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Pre- and postsynaptic inhibitory control in the spinal cord dorsal horn

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Abstract

Sensory information transmitted to the spinal cord dorsal horn is modulated by a complex network of excitatory and inhibitory interneurons. The two main inhibitory transmitters, GABA and glycine, control the flow of sensory information mainly by regulating the excitability of dorsal horn neurons. A presynaptic action of GABA has also been proposed as an important modulatory mechanism of transmitter release from sensory primary afferent terminals. By inhibiting the release of glutamate from primary afferent terminals, activation of presynaptic GABA receptors could play an important role in nociceptive and tactile sensory coding, while changes in their expression or function could be involved in pathological pain conditions, such as allodynia.

Keywords

dorsal horn; pain; GABA; glycine; inhibition

Introduction

The superficial and deep laminae of the spinal cord dorsal horn are under strong inhibitory control, importantly exerted by two neurotransmitters, gamma-aminobutyric acid (GABA) and glycine. These transmitters, released by both local interneurons and inhibitory descending fibers, bind to their cognate anion permeable receptors, GABA_A and glycine, respectively. GABA additionally binds to its G protein-coupled receptor, the GABA_B receptor. Activation of GABA and glycine receptors depresses neuronal excitation through

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Conflicts of interest

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hyperpolarization of the postsynaptic membrane and/or activation of a shunting conductance. Indeed, application of bicuculline or strychnine (blockers of GABA_A and glycine receptors, respectively) to a spinal cord slice preparation shows the effect of ambient GABA and glycine on excitability of dorsal horn neurons (Fig. 1). As we will describe subsequently, GABA can also directly decrease glutamate release from primary afferent fibers (PAFs).¹ Behavioral studies in rodents have shown that intrathecal administration of bicuculline or strychnine induces nocifensive responses and lowers nociceptive threshold in rats, while injection of GABA or glycine is antinociceptive under most circumstances.²⁻⁴ Furthermore, enhancing GABA_A receptor function by spinal application of GABA or a positive allosteric modulator, such as midazolam, depresses noxious stimulus-evoked activity in spinal cord neurons.^{5,6} Loss of synaptic inhibition is widely accepted as an important factor contributing to the generation and maintenance of chronic pain. Therefore, we will consider recent advances in our understanding of inhibition in the spinal cord dorsal horn.

Region-specific inhibition by GABA and glycine in the spinal cord dorsal horn

The relative contributions of GABA and glycine to the control of the flow of information in the dorsal horn varies among different laminae and somatosensory modalities. Immunohistochemical studies and, more recently, transgenic mice in which enhanced green fluorescent protein (eGFP) is expressed under the promoter of the gene encoding the enzyme glutamic acid decarboxylase 67 (GAD67), involved in the synthesis of GABA (GAD67-eGFP mice), have shown that GABAergic interneurons are abundant in the spinal cord dorsal horn. This is especially true in superficial laminae,⁷⁻¹⁰ where nociceptive fibers terminate. A subpopulation of GABAergic interneurons also express glycine (33%, 43%, and 64% of GABAergic neurons in lamina I, II, and III, respectively, express glycine).^{10,11} *In situ* hybridization studies and observations from glycine transporter 2 (GlyT2)-eGFP mice have shown that glycinergic neurons are more abundant in the deeper dorsal layers (laminae III-V)^{12,13} that receive tactile sensory inputs as well as some nociceptive inputs.

Analysis of miniature inhibitory postsynaptic currents (mIPSCs) mediated by GABA_A and glycine receptors, reflecting quantal release of these transmitters, has confirmed that the contribution of GABA and glycine to fast synaptic inhibition changes between different laminar regions of the dorsal horn. GABAergic mIPSCs seem to predominate in laminae I and II outer (IIo), while glycine mIPSCs play a dominant role in lamina III.¹⁴ Our group has recently characterized two populations of inhibitory interneurons in GAD67-eGFP mice. One group of neurons predominantly receives strongly bicuculline-sensitive mIPSCs with slower decay kinetics (GABA-dominant) while another class of inhibitory neurons predominantly receives fewer bicuculline-sensitive mIPSCs with fast decay kinetics (glycine-dominant).¹⁵ Consistent with previous studies,^{14,16,17} inhibitory interneurons are mainly GABA-dominant in laminae I-IIo, while glycine-dominant neurons are prevalent at the lamina II-III border, as illustrated in Figure 2.

Extrasyaptic GABA_A and glycine receptors mediate tonic currents in dorsal horn neurons, 18–20 where they play important roles in regulating neuronal excitability. We have shown in mature mice that tonic GABA currents predominate in GABA-dominant neurons, while tonic glycine currents are critical in regulating the inhibitory tone of glycine-dominant neurons at the lamina II–III border.¹⁵ This border area receives inputs from low-threshold mechanosensitive afferents and is critically involved in the generation of dynamic mechanical allodynia.²¹ Excitatory interneurons expressing the γ isoform of protein kinase C (PKC γ), located at the ventral border of inner lamina II (lamina Ili), and in lamina III, are essential contributors to mechanical allodynia.²² These PKC γ -expressing interneurons receive projections from low-threshold mechanoreceptors²³ and are normally inhibited by glycinergic interneurons.²⁴ Removal of glycine inhibition allows activation of a polysynaptic excitatory pathway triggered by low-threshold mechanical input, leading to the excitation of nociceptive-specific projection neurons in the superficial dorsal horn.²¹ This is similar to the polysynaptic excitatory pathway between low-threshold A β fibers and lamina I projection neurons observed in the presence of bicuculline and strychnine in another study.²⁵ Further experiments will be required to assess the contribution of synaptic and tonic glycine currents in controlling the excitability of inhibitory and excitatory interneurons, both in control animals and in animal models of chronic pain.

Presynaptic modulation of primary afferent terminals

GABA_A receptors and primary afferent depolarization

GABA has long been known to be one of the inhibitory transmitters mediating presynaptic inhibition of excitatory transmission in the spinal cord, acting through both ionotropic (GABA_A) and G protein-coupled receptors (GABA_B). In 1957, Frank and Fuortes²⁶ first proposed the concept of presynaptic inhibition, based on the observation that muscle afferent volleys depressed the size of the monosynaptic excitatory postsynaptic potential of spinal motoneurons produced by other muscle afferents without any changes in the membrane potential or excitability of those motoneurons. This form of presynaptic inhibition is caused by depolarization of the primary afferent terminals (PAD) and is strongly depressed by GABA_A receptor antagonists, such as bicuculline, suggesting that GABAergic interneurons can be involved in this mechanism through a polysynaptic circuit.^{27–29}

Histological evidence supporting this hypothesis has been provided by several studies. GABAergic interneurons form axo-axonic synapses in both the ventral and dorsal horns, not only on group Ia, Ib, and II muscle PAFs, but also on cutaneous afferents, particularly those of large diameter.³⁰ As shown in Figure 3, the stimulation of sensory cutaneous afferents could activate a disynaptic circuit in the dorsal horn, producing release of GABA from inhibitory interneurons, causing decrease of glutamate release from the PADs. Electron microscopy studies performed on the superficial dorsal horn from spinal cord preparations of different mammalian species have demonstrated the presence of complex synaptic structures called glomeruli. These are formed by a central terminal of either myelinated or unmyelinated PAFs, and several dendrites and axons.^{11,31} Axon terminals presynaptic to central terminals of unmyelinated PAFs are predominantly GABAergic, while the majority of those contacting terminals of myelinated PAFs are both GABAergic and glycinergic.³² In

lamina III of the rat spinal cord, axons containing GABA, or GABA plus glycine, form a synaptic triadic arrangement, contacting both the terminal of a hair follicle myelinated afferent and a postsynaptic dendrite.³³ Parvalbumin-expressing inhibitory interneurons, which predominate in lamina III, have recently been shown to be involved in axo-axonic synapses with nonnociceptive A δ down hair afferents belonging to synaptic glomeruli, or with larger myelinated fibers such as hair follicle afferents.³⁴

The mechanisms of presynaptic inhibition mediated by PAD have largely been elucidated.³⁰ Primary sensory neurons exhibit a higher intracellular concentration of chloride than central neurons. This is due to the high expression of the transporter NKCC1, which transports Cl⁻, Na⁺, and K⁺ into the cell, and low expression of KCC2, which transports Cl⁻ and K⁺ out of the cell.³⁵⁻³⁷ For this reason, the chloride equilibrium potential in dorsal root ganglion neurons (DRGs) is about -30 mV. Thus, the opening of GABA_A receptors causes the efflux of Cl⁻ and depolarization of the terminals. Inactivation of voltage-dependent sodium and calcium channels, caused by the terminal depolarization³⁸ and the shunting effect due to opening of GABA_A receptors, impairs the propagation of action potentials along PAFs into the terminals, and decreases the release of glutamate. Suprathreshold depolarizations can potentially produce the opposite effect by eliciting action potentials in spinal PAF terminals, possibly triggering dorsal root reflexes that may contribute to neurogenic inflammation.³⁹ The upregulation of the GABA system on DRG neurons⁴⁰ and/or the increase of NKCC1 transporter activity⁴¹ could contribute to the shift from presynaptic inhibition to dorsal reflexes and pain sensitization.

Studies performed during the last decade have suggested the need for additional mechanisms for PAD. PAF terminals express other synaptic receptors able to depolarize the terminals. Glutamatergic ionotropic AMPA, NMDA, and kainate receptors have been detected at the central terminals of PAFs. Activation of these receptors causes depolarization of PAFs in the rat spinal cord.⁴²⁻⁴⁴ Furthermore, the observation that PAD is not completely blocked by inhibition of synaptic transmission suggests that it could be partly mediated by either spillover of transmitters released from PAFs or a dendroaxonic reciprocal synaptic microcircuit.^{45,46} At the glomerular level, several dendrites contacting PAF central terminals contain clear vesicles, so these could be involved in the generation of PAD.^{10,47}

The subunit composition of presynaptic GABA_A receptors expressed on PAF terminals has recently been investigated. Central terminals of primary afferents in mouse dorsal horn express four α subunits ($\alpha 1-3$, $\alpha 5$), with a prevalence of $\alpha 2$ or $\alpha 3$ on C-fibers, while myelinated A δ and A β fibers are co-labeled in roughly equal proportion with each subunit.⁴⁸ Mice with nociceptors (DRG neurons expressing SNS [or Nav1.8] channels) selectively lacking the GABA_A $\alpha 2$ subunits exhibit reduced potentiation of dorsal root potentials and impaired thermal and mechanical antihyperalgesia by diazepam in a model of inflammatory pain,⁴⁹ confirming a role of the $\alpha 2$ subunit in regulating sensitization in models of inflammatory and neuropathic pain.^{50,51}

Although glycine is present in several axon terminals of GABAergic neurons presynaptic to PAFs (see previous discussion), strychnine does not block PAD or presynaptic inhibition,^{27,52,53} and glycine does not directly depolarize PAFs.⁵⁴ Furthermore, glycine receptors have

not been detected on PAF terminals,⁵⁵ so the putative presynaptic role of glycine on PAF central terminals is still controversial. It is possible that the major site of action of glycine, released by inhibitory interneurons, is on postsynaptic dendrites, belonging to glomeruli or triadic arrangements.

GABA_B receptors

Metabotropic GABA_B receptors are expressed both in DRGs and spinal cord dorsal horn, particularly in the superficial laminae.^{56,57} Their activation produces antinociceptive effects: treatment with the GABA_B agonist baclofen induces dose-dependent inhibition of C-fiber and pinch-evoked activity of rat wide dynamic range neurons *in vivo*,⁵⁸ and reverses hypersensitivity of these neurons to mechanical stimuli after spinal cord ischemia.⁵⁹

Exogenous activation of GABA_B receptors by baclofen inhibits glutamate and substance P release from PAFs in spinal cord dorsal horn⁶⁰ by acting on presynaptic voltage-dependent calcium channels. A postsynaptic effect of baclofen has also been observed in rat superficial and deep dorsal horn, consisting of the generation of an outward current mediated by potassium channels.^{57,61,62} GABA_B receptors are also involved in the depression of GABA release from dorsal horn neurons: in lamina I, paired pulse depression of evoked IPSCs is decreased by an antagonist of GABA_B receptors, while it is not affected by GABA_A antagonists.⁶³ Because GABA_B receptors have a higher sensitivity for GABA than GABA_A receptors do, they could be activated even under conditions of low extracellular concentrations of GABA. The endogenous effect of GABA_B receptors in modulating glutamate release from PAFs in lamina II has recently been investigated:⁶⁴ blockade of GABA_B receptors facilitates the evoked action potential-dependent synaptic responses and increases neuronal excitability after dorsal root stimulation.

Function of GABA-mediated presynaptic inhibition in the dorsal horn, and future perspectives

The first synapse in the somatosensory pathway, that is, the synapse between nociceptive or tactile PAFs and dorsal horn neurons, is modulated by several mechanisms of presynaptic inhibition. Here we have illustrated some aspects of the inhibition mediated by GABA receptors. Both ionotropic GABA_A and metabotropic GABA_B receptors exert an inhibitory action on glutamate release from PAFs, involving different cellular mechanisms. Despite the large number of studies regarding GABA-mediated presynaptic inhibition in dorsal horn, several aspects remain to be elucidated.

Synaptic responses generated by glutamate release from PAFs onto dorsal horn neurons are known to undergo a strong short-term depression (see Ref. 65). We have observed that, in rat lamina III, the level of depression is variable from one postsynaptic cell to another (unpublished observation). This likely reflects (1) different PAF properties and/or (2) different synaptic circuits recruited by dorsal root stimulation. The roles of GABA_A and GABA_B receptors in short-term depression have not been established. We hypothesize that during PAF repetitive stimulation, the activation of presynaptic GABA_A and GABA_B receptors could contribute to shaping the pattern of postsynaptic response amplitudes, both

in superficial and deep dorsal horn. Application of bicuculline attenuates paired pulse depression of postsynaptic responses evoked on dorsal horn neurons by low-threshold afferent stimulation *in vivo*.⁶⁶ Preliminary results obtained in our laboratories indicate that the GABA_A agonist muscimol is able to modulate glutamate release from Aβ fibers onto rat spinal lamina III neurons and increase the paired pulse ratio of evoked EPSCs. Thus, synaptic depression at central terminals of PAFs is modified by receptor-mediated presynaptic inhibition. This could, in turn, affect the firing pattern of dorsal horn neurons and the sensory coding process. A characterization of the postsynaptic neurons involved in this mechanism (i.e., inhibitory versus excitatory interneurons) would also be critical in understanding the organization and function of presynaptic inhibition in the dorsal horn.

Another important consideration is how presynaptic modulation is affected by the plastic changes that occur during chronic pain. Some studies suggest that peripheral inflammation induces an increase of PAD on PAFs (both nociceptive and nonnociceptive). Application of inflammatory agents on DRGs induces a rapid increase of intracellular Cl⁻ concentration through the upregulation of the NKCC1 transporter.⁶⁷ After persistent peripheral inflammation, GABA-induced depolarization on DRGs increases, partially due to the inhibition of voltage-dependent potassium currents⁴⁰ and to enhanced GABA_A receptor function⁶⁷ in addition to elevated intracellular chloride. The potentiation of PAD during inflammatory pain could contribute to hyperalgesia by enhancing dorsal root reflexes in nociceptors.^{39,68,69}

Modulation of PAD on low-threshold afferents could be involved in the generation of allodynia. If glutamate release from low-threshold mechanoreceptors increased due to augmented excitability of presynaptic terminals, this could help drive the excitatory polysynaptic pathway revealed with use of GABA_A and glycine receptor antagonists, as proposed by Torsney and MacDermott (Fig. 3). However, enhanced PAD could also mediate enhanced presynaptic inhibition. Correspondingly, a recent study has proposed an opposite role for PAD in inflammatory pain.⁴⁹ Mice lacking benzodiazepine-sensitive α2-GABA_A receptors in primary nociceptors showed reduced antihyperalgesia in response to intrathecally injected diazepam in an inflammatory pain model. These results suggest that facilitation of GABA_A receptor activation on spinal nociceptor terminals could exert an analgesic action. A more extensive characterization of the dorsal horn synaptic circuits and neuronal types involved in PAD and presynaptic inhibition will be important to clarify these discrepancies and understand the role of presynaptic GABA_A receptors in chronic pain.

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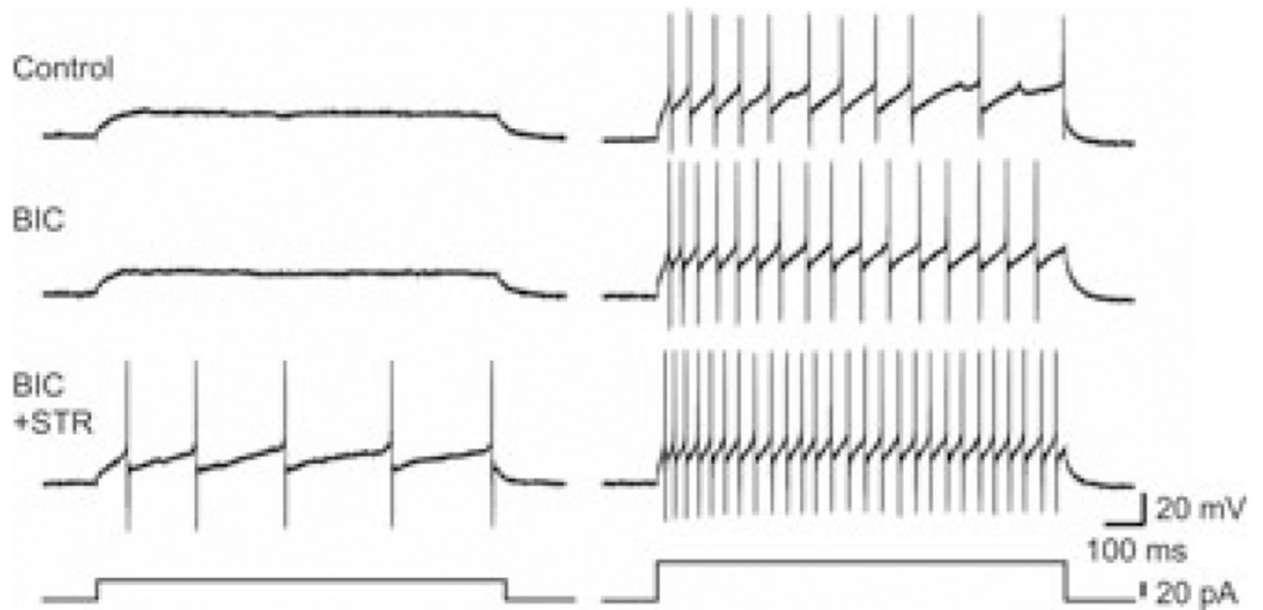


Figure 1. Blockade of GABA_A and glycine receptors with bicuculline and strychnine enhances excitability of inhibitory neurons in mouse dorsal horn. Shown are examples of action potentials induced by current injection under three conditions: (1) control; NBQX [10 μ M] and AP5 [50 μ M]; (2) BIC; NBQX, AP5, and bicuculline [10 μ M]; and (3) BIC+STR; NBQX, AP5, bicuculline, and strychnine [1 μ M]. Resting membrane potential was kept at -65 mV. Left panel shows responses to a small current injection in the three conditions, and the right panel shows responses to a larger current injection. Bottom traces show injected currents. Taken with permission from Ref. 15.

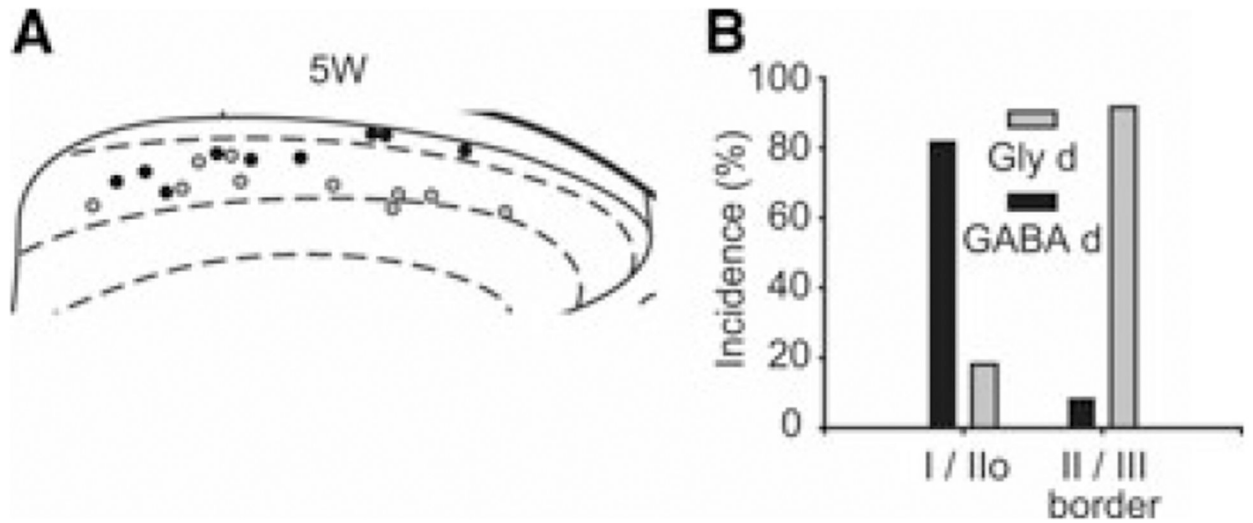


Figure 2.

GAD67-eGFP⁺ neurons have regionally distinct properties of synaptic inhibitory input. Part A shows soma locations of glycine-dominant (Gly-d, gray circles) and GABA-dominant (GABA-d, black circles) neurons recorded from post-natal day 29–32 (5W) mice. Right and upper sides of the schematic diagram show lateral and dorsal edges of the dorsal horn, respectively. Laminae I, II, and III are separated by dotted lines. (B) Gly-d neurons are the major population at the lamina II/III border ($n = 12$), while GABA-d neurons (black bars) are the major population in lamina I and Ilo ($n = 11$). Bars indicate the incidence of neurons located in laminae I/Ilo, and at the laminae II/III border. Taken with permission from Ref. 15.

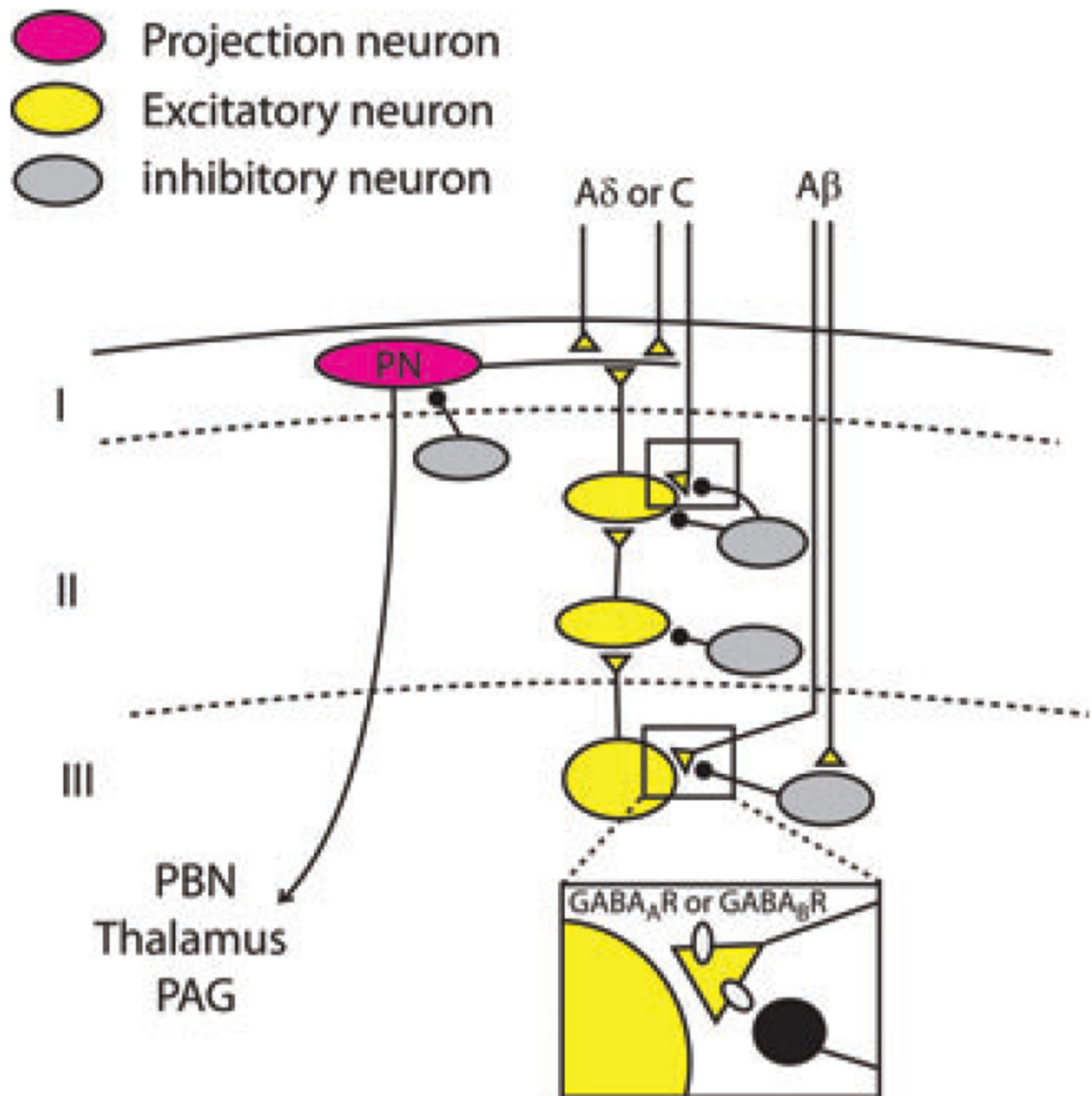


Figure 3. Schematic representation of a proposed polysynaptic excitatory pathway connecting lamina III to lamina I projection neurons. This pathway is normally under powerful inhibitory control mediated by GABA and glycine released from inhibitory neurons. Presynaptic inhibition mediated by GABA_A or GABA_B receptors in the spinal cord dorsal horn is illustrated in detail for the A β fiber synaptic terminal onto a lamina III neuron, but also occurs on nociceptor PAF terminals. Stimulation of cutaneous tactile fibers induces GABA release from inhibitory interneurons, causing the activation of GABA_A or GABA_B receptors expressed on primary afferent terminals and the inhibition of glutamate release onto lamina III–IV neurons.