Published in final edited form as:
Eur J Pain. 2013 April ; 17(4): 505–513. doi:10.1002/j.1532-2149.2012.00221.x.

Spinal 5-HT3 receptors facilitate behavioral hypersensitivity induced by elevated calcium channel alpha-2-deltal-1 protein

Eric Y. Chang[1,*], **Xiaoguang Chen**[1,3,*], **Amandeep Sandhu**[1], **Chun-Ying Li**[1], and **Z. Davi Luo**[1,2]

[1]Department of Anesthesiology & Perioperative Care School of Medicine, University of California, Irvine, USA

[2]Department of Pharmacology, School of Medicine, University of California, Irvine, USA

[3]Department of Anesthesiology, The First Hospital of China Medical University, Shenyang, China

Abstract

Background—Peripheral nerve injury induces upregulation of the calcium channel alpha-2-delta-1 proteins in the dorsal root ganglia and dorsal spinal cord that correlates with neuropathic pain development. Similar behavioral hypersensitivity was also observed in injury-free transgenic mice (TG) over-expressing the alpha-2-delta-1 proteins in neuronal tissues. To investigate pathways regulating alpha-2-delta-1 protein-mediated behavioral hypersensitivity, we examined whether spinal serotonergic 5-HT3 receptors are involved similarly in the modulation of behavioral hypersensitivity induced by either peripheral nerve injury in a nerve injury model or neuronal alpha-2-delta-1 over-expression in the TG model.

Methods—The effects of blocking behavioral hypersensitivity in these two models by intrathecal or systemic injections of 5-HT3 receptor antagonist, ondansetron, were compared.

Results—Our data indicated that the TG mice displayed similar behavioral hypersensitivities to non-painful mechanical stimulation (tactile allodynia) and painful thermal stimulation (thermal hyperalgesia) as that observed in the nerve injury model. Interestingly, tactile allodynia and thermal hyperalgesia in both models can be blocked similarly by intrathecal, but not systemic, injection of ondansetron.

Conclusions—Our data suggest that spinal 5-HT3 receptors are likely play a role in alpha-2-delta-1-mediated behavioral hypersensitivities through a descending serotonergic facilitation.

Corresponding author: Z. David Luo, Department of Anesthesiology and Perioperative Care, School of Medicine, University of California Irvine Medical Center, Rm 227, Bldg 55, 101 The City Drive South, Orange, CA 92868, USA, Phone: (714) 456-7962, Fax: (714) 456-7903, zluo@uci.edu.
*Contributed equally to the study.

Conflicts of interest: None declared.

Author contributions: In addition to the following contributions from each author, all authors discussed the results, commented on the manuscript and approved for the submission.
Chang, Eric - contributed to conception, design of the study, data acquisition, analysis, and interpretation, drafting, editing the manuscript.
Chen, Xiaoguang - contributed to conception, design of the study, data acquisition, analysis, and interpretation.
Sandhu, Amandeep - contributed to study design, data acquisition, analysis, and interpretation.
Li, Chun-Ying - contributed to study design, data acquisition, analysis, and interpretation.
Luo, Z. David - contributed to conception, design, and overall supervision of the study. Also performed data analysis, interpretation, drafting, and revising the manuscript.

Introduction

The development of efficacious and safe medications for neuropathic pain management relies on our better understanding of neuropathic pain mechanisms. Data from previous studies have indicated that peripheral nerve injury causes upregulation of the calcium channel alpha-2-delta-1 subunit proteins ($Ca_v\alpha_2\delta_1$) in dorsal root ganglia and dorsal spinal cord that plays a critical role in neuropathic pain development (Boroujerdi et al., 2008; Li et al., 2004; Li et al., 2006; Luo et al., 2002; Luo et al., 2001; Newton et al., 2001; Nguyen et al., 2009). It has been reported that a descending serotonergic modulatory pathway facilitates spinal nerve injury induced behavioral hypersensitivity through 5-HT3 receptors in dorsal spinal cord (Bee and Dickenson, 2008; Suzuki et al., 2004; Zhuo and Gebhart, 1991). Interestingly, activation of descending 5-HT3 facilitatory drive is required for the efficacy of pregabalin, an anti-neuropathic pain drug that binds to $Ca_v\alpha_2\delta_1$ (Gee et al., 1996), in a neuropathic pain model (Bee and Dickenson, 2008), suggesting that injury-induced dysregulation of $Ca_v\alpha_2\delta_1$ may mediate neuropathic pain states through a 5-HT3 receptor dependent pathway. Since nerve injury also leads to dysregulation of a multitude of genes (Costigan et al., 2002; Kim et al., 2009; Valder et al., 2003; Wang et al., 2002), it is not clear if $Ca_v\alpha_2\delta_1$ upregulation is an essential and necessary factor in this neuropathic pain pathway. Therefore, we have generated a $Ca_v\alpha_2\delta_1$ overexpressing transgenic mouse line in which $Ca_v\alpha_2\delta_1$ gene overexpression in neuronal tissue alone is sufficient to drive dorsal horn neuron sensitization and behavioral hypersensitivities, mimicking that observed in spinal nerve injured neuropathic pain models (Li et al., 2006; Nguyen et al., 2009). To determine if $Ca_v\alpha_2\delta_1$-mediated behavioral hypersensitivities in the transgenic model involve a 5-HT3 receptor-dependent facilitatory pathway similar to that in the spinal nerve ligation neuropathic pain model (Bee and Dickenson, 2008; Suzuki et al., 2004), we compared the effects of a 5-HT3 receptor antagonist, ondansetron, in blocking behavioral hypersensitivities observed in the $Ca_v\alpha_2\delta_1$ transgenic mouse model and unilateral spinal nerve ligation injury (SNL) mouse model.

Methods

Transgenic mice

$Ca_v\alpha_2\delta_1$ over-expressing transgenic mice (TG) were generated as described previously (Li et al., 2006). These mice were fertile, had normal growth rate, grooming, social interactions and showed no signs of ataxia, motor function defects, tremor, seizure, or other abnormalities (Li et al., 2006). Only adult male TG mice and their wild type (WT) littermates were used for the experiments.

Neuropathic lesions

The unilateral spinal nerve ligation (SNL) surgery was performed as described (Kim and Chung, 1992). Briefly, under isoflurane anesthesia, the mouse left L4 spinal nerve (Rigaud et al., 2008) were exposed and a tight ligation with a silk suture was made between the DRG and the conjunction where spinal nerves form the sciatic nerve. In sham operations, the same procedure was performed except that L4 spinal nerve was not ligated.

All animal care and experimental procedures were performed based on protocols approved by the Institutional Animal Care Committee of the University of California Irvine.

Drug injection

Ondansetron was dissolved in sterile saline, and injected either intraperitoneally (300 μL/mouse) or intrathecally (5 μL/mouse) between lumbar regions 4–5 (Nguyen et al., 2009). In the case of repetitive drug injections into the same group of animals, at least a drug-free

period of one day was introduced after the previous drug effect, if any, had completely dissipated (which occurred within a 3 hr post injection period).

Behavioral test

Hindpaw sensitivities to mechanical and thermal stimuli were tested blindly as described before and post drug treatments (Li et al., 2006).

Mechanical sensitivity—After acclimation for 1 hr in a clearplastic cage with a wire mesh bottom, the mice were tested for 50% paw withdrawal thresholds (PWT) to von Frey filament (Stoelting, Wood Dale, IL) stimulation using a modified up-down method (Dixon, 1980). Briefly, a set of filaments (starting with one that has a buckling weight of 0.41 g) was perpendicularly applied to the hindpaw plantar surface, in a consecutive order, with a slightly bending force. Lifting of the hindpaw within 5 s was considered a positive response and led to the application of the next weaker filament. Absence of a paw lifting after 5 s was considered a negative response and led to the use of the next filament with increasing weight. The scores of six measurements, starting from the one prior to the first change in response, were used to calculate the 50% paw withdrawal thresholds as described (Li et al., 2004; Luo et al., 2002; Luo et al., 2001). In the case that four consecutive positive or three consecutive negative responses had occurred, a score of 0.01 g or 3 g, respectively, was assigned. Paw withdrawal thresholds from each hindpaw were recorded separately. Data from the injured and uninjured side in the sham or SNL groups or averaged from both hindpaws of the injury-free $Ca_v\alpha_2\delta_1$ TG and WT mice were used for comparing behavioral sensitivities between the injury and nonjury side in SNL or sham mice or between injury-free TG and WT mice, respectively, before or after systemic and intrathecal drug treatments.

Hot box test—Mouse hindpaw withdrawal latencies (PWL) to thermal stimuli were examined in a modified Hargreaves-type thermal testing device (Hargreaves et al., 1988). Briefly, after acclimation for at least 30 min in individual boxes on the glass surface of the hot box maintained at 30 °C, mouse planter surface of the hindpaw was aligned to a radiant light source underneath the glass surface. A timer was activated when the light source was turned on, and turned off when paw withdrawal from the light source occurred or at 20 s of light stimulation that turned off the light bulb. Paw withdrawal latencies from each hindpaw were recorded separately. Data from the injured and uninjured side in the sham or SNL groups or averaged from both hindpaws of the injury-free $Ca_v\alpha_2\delta_1$ TG and WT mice were used for comparing behavioral sensitivities between the injury and nonjury side in SNL or sham mice or between injury-free TG and WT mice, respectively, before or after systemic and intrathecal drug treatments.

Statistic analysis

Significant changes were determined by the two-way ANOVA followed by Bonferroni post-test analysis. A p value < 0.05 was considered statistically significant.

Results

Intrathecal, but not intraperitoneal, treatment with 5-HT3 receptor antagonist reversed tactile allodynia in SNL and TG mice

As shown in Fig. 1, over-expression of $Ca_v\alpha_2\delta_1$ in neuronal cells of the TG mice induced a similar level of reduction in paw withdrawal thresholds to innocuous mechanical stimulation (tactile allodynia) as that in the injury side of the unilateral SNL injury model (before drug treatments), suggesting that elevated level of $Ca_v\alpha_2\delta_1$ that occurs in both models may play a similar role in mediating tactile allodynia. Since a spinal 5-HT3 receptor mediated pathway is involved in tactile allodynia processing in the SNL model (Dogrul et al., 2009), we

studied if this pathway is modulated by increased spinal $Ca_v\alpha_2\delta_1$ in the SNL model by comparing whether intrathecal treatment with ondansetron can affect behavioral hypersensitivities similarly in both the SNL and $Ca_v\alpha_2\delta_1$ TG mouse models. As shown in Fig. 1A, intrathecal ondansetron could block $Ca_v\alpha_2\delta_1$ induced tactile allodynia in a dose-dependent and reversible manner in the TG mice compared with that in the age- and sex-matched WT littermates. A complete reversal of allodynia was achieved by 10 μg/mouse, which is consistent with the reported effective dose in blocking central sensitization (Rahman et al., 2004; Suzuki et al., 2004) and behavioral hypersensitivity (Green et al., 2000) in animal models. The anti-allodynic effects of 10 μg/mouse intrathecal ondansetron were fast-in onset, peaked at 30 min post injection, and lasted for approximately two hrs. In contrast, a similar intrathecal injection with 0.1 μg/mouse ondansetron or sterile saline was without effect, and the anti-allodynic effects of 1.0 μg/mouse ondansetron was between that of 0.1 μg/mouse and 10 μg/mouse. As indicated in Fig. 1B, similar dose-and time-dependent tactile allodynia reversals by intrathecal injection of ondansetron were observed in the SNL model. Baseline behavioral sensitivity in the WT mice and non-injury side of the SNL mice was not affected by the highest dose of ondansetron, nor saline. As shown in Fig. 2, intraperitoneal injections with the effective intrathecal dose, 10 μg/mouse, of ondansetron in the $Ca_v\alpha_2\delta_1$ TG (Fig. 2A) and SNL (Fig. 2B) mice failed to reverse tactile allodynia significantly in these models. Similar negative effects in allodynia reversal were observed in $Ca_v\alpha_2\delta_1$ TG mice injected with a three-times higher intraperitoneal ondansetron dose (30 μg/mouse, data not shown), suggesting that the negative effect was not likely due to insufficient systemic dosing.

Intrathecal, but not intraperitoneal, treatment with 5-HT3 receptor antagonist reversed thermal hyperalgesia in SNL and TG mice

Over-expression of neuronal $Ca_v\alpha_2\delta_1$ in the TG mice also induced a reduced hindpaw withdrawal latency to thermal stimuli (thermal hyperalgesia) (Fig. 3A) similar to that in the injury side of the SNL, but not sham, injury model (Fig. 3B), suggesting that elevated level of $Ca_v\alpha_2\delta_1$ that occurs in both models may play a critical role in mediating thermal hyperalgesia. To determine whether a similar spinal 5-HT3 receptor mediated pathway is also involved in thermal hyperalgesia processing in these models, we examined if treatment with ondansetron could reverse thermal hyperalgesia similarly in both models. As shown in Fig. 3A, intrathecal ondansetron at the dose (10 μg/mouse) that was effective in reversing tactile allodynia, could also reverse thermal hyperalgesia in the TG mice without affecting the baseline sensitivity in age- and sex-matched WT littermates. Similar intrathecal injection with sterile saline was without effects (data not shown). As indicated in Fig. 3B, a similar intrathecal ondansetron treatment in the SNL model led to a similar thermal hyperalgesia reversal without affecting the baseline behavioral sensitivity in the sham control or non-injury side of SNL mice. In contrast, intraperitoneal injections with the same dose of ondansetron in the $Ca_v\alpha_2\delta_1$ TG (Fig. 4A) or the SNL (Fig. 4B) mice failed to significantly reverse thermal hyperalgesia.

Discussion

While spinal cord 5-HT3 receptors play a critical role in facilitating tactile allodynia in the SNL model (Bee and Dickenson, 2008; Dogrul et al., 2009; Leong et al., 2011; Suzuki et al., 2004), the detail mechanism underlying this pathway in neuropathic pain processing is not clear. It has been shown that pregabalin, a drug that binds to the $Ca_v\alpha_2\delta_1$ proteins, can block the facilitation mediated by spinal 5-HT3 receptors (Bee and Dickenson, 2008), suggesting that $Ca_v\alpha_2\delta_1$ is involved in the regulation of this facilitation pathway. However, this has not been directly proven. Since nerve injury can also induce dysregulation of other genes (Kim et al., 2009; Valder et al., 2003; Wang et al., 2002), the involvement of $Ca_v\alpha_2\delta_1$ in this

facilitation pathway could be indirect. We tested this hypothesis by comparing data from the SNL neuropathic pain model with that from injury-free $Ca_v\alpha_2\delta_1$ overexpressing TG mice, which also display similar behavioral hypersensitivity as the SNL model but without any influence from other injury factors (Li et al., 2006). Our data have indicated that intrathecal, but not systemic, administration of the 5-HT3 receptor antagonist - ondansetron results in a similar dose- and time-dependent reversal of behavioral hypersensitivities in both models, supporting that $Ca_v\alpha_2\delta_1$ protein, which is a common factor elevated in both models, plays a critical role in 5-HT3 receptor mediated facilitation in neuropathic pain processing at the spinal cord level.

In contrast to other serotonin receptor subtypes that are G-protein-coupled, 5-HT3 receptors are excitatory ionotropic receptors that enhance neurotransmitter release from spinal dorsal horn neurons due to activation of the descending facilitatory serotenergic pathway from the rostral ventromedial medulla (RVM) (Farber et al., 2004; Suzuki et al., 2002). 5-HT3 receptors have been found on terminals of glutamate-releasing myelinated primary afferent fibers, excitatory interneurons, and NK1 receptor expressing projection neurons in lamina I/III (Conte et al., 2005; Kidd et al., 1993; Zeitz et al., 2002). It has been a controversial issue regarding whether serotonergic descending fibers from brain stem are inhibitory or facilitatory in modulating pain processing at the spinal cord level. Originally, it has been proposed that the serotonergic descending modulatory pathway is inhibitory (Basbaum and Fields, 1984) until the discovery of facilitation activity of the serotonergic descending pathway in pain processing (Suzuki et al., 2002; Zhuo and Gebhart, 1991). Findings from Leong et al (2011) recently shed some light on this issue by demonstrating that peripheral nerve injury (SNL) leads to loss of serotonergic inhibitory tone, most likely due to death of inhibitory serotonergic neurons in the RVM. However, the remaining serotonergic descending pathway becomes facilitatory. They concluded that injury-induced loss of brain stem inhibitory neurons shifts the balance of descending serotonergic modulation from inhibitory to facilitatory. Our data are consistent with their conclusions.

Since 5-HT3 receptors are expressed in both central (Yakel and Jackson, 1988) and peripheral (Fozard, 1984) neurons, the lack of inhibitory effects after systemic ondansetron administration suggests that 5-HT3 receptor-mediated facilitation is mainly at the spinal, but less likely at the supraspinal and peripheral levels. In addition, our data indicate that intrathecal ondansetron treatments in the WT and sham SNL mice do not affect significantly behavioral sensitivities to mechanical and thermal stimuli in these animals. This is consistent with findings from spinal cord recordings in normal animals (Green et al., 2000; Rahman et al., 2004), supporting a state-dependency of the drug action. Together, these data suggest that there is minimal 5-HT3 receptor mediated facilitation at the spinal cord level in the absence of injury-induced plasticity changes as that seen in the SNL model, or of elevated $Ca_v\alpha_2\delta_1$ protein level as that seen in the TG mouse model, respectively. However, under certain pain-inducing conditions, such as peripheral nerve injury and/or elevated $Ca_v\alpha_2\delta_1$ levels in the sensory pathway, 5-HT3 receptor-mediated facilitation at the spinal cord level is activated.

It is not clear why this 5-HT3 receptor-mediated facilitatory pathway was not detectable in the rat SNL model treated with up to 100 μg/rat of intrathecal ondansetron in a recent study (Peters et al., 2010). Overall species differences may not be the major factor since ondansetron sensitive, 5-HT3 receptor mediated facilitation at the spinal cord level of the same rat model was detectable with a lower dose of intrathecal ondansetron in another study (Dogrul et al., 2009). In this study with mouse models, we observed complete reversal of tactile allodynia and thermal hyperalgesia with intrathecal ondansetron at a dose higher than that used in the latter study, but similar to the maximal dose used in the former study. The onset time of reversal in behavioral hypersensitivity in our study is faster than that in the rat

SNL model (Dogrul et al., 2009). It is possible that, in addition to other potential factors, such as sources and/or strains of experimental animals, local environment, variations in surgical procedures, and behavioral testing, that may contribute to the differences (Chesler et al., 2002), species differences in pharmacokinetics and pharmacodynamics may also contribute to this discrepancy.

Neuropathic pain models that involve altered influence of descending serotonergic activity on spinal 5-HT3 receptors may mimic conditions in human neuropathic pain states that respond to 5-HT3 receptor blockers. However, a possible anti-nociceptive role of 5-HT3 receptor blockers has not been consistently supported by clinical data. Compared to placebo, a bolus intravenous injection of ondansetron has been shown to alleviate the overall pain experience by neuropathic pain patients (McCleane et al., 2003). Another study in postoperative pain after laminectomy did not show any difference in the amount of analgesic use for break-through pain control between patients that used intravenous ondansetron or saline control (Derbent et al., 2005). In a randomized, double-blind, placebo-controlled study of 15 patients with neuropathic pain associated with peripheral neuropathy, intraveous ondansetron had no influence on the intensity of brush-evoked or spontaneous ongoing pain in these patients when compared with saline control (Tuveson et al., 2011). The inconsistency in the effects of ondansetron in pain relief may result from differences in administration routes of ondanstetron, as supported by our findings that 5-HT3 receptor mediated facilitation at the spinal level can be reversed by intrathecal, but not systemic, administration of ondansetron.

How does injury-induced $Ca_v\alpha_2\delta_1$ proteins at the spinal cord level contribute to 5-HT3 receptor-mediated facilitation? Previous findings indicate that peripheral nerve injury leads to an increased $Ca_v\alpha_2\delta_1$ expression in dorsal root ganglion (DRG) sensory neurons that results in elevated $Ca_v\alpha_2\delta_1$ proteins at the pre-synaptic sensory fiber terminals in dorsal spinal cord (Bauer et al., 2009; Li et al., 2004). This neuroplasticity can cause spinal neuron sensitization and behavioral hypersensitivity through enhanced pre-synaptic excitatory neurotransmitter release at the spinal level (Zhou et al., 2012), similar to that mediated by neuronal $Ca_v\alpha_2\delta_1$ over-expression (Nguyen et al., 2009; Zhou et al., 2012). Since 5-HT3 receptor mediated facilitatory input into dorsal spinal cord is required for the anti-neuropathic pain effects of gabapentin to occur in the SNL model, it has been proposed that spinal interactions of 5-HT3 and $Ca_v\alpha_2\delta_1$ are permissive for the anti-neuropathic pain actions of gabapentin (Rahman et al., 2009; Suzuki et al., 2005).

Even though pharmacology data may not allow the exclusion of possible involvement from other pathways since gabapentin may have effects independent from its binding to the $Ca_v\alpha_2\delta_1$ protein (Taylor, 2009), our data however have demonstrated that 5-HT3 receptor-mediated spinal facilitation also mediates similar behavioral hypersensitivities in the $Ca_v\alpha_2\delta_1$ TG mice, which also display similar spinal pre-synaptic hyperexcitability as the SNL mice (Nguyen et al., 2009; Zhou et al., 2012). Therefore, our data confirm a critical involvement of spinal $Ca_v\alpha_2\delta_1$ in the 5-HT3 receptor-mediated serotonergic circuits regulating central sensitization, behavioral hypersensitivities and gabapentin efficacy in neuropathic pain states. It seems that in the absence of injury/inflammatory insults that could trigger spinal 5-HT3 receptor activation (as in the case of injury-free $Ca_v\alpha_2\delta_1$ TG mice), increased pre-synaptic excitatory input by elevated $Ca_v\alpha_2\delta_1$ levels is sufficient to activate this spinal-supraspinal descending serotonergic facilitatory pathway, presumably through activation of spinal post-synaptic NK1 receptor expressing neurons (Suzuki et al., 2005). This in turn activates spinal 5-HT3 receptors that facilitates central sensitization and behavioral hypersensitivities. As summarized in Fig. 5, these spinal-supraspinal serotonergic circuits are likely turned on constantly in the SNL model after the onset of $Ca_v\alpha_2\delta_1$ upregulation that requires days to complete (Li et al., 2004; Luo et al., 2001). Eeven though

SNL also leads to substantial down-regulation of 5-HT3 receptor in injured DRG (Kim et al., 2009; Valder et al., 2003; Wang et al., 2002) but no change in spinal cord (Wang et al., 2002), this neuroplasticity may result in reduced 5-HT3 receptor levels in affected afferent central terminals in spinal dorsal horn. Thus, $Ca_v\alpha_2\delta_1$ upregulation post peripheral nerve injury may drive central sensitization and neuropathic pain states in neuropathy models mainly through activation of 5-HT3 receptors on excitatory interneurons and projection neurons by this serotenergic descending facilitation pathway.

Acknowledgments

Funding Sources: Supported in part by NIH grants: NS064341 and DE021847 (Z.D. Luo). E. Y. Chang is supported by a NIH K12 fellowship (K12HD001097) from the Eunice Kennedy Shriver National Institute Of Child Health & Human Development.

References

Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annu Rev Neurosci. 1984; 7:309–338. [PubMed: 6143527]

Bauer CS, Nieto-Rostro M, Rahman W, Tran-Van-Minh A, Ferron L, Douglas L, Kadurin I, Sri Ranjan Y, Fernandez-Alacid L, Millar NS, et al. The increased trafficking of the calcium channel subunit alpha2delta-1 to presynaptic terminals in neuropathic pain is inhibited by the alpha2delta ligand pregabalin. J Neurosci. 2009; 29:4076–4088. [PubMed: 19339603]

Bee LA, Dickenson AH. Descending facilitation from the brainstem determines behavioural and neuronal hypersensitivity following nerve injury and efficacy of pregabalin. Pain. 2008; 140:209–223. [PubMed: 18809257]

Boroujerdi A, Kim HK, Lyu YS, Kim DS, Figueroa KW, Chung JM, Luo ZD. Injury discharges regulate calcium channel alpha-2-delta-1 subunit upregulation in the dorsal horn that contributes to initiation of neuropathic pain. Pain. 2008; 139:358–366. [PubMed: 18571852]

Chesler EJ, Wilson SG, Lariviere WR, Rodriguez-Zas SL, Mogil JS. Identification and ranking of genetic and laboratory environment factors influencing a behavioral trait, thermal nociception, via computational analysis of a large data archive. Neurosci Biobehav Rev. 2002; 26:907–923. [PubMed: 12667496]

Conte D, Legg ED, McCourt AC, Silajdzic E, Nagy GG, Maxwell DJ. Transmitter content, origins and connections of axons in the spinal cord that possess the serotonin (5-hydroxytryptamine) 3 receptor. Neuroscience. 2005; 134:165–173. [PubMed: 15975728]

Costigan M, Befort K, Karchewski L, Griffin RS, D'Urso D, Allchorne A, Sitarski J, Mannion JW, Pratt RE, Woolf CJ. Replicate high-density rat genome oligonucleotide microarrays reveal hundreds of regulated genes in the dorsal root ganglion after peripheral nerve injury. BMC Neurosci. 2002; 3:16. Print 2002 Oct 2025. [PubMed: 12401135]

Derbent A, Uyar M, Demirag K, Uyer M, Kurtoglu E, Goktay A. Can antiemetics really relieve pain? Adv Ther. 2005; 22:307–312. [PubMed: 16418140]

Dixon WJ. Efficient analysis of experimental observations. Annual Review of Pharmacology and Toxicology. 1980; 20:441–462.

Dogrul A, Ossipov MH, Porreca F. Differential mediation of descending pain facilitation and inhibition by spinal 5HT-3 and 5HT-7 receptors. Brain Res. 2009; 1280:52–59. [PubMed: 19427839]

Farber L, Haus U, Spath M, Drechsler S. Physiology and pathophysiology of the 5-HT3 receptor. Scand J Rheumatol Suppl. 2004; 119:2–8. [PubMed: 15515404]

Fozard JR. Neuronal 5-HT receptors in the periphery. Neuropharmacology. 1984; 23:1473–1486. [PubMed: 6527747]

Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R, Woodruff GN. The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. J Biol Chem. 1996; 271:5768–5776. [PubMed: 8621444]

Green GM, Scarth J, Dickenson A. An excitatory role for 5-HT in spinal inflammatory nociceptive transmission; state-dependent actions via dorsal horn 5-HT(3) receptors in the anaesthetized rat. Pain. 2000; 89:81–88. [PubMed: 11113296]

Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain. 1988; 32:77–88. [PubMed: 3340425]

Kidd EJ, Laporte AM, Langlois X, Fattaccini CM, Doyen C, Lombard MC, Gozlan H, Hamon M. 5-HT3 receptors in the rat central nervous system are mainly located on nerve fibres and terminals. Brain Res. 1993; 612:289–298. [PubMed: 8330206]

Kim DS, Figueroa KW, Li KW, Boroujerdi A, Yolo T, Luo ZD. Profiling of dynamically changed gene expression in dorsal root ganglia post peripheral nerve injury and a critical role of injury-induced glial fibrillary acidic protein in maintenance of pain behaviors [corrected]. Pain. 2009; 143:114–122. [PubMed: 19307059]

Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain. 1992; 50:355–363. [PubMed: 1333581]

Leong ML, Gu M, Speltz-Paiz R, Stahura EI, Mottey N, Steer CJ, Wessendorf M. Neuronal loss in the rostral ventromedial medulla in a rat model of neuropathic pain. J Neurosci. 2011; 31:17028–17039. [PubMed: 22114272]

Li CY, Song YH, Higuera ES, Luo ZD. Spinal dorsal horn calcium channel alpha2delta-1 subunit upregulation contributes to peripheral nerve injury-induced tactile allodynia. J Neurosci. 2004; 24:8494–8499. [PubMed: 15456823]

Li CY, Zhang XL, Matthews EA, Li KW, Kurwa A, Boroujerdi A, Gross J, Gold MS, Dickenson AH, Feng G, Luo ZD. Calcium channel alpha2delta1 subunit mediates spinal hyperexcitability in pain modulation. Pain. 2006; 125:20–34. [PubMed: 16764990]

Luo ZD, Calcutt NA, Higuera ES, Valder CR, Song YH, Svensson CI, Myers RR. Injury type-specific calcium channel alpha 2 delta-1 subunit up-regulation in rat neuropathic pain models correlates with antiallodynic effects of gabapentin. J Pharmacol Exp Ther. 2002; 303:1199–1205. [PubMed: 12438544]

Luo ZD, Chaplan SR, Higuera ES, Sorkin LS, Stauderman KA, Williams ME, Yaksh TL. Upregulation of dorsal root ganglion (alpha)2(delta) calcium channel subunit and its correlation with allodynia in spinal nerve-injured rats. J Neurosci. 2001; 21:1868–1875. [PubMed: 11245671]

McCleane GJ, Suzuki R, Dickenson AH. Does a single intravenous injection of the 5HT3 receptor antagonist ondansetron have an analgesic effect in neuropathic pain? A double-blinded, placebo-controlled cross-over study. Anesth Analg. 2003; 97:1474–1478. [PubMed: 14570668]

Newton RA, Bingham S, Case PC, Sanger GJ, Lawson SN. Dorsal root ganglion neurons show increased expression of the calcium channel alpha2delta-1 subunit following partial sciatic nerve injury. Brain Res Mol Brain Res. 2001; 95:1–8. [PubMed: 11687271]

Nguyen D, Deng P, Matthews EA, Kim DS, Feng G, Dickenson AH, Xu ZC, Luo ZD. Enhanced pre-synaptic glutamate release in deep-dorsal horn contributes to calcium channel alpha-2-delta-1 protein-mediated spinal sensitization and behavioral hypersensitivity. Mol Pain. 2009; 5:6. [PubMed: 19216737]

Peters CM, Hayashida K, Ewan EE, Nakajima K, Obata H, Xu Q, Yaksh TL, Eisenach JC. Lack of analgesic efficacy of spinal ondansetron on thermal and mechanical hypersensitivity following spinal nerve ligation in the rat. Brain Res. 2010; 1352:83–93. [PubMed: 20637741]

Rahman W, Bauer CS, Bannister K, Vonsy JL, Dolphin AC, Dickenson AH. Descending serotonergic facilitation and the antinociceptive effects of pregabalin in a rat model of osteoarthritic pain. Mol Pain. 2009; 5:45. [PubMed: 19664204]

Rahman W, Suzuki R, Rygh LJ, Dickenson AH. Descending serotonergic facilitation mediated through rat spinal 5HT3 receptors is unaltered following carrageenan inflammation. Neurosci Lett. 2004; 361:229–231. [PubMed: 15135935]

Rigaud M, Gemes G, Barabas ME, Chernoff DI, Abram SE, Stucky CL, Hogan QH. Species and strain differences in rodent sciatic nerve anatomy: implications for studies of neuropathic pain. Pain. 2008; 136:188–201. [PubMed: 18316160]

Suzuki R, Morcuende S, Webber M, Hunt SP, Dickenson AH. Superficial NK1-expressing neurons control spinal excitability through activation of descending pathways. Nat Neurosci. 2002; 5:1319–1326. [PubMed: 12402039]

Suzuki R, Rahman W, Hunt SP, Dickenson AH. Descending facilitatory control of mechanically evoked responses is enhanced in deep dorsal horn neurones following peripheral nerve injury. Brain Res. 2004; 1019:68–76. [PubMed: 15306240]

Suzuki R, Rahman W, Rygh LJ, Webber M, Hunt SP, Dickenson AH. Spinal-supraspinal serotonergic circuits regulating neuropathic pain and its treatment with gabapentin. Pain. 2005; 117:292–303. [PubMed: 16150546]

Taylor CP. Mechanisms of analgesia by gabapentin and pregabalin--calcium channel alpha2-delta [Cavalpha2-delta] ligands. Pain. 2009; 142:13–16. [PubMed: 19128880]

Tuveson B, Leffler AS, Hansson P. Ondansetron, a 5HT3-antagonist, does not alter dynamic mechanical allodynia or spontaneous ongoing pain in peripheral neuropathy. Clin J Pain. 2011; 27:323–329. [PubMed: 21178594]

Valder CR, Liu JJ, Song YH, Luo ZD. Coupling gene chip analyses and rat genetic variances in identifying potential target genes that may contribute to neuropathic allodynia development. J Neurochem. 2003; 87:560–573. [PubMed: 14535940]

Wang H, Sun H, Della Penna K, Benz RJ, Xu J, Gerhold DL, Holder DJ, Koblan KS. Chronic neuropathic pain is accompanied by global changes in gene expression and shares pathobiology with neurodegenerative diseases. Neuroscience. 2002; 114:529–546. [PubMed: 12220557]

Yakel JL, Jackson MB. 5-HT3 receptors mediate rapid responses in cultured hippocampus and a clonal cell line. Neuron. 1988; 1:615–621. [PubMed: 3272181]

Zeitz KP, Guy N, Malmberg AB, Dirajlal S, Martin WJ, Sun L, Bonhaus DW, Stucky CL, Julius D, Basbaum AI. The 5-HT3 subtype of serotonin receptor contributes to nociceptive processing via a novel subset of myelinated and unmyelinated nociceptors. J Neurosci. 2002; 22:1010–1019. [PubMed: 11826129]

Zhou, C.; Chen, X.; Li, K-W.; Luo, Z. Electrophysiological characterization of dorsal horn neuron sensitization by elevated calcium channel alpha-2-delta-1 subunit proteins. 2012. Submitted

Zhuo M, Gebhart GF. Spinal serotonin receptors mediate descending facilitation of a nociceptive reflex from the nuclei reticularis gigantocellularis and gigantocellularis pars alpha in the rat. Brain Res. 1991; 550:35–48. [PubMed: 1888999]

Bulleted Statements

What's already known about this topic?

- It is known that either peripheral nerve injury or elevated calcium channel alpha-2-deltal-1 protein expression leads to behavioral hypersensitivity, and descending activation of 5-HT3 receptors in spinal cord facilitates injury-induced pain states.

What does this study add?

- Data from this study added that elevated alpha-2-deltal-1 protein is a critical contributor to spinal 5-HT3 receptor-facilitated pain states post peripheral nerve injury.

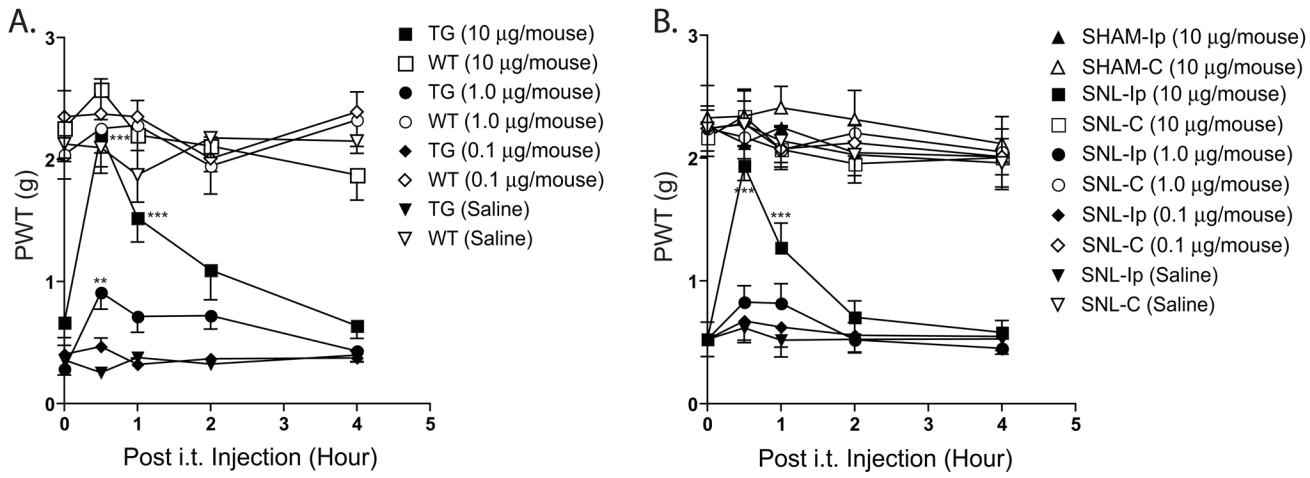

Figure 1. Intrathecal administration of 5-HT3 receptor antagonist ondansetron reversed tactile allodynia similarly in $Ca_v\alpha_2\delta_1$ TG and SNL mice in a dose-dependent manner

Hindpaw withdrawal thresholds to von Frey filament stimulation were examined before and after intrathecal ondansetron treatments in injury-free $Ca_v\alpha_2\delta_1$ TG, WT mice, and SNL or sham mice at least one-week post injury. **A**. intrathecal treatments with saline or ondansetron doses as indicated in WT and $Ca_v\alpha_2\delta_1$ TG mice. **B.** intrathecal treatments with saline or ondansetron doses as indicated in sham and SNL mice. Ip - ipsilateral to the injury; C – contralateral to the injury. Data presented are the Means ± SEM from at least eight mice in each group. ** $P < 0.01$, *** $P < 0.001$ compared with the pre-treatment level by two-way ANOVA with Bonferroni post-test.

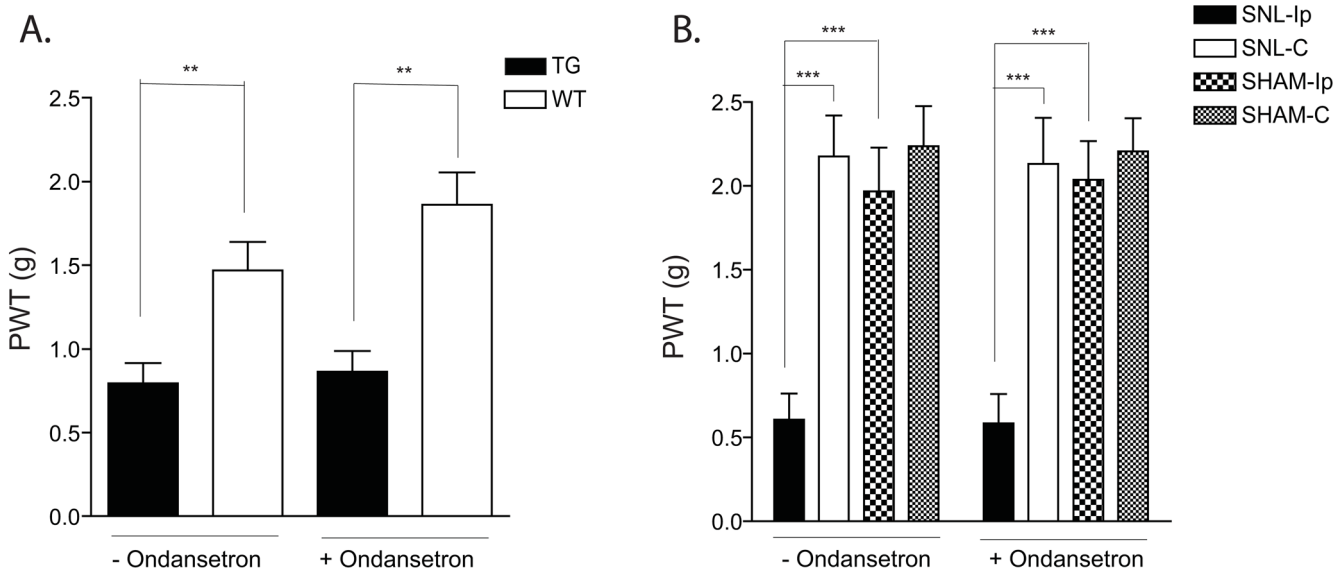

Figure 2. Systemic administration of 5-HT3 receptor antagonist ondansetron failed to reverse tactile allodynia in $Ca_v\alpha_2\delta_1$ TG and SNL mice

Hindpaw withdrawal thresholds to von Frey filament stimulation were examined before and one hour after intraperitoneal ondansetron treatments in injury-free $Ca_v\alpha_2\delta_1$ TG, WT mice, and SNL or sham mice at least one-week post injury. **A**. intraperitoneal treatments with ondansetron (10 μg/mouse) in WT and $Ca_v\alpha_2\delta_1$ TG mice. **B.** intraperitoneal treatments with ondansetron (10 μg/mouse) in sham and SNL mice. Ip - ipsilateral to the injury; C – contralateral to the injury. Data presented are the Means ± SEM from at least seven mice in each group. ** $P < 0.01$, *** $P < 0.001$ by two-way ANOVA with Bonferroni post-test.

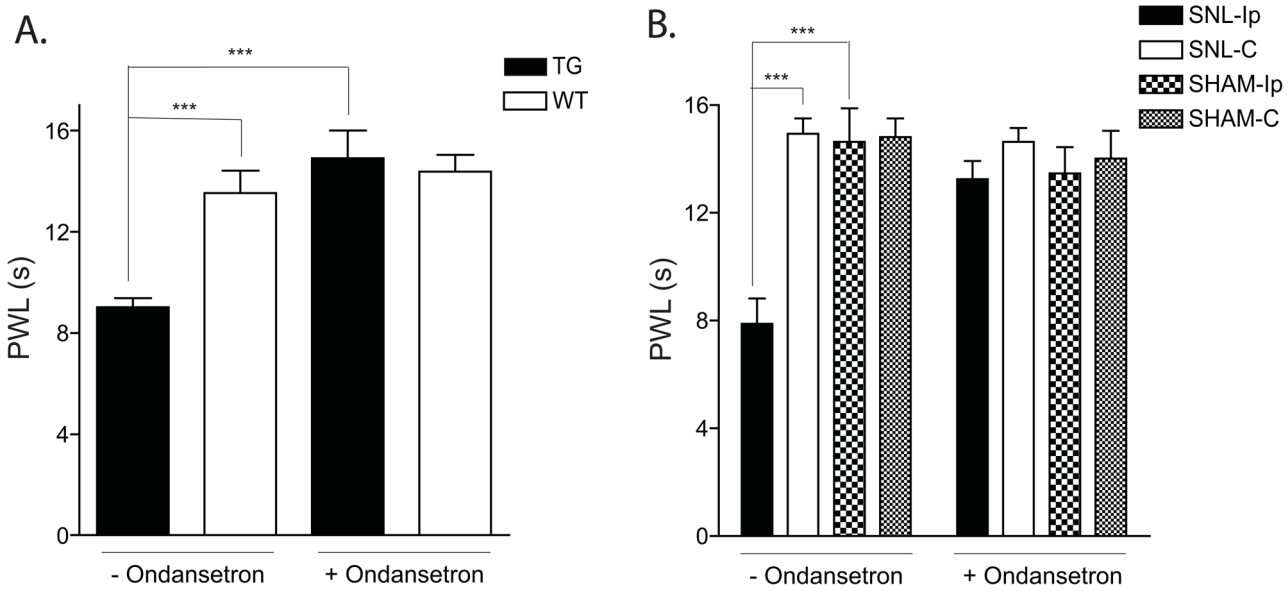

Figure 3. Intrathecal administration of 5-HT3 receptor antagonist ondansetron reversed thermal hyperalgesia similarly in $Ca_v\alpha_2\delta_1$ TG and SNL mice

Hindpaw withdrawal latency to thermal stimulation was examined before and 30 min after intrathecal ondansetron treatments in injury-free $Ca_v\alpha_2\delta_1$ TG, WT mice, and SNL or sham mice at least one-week post injury. **A**. intrathecal treatments with (10 μg/mouse) or without ondansetron in WT and $Ca_v\alpha_2\delta_1$ TG mice. **B.** intrathecal treatments with (10 μg/mouse) or without ondansetron in sham and SNL mice. Ip - ipsilateral to the injury; C – contralateral to the injury. Data presented are the Means ± SEM from at least eight mice in each group. *** $P < 0.001$ by two-way ANOVA with Bonferroni post-test.

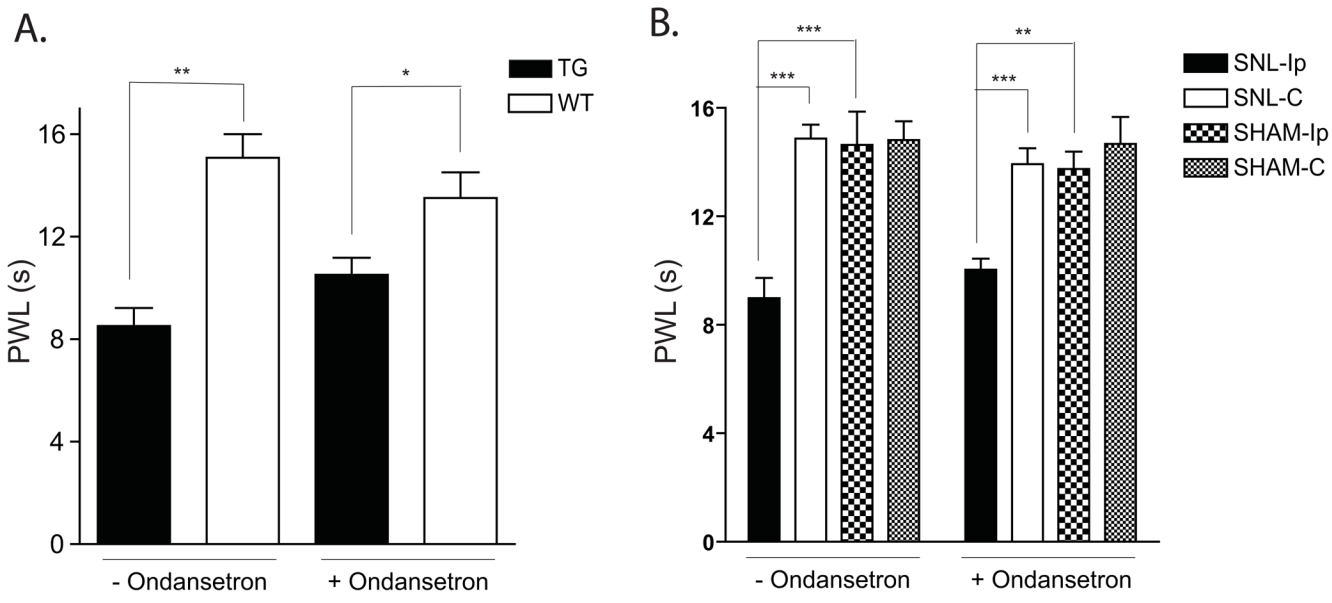

Figure 4. Systemic administration of 5-HT3 receptor antagonist ondansetron failed to reverse thermal hyperalgesia in $Ca_v\alpha_2\delta_1$ TG and SNL mice

Hindpaw withdrawal latency to thermal stimulation was examined before and one hour after intraperitoneal ondansetron treatments in injury-free $Ca_v\alpha_2\delta_1$ TG, WT mice, and SNL or sham mice at least one-week post injury. **A**. intraperitoneal treatments with (10 μg/mouse) or without ondansetron in WT and $Ca_v\alpha_2\delta_1$ TG mice. **B.** intraperitoneal treatments with (10 μg/mouse) or without ondansetron in sham and SNL mice. Ip - ipsilateral to the injury; C – contralateral to the injury. Data presented are the Means ± SEM from at least seven mice in each group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by two-way ANOVA with Bonferroni post-test.

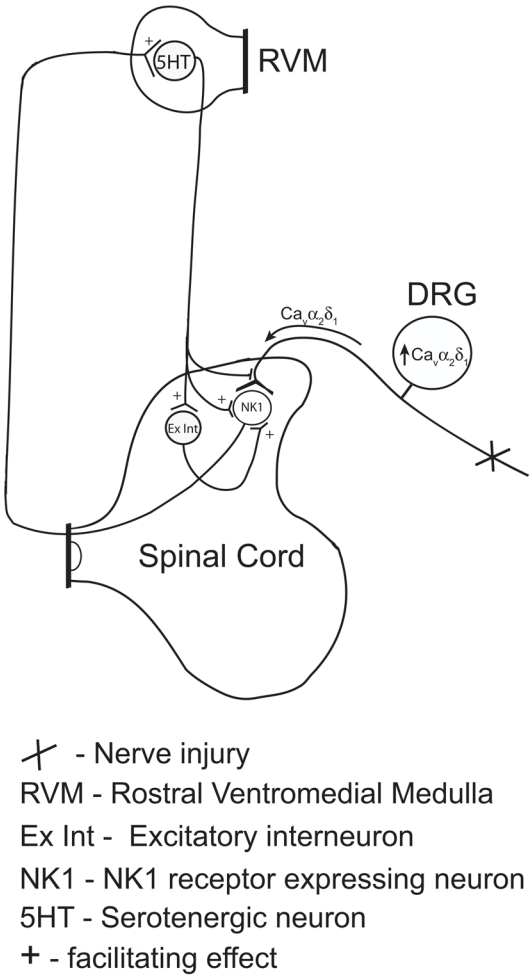

Fig. 5. Schematic illustration of the proposed mechanism

Peripheral nerve injury induced $Ca_v\alpha_2\delta_1$ upregulation in DRG leads to increased $Ca_v\alpha_2\delta_1$ translocation to the dorsal spinal cord pre-synaptic terminals through the central axons of sensory neurons. This results in increased pre-synaptic excitatory input to activate spinal post-synaptic NK1 receptor expressing neurons (Suzuki et al., 2005), leading to activation of spinal-supraspinal descending serotonergic facilitatory pathway located in RVM. This in turn can activate spinal 5-HT3 receptors on primary afferent fiber terminals, excitatory interneurons, and NK1 receptor expressing projection neurons (Conte et al., 2005; Kidd et al., 1993; Zeitz et al., 2002). Since spinal nerve injury also leads to substantial 5-HT3 receptor down-regulation in injured DRG (Kim et al., 2009; Valder et al., 2003; Wang et al., 2002) that may result in reduced levels of 5-HT3 receptors and facilitatory effects in affected afferent central terminals. Thus, this facilitatory pathway may lead to central sensitization and behavioral hypersensitivity mainly through activation of spinal 5-HT3 receptors on excitatory interneurons, and projection neurons.