INVITED REVIEW

The role of spinal cord extrasynaptic α5GABA<sub>A</sub> receptors in chronic pain

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Abstract
Chronic pain is an incapacitating condition that affects a large population worldwide. Until now, there is no drug treatment to relieve it. The impairment of GABAergic inhibition mediated by GABA<sub>A</sub> receptors (GABA<sub>A</sub>R) is considered a relevant factor in mediating chronic pain. Even though both synaptic and extrasynaptic GABA<sub>A</sub> inhibition are present in neurons that process nociceptive information, the latter is not considered relevant as a target for the development of pain treatments. In particular, the extrasynaptic α<sub>5</sub>GABA<sub>A</sub>Rs are expressed in laminae I-II of the spinal cord neurons, sensory neurons, and motoneurons. In this review, we discuss evidence showing that blockade of the extrasynaptic α<sub>5</sub>GABA<sub>A</sub>Rs reduces mechanical allodynia in various models of chronic pain and restores the associated loss of rate-dependent depression of the Hoffmann reflex. Furthermore, in healthy animals, extrasynaptic α<sub>5</sub>GABA<sub>A</sub>R blockade induces both allodynia and hyperalgesia. These results indicate that this receptor may have an antinociceptive and pronociceptive role in healthy and chronic pain-affected animals, respectively. We propose a hypothesis to explain the relevant role of the extrasynaptic α<sub>5</sub>GABA<sub>A</sub>Rs in the processing of nociceptive information. The data discussed here strongly suggest that this receptor could be a valid pharmacological target to treat chronic pain states.

KEYWORDS
extrasynaptic GABA<sub>A</sub> receptors, GABA<sub>A</sub> and GABA<sub>B</sub> receptors, Hoffmann reflex, Pain
1 | INTRODUCTION

The circuit processing nociceptive information located mainly in laminae I–III of the spinal cord’s dorsal horn contains, among other cells, projection neurons, GABAAergic, glycineergic, and glutamatergic interneurons, Aβ, Aδ, and C primary afferent fibers, as well as descending afferent fiber terminals (Todd, 2010). Under normal conditions, projection neurons are activated mainly by nociceptive primary afferent fibers generating a withdrawal reaction from the noxious stimulus. The excitability of projection neurons is regulated by a vast repertoire of channels and receptors, including the GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs). Most GABA<sub>A</sub>Rs mediate synaptic communication and are located in the underlying postsynaptic density. These receptors can be activated by GABA release from presynaptic vesicles, increasing the membrane permeability to chloride and bicarbonate ions by a vast repertoire of channels and receptors, including the GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs). Most GABA<sub>A</sub>Rs mediate synaptic communication and are located in the underlying postsynaptic density. These receptors can be activated by GABA release from presynaptic vesicles, increasing the membrane permeability to chloride and bicarbonate ions for brief periods (<100 ms), producing inhibitory (IPSC) or excitatory (EPSC) postsynaptic currents in mature and immature and sensory neurons, respectively. Though a subpopulation of GABA<sub>A</sub>Rs is present in somatic, dendritic, and axonal membranes, they are not in opposition to presynaptic terminals. Extrasynaptic receptors mediate an alternative form of inhibition that modulates the neurons’ excitability by a persistent increase in conductance and tonic shunt (Brickley et al., 1996; Farrant & Nusser, 2005; Kullmann et al., 2005). The transmitter release from nociceptive and non-nociceptive primary afferents is under presynaptic GABAAergic control through the axo-axonic synapses (Rudomin & Schmidt, 1999). According to the gate theory, this presynaptic inhibition regulates the excitatory input onto projection neurons preventing its activation (Melzack & Wall, 1965).

Based on its pharmacological properties, GABA<sub>A</sub>Rs can be divided into sensitive and nonsensitive to benzodiazepines. This feature is related to the subunit composition of the receptor because the action of these drugs is mediated by a specific binding site located in the α<sub>2,3,5</sub> and γ subunits. GABA<sub>A</sub>Rs containing α<sub>6</sub> or α<sub>β</sub> subunits do not bind benzodiazepines and are associated with the δ subunit (Rudolph & Knoflach, 2011; Zeilhofer et al., 2012). The side effects of benzodiazepines, such as sedation and addiction after long-term use, are considered important issues in developing new GABA<sub>A</sub>Rs subtype-selective compounds to overcome the limitations of classical benzodiazepines (Zeilhofer et al., 2012).

2 | GABA<sub>A</sub> RECEPTORS IN THE SPINAL CORD

Immunohistochemical, in situ hybridization, and functional studies (Alvarez et al., 1996; Boihlhalter et al., 1996; Ma et al., 1993) have demonstrated the expression and localization of α<sub>2,3,5</sub>GABA<sub>A</sub>Rs and δ subunit-containing GABA<sub>A</sub>Rs in the dorsal horn neurons. In particular, the α<sub>5</sub>GABA<sub>A</sub>R and those containing the δ subunit are expressed extrasynaptically in neurons of laminae I and II mediating a tonic current (Bonin et al., 2011; Perez-Sanchez et al., 2017; Takahashi et al., 2006; Todd, 2010). Likewise, primary afferent fibers also express synaptic α<sub>5</sub>GABA<sub>A</sub>Rs (Witschi et al., 2011) and extrasynaptic α<sub>5</sub>GABA<sub>A</sub>Rs that mediate a phasic and tonic depolarization, respectively (Bravo-Hernández et al., 2016; Hernández-Reyes et al., 2019; Lucas-Osma et al., 2018). Moreover, α<sub>5</sub>GABA<sub>A</sub>R mRNA and protein are present in sciatic nerve, dorsal root, and dorsal root ganglion (DRG) neurons (Bravo-Hernández et al., 2016; Loeza-Alcocer et al., 2013). α<sub>5</sub>GABA<sub>A</sub>Rs are found in primary afferent terminals co-localizing with peptidergic terminals in lamina IIo, non-peptidergic terminals in lamina Iii, and with myelinated terminals in lamina III (Paul et al., 2012). Besides, α<sub>5</sub>GABA<sub>A</sub>Rs have been found in glutamatergic and GABAAergic neurons of layers II–V of the spinal dorsal horn (Boihlhalter et al., 1996; Ma et al., 1993), motoneurons (Alvarez et al., 1996; Canto-Bustos et al., 2017), and ventral horn interneurons (Castro et al., 2011). There is evidence that the most profuse subunits along the laminae of the spinal cord are α<sub>5</sub>γ<sub>2</sub>β<sub>2,3</sub>δ subunits (Alvarez et al., 1996; Boihlhalter et al., 1996; Paul et al., 2012).

3 | PAMS AND NAMS TO TREAT CHRONIC PAIN

The GABAAergic inhibition, mediated by GABA<sub>A</sub>Rs, in the spinal cord is so relevant that its blockade with bicuculline, an antagonist of GABA<sub>A</sub>Rs, produces allodynia and hyperalgesia in healthy rodents (Roberts et al., 1986). Consequently, based on this fact, the development of chronic and neuropathic pain is considered a result of a loss of GABA<sub>A</sub> inhibition in the spinal cord (Bonin & De Konick, 2013; Munro et al., 2011; Zeilhofer et al., 2012). Many mechanisms have been described to explain the sensitization at the spinal dorsal horn produced by the loss of synaptic GABA<sub>A</sub> inhibition. One of these studies shows a substantial increase in polysynaptic input onto lamina II neurons in the presence of bicuculline (Baba et al., 2003). Another interesting study was performed by recording second-order lamina I neurons, which express neurokinin 1 receptors and receive sensory excitatory synaptic inputs exclusively from C and Aδ nociceptors. In the presence of GABA<sub>A</sub>R and GlyR antagonists, polysynaptic inputs appeared in response to Aβ fiber activation (Torsney & MacDermott, 2006). Similar anomalous synaptic inputs were recorded from neurons of the substantia gelatinosa in transverse spinal cord slices from animals with chronic pain (Baba et al., 1999). The presence of this polysynaptic excitatory input has been proposed to underlie allodynia activated in vivo after the intrathecal application of bicuculline or
strychnine (Zeilhofer et al., 2015). These studies indicate the relevance of GABA<sub>A</sub> synaptic inhibition as a pharmacological target for reversing pathological pain states. Taking into consideration, all these evidence by transgenic mice with mutated α subunits, a positive allosteric modulators (PAM) battery of α<sub>2,3,5</sub>GABA<sub>A</sub>R with less sedating benzodiazepines action has been developed to restore the loss of synaptic GABA<sub>A</sub> inhibition to relieve pain (Bonin & De Koninck, 2013; Knabl et al., 2008; Munro et al., 2011; Zeilhofer et al., 2012). In parallel, the use of point-mutated mice has shown that the ablation of the α<sub>2</sub>GABA<sub>A</sub>R has a strong antihyperalgesic effect with reduced side effects in different pain models (Ralvenius et al., 2011; Zeilhofer et al., 2012). In addition, the pharmacological targeting of this receptor could prevent the development of tolerance.

All these studies indicate that the different subtypes of benzodiazepine-sensitive GABA<sub>A</sub>Rs contribute to spinal antihyperalgesia with the rank order α<sub>2</sub> > α<sub>3</sub> > α<sub>5</sub> > α<sub>1</sub>. The antihyperalgesic action of benzodiazepines has been tested in three pain models, that is, zymosan A, chronic constriction injury and formalin test (Zeilhofer et al., 2015). In addition, it has been reported that α<sub>5</sub>GABA<sub>A</sub>Rs activation, via the administration of PAMs, does not induce analgesia in inflammatory and neuropathic pain models (Munro et al., 2011; Zeilhofer et al., 2012).

4 | PRONOCICEPTIVE ACTION OF α<sub>5</sub>GABA<sub>A</sub>R IN DIABETIC NEUROPATHY

Investigating the mechanisms underlying hyperalgesia developed by the formalin test in diabetic rats, it was found a paradoxical reduction of glutamate, the increase of GABA, and downregulation in the expression of the K<sup>+</sup>-Cl<sup>-</sup> co-transporter 2 (KCC2) in the laminae I-II of the dorsal horn spinal cord (Jolivalt et al., 2008; Morgado et al., 2008). Unexpectedly bicuculline reduced formalin-evoked flinching and alleviated tactile allodynia (Jolivalt et al., 2008), suggesting that reduced KCC2 expression in parallel with the increased GABA release contribute to the allodynia and hyperalgesia in diabetics (Jolivalt et al., 2008). The relevant role of GABA<sub>A</sub>Rs in diabetic neuropathy is highlighted by the evidence that in dorsal horn neurons, even when subjected to intense glycinergic inhibition (Yoshimura & Nishi, 1995), the blockade of GABA<sub>A</sub>Rs is enough to reduce allodynia and produce it in healthy animals, respectively. Interestingly, some of the molecular and cellular alterations produced in the spinal cord in diabetic neuropathy are quite similar to that reported in nerve injury models (CoulI et al., 2003). In this case, KCC2 is also downregulated by the binding of BDNF to TrK1 receptors producing the depolarization of the E<sub>Cl</sub> to a level high enough to generate action potentials when GABA<sub>A</sub>Rs are activated (CoulI et al., 2003, 2005). Therefore, KCC2 downregulation in diabetic neuropathy might also produce a switch from GABA<sub>A</sub> inhibition to the excitation of laminae I-II neurons involved in the nociceptive processing information as in peripheral nerve injury. Unexpectedly, but in accordance with Jolivalt et al. (2008), our results have shown that the blockade of the α<sub>5</sub>GABA<sub>A</sub>Rs is enough to reverse mechanical allodynia in streptozotocin (STZ)-induced diabetic rats (Hernández-Reyes et al., 2019), suggesting that this receptor contribute significantly to pain in diabetic neuropathy.

5 | THE ANTINOCICEPTIVE AND PRONOCICEPTIVE ROLE OF α<sub>5</sub>GABA<sub>A</sub>R IN CHRONIC INFLAMMATION AND NEUROPATHIC PAIN

It is well known that the tonic current mediated by extrasynaptic GABA<sub>A</sub>Rs has great relevance in physiological and pathological events both at the level of individual neurons and in neural networks (Farrant & Nusser, 2005). Even though the α<sub>5</sub>GABA<sub>A</sub>Rs are expressed in laminae I-II neurons of the network that processes nociception, their role in chronic and neuropathic pain is unknown. Only one study carried out in Gabra<sub>5</sub><sup>−/−</sup> mice shows that these receptors mediate a tonic current in those neurons. This work also concludes that α<sub>5</sub>GABA<sub>A</sub>Rs do not modulate acute nociception but play a pronociceptive role at the late inflammation stages (Perez-Sanchez et al., 2017). Interestingly, intrathecal administration of L-655,708 (α<sub>5</sub>GABA<sub>A</sub>R inverse agonist) decreases pain threshold in naïve rats (De la Luz-Cuellar et al., 2019; Franco-Enzástiga et al., 2021; Hernández-Reyes et al., 2019) and mice (Xue et al., 2017). In contrast, L-655,708 prevents and reverses long-lasting allodynia induced by formalin, Freund’s adjuvant, nerve injury (Bravo-Hernández et al., 2016; 2014), and reserpine-induced pain-type fibromyalgia (De la Luz-Cuellar et al., 2019). Indeed, a siRNA against α<sub>5</sub>GABA<sub>A</sub>R reduces reserpine-induced allodynia in female rats (De la Luz-Cuellar et al., 2019). Interestingly, as intrathecal L-655-708 (Hernández-Reyes et al., 2019), the siRNA against α<sub>5</sub>GABA<sub>A</sub>R induces tactile allodynia in naïve rats certifying that spinal α<sub>5</sub>GABA<sub>A</sub>Rs have an antinociceptive and pronociceptive role in healthy and chronic pain, respectively.

6 | ROLE OF α<sub>5</sub>GABA<sub>A</sub>R IN PRIMARY AFFERENTS, THE HOFFMANN REFLEX, AND CHRONIC INFLAMMATION

The α<sub>5</sub>GABA<sub>A</sub>Rs are expressed in primary afferent fibers (Bravo-Hernández et al., 2016; Hernández-Reyes et al., 2019; Loeza-Alcocer et al., 2013; Lucas-Osma et al., 2018),
ventral horn interneurons (Castro et al., 2011), and moto-neurons (Canto-Bustos et al., 2017) where they are activated by the endogenous GABA reducing its excitability tonically. It is well known that the presynaptic inhibition of low threshold primary afferents, associated with depolarization (PAD), is mediated by the activation of synaptic α5GABAARs (Witschi et al., 2011) and depresses the monosynaptic reflex (MSR) (Rudomin & Schmidt, 1999). Interestingly, L-655,708 facilitated the MSR without affecting the PAD, suggesting that besides synaptic α5GABAARs also extrasynaptic α5GABAARs are involved in motor control (Canto-Bustos et al., 2017; Loeza-Alcocer et al., 2013). The role of α5GABAARs in motor control has been evidenced by the Hoffmann reflex (HR). The property of HR, known as rate-dependent depression (RDD), has been proposed as a biomarker to detect the presence of neuropathic pain (Marshall et al., 2017). As shown in rats in humans, the RDD is also impaired in diabetic neuropathy (Hernández-Reyes et al., 2019; Lee-Kubli & Calcutt, 2014; Marshall et al., 2017). Interestingly, bicuculline restores the loss of RDD, suggesting that the disruption of GABAergic inhibition mediated by GABAARs is involved in the impairment of this property (Jolivalt et al., 2008; Lee-Kubli & Calcutt, 2014). We have shown that the intrathecal application of L-655,708 abolishes RDD in healthy animals and reestablished it in neuropathic diabetic rats in parallel with the reduction of tactile allodynia (Figure 1, Hernández-Reyes et al., 2019). This result demonstrates that the impairment of RDD is mediated by extrasynaptic α5GABAARs.

There is evidence that formalin- or capsaicin-induced secondary hyperalgesia is associated with the activation of antinocicptive action potentials in sensory neurons produced by the facilitated PAD (inflammatory conditions). This phenomenon, known as dorsal root reflex (DRR), propagates retrogradely to the peripheral terminal, where it evokes the release of inflammatory neuropeptides (substance P and CGRP) (Willis, 1999), contributing to hyperalgesia (Cervero et al., 2003). In this context, instead of activating GABAARs, it was proposed to induce analgesia by blocking them. In line with this, the α5-selective negative allosteric modulator (NAM) α5IA-II was evaluated in the formalin and carrageenan tests. In both cases, it reverses mechanical hypersensitivity and weight-bearing deficits. In contrast, it did not affect nociception in a neuropathic pain model (Munro et al., 2011). Unexpectedly, in the turtle, we showed that L-655,708 depresses the DRRs without affecting PAD, suggesting that extrasynaptic α5GABAARs might be tonically depolarizing the primary afferents to reach the threshold to activate the DRRs (Loeza-Alcocer et al., 2013). In agreement with this result, the peripheral and intrathecal pretreatment or post-treatment with L-655,708 prevents and reverses the long-lasting allodynia and hyperalgesia in the formalin-induced test and restores the loss RDD of the HR (Bravo-Hernández et al., 2016).

7 | HOW SELECTIVE IS L-655,708?

The pharmacological approach to investigate the function of α5GABAARs has the disadvantage of the differential selectivity of L-655,708 for the GABAARs. It displays more selectivity for the α5 subunit-containing receptors than for those containing α1, α2, α3, and α6 subunits (Quirk et al., 1996). To circumvent this issue, we have used the excitability Wall-test in healthy rats. For this, the L1 vertebrae segment was removed to expose the lumbar enlargement, and the antidromic compound action potential (test cAP) recorded in the tibial nerve was evoked by the spinal cord electrical stimulation and was conditioned by the electrical stimulation of the sural and peroneal nerves. The test and the conditioned responses indicate the phasic and the tonic excitability of primary afferent fibers, respectively. Interestingly, the phasic excitability of primary afferent fibers mediated by the activation of synaptic α5GABAARs was not affected by L-655,708, while the tonic excitability mediated by extrasynaptic α5GABAARs was increased (Figure 2; Hernández-Reyes et al., 2019). Therefore, this result, together with the action of the α5GABAAR siRNA in healthy and reserpine-induced fibromyalgia (De la Luz-Cuellar et al., 2019), confirms the antinociceptive and pronociceptive role of α5GABAARs in healthy and chronic pain conditions, respectively.

8 | WHY IS THE α5GABAAR SO RELEVANT IN HEALTH AND PAIN?

α5GABAARs are expressed in laminae I and II neurons, where they mediate a tonic inhibitory current in healthy animals (Perez-Sanchez et al., 2017). KCC2 is downregulated in the dorsal horn of rodents with nerve injury neuropathy (Coull et al., 2003) and diabetic neuropathy (Jolivalt et al., 2008). Therefore, the action of GABAAR might be switching from inhibition into excitation, which has been shown in nerve injury (Coull et al., 2003). Given that the action of extrasynaptic GABAARs is about six times more intense than the synaptic receptors (Ataka & Gu, 2006), we have hypothesized that the extrasynaptic spinal α5GABAARs might be tonically hyperpolarizing the projection neurons in the health condition, while in chronic pain they might be causing a tonic depolarization of the cell membrane. In the first case, they prevent the excitatory synaptic inputs of the low threshold afferent fibers from activating the projection neurons of laminae I-II (Baba et al., 2003; Torsney & MacDermott, 2006), and in the second condition, the gate opens, allowing the fibers mentioned above to activate the neurons (Baba et al., 1999; Figure 3).

Although we have shown that α5GABAARs have a pronociceptive role in several models of chronic pain, there remains an unanswered question: after blocking these receptors, what
inhibits the projection neurons, preventing them from being stimulated by the activation of the low-threshold afferent fibers? One possibility is the participation of the GABA_B receptors (GABABRs). The expression of GABABRs in dorsal horn neurons and primary afferent fibers in the spinal circuitry processing nociceptive information is well documented (Malcangio 2018; Schuler et al., 2001; Towers et al., 2000). Indeed, GABA_BRs are tonically active inhibiting transmitter release (Peshori et al., 1988). At postsynaptic neurons, they control neuronal excitability. Moreover, activation of these receptors with baclofen inhibits the plateau properties, wind-up, and post-discharge induced by dorsal root stimulation in dorsal horn neurons even in the presence of tetrodotoxin (Russo et al., 1998) or by noxious mechanical stimulation in vivo (Reali et al., 2011). Lee-Kubli et al. (2021) confirmed that the GABA_B Rs in conjunction with the GABA_A Rs contribute to the inhibitory circuitry involved in the modulation of RDD of the HR and tactile allodynia in diabetic rats (Lee-Kubli et al., 2021).

Interestingly, phaclofen, an antagonist of the GABA_B Rs, but not bicuculline, impaired the RDD in 4-week diabetic rats, suggesting that in these animals the normal RDD is mediated by functional GABABRs. It is well known that in 8-week diabetic rats, bicuculline and L-655708 restored the impaired RDD and reversed the mechanical allodynia (Hernández-Reyes et al., 2019; Jolivalt et al., 2008). However, unexpectedly, the administration of phaclofen in these rats reverted the restoration of RDD by bicuculline. In addition, the intrathecal application of baclofen alleviated mechanical allodynia in diabetic rats. These results suggested to the authors that GABA_B Rs, together with GABA_A Rs, are mediating the GABAergic inhibition in the dorsal horn of the spinal cord.
where nociceptive information is processed. Consequently, if GABA$_A$Rs are closing the gate to the excitatory input of low-threshold afferent fibers on projection neurons, it would be interesting to know whether intrathecal administration of a GABA$_B$ receptor antagonist restores mechanical allodynia in diabetic rats after having removed it by blocking α$_5$GABA$_A$Rs receptors.

9 | EXTRASYNAPTIC α$_5$GABA$_A$ RECEPTORS REGULATION

Previous pharmacological studies have reported sex differences in α$_5$GABA$_A$Rs function. PAMs of α$_5$GABA$_A$Rs reduce stress-induced behaviors in females but not in male rodents (Piantadosi et al., 2016). More recently, our group reported a sex-dependent effect of α$_5$GABA$_A$Rs activation in chronic pain (De la Luz-Cuellar et al., 2019; Franco-Enzáñstiga et al., 2021). We found that α$_5$GABA$_A$Rs mRNA and protein changes in DRG and spinal cord are modulated in a sex-dependent way. Nerve injury increases the expression of α$_5$GABA$_A$R in female DRG and spinal cord in female rodents, but not in males (Franco-Enzáñstiga et al., 2021). These changes are activated by DNA methylation in the CpG island of Gabra5 in males but not females. In contrast, the expression and function of spinal α$_5$GABA$_A$Rs in females is associated with the presence of 17β-estradiol (Franco-Enzáñstiga et al., 2021).

10 | CONCLUSIONS

Multiple and irreversible plastic changes involving non-neuronal and neuronal cells in the spinal cord circuits...
processing nociceptive information convey by sensory neurons underlie chronic and neuropathic pain. Between the complexity of adaptations in the function of the nervous system, the loss of inhibition mediated by synaptic GABA$_A$Rs has been identified as a target for developing drugs to relieve pain. Reestablishing GABAergic inhibition without knowing where and how the inhibition is lost represents a very complex issue. The reversion of allodynia by blocking the $\alpha_5$GABA$_A$Rs in pain induced by diabetes, nerve injury, fibromyalgia, and chronic inflammation indicates that the spinal inhibitory dysfunction might be related to a downregulation of KCC2 transporter affecting the homeostasis of Cl$^-$ in projection neurons, where the tonic current through this receptor might be switched from inhibition to excitation. Therefore, blocking $\alpha_5$GABA$_A$Rs may be a feasible strategy to treat chronic pain.

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CONFLICTS OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
All authors read, improved, and approved the manuscript. V.G.-S., and R.F., and R.D.-L., wrote the manuscript.

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REFERENCES

Alvarez, F. J., Taylor-Blake, B., Fryffe, R. E., De Blas, A. L., & Light, A. R. (1996). Distribution of immunoreactivity for the β2 and β3 subunits of the GABA_A receptor in the mammalian spinal cord. *Journal of Comparative Neurology*, 365, 392–412.

Ataka, T., & Gu, J. G. (2006). Relationship between tonic inhibitory currents and phasic inhibitory activity in the spinal cord lamina II region of adult mice. *Molecular Pain*, 2, 36. https://doi.org/10.1186/1744-8069-2-36

Baba, H., Doubell, T. P., & Woolf, C. J. (1999). Peripheral inflammation facilitates Aβ fiber-mediated synaptic input to the substantia gelatosa of the adult rat spinal cord. *Journal of Neuroscience*, 9(2), 859–867. https://doi.org/10.1523/JNEUROCSCI.19-02-00859.1999

Baba, H., Ji, R.-R., Kohno, T., Moore, K. A., Ataka, T., Wakai, A., Okamoto, M., & Woolf, C. J. (2003). Removal of GABAergic inhibition facilitates polysynaptic A fiber-mediated excitatory transmission to the superficial spinal dorsal horn. *Molecular Cell Neuroscience*, 24, 818–830. https://doi.org/10.1016/S1044-7431(03)00236-7

Bohnhalter, S., Weinmann, O., Mohler, H., & Fritschy, J. M. (1996). Laminar compartmentalization of GABA_A receptor subtypes in the spinal cord: an immunohistochemical study. *Journal of Neuroscience*, 16, 283–297. https://doi.org/10.1523/JNEUROSCI.16-01-00283.1996

Bonin, R. P., & De Koninck, Y. (2013). Restoring ionotropic inhibition as an analgesic strategy. *Neuroscience Letters*, 557, 43–51. https://doi.org/10.1016/j.neulet.2013.09.047

Bonin, R. P., Labrakasis, C., Eng, D. G., Whissell, P. D., De Koninck, Y., & Orser, B. A. (2011). Pharmacological enhancement of delta-subunit-containing GABA_A receptors that generate a tonic inhibitory conductance in spinal neurons attenuates acute nociception in mice. *Pain*, 152, 1317–1326.

Bravo-Hernández, M., Corleto, J. A., Barragán-Iglesias, P., González-Ramírez, R., Pineda-Farias, J. B., Felix, R., Calcutt, N. A., Delgado-Lezama, R., Marsala, M., & Granados-Soto, V. (2016). The α5 subunit containing GABA_A receptors contribute to chronic pain. *Pain*, 157, 613–626. https://doi.org/10.1007/pain.0000000000410

Bravo-Hernández, M., Feria-Morales, L. A., Torres-López, J. E., Cervantes-Durán, C., Delgado-Lezama, R., Granados-Soto, V., & Rocha-González, H. I. (2014). Evidence for the participation of peripheral α5 subunit-containing GABA_A receptors in GABA_A agonists-induced nociception in rats. *European Journal of Pharmacology*, 734, 91–97. https://doi.org/10.1016/j.ejphar.2014.03.051

Brickley, S. G., Cull-Candy, S. G., & Farrant, M. (1996). Development of a tonic form of synaptic inhibition in rat cerebellar granule cells resulting from persistent activation of GABA_A receptors. *Journal of Physiology*, 497, 753–759. https://doi.org/10.1113/jphysiol.1996.sp021806

Canto-Bustos, M., Loeca-Alcocer, E., Cuellar, C. A., Osuna, P., Elias-Viñas, D., Granados-Soto, V., Manjarrez, E., Felix, R., & Delgado-Lezama, R. (2017). Tonically active α5GABA_A receptors reduce motoneuron excitability and decrease the monosynaptic reflex. *Frontiers in Cell Neuroscience*, 11, 283. https://doi.org/10.3389/fncel.2017.00283

Castro, A., Aguilar, J., González-Ramírez, R., Loeca-Alcocer, E., Canto-Bustos, M., Felix, R., & Delgado-Lezama, R. (2011). Tonic inhibition in spinal ventral horn interneurons mediated by α5 subunit-containing GABA_A receptors. *Biological and Biophysical Research Communication*, 412(1), 26–31. https://doi.org/10.1016/j.bbrc.2011.07.026

Cervero, F., Laird, J. M. A., & García-Nicas, E. (2003). Secondary hyperalgesia and presynaptic inhibition: an update. *European Journal of Pain*, 7, 345–351. https://doi.org/10.1016/S1090-3801(03)00047-8

Coull, J. A., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., Gravel, C., Salter, M. W., & De Koninck, Y. (2005). BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature*, 438(7070), 1017–1021.

Coull, J. A., Boudreau, D., Bachand, K., Prescott, S. A., Nault, F., Sik, A., De Koninck, P., & De Koninck, Y. (2003). Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. *Nature*, 424, 938–942.

De la Luz-Cuellar, Y. E., Rodríguez-Palma, E. J., Franco-Enzástiga, U., Salinas-Abarca, A. B., Delgado-Lezama, R., & Granados-Soto, V. (2019). Blockade of α5GABA_A receptors differentially reduces reserpine-induced fibromyalgia-type pain in female rats. *European Journal of Pharmacology*, 858, 1–12.

Farrall, M., & Nusser, Z. (2005). Variations on an inhibitory theme: phasic and tonic activation of GABA_A receptors. *Nature Review Neuroscience*, 6, 215–229. https://doi.org/10.1038/nrn1625

Franco-Enzástiga, U., García, G., Murbartián, J., González-Barrios, R., Salinas-Abarca, A. B., Sánchez-Hernández, B., Tavares-Ferreira, D., Herrera, L. A., Barragán-Iglesias, P., Delgado-Lezama, R., Price, T. J., & Granados-Soto, V. (2021). Sex-dependent pronociceptive role of spinal α5GABA_A receptor and its epigenetic regulation in neuropathic rodents. *Journal of Neurochemistry*, 156(6), 897–916.

Hernández-Reyes, J. E., Salinas-Abarca, A. B., Vidal-Cantú, G. C., Raya-Tafolla, G., Elias-Viñas, D., Granados-Soto, V., & Delgado-Lezama, R. (2019). α5-GABA_A receptors play a pronociceptive role and avoid the rate-dependent depression of the Hoffmann reflex in diabetic neuropathic pain and reduce primary afferent excitability. *Pain*, 160, 1448–1458. https://doi.org/10.1007/j. pain.000000000001515

Jolivalt, C. G., Lee, C. A., Ramos, K. M., & Calcutt, N. A. (2008). Allodynia and hyperalgesia in diabetic rats are mediated by GABA and depletion of spinal potassium-chloride co-transporters. *Pain*, 140, 48–57. https://doi.org/10.1016/j.pain.2008.07.005

Knabl, J., Witschi, R., Hösl, K., Reinold, H., Zeilhofer, U. B., Ahmadi, S., Brockhaus, J., Sergejeva, M., Hess, A., Brune, K., Fritschy, J. M., Rudolph, U., Möhler, H., & Zeilhofer, H. U. (2008). Reversal of pathological pain through specific spinal GABA_A receptor subtypes of pathological pain through specific spinal GABA_A receptor subtypes. *Nature*, 451, 330–334. https://doi.org/10.1038/nature06493

Kullmann, D. M., Ruiz, A., Rusakov, D. M., Scott, R., Semyanov, A., & Walker, M. C. (2005). Presynaptic, extrasynaptic and axonal GABA_A receptors in the CNS: where and why? *Progress in Biophysics & Molecular Biology*, 87(1), 33–46. https://doi.org/10.1016/j.pbiomolbio.2004.06.003

Lee-Kubli, C. A., & Calcutt, N. A. (2014). Altered rate-dependent depression of the spinal H-reflex as an indicator of spinal disinhibition in models of neuropathic pain. *Pain*, 155, 250–260. https://doi.org/10.1016/j.pain.2013.10.001

Lee-Kubli, C. A., Zhou, X., Jolivalt, C. G., & Calcutt, N. A. (2021). Pharmacological modulation of rate-dependent depression of the spinal H-reflex predicts therapeutic efficacy against painful diabetic neuropathy. *Diagnostics* (Basel), 11, 283.
Loeza-Alcocer, E., Canto-Bustos, M., Aguilar, J., González-Ramírez, R., Felix, R., & Delgado-Lezama, R. (2013). α5GABA<sub>A</sub> receptors mediate primary afferent fiber tonic excitability in the turtle spinal cord. *Journal of Neurophysiology*, 110, 2175–2184.

Lucas-Osma, A. M., Li, Y., Lin, S., Black, S., Singla, R., Fouad, K., Ferich, K. K., & Bennett, D. J. (2018). Extrasynaptic α5GABA<sub>A</sub> receptors on proprioceptive afferents produce a tonic depolarization that modulates sodium channel function in the rat spinal cord. *Journal of Neurophysiology*, 120, 2953–2974.

Ma, W., Saunders, P. A., Somogyi, R., Poulter, M. O., & Barker, J. L. (1993). Ontogeny of GABA<sub>A</sub> receptor subunit mRNAs in rat spinal cord and dorsal root ganglia. *Journal of Comparative Neurology*, 338(3), 337–359. https://doi.org/10.1002/cne.903380303

Malcangio, M. (2018). GABA<sub>A</sub> receptors and pain. *Neuropsychopharmacology*, 136, 102–105.

Marshall, A. G., Lee-Kubli, C., Azmi, S., Zhang, M., Ferdousi, M., Mixcoati-Zecuatl, T., Petropoulos, I. N., Ponirakis, G., Fineman, M. S., Fadavi, H., Frizzi, K., Tavakoli, M., Jezierska, M., Jolivalt, C. G., Boulton, A. J. M., Efron, N., Calcutt, N. A., & Malik, R. A. (2017). Spinal disinhibition in experimental and clinical painful diabetic neuropathy. *Diabetes*, 66, 1380–1390. https://doi.org/10.2337/db16-1181

Melzack, R., & Wall, P. D. (1965). Pain mechanisms: A new theory. *Science*, 150, 971–979.

Morgado, C., Pinto-Ribeiro, F., & Tavares, I. (2008). Diabetes affects the expression of GABA and potassium chloride cotransporter in the spinal cord: A study in streptozotocin diabetic rats. *Neuroscience Letters*, 438(1), 102–106. https://doi.org/10.1016/j.neulet.2008.04.032

Munro, G., Erichsen, H. K., Rae, M. G., & Mirza, N. R. (2011). A question of balance–positive versus negative allosteric modulation of GABAA receptor subtypes as a driver of analgesic efficacy in rat models of inflammatory and neuropathic pain. *Neuropsychopharmacology*, 61, 121–132. https://doi.org/10.1016/j.neuropsychopharmacology.2011.03.017

Paul, J., Zeilhofer, H. U., & Fritschy, J. M. (2012). Selective distribution of GABA<sub>A</sub> receptor subtypes in mouse spinal dorsal horn neurons and primary afferents. *Journal of Comparative Neurology*, 520, 3895–3911.

Perez-Sanchez, J., Lorenzo, L. E., Lecker, I., Zurek, A. A., Labrakakis, C., Bridgewater, E. M., Orser, B. A., De Koninck, Y., & Bonin, R. P. (2017). α<sub>5</sub>GABA<sub>A</sub> receptors mediate tonic inhibition in the spinal cord dorsal horn and contribute to the resolution of hyperalgesia. *Journal of Neuroscience Research*, 95, 1307–1318.

Peshori, K. R., Collins, W. F., & Mendell, L. M. (1988). EPSP amplitude modulation at the rat la-alpha motoneuron synapse: Effects of GABA<sub>B</sub> receptor agonists and antagonists. *Journal of Neurophysiology*, 79, 181–189.

Piantadosi, S. C., French, B. J., Poe, M. M., Timić, T., Marković, B. D., Pabba, M., Seney, M. L., Oh, H., Orser, B. A., Savić, M. M., Cook, J. M., & Sibille, E. (2016). Sex-dependent anti-stress effect of an α<sub>5</sub> subunit containing GABA<sub>A</sub> receptor positive allosteric modulator. *Frontiers in Pharmacology*, 7, 446. https://doi.org/10.3389/fphar.2016.00446

Quirk, K. P., Blurton, P., Fletcher, S., Leeson, P., Tang, F., Mellilo, D., Ragan, C. I., & McKernan, R. M. (1996). [HJL]-655,708, a novel ligand selective for the benzodiazepine site of GABA<sub>A</sub> receptors which contain the α<sub>1</sub> subunit. *Neuropsychopharmacology*, 35, 1331–1335.

Ravvenius, W. T., Benke, D., Acuña, M. A., Rudolph, U., & Zeilhofer, H. U. (2015). Analgesia and unwanted benzodiazepine effects in point-mutated mice expressing only one benzodiazepine-sensitive GABA<sub>A</sub> receptor subtype. *Nature Communications*, 6, 6803.

Reali, C., Fossat, P., Landry, M., Russo, R. E., & Nacy F. (2011). Intrinsic membrane properties of spinal dorsal horn neurons modulate nociceptive information processing in vivo. *Journal of Physiology*, 589, 2733–2743.

Roberts, L. A., Beyer, C., & Komisaruk, B. R. (1986). Nociceptive responses to altered GABAergic activity at the spinal cord. *Life Sciences*, 39, 1667–1674. https://doi.org/10.1016/0022-2836(86)90164-5

Rudolph, U., & Knoflach, F. (2011). Beyond classical benzodiazepines: Novel therapeutic potential of GABA<sub>A</sub> receptor subtypes. *Nature Review Drug Discovery*, 10(9), 685–697.

Rudomin, P., & Schmidt, R. F. (1999). Presynaptic inhibition in the vertebral spinal cord revisited. *Experimental Brain Research*, 129, 1–37. https://doi.org/10.1007/s002210050933

Russo, R. E., Nagy, F., & Houngsaard, J. (1998). Inhibitory control of plateau properties in dorsal horn neurons in the turtle spinal cord in vitro. *Journal of Physiology*, 506, 795–808.

Schuler, V., Lüsher, C., Blanchet, C., Klix, N., Sansig, G., Klebs, K., Schmutz, M., Heid, J., Gentry, C., Urban, L., Fox, A., Spooren, W., Jaton, A. L., Vigoureit, J., Pozza, M., Kelly, P. H., Mosbacher, J., Froestl, W., Käslin, E., … Bestler, B. (2001). Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postynaptic GABA(B) responses in mice lacking GABA(B1). *Neuron*, 31, 47–58.

Takahashi, A., Mashimo, T., & Uchida, I. (2006). GABAergic tonic inhibition of substantia gelatinosa neurons in mouse spinal cord. *NeuroReport*, 17(12), 1313–1315. https://doi.org/10.1097/01. nmr.0000230515.86090.bc

Todd, A. J. (2010). Neuronal circuitry for pain processing in the dorsal horn. *Nature Review Neuroscience*, 11, 823–836. https://doi.org/10.1038/nn2947

Torsney, C., & MacDermott, A. B. (2006). Disinhibition opens the gate to pathological pain signaling in superficial neurokinin 1 receptor-expressing neurons in rat spinal cord. *Journal of Neuroscience*, 26, 1833–1843. https://doi.org/10.1523/JNEUROSCI.4584-05.2006

Towers, S., Princivalle, A., Billinton, A., Edmunds, M., Bestler, B., Urban, L., Castro-Lopes, J., & Bowery, N. G. (2000). GABA<sub>B</sub> receptor protein and mRNA distribution in rat spinal cord and dorsal root ganglia. *European Journal of Neuroscience*, 12, 3201–3210.

Willis, W. D. (1999). Dorsal root potentials and dorsal root reflexes: a double-edged sword. *Experimental Brain Research*, 124, 395–421. https://doi.org/10.1007/s002210050637

Witschi, R., Punnakkal, P., Paul, J., Walczak, J. S., Cervero, F., Fritschy, J. M., Kaner, R., Keist, R., Rudolph, U., & Zeilhofer, H. U. (2011). Presynaptic α<sub>5</sub>-GABA<sub>A</sub> receptors in primary afferent depolarization and spinal pain control. *Journal of Neuroscience*, 31, 8134–8142.

Xue, M., Liu, J. P., Yang, Y. H., Suo, Z. W., Yang, X., & Hu, X. D. (2017). Inhibition of α<sub>5</sub> subunit-containing GABA<sub>A</sub> receptors facilitated spinal nociceptive transmission and plasticity. *European Journal of Pain*, 21, 1061–1071.
Yoshimura, M., & Nishi, S. (1995). Primary afferent-evoked glycine- and GABA-mediated IPSPs in substantia gelatinosa neurones in the rat spinal cord in vitro. *The Journal of Physiology, 482*, 29–38. https://doi.org/10.1113/jphysiol.1995.sp020497

Zeilhofer, H. U., Benke, D., & Yevenes, G. E. (2012). Chronic pain states: pharmacological strategies to restore diminished inhibitory spinal pain control. *Annual Review of Pharmacology and Toxicology, 52*, 111–133. https://doi.org/10.1146/annurev-pharmtox-010611-134636

Zeilhofer, H. U., Ralvenius, W. T., & Acuña, M. A. (2015). Restoring the spinal pain gate: GABA<sub>A</sub> receptors as targets for novel analgesics. *Advances in Pharmacology, 73*, 71–96.

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