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## Neuronal nicotinic receptors as analgesic targets: It's a winding road

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### Abstract

Along with their well known role in nicotine addiction and autonomic physiology, neuronal nicotinic receptors (nAChRs) also have profound analgesic effects in animal models and humans. This is not a new idea, even in the early 1500s, soon after tobacco was introduced to the new world, its proponents listed pain relief among the beneficial properties of smoking. In recent years, analgesics that target specific nAChR subtypes have shown highly efficacious antinociceptive properties in acute and chronic pain models. To date, the side effects of these drugs have precluded their advancement to the clinic. This review summarizes the recent efforts to identify novel analgesics that target nAChRs, and outlines some of the key neural substrates that contribute to these physiological effects. There remain many unanswered mechanistic questions in this field, and there are still compelling reasons to explore neuronal nAChRs as targets for the relief of pain.

### Keywords

Nicotinic; Pain; Nociception; Analgesia

## 1. Introduction

The last two decades have seen an increase in the use of opioid drugs for the treatment of chronic non-cancer pain in the United States. Although opioid analgesics are highly efficacious [1], the associated side effects limit their usefulness [2]. In addition, opioids have high abuse liability [3]. Although the majority of chronic pain patients do not abuse prescription opioids [3], as noted by Compton and Volkow, the increases in opioid pain prescriptions and opioid abuse have been correlated [4]. Thus, there is a strong need for effective chronic pain treatments that lack the side effects of opioids.

### 1.1. Nicotine and analgesia

Nicotinic acetylcholine receptors (nAChRs) may represent viable targets for novel analgesics. Although the pain-relieving properties of nicotinic acetylcholine receptors (nAChRs) are relatively recent findings, the link between nicotine and analgesia is not new.

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Oviedo y Valdes, a Spanish historian, first reported that tobacco relieved pain caused by syphilis in the early 16th century [5]. However, nicotine, a major constituent of tobacco, was not linked with analgesia until the 20th century when nicotine application was found to produce antinociception in cats [6]. More recent research has revealed that nicotine microinjection into various brainstem regions could be either pronociceptive or antinociceptive [7]. In addition, nicotine-induced antinociception can be blocked by mecamylamine, a nonselective neuronal nAChR antagonist [8]. The analgesic effect of nicotine has also been replicated in humans, as nicotine reduces acute pain experienced from a cold pressor test in both smokers and ex-smokers [9]. Although these findings supported developing nicotine as an analgesic, evidence showing its addictive effects raised concern [10,11]. Furthermore, repeated administration of nicotine induces tolerance to antinociceptive effects in rats [12]. Despite these concerns, the focus on nAChRs as analgesic targets has become an active area of investigation.

## 1.2. nAChR-mediated antinociception

In 1974, while conducting experiments in Ecuador, John Daly and Charles Myers found that a frog skin extract from *Phyllobates anthonyi* induces a Straub tail response, indicative of opioid expression in the extract [13,14]. However, Daly later showed naloxone, an opioid antagonist, did not block the extract-induced Straub-tail response [13,15]. Eventually the active compound, known as epibatidine [15], was isolated and subsequently shown to induce nAChR-mediated antinociception in rodents [16,17]. Epibatidine is a nAChR agonist that binds to several nAChR subtypes, including  $\alpha 7$ ,  $\alpha 4\beta 2$ , and the neuromuscular  $\alpha 1\beta 1\delta\gamma$  subtype [18]. Antinociceptive efficacy of epibatidine is similar to morphine with 100-fold higher potency in the hot-plate test [15]. Although epibatidine displays promising antinociceptive effects, it also induces adverse side effects, including dose-dependent decreases in body temperature and locomotor activity [16]. In addition, epibatidine increases blood pressure in rodents, which may show sensitization, as repeated administration can transform nonlethal doses into lethal doses [18]. The nonselective nAChR agonist properties of epibatidine raise the possibility that distinct subsets of nAChRs mediate its antinociceptive vs. adverse effects [19].

## 2. nAChRs as analgesic targets

### 2.1. $\alpha 4\beta 2$ nAChRs

Researchers at Abbott labs initially focused on  $\alpha 4\beta 2$  nAChR agonists with the goal of developing treatments for Alzheimer's disease [13,14]. However, they discovered structural similarities between their candidate drugs and epibatidine [13]. This led to the development of ABT-594 [13], a compound closely related to epibatidine, with higher selectivity for  $\alpha 4\beta 2$  nAChRs [19]. ABT-594 produces antinociception that is more potent than either nicotine or morphine, with equal or higher efficacy in multiple preclinical pain models (acute and chronic) [19–21]. Similar to the antinociceptive effects of nicotine and epibatidine, ABT-594-induced antinociception is also blocked by mecamylamine [19,20]. Furthermore, ABT-594 does not perturb the cardiovascular system, particularly when compared to epibatidine [22], and does not cause signs of physical dependence in mice. Thus, the preclinical data supported the view that ABT-594 could be a promising therapeutic drug for

chronic pain patients. However, drug development was hindered by phase II clinical trial findings. Low doses of ABT-594, which were well-tolerated by the patients, did not have significant analgesic effects [23]. Higher ABT-594 doses (150 to 300  $\mu\text{g}$ ) significantly reduced pain, however this was accompanied by significant adverse effects (e.g. nausea, dizziness, vomiting) relative to placebo among patients in three different treatment groups for neuropathic pain [23]. Furthermore, patient withdrawal frequency directly correlated with increasing dose [23]. Despite analgesic efficacy comparable to opioid drugs, the prevalence and severity of adverse effects precluded further ABT-594 drug development.

Despite this setback, targeting a specific nAChR subtype may retain the analgesic properties of ABT-594 or epibatidine without the problematic side effects. Two approaches come to mind, if we focus on the major nAChR subtypes: either target non- $\alpha 4\beta 2$  nAChR subtypes, or optimize  $\alpha 4\beta 2$  nAChR activation to minimize the adverse effects. A recent study reported that intra-cerebroventricular (i.c.v.) administration of a selective  $\alpha 4\beta 2$  agonist reduced the allodynic effects of splanchnic nerve ligation, which was attributed to nAChR binding in thalamus [24]. Antiallodynic effects are seen with a 5 nmol injection, but not a 50 nmol injection, which supports the idea that receptor desensitization can reverse therapeutic effects at high agonist doses.

## 2.2. $\alpha 5$ -containing and $\alpha 3\beta 4$ nAChRs

Residual nAChR-mediated analgesia occurs in mice lacking the  $\alpha 4$ , or  $\beta 2$  nAChR subunit [25]. Therefore, other nAChR subtypes likely contribute to this behavior. Potential candidates include  $\alpha 5$ -containing nAChRs,  $\alpha 3\beta 4$ , and  $\alpha 7$  homomeric receptors. The  $\alpha 5$  subunits do not form functional receptors alone or in heteromeric complexes with beta subunits. They can be incorporated with  $\alpha 4\beta 2$ ,  $\alpha 3\beta 4$ , or  $\alpha 3\beta 2$  nAChR subtypes, where they alter both pharmacology and physiology of these receptor complexes [26–28]. Mice that lack the  $\alpha 5$  subunit display lower antinociceptive potency and efficacy of nicotine relative to wildtype animals [29]. Furthermore, epibatidine-induced antinociception is completely absent in  $\alpha 5$ -KO mice [29]. However, more work is needed to evaluate the contribution of the  $\alpha 5$  nAChR subunit to antinociception in other preclinical models of pain. The  $\alpha 3\beta 4$  nAChR subtype is highly expressed in autonomic ganglia, and a recent study demonstrated that the selective antagonist conotoxin AuIB, has no effect on nicotine-mediated antinociception [30].

## 2.3. $\alpha 7$ nAChRs

In addition to  $\alpha 4\beta 2$ , the  $\alpha 7$  homomeric nAChR subtype is another target for analgesia. Systemic and intrathecal injection of choline, an  $\alpha 7$  nAChR selective agonist, produces antinociception [31–33]. There is also evidence for an analgesic role for supraspinal  $\alpha 7$  containing nAChRs as injection of a choline analog i.c.v. produces antinociception [34,35]. Potential sites of action for  $\alpha 7$ -mediated analgesia are discussed in more detail below.

Despite these encouraging results, there are mixed reports on  $\alpha 7$  nAChR-mediated analgesia. In recent findings by Gao et al. [36] administration of some  $\alpha 7$  agonists result in antinociception, while others have no effect. Animals treated with Compound Q ((S)-5-(pyridin-2-yl)-N-(quinuclidin-3-yl)thiophene-2-carboxamide), a selective  $\alpha 7$  nAChR

agonist, display antinociception at 50 mg/kg, which is intriguing as Compound Q does not penetrate the blood brain barrier [36]. Thus, peripheral  $\alpha 7$  nAChRs could contribute to  $\alpha 7$ -mediated analgesia.

A subset of  $\alpha 7$  nAChR agonists also antagonize spinal 5-HT<sub>3</sub> receptor activity [37,38]. After inflammation or injury, 5-HT<sub>3</sub> receptor activation contributes to nociceptive facilitation via supraspinal descending pathways [39,40]. Considering that tropisetron, an  $\alpha 7$  agonist in this subset, has shown clinical efficacy in fibromyalgia patients [41], the activation of  $\alpha 7$  nAChRs along with inhibition of 5-HT<sub>3</sub>Rs, could lead to a synergistic antinociceptive effect. In addition,  $\alpha 7$  activation inhibits serotonin release in the dorsal spinal cord [42], which could further reduce 5-HT signaling and potentially enhance antinociception [43].

#### 2.4. nAChR positive allosteric modulators

An alternative approach to activating nAChRs is to utilize positive allosteric modulators (PAMs), molecules that enhance agonist-evoked response by reducing energy required for a receptor to move from resting to active state [44]. PAMs can be classified into two categories: Type I that enhance receptor function but do not affect desensitization properties, and Type II that reduce receptor desensitization, thereby prolonging the receptor activation [44]. PNU-120596, a Type II  $\alpha 7$  PAM, reduces nociceptive behavior more effectively than NS-1738, a Type I  $\alpha 7$  PAM, in a variety of preclinical nociceptive tests 6 hours post-injury [45]. This observation suggests that endogenous cholinergic signaling induces significant receptor desensitization and that limiting the transition to that state can inhibit nociceptive signaling. Not surprisingly, co-application of PNU-120596 with an  $\alpha 7$  nAChR agonist enhanced the antinociceptive effects relative to either the PAM or  $\alpha 7$  agonist alone [32]. In addition, systemic injection of MLA, an  $\alpha 7$ -selective antagonist reverses  $\alpha 7$  PAM – mediated antinociception [45]. These data suggest that combined  $\alpha 7$  Type II PAM and agonist application could be an effective treatment for inflammatory pain.

Another useful approach involves developing novel drugs that target  $\alpha 4\beta 2$  nAChRs more selectively, to potentially minimize adverse effects caused by ABT-594. The advantage with this approach is the analgesic effects of  $\alpha 4\beta 2$  receptors has already been demonstrated in humans [23]. A-366833 is a recently developed  $\alpha 4\beta 2$ -selective agonist that produces substantial antinociceptive effects in acute, neuropathic and inflammatory models of pain [46,47]. Furthermore, it does not induce emesis in ferrets at concentrations well above the therapeutic dose (ferrets are excellent animal models for emesis) [47]. These findings contrast with observations of Gao et al. [36] who found no antinociceptive effects of systemic administration of the selective  $\alpha 4\beta 2$  agonist, ispronicline in preclinical pain models.

PAMs provide an alternate strategy for activating  $\alpha 4\beta 2$  nAChRs. Recent findings demonstrate the therapeutic potential of a combined administration of a Type 1 PAM, NS9283 with ABT-594 [48]. NS9283 increases acetylcholine currents without affecting desensitization kinetics [49]. In preclinical pain models, NS9283 enhances ABT-594 potency [48,50]. In addition, NS9283 does not shift emetic threshold [48]. This is important, as it shows that the combination of ABT-594 and NS9283 increases the therapeutic window for analgesic effects of both drugs. Gao et al. [36] tested a different PAM, Compound 2B,

and found a decrease in nociceptive behaviors, albeit at extremely high doses [36]. Combined with the observation that NS9283 administration does not reduce mechanical allodynia in the spinal nerve ligation model [48], these results raise concerns about the therapeutic efficacy and potency for PAM-only administration. However, a PAM-plus-agonist approach could be therapeutically effective for multiple nAChR subtypes, including in  $\alpha 7$  and  $\alpha 4\beta 2$  nAChRs.

### 3. Neural substrates for nAChR-dependent analgesia

Despite the pharmacological and behavioral evidence supporting nAChR-mediated analgesia, remarkably little is known about its underlying cellular mechanisms. Nicotinic receptors are found along ascending as well as descending nociceptive pathways and have been especially well characterized in the spinal cord dorsal horn and in sensory nerve afferents. In addition to the spinal cord, supraspinal areas are also critical sites of action for nicotinic analgesics, yet we know even less about the contribution and underlying mechanisms of nAChRs in these areas.

#### 3.1. Peripheral nAChRs

Nicotinic receptors are at the periphery at sites of injury where inflammation is initiated. Dorsal root ganglia (DRG) neurons predominantly express  $\alpha 7$  nAChRs, which are especially prevalent in large diameter cells in neonates. This likely includes both A $\delta$ , A $\beta$  populations carrying nociceptive and non-nociceptive sensory information [51,52]. Here,  $\alpha 7$  nAChRs enhance glutamate release probability and increase the likelihood of LTP in the dorsal horn [53]. Compared to neonates,  $\alpha 7$  nAChR-expressing DRGs in older rats are exclusively found in unmyelinated (C fiber) nociceptors, although these likely include multiple nociceptive modalities [54]. Among these are both peptidergic and nonpeptidergic neurons, including some that bind IB4 and co-express  $\alpha 3\beta 4\alpha 5$  [54] (Fig. 1).

In contrast to C fibers, myelinated nociceptors do not express  $\alpha 7$ , but rather contain  $\alpha 3\beta 4\alpha 5$  or  $\alpha 3\beta 4$  nAChR subtypes [54]. Human unmyelinated nociceptor axons contain nAChRs that increase excitability with pharmacology suggesting the  $\alpha 3\beta 4\alpha 5$  subtype. These nAChRs are pronociceptive because nAChR antagonist mecamylamine and amitriptyline, a drug used to treat neuropathic pain, both inhibit nicotine-induced enhancement of excitability in a similar manner [55,56]. These  $\alpha 3\beta 4\alpha 5$  expressing sensory neurons are likely to underlie the reported pronociceptive effects of  $\alpha 5$  subunit [57] (Fig. 1).

Peripheral (intraplantar) application of nicotinic agonists selective for the  $\alpha 4\beta 2$  or  $\alpha 7$  subtype is antinociceptive [19,58,59]. Nicotinic receptors on peripheral terminals may contribute to these effects, although direct evidence for this is lacking. This antinociceptive effect is surprising, given the excitatory effects of activating  $\alpha 7$ ,  $\alpha 3\beta 4\alpha 5$  and  $\alpha 3\beta 4\alpha$  nAChRs on nociceptive DRG neurons that support a pronociceptive role. The mechanism for this disparity is not clear and requires further study.

Especially with respect to  $\alpha 7$  antinociception, non neuronal cells at the periphery are also likely to contribute to antinociception after injury or inflammation. In nerve injury or inflammatory pain models,  $\alpha 7$ -mediated antihyperalgesia is associated with a decrease in the

immune response at the periphery [60–62]. Cytokine release is inhibited by  $\alpha 7$  activation on macrophages [63]. In addition to macrophages, other non neuronal peripheral cells such as keratinocytes and lymphocytes also express  $\alpha 7$  and their anti-inflammatory effects likely contribute to reported antinociception [64]. These anti-inflammatory effects would further support therapeutic strategies aimed at  $\alpha 7$  nAChRs, but further investigation is needed as to the extent of immune suppression in the long term.

Another nAChR subtype expressed in the periphery is the  $\alpha 9/\alpha 10$  subtype. DRG neurons as well as lymphocytes express  $\alpha 9/\alpha 10$  [65,66]. Alpha-conotoxins RgIA and Vc1.1 are highly selective antagonists to this subtype compared to other nAChRs. Although efficacy in neuropathic pain models is demonstrated, clinical drug development is hindered by controversy over mechanism of action. Activation of presynaptic GABA<sub>B</sub> receptors on sensory afferents leads to N-type Ca<sup>2+</sup> inhibition, which also contributes to the analgesic mechanism [67]. Interestingly, RgIA is 300-fold less potent for human  $\alpha 9/\alpha 10$  nAChRs than those of the rat [68]. Nevertheless, RgIA and Vc1.1 still hold promise as analgesics due to their independent effects on GABA<sub>B</sub> receptors and anti-inflammatory effects [69].

### 3.2. Spinal cord dorsal horn

Intrathecal nicotine or epibatidine produce dose-dependent antinociception in the tail flick test [31,70]. These findings are contradicted by other reports either demonstrating no effect on acute nociception with intrathecal nicotine or epibatidine [71,72] algogenic responses [73], or antinociception only after nerve injury [74]. These mixed results from intrathecal administration reflect the diversity of nAChR subtypes and their effects among incoming sensory afferents and within spinal circuitry.

In the neonate, a subset of dorsal horn (DH) neurons contain somatic  $\alpha 7$  nAChRs, and an equal number contain non- $\alpha 7$  nAChRs, with very little overlap of these nAChR subtypes. Local excitatory DH neurons as well as NK1 receptor-positive projection neurons have transcripts for  $\alpha 3$ ,  $\beta 2$ , and  $\alpha 7$  with associations between the three transcripts in the mouse [75], which supports a pronociceptive role for spinal  $\alpha 7$  in the neonate. However, these findings have not been confirmed in the adult. In fact, in adult mice, intrathecal agonists to  $\alpha 7$  nAChRs are antinociceptive in both acute and persistent inflammatory models [32,76]. Furthermore,  $\alpha 7$  antinociception is demonstrated in a model of colitis [77]. Thus, the preponderance of evidence supports the idea that  $\alpha 7$  nAChRs on spinal neurons are antinociceptive, not pronociceptive (Fig. 1).

In contrast to sensory afferents, local inhibitory interneurons in the spinal cord dorsal horn contain transcripts for  $\alpha 4\beta 2$  nAChRs [75], and express functional  $\alpha 4\beta 2$  nAChRs at synaptic terminals where they enhance GABAergic drive onto DH neurons, possibly contributing to analgesic effects of nicotine [78]. Activation of these  $\alpha 4\beta 2$  nAChRs also reduces stimulus-driven LTD of inhibitory inputs, thereby preserving normal balance of inhibitory drive to prevent pathological excitation [78].

In the spinal cord glycinergic transmission is another key inhibitory regulator of nociception. Both GABA and glycinergic neurons contain transcripts for  $\alpha 4\beta 2$  nAChRs [75]. Glycinergic transmission is enhanced by presynaptic  $\alpha 4\beta 2$  nAChRs in spinal slices and in mechanically



dissociated spinal lamina II neurons [79]. However, spinal  $\alpha 4\beta 2$  nAChRs are not merely analgesic targets, but actually play an important role in maintaining normal sensory thresholds. GABAergic and glycinergic drive is tonically enhanced by endogenous acetylcholine [80] (Fig. 1). Furthermore, mechanical and thermal thresholds are lower in  $\beta 2$  knockout mice [81].

It is possible that other nAChR subtypes exist in spinal circuitry that are yet to be identified. In the adult rat, nAChRs have been demonstrated on inhibitory terminals in the superficial dorsal horn that are not sensitive to selective antagonists of  $\alpha 4\beta 2$  or  $\alpha 7$  subtypes [82]. Other subunits found among spinal inhibitory neurons whose nociceptive impact is not well-understood include  $\alpha 6$  or  $\alpha 5$ , which may co-assemble with  $\alpha 4\beta 2$  nAChRs [75]. In addition, a subset of inhibitory neurons contain transcripts for the  $\alpha 2$  subunit, a potentially promising therapeutic target, since these may be rare in supraspinal areas.

### 3.3. Supraspinal nAChRs

In addition to local spinal inhibitory influences, rostral (supraspinal) areas also exert strong influences on nociception and some of these areas contain nAChRs. The brainstem rostral ventromedial medulla (RVM) is the proposed site of action for the analgesic effects of the selective  $\alpha 4\beta 2$  agonist, ABT-594, as the effects are nearly abolished in  $\alpha 4$  or  $\beta 2$  null mice [25] and are accompanied by increased c-fos expression in RVM neurons [19,83]. It is the activation of  $\alpha 4\beta 2$  in the RVM, not its desensitization that produces antinociception, as the effect is blocked by pretreatment with the nAChR antagonist mecamylamine, but the antagonist alone had no effect [19].

Despite these compelling findings, the cellular mechanisms of nAChR analgesia in the RVM are not well understood. Serotonin signaling is implicated in these effects, as  $\alpha 4$  immunostaining overlaps with markers for serotonin in the RVM, and  $\alpha 4$  staining is reduced after selective ablation of serotonergic neurons [83,84]. We do not know how serotonergic RVM neurons exert these effects. In addition, it is clear that  $\alpha 4\beta 2$  on serotonergic neurons in the dorsal raphe as well as noradrenergic cell groups also contribute to systemic effects of ABT-594 [83,84] (Fig. 2). Finally, a spinal  $\alpha 4\beta 2$  contribution after nerve injury cannot be ruled out [74,80,81,85].

In addition to  $\alpha 4\beta 2$  nAChRs, local inhibitory RVM neurons can express  $\alpha 7$  nAChRs and the role these play in nociceptive modulation is not known [86]. The RVM exerts complex influences on nociception and can actually facilitate nociception, in part via serotonergic projections [40,87,88]. Spinal serotonin release via descending projections is also modulated by multiple subtypes of nAChRs residing on serotonergic terminals and their axo-axonal inputs from local interneurons [42]. Further study is needed to determine exactly how brainstem nAChRs fit into nociceptive facilitatory vs. inhibitory pathways and how this changes after injury.

Intra-cerebroventricular administration of choline, an  $\alpha 7$  nAChR selective agonist, is antinociceptive in acute, inflammatory and neuropathic pain and an  $\alpha 7$ -selective antagonist blocks these effects [33–35,89]. Thus supraspinal  $\alpha 7$  nAChRs are antinociceptive, but the sites of action are not known. A strong candidate is the midbrain periaqueductal gray (PAG),

which relays information from limbic and homeostatic centers (hypothalamus and amygdala) to the spinal cord via the RVM. The PAG contains nAChRs including  $\alpha 7$  and locally administered nicotinic agonists produce antinociception [90,91]. Interestingly, our recent findings suggest that  $\alpha 7$  nAChRs are expressed on a distinct population of PAG-RVM projection neurons from those expressing somatic  $\mu$  opioid receptors (unpublished material). In dissociated PAG neurons  $\alpha 4\beta 2$  nAChRs on GABAergic terminals enhance release [92]. It will be important to confirm this in older animals and identify the neurons affected. In addition, it is unknown whether excitatory synaptic transmission is also modulated by nicotinic receptors in this area. Although there is evidence for  $\alpha 2$  nAChR subunit mRNA in the PAG a nociceptive role for these has not been demonstrated [93]. We know surprisingly little about the distribution of nicotinic subtypes in the PAG, and whether they function to modulate descending facilitatory or inhibitory nociceptive pathways.

#### 4. Conclusions

In summary, nAChRs mediate antinociception via various receptor subtypes distributed among multiple central and peripheral nociceptive areas. This diversity of neural substrates and nAChR subtypes provides a rich environment for analgesic drug development. This richness also carries cost, as unwanted side effects have sidelined some of the strongest candidate nicotinic analgesic drugs. Perhaps the combination of agonists with selective positive allosteric modulators will provide an efficacious strategy for targeting specific receptor populations and optimizing their physiological impact. Although our knowledge base has improved in recent years, the limits of our understanding about the sites of action and cellular mechanisms involved in nicotinic analgesia is remarkable, particularly in supraspinal areas. Successful analgesic drug development will require better understanding of these neural circuits, how they are modified by chronic pain, and how nAChRs can be exploited to suppress pain signaling.

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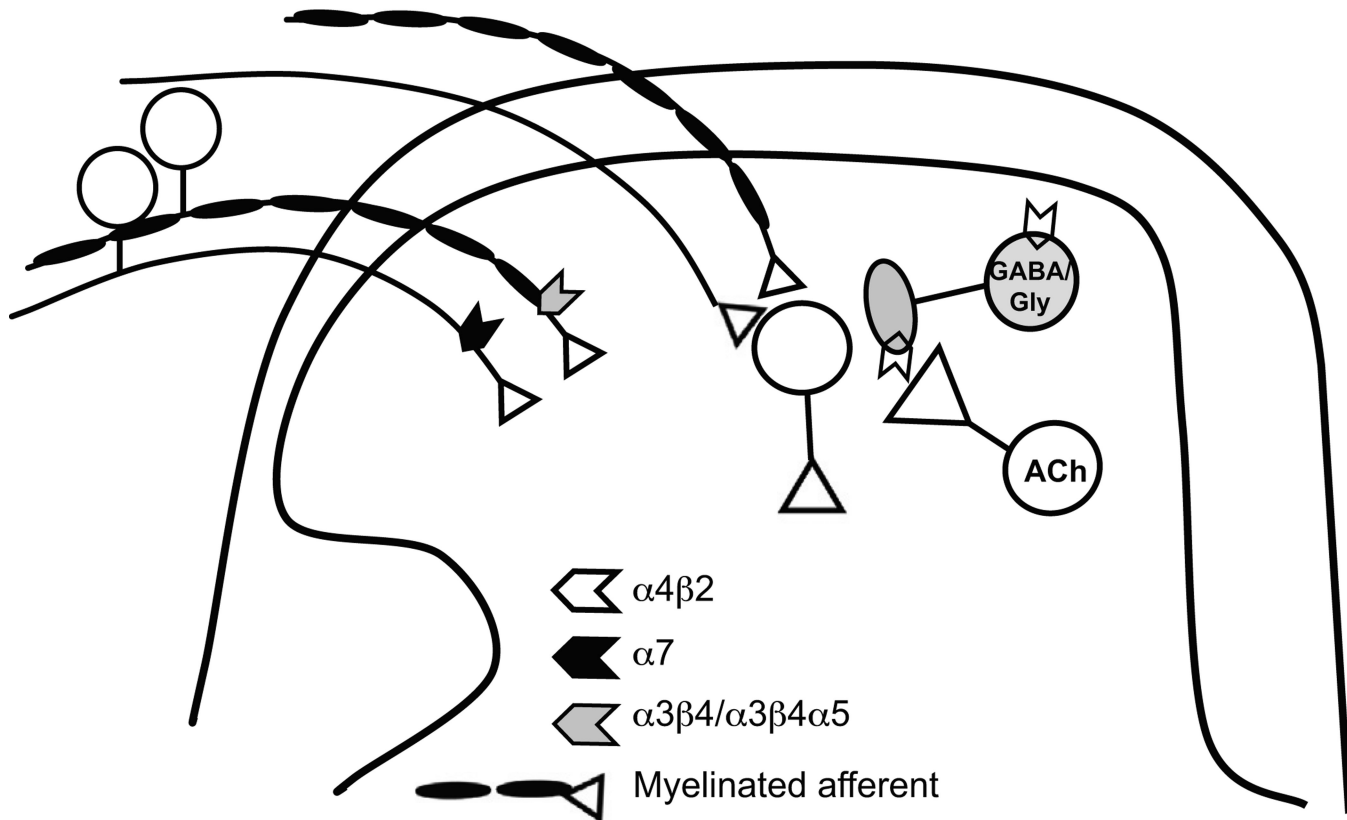
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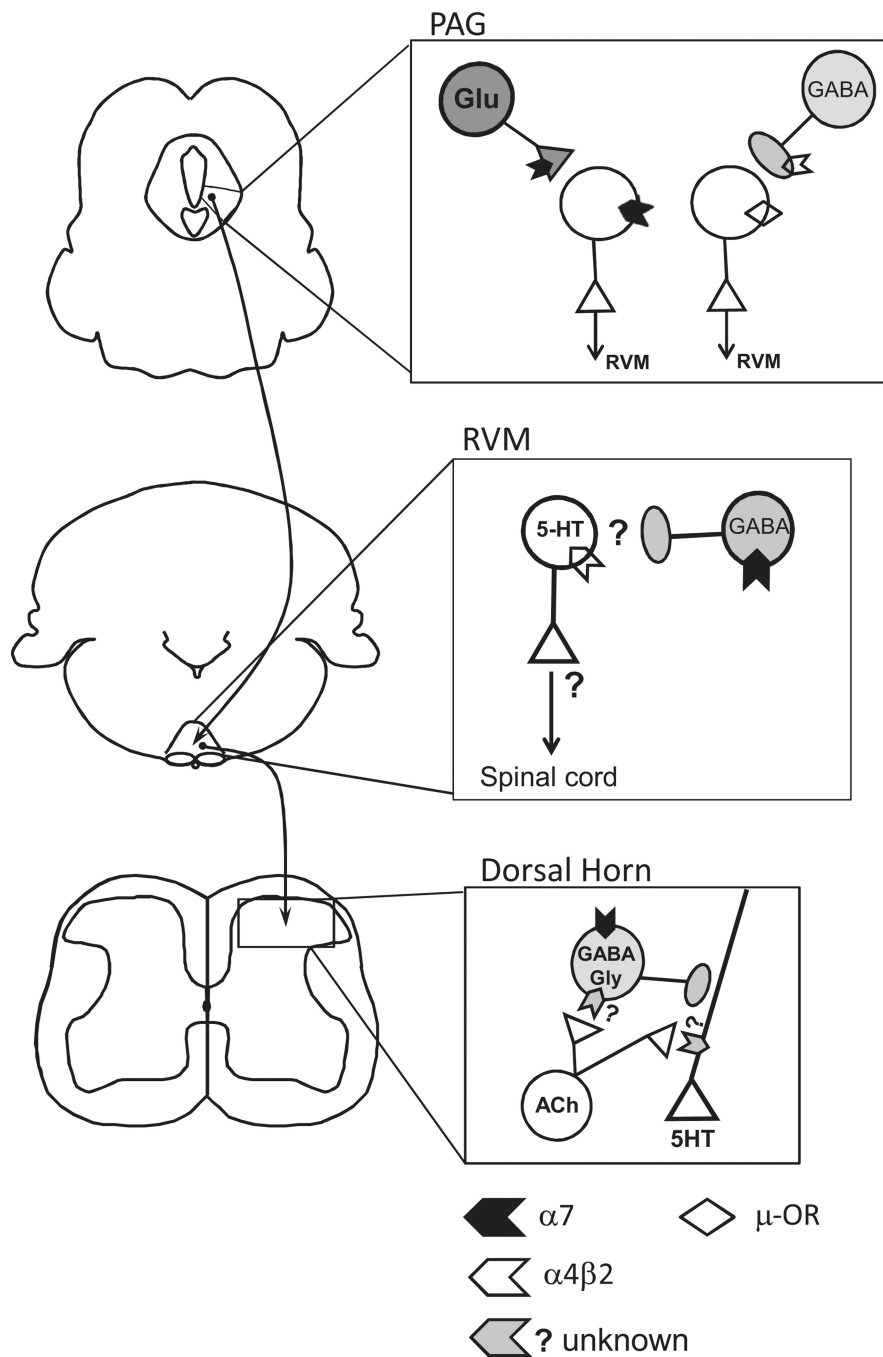
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# Nociceptive afferents



**Fig. 1.** Nicotinic receptors modulate ascending nociceptive information. Myelinated nociceptive inputs to the spinal cord dorsal horn express  $\alpha 3\beta 4/\alpha 3\beta 4\alpha 5$  nAChRs, while unmyelinated nociceptive afferents express  $\alpha 7$  nAChRs [54,55]. Both may act to enhance nociception. By contrast, tonic cholinergic modulation of GABA and glycine transmission via  $\alpha 4\beta 2$  nAChRs is important for setting baseline inhibitory tone and the influence of incoming nociceptive activity.





**Fig. 2.** Nicotinic receptors modulate descending pain modulatory pathways. PAG: Our recent finding indicates that a subset of ventrolateral PAG (vlPAG) neurons express  $\alpha 7$  nAChRs on their somata ( $\alpha 7$ ), and that this population was distinct from neurons that express  $\mu$ -opioid receptors ( $\mu$ -OR). Specifically, 50% (33/69) of vlPAG projection neurons responded to acetylcholine (ACh). Responses were blocked by the  $\alpha 7$ -selective antagonist MLA ( $n = 15$ ). In a separate experiment, we observed 4 response classes among vlPAG projection neurons: nAChR-only responses (8/41).  $\mu$ -OR– response accompanied by weak/no nAChR response

(13/41), both nAChR and  $\mu$ -OR responses (4/41), or no response of either type (16/41) (unpublished observations). In addition, activation of presynaptic  $\alpha 7$  nAChRs increased glutamatergic drive to nAChR+neurons that project to the RVM. By contrast, nicotine-induced modulation of GABAergic inputs to nAChR+vIPAG projection neurons was rare. In the right panel, we hypothesize that presynaptic nAChRs modulate inhibitory drive to a majority of vIPAG projection neurons that lack the somatic nAChR. The presynaptic nAChR that mediates this phenomenon has not been characterized but is likely to be  $\alpha 4\beta 2$ , based on previous work in the PAG [92]. We propose that presynaptic and somatic  $\alpha 7$  nAChRs contribute to excitation of putative pain-inhibiting neurons in the vIPAG. This could be a supraspinal mechanism for  $\alpha 7$  nAChR-mediated analgesia. RVM: Although the RVM is a well-documented site for supraspinal analgesia, the underlying circuitry is still not well-understood. Immunohistochemistry studies show that  $\alpha 4\beta 2$  nAChR subtype and 5-HT markers are co-localized [83]. However, electrophysiological approaches have not verified this finding. In addition, the specific projection targets of  $\alpha 4\beta 2$ -containing 5HT RVM neurons are not known, particularly within the context of descending pain modulatory pathways.  $\alpha 7$  nAChRs are expressed on GABA interneurons in the RVM [86]. Although the RVM is implicated in nicotinic analgesia via behavioral studies, more work is needed to investigate the cellular mechanisms at the circuit level. Spinal Dorsal Horn: Tonic endogenous cholinergic tone modulates serotonin release from descending inputs, which is mediated by nAChRs that are neither  $\alpha 4\beta 2$  nor  $\alpha 7$ . These receptors are located on descending serotonergic inputs, as well as on neighboring GABAergic interneurons that make axo-axonic contacts with serotonergic terminals. Additional modulation of 5HT release via a non-tonic mechanism is mediated by  $\alpha 7$  nAChRs located on the somata of GABAergic interneurons that make axo-axonic contacts with descending serotonergic input. The impact of this modulation on nociception in normal or pathological states needs further investigation (modified from [42]).