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Themed Section: Recent Advances in Targeting Ion Channels to Treat Chronic Pain

REVIEW ARTICLE

Injury-induced maladaptation and dysregulation of calcium channel $\alpha_2\delta$ subunit proteins and its contribution to neuropathic pain development

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Voltage-gated calcium channels (VGCCs) play important roles in physiological functions including the modulation of neurotransmitter release, neuronal network activities, intracellular signalling pathways and gene expression. Some pathological conditions, including nerve injuries, can cause the dysregulation of VGCCs and their subunits. This in turn can lead to a functional maladaptation of VGCCs and their subunits, which can contribute to the development of disorders such as pain sensations. This review has summarized recent findings related to maladaptive changes in the dysregulated VGCC $\alpha_2\delta_1$ subunit ($Ca_v\alpha_2\delta_1$) with a focus on exploring the mechanisms underlying the contribution of $Ca_v\alpha_2\delta_1$ to pain signal transduction. At least under neuropathic pain conditions, the dysregulated $Ca_v\alpha_2\delta_1$ can modulate VGCC functions as well as other plasticity changes. The latter includes abnormal excitatory synaptogenesis resulting from its interactions with injury-induced extracellular matrix glycoprotein molecule thrombospondins, which is independent of the VGCC functions. Blocking $Ca_v\alpha_2\delta_1$ with gabapentinoids can reverse neuropathic pain significantly with relatively mild side effects, but only in a small population of neuropathic pain patients due to reasons yet to be explored. There are emerging data suggesting that early preventive treatment with gabapentinoids can prevent aberrant excitatory synapse formation and the development of chronic pain. If these findings are confirmed clinically, this could be an attractive approach for neuropathic pain management.

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Abbreviations

DRG, dorsal root ganglion; HVA, high voltage-activated; LVA, low voltage-activated; TSP4, thrombospondin-4; VGCC, voltage-gated calcium channels

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Introduction

Under physiological conditions, nociceptive sensations primarily serve as a protective mechanism for our body. A stimulus detected by sensory nerve endings evokes action potentials that propagate along the primary afferent nerve fibres up to the soma of dorsal root ganglion (DRG) neurons and then through their central axons to the first relay in the dorsal spinal cord. As a sensory information processing hub referring periphery sensory information to the brain, the spinal dorsal horn sensory circuitry is critically involved in processing sensory information locally and sending it through ascending tracts to the higher CNS for further processing. The brain then sends descending signals to the spinal cord through descending pathways to modulate sensory and motor neuron circuitry activities. However, under pathological conditions, damage to the nervous system can induce plasticity changes in the sensory pathway so that its activation thresholds are lowered. As a consequence, exaggerated pain perception can occur in the absence of painful stimuli or with mild painful stimulation. This kind of abnormal sensation gives rise to painful phenomena including 'allodynia' (i.e. painful perception to an innocuous stimulus) and 'hyperalgesia' (exacerbated pain perception to a mildly noxious stimulus), collectively called pain states here. Among the factors that play critical roles in pain development after nerve injuries (neuropathic pain states), voltage-gated calcium channels (**VGCCs**) contribute to this process through the modulation of important pathophysiological functions. These include the release of excitatory neurotransmitters (Lee, 2013), calcium-dependent enzyme activation (Park and Luo, 2010), effects on gene expression (Perret and Luo, 2009; Park and Luo, 2010; Wheeler *et al.,* 2012) and other short- and long-term plasticity changes in the spinal dorsal horn (Russo and Hounsgaard, 1994; Naka *et al.,* 2013; Youn *et al.,* 2013; Liu and Zhou, 2015). In addition, injury-induced dysregulation of VGCC subunits, such as $\alpha_2\delta_1$ ($Ca_v\alpha_2\delta$), may also contribute to pain signal transduction by modulating additional neural plasticity such as abnormal synaptogenesis (Eroglu *et al.,* 2009; Park *et al.,* 2016). These changes can alter the activities of the neural network that fundamentally contribute to the cellular underpinning of chronic pain development.

Voltage-gated calcium channels and accessory subunits

So far, 10 VGCC subtypes have been identified that are divided broadly into two categories based on their thresholds of activation by membrane depolarization: high voltage-activated (HVA) and low voltage-activated (LVA) channels (Benarroch, 2010; Simms and Zamponi, 2014; Zamponi *et al.,* 2015). The HVA channel family can be further subdivided, based on their pharmacological and functional characteristics, into L-, N-, P/Q- and R-types, and the LVA channels are mainly T-type VGCCs. Structurally, all VGCCs contain a pore-forming α_1 subunit that determines their main biophysical properties. There are three major families of α_1 subunits (Ca_v1, Ca_v2 and Ca_v3), which can undergo alternative splicing in a tissue-, age- and pathology-dependent manner (Lipscombe *et al.,* 2013). In addition, the HVA channels are heteromultimers with three auxiliary subunits, $Ca_v\beta$, $Ca_v\alpha_2\delta$ and $Ca_v\gamma$. Four $Ca_v\beta$ subunits ($Ca_v\beta_1$–β_4), four $Ca_v\alpha_2\delta$ subunits ($Ca_v\alpha_2\delta_{-4}$) and eight $Ca_v\gamma$ subunits ($Ca_v\gamma_{1-8}$) have been identified. Unlike the HVA channels that require co-assembly with auxiliary calcium channel subunits to function, the LVA channels appear as α_1 subunit monomers that can be functionally regulated by the auxiliary subunits (Dubel *et al.,* 2004). However, other studies reported that auxiliary subunits of calcium channels have little or no modulatory effect on LVA channel currents (Perez-Reyes, 2003; Turner and Zamponi, 2014).

The functions and details of the $Ca_v\alpha_1$, $Ca_v\beta$ and $Ca_v\gamma$ subunits are reviewed and summarized in other chapters in this issue and recent reviews (Simms and Zamponi, 2014; Zamponi *et al.,* 2015; Campiglio and Flucher, 2015). In addition, the structural feature, regulation and function of different types of VGCCs and their implications in neurological diseases including pain, migraine, epilepsy, cerebellar ataxia, Parkinson disease and hypertension have been the subjects of numerous recent reviews (Perret and Luo, 2009; Park and Luo, 2010; Cain and Snutch, 2011; Vink and Alewood, 2012; Simms and Zamponi, 2014; Zamponi *et al.,* 2015; Zamponi, 2016). In this review, we focus on recent findings related to maladaptive and dysregulation of $Ca_v\alpha_2\delta$ and its potential contribution to pain development, as well as prospective views of blocking $Ca_v\alpha_2\delta$ in neuropathic pain development and management.

$Ca_v\alpha_2\delta$ subunits

Each $Ca_v\alpha_2\delta$ subunit consists of the $Ca_v\alpha_2$ and $Ca_v\delta$ peptides that are encoded by the same gene, post-translationally cleaved and then linked by disulfide-bounds (Ellis *et al.,* 1988). Site-directed mutagenesis data have indicated that a single intermolecular disulfide bound between cysteine residue 404 in the von Willebrand factor A (VWA) domain of $Ca_v\alpha_2$ and cysteine residue 1047 in the extracellular domain of $Ca_v\delta$ is critical for the structural and functional integrity of the $Ca_v\alpha_2\delta$ protein (Calderon-Rivera *et al.,* 2012). Recently, data from cryo-electron microscopy reveal that the primary sequence of $Ca_v\alpha_2\delta$ contains four tandem cache domains and one VWA domain that are intertwined (Wu *et al.,* 2016). In addition, there are four disulfide bounds between the $Ca_v\alpha_2$ and $Ca_v\delta$ peptides and two within the $Ca_v\delta$ peptide (Wu *et al.,* 2016). In combination with the site-directed mutagenesis data, it is likely that each of these disulfide bounds is mutually critical in stabilizing the calcium channel complex and for its functions. Data from biochemical characterization of $Ca_v\alpha_2\delta$ proteins support the view that $Ca_v\alpha_2\delta$ is attached to the extracellular leaflet of the plasma membrane *via* a glycophosphatidylinositol anchor (Davies *et al.,* 2010), which seems to be supported by structural data revealed by cryo-electron microscopy (Wu *et al.,* 2016). The known functions of $Ca_v\alpha_2\delta$ subunits include promoting and stabilizing cell surface expression and modulating functions of VGCC (Dolphin, 2012; Dolphin, 2013).

Calcium channel $Ca_v\alpha_2\delta_1$ subunit as a therapeutic target in pain management

Pharmacological and biochemical studies

Data from biochemical studies have indicated that gabapentinoids, including **gabapentin** (Neurontin; Pfizer) and **pregabalin** (Lyrica; Pfizer), bind to both $Ca_v\alpha_2\delta_1$ and $Ca_v\alpha_2\delta_2$ subunit proteins (Gee *et al.*, 1996; Gong *et al.*, 2001; Marais *et al.*, 2001b; Li *et al.*, 2011; Kukkar *et al.*, 2013; Verma *et al.*, 2014; Landmark *et al.*, 2015). Gabapentinoids are widely used in management of neuropathic pain, including that derived from diabetic neuropathy, nerve injuries, drugs and radiation-induced neuropathies, as well as post-herpetic and trigeminal neuralglia (Johnson and Rice, 2014; Moore *et al.*, 2014; Zamponi *et al.*, 2015). However, only a significant up-regulation of $Ca_v\alpha_2\delta_1$, but not $Ca_v\alpha_2\delta_2$, has been observed in DRG/spinal cord of neuropathic pain models (Luo *et al.*, 2001; 2002; Valder *et al.*, 2003; Bauer *et al.*, 2009), supporting the concept that the anti-hyperalgesic properties of gabapentinoids are mediated by their binding to the $Ca_v\alpha_2\delta_1$ subunit. This is confirmed by point mutation of the binding site for gabapentinoids on $Ca_v\alpha_2\delta_1$ in a transgenic mouse line, which diminishes $Ca_v\alpha_2\delta_1$ binding and the anti-hyperalgesic properties of gabapentinoids (Field *et al.*, 2006).

Genetic studies

One way to confirm that $Ca_v\alpha_2\delta_1$ has a role in pain processing is to use $Ca_v\alpha_2\delta_1$ knockout mice to generate pain research models, which certainly come with limitations, or correlate abnormal sensation deficits with $Ca_v\alpha_2\delta_1$ genetic defects in patients. Table 1 summarizes findings of the studies done in $Ca_v\alpha_2\delta$ genetic-knockout mouse models or from patients with genetic defects regarding a potential role for each $Ca_v\alpha_2\delta$ subunit in pain signal transduction. Even though $Ca_v\alpha_2\delta_1$ knockout is conceived as embryonic lethal (Simms and Zamponi, 2014), it has been reported that mice with a targeted deletion of $Ca_v\alpha_2\delta_1$ (Fuller-Bicer *et al.*, 2009) display normal sensitivity to thermal stimulation but reduced sensitivity to mechanical and cold stimuli that correlates with reduced responses of dorsal horn wide dynamic range neurons to stimuli (Fuller-Bicer *et al.*, 2009; Patel *et al.*, 2013). In addition, the onset time of mechanical hypersensitivity is delayed, and the anti-hyperalgesic activity of gabapentinoids after peripheral nerve injury is absent in this line of mice (Patel *et al.*, 2013). These findings confirm that the induction of $Ca_v\alpha_2\delta_1$ is critical for the initial development of neuropathic pain states after nerve injury (Boroujerdi *et al.*, 2008) and that $Ca_v\alpha_2\delta_1$ does indeed mediate the anti-hyperalgesic properties of gabapentinoids (Field *et al.*, 2006), as reported previously. Peripherally, general $Ca_v\alpha_2\delta_1$ knockout results in a significant reduction in calcium entry per action potential, and in the duration and frequency of action potentials in DRG sensory neurons (Margas *et al.*, 2016). However, linking a direct causal role of $Ca_v\alpha_2\delta_1$ to these electrophysiological and behavioural changes in this mouse line is difficult because we cannot rule out the contributions from other compensatory factors and/or developmental changes due to general embryonic knockout of $Ca_v\alpha_2\delta_1$. It is conceivable that general knockout of $Ca_v\alpha_2\delta_1$ can affect a wide range of

Table 1

Genetic studies regarding the role of $Ca_v\alpha_2\delta$ subunits in pain signal transduction

$Ca_v\alpha_2\delta$ isoform	Genetic modification	Sensory phenotype	Reference
$Ca_v\alpha_2\delta_1$	General (embryonic) knockout in mice	↓ Mechanical/cold sensitivity	Fuller-Bicer *et al.*, 2009
		Delayed mechanical hypersensitivity onset after nerve injury	Patel *et al.*, 2013
	Natural aberrations/mutations in patients	Not reported	Vergult *et al.*, 2015
			Hino-Fukuyo *et al.*, 2015
$Ca_v\alpha_2\delta_2$	Spontaneous mutations in mice	Not reported	Barclay *et al.*, 2001; Brodbeck *et al.*, 2002
			Donato *et al.*, 2006
			Brill *et al.*, 2004
	Targeted disruption in mice		Ivanov *et al.*, 2004
	Homozygous mutations in patients		Edvardson *et al.*, 2013
			Pippucci *et al.*, 2013
$Ca_v\alpha_2\delta_3$	Spontaneous mutations in Drosophila	↓ Acute heat pain sensitivity	Neely *et al.*, 2010
	Deletion by homologous recombination in mice	Delayed inflammatory thermal hyperalgesia	
	Single-nucleotide polymorphisms in patients	↓ Low-back pain sensitivity	
	Splice site mutation	Not reported	Iossifov *et al.*, 2012
$Ca_v\alpha_2\delta_4$	Spontaneous homozygous frame shift mutation in mice	Not reported	Wycisk *et al.*, 2006a
	Homozygous nucleotide substitution in patients		Wycisk *et al.*, 2006b

important organ development and physiological functions due to the importance of $Ca_v\alpha_2\delta_1$ in VGCC trafficking, membrane expression and in modulating different functions of VGCCs (Hendrich *et al.,* 2008; Cassidy *et al.,* 2014). With the application of Cre-induced recombination technology, sensory neuron-type specific deletion of $Ca_v\alpha_2\delta_1$ (Park *et al.,* 2016) allows us to study the contribution of $Ca_v\alpha_2\delta_1$ up-regulation in a subpopulation of sensory neurons to modality-specific processing of neuropathic pain states.

Genomic aberration of the CACNA2D1 gene encoding $Ca_v\alpha_2\delta_1$ has been identified in total blood DNA of three patients with epilepsy and intellectual disability, supporting a potential contribution of $Ca_v\alpha_2\delta_1$ to these clinical phenotypes (Vergult *et al.,* 2015). Similarly, another study has identified mutations in $Ca_v\alpha_2\delta_1$ from peripheral blood DNA of patients with early-onset epileptic encephalopathy (West syndrome) (Hino-Fukuyo *et al.,* 2015). Unfortunately, abnormal behavioural sensitivity has not been reported or tested in these patients. However, $Ca_v\alpha_2\delta_1$ over-expression in mouse neuronal tissues has been reported to cause brain epileptiform activity associated with behavioural arrests (Faria *et al.,* 2017) and spinal synaptic hyperexcitability associated with behavioural hypersensitivity to mechanical/thermal/cold stimuli (Li *et al.,* 2006; Zhou and Luo, 2013; Zhou and Luo, 2015). These data support a role for $Ca_v\alpha_2\delta_1$ in mediating the neural circuitry hyperexcitability associated with both epileptic and pain phenotypes.

Since gabapentinoids bind to both $Ca_v\alpha_2\delta_1$ and $Ca_v\alpha_2\delta_2$ subunits and show antiepileptic, anxiolytic and antihyperalgesic efficacy in preclinical studies, a recent study was well-designed to distinguish the role of $Ca_v\alpha_2\delta_1$ and $Ca_v\alpha_2\delta_2$ in the anticonvulsant activity of gabapentinoids. Mutations of the binding site for gabapentinoids in $Ca_v\alpha_2\delta_1$, but not in $Ca_v\alpha_2\delta_2$, result in a significant reduction in the anticonvulsant efficacy of pregabalin compared with wild-type controls and the $Ca_v\alpha_2\delta_2$ mutants (Lotarski *et al.,* 2014). Similarly, these mouse mutants were used to reveal the association of drug binding to $Ca_v\alpha_2\delta_1$, but not $Ca_v\alpha_2\delta_2$, with the anxiolytic-like activity of gabapentinoids (Lotarski *et al.,* 2011). Together with other findings indicating a role for $Ca_v\alpha_2\delta_1$ in pain signal transduction and in mediating the antihyperalgesic activity of gabapentinoids, findings from these genetic studies support the involvement of $Ca_v\alpha_2\delta_1$ in mediating pain signal transduction, epileptic and anxiolytic activities as well as the inhibitory effects of gabapentinoids on these disorders.

Genetic studies on other $Ca_v\alpha_2\delta$ isoforms

Naturally occurring mutations in the CACNA2D2 gene encoding $Ca_v\alpha_2\delta_2$ in ducky (Barclay *et al.,* 2001; Brodbeck *et al.,* 2002), ducky^{2j} (Donato *et al.,* 2006) and entla (Brill *et al.,* 2004) mice as well as targeted $Ca_v\alpha_2\delta_2$ knockout (Ivanov *et al.,* 2004) result in reduced calcium current densities, gabapentinoid binding sites and ataxia and epileptic phenotypes. Two recent clinical studies have identified different homozygous mutations in the CACNA2D2 gene that correlate with epileptic encephalopathy (Edvardson *et al.,* 2013; Pippucci *et al.,* 2013), supporting the possibility that $Ca_v\alpha_2\delta_2$ may play a role in mediating epileptic activities. Even though abnormalities in both $Ca_v\alpha_2\delta_1$ and $Ca_v\alpha_2\delta_2$ correlate with the development of epileptic activity, gabapentinoids' anti-seizure effect is likely to be mediated through their binding to $Ca_v\alpha_2\delta_1$, but not $Ca_v\alpha_2\delta_2$, because the anti-seizure efficacy of pregabalin is reduced in mice with a gabapentinoid binding site mutation in $Ca_v\alpha_2\delta_1$, but not in $Ca_v\alpha_2\delta_2$ (Lotarski *et al.,* 2014). Further mechanistic studies regarding the relative contribution of $Ca_v\alpha_2\delta_1$ and $Ca_v\alpha_2\delta_2$ to brain neural circuitry hyperexcitability in a cell type-specific manner may provide answers to this discrepancy. Abnormal nociceptive sensitivity was not reported in these studies (Table 1).

A genome-wide screen study in drosophila has identified the CACNA2D3 gene encoding $Ca_v\alpha_2\delta_3$ as an evolutionarily conserved pain gene mediating thermal hyperalgesia (Neely *et al.,* 2010). Deletion of $Ca_v\alpha_2\delta_3$ with homologous recombination in mice results in deficits in acute heat pain sensitivity and delayed thermal hyperalgesia induced by inflammation (Neely *et al.,* 2010). In addition, single-nucleotide polymorphisms within or close to the human CACNA2D3 gene are shown to be associated with reduced thermal pain sensitivity in healthy volunteers, and with chronic low-back pain in patients (Neely *et al.,* 2010). Data from functional imaging in $Ca_v\alpha_2\delta_3$ mutant mice indicate that thermal pain signal transmission from the thalamus to higher pain centres is impaired (Neely *et al.,* 2010). Since $Ca_v\alpha_2\delta_3$ is mainly expressed in the brain, and it is not a binding site for gabapentinoids (Marais *et al.,* 2001a), these findings support a role for $Ca_v\alpha_2\delta_3$ in transmitting pain signals in the brain through a mechanism that could be insensitive to gabapentinoids. Interestingly, sensory (noxious thermal and tactile) stimulation can trigger strong cross-activation of brain regions related to hearing, vision and olfaction in $Ca_v\alpha_2\delta_3$ mutant mice (Neely *et al.,* 2010), supporting a role for $Ca_v\alpha_2\delta_3$ in maintaining normal sensory signal distinction in the brain. In addition, splice site mutation of the human CACNA2D3 gene might be involved in autism spectrum disorders (Iossifov *et al.,* 2012). However, sensory testing is not reported in these studies (Table 1).

As $Ca_v\alpha_2\delta_4$ is the major $Ca_v\alpha_2\delta$ subunit expressed in mouse retina cells (Knoflach *et al.,* 2013), mutations in the CACNA2D4 gene encoding $Ca_v\alpha_2\delta_4$ result in loss of retina signalling, abnormal synapse morphology and dysfunction in mouse rods and cones (Wycisk *et al.,* 2006a), as well as autosomal recessive cone dystrophy associated with night blindness in patients (Wycisk *et al.,* 2006b). Abnormal nociceptive sensitivity was not reported or tested in these studies (Table 1).

Potential mechanisms underlying $Ca_v\alpha_2\delta_1$ dysregulation in pain signal transduction and the anti-hyperalgesic effects of gabapentinoids

$Ca_v\alpha_2\delta_1$ expression is up-regulated in DRG (and trigeminal ganglia) and spinal cord in some neuropathic pain models derived from mechanical nerve injury, spinal cord injury, diabetic or chemical neuropathies that correlate with the onset and maintenance of neuropathic pain states (Luo *et al.,* 2001; 2002; Newton *et al.,* 2001; Yusaf *et al.,* 2001; Li *et al.,* 2004; 2014b; Boroujerdi *et al.,* 2008; 2011). The $Ca_v\alpha_2\delta_1$

ligand gabapentinoids can reverse neuropathic pain states in these animal models (Hwang and Yaksh, 1997; Luo *et al.*, 2002; Boroujerdi *et al.*, 2011; Li *et al.*, 2014b). In addition, transgenic mice overexpressing $Ca_v\alpha_2\delta_1$ in neuronal tissues, including sensory and spinal cord neurons, display neuropathic pain states such as tactile allodynia and thermal hyperalgesia, in the absence of any nerve injury, that can be blocked by gabapentinoids (Li *et al.*, 2006). Together, these findings support the view that injury-induced $Ca_v\alpha_2\delta_1$ up-regulation may play a critical role in neuropathic pain development.

Data from detailed mechanistic investigations indicate that nerve injury-induced $Ca_v\alpha_2\delta_1$ expression occurs predominantly in DRG sensory neurons, then undergoes axonal translocation to presynaptic terminals in spinal dorsal horn (Figure 1), since the axonal transport can be interrupted by cutting the dorsal root connecting injured DRG and dorsal spinal cord through dorsal rhyzotomy (Li *et al.*, 2004). Subsequently, this axonal transport of an elevated expression of $Ca_v\alpha_2\delta_1$ from DRG neurons to dorsal spinal cord was independently confirmed. Data from electron microscopy show directly that injury-induced $Ca_v\alpha_2\delta_1$ in DRG is indeed trafficking to pre-synaptic terminals of sensory fibres in dorsal spinal cord, and this process can be blocked by gabapentinoids (prebabalin) (Bauer *et al.*, 2009). Thus, injury-induced increased expression of $Ca_v\alpha_2\delta_1$ probably regulates sensory pathway sensitivity peripherally and centrally.

At both the peripheral and central levels, it is possible that different $Ca_v\alpha_2\delta_1$ isoforms play distinct roles in mediating pain states. Early reports have indicated that peripheral nerve injury induces the up-regulation of distinct forms of $Ca_v\alpha_2\delta_1$ in DRG and spinal cord that correlates with the development of neuropathic pain states (Luo *et al.*, 2001). In addition, injury-induced DRG $Ca_v\alpha_2\delta_1$ isoforms differ from that detected in other tissues such as spinal cord, brain and skeletal muscle as they have unique migration rates in Western blots and different glycosylation patterns (Luo, 2000; Luo *et al.*, 2001). These findings suggest that $Ca_v\alpha_2\delta_1$ may have tissue-specific functions and injury-induced $Ca_v\alpha_2\delta_1$ up-regulation may modulate pain processing in a tissue type-specific manner. Recently, it was reported that DRG $Ca_v\alpha_2\delta_1$ isoforms are indeed derived from alternative splicing (Lana *et al.*, 2014). In addition, a minor splice variant ($\Delta A + B\Delta C$) of $Ca_v\alpha_2\delta_1$ is preferentially up-regulated in small DRG neurons after spinal nerve ligation injury, which also enhances calcium currents as it is the major splice variant of DRG $Ca_v\alpha_2\delta_1$, but has significantly reduced affinity for gabapentin (Lana *et al.*, 2014). It is speculated that differential expression of specifically spliced $Ca_v\alpha_2\delta_1$ variants in neuropathic pain patients may account for the variations in efficacy of pain relief by gabapentinoids observed clinically (Lana *et al.*, 2014).

At the peripheral level, dysregulated $Ca_v\alpha_2\delta_1$ can regulate DRG neuron activities through the modulation of calcium channel activities (Li *et al.*, 2006; D'Arco *et al.*, 2015). At the spinal cord level, it can increase presynaptic excitatory input into superficial and deep dorsal horn that regulates dorsal horn neuron excitabilities (Nguyen *et al.*, 2009; Zhou and Luo, 2013, 2015). Since different VGCC blockers have distinct inhibitory effects on $Ca_v\alpha_2\delta_1$-mediated nociception, it is likely that $Ca_v\alpha_2\delta_1$ dysregulation contributes to pain processing at least partially through modulation of pathways associated with specific VGCCs (Chang *et al.*, 2015).

Data from *in vitro* studies indicate that $Ca_v\alpha_2\delta_1$ can increase cell-surface and synaptic expression of VGCCs, including the P/Q-type (**Ca_v2.1**) (Hendrich *et al.*, 2008; Hoppa *et al.*, 2012) and N-type (**Ca_v2.2**) (Cassidy *et al.*, 2014) calcium channels. Calcium entry through these channels predominantly drives synaptic transmission and initiates fast release of classical neurotransmitters (Catterall, 2011; Campiglio and Flucher, 2015). Mechanistically, it has been shown that increased transient $Ca_v\alpha_2\delta_1$ expression in cultured rat DRG neurons leads to increased membrane expression of N-type (Ca_v2.2) VGCC. This plasticity couples to a prolonged Ca^{2+} signal evoked by membrane depolarization and activity-dependent slowing of axonal mitochondrial transport, both of which can be inhibited by treatments with N-type VGCC blockers. These findings suggest that increased $Ca_v\alpha_2\delta_1$ expression modulates sensory neuron responses to stimulation through the regulation of mitochondrial calcium buffering, which is mediated by N-type VGCCs (D'Arco *et al.*, 2015). Interestingly, chronic treatment with gabapentinoids can reduce the densities of $Ca_v\alpha_2\delta_1$ and Ca_v2 (Ca_v2.1 and Ca_v2.2) subunits in plasma membrane and inhibit excitatory presynaptic transmission (Hendrich *et al.*, 2008, 2012; Bauer *et al.*, 2009, 2010; Cassidy *et al.*, 2014), presumably by interfering with $Ca_v\alpha_2\delta_1$ trafficking process through disrupting the recycling of $Ca_v\alpha_2\delta_1$ to the plasma membrane (Tran-Van-Minh and Dolphin, 2010). This is supported by *in vitro* findings that the membrane surface expression of Ca_v2.2 channels is reduced and become insensitive to gabapentin blockade when co-expressed with a $Ca_v\alpha_2\delta_1$ mutant lacking the binding site for gabapentinoids (Cassidy *et al.*, 2014). $Ca_v\alpha_2\delta_1$ can also enhance the expression of T-type calcium channels in plasma membrane without changing their electrophysiological properties (Dubel *et al.*, 2004). However, $Ca_v\alpha_2\delta_1$ has also been found to have little or no modulatory effect on T-type channel currents (Perez-Reyes, 2003; Turner and Zamponi, 2014), and gabapentin failed to affect VGCC trafficking or function in cultured hippocampal neurons (Brown and Randall, 2005; Hoppa *et al.*, 2012).

Recent data from biochemical studies suggest that an elevation in $Ca_v\alpha_2\delta_1$ promotes presynaptic neurotransmitter release by modulating presynaptic VGCC abundance and Ca^{2+} influx-driven exocytosis. While the former can be explained by the ability of $Ca_v\alpha_2\delta_1$ to promote VGCC trafficking, the latter is believed to be mediated through an extracellular metal ion-dependent adhesion site within the VWA domain of $Ca_v\alpha_2\delta_1$ (Hoppa *et al.*, 2012; Korber and Kuner, 2016). These findings provide mechanistic insights, revealing how $Ca_v\alpha_2\delta$ plays a significant pathological role in modulating the release of numerous neurotransmitters, including pain-inducing excitatory neurotransmitters and peptides at the spinal cord level. This can be one of the primary targets of gabapentinoids in pain modulation.

While alterations in VGCC trafficking may lead to changes in VGCC functions, and subsequently changes in sensory neuron excitability or presynaptic neurotransmitter release, it is unable to explain the fast actions of gabapentinoids in reversing pain states in animal models, which occur within 1 h of administration (Hwang and Yaksh, 1997; Luo *et al.*, 2002). Recently, it was reported that $Ca_v\alpha_2\delta_3$

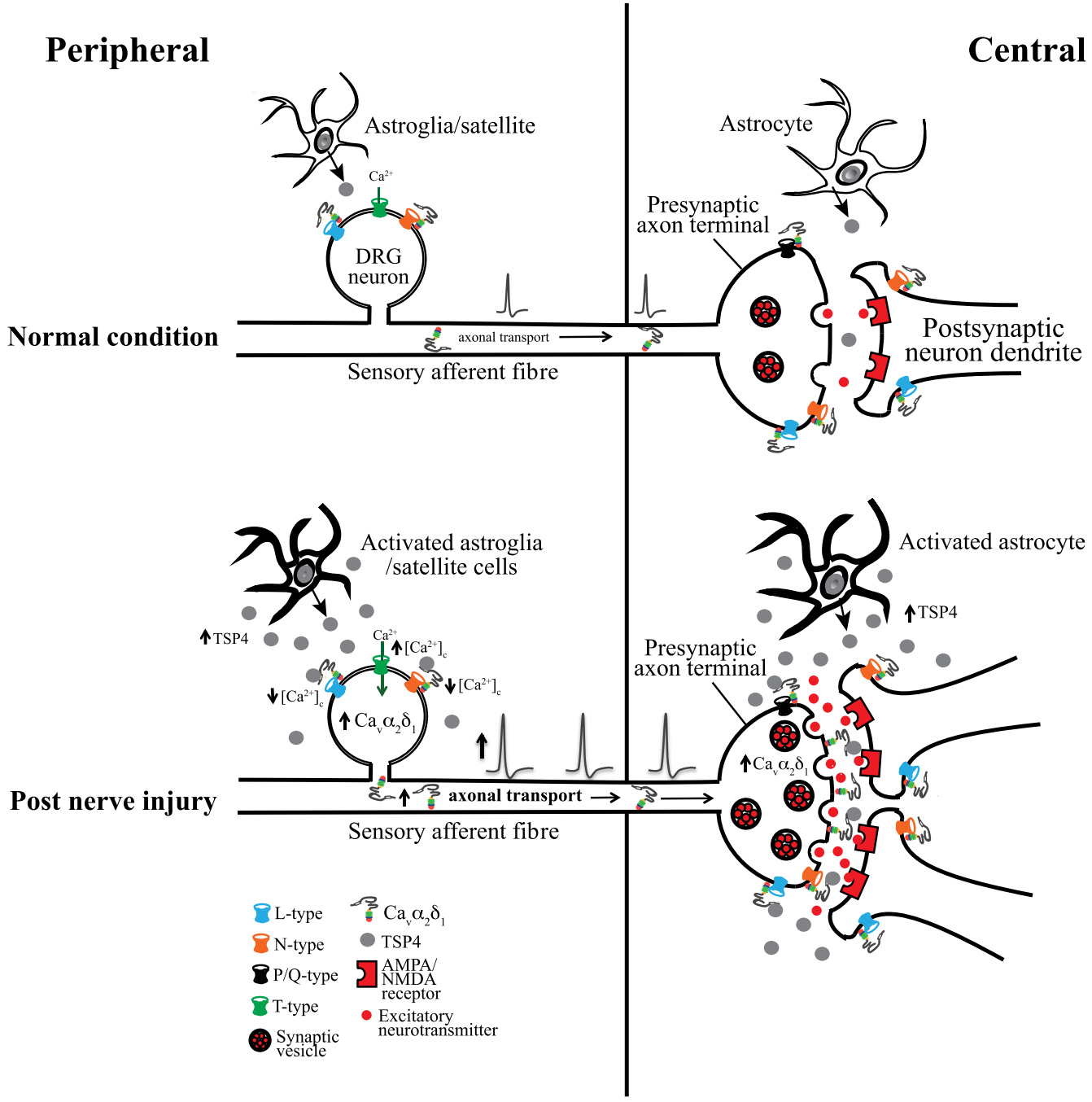

Figure 1

Cartoon illustration of injury-induced peripheral and central plasticity contributing to sensory sensitization and pain states. Nerve injury induces an up-regulation of $Ca_v\alpha_2\delta_1$ in DRG that undergoes axonal transport to the presynaptic terminal of injured sensory fibres in the dorsal spinal cord. Nerve injury also leads to increased TSP4 expression and secretion from activated DRG neurons/non-neuronal cells and spinal cord astrocytes. Peripherally, the elevated TSP4/$Ca_v\alpha_2\delta_1$ can interact to modulate VGCC currents and intracellular calcium levels, which may cause sensory neuron/afferent activation and sensitization of presynaptic terminals. Centrally, elevated TSP4/$Ca_v\alpha_2\delta_1$ can interact to promote presynaptic excitatory neurotransmitter release and excitatory synapse formation, which together can lead to activation of postsynaptic dorsal horn neurons and central sensitization.

is required for the rapid induction and sustained maintenance of presynaptic homeostatic potentiation, a form of neuroplasticity in which the presynaptic neurotransmitter release apparatus is modulated in response to postsynaptic perturbations that may occur in neurological disorders (Wang *et al.*, 2016). It appears that extracellular $Ca_v\alpha_2\delta_3$ residing in the presynaptic release sites of the synaptic cleft serves as a transsynaptic homeostatic signalling molecule that relays retrograde information to presynaptic cytoplasm to modulate active zone activities, presumably through

interactions with other proteins. This plasticity seems to be independent of its ability to regulate VGCC abundance (Wang *et al.,* 2016). If this plasticity and its sensitivity to blockade by gabapentinoids are confirmed in neuropathic pain conditions, it may underlie a new mechanism through which gabapentinoids reverse neuropathic pain states so promptly. Identification of $Ca_v\alpha_2\delta_3$ as a pain gene (Neely *et al.,* 2010) (Table 1) supports this notion. However, there is no evidence so far indicating that $Ca_v\alpha_2\delta_3$ is a binding site for gabapentinoids, or is up-regulated after nerve injuries. In fact, $Ca_v\alpha_2\delta_3$ mRNA is significantly reduced in injured DRG after nerve injury (Bauer *et al.,* 2009). More detail studies may help to distinguish the relative contribution of $Ca_v\alpha_2\delta_1$ and $Ca_v\alpha_2\delta_3$ to pain signal transduction and the anti-hyperalgesic actions of gabapentinoids.

In addition, it has been reported that gabapentin can block the interactions between $Ca_v\alpha_2\delta_1$ and synaptogenic thrombospondin-4 (TSP4) (Park *et al.,* 2016), which is also up-regulated in DRG and spinal cord after peripheral nerve injury (Kim *et al.,* 2012; Pan *et al.,* 2016). This leads to diminished dorsal horn neuron hypersensitivity and behavioural hypersensitivity within 1 h (Park *et al.,* 2016). This fast action of gabapentin may provide another possible explanation about the fast anti-hyperalgesic actions of gabapentinoids.

Current states of gabapentinoids in the management and prevention of neuropathic pain states

Gabapentinoids provide pain relief mainly in a pathophysiological state-dependent manner; they are only effective in alleviating pathological pain sensations but do not affect acute physiological noxious sensations (Field *et al.,* 1997b; Stanfa *et al.,* 1997; Dickenson *et al.,* 2005). These findings suggest that gabapentinoids most likely work on a sensitized sensory pathway related to $Ca_v\alpha_2\delta_1$ up-regulation. This may explain why gabapentinoids only provide significant pain relief in about one third of neuropathic pain patients (Moore *et al.,* 2014), since it is conceivable that pathological changes induced by $Ca_v\alpha_2\delta_1$ up-regulation may not be present in all neuropathic pain patients non-selectively. This is supported by data from animal model studies indicating that gabapentin is only effective in reversing neuropathic pain states associated with detectable $Ca_v\alpha_2\delta_1$ up-regulation in dorsal spinal cord and/or DRG (Luo *et al.,* 2002). Gabapentinoids have relatively mild side effects, which include dizziness, somnolence and ataxia. The latter is believed to be mediated through an effect on calcium channel trafficking (Jun *et al.,* 1999; Mark *et al.,* 2011) and by binding to $Ca_v\alpha_2\delta_2$ (Barclay *et al.,* 2001; Ivanov *et al.,* 2004).

Based on evidence from randomized clinical trials (RCTs), expert panels from the Neuropathic Pain Special Interest Group of the International Association for the Study of Pain (Dworkin *et al.,* 2007), European Federation of Neurological Societies (Attal *et al.,* 2006) and Canadian Pain Society (Moulin *et al.,* 2007) recommended, almost simultaneously, that gabapentinoids should be one of the first line medication classes for neuropathic pain management in 2006–2007. Recent updated reviews for using gabapentinoids in neuropathic pain management are summarized in Table 2. Depending on the aetiology of a neuropathic pain condition, the conclusions from these reviews still confirm that gabapentinoids can be used as first-line medications or are recommended for the management of neuropathic pain derived from certain aetiologies (Dworkin *et al.,* 2010; Vargas-Espinosa *et al.,* 2012; Hershman *et al.,* 2014; Finnerup *et al.,* 2015; Loh *et al.,* 2016). Findings from these reviews also point out the need for more studies for dose-standardization, to assess their adverse effects and their suitability for neuropathic pain derived from other aetiologies such as cancer, chemotherapy-induced peripheral neuropathies, and combination therapies, so that better drug efficacy can be achieved and/or their side effects reduced (Dworkin *et al.,* 2010; Chaparro *et al.,* 2012; Vargas-Espinosa *et al.,* 2012; Hershman *et al.,* 2014; Guan *et al.,* 2016).

Even though most animal model studies confirm that gabapentinoids are effective at reversing pain states, clinical data for the efficacy of gabapentinoids in acute and persistent pain relief are not consistent. While some studies show that gabapentinoids are effective in pain relief after surgeries, this comes at the expense of increased adverse effects (Buvanendran *et al.,* 2010; Engelman and Cateloy, 2011; Clarke *et al.,* 2012; Eipe *et al.,* 2015; Mishriky *et al.,* 2015). Other studies do not show similar benefits of gabapentinoid treatment (Lunn *et al.,* 2015; Kharasch and Eisenach, 2016; Martinez *et al.*, 2017a,b). This could be due to the fact that for various reasons negative data from animal model studies are often not getting published, but that is less likely to be the case for most clinical trials. These discrepancies in clinical studies has lead to the suggestion that more studies in different surgical models and specific patient populations are needed to explore the benefits of a surgical model-specific application of gabapentinoids (Fletcher and Martinez, 2015).

One way to optimize the pharmacological benefits and reduce the adverse effects of gabapentinoids in pain management is to lower the dose of gabapentinoids by using them as a combination treatment. A few studies have addressed this option with inconsistent outcomes, as summarized in the reviews presented in Table 2. Alternatively, pre-emptive treatment with gabapentinoids has been shown to be efficacious in blocking post-operative pain development in patients (Field *et al.,* 1997a; Clivatti *et al.,* 2009; Mardani-Kivi *et al.,* 2013; Ravindran, 2014; Hwang *et al.,* 2015). However, few studies have addressed the underlying mechanism of the preventive effects of gabapentinoids in chronic pain development. In another study reported in this issue, we tested the hypothesis that early low-dose gabapentin treatment can prevent injury-induced neuropathic pain development by inhibiting abnormal excitatory synaptogenesis between sensory and spinal cord neurons (Yu *et al.,* 2017).

Synaptogenic effects of $Ca_v\alpha_2\delta_1$/TSP4 in chronic pain processing

At the dorsal spinal cord level, the interactions of the up-regulated $Ca_v\alpha_2\delta_1$ with TSP4 post injury can lead to aberrant excitatory synaptogenesis that is highly likely to contribute to the development of chronic neuropathic pain, as illustrated in Figure 1 and is supported by the following findings from animal studies. Peripheral nerve injury induces an up-

Table 2

Recommendation of gabapentinoids for neuropathic pain management

Review criteria/strength	Neuropathic pain-inducing condition	Recommendation	Reference
Randomized clinical trials (RCTs)/systemic reviews/ pooled analysis/retrospective study	Post-herpetic neuralgia	Type A recommendation from consistent, good quality patient-oriented evidence More studies are needed for standardizing doses and assessing adverse effects	Vargas-Espinosa *et al.*, 2012
RCTs ≥1 week, combination pharmacotherapy, neuropathic pain in cancer patients	Cancer	Further studies are needed	Guan *et al.*, 2016
Double-blind, RCTs comparing two or more drug combinations to placebo and/or ≥1 other comparator	Varies	More studies are needed for two-drug combinations including comparisons with placebo and both single-agent components in neuropathic pain treatments	Chaparro *et al.*, 2012
Review established guidelines (Dworkin *et al.*, Pain 2007) and resent RCT studies for development of future recommendations	Varies	Single medication therapy: First-line medication Combination therapy: additional studies are needed to develop specific combination therapies.	Dworkin *et al.*, 2010
Recent RCTs	Chemotherapy-induced peripheral neuropathies (CIPN)	Reasonable to try for selective CIPN pain patients who should be informed about limited scientific evidence, potential benefits, harms and costs.	Hershman *et al.*, 2014
Expert opinion on clinical experience, side effect profile, effectiveness in other neuropathic pain conditions, other relevant factors	Spinal cord injury	First line therapy	Loh *et al.*, 2016
Update of evidence-based recommendations/systemic review/ double-blind RCTs, including unpublished clinical trials, and meta-analysis used Numbers needed to treat for 50% pain relief and assessed publication bias	Varies	First line therapy	Finnerup *et al.*, 2015

regulation of both $Ca_v\alpha_2\delta_1$ and TSP4 in the DRG and spinal cord that precedes the onset and correlates with the duration of neuropathic pain states (Luo *et al.*, 2001; 2002; Newton *et al.*, 2001; Li *et al.*, 2004, 2014a; Kim *et al.*, 2012; Pan *et al.*, 2015). Blocking injury-induced $Ca_v\alpha_2\delta_1$ up-regulation (Boroujerdi *et al.*, 2008; Park *et al.*, 2016) or genetic ablation of TSP4 (Kim *et al.*, 2012) prevents the onset and development of injury-induced pain states. Activation of a $Ca_v\alpha_2\delta_1$/TSP4 dependent pathway at the spinal cord level leads to sensitization of spinal dorsal horn neurons through enhanced presynaptic excitatory neurotransmission and aberrant excitatory synapse formation that are known to contribute to neuropathic pain development after peripheral nerve injuries (Zhou and Luo, 2013; 2015; Park *et al.*, 2016). Treatments that block injury-induced pain states, such as genetic ablation of either $Ca_v\alpha_2\delta_1$ or TSP4 alone, also block injury-induced aberrant excitatory synaptogenesis (Park *et al.*, 2016). Furthermore, nerve injury-induced TSP4 seems to affect VGCC subtypes in sensory neurons differently, resulting in decreased HVA and increased LVA calcium currents, by interacting with $Ca_v\alpha_2\delta_1$ (Pan *et al.*, 2016). Together, these findings strongly support a critical role for injury-induced $Ca_v\alpha_2\delta_1$/TSP4 in chronic neuropathic pain processing, mediated through the promotion of aberrant excitatory synapse formation and presynaptic neurotransmission in the dorsal spinal cord. The exact location of $Ca_v\alpha_2\delta_1$/TSP4 interactions *in vivo* remains to be established since data from a recent *in vitro* co-transfection study suggest that $Ca_v\alpha_2\delta_1$/TSP4 interactions occur intracellularly (Lana *et al.*, 2016).

New prospects of targeting $Ca_v\alpha_2\delta_1$ for neuropathic pain treatment and prevention

The translational value of these findings suggest that blocking injury-induced synapse formation may lead to the prevention or reversal of neuropathic pain states post-nerve

injuries. While genetic ablation or biochemical knockdown of $Ca_v\alpha_2\delta_1$ or TSP4 are powerful tools in mechanistic research with animal models, these approaches are not practical currently for preventing/treating neuropathic pain states in patients. Blocking injury-induced abnormal synapse formation by small molecule drugs can be an attractive approach in preventing/managing neuropathic pain development after nerve injuries. Since gabapentinoids can block the $Ca_v\alpha_2\delta_1$/TSP4-induced aberrant excitatory synaptogenesis and development of pain states (Park *et al.*, 2016), using these drugs to block abnormal synapse formation could be a novel approach for chronic neuropathic pain management. Unfortunately, recent *in vitro* data indicate that late treatments, starting after the initiation of synapse formation, with gabapentinoids cannot reverse already formed excitatory synapses (Eroglu *et al.*, 2009; Yu *et al.*, 2017). Thus, the acute effects of gabapentinoids in blocking neuropathic pain states are unlikely to be due to reversing abnormal synapse formation.

Can early gabapentinoid treatment prevent abnormal synapse formation and pain state development? To address this question, we have tested the effects of gabapentin in synaptogenesis between cultured DRG sensory neurons and spinal cord neurons *in vitro* and in TSP4 injected mice *in vivo* (Yu *et al.*, 2017). Our data indicate that pre-emptive gabapentin treatment at a low-dose can prevent TSP4-induced excitatory synapse formation *in vitro*. However, delayed gabapentin treatment for 2 days after TSP4 addition, when excitatory synapses have been formed, has no anti-synaptogenic effects. These findings were confirmed *in vivo* by analysing synapse formation in the dorsal spinal cord of mice 4 days after bolus intrathecal TSP4 injection, which is known to cause pain-like behavioural hypersensitivity (Kim *et al.*, 2012; Park *et al.*, 2016). Early daily treatment with a sub-anti-hyperalgesic dose of gabapentin blocks TSP4-induced behavioural hypersensitivity and *in vivo* excitatory synapse formation. However, delayed gabapentin treatment starting 2 days later is not effective in blocking TSP4-induced pain states and excitatory synapse formation *in vivo* (Yu *et al.*, 2017). These findings confirm that early blocking of the activation of the TSP4/$Ca_v\alpha_2\delta_1$-dependent pathway by a low dose of gabapentin may prevent aberrant excitatory synapse formation in the spinal cord resulting from TSP4/$Ca_v\alpha_2\delta_1$ up-regulation, such as in the case post-peripheral nerve injury. Clinical validation of these results may lead to this approach being used to prevent the development of pain states.

Conclusions

Maladaptive changes in the expression and functions of the VGCC $Ca_v\alpha_2\delta_1$ subunit post injuries in the peripheral and central nervous systems can contribute to chronic pain development through pathological changes associated with or independent of VGCC functions. Gabapentinoids, by acting as $Ca_v\alpha_2\delta_1$ ligands, can reverse pain states significantly in a subpopulation of neuropathic pain patients through the various potential mechanisms discussed, some of which remain to be validated. Emerging data suggest that early preventive treatment with gabapentinoids can prevent aberrant excitatory synapse formation and pain state development, which, if confirmed clinically, could be an attractive approach for preventing neuropathic pain development.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015).

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Conflict of interest

The authors declare no conflicts of interest.

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