The Anti-Allodynic Gabapentinoids: Myths, Paradoxes and Acute Effects.

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Abstract
The gabapentinoids (pregabalin and gabapentin) are first line treatments for neuropathic pain. They exert their actions by binding to the $\alpha_2\delta$ accessory subunits of voltage-gated $\text{Ca}^{2+}$ channels. Because these subunits interact with critical aspects of the neurotransmitter release process, gabapentinoid binding prevents transmission in nociceptive pathways. Gabapentinoids also reduce plasma membrane expression of voltage-gated $\text{Ca}^{2+}$ channels but this may have little direct bearing on their therapeutic actions.

In animal models of neuropathic pain, gabapentinoids exert an anti-allodynic action within 30 min but most of their \textit{in vitro} effects are 30-fold slower, taking at least 17 hrs to develop. This difference may relate to increased levels of $\alpha_2\delta$ expression in the injured nervous system. Thus, in situations where $\alpha_2\delta$ is experimentally upregulated \textit{in vitro}, gabapentinoids act within minutes to interrupt trafficking of $\alpha_2\delta$ subunits to the plasma membrane within nerve terminals. When $\alpha_2\delta$ is not upregulated, gabapentinoids act slowly to interrupt trafficking of $\alpha_2\delta$ protein from cell bodies to nerve terminals. This improved understanding of the mechanism of gabapentinoid action is related to their slowly-developing actions in neuropathic pain patients, to the concept that different processes underlie the onset and maintenance of neuropathic pain and to the use of gabapentinoids in management of postsurgical pain.

Key Words
Neuropathic Pain, Alpha-2-delta ligand, Calcium Channels, Neurotransmitter Release, Time course.
Introduction

By signaling actual or potential tissue damage, pain protects from injury and enables survival and procreation. By contrast, injury to the somatosensory system can produce maladaptive ‘neuropathic’ pain that lasts for months or years after any injury has healed (Moulin et al., 2014; Costigan et al., 2009). This ‘disease of pain’ has a 1.5 - 3% prevalence within the general population (Taylor, 2006; Gilron et al., 2006; Torrance et al., 2013; Torrance et al., 2006), suggesting that as many as 210,000,000 people are afflicted worldwide. Neuropathic pain can be associated with diabetic, post-herpetic or HIV-related neuropathies, with multiple sclerosis or fibromyalgia as well as with traumatic nerve, spinal cord or brain injury (including stroke). It is characterized by touch-induced pain (alldynia), heightened responses to noxious stimuli (hyperalgesia) and may be associated with an ongoing “burning” pain (causalgia) and “electric shock-like” bouts of spontaneous pain that are independent of sensory activation.

Neuropathic pain is poorly responsive to traditional analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs) and to opioids, and this can lead to the over-prescription and abuse. First line treatment thus includes the anti-allodynic gabapentinoids, pregabalin and gabapentin (Moulin et al., 2014; Finnerup et al., 2015; Moulin et al., 2007; Martinotti et al., 2013). Here we will review the current understanding of gabapentinoid effectiveness in neuropathic pain. In so doing, we hope to dispel some misconceptions relating to their mechanism of action and to resolve two paradoxes relating to their time course of effect.

Sensory Processing in Neuropathic Pain

Many laboratory studies of neuropathic pain focus primarily on the consequences of chronic peripheral nerve injury in rodents (Kim et al., 1997; Decosterd & Woolf, 2000). This leads to mechanical allodynia which is initiated by ectopic discharges in primary afferent fibres (Wall & Devor, 1983; Pitcher & Henry, 2008; Dib-Hajj et al., 2010; Waxman & Zamponi, 2014; Sandkuhler, 2009; von Hehn et al., 2012) and “central sensitization” wherein neurons in the nociceptive circuitry of the spinal dorsal horn become susceptible to activation by innocuous peripheral stimuli (Dalal et al., 1999; Baranauskas & Nistri, 1998; Woolf, 1983; Sandkuhler, 2009; Berger et al., 2011). Central sensitization has been described as a “pathological learning process” (Woolf, 1983) and several papers explore the relationship between central sensitization and classical neuronal learning processes (Fenselau et al., 2011; Ruscheweyh et al., 2011).
Aberrations in sensory processing at the level of the spinal dorsal horn are marked by increases in the release of excitatory neurotransmitters, an increase in excitatory synaptic drive, a decrease in inhibitory synaptic drive as well as suppression of GABA and/or glycine-mediated post synaptic inhibition (Sandkuhler, 2009; Leitner et al., 2013; Coull et al., 2003; Coull et al., 2005; Balasubramanyan et al., 2006; Lu et al., 2009; Chen et al., 2009; Lee & Prescott, 2015). Many of these effects are driven by release of brain-derived neurotrophic factor (BDNF) from activated microglia (Biggs et al., 2010; Coull et al., 2005; Lu et al., 2009) as well as injury-induced T-cell infiltration (von Hehn et al., 2012) and the actions of pro-inflammatory cytokines and chemokines (Grace et al., 2014).

Neuropathic pain is also associated with changes in thalamic, limbic, autonomic and cortical structures including changes in the size of areas involved in sensory and affective processing and changes in the release of excitatory and inhibitory neurotransmitters (Gustin et al., 2012; Masocha, 2015; Zhuo, 2008; Xu et al., 2008; Lin et al., 2014). This so called “pain matrix” includes the medial prefrontal cortex, nucleus accumbens, anterior cingulate cortex, insula, amygdala, periaqueductal gray, locus coerulus, and rostroventral medulla (von Hehn et al., 2012). It is also generally accepted that processes responsible for the initiation of neuropathic pain may differ from those responsible for its long-term maintenance. Microglia activation may be primarily associated with pain onset and astrocytes may contribute to the persistence of pain over periods of months and years (Zhang & de Koninck, 2006; Grace et al., 2014).

Therapies for neuropathic pain attempt to counteract the resultant enduring changes in neuronal excitability. Partial success may be achieved using the gabapentinoids (pregabalin and gabapentin) and/or serotonin/noradrenaline uptake inhibitors and/or topical capsaicin application (Finnerup et al., 2015; Moulin et al., 2014; Sindrup & Jensen, 1999). Unfortunately, these first line treatments are far from universally effective. Gabapentinoids bring relief in only 35% of patients (Moore et al., 2014).

The Gabapentinoids

Gabapentin was designed as a GABA mimetic with increased lipophilicity so as to improve access to the central nervous system. Since many forms of epilepsy involve dysfunctional GABAergic transmission, gabapentin was first studied as an anti-convulsant (Kondo et al.,
It was approved by the FDA in 1993 as an adjunct therapy for partial seizures in patients above the age of 12. However, it was later approved for children aged 3 to 12 in 2000 for the same indication and in 2004, gabapentin was approved for the treatment of post-herpetic neuralgia in adults (Mack, 2003). Since gabapentin came on to the market, there has been widespread off-label use for bipolar disorder, attention deficit hyperactivity disorder (ADHD), restless leg syndrome, drug and alcohol withdrawal seizures and sleep disorders (Mack, 2003). It has also been shown that the paradoxical effect of long-term opioid administration which can leading to opioid-induced hyperalgesia, can be mitigated by gabapentin treatment (Stoicea et al., 2015).

A recent meta-analysis concluded that gabapentin produces meaningful pain reduction of at least 50% compared to placebo in cases of post-herpetic neuralgia and painful diabetic neuropathy, however, information regarding usefulness in other pain conditions is inconclusive (Moore et al., 2014). It was also found that 66% of patients taking gabapentin experienced an adverse event which included dizziness or drowsiness and less commonly, gait disturbance or peripheral oedema (Moore et al., 2014).

A second gabapentinoid, pregabalin (S(+)-3-isobutyl GABA) was also developed as a GABA mimetic as a successor to gabapentin (Figure 1) (Tzellos et al, 2010; McClelland et al, 2004). It was approved in 2004 for treatment of epilepsy and neuropathic pain syndromes, namely painful diabetic neuropathy (Dworkin & Kirkpatrick, 2005). In addition, pregabalin was approved for the treatment of anxiety disorders in Europe (Tzellos et al, 2010).

Despite their structural similarity to GABA (Figure 1) neither pregabalin nor gabapentin bind strongly to GABA_A or GABA_B receptors (Lanneau et al., 2001; Moore et al., 2002; Sutton et al., 2002; Li et al., 2011). Gabapentinoids also do not affect GABA uptake, synthesis or metabolism (Taylor et al., 2007). The first insight into their mechanism of action came 20 years ago when Gee et al (Gee et al., 1996) isolated and sequenced a protein that bound gabapentin from porcine brain and identified it as the α2δ-1 subunit of voltage-gated calcium channels or Ca_α2δ1. Gabapentinoids are thus referred to as α2δ ligands (Dooley et al., 2007). The physiological role of α2δ subunits and their likely involvement in the etiology of neuropathic pain is covered in the next few paragraphs.
Voltage-Gated Calcium Channels and their α2δ Accessory Subunits

A detailed description of voltage-gated calcium channels is beyond the scope of this review, but may be found in two recent reviews (Simms & Zamponi, 2014; Zamponi, 2015b). Briefly, these channels encompass high-voltage activated (HVA) L-types (Cav1.1, Cav1.2, Cav1.3, and Cav1.4); P/Q-type (Cav2.1), N-type (Cav2.2), and R-type (Cav2.3) as well as T-type (low-voltage-activated, LVA) Ca\textsuperscript{2+} channels (Cav3.1, Cav3.2, Cav3.3) (Zamponi, 2015a). Influx of Ca\textsuperscript{2+} via high-voltage-activated (HVA) Ca\textsuperscript{2+} channels triggers neurotransmitter release from presynaptic vesicles and thereby determines neuronal network excitability. The importance of HVA-Ca\textsuperscript{2+} channels in neuropathic pain is illustrated by the clinical effectiveness of the N-type Ca\textsuperscript{2+} channel blocker ziconotide (Zamponi et al., 2015) and as will discussed below, the relationship between HVA-Ca\textsuperscript{2+} channel function and the actions of gabapentinoids.

Voltage-gated Ca\textsuperscript{2+} channels consist of five subunits: the α\textsubscript{1} pore-forming subunit and auxiliary subunits α\textsubscript{2}, β, δ and γ (Figure 2, reviewed in Zamponi et al., 2015). The main subtype found in presynaptic terminals is Ca\textsubscript{v}2 (Westenbroek et al., 1992). Ca\textsubscript{v}2.1 and Ca\textsubscript{v}2.2 both contain a synaptic protein interaction site (synprint) that interacts with SNARE proteins (syntaxin and SNAP-25) (Rettig et al., 1996; Sheng et al., 1994). By this mechanism, channels can be closely associated with synaptic vesicles that govern release of neurotransmitter.

The α2δ subunits, which bind and mediate the effects of gabapentinoids (Field et al., 2006; Bauer et al., 2010a), are multifunctional and are expressed in the plasma membrane in multimeric complexes with mature HVA-Ca\textsuperscript{2+} channels. T-type (LVA) Ca\textsuperscript{2+} channels (Cav3.1, Cav3.2, Cav3.3) do not appear to associate directly with α2δ proteins. (Dolphin, 2013; Lacinova et al., 2000).

Figure 2 Near Here

Four different mammalian genes encode the α2δ subunits: \textit{CACNA2D1-CACNA2D4} (Whittaker and Hynes 2002). Of the four available types (α2δ–1 through α2δ–4) (Dolphin, 2012), the α2δ–1 subunit is expressed in primary afferent nerve fibres and is crucial for release of excitatory neurotransmitter from their terminals in the spinal dorsal horn (Hoppa et al., 2012). α2δ subunits are glycosylphatidylinositol (GPI)-anchored (Figure 2 (Davies et al., 2010). α2δ-1 subunits have been shown to increase Ca\textsubscript{v}2 plasma membrane expression suggesting that part of the role of α2δ is in trafficking of channel complexes (Cassidy et al., 2014). While T-type (Ca,v3)
channels do not require $\alpha_2\delta$ to be expressed, their expression is enhanced by the presence of $\alpha_2\delta$ (Zamponi et al., 2015).

**$\alpha_2\delta$ Subunits and Neuropathic Pain**

Deletion of the $\alpha_2\delta$–1 gene in animal models delays mechanical hypersensitivity in response to peripheral nerve damage and impedes functional expression of pore forming Ca\textsubscript{v}2.2 $\alpha$–subunits in the plasma membrane of the cell bodies of dorsal root ganglion (DRG) neurons (Patel et al., 2013). By contrast, transgenic mice engineered to overexpress $\alpha_2\delta$–1 display increased HVA-Ca\textsuperscript{2+} channel current ($I_{Ca}$) in DRG neurons as well as pain behaviours and prolonged dorsal horn neuronal responses to mechanical and thermal stimulation in the periphery (Li et al., 2006). It has also been shown that injury-induced discharges that contribute to the initiation of neuropathic pain are involved in the up-regulation of $\alpha_2\delta$–1 levels in the spinal dorsal horn (Boroujerdi et al., 2008).

Increased expression of $\alpha_2\delta$–1 following nerve injury has thus been strongly implicated in the etiology of neuropathic pain (Li et al., 2006; Zhou & Luo, 2015; Boroujerdi et al., 2011) and binding of gabapentinoids to this subunit likely plays a major role in their anti-allodynic actions (Hendrich et al., 2008; Bauer et al., 2009; Boroujerdi et al., 2011; Luo et al., 2002; Zamponi et al., 2015).

**Mechanism of Gabapentinoid Action**

Gabapentinoids are transported into the neuronal cytoplasm via a neutral amino acid transporter (Su et al., 1995; Cheng & Chiou, 2006) where they bind to $\alpha_2\delta$-1 (Field et al., 2006; Bauer et al., 2009). Interruption of the interaction of $\alpha_2\delta$–1 with pore-forming $\alpha$-subunits of HVA Ca\textsuperscript{2+} channels reduces their trafficking and the appearance of functional channels at the cell surface. This likely involves impediment of the action of a positive regulator of trafficking such as isoleucine (Hendrich et al., 2008; Zamponi et al., 2015). This has led to the assumption that gabapentinoids also interrupt trafficking of pore forming $\alpha$–subunits over a longer distance as they are gradually transported from cell bodies of sensory neurons to primary afferent terminals. The resulting decrease in channel availability would be expected to decrease depolarization-induced Ca\textsuperscript{2+} influx and this has been suggested to reduce neurotransmitter release (Field et al., 2006; Cheng
& Chiou, 2006; Bauer et al., 2010a; Fink et al., 2000; Yang et al., 2014). As will be outlined below, this assumption seems to be invalid (Biggs et al., 2014; Hoppa et al., 2012).

**Myth – Gabapentinoids Reduce Neurotransmitter Release by Decreasing Expression of HVA Ca\(^{2+}\) Channels on Nerve Terminals.**

Drug or neurotransmitter modulation of HVA-Ca\(^{2+}\) channels on the cell bodies of DRG neurons has for many years been used as a model to predict their action at primary afferent terminals within the dorsal horn (Dunlap & Fischbach, 1981). This concept is illustrated in Fig 3a. The assumption has been made that any drug that reduces HVA-I\(_{Ca}\) in DRG cell bodies will exert the same effect on Ca\(^{2+}\) channels at nerve terminals and that this will be reflected as reduction in neurotransmitter release. This is not the case for gabapentinoids (Biggs et al., 2014). Incubation of cultured DRG neurons for 3-4d with 10\(\mu\)M pregabalin reduces HVA-I\(_{Ca}\) in the cell bodies of small, putative nociceptive, “IB4 negative” neurons to 67% of their control amplitude. This is illustrated in the current-voltage relationship for HVA-Ca\(^{2+}\) channels (Fig 3b). By contrast, acute application of a low concentration of Mn\(^{2+}\) is considerably more effective; 200\(\mu\)M Mn\(^{2+}\) reduces HVA-I\(_{Ca}\) to 8% of its control amplitude (Fig 3c). A typical recording of HVA Ca\(^{2+}\) current illustrating the strong effect of 200\(\mu\)M Mn\(^{2+}\) is illustrated in Fig 3d. In the dorsal horn however, 200\(\mu\)M Mn\(^{2+}\), failed to affect synaptic activity in **substantia gelatinosa** neurons as monitored by the amplitude of spontaneous excitatory postsynaptic currents (sEPSCs; Figs 3a, e and g). Despite its relatively small effect on HVA-I\(_{Ca}\) in DRG cell bodies, 5-6d exposure of **substantia gelatinosa** neurons in organotypic culture to 10\(\mu\)M pregabalin has a clear depressant effect on synaptic transmission as demonstrated by a significant reduction in the amplitude of sEPSCs (Fig 3f). The summarized findings presented in Fig 3g show that 200\(\mu\)M Mn\(^{2+}\) is more effective in blocking HVA-Ca\(^{2+}\) channels than 10\(\mu\)M pregabalin whereas 10\(\mu\)M pregabalin is more effective than 200\(\mu\)M Mn\(^{2+}\) in blocking neurotransmitter release. The moderate effect of pregabalin on HVA-I\(_{Ca}\) in DRG cell bodies (Fig 3b) is therefore insufficient to explain its ability to reduce transmitter release in the spinal dorsal horn (Fig 3f)

*Figure 3 Near Here*
These findings may be explained in terms of the results of Hoppa et al (2012) that gabapentinoid inhibition of neurotransmitter release reflects interruption of the ability of α2δ to facilitate interaction of HVA-Ca\(^{2+}\) channels with neurotransmitter release sites. Additional evidence for a direct action on the release process is provided by the recent observation that gabapentin reduces the frequency of miniature EPSCs (mEPSCs) in the dorsal horn (Zhou & Luo, 2015; Zhou & Luo, 2014). Since mEPSCs are recorded in the presence of tetrodotoxin, they reflect transmitter release that is independent of depolarization and hence the entry of Ca\(^{2+}\)-via HVA-Ca\(^{2+}\) channels. Thus gabapentin exerts its effect by a mechanism that is distinct from reduced expression of HVA-Ca\(^{2+}\) channels in plasma membrane of nerve terminals.

The modest effect of Mn\(^{2+}\) on transmitter release may be explained by the classical 3-4\(^{th}\) power relationship between Ca\(^{2+}\) influx and release (Dodge & Rahamimoff, 1967); even though the amount of Ca\(^{2+}\) entering terminals is reduced in the presence of Mn\(^{2+}\), this is sufficient to support substantial neurotransmitter release. This possibility is supported by the observation that overexpression of pore-forming Ca\(_{\text{v}}\)2.2 channels in hippocampal neurons fails to increase EPSC size and the suggestion that the strength of neurotransmission is saturated with regard to levels of Ca\(^{2+}\) channel expression (Cao & Tsien, 2010).

**Do Gabapentinoids Impede Trafficking of Pore Forming Alpha Subunits from Cell Bodies to Nerve Terminals?**

The actions of gabapentinoids on DRG or dorsal horn neurons take at least 17h to develop in vitro (Heblich et al., 2008; Hendrich et al., 2008; Hendrich et al., 2012; Biggs et al., 2014). This is consistent with the suggestion that α2δ ligands prevent the transport of newly synthesized pore-forming Ca\(^{2+}\) channel α–subunits from the cell body of DRG neurons to their terminals in the dorsal horn. This is further supported by the observation that long-term gabapentinoid exposure limits the expression of functional HVA-Ca\(^{2+}\) channels in the plasma membrane of cell bodies of DRG neurons (Hendrich et al., 2008; Biggs et al., 2014).

However, in the light of the previous discussion, decreased expression of functional Ca\(^{2+}\) channels at nerve terminals may be of little consequence, as their blockade by Mn\(^{2+}\) has surprisingly little effect on neurotransmitter release (Fig 3e). Also, since α2δ participates directly in the neurotransmitter release process per se, (Hoppa et al., 2012; Zhou & Luo, 2015; Zhou & Luo, 2014) the slowly developing effects of gabapentinoids may reflect inhibition of trafficking of
α2δ-1 subunits, as opposed to pore forming α subunits, from cell bodies to terminals. This alone would be expected to reduce neurotransmitter release by impeding interaction of HVA-Ca\(^{2+}\) channels with the release process. This possibility is supported by the findings of Bauer et al. (2009) who showed in nerve injured animals, where α2δ-1 is upregulated, its trafficking to primary afferent terminals is prevented by chronic pregabalin treatment.

Slowly developing actions of gabapentinoids in vitro do not correlate with their rapid actions in animal models in vivo where antiallodynic effects can be seen within 30min of IP injection (Kumar et al., 2013; Field et al., 2006; Patel et al., 2001). We thus define “rapid” effects as those occurring with 30-60min to distinguish them from “slow” effects that take 10-20 h to develop. The paradoxical difference between the rapid in vivo and slow in vitro actions of gabapentinoids is discussed in next section.

**Paradox 1 - Time Course of Gabapentinoid Action in Animal Models; In vitro Versus in vivo.**

A single intraperitoneal injection of 100 mg/kg gabapentin suppresses mechanical allodynia (Fox et al., 2003) and other signs of neuropathic pain in animal models within 30 - 60min (Kumar et al., 2013; Field et al., 2006; Patel et al., 2001) yet, as mentioned, most reported actions of gabapentinoids on neurons in vitro are ~30 fold slower, taking 17 hours or more to develop (Hendrich et al., 2012; Biggs et al., 2015; Biggs et al., 2014). For additional detail see table 1. Fig 4a shows the reduction of withdrawal threshold for mechanical (von Frey filament) stimulation seen in rats subject to chronic constriction injury (CCI) of their sciatic nerve; lowered mechanical thresholds are indicative of allodynia and hyperalgesia (Kim et al., 1997). Intraperitoneal injections of 100mg/kg gabapentin rapidly and reversibly eliminate these signs and increase mechanical withdrawal threshold towards that seen in uninjured animals.

*Figure 4 and Table 1 Near Here*

In line with our observations with pregabalin (Fig 3b and f) and the consensus that neuronal actions of gabapentinoids in vitro take 17 hr or more to develop (Heblich et al., 2008; Hendrich et al., 2008; Hendrich et al., 2012; Biggs et al., 2015; Biggs et al., 2014; Zamponi et al., 2015), we found that 3-4d exposure to a therapeutically relevant concentration of 100 μM gabapentin
(Kushnir et al., 1999) significantly reduced HVA I_{ca} in DRG neurons (p<0.01) whereas acute exposure was without effect (Biggs et al., 2014). These findings are summarized in Fig 4d. A similar slowly-developing effect of gabapentin was also seen on dorsal horn excitability, but acutely applied drug was also without effect (Fig 4c). Spinal cord neurons in organotypic slice culture (Lu et al., 2006; Biggs et al., 2012) were challenged with 35 mM K^+ for 90s and this evoked a large increase in intracellular Ca^{2+} as monitored by confocal Ca^{2+} imaging using Ca^{2+} indicator Fluo-4 AM. This response, which was used as an overall index of dorsal horn excitability, was significantly reduced in slices exposed to gabapentin for 5-6 days (Fig 4c) but was unaffected by acute exposure to 100 μM gabapentin. (Biggs et al., 2014). Sample recordings of the effect of 5d GBP exposure on Fluo-4 fluorescence are shown in Fig 4d and e.

One likely reason for this temporal discrepancy between in vivo and in vitro drug actions is that many in vitro studies have been done on neurons from uninjured, control animals (Moore et al., 2002; Hendrich et al., 2012; Biggs et al., 2014) whereas the rapidly developing behavioral effects are done on nerve injured animals where α2δ–1 is upregulated (Field et al., 2006; Kumar et al., 2013; Narita et al., 2012). Other work in either neuropathic animals (Patel et al., 2000; Coderre et al., 2005) or in situations where α2δ–1 is upregulated (Li et al., 2006) have revealed rapidly developing effects of acutely-applied gabapentinoids (Zhou & Luo, 2015).

From the available literature, we suggest the following explanation. α2δ–1 subunits are complexed with pore forming α–subunits and accessory β–subunits in post-Golgi compartments of the endoplasmic reticulum and Golgi apparatus of neuronal cell bodies (Canti et al., 2005; Tran-Van-Minh & Dolphin, 2010). In primary afferent terminals, channel complexes comprising α2δ–1, α–subunits and β -subunits are transported and inserted into the plasma membrane (Heblich et al., 2008). This enables plasma membrane expression of HVA-Ca^{2+} channels but more importantly, enables their coupling to the neurotransmitter release machinery (Hoppa et al., 2012). The channel complexes are then removed from the plasma membrane by endocytosis (Bauer et al., 2009; Tran-Van-Minh & Dolphin, 2010; Dolphin, 2012) into early endosomes where they are targeted for recycling or degradation. In the control or uninjured situation, where levels of α2δ–1 are low, cycling of protein to and from the plasma membrane in nerve terminals may be a relatively
slow overall process (Fig 5a). However this process may become much more rapid when $\alpha_2\delta$–1 is upregulated either experimentally or as result of nerve injury as new protein is inserted into the plasma membrane (Tran-Van-Minh & Dolphin, 2010) (Fig 5b). Since gabapentinoids do not appear to affect the rate of endocytosis (Tran-Van-Minh & Dolphin, 2010), this renders surface expression of $\alpha_2\delta$–1 more labile and susceptible to inhibition by gabapentinoids which may be capable of exerting their effects within minutes (Tran-Van-Minh & Dolphin, 2010) rather than hours (Hendrich et al., 2008; Heblich et al., 2008; Biggs et al., 2015; Biggs et al., 2014) (Fig 5c).

In uninjured nerves, where $\alpha_2\delta$–1 is not upregulated, gabapentinoid impediment of trafficking of $\alpha_2\delta1$–HVA I, Ca complexes from cell bodies to nerve terminals (Bauer et al., 2009) will cause gradual depletion of their surface expression as the rate of endocytosis exceeds the rate of replenishment (Fig 5d); a processes which appears to take 17 hours or more to occur (Heblich et al., 2008).

Recent data from our laboratory are consistent with this mechanism. We find gabapentinoids have limited acute effects on dorsal horn neurons in spinal cord slices isolated from sham operated animals whereas profound suppression of synaptic transmission and excitability can be observed in neurons in slices from nerve injured animals (Alles et al., 2015). Similar rapid effects of gabapentin on excitatory synaptic transmission in both deep dorsal horn neurons (Zhou & Luo, 2015) and in superficial laminae (Zhou & Luo, 2014) have been seen in $\alpha_2\delta1$-overexpressing transgenic mice. The drugs thus act slowly in non-injured neurons and rapidly in injured neurons.

**An Analogy to Explain Gabapentinoid Action.**

Let us assume the axon from the cell body is analogous to a highway into a city, the nerve terminal is the city, cars are $\alpha_2\delta$–1 subunits and gabapentinoids represent roadworks. Under normal conditions, roadworks on the highway will eventually decrease the number of cars getting to the city so the supply of cars will very slowly run out (slowly developing effects of gabapentinoids on uninjured nerves *in vitro* Fig 5d). Similarly, if there are roadworks in the city, late at night or a on a Sunday, this won’t have much effect on the movements of the few cars ($\alpha_2\delta$–1 subunits) that are active (limited acute effect of gabapentinoids in uninjured nerves). By contrast at rush hour (equivalent to the nerve injury situation Fig 5b) where there are many more active $\alpha_2\delta$–1 subunits in the nerve terminals (cars in the city), gabapentinoids (roadworks) will
have a much more rapid and profound action (Fig 5c). “Gridlock” may account for their rapid action in terminals after nerve injury. This analogy may also explain the rapid reversibility of gabapentinoid action *in vivo* (Yang et al., 2014) (see also Fig 4a). Removal of drug would alleviate the “gridlock” in the active terminal and allow the resumption of rapid cycling of α2δ−1 into the plasma membrane. Thus re-enabling interaction of HVA-Ca2+ channels with neurotransmitter release sites

**Additional Factors that Contribute to the Rapid Action of Gabapentinoids *in vivo***

In the preceding sections we have considered the actions of gabapentinoids in the dorsal horn as this is a major site of nociceptive processing. Because gabapentinoids are effective as anticonvulsants (Sivenius *et al.*, 1991;Kondo *et al.*, 1991;Dworkin & Kirkpatrick, 2005) and anxiolytics (Singh *et al.*, 1996) they obviously exert actions, at many, if not all levels of the central nervous system. Such effects likely involve interactions with various types of α2δ subunit (Dolphin, 2012). That rapid central effects may contribute to the overall antiallodynic actions of gabapentinoids *in vivo* is supported by a study using 8 F-fluorodeoxyglucose-positron emission tomography (Lin *et al.*, 2014). These authors showed that spared nerve injury-induced increases of glucose metabolism in thalamus, cerebellar vermis and medial prefrontal cortex and that these changes were attenuated by acute gabapentin treatment.

Gabapentinoids may also exert acute *in vivo* effects as a result of inhibition of descending serotonergic facilitation of nociceptive processing (Suzuki *et al.*, 2005;Rahman *et al.*, 2009). Such acute effects would obviously be absent in *ex vivo* spinal cord slices or in organotypic cultures yet would be present *in vivo*. Interestingly, this effect does not appear to involve effects of gabapentin on serotonin release but may rather include acute interaction and inhibition of 5HT3 receptors (Suzuki *et al.*, 2005). The precise mechanism and the possible role of α2δ in this interaction remains to be elucidated.

An additional central mechanism that would be absent in acutely isolated spinal cord slices relates to the observation that gabapentin acutely (30 – 90 min) increases glutamate levels in the *locus coeruleus* (Suto *et al.*, 2014). This reflects acute inhibition of the astroglial glutamate transporter (GLT-1/EAAT2). Since the *locus coeruleus* provides a descending inhibitory noradrenergic input to the dorsal horn (Tanabe *et al.*, 2005), this effect would be expected to reduce excitability and to impede the transfer of nociceptive information.
Since ectopic activity in damaged peripheral nerves is required to drive central sensitization at the level of the dorsal horn (Pitcher & Henry, 2008; Vaso et al., 2014) and there are reports of acute gabapentioid actions on peripheral nerves (Pan et al., 1999; Yang et al., 2009) such actions may also contribute to the appearance of rapid drug effects in vivo.

**The Role of α2δ as a Thrombospondin Receptor.**

Interestingly, α2δ-1, which is expressed extracellularly in mature Ca\(^{2+}\) channels (Figure 2) (Dolphin, 2013; Hendrich et al., 2008; Dolphin, 2012), has been implicated as a receptor for a group of neurotrophins known as thrombospondins (Eroglu et al., 2009; Risher & Eroglu, 2012). All five members of this group (TSP 1-5) are secreted matrix proteins and all have been implicated in excitatory synaptogenesis (Eroglu et al., 2009; Christopherson et al., 2005). One member of this group, thrombospondin 4 (TSP4) has been implicated in the etiology of neuropathic pain (Kim et al., 2012; Pan et al., 2015). TSP4 is expressed in astrocytes and is upregulated in the injury side of dorsal spinal cord and this correlates with the development of signs of neuropathic pain. TSP4 blockade by intrathecally delivered antibodies, antisense oligodeoxynucleotides, or inactivation of the TSP4 gene reverses or prevents behavioral hypersensitivity. Intrathecal injection of TSP4 protein into naive rats increases the frequency of mEPSCs in dorsal horn neurons (Kim et al., 2012), suggesting an increased excitatory presynaptic input that would be consistent with behavioral hypersensitivity.

Seven days of gabapentinoid treatment has been shown to decrease synapse formation in cortical structures (Eroglu et al., 2009). Since both neuropathic pain and α2δ subunits have been associated with excitatory synaptogenesis (Crosby et al., 2015; Li et al., 2014; Bauer et al., 2010b), gabapentinoid interaction with α2δ–1 to antagonize the actions of thrombospondins may contribute to some of its more slowly developing effects. It is not however an exclusive mechanism for three reasons.

1. Astrocytes secrete TSPs to increase synapse number (Christopherson et al., 2005) but we have seen slowly developing, neuron-subtype specific effects from in vitro experiments in neuron enriched cultures of DRG neurons which do not contain astrocytes (Biggs et al., 2014). Thus, the presence of thrombospondin is not needed for gabapentinoid action. Data shown in Fig 3b were obtained from such cultures.
2. Since the effects of gabapentinoids are prevented following blockade of uptake into the neuronal cytoplasm by of 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) (Hendrich et al., 2008; Biggs et al., 2015; Biggs et al., 2014), it would seem unlikely that they act exclusively at an extracellular binding site on mature HVA-Ca\(^{2+}\) channels.

3. Because actions of gabapentinoids can be observed within minutes of application under appropriate experimental conditions both \textit{in vivo} (Kumar et al., 2013; Narita et al., 2012; Coderre et al., 2005) and \textit{in vitro} (Alles et al., 2015; Zhou & Luo, 2015; Zhou & Luo, 2014) these effects are unlikely to reflect impairment of the slow process of synaptogenesis. This idea is supported by the observation of Kim \textit{et al} (2012) that TSP-4 takes at least 4 days to increase synaptic transmission within the dorsal horn of the spinal cord.

\textit{Paradox 2 - Rapid Effects in Animals but Slow Effects in People?}

If it is accepted that the rapidity of onset of gabapentinoid action \textit{in vitro} is directly related to the level of \(\alpha_2\delta-1\) expression and drug effects emerge within 30 min in animal models, why is it commonly reported that the drug effects take many days to appear in the clinic (Cheshire, 2002; Sharma \textit{et al}., 2010; Parsons \textit{et al}., 2015; Gottrup \textit{et al}., 2004)? One possibility is that in patients presenting with chronic neuropathic pain, \(\alpha_2\delta\) is no longer upregulated and other maladaptive process have taken over the maintenance of central sensitization (Figure 6). This idea is congruent with the likelihood that the processes that maintain neuropathic pain differ from those that initiate it. Thus, gabapentinoids may only act to slowly shut off the supply of \(\alpha_2\delta\) subunits to nerve terminals in neuropathic pain patients. For example, when patients receive gabapentinoids to alleviate pain associated with diabetic or other neuropathies, there is no way of knowing when the initial precipitating nerve injury events occurred; they are in the “maintenance phase” of neuropathic pain. Our search of the literature revealed no information about the persistence of injury-induced \(\alpha_2\delta\) upregulation in either animal models or in patients. Such studies are urgently required to understand the protracted action of gabapentinoids in the clinic. Since gabapentinoids are not universally effective, and as many as 50% of treated patients do not experience pain relief with gabapentin (Moore \textit{et al}., 2014), it would be interesting to know whether \(\alpha_2\delta-1\) levels in individual patients would predict drug efficacy.

\textit{Figures 6 and 7 Near Here}
Another likely factor relates to differences in measuring “pain” in animals versus people (Mogil, 2009; Mogil & Crager, 2004). Drug-induced increases in withdrawal threshold to tactile stimuli in neuropathic animals may simply reflect attenuation of spinal reflexes. For this reason, preclinical evaluations of anti-allodynic effectiveness are moving towards “operant” models (Xie et al., 2014; Yezierski et al., 2013). In this situation, the animal needs to make a decision based on the cortical processing of a noxious stimulus. For example, rats will naturally select for a covered, darkened environment to avoid predators. If a rat is nerve injured, a mildly warm stimulus will produce thermal hyperalgesia. In an operant test, the animal is given the choice of being on a warm surface in the dark or a cool surface in the light. If the animal is experiencing thermal hyperalgesia, it would be expected to spend more time in the light than in dark; a very different response from a naïve animal (see Figure 7). To the best of our knowledge, relatively few studies of gabapentinoid action in operant pain models have been done (Yezierski et al., 2013; Munro et al., 2007; Park et al., 2015). Further studies of this type may better align findings in animals with experience in the clinic.

There is also a good deal of interest in the use of gabapentinoids in post-surgical pain (Eipe et al., 2015). In general, the effectiveness is rather variable but rapidly developing effects have been reported (Schmidt et al., 2013). Our model posits that rapid effects of gabapentinoids will only occur under conditions where α2δ−1 is upregulated. Interestingly, surgery itself (in the absence of deliberate nerve injury) has been reported to increase α2δ−1 (Bauer et al., 2009) and it has also been suggested that injury-induced discharge in primary afferent fibres, as may occur during surgical manipulation, can upregulate α2δ−1 (Boroujerdi et al., 2008).

**Detailed Actions of Gabapentinoids in the Dorsal Horn**

Acute and slowly developing anti-allodynic effects of gabapentinoids involve attenuation of neurotransmitter release from primary afferent terminals and perhaps from other central and spinal sites (Biggs et al., 2014; Zhou & Luo, 2015; Moore et al., 2002; Coderre et al., 2005). Early studies on the effects of gabapentinoids did not address the effect on specific cell types within the spinal dorsal horn (Moore et al., 2002). If gabapentinoids were to inhibit inhibitory neurons the resultant disinhibition would tend to increase overall excitability. This would be inconsistent with both their anti-allodynic action and their overall depressant effect on spinal cord excitability (Fig
Most tonic firing, low threshold, neurons in *substantia gelatinosa* exhibit a GABAergic phenotype and most high threshold, delay firing neurons are glutamatergic (Yasaka et al., 2010; Punnakkal et al., 2014; Schoffnegger et al., 2006). In our study of long-term actions of pregabalin and gabapentin on *substantia gelatinosa* neurons in organotypic culture, we found that synaptic input to putative excitatory neurons was reduced preferentially (Biggs et al., 2014) and this effect was only seen when drugs were present for 5-6 days. The basis of this nerve terminal selectivity of gabapentinoid action within the dorsal horn needs to be elucidated. One possibility may be that the excitatory terminals onto inhibitory *substantia gelatinosa* have reduced expression of the neutral amino acid transporter.

Gabapentinoids select for excitatory transmission in another way (Zhou & Luo, 2015) as unlike mEPSCs, miniature IPSCs (inhibitory postsynaptic currents) in spinal neurons were unaffected by the drug. This finding is congruent with the observation that α2δ−1 subunits preferentially localize with excitatory rather than inhibitory terminals in the spinal cord (Bauer et al., 2009).

**Conclusions.**

1. Although gabapentinoids are sometimes classified as “calcium channel blocking agents”, this does not really reflect their mechanism of action. They are more accurately described as drugs that depress neuronal excitability by a variety of mechanisms following their interaction with multifunctional α2δ proteins.

2. Both in the laboratory and in the clinic, some actions of gabapentinoids develop within less than 30 minutes whereas others take days or weeks to appear. It is suggested that the level of the gabapentinoid binding protein, α2δ−1 in nerve terminals directly dictates the rate of onset of gabapentinoid action in the laboratory and possibly within the clinic. This opens up the possibility for a personalized method of prescription of the gabapentinoids depending on α2δ−1 expression profile. It is possible that α2δ is no longer upregulated in many patients who have endured neuropathic pain for periods of months or years. This may account for the 35% success rate observed with gabapentin in the clinical setting.
Acknowledgement

We thank Dr. Nataliya Bukhanova for carrying out the behavioral experiments illustrated in Fig 4a,
Table 1. Summary of rapid *in vivo* and slow *in vitro* actions of gabapentinoids in animal models of neuropathic pain.

<table>
<thead>
<tr>
<th>Rapid effects occurring in 30-60min <em>in vivo</em></th>
<th>Slow effects taking more than 10h <em>in vitro</em></th>
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<tr>
<td>Patel <em>et al</em> 2001, Gabapentin (100mg/kg) increases paw withdrawal threshold in an inflammatory pain model within 1 hour of IP injection</td>
<td>Heblich <em>et al</em> (2008), 1mM gabapentin inhibited current through Ca$^{2+}$ channels expressed in TsA201 cells within 17 – 20h but not within 3-6h</td>
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<tr>
<td>Fox <em>et al</em> (2003) Gabapentin produced significant dose-related reversal of tactile allodynia in the rat following a single administration.</td>
<td>Hendrich <em>et al</em> (2008) 1mM gabapentin inhibited current through Ca$^{2+}$ channels expressed in TsA201 cells or native currents in rat DRG neurons within 40h but not acutely</td>
</tr>
<tr>
<td>Coderre <em>et al</em> (2005). Gabapentin (300mg/kg) reduces neuropathic pain by inhibiting the spinal release of glutamate.</td>
<td>Hendrich <em>et al</em> (2012) 40-48h exposure to pregabalin (100$\mu$M) inhibits synaptic transmission between rat dorsal root ganglion and dorsal horn neurons in culture</td>
</tr>
<tr>
<td>Field <em>et al</em> 2006, Pregabalin (30 or 100mg/kg) or Gabapentin (100 or 300mg/kg) increases paw withdrawal threshold in a neuropathic pain model within 1 hour of IP injection</td>
<td>Biggs <em>et al</em> (2014) Exposure to 10$\mu$M PGB for 5–6 days reduced maximal HVA I$_{Na}$ density in small IB4 positive DRG neurons.</td>
</tr>
<tr>
<td>Kumar <em>et al</em> (2013), Attenuation of facial hypersensitivity and noxious stimulus-evoked release of glutamate in medullary dorsal horn in a rodent model of trigeminal neuropathic pain within 30 min of IP injection of 1 or 25mg/kg pregabalin</td>
<td>Biggs <em>et al</em> (2014, 2015). Studies on rat spinal cord in organotypic culture, decreased excitability and excitatory synaptic transmission following 5-6d exposure to 10$\mu$M pregabalin or 100$\mu$M gabapentin.</td>
</tr>
<tr>
<td>This review, Fig 4a, increase in paw withdrawal threshold in rats subject to CCI following IP injection of 100mg/kg gabapentin.</td>
<td></td>
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</tbody>
</table>
**Figure Legends**

**Figure 1. Structure of GABA and the Gabapentinoid Drugs for Neuropathic Pain.**

**Figure 2. The Voltage-gated Calcium Channel.** Diagram to illustrate the structure of HVA Ca$^{2+}$ channels showing interactions of the pore-forming α₁ subunit and the auxiliary α₂δ, β and γ subunits. The α₂ accessory subunit is entirely extracellular and is linked via a disulfide bridge to the δ subunit which is mainly extracellular with a short transmembrane and intracellular domain. The β subunit is entirely intracellular.

**Figure 3. Differential effects of Mn$^{2+}$ and pregabalin (PGB) on HVA-I$_{Ca}$ and nerve terminal activity.** a. Diagram to illustrate the concept that study of voltage gated Ca$^{2+}$ channels in dorsal root ganglion (DRG) neuronal cell bodies may be used as a model for inaccessible Ca$^{2+}$ channels on primary afferent terminals. Release of glutamate (yellow circles) from primary afferent terminals generates excitatory postsynaptic currents (EPSC’s) in dorsal horn neurons. Data records at right show spontaneous EPSC’s (sEPSC) recorded in a substantia gelatinosa neuron in the dorsal horn of a rat spinal cord in the absence and presence of 200μM Mn$^{2+}$. b. Effect of 3-4d exposure to 10μM pregabalin on HVA-I$_{Ca}$ density – voltage relationship of small IB4 negative DRG neurons (Ba$^{2+}$ was used as charge carrier). c. Effect of 200μM Mn$^{2+}$on current density-voltage relationship of HVA-I$_{Ca}$ in a small DRG neuron. Note that current is reduced to <10% of its control value. d. Recording of HVA-I$_{Ba}$ at -10mV from a small DRG neuron prior to and after the addition of 200μM Mn$^{2+}$. $V_h = -100$mV, voltage command shown in lower trace. Note strong suppression of current by 200μM Mn$^{2+}$. e. Superimposed cumulative probability plots to show lack of effect of 200μM Mn$^{2+}$ on amplitude of sEPSC’s in neurons from substantia gelatinosa region of a spinal cord. Data pooled from 5 neurons. f. Cumulative probability plots of sEPSC amplitude from high threshold, putative excitatory substantia gelatinosa neurons in organotypic culture replotted (with permission) from data of Biggs et al (2014). Comparison of control neurons with those exposed to 10μM pregabalin for 5-6d. ($p<0.0001$, Kolgomorov-Smirnov test). g. Replotting of data from b and c and from Biggs et al (2014) as percentage changes to show that 200μM Mn$^{2+}$ is far more effective than 10μM pregabalin in attenuating HVA-I$_{Ca}$ in DRG cell
bodies. 10µM pregabalin attenuates sEPSC amplitude in substantia gelatinosa yet 200 µM Mn²⁺ is almost without effect.

**Figure 4.** Acute versus chronic effects of gabapentin (GBP) on HVA-I\(_{\text{Ca}}\) in DRG neurons and dorsal horn excitability. a. Effects of chronic constriction injury (CCI, see Balasubramanyan et al., 2006) on mechanical allodynia measured from withdrawal thresholds to paw stimulation with von Frey filaments. Black downward triangles designate intraperitoneal injections of 100mg/kg gabapentin (GBP). Note rapid increase in withdrawal threshold indicating suppression of allodynia and return of allodynia within 2h of discontinuation of drug injections. Experiments done on 4 sham operated animals, 11 animals treated with gabapentin and 11 animals treated with vehicle (IP saline injection). *Inset* is replot of 30 minute time point data from main graph to further illustrate the rapid onset of gabapentin effect. b. Comparison of acute and chronic effects of 100µM gabapentin (GBP) on HVA-I\(_{\text{Ca}}\) in small, putative nociceptive, IB4 negative DRG neurons. Drug was applied to 6 neurons for 20min. and no significant effect was seen. By contrast, exposure of cultured DRG neurons to 100µM gabapentin for 3-4d produced a significant reduction in current (data from 5 control neurons and 6 exposed to drug, \(p<0.01\)). c. Lack of effect of acutely applied gabapentin (100µM) on spinal cord excitability as monitored by Ca²⁺ response to 35mM K⁺ challenge (n=13) and significant effect (\(p<0.01\)) seen with 5d gabapentin exposure. Data from 40 control neurons and 27 exposed to drug. Experiments were done by confocal Ca²⁺ imaging of substantia gelatinosa neurons in organotypic culture and were derived from previously published data (Biggs et al., 2014). d and e Typical neuronal Ca²⁺ responses to 35mM K⁺ in a control slice or in one treated for 5d with 100µM gabapentin. AU = arbitrary units of Fluo-4 AM (Ca²⁺ indicator) fluorescence

**Figure 5.** Schematic representation of a dorsal root ganglion neuron and its terminal in the spinal dorsal horn to explain rapid and slowly developing effects of gabapentinoids. a. The α–subunits of HVA Ca²⁺ channels associate with α2δ–1 subunits and both are trafficked to the nerve terminal. α2δ–1 is responsible for trafficking channels to the plasma membrane thereby setting the abundance of functional HVA-Ca²⁺ channels and enabling their interaction with the neurotransmitter release machinery. Expressed channels are removed from the membrane by endocytosis into endosomes where they are targeted for recycling or degradation. With low,
physiological levels of \( \alpha 2\delta-1 \), the recycling of \( Ca^{2+} \) channels proceeds relatively slowly. 

b. Schematic representation of a primary afferent terminal when \( \alpha 2\delta-1 \) levels are increased. The terminal becomes much more “busy”, more \( Ca^{2+} \) channels may be targeted to the release machinery and neurotransmitter release is increased. The rate of channel turnover at the plasma membrane is assumed to be increased. 

c. In the presence of gabapentinoids, the rapid forward trafficking of HVA \( Ca^{2+} \) to the plasma membrane and release sites is decreased. Interruption of this rapid process may account for rapid acute effects of gabapentinoids in situations where \( \alpha 2\delta1 \) is upregulated. 

d. Diagram to illustrate the slowly developing actions of gabapentinoids seen in naïve animals. Impaired trafficking of \( \alpha 2\delta-1 \) subunits and \( \alpha- \) subunits gradually depletes them at release sites and neurotransmitter release declines over a period of many hours.

**Figure 6. Scheme to illustrate predicted changes in \( \alpha 2\delta-1 \) levels following nerve injury.** In the days or weeks following injury, \( \alpha 2\delta \) levels are increased and gabapentinoids act rapidly. Although allodynia may persist indefinitely after injury, \( \alpha 2\delta-1 \) levels may return to control levels, this would predict slowly developing effects of gabapentinoids that may parallel the clinical situation.

**Figure 7. Diagram to Illustrate an Operant Model for Pain Assessment in Rodents.** Normal rats will avoid an open, well-lit environment to avoid exposure to potential predators. If a darkened environment with a warm floor is provided, nerve injured rats will risk venturing into the open, well-lit environment to avoid thermal hyperalgesia they would experience in the dark. In other words, the animals have to decide whether they would prefer exposure to potential predators to avoid thermal hyperalgesia.
References


Gabapentin

Pregabalin

GABA
a) Voltage gated Calcium Channels In DRG cell body

b) Long-term Effect of PGB

c) Effect of 200μM Mn²⁺

d) Current Recordings

DRG HVA I_Ca

sEPSC in Dorsal Horn Neurons

e) No Effect of 200μM Mn²⁺

f) Long-term Effect of PGB

g) Comparison DRG and Dorsal Horn sEPSC's

Cumulative Fraction

http://mc.manuscriptcentral.com/nro
a) GBP rapidly and reversibly attenuates mechanical allodynia

b) Effect of GBP on HVA \( I_{Ca} \)

c) Effect of GBP on Dorsal Horn Excitability

d) Control

e) 5d 100\mu M GBP
a. Normal drug free conditions

- Ca\textsuperscript{2+} channel α2δ-1 subunit
- Cell body in DRG
- Endosome
- Voltage gated Calcium Channels
- Slow net recycling of α2δ-1 associated channels between membrane and endosomes
- Neurotransmitter release machinery
- Neurotransmitter release

b. Nerve terminal after injury

- Ca\textsuperscript{2+} channel α2δ-1 subunit
- Voltage gated Calcium Channels
- Rapid net recycling of α2δ-1 associated channels between membrane and endosomes
- Neurotransmitter release increased


c. Rapid gabapentinoid effect at nerve terminal after injury

- Gabapentinoid entry via transporter
- Voltage gated Calcium Channels
- Ca\textsuperscript{2+} channel α2δ-1 subunit
- Endocytosis still active
- Primary Afferent Terminal in Dorsal Horn
- Neurotransmitter release decreased
- Forward trafficking of Ca\textsuperscript{2+} channels to release sites blocked


d. Slow gabapentinoid effect on control neuron

- Ca\textsuperscript{2+} channel α2δ-1 subunit
- Gabapentinoid
- Endosome
- Voltage gated Calcium Channels
- Slow net recycling of α2δ-1 associated channels between membrane and endosomes
- Neurotransmitter release slowly compromised as supply of α2δ-1 runs out