Send Orders for Reprints to reprints@benthamscience.ae

Current Neuropharmacology, **2018**, *16*, **415-425**

REVIEW ARTICLE

New Insights on Neuronal Nicotinic Acetylcholine Receptors as Targets for Pain and Inflammation: A Focus on α7 nAChRs

Deniz Bagdas[a,b,*], Mine S. Gurun[c], Pamela Flood[d], Roger L. Papke[e] and M. Imad Damaj[a]

[a]*Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, Virginia, USA;* [b]*Experimental Animals Breeding and Research Center, Faculty of Medicine, Uludag University, Bursa, Turkey;* [c]*Department of Pharmacology, Faculty of Medicine, Uludag University, Bursa, Turkey;* [d]*Department of Anesthesiology, Perioperative and Pain Medicine, Stanford University, Palo Alto, California, USA;* [e]*Department of Pharmacology and Therapeutics, University of Florida, Gainesville, Florida, USA*

ARTICLE HISTORY

Received: May 04, 2017
Revised: June 20, 2017
Accepted: August 16, 2017

DOI:
10.2174/1570159X15666170818102108

Abstract: ***Background*:** Nicotine and nicotinic acetylcholine receptors (nAChRs) have been explored for the past three decades as targets for pain control. The aim of this review is to introduce readers particularly to α7 nAChRs in a perspective of pain and its modulation.

***Methods*:** Developments for α7 nAChR modulators and recent animal studies related to pain are reviewed.

***Results*:** Accumulating evidences suggest that selective ligands for α7 nAChRs hold promise in the treatment of chronic pain conditions as they lack many of side effects associated with other nicotinic receptor types.

***Conclusion*:** This review provides the reader recent insights on α7 nAChRs from structure and function to the latest findings on the pharmacology and therapeutic targeting of these receptors for the treatment of pain and inflammation.

Keywords: α7 nicotinic acetylcholine receptors, nicotine, pain, inflammation, modulators, anti-inflammatory, agonists.

1. INTRODUCTION

Nicotine and other ligands that act on nicotinic acetylcholine receptors (nAChR) have been explored for the past three decades as a strategy for pain control. These receptors are widely expressed throughout the central and peripheral nervous system as well as on immune cells. Despite encouraging results with many selective α4β2* agonists in animal models of pain, human studies showed a narrow therapeutic window between analgesic efficacy and toxicity associated with the use of these agonists as analgesics (For a recent chapter see [1]). However, several recent developments have potentially opened new windows of opportunity in the use of nicotinic agents for analgesia. α7 nAChR agonists have shown to be beneficial for central nervous system disorders characterized by cognitive deficits, such as Alzheimer's disease and schizophrenia [2]. In addition, accumulating evidences suggest that agonists and modulators of nicotinic receptors that contain the α7 subunit hold promise for the treatment of chronic inflammatory pain conditions.

*Address correspondence to this author at the Department of Pharmacology and Toxicology, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, VA 23298-0613; Tel/Fax: +1-804-828-9256; E-mail: dbagdas@vcu.edu

1.1. Alpha7 Nicotinic Acetylcholine Receptor Structure and Modulation

The nAChRs are pentameric assemblies of subunits configured around a central ion channel pore that can be gated by the binding of ligands (orthosteric agonists) to sites in the N-terminal extracellular domains at the interface between subunits. The first nAChR to be studied and isolated were those of the neuromuscular junction and the homologous electric organ of the Torpedo ray. These receptors evolved to rapidly and efficiently activate when stimulated with a large increase in acetylcholine (ACh) concentration [3]. These muscle-type receptors were found to have four different types of subunits, referred to as α1, β1, γ and δ, with two α1 subunits per receptor. For the most part, each subunit had a relatively well-conserved structure, with three transmembrane domains following the N-terminal extracellular domain, a more variable intracellular domain [4], followed by a fourth transmembrane domain. The α1 subunits contained a pair of disulfide-linked vicinal cysteines, and all of the subunits contained a disulfide-constrained loop in a region later shown to be at the interface between the extracellular and transmembrane domains. This hallmark "Cys-loop" was ultimately found in all nAChR subunits subsequently cloned, as well as in homologous receptors activated by GABA, gly-

1570-159X/18 $58.00+.00

cine, or serotonin, identifying the Cys-loop superfamily of ligand-gated ion channels (LGIC).

The family of nAChR subunits was ultimately expanded to include one additional alternative muscle subunit, epsilon, and twelve additional subunits cloned from neuronal tissues. The neuronal subunits were classified as alpha subunits (α2 - α10, although α8 is found only in birds) if they contained vicinal cysteines homologous to those in α1, or as β subunits (β2 - β4) if they did not. Each subunit has a unique pattern of expression associated with diverse functions for nAChR in different tissues and brain structures [5]. Most nAChR subtypes are heteromers containing both α and non-alpha subunits with two orthosteric agonist-binding sites contributed by α subunits in each receptor, as in the muscle-type receptor. In the rodent brain the predominant nAChRs contain combinations of α4 and β2 subunits, and these receptors were shown to bind radiolabeled ACh or nicotine with high affinity. It was also shown that a second class of nAChR is present in brain that did not bind ACh or nicotine with high affinity but did bind the neuromuscular receptor blocker α-bungarotoxin [6]. These were subsequently shown to be associated with the expression of the α7 subunit, which could form receptors without the co-expression of a beta subunit [7].

Homomeric α7 nAChR lacks the specializations for synaptic function that are present in muscle-type and ganglionic nAChR [3]. The absence of non-alpha subunits modifies the agonist binding site, reducing high-affinity agonist binding to desensitized states and increasing sensitivity to the ubiquitous ACh precursor choline. In regard to functioning as a homopentamer, α7 nAChR is like the proton-gated channels *Gloeobacter* ligand-gated ion channel (GLIC) [8] and *Erwinia* ligand-gated ion channel (ELIC) [9] found in bacteria, which are believed to be homologs of the vertebrate Cys-loop LGIC.

The unique cellular and subcellular α7 expression patterns indicate special roles for this receptor subtype. This unique pattern includes wide spread expression in non-neuronal cells, including cells of the immune system where α7 has been uniquely implicated in regulating the cholinergic anti-inflammatory pathway. Additionally, due to its ability to be activated by choline in addition to ACh and its rapid concentration-dependent desensitization of ion channel currents, α7 nAChR will respond in a very different way from other nAChR to endogenous cholinergic signals, including paracrine signals in peripheral tissues.

Open α7 ion channels have high calcium permeability [7]. While the high calcium permeability of NMDA-type glutamate receptors is responsible for their key role in synaptic plasticity, it has been implicated to lead to the potential for excitotoxic activation, in the case of α7. This feature is offset by the fact that normally the open probability of the α7 receptor channel is extremely low compared to other nAChR [10,11]. Changes in intracellular calcium concentration subsequent to α7 stimulation are typically more due to release of calcium from intracellular stores rather than calcium influx through the α7 channels [12], suggesting a metabotropic-like function for α7 nAChR. This may especially be the case for non-neuronal cells, where no α7-mediated ionic currents can be detected [13-15].

The identification of cholinergic anti-inflammatory activity mediated by α7 nAChR in cells of the immune system [16-18] has drawn attention to the likelihood that ligand-induced conformational changes of α7 receptors are global and apparently encompass changes in signaling associated with the receptor's interactome [19] and potentially with G-protein-mediated signals [20]. While the prokaryotic Cys-loop receptor homologs lack any intracellular domains, the vertebrate nAChR subunits show remarkable diversity and specialization in their intracellular domains, and the unique intracellular domain of α7 receptors has been especially well conserved throughout the evolution of vertebrates [4].

As noted above, the unique configuration of the α7 orthosteric agonist binding between pairs of identical, rather than specialized subunits, allows for these receptors to be effectively targeted by multiple classes of selective agonists [21]. The presence of five potential agonist-binding sites per receptor also permits multiple types of ligand-induced conformational states based on the level of binding site occupancy. Data suggest that only relatively low levels of binding site occupancy effectively promote channel activation (albeit with low probability) [22-24] and that higher levels of agonist occupancy preferentially induce nonconducting (*i.e*. desensitized) states that are far more stable than the open channel state. The existence of these unique nonconducting states has been confirmed by the activity of α7-selective positive allosteric modulators (PAMs), which, at least in the case of efficacious type II PAMs like PNU-120596 [25], can destabilize the nonconducting conformations and couple them to unique open-channel states [11, 26, 27]. The identification of diverse classes of α7-selective PAMs [25] opened up a new avenue for α7-directed drug development [28] and ultimately led to the identification of another novel class of α7-channel activators, ago-PAMs, including 4-BP-TQS [29], its active isomer GAT107 [30], and more recently, the structurally unrelated B-973 [31], that function both as PAMs and allosteric agonists. The allosteric agonism has been hypothesized to be associated with a specific allosteric activation site in the extracellular domain [32].

Agonists, PAMs and ago-PAMs are all capable, to varying degrees, of activating α7 ion channel currents, although for a PAM that activation is conditional on the coincident presence of an agonist. All of these currents can be antagonized by both competitive and non-competitive antagonists, for example MLA and mecamylamine, respectively. However, the conducting states activated in the presence of a PAM differ subtly in their sensitivity to specific antagonists [27] than those activated by orthosteric agonist in the absence of a PAM. Additionally, molecules have been identifies which specifically antagonize the effects of PAMs with little or no effects on activation by ordinary orthosteric agonists [33]. Conversely, silent agonists [34], which produce channel activation in the presence of a PAM, behave as very low efficacy partial agonists in the absence of a PAM.

While the primary focus for the use of α7-PAMs has been as tools to overcome the intrinsic limitations to α7

channel activation, they have also been used to investigate the induction of nonconducting conformational states of the receptor that may play important roles in the metabotropic functions of α7 receptors. As noted above, while the open-channel state of α7 is very unstable, the nonconducting states that can be converted to conducting states by the activity of PAMs are far more stable. This has allowed the use of PAMs to identify "silent agonists", ligands that bind to a site that overlaps the agonist binding site [34], do not produce significant activation when applied alone, but will activate currents in the presence of a PAM. NS6740 was the first silent agonist introduced in the literature. It was reported to be an α7 ligand that required a PAM to produce channel activation. NS6740 was later shown to be very effective at inducing stable nonconducting states of the receptor and also to be very effective at reducing the behavioral manifestations of pain in several animal models. While NS6740 was ineffective in an animal model of cognition, it was subsequently shown that both NS6740 and GTS-21, a low-efficacy α7-selective partial agonist, were effective modulators of immune signaling in microglia [35]. Interestingly, the α7 ago-PAM GAT107, which also induces stable nonconducting states, had a profile similar to that of NS6740 in the same pain models [36]. The long-term effects of NS6740 on ACh-evoked responses are desensitizing [37], while those of GAT107 are potentiating [30], suggesting that the anti-inflammatory activity reducing the manifestations of pain may be specifically associated with the nonconducting states of the receptor.

1.2. Alpha7 Nicotinic Acetylcholine Receptor Expression

α7 nAChR is present in supraspinal and spinal pain-transmission pathways [7, 38-42]. Autoradiographic analyses showed that α7 nAChR binding sites were numerous within the substantia gelatinosa in rat [43] and human [40] spinal cord, and these sites were reduced following dorsal rhizotomy [44]. α7 nAChRs have been shown to be expressed on immune and non-immune cytokine-producing cells such as macrophages [17, 45, 46], microglia and keratinocytes [17, 39, 47-49]. They are also expressed in monocytes [50-52], T-cells [53, 54], and B-cells [55, 56]. α7 nAChRs that are expressed on macrophages, which are key immune cells involved in the initiation, maintenance, resolution and modulation of inflammatory processes [15, 39, 45]. For example, they regulate cytokine release [17, 57]. Another important expression localization for these receptors is microglia, which can remain in the activated state for a prolonged period of time to evoke secretion of various inflammatory factors [58-60].

Both RIC-3, and a newly identified accessory protein NACHO [61] have been shown to be crucial regulators of α7 functional expression on both cellular and subcellular levels. While the only known function for NACHO has been as a regulator of α7, RIC-3 has been more broadly implications as a regulator of other cys-loop receptors [61-65].

1.3. Anti-inflammatory Signaling of alpha7 Nicotinic Acetylcholine Receptors

α7 nAChR endogenous ligands, ACh and choline, are closely associated with controlling immune cell functions, attenuation of pro-inflammatory cytokine production and inhibition of the inflammatory process *via* activation of α7 nAChRs [15, 66]. This neurophysiological mechanism decreases inflammation by reducing cytokine synthesis *via* release of ACh in organs of the reticulo-endothelial system, such as the lungs, spleen, liver, kidneys and gastrointestinal tract [67]. It has been revealed that α7 nAChRs are implicated in modulating tumor necrosis factor (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-18 (IL-18), high mobility group box 1 (HMGB1) and some other pro-inflammatory cytokines without affecting the anti-inflammatory cytokine interleukin-10 (IL-10) [39,66,68-70]. Indeed, a critical role for α7 nAChR as a peripheral component in cholinergic anti-inflammatory pathway has been demonstrated using α7 subunit knockout (KO) mice [17]. Intraplantar complete Freund's adjuvant or inactivated and dried mycobacteria, which induces chronic inflammation and inflammatory pain, injection increased more edema, hyperalgesia and allodynia in the α7 KO mice compared with the wild-type (WT) littermates [71]. ACh and nicotine pre-treatment inhibited lipopolysaccharide (LPS)-induced TNF-α release in murine-derived microglial cells through α7 nAChR activation [60]. Another study also showed that pre-treatment with ACh inhibited LPS-induced matrix metalloproteinase 9 (MMP-9) production and macrophage migration *in vitro* [72]. It has been reported that activation of these receptors by an agonist attenuated TNF-α and IL-1β levels in human whole blood activated by exposure to endotoxin [50] and microglial α7 nAChR activation reduced TNF-α release but not IL-1β [58]. Choline has also been shown to modulate TNF-α release *via* α7 nAChR-mediated signaling [73]. CDP-choline is an endogenously synthesized nucleotide which rapidly metabolizes to choline and cytidine/uridine. Consistently, exogenous administration of CDP-choline results in elevations in plasma and tissue levels of choline [74, 75]. When CDP-choline, also a source for the synthesis of the cholinergic neurotransmitter ACh, was locally administered, it reduced the production of the TNF-α, reduced edema and reversed the mechanical hyperalgesia through α7 nAChR, suggesting that the local application of α7 nAChR activators may provide a tool to reduce the local inflammation and pain [76]. Nuclear translocation of nuclear factor-κB (NF-κB) is the main factor of immune cell activation and proinflammatory cytokines expression [77]. It has been shown that α7 nAChR activation decreases NF-κB activity and reduces inflammatory response [17, 51]. It has been suggested that different mechanisms effect α7 nAChR activation on NF-κB activity [51, 78]. One possible mechanism is the recruitment of Janus kinase-2 (Jak2) to the α7 nAChR and activation of Jak2, thereby initiating the anti-inflammatory signal transducer and activator of transcription 3 (STAT3) and suppressor of cytokine signaling 3 (SOCS3) signaling cascade. Vagus nerve stimulation has been shown to attenuate macrophage activation through activating the Jak2-STAT3 signaling pathway and STAT3 phosphorylation, a potential negative regulator of inflammatory responses, depends on α7 nAChR activation [78, 79]. In addition, α7 nAChR activation leads to suppression of the phosphorylation of I kappa B (I-κB) which results in inhibition of transcriptional activity of NF-κB and inhibition of proinflammatory mediators [51].

Another possible mechanism may result from an interaction between α7 nAChRs and toll-like receptor 4 (TLR4) through myeloid differentiation factor 88 (MyD88) and NF-κB. MyD88, a key intracellular reactive protein for TLR4, could activate I-κB kinases (IKK) and induce NF-κB translocation [80]. Nicotine has been shown to decrease the expression of MyD88 and to interfere NF-κB transcription in an α7 nAChR antagonist α-bungarotoxin dependent manner. Consequently, nicotine reduced TNF-α expression through α7 nAChR/MyD88/NF-κB pathway [81]. Many other downstream pathways may involve α7 nAChRs and TLR4 interaction, such as overexpression of IL-1 receptor-associated kinase M (IRAK-M), a negative regulator of TLR4, in macrophages resulted in decreased TNF-α production through a Jak2-STAT3 signaling pathway by α7 nAChR activation [82]. HMGB1, a cytokine mediator of inflammation, has been reported to upregulate α7 nAChR expression [83]. In addition, vagus nerve stimulation or cholinergic receptor agonists have been suggested to inhibit the activation of NACHT, LRR, PYD domains-containing protein 3 (NLRP3) inflammasome, a key modulator of innate immunity, whereas genetic deletion of α7 nAChR enhances inflammasome activation. Mitochondrial α7 nAChR activation suppressed the release of mitochondrial DNA, an NLRP3 ligand, as a result IL-1β and HMGB1 expressions were suppressed [84]. It has also been shown that α7 nAChR gene expression was increased in septic patients' peripheral blood mononuclear cells and these patients responded dynamically to the inflammatory challenge; besides a group of patients with low levels of α7 expression had a defective response to infection and showed worst clinical outcome with respect to inflammation [85]. This is also suggestive that there might be bilateral interactions between α7 nAChR and inflammatory mediators. With regard to these results, α7 gene expression level in septic patients might be used to assess cholinergic anti-inflammatory pathway activity as a clinical marker. Furthermore, ligands for α7 nAChRs might open new strategies to monitor and cure inflammatory diseases such as ulcerative colitis, sepsis, acute pancreatitis and asthma. Therefore, these receptors present an alternative therapeutic strategy for modulation of inflammation-based syndromes.

1.4. Effects of alpha7 Nicotinic Acetylcholine Receptors Agonists and Modulators in Rodents Pain Models

Animal studies have shown that α7 nAChR agonists such as choline, compound B [(R)-N-(1- azabicyclo[2.2.2]oct-3-yl)(5-(2-pyridyl)thiopene-2-carboxamide)], JN403, PHA-543613 and AR-R17779 exhibit anti-inflammatory effects in various inflammatory pain models in rodents [86-90]. In addition, they were found to be active in chronic neuropathic pain models [91-93]. For example, repeated administration of a selective α7 agonist decreases allodynia in a chronic neuropathic pain model in the rat and reverses signs of neuroinflammation and neurodegeneration (macrophage infiltration, decreases in axon compactness and diameter with a significant loss of myelin sheaths) [93]. Not limited to direct α7 nAChR agonists, CDP-choline which is an intermediate in the biosynthesis of phosphatidylcholine and leads to an increase in choline levels, has been reported as effective to modulate nerve injury- and chemotherapy-induced neuropathic pain through α7 nAChRs [94, 95]. The partial agonist GTS-21 has been reported to have antinociceptive properties in a mouse model of postoperative pain [50]. Stimulation of cholinergic anti-inflammatory pathways resulted in attenuation of neuroinflammation in a tibial fracture model of rats through α7 nAChR [96]. Choline has been also reported to attenuate a model of postoperative pain in mice [97]. Choline and nicotine have been shown to enhance inhibitory synaptic transmission in the dorsal horn neurons of spinals cords of rats [98]. However, there are some limitations to the development α7 nAChR agonists for the treatment of pain, such as receptor selectivity issues (cross-reactivity with 5-HT3 receptors, which have high homology with α7 nAChRs, and possible related adverse effects), overactivation and possible desensitization of the receptor. In addition, while α7 nAChR agonists have shown beneficial effects in chronic pain models in some studies, this effect was not consistently seen in others [99]. Efficacy of various α7 nAChRs ligands in various animal models of pain is shown in Table **1**. Therefore, alternative approaches to overcome these limitations associated with direct nAChR agonists were developed in the last decade.

In addition to agonists, α7 nAChRs PAMs also provide a unique strategy for rational drug design and discovery. As discussed above, PAMs for α7 nAChRs increase the potency and/or maximal efficacy of endogenous (ACh and choline) or exogenous agonists for the α7 nAChRs without directly activating α7 nAChRs. Recently, several structurally distinct and selective α7 nAChR PAMs were identified and classified as type I and type II based on their electrophysiological properties [100, 101]. Type I PAMs increase agonist response with little or no effect on desensitization of α7 nAChRs, whereas type II PAMs increase agonist response and slow down the apparent desensitization profile of the agonist response [28]. It has been suggested that PAMs may modulate conformational states for both channel activity and ion channel-independent signaling [27]. Importantly, it has been reported that in contrast to α7 agonists, α7 nAChR PAMs do not induce upregulation of α7 nAChR expression in the brain *in vivo* [102].

Both PAM types have been recently tested *in vivo* for their efficacy in animal models of inflammatory and neuropathic pain. Type II but not type I PAMs were shown to be efficacious in these models [92, 103]. This is different from rodent models of cognition and memory, where both type I and type II PAMs for the α7 nAChRs showed cognitive enhancement. There are several examples of antinociceptive, antiallodynic and antihyperalgesic activities of type II PAMs. PNU-120596, an α7 nAChR type II PAM, has well described activity in tonic, inflammatory and chronic pain models in rodents [92, 103, 104]. Recently, another α7 nAChR type II PAM, 2,4,2',5'-tetrahydroxychalcone, has been found active against complete Freund's adjuvant-induced inflammatory pain in rats [105]. In addition, 3-furan-2-yl-N-p-tolyl-acrylamide, an α7 nAChR type II PAM agent reduces neuropathic and inflammatory pain outcomes in mice in a dose and time dependent manner [92,106]. Different mechanisms may underlie PAM-induced anti-inflammatory effects.

Table 1. Efficacy of α7 neuronal nicotinic acetylcholine receptor ligands in various animal models of pain.

Compound	Ligand Type	Animal Model	Animal	Response	Refs.
Choline	Agonist	Murine endotoxemia and sepsis	Mice	Reduced systemic TNF levels, suppressed HMGB1 release	[73]
Choline	Agonist	Tail flick test	Mice	Antinociceptive	[87]
Choline	Agonist	Incisional postoperative pain	Mice	Reduced thermal hyperalgesia and mechanical allodynia	[97]
CDP-choline	Source of choline	Carrageenan	Rats	Reduced mechanical hyperalgesia decreased TNF-α in the paw	[76]
CDP-choline	Source of choline	Oxaliplatin-induced CIPN	Rats	Reduced mechanical hyperalgesia	[94]
CDP-choline	Source of choline	CCI	Rats	Reduced mechanical hyperalgesia	[95]
JN403	Agonist	CFA and partial sciatic nerve ligation models Tail flick test	Rats	Reduced mechanical hyperalgesia Lack of effect	[86]
DMXB and 4-OH-DMXB	Partial agonist	Tail flick test	Mice	Lack of effect by itself blocked choline-induced antinociception	[87]
Compound-B	Agonist	CFA	Rats, mice	Reduced mechanical hyperalgesia	[88]
Compound-B	Agonist	Carrageenan and CFA Formalin models	Rats	Reduced mechanical hyperalgesia Attenuated pain behavior in formalin test	[104]
Nicotine	Agonist	CIA model	Mice	Ameliorated arthritis and reduced synovial inflammation	[89]
AR-R17779	Agonist	CIA model	Mice	Ameliorated arthritis and reduced synovial inflammation	[89]
TC-7020	Agonist	CCI	Rats	Reduced mechanical allodynia	[91]
PHA-543613	Agonist	CCI	Mice	Reduced mechanical allodynia	[92]
PNU-282987	Agonist	CCI	Rats	Reduced mechanical hyperalgesia	[93]
NS1738	Type I PAM	Carrageenan CCI	Mice	Reduced thermal hyperalgesia Lack of effect	[92]
NS1738	Type I PAM	Hot plate and tail-flick tests Formalin model	Mice	Lack of effect Lack of effect	[103]
PNU-120596	Type II PAM	Carrageenan CCI	Mice	Reduced thermal hyperalgesia reduced thermal hyperalgesia and mechanical allodynia potentiated effects of PHA-543613	[92]
PNU-120596	Type II PAM	Carrageenan and CFA Formalin model	Rats	Reduced mechanical hyperalgesia and weight-bearing deficit lack of effect	[104]
PNU-120596	Type II PAM	Hot plate and tail-flick tests formalin model	Mice	Lack of effect Attenuated pain behavior in formalin test	[103]
2,4,2',5'-tetrahydroxychalcone	Type II PAM	CFA	Rats	Lack of effect on thermal hyperalgesia reduced mechanical hyperalgesia	[105]
3-furan-2-yl-N-p-tolyl-acrylamide	Type II PAM	Carrageenan CFA CCI CPA	Mice	Reduced mechanical allodynia by itself and potentiated antiallodynic effect of choline Reduced thermal hyperalgesia Reduced mechanical allodynia Reversed negative affective behaviors	[106]

(Table 1). contd….

Compound	Ligand Type	Animal Model	Animal	Response	Refs.
GAT107	Ago-PAM (allosteric agonist and type II PAM)	Tail flick and hot plate tests Formalin model CFA and CCI models LPS Acetic acid induced visceral stretching and CPA	mice	Lack of effect antinociceptive reduced thermal hyperalgesia and mechanical allodynia reduced mechanical allodynia decreased stretching behavior and reversed negative affective behaviors	[36]
NS6740	Silent agonist	Formalin model CCI CPA	mice	Attenuated pain behavior in formalin test reduced mechanical allodynia reversed negative affective behaviors	[37]
PMP-072	Silent agonist	CIA	mice	Reduced arthritis	[109]

CCI—Chronic constrictive nerve injury, CFA—Complete Freund's adjuvant, CIA—collagen-induced arthritis, CIPN—chemotherapy-induced peripheral neuropathy, CPA—Conditioned place aversion, PAM—positive allosteric modulator, PSNL—Partial sciatic nerve ligation, SNL—Spinal nerve ligation.

PNU120596 reduced TNF- α and IL-6 within the hind paw edema in a rat model of the carrageenan test [104]. The activation of spinal extracellular signal-regulated kinase-1/2 (ERK1/2) pathways are the likely one possible post receptor signaling mechanism for the antinociceptive effect of PNU-120596 in the formalin test [92]. Although, α7 nAChR modulation attenuates inflammatory and neuropathic pain, they are mostly lack of efficacy for acute pain [36, 37, 103]. Additional studies to fully clarify the analgesic-like properties of α7 nAChR are necessary in animal models of chronic pain.

An alternative therapeutic approach involves allosteric agonist and allosteric modulators called ago-PAMs. While PAMs are thought to bind to a distinct binding site from the orthosteric site and thus lack intrinsic agonist activation, recent studies have reported that some molecules show dual activity as allosteric modulators and allosteric agonists [33,107]. GAT107 is a potent α7 nAChR type II PAM with intrinsic allosteric agonist activities suggesting it is an ago-PAM for α7 nAChRs [107]. In several mouse models of chronic inflammatory and neuropathic pain, GAT107 attenuated pain behavior and showed anti-inflammatory effects. Furthermore, intrathecal, but not intraplantar, injections of GAT107 reversed nociception in the complete Freund's adjuvant-induced inflammatory pain model, suggesting a spinal mechanism of action. Immunohistochemical evaluation of the lumbar spinal cord dorsal horn of mice treated with complete Freund's adjuvant showed an increase in the expression of astrocyte-specific glial fibrillary acidic protein (GFAP) and phosphorylated p38-mitogen-activated-kinase (p-p38MAPK). Treatment with intrathecal GAT107 robustly attenuated these inflammatory changes. These findings suggest that α7 ago-PAMs represent a novel and effective pharmacological strategy for treating inflammatory and neuropathic pain in mice [36].

An additional new class of selective ligand has been identified for α7 nAChR. While direct α7 nAChR agonists bind to the receptor on the orthosteric binding site to activate the channel, ligands called "silent agonists" also bind the orthosteric binding site of the receptor, but preferentially induce a nonconducting state associated with desensitization that can none the less modulate signal transduction [108, 109]. Recent studies showed that α7 nAChR-selective silent agonism may provide a novel strategy for pain management. NS6740, α7 silent agonist, induces nonconducting states of the receptor. NS6740 modulates the inflammatory responses of microglia cells *in vitro* and it is effective in both chronic constriction nerve injury-induced neuropathic pain and formalin model of tonic inflammatory pain [37]. Another α7 nAChR silent agonist PMP-072 has also been shown to have anti-inflammatory effects in collagen-induced arthritis in mice [109]. The behavioral and pharmacological profile of PMP-072 and NS6740 in these models are consistent with the induction of nonconducting conformational states of the receptor which confirmed *in vitro* studies. Indeed, data obtained from α7 nAChR silent agonist R-47 (another name of compound PMP-072) support the hypothesis that the anti-inflammatory effects of silent agonism was mediated by a signal transduction pathway that was independent of ion current [110]. α7 nAChRs are downregulated in dorsal root ganglion and spinal cord of rats by oxaliplatin treatment [111] suggesting that α7 nAChRs might have modulatory role in chemotherapy-induced neuropathic pain. In support of this hypothesis, another study showed that α7 nAChR activation protected neurons from oxaliplatin-induced toxicity through a mechanism related to the neuroprotective functions of astrocytes [112]. Ligands that favor non-conducting states are favorable compared to α7 full agonists that enhanced tumor growth in small cell lung cancer cell lines [113], and in mouse model of lung cancer [114]. Because of these considerations, α7 agonists have limited potential for the treatment of chemotherapy-induced neuropathic pain. Targeting alternative conformational states of α7 nAChRs, such as silent agonism, may provide a better therapeutic approach for this common type of neuropathic pain. The recent characterization of α7 nAChR silent agonists has created new opportunities for targeting these receptors as analgesic agents for cancer and chemotherapy-induced peripheral neuropathy. It is very possible that α7 silent agonists may be more efficacious to prevent or attenuate the chemotherapy-

induced neuropathic pain. Further pre-clinical behavioral studies will clarify the effects of silent agonists potentially influencing their future clinical development.

Pain has been described as a multi-dimensional state composed of sensory, affective, and cognitive components [115-117]. As such, pain states that require clinical intervention are often accompanied by changes in affective behaviors that have complex interplay with the nociceptive aspects of the pain response [118-121]. A positive feature of α7 nAChR ligands is that in contrast to opioids, they appear to improve pain related affective behaviors in preclinical models. The conditioned place aversion model [122] was used to test the activities of several α7 nAChR. The type II PAM 3-furan-2-yl-N-p-tolyl-acrylamide, silent agonist NS6740 and ago-PAM GAT107 were effective to reverse negative affective behaviors associated with visceral pain induced by acetic acid in mice [36, 37, 106]. These findings suggest that α7 nAChR modulation may serve an efficacious strategy to treat both the sensory and affective components of pain.

CONCLUSION

In summary, many of immune cells and non-immune neuronal cells have been reported to express α7 nAChR subtype. This receptor subtype affords a critical role on the initiation, maintenance and modulation inflammation in addition to direct neuronal signaling. Exploring different pathways to the activation and/or modulation of α7 nAChRs and determining downstream signaling pathways will provide data critical to develop beneficial α7 nAChR ligands for translational research on pain and inflammation.

LIST OF ABBREVIATIONS

nAChR	=	Nicotinic acetylcholine receptors
ACh	=	Acetylcholine
LGIC	=	Ligand-gated ion channels
GLIC	=	*Gloeobacter* ligand-gated ion channel
ELIC	=	*Erwinia* ligand-gated ion channel
PAMs	=	Positive allosteric modulators
TNF-α	=	Tumor necrosis factor
IL-1	=	Interleukin-1
IL-6	=	Interleukin-6
IL-18	=	Interleukin-18
HMGB1	=	High mobility group box 1
IL-10	=	Interleukin-10
LPS	=	Lipopolysaccharide
MMP-9	=	Matrix metalloproteinase 9
NF-κB	=	Nuclear translocation of nuclear factor-κB
Jak2	=	Janus kinase-2
STAT3	=	Signal transducer and activator of transcription 3
SOCS3	=	Suppressor of cytokine signaling 3
I-κB	=	I kappa B
TLR4	=	Toll-like receptor 4
MyD88	=	Myeloid differentiation factor 88
IKK	=	I-κB kinases
IRAK-M	=	IL-1 receptor-associated kinase M
NLRP3	=	NACHT, LRR, PYD domains-containing protein 3
ERK1/2	=	Extracellular signal-regulated kinase-1/2
GFAP	=	Astrocyte-specific glial fibrillary acidic protein
p-p38MAPK	=	Phosphorylated p38-mitogen-activated-kinase

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This work was supported by grants from National Institute of Health [R01-CA206028] to MID, [GM57481] to RLP and from Research Fund of Uludag University [KUAP (T) 2016/15 and 2016/16) to MSG.

REFERENCES

[1] Damaj, M.I.; Freitas, K.; Bagdas, D.; Flood, P. Nicotinic receptors as targets for novel analgesics and anti-inflammatory drugs. In: R.A.J. Lester (Ed.), Nicotinic Recept., Springer New York, New York, NY, **2014**, pp. 239-254. doi:10.1007/978-1-4939-1167-7_12.

[2] Kalkman, H.O.; Feuerbach, D. Modulatory effects of α7 nAChRs on the immune system and its relevance for CNS disorders. *Cell Mol. Life Sci.*, **2016**, *73*, 2511-30. doi: 10.1007/s00018-016-2175-4.

[3] Papke, R.L. Merging old and new perspectives on nicotinic acetylcholine receptors. *Biochem. Pharmacol.,* **2014**, *89*, 1-11. doi:10.1016/j.bcp.2014.01.029.

[4] Stokes, C.; Treinin, M.; Papke, R.L. Looking below the surface of nicotinic acetylcholine receptors. *Trends Pharmacol. Sci.,* **2015**, *36,* 514-523. doi:10.1016/j.tips.2015.05.002.

[5] Millar, N.S.; Gotti, C. Diversity of vertebrate nicotinic acetylcholine receptors. *Neuropharmacology*, **2009**, *56*, 237-246. doi:10.1016/j.neuropharm.2008.07.041.

[6] Clarke, P.B.; Hamill, G.S.; Nadi, N.S.; Jacobowitz, D.M.;Pert, A. 3H-nicotine- and 125I-alpha-bungarotoxin-labeled nicotinic receptors in the interpeduncular nucleus of rats. II. Effects of habenular deafferentation. *J. Comp. Neurol*., **1986**, *251*, 407-413. doi:10.1002/cne.902510311.

[7] Seguela, P.; Wadiche, J.; Dineley-miller, K.; Dani, J.A.J.; Patrick, J. Molecular cloning, functional properties, and distribution of rat brain α7: a nicotinic cation channel highly permeable to calcium. *J. Neurosci.* **1993**, *13*, 596-604.

[8] Bocquet, N.; Nury, H.; Baaden, M.; Le Poupon, C.; Changeux, J.-P.; Delarue, M.; Corringer, P.-J. X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. *Nature,* **2009**, *457*, 111-4. doi:10.1038/nature07462.

[9] Hilf, R.J.C.; Dutzler, R. X-ray structure of a prokaryotic pentameric ligand-gated ion channel. *Nature*, **2008**, *452*, 375-379. doi:10.1038/nature06717.

[10] Williams, D.K.; Peng, C.; Kimbrell, M.R.; Papke, R.L. Intrinsically low open probability of α7 nicotinic acetylcholine receptors can be overcome by positive allosteric modulation and serum factors leading to the generation of excitotoxic currents at physiological temperatures. *Mol. Pharmacol.*, **2012**, *82*, 746-759. doi:10.1124/mol.112.080317.

[11] Williams, D.K.; Wang, J.; Papke, R.L. Investigation of the molecular mechanism of the α7 nicotinic acetylcholine receptor positive allosteric modulator PNU-120596 provides evidence for two distinct desensitized states. *Mol. Pharmacol.*, **2011** *80,* 1013-1032. doi:10.1124/mol.111.074302.

[12] Gilbert, D.; Lecchi, M.; Arnaudeau, S.; Bertrand, D.; Demaurex, N. Local and global calcium signals associated with the opening of neuronal alpha7 nicotinic acetylcholine receptors. *Cell Calcium*, **2009**, *45,* 198-207. doi:10.1016/j.ceca.2008.10.003.

[13] Egleton, R.D.; Brown, K.C.; Dasgupta, P. Nicotinic acetylcholine receptors in cancer: multiple roles in proliferation and inhibition of apoptosis. *Trends Pharmacol. Sci.,* **2008**, *29,* 151-158. doi:10.1016/j.tips.2007.12.006.

[14] Arredondo, J.; Chernyavsky, A.I.; Jolkovsky, D.L.; Pinkerton, K.E.;Grando, S.A. Receptor-mediated tobacco toxicity: cooperation of the Ras/Raf-1/MEK1/ERK and JAK-2/STAT-3 pathways downstream of alpha7 nicotinic receptor in oral keratinocytes. *FASEB J.*, **2006**, *20*, 2093-2101. doi:10.1096/fj.06-6191com.

[15] de Jonge, W.J.;Ulloa, L. The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation. *Br. J. Pharmacol.,* **2007**, *151*, 915-929. doi:10.1038/sj.bjp.0707264.

[16] Pavlov, V.A.; Ochani, M.; Yang, L.-H.; Gallowitsch-Puerta, M.; Ochani, K.; Lin, X.; Levi, J., Parrish, W.R.; Rosas-Ballina, M.; Czura, C.J.; Larosa, G.J.; Miller, E.J.; Tracey, K.J.; Al-Abed, Y. Selective alpha7-nicotinic acetylcholine receptor agonist GTS-21 improves survival in murine endotoxemia and severe sepsis. *Crit. Care Med*., **2007**, *35,* 1139-1144. doi:10.1097/01.CCM.0000259381.56526.96.

[17] Wang, H.;Yu, M.;Ochani, M., Amella, C.A.;Tanovic, M.; Susarla, S.; Li, J.H.; Wang, H.; Yang, H.; Ulloa, L.; Al-Abed, Y.; Czura, C.J.; Tracey, K.J. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature*, **2003**, *421*, 384-388. doi:10.1038/nature01339.

[18] Tracey, K.J. Physiology and immunology of the cholinergic antiinflammatory pathway. *J. Clin. Invest.,* **2007**, *117,* 289-296. doi:10.1172/JCI30555.

[19] Paulo, J.A.; Brucker, W.J.; Hawrot, E. Proteomic analysis of an alpha7 nicotinic acetylcholine receptor interactome. *J. Proteome Res.,* **2009**, *8*, 1849-58. doi:10.1021/pr800731z.

[20] King, J.R.; Kabbani, N. Alpha 7 nicotinic receptor coupling to heterotrimeric G proteins modulates RhoA activation, cytoskeletal motility, and structural growth. *J. Neurochem.,* **2016**, *138,* 532-545. doi:10.1111/jnc.13660.

[21] Horenstein, N.A.; Leonik, F.M.; Papke, R.L. Multiple pharmacophores for the selective activation of nicotinic alpha7-type acetylcholine receptors. *Mol. Pharmacol.,* **2008**, *74*, 1496-1511. doi:10.1124/mol.108.048892.

[22] Papke, R.L.; Porter P.J.K. Comparative pharmacology of rat and human alpha7 nAChR conducted with net charge analysis. *Br. J. Pharmacol.*, **2002**, *137,* 49-61. doi:10.1038/sj.bjp.0704833.

[23] Uteshev, V.V.; Meyer, E.M.; Papke, R.L. Activation and inhibition of native neuronal alpha-bungarotoxin-sensitive nicotinic ACh receptors. *Brain Res.,* **2002**, *948,* 33-46. http://www.ncbi.nlm.nih.gov/pubmed/12383953 (accessed April 30, 2017).

[24] Williams, D.K.; Stokes, C.; Horenstein, N.A.; Papke, R.L. The effective opening of nicotinic acetylcholine receptors with single agonist binding sites. *J. Gen. Physiol.,* **2011**, *137*, 369-384. doi:10.1085/jgp.201010587.

[25] Grønlien, J.H.; Håkerud, M.; Ween, H.;Thorin-Hagene, K.; Briggs, C.A.; Gopalakrishnan, M.; Malysz, J. Distinct profiles of alpha7 nAChR positive allosteric modulation revealed by structurally diverse chemotypes. *Mol. Pharmacol.,* **2007**, *72,* 715-724. doi:10.1124/mol.107.035410.

[26] Andersen, N.D.; Nielsen, B.E.; Corradi, J.; Tolosa, M.F.; Feuerbach, D.; Arias, H.R.; Bouzat, C. Exploring the positive allosteric modulation of human α7 nicotinic receptors from a single-channel perspective. *Neuropharmacology,* **2016**, *107,* 189-200. doi:10.1016/j.neuropharm.2016.02.032.

[27] Peng, C.; Kimbrell, M.R.; Tian, C.; Pack, T.F.; Crooks, P.A.; Fifer, E.K.; Papke, R.L. Multiple modes of α7 nAChR noncompetitive antagonism of control agonist-evoked and allosterically enhanced currents. *Mol. Pharmacol.,* **2013**, *84,* 459-475. doi:10.1124/mol.113.086462.

[28] Williams, D.K.; Wang, J.; Papke, R.L. Positive allosteric modulators as an approach to nicotinic acetylcholine receptor-targeted therapeutics: Advantages and limitations. *Biochem. Pharmacol.,* **2011**, *82,* 915-930. doi:10.1016/j.bcp.2011.05.001.

[29] Gill, J.K.; Savolainen, M.; Young, G.T.; Zwart, R.; Sher, E.; Millar, N.S. Agonist activation of alpha7 nicotinic acetylcholine receptors *via* an allosteric transmembrane site. *Proc. Natl. Acad. Sci. U. S. A.,* **2011**, *108,* 5867-5872. doi:10.1073/pnas.1017975108.

[30] Papke, R.L.; Horenstein, N.A.; Kulkarni, A.R.; Stokes, C.; Corrie, L.W.; Maeng, C.Y.; Thakur, G.A. The activity of GAT107, an allosteric activator and positive modulator of α7 nicotinic acetylcholine receptors (nAChR), is regulated by aromatic amino acids that span the subunit interface. *J. Biol. Chem.* **2014**, *289,* 4515-4531. doi:10.1074/jbc.M113.524603.

[31] Post-Munson, D.J.; Pieschl, R.L.; Molski, T.F.; Graef, J.D.; Hendricson, A.W.; Knox, R.J.; McDonald, I.M.; Olson, R.E.; Macor, J.E.; Weed, M.R.; Bristow, L.J.; Kiss, L.; Ahlijanian, M.K.; Herrington, J. B-973, a novel piperazine positive allosteric modulator of the α7 nicotinic acetylcholine receptor. *Eur. J. Pharmacol.,* **2017**, *799,* 16-25. doi:10.1016/j.ejphar.2017.01.037.

[32] Horenstein, N.A.; Papke, R.L.; Kulkarni, A.R.; Chaturbhuj, G.U.; Stokes, C.; Manther, K.; Thakur, G.A. Critical molecular determinants of α7 nicotinic acetylcholine receptor allosteric activation: Separation of direct allosteric activation and positive allosteric modulation. *J. Biol. Chem.,* **2016**, *291*, 5049-5067. doi:10.1074/jbc.M115.692392.

[33] Gill, J.; Dhankher, P.; Sheppard, T.; Sher, E.; Millar, N. A series of α7 nicotinic acetylcholine receptor allosteric modulators with close chemical similarity but diverse pharmacological properties. *Mol. Pharmacol.,* **2012**, *81*, 710-718. doi:10.1124/mol.111.076026.

[34] Papke, R.L.; Chojnacka, K.; Horenstein, N.A. The minimal pharmacophore for silent agonism of alpha7 nAChR. *J. Pharmacol. Exp. Ther.,* **2014**, *350*, 665-680. doi:10.1124/jpet.114.215236.

[35] Thomsen, M.S.; Mikkelsen, J.D. The α7 nicotinic acetylcholine receptor ligands methyllycaconitine, NS6740 and GTS-21 reduce lipopolysaccharide-induced TNF-α release from microglia. *J. Neuroimmunol.*, **2012**, *251*, 65-72. doi:10.1016/j.jneuroim.2012.07.006.

[36] Bagdas, D.; Wilkerson, J.L.; Kulkarni, A.; Toma, W.; AlSharari, S.; Gul, Z.; Lichtman, A.H.; Papke, R.L.; Thakur, G.A.;Damaj, M.I. The α7 nicotinic receptor dual allosteric agonist and positive allosteric modulator GAT107 reverses nociception in mouse models of inflammatory and neuropathic pain. *Br. J. Pharmacol.*, **2016**, *173*, 2506-2520. doi:10.1111/bph.13528.

[37] Papke, R.L.; Bagdas, D.; Kulkarni, A.R.; Gould, T.; AlSharari, S.D.; Thakur, G.A.; Damaj, M.I. The analgesic-like properties of the alpha7 nAChR silent agonist NS6740 is associated with non-conducting conformations of the receptor. *Neuropharmacology,* **2015**, *91,* 34-42. doi:10.1016/j.neuropharm.2014.12.002.

[38] Wang, Y.; Su, D.M.; Wang, R.H.; Liu, Y.; Wang, H. Antinociceptive effects of choline against acute and inflammatory pain. *Neuroscience*, **2005**, *132,* 49-56. doi:10.1016/j.neuroscience.2004.12.026.

[39] Pavlov, V.A.; Tracey, K.J. eural regulators of innate immune responses and inflammation. *Cell. Mol. Life Sci.,* **2004**, *61*, 2322-2331. doi:10.1007/s00018-004-4102-3.

[40] Gillberg, P.G.;Aquilonius, S.M. Cholinergic, opioid and glycine receptor binding sites localized in human spinal cord by *in vitro* autoradiography. Changes in amyotrophic lateral sclerosis. *Acta Neurol. Scand.*, **1985**, *72*, 299-306. http://www.ncbi.nlm.nih.gov/pubmed/2998142 (accessed April 11, 2017).

[41] Cordero-Erausquin, M.; Changeux, J.P. Tonic nicotinic modulation of serotoninergic transmission in the spinal cord. *Proc. Natl. Acad. Sci. U. S. A.,* **2001**, *98,* 2803-2807. doi:10.1073/pnas.041600698.

[42] Cordero-Erausquin, M.; Pons, S.; Faure, P.; Changeux, J.P. Nicotine differentially activates inhibitory and excitatory neurons in the dorsal spinal cord. *Pain,* **2004**, *109,* 308-318. doi:10.1016/j.pain.2004.01.034.

[43] Hunt, S.; Schmidt, J. Some observations on the binding patterns of alpha-bungarotoxin in the central nervous system of the rat. *Brain*

Res. **1978**, *157*, 213-232. http://www.ncbi.nlm.nih.gov/pubmed/719523 (accessed April 11, 2017).

[44] Gillberg, P.G.; Wiksten, B. Effects of spinal cord lesions and rhizotomies on cholinergic and opiate receptor binding sites in rat spinal cord. *Acta Physiol. Scand.,* **1986**, *126,* 575-82. doi:10.1111/j.1748-1716.1986.tb07857.x.

[45] De Lucas-Cerrillo, A.M.; Maldifassi, M.C.; Arnalich, F.;Renart, J., Atienza, G.; Serantes, R.; Cruces, J.S.; Sánchez-Pacheco, A.; Andrés-Mateos, E.; Montiel, C. Function of partially duplicated human α7 nicotinic receptor subunit CHRFAM7A gene: Potential implications for the cholinergic anti-inflammatory response. *J. Biol. Chem.,* **2011**, *286,* 594-606. doi:10.1074/jbc.M110.180067.

[46] Borovikova, L.V.; Ivanova, S.; Zhang, M.; Yang, H.; Botchkina, G.I.; Watkins, L.R.; Wang, H.; Abumrad, N.; Eaton, J.W.;Tracey, K.J. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature*, **2000**, *405,* 458-462. doi:10.1038/35013070.

[47] Wessler, I.; Kirkpatrick, C.J. Acetylcholine beyond neurons: the non-neuronal cholinergic system in humans. *Br. J. Pharmacol.*, **2009**, *154*, 1558-1571. doi:10.1038/bjp.2008.185.

[48] Gahring, L.C.; Rogers, S.W. Neuronal nicotinic acetylcholine receptor expression and function on nonneuronal cells. *AAPS J.*, **2005**, *7*, E885-E894. doi:10.1208/aapsj070486.

[49] Sharma, G.;Vijayaraghavan, S. Nicotinic receptor signaling in nonexcitable cells. *J. Neurobiol.,* **2002**, *53,* 524-534. doi:10.1002/neu.10114.

[50] Rosas-Ballina, M.;Goldstein, R.S.;Gallowitsch-Puerta, M.; Yang, L.; Valdés-Ferrer, S.I.; Patel, N.B.; Chavan, S.; Al-Abed, Y.;Yang, H.; Tracey, K.J. The selective alpha7 agonist GTS-21 attenuates cytokine production in human whole blood and human monocytes activated by ligands for TLR2, TLR3, TLR4, TLR9, and RAGE. *Mol. Med.,* **2009**, *15,* 195-202. doi:10.2119/molmed.2009.00039.

[51] Yoshikawa, H.; Kurokawa, M.; Ozaki, N.;Nara, K., Atou, K.; Takada, E.; Kamochi, H., Suzuki, N. Nicotine inhibits the production of proinflammatory mediators in human monocytes by suppression of I-κB phosphorylation and nuclear factor-κB transcriptional activity through nicotinic acetylcholine receptor α7. *Clin. Exp. Immunol.,* **2006**, *146*, 116-123. doi:10.1111/j.1365-2249.2006.03169.x.

[52] Hamano, H.K,R.; Takahashi, H.; Iwagaki, T.;Yoshino, M.; Nishibori, N. Tanaka, Stimulation of alpha7 nicotinic acetylcholine receptor inhibits CD14 and the toll-like receptor 4 expression in human monocytes., Shock. 26 (2006) 358-64. doi:10.1097/01.shk.0000228168.86845.60.

[53] De Rosa, M.J.; Dionisio, L.; Agriello, E.; Bouzat, C.; del C.; Esandi, M. Alpha 7 nicotinic acetylcholine receptor modulates lymphocyte activation. *Life Sci.,* **2009**, *85*, 444-449. doi:10.1016/j.lfs.2009.07.010.

[54] Razani-Boroujerdi, S.; Boyd, R.T.; Dávila-García, M.I.; Nandi, J.S.; Mishra, N.C.;Singh, S.P.; Pena-Philippides, J.C.; Langley, R.; Sopori, M.L. T cells express alpha7-nicotinic acetylcholine receptor subunits that require a functional TCR and leukocyte-specific protein tyrosine kinase for nicotine-induced Ca^{2+} response. *J. Immunol.,* **2007**, *179,* 2889-98. http://www.ncbi.nlm.nih.gov/pubmed/17709503 (accessed April 12, 2017).

[55] Skok, M.V.; Kalashnik, E.N.; Koval, L.N.; Tsetlin, V.I.; Utkin, Y.N.; Changeux, J.-P.; Grailhe, R. Functional nicotinic acetylcholine receptors are expressed in B lymphocyte-derived cell lines. *Mol. Pharmacol.*, **2003**, *64*, 885-9. doi:10.1124/mol.64.4.885.

[56] Kawashima, K.; Yoshikawa, K.; Fujii, Y.X.; Moriwaki, Y.; Misawa, H. Expression and function of genes encoding cholinergic components in murine immune cells. *Life Sci.,* **2007**, *80*, 2314-9. doi:10.1016/j.lfs.2007.02.036.

[57] Zdanowski, R.; Krzyżowska, M.; Ujazdowska, D.; Lewicka, A.; Lewicki, S. Role of α7 nicotinic receptor in the immune system and intracellular signaling pathways. *Cent. J. Immunol. / Polish Soc. Immunol. Elev. Other Cent. Immunol. Soc.,* **2015**, *40*, 373-9. doi:10.5114/ceji.2015.54602.

[58] De Simone, R.; Ajmone-Cat, M.A.; Carnevale, D.; Minghetti, L. Activation of α7 nicotinic acetylcholine receptor by nicotine selectively up-regulates cyclooxygenase-2 and prostaglandin E2 in rat microglial cultures. *J. Neuroinflamm.,* **2005**, *2,* 4. doi:10.1186/1742-2094-2-4.

[59] Parada, E.; Egea, J.; Buendia, I.; Negredo, P.;Cunha, A.C.; Cardoso, S.;Soares, M.P.;López, M.G. The microglial α7-acetylcholine nicotinic receptor is a key element in promoting neuroprotection by inducing heme oxygenase-1 *via* nuclear factor erythroid-2-related factor 2. *Antioxid. Redox Signal,* **2013**, *19*, 1135-48. doi:10.1089/ars.2012.4671.

[60] Shytle, R.D.; Mori, T.; Townsend, K.; Vendrame, M.; Sun, N.;, Zeng, J., Ehrhart, J., Silver, A.A., Sanberg, P.R., Tan, J. Cholinergic modulation of microglial activation by α7 nicotinic receptors. *J. Neurochem.,* **2004**, *89,* 337-343. doi:10.1046/j.1471-4159.2004.02347.x.

[61] Matta, J.A.; Gu, S.; Davini, W.B.; Lord, B.; Siuda, E.R.; Harrington, A.W., Bredt, D.S. NACHO mediates nicotinic acetylcholine receptor function throughout the brain. *Cell Rep.,* **2017***, 25,* 688-696. doi: 10.1016/j.celrep.2017.04.008.

[62] Ben-David, Y.; Treinin, M. Regulation of RIC-3 and of nAChR expression. *Oncotarget,* **2017**, *8*, 5662-5663. doi: 10.18632/oncotarget.13925.

[63] Dau, A.; Komal, P.; Truong, M.; Morris, G.; Evans, G.; Nashmi, R. RIC-3 differentially modulates α4β2 and α7 nicotinic receptor assembly, expression, and nicotine-induced receptor upregulation. *BMC Neurosci.,* **2013**, *14*, 47. doi:10.1186/1471-2202-14-47.

[64] Alexander, J.K.; Sagher, D.; Krivoshein, A.V.; Criado, M.; Jefford, G.; Green, W.N. RIC-3 promotes alpha7 nicotinic receptor assembly and trafficking through the ER subcompartment of dendrites. *J Neurosci.,* **2010**, *30,* 10112-10126. doi:10.1523/JNEUROSCI.6344-09.2010.

[65] Walstab, J.; Hammer, C.; Lasitschka, F.; Möller, D.; Connolly, C.N.; Rappold, G.; Brüss, M.; Bönisch, H.; Niesler, B. RIC-3 exclusively enhances the surface expression of human homomeric 5-hydroxytryptamine type 3A (5-HT3A) receptors despite direct interactions with 5-HT3A, -C, -D, and -E subunits. *J. Biol. Chem.,* **2010**, *285,* 26956-26965. doi: 10.1074/jbc.M110.122838.

[66] Czura, C.J.; Friedman, S.G.; Tracey, K.J. Neural inhibition of inflammation: the cholinergic anti-inflammatory pathway. *J. Endotoxin Res.*, **2003**, *9*, 409-413. doi:10.1179/096805103225002755.

[67] Bencherif, M.; Lippiello, P.M.;Lucas, R.; Marrero, M.B. Alpha7 nicotinic receptors as novel therapeutic targets for inflammation-based diseases. *Cell. Mol. Life Sci.,* **2011**, *68*, 931-949. doi:10.1007/s00018-010-0525-1.

[68] Bernik, T.R.; Friedman, S.G.; Ochani, M.; DiRaimo, R.; Ulloa, L.; Yang, H.; Sudan, S.; Czura, C.J.; Ivanova, S.M.; Tracey, K.J. Pharmacological stimulation of the cholinergic antiinflammatory pathway., J. Exp. Med. 195 (2002) 781-788. doi:10.1084/jem.20011714.

[69] Pavlov, V.A. Cholinergic modulation of inflammation. *Int. J. Clin. Exp. Med.,* **2008**, *1*, 203-212.

[70] Pavlov, V.A.; Tracey, K.J. The cholinergic anti-inflammatory pathway. *Brain. Behav. Immun.,* **2005**, *19,* 493-499. doi:10.1016/j.bbi.2005.03.015.

[71] AlSharari, S.D.; Freitas, K.; Damaj, M.I. Functional role of alpha7 nicotinic receptor in chronic neuropathic and inflammatory pain: Studies in transgenic mice. *Biochem. Pharmacol.,* **2013***, 86*, 1201-1207. doi:10.1016/j.bcp.2013.06.018.

[72] Yang, Y.-H.; Li, D.-L.; Bi, X.-Y.; Sun, L.; Yu, X.-J.; Fang, H.-L.; Miao, Y.;Zhao, M., He, X.; Liu, J.-J.; Zang, W.-J. Acetylcholine inhibits LPS-induced MMP-9 production and cell migration *via* the a7 nAChR-JAK2/STAT3 pathway in RAW264.7 cells. *Cell. Physiol. Biochem*., (**2015**, *36*, 2025-2038. doi:10.1159/000430170.

[73] Parrish, W.R.; Rosas-Ballina, M.; Gallowitsch-Puerta, M.; Ochani, M.; Ochani, K.;Yang, L.-H.; Hudson, L.; Lin, X.;Patel, N.; Johnson, S.M.;Chavan, S.; Goldstein, R.S.; Czura, C.J.; Miller, E.J.; Al-Abed, Y.; Tracey, K.J.; Pavlov, V.A. Modulation of TNF release by choline requires α7 subunit nicotinic acetylcholine receptor-mediated signaling. *Mol. Med.,* **2008**, *14,* 567-574. doi:10.2119/2008-00079.Parrish.

[74] Lopez, G-Coviella, I.; Agut, J.; Von Borstel, R.;Wurtman, R.J. Metabolism of cytidine (5')-diphosphocholine (cdp-choline) following oral and intravenous administration to the human and the rat. *Neurochem. Int.,* **1987**, *11*, 293-297.

[75] Lopez G-Coviella, I.; Agut, J.; Savci, V.; Ortiz, J.A.; Wurtman, R.J. Evidence that 5'-cytidinediphosphocholine can affect brain phospholipid composition by increasing choline and cytidine plasma levels. *J. Neurochem.,* **1995***, 65,* 889-894.

[76] Gurun, M.S.; Parker, R.; Eisenach, J.C.; Vincler, M. The effect of peripherally administered cdp-choline in an acute inflammatory

pain model: The role of α7 nicotinic acetylcholine receptor. *Anesth. Anal.,* **2009** *108,* 1680-1687. doi:10.1213/ane.0b013e31819dcd08.

[77] Lawrence, T. The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb. Perspect. Biol.,* **2009**, *1*, 1-10. doi:10.1101/cshperspect.a001651.

[78] De Jonge, W.J.; Van der Zanden, E.P.; The, F.O.;Bijlsma, M.F.; Van Westerloo, D.J.; Bennink, R.J.; Berthoud, H.-R.; Uematsu, S.; Akira, S.; Van den Wijngaard, R.M.; Boeckxstaens, G.E. Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway. *Nat. Immunol.,* **2005**, *6,* 844-851. doi:10.1038/ni1229.

[79] Kox, M.; Pompe, J.C.; Peters, E.; Vaneker, M.; Van Der Laak, J.W. Van Der Hoeven, J.G.; Scheffer, G.J.; Hoedemaekers, C.W.; Pickkers, P. α7 Nicotinic acetylcholine receptor agonist GTS-21 attenuates ventilator-induced tumour necrosis factor-α production and lung injury. *Br. J. Anaesth.,* **2011**, *107,* 559-566. doi:10.1093/bja/aer202.

[80] O'Neill, L.A.J. The role of MyD88-like adapters in Toll-like receptor signal transduction. *Biochem. Soc. Trans.,* **2003**, *31,* 643-647. doi:10.1042/.

[81] Li, Q.; Zhou, X.-D.; Kolosov, V.P.; Perelman, J.M. Nicotine reduces TNF-α expression through a α7 nAChR/MyD88/NF-κB pathway in HBE16 airway epithelial cells. *Cell. Physiol. Biochem.,* **2011**, *27,* 605-612. doi:10.1159/000329982.

[82] Maldifassi, M.C.; Atienza, G.; Arnalich, F.; López-Collazo, E.; Cedillo, J.L.; Martín-Sánchez, C.; Bordas, A.; Renart, J.;Montiel, C. A New IRAK-M-mediated mechanism implicated in the anti-inflammatory effect of nicotine *via* α7 nicotinic receptors in human macrophageS. *PLoS One,* **2014**, *9,* e108397. doi:10.1371/journal.pone.0108397.

[83] Holmes, C.J.; Plichta, J.K.; Gamelli, R.L.; Radek, K.A. Burn injury alters epidermal cholinergic mediators and increases HMGB1 and caspase 3 in autologous donor skin and burn margin. *Shock*, **2016**, *47*, 175-183. doi:10.1097/SHK.0000000000000752.

[84] Lu, B.; Kwan, K.; Levine, Y.A.; Olofsson, P.S.; Yang, H.; Li, J.; Joshi, S.; Wang, H.; Andersson, U.; Chavan, S.S.; Tracey, K.J. α7 nicotinic acetylcholine receptor signaling inhibits inflammasome activation by preventing mitochondrial DNA release. *Mol. Med.,* **2014**, *20,* 350-358. doi:10.2119/molmed.2013.00117.

[85] Cedillo, J.L.; Arnalich, F.; Martín-Sánchez, C.; Quesada, A.; Rios, J.J.; Maldifassi, M.C.; Atienza, G.; Renart, J.; Fernández-Capitán, C.; García-Rio, F.; López-Collazo, E.; Montiel, C. Usefulness of α7 nicotinic receptor messenger RNA levels in peripheral blood mononuclear cells as a marker for cholinergic antiinflammatory pathway activity in septic patients: Results of a pilot study. *J. Infect. Dis.,* **2015**, *211,* 146-155. doi:10.1093/infdis/jiu425.

[86] Feuerbach, D.; Lingenhoehl, K.; Olpe, H.R.; Vassout, A.; Gentsch, C.; Chaperon, F.; Nozulak, J.; Enz, A.; Bilbe, G.; McAllister, K.; Hoyer, D. The selective nicotinic acetylcholine receptor α7 agonist JN403 is active in animal models of cognition, sensory gating, epilepsy and pain, *Neuropharmacology,* **2009**, *56*, 254-263. doi:10.1016/j.neuropharm.2008.08.025.

[87] Damaj, M.I.; Meyer, E.M.; Martin, B.R. The antinociceptive effects of alpha7 nicotinic agonists in an acute pain model. *Neuropharmacology,* **2000**, *39,* 2785-2791. doi:S0028390800001398 [pii].

[88] Medhurst, S.J.; Hatcher, J.P.; Hille, C.J.; Bingham, S.; Clayton, N.M.; Billinton, A.; Chessell, I.P. Activation of the α7-nicotinic acetylcholine receptor reverses complete freund adjuvant-induced mechanical hyperalgesia in the rat *via* a central site of action. *J. Pain*, **2008**, *9*, 580-587. doi:10.1016/j.jpain.2008.01.336.

[89] Van Maanen, M.A.; Lebre, M.C.;Van Der Poll, T.; LaRosa, G.J.; Elbaum, D.; Vervoordeldonk, M.J.; Tak, P.P. Stimulation of nicotinic acetylcholine receptors attenuates collagen-induced arthritis in mice. *Arthritis Rheum.,* **2009**, *60*, 114-122. doi:10.1002/art.24177.

[90] Marrero, M.B.; Bencherif, M.; Lippiello, P.M.; Lucas, R. Application of alpha7 nicotinic acetylcholine receptor agonists in inflammatory diseases: An overview. *Pharm. Res.*, **2011**, *28*, 413-416. doi:10.1007/s11095-010-0283-7.

[91] Loram, L.C.; Taylor, F.R.; Strand, K.A.; Maier, S.F.; Speake, J.D.; Jordan, K.G.; James, J.W.; Wene, S.P.; Pritchard, R.C.; Green, H.;Van Dyke, K., Mazarov, A., Letchworth, S.R., Watkins, L.R. Systemic administration of an alpha-7 nicotinic acetylcholine agonist reverses neuropathic pain in male sprague dawley rats. *J. Pain,* **2012**, *13,* 1162-1171. doi:10.1016/j.jpain.2012.08.009.

[92] Freitas, K.; Ghosh, S.; Carroll, F.I.; Lichtman, A.H.; Damaj, M.I. Effects of alpha 7 positive allosteric modulators in murine inflammatory and chronic neuropathic pain models. *Neuropharmacology,* **2013**, *65,* 156-164. doi:10.1016/j.neuropharm.2012.08.022.

[93] Pacini, A.; Di Cesare Mannelli, L.; Bonaccini, L.; Ronzoni, S.; Bartolini, A.; Ghelardini, C. Protective effect of alpha7 nAChR: Behavioural and morphological features on neuropathy. *Pain.,* **2010**, *150,* 542-549. doi:10.1016/j.pain.2010.06.014.

[94] Kanat, O.; Bagdas, D.; Ozboluk, H.Y.; Gurun, M.S. Preclinical evidence for the antihyperalgesic activity of CDPcholine in oxaliplatin-induced neuropathic pain. *J. B.U.ON.,* **2013**, *18,* 1012-1018.

[95] Bagdas, D.; Sonat, F.A.; Hamurtekin, E.; Sonal, S.; Gurun, M.S. The antihyperalgesic effect of cytidine-5'-diphosphate-choline in neuropathic and inflammatory pain models. *Behav. Pharmacol.,* **2011**, *22,* 589-598. doi:10.1097/FBP.0b013e32834a1efb.

[96] Zhu, Y., Peng, K., Meng, X., Ji, F. Attenuation of neuroinflammation by dexmedetomidine is associated with activation of a cholinergic anti-inflammatory pathway in a rat tibial fracture model. *Brain Res.,* **2016**, *1644,* 1-8. doi:10.1016/j.brainres.2016.04.074.

[97] Rowley, T.J.; McKinstry, A.; Greenidge, E.; Smith, W.;Flood, P. Antinociceptive and anti-inflammatory effects of choline in a mouse model of postoperative pain. *Br. J. Anaesth.*, **2010**, *105,* 201-207. doi:10.1093/bja/aeq113.

[98] Takeda, D.; Nakatsuka, T.; Gu, J.G.; Yoshida, M. The activation of nicotinic acetylcholine receptors enhances the inhibitory synaptic transmission in the deep dorsal horn neurons of the adult rat spinal cord. *Mol. Pain.,* **2007**, *3,* 26. doi:10.1186/1744-8069-3-26.

[99] Gao, B.; Hierl, M.; Clarkin, K.; Juan, T.; Nguyen, H.;Van Der Valk, M.; Deng, H.; Guo, W.; Lehto, S.G.; Matson, D.; McDermott, J.S.; Knop, J.; Gaida, K.; Cao, L.; Waldon, D.; Albrecht, B.K.; Boezio, A.A.; Copeland, K.W.; Harmange, J.C.; Springer, S.K.; Malmberg, A.B.; McDonough, S.I. Pharmacological effects of nonselective and subtype-selective nicotinic acetylcholine receptor agonists in animal models of persistent pain. *Pain,* **2010**, *149,* 33-49. doi:10.1016/j.pain.2010.01.007.

[100] Hurst, R.S.; Hajós, M.; Raggenbass, M.; Wall, T.M.; Higdon, N.R.; Lawson, J.A.; Rutherford-Root, K.L.; Berkenpas, M.B.; Hoffmann, W.E., Piotrowski, D.W., Groppi, V.E., Allaman, G., Ogier, R.; Bertrand, S.; Bertrand, D.; Arneric, S.P. A novel positive allosteric modulator of the alpha7 neuronal nicotinic acetylcholine receptor: *in vitro* and *in vivo* characterization. *J. Neurosci.,* **2005**, *25,* 4396-4405. doi:10.1523/JNEUROSCI.5269-04.2005.

[101] Timmermann, D.B.; Grønlien, J.H.; Kohlhaas, K.L.; Nielsen, E.Ø.; Dam, E., Jørgensen, T.D., Ahring, P.K., Peters, D., Holst, D., Chrsitensen, J.K.; Malysz, J.; Briggs, C.A.; Gopalakrishnan, M.; Olsen, G.M. An allosteric modulator of the α7 nicotinic acetylcholine receptor possessing cognition-enhancing properties *in vivo*. *J. Pharmacol. Exp. Ther.,* **2007**, *323,* 294-307. doi:10.1124/jpet.107.120436.vidual.

[102] Christensen, D.Z.; Mikkelsen, J.D.; Hansen, H.H.; Thomsen, M.S. Repeated administration of 7 nicotinic acetylcholine receptor (nAChR) agonists, but not positive allosteric modulators, increases 7 nAChR levels in the brain. *J. Neurochem.,* **2010**, *114,* 1205-1216. doi:10.1111/j.1471-4159.2010.06845.x.

[103] Freitas, K.; Carroll, F.I.; Damaj, M.I. The antinociceptive effects of nicotinic receptors α7-positive allosteric modulators in murine acute and tonic pain models. *J. Pharmacol. Exp. Ther.,* **2013**, *344,* 264-75. doi:10.1124/jpet.112.197871.

[104] Munro, G.; Hansen, R.R.; Erichsen, H.K.; Timmermann, D.B.; Christensen, J.K.; Hansen, H.H. The α7 nicotinic ACh receptor agonist compound B and positive allosteric modulator PNU-120596 both alleviate inflammatory hyperalgesia and cytokine release in the rat. *Br. J. Pharmacol.,* **2012**, *167,* 421-435. doi:10.1111/j.1476-5381.2012.02003.x.

[105] Balsera, B.; Mulet, J.; Fernández-Carvajal, A.; La Torre-Martínez, R.D.; Ferrer-Montiel, A.; Hernández-Jiménez, J.G.; Estévez-Herrera, J.; Borges, R.; Freitas, A.E.; López, M.G.; García-López, M.T.; González-Muñiz, R.; Pérez de Vega, M.J.; Valor, L.M.; Svobodová, L.; Sala, S.; Sala, F.; Criado, M. Chalcones as positive allosteric modulators of α7 nicotinic acetylcholine receptors: A new target for a privileged structure. *Eur. J. Med. Chem.*, **2014**, *86,* 724-739. doi:10.1016/j.ejmech.2014.09.039.

[106] Bagdas, D.; Targowska-Duda, K.M.; López, J.J.; Perez, E.G.; Arias, H.R.; Damaj, M.I. The antinociceptive and antiinflammatory properties of 3-furan-2-yl-N-p-tolyl-acrylamide, a positive allosteric modulator of α7 nicotinic acetylcholine receptors in mice. *Anesth. Analg.,* **2015**, *121,* 1369-1377. doi:10.1213/ANE.0000000000000902.

[107] Thakur, G.A.; Kulkarni, A.R.; Deschamps, J.R.; Papke, R.L. Expeditious synthesis, enantiomeric resolution, and enantiomer functional characterization of (4-(4-bromophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide (4BP-TQS): an allosteric agonist-positive allosteric modulator of α7 nicotinic ac. *J. Med. Chem.,* **2013**, *56*, 8943-8947. doi:10.1021/jm401267t.

[108] Chojnacka, K.; Papke, R.L.; Horenstein, N.A. Synthesis and evaluation of a conditionally-silent agonist for the α7 nicotinic acetylcholine receptor. *Bioorg. Med. Chem. Lett.,* **2013**, *23,* 4145-9. doi:10.1016/j.bmcl.2013.05.039.

[109] Van Maanen, M.A.; Papke, R.L.; Koopman, F.A.; Koepke, J.; Bevaart, L.; Clark, R.; Lamppu, D.; Elbaum, D.; LaRosa, G.J.; Tak, P.P.; Vervoordeldonk, M.J. Two novel α7 nicotinic acetylcholine receptor ligands: *in vitro* properties and their efficacy in collagen-induced arthritis in mice. *PLoS One,* **2015**, *10,* e0116227. doi:10.1371/journal.pone.0116227.

[110] Clark, R.B.; Lamppu, D.; Libertine, L.; Mcdonough, A.; Kumar, A.; Larosa, G.; Rush, R.; Elbaum, D. Discovery of Novel 2‐((Pyridin-3-yloxy)methyl)piperazines as α 7 Nicotinic Acetylcholine Receptor Modulators for the Treatment of Inflammatory Disorders. *J Med Chem.,* **2014**, *57,* 3966-3983. doi: 10.1021/jm5004599.

[111] Di Cesare Mannelli, L.; Pacini, A.; Matera, C.; Zanardelli, M.; Mello, T.; De Amici, M.; Dallanoce, C.; Ghelardini, C. Involvement of α7 nAChR subtype in rat oxaliplatin-induced neuropathy: Effects of selective activation. *Neuropharmacology,* **2014**, *79,* 37-48. doi:10.1016/j.neuropharm.2013.10.034.

[112] Di Cesare Mannelli, L.; Tenci, B.; Zanardelli, M.; Failli, P.; Ghelardini, C. α7 nicotinic receptor promotes the neuroprotective functions of astrocytes against oxaliplatin neurotoxicity. *Neural Plast.,* **2015**, *2015,* 396908. doi:10.1155/2015/396908.

[113] Plummer, H.K.; Dhar, M.; Schuller, H.M. Expression of the α7 nicotinic acetylcholine receptor in human lung cells. *Respir. Res.,* **2005**, *6,* 29. doi:10.1186/1465-9921-6-29.

[114] Davis, R.; Rizwani, W.; Banerjee, S.; Kovacs, M.; Haura, E.; Coppola, D.; Chellappan, S. Nicotine promotes tumor growth and metastasis in mouse models of lung cancer. *PLoS One,* **2009**, *4,* e7524. doi:10.1371/journal.pone.0007524.

[115] Apkarian, A.V.; Sosa, Y.; Krauss, B.R.; Thomas, P.S.; Fredrickson, B.E.; Levy, R.E.; Harden, R.N.; Chialvo, D.R. Chronic pain patients are impaired on an emotional decision-making task. *Pain,* **2004**, *108,* 129-136. doi:10.1016/j.pain.2003.12.015.

[116] Ji, G.; Sun, H.; Fu, Y.; Li, Z.; Pais-Vieira, M.; Galhardo, V.; Neugebauer, V. Cognitive impairment in pain through amygdala-driven prefrontal cortical deactivation. *J. Neurosci.,* **2010**, *30,* 5451-5464. doi:10.1523/JNEUROSCI.0225-10.2010.

[117] Neugebauer, V.; Galhardo, V.; Maione, S.; Mackey, S.C. Forebrain pain mechanisms. *Brain Res. Rev.,* **2009**, *60*, 226-242. doi:10.1016/j.brainresrev.2008.12.014.

[118] Hummel, M.; Lu, P.; Cummons, T.A.; Whiteside, G.T. The persistence of a long-term negative affective state following the induction of either acute or chronic pain. *Pain,* **2008**, *140,* 436-445. doi:10.1016/j.pain.2008.09.020.

[119] Mogil, J.S. Animal models of pain: progress and challenges. *Nat. Rev. Neurosci.,* **2009**, *10,* 283-294. doi:10.1038/nrn2606.

[120] Joshi, S.K., Honore, P. Animal models of pain for drug discovery. *Expert Opin. Drug Discov.,* **2006**, *1,* 323-34. doi:10.1517/17460441.1.4.323.

[121] Whiteside, G.T.; Pomonis, J.D.; Kennedy, J.D. An industry perspective on the role and utility of animal models of pain in drug discovery. *Neurosci. Lett.,* **2013**, *557,* 65-72. doi:10.1016/j.neulet.2013.08.033.

[122] Bagdas, D.; Muldoon, P.P.; Alsharari, S.; Carroll, F.I.; Negus, S.S.; Damaj, M.I. Expression and pharmacological modulation of visceral pain-induced conditioned place aversion in mice. *Neuropharmacology,* **2016**, *102,* 236-243. doi:10.1016/j.neuropharm.2015.11.024.