C, *et al.* ZC88, a novel N-type calcium channel blocker from 4-amino-piperidine derivatives state-dependent inhibits $Ca_v2.2$ calcium channels. Brain Res 2015, 1605: 12–21.
68. Abbadie C, McManus OB, Sun SY, Bugianesi RM, Dai G, Haedo RJ, *et al.* Analgesic effects of a substituted N-triazole oxindole (TROX-1), a state-dependent, voltage-gated calcium channel 2 blocker. J. Pharmacol Exp Ther 2010, 334: 545–555.
69. Meng G, Wu N, Zhang C, Su RB, Lu XQ, Liu Y, *et al.* Analgesic activity of ZC88, a novel N-type voltage-dependent calcium channel blocker, and its modulation of morphine analgesia, tolerance and dependence. Eur J Pharmacol 2008, 586: 130–138.
70. Patel R, Rutten K, Valdor M, Schiene K, Wigge S, Schunk S, *et al.* Electrophysiological characterization of activation state-dependent Ca(v)2 channel antagonist TROX-1 in spinal nerve injured rats. Neuroscience 2015, 297: 47–57.
71. Sairaman A, Cardoso FC, Bispat A, Lewis RJ, Duggan PJ, Tuck KL. Synthesis and evaluation of aminobenzothiazoles as blockers of N- and T-type calcium channels. Bioorg Med Chem 2018, 26: 3046–3059.
72. Yamaguchi S, Okamura Y, Nagao T, Adachi-Akahane S. Serine residue in the IIIS5-S6 linker of the L-type Ca^{2+} channel alpha 1C subunit is the critical determinant of the action of dihydropyridine Ca^{2+} channel agonists. J Biol Chem 2000, 275: 41504–41511.
73. Fossat P, Dobremez E, Bouali-Benazzouz R, Favereaux A, Bertrand SS, Kilk K, *et al.* Knockdown of L calcium channel subtypes: differential effects in neuropathic pain. J Neurosci 2010, 30: 1073–1085.
74. Kang S, Cooper G, Dunne SF, Dusel B, Luan CH, Surmeier DJ, *et al.* $Ca_v1.3$-selective L-type calcium channel antagonists as potential new therapeutics for Parkinson's disease. Nat Commun 2012, 3: 1146.
75. Ortner NJ, Bock G, Vandael DH, Mauersberger R, Draheim HJ, Gust R, *et al.* Pyrimidine-2,4,6-triones are a new class of voltage-gated L-type Ca^{2+} channel activators. Nat Commun 2014, 5: 3897.
76. Lee S. Pharmacological inhibition of voltage-gated Ca(2+) channels for chronic pain relief. Curr Neuropharmacol 2013, 11: 606–620.
77. Terashima T, Xu Q, Yamaguchi S, Yaksh TL. Intrathecal P/Q- and R-type calcium channel blockade of spinal substance P release and c-Fos expression. Neuropharmacology 2013, 75: 1–8.
78. Matthews EA, Bee LA, Stephens GJ, Dickenson AH. The Cav2.3 calcium channel antagonist SNX-482 reduces dorsal horn neuronal responses in a rat model of chronic neuropathic pain. Eur J Neurosci 2007, 25: 3561–3569.
79. Nebe J, Vanegas H, Neugebauer V, Schaible HG. Omega-agatoxin IVA, a P-type calcium channel antagonist, reduces nociceptive processing in spinal cord neurons with input from the inflamed but not from the normal knee joint–an electrophysiological study in the rat in vivo. Eur J Neurosci 1997, 9: 2193–2201.
80. Pringos E, Vignes M, Martinez J, Rolland V. Peptide neurotoxins that affect voltage-gated calcium channels: a close-up on ω-agatoxins. Toxins 2011, 3: 17–42.
81. Fukuizumi T, Ohkubo T, Kitamura K. Spinally delivered N-, P/Q- and L-type Ca2+-channel blockers potentiate morphine analgesia in mice. Life Sci. 2003, 73: 2873–2881.
82. Dalmolin GD, Silva CR, Rigo FK, Gomes GM, Cordeiro Mdo N, Richardson M, *et al.* Antinociceptive effect of Brazilian armed spider venom toxin Tx3-3 in animal models of neuropathic pain. Pain 2011, 152: 2224–2232.
83. Anliker B, Abel T, Kneissl S, Hlavaty J, Caputi A, Brynza J, *et al.* Specific gene transfer to neurons, endothelial cells and

hematopoietic progenitors with lentiviral vectors. Nat Methods 2010, 7: 929–935.
84. Clauw D. Hijacking the endogenous opioid system to treat pain: who thought it would be so complicated? Pain 2017, 158: 2283–2284.
85. Fitzcharles MA, Clauw DJ, Ste-Marie PA, Shir Y. The dilemma of medical marijuana use by rheumatology patients. Arthritis Care Res (Hoboken). 2014, 66: 797–801.
86. Yokoyama K, Kurihara T, Makita K, Tanabe T. Plastic change of N-type Ca channel expression after preconditioning is responsible for prostaglandin E2-induced long-lasting allodynia. Anesthesiology 2003, 99: 1364–1370.
87. Lu SG, Zhang XL, Luo ZD, Gold MS. Persistent inflammation alters the density and distribution of voltage-activated calcium channels in subpopulations of rat cutaneous DRG neurons. Pain 2010, 151: 633–643.
88. Cizkova D, Marsala J, Lukacova N, Marsala M, Jergova S, Orendacova J, *et al.* Localization of N-type Ca^{2+} channels in the rat spinal cord following chronic constrictive nerve injury. Exp Brain Res 2002, 147: 456–463.
89. Boroujerdi A, Kim HK, Lyu YS, Kim DS, Figueroa KW, Chung JM, *et al.* Injury discharges regulate calcium channel alpha-2-delta-1 subunit upregulation in the dorsal horn that contributes to initiation of neuropathic pain. Pain 2008, 139: 358–366.
90. Leo M, Schmitt LI, Erkel M, Melnikova M, Thomale J, Hagenacker T. Cisplatin-induced neuropathic pain is mediated by upregulation of N-type voltage-gated calcium channels in dorsal root ganglion neurons. Exp Neurol 2017, 288: 62–74.
91. Qian A, Song D, Li Y, Liu X, Tang D, Yao W, *et al.* Role of voltage gated Ca2+ channels in rat visceral hypersensitivity change induced by 2,4,6-trinitrobenzene sulfonic acid. Mol Pain 2013, 9: 15.
92. Nishimoto R, Kashio M, Tominaga M. Propofol-induced pain sensation involves multiple mechanisms in sensory neurons. Pflugers Arch. 2015, 467: 2011–2020.
93. Alles SR, Garcia E, Balasubramanyan S, Jones K, Tyson JR, Joy T, *et al.* Peripheral nerve injury increases contribution of L-type calcium channels to synaptic transmission in spinal lamina II: Role of α2δ-1 subunits. Mol Pain 2018, 14: 1744806918765806.
94. Dobremez E, Bouali-Benazzouz R, Fossat P, Monteils L, Dulluc J, Nagy F, *et al.* Distribution and regulation of L-type calcium channels in deep dorsal horn neurons after sciatic nerve injury in rats. Eur J Neurosci 2005, 21: 3321–3333.
95. Kawashiri T, Egashira N, Kurobe K, Tsutsumi K, Yamashita Y, Ushio S, *et al.* L type Ca^{2+} channel blockers prevent oxaliplatin-induced cold hyperalgesia and TRPM8 overexpression in rats. Mol Pain 2012, 8: 7.
96. Gandla J, Lomada SK, Lu J, Kuner R, Bali KK. miR-34c-5p functions as pronociceptive microRNA in cancer pain by targeting $Ca_v2.3$ containing calcium channels. Pain 2017, 158: 1765–1779.
97. Diaz A, Dickenson AH. Blockade of spinal N- and P-type, but not L-type, calcium channels inhibits the excitability of rat dorsal horn neurones produced by subcutaneous formalin inflammation. Pain 1997, 69: 93–100.
98. Yusaf SP, Goodman J, Gonzalez IM, Bramwell S, Pinnock RD, Dixon AD, *et al.* Streptozocin-induced neuropathy is associated with altered expression of voltage-gated calcium channel subunit mRNAs in rat dorsal root ganglion neurons. Biochem Biophys Res Commun 2001, 289: 402–406.