Pharmacological Research 190 (2023) 106735

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

The α9α10 acetylcholine receptor: A non-neuronal nicotinic receptor☆

Ana Belén Elgoyhen *

Instituto de Investigaciones en Ingeniería Genética y Biología Molecular "Dr. Héctor N. Torres" (INGEBI), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Vuelta de Obligado 2490, Buenos Aires 1428, Argentina

ARTICLE INFO

Keywords:
Nicotinic receptors
Ligand-gated channels
Hair cells
Cochlea
Hearing

ABSTRACT

Within the superfamily of pentameric ligand-gated ion channels, cholinergic nicotinic receptors (nAChRs) were classically identified to mediate synaptic transmission in the nervous system and the neuromuscular junction. The α9 and α10 nAChR subunits were the last ones to be identified. Surprisingly, they do not fall into the dichotomic neuronal/muscle classification of nAChRs. They assemble into heteropentamers with a well-established function as canonical ion channels in inner ear hair cells, where they mediate central nervous system control of auditory and vestibular sensory processing. The present review includes expression, pharmacological, structure-function, molecular evolution and pathophysiological studies, that define receptors composed from α9 and α10 subunits as distant and distinct members within the nAChR family. Thus, although α9 and α10 were initially included within the neuronal subdivision of nAChR subunits, they form a distinct clade within the phylogeny of nAChRs. Following the classification of nAChR subunits based on their main synaptic site of action, α9 and α10 should receive a name in their own right.

1. Introduction

A wide spectrum of ion channel families has evolved over billions of years and plays fundamental roles in all domains of life. Included in this wide variety, the superfamily of pentameric ligand-gated ion channels (pLGICs) is the largest and most functionally diversified within those ion channels which are gated in response to ligand binding [1,2]. The discovery of pLGICs in bacterial species and Archaea indicates an ancient origin for this receptor superfamily [1–4]. In vertebrates, pLGICs are named the Cys-loop family, since all member subunits contain a disulfide cysteine bridge in the extracellular domain which closes a loop comprising 13 amino acids [1,5–7]. This Cys-loop family includes the nicotinic acetylcholine receptors (nAChRs), serotonin type 3 receptors (5-HT_3), gamma aminobutyric acid type A receptors ($GABA_A$) and glycine receptors [1,2,8].

Vertebrate nAChRs are non-selective cation channels. To date, 19 different nAChR subunits have been described: α1- α10, β1- β4, γ, δ and ε [1,9,10], including α11 and β5 only identified in some fish species [11]. The vertebrate nAChRs have been classically divided into two subgroups, following their main expression pattern: neuronal and muscle nAChRs. The last nAChR subunits to be identified were α9 and α10, whose main function has been deciphered in inner ear mechanosensory hair cells and lateral line systems [12–18]. The general structure of nAChRs is described in other manuscripts within this special issue and will not be covered here. The present review includes expression, pharmacological, structure-function, molecular evolution and pathophysiological studies, that define receptors composed from α9 and α10 subunits as distant and distinct members within the nAChR family.

2. Expression of α9 and α10 nAChR subunits

The α9 subunit was first isolated from a rat olfactory epithelium cDNA library [17]. Early on, in situ hybridization analysis localized α9 to rat cochlear [17,19] and vestibular hair cells [20–22], the nasal (non-neuronal) epithelium, the pars tuberalis of the pituitary [17], and bone marrow [23]. The α10 subunit was first isolated from a rat cochlear

Abbreviations: ACh, acetylcholine; pLGICs, pentameric ligand-gated ion channels; nAChRs, nicotinic cholinergic receptors; 5-HT_3,, serotonin type 3 receptors, $GABA_A$, gamma aminobutyric acid type A receptors; MOC, medial olivocochlear; OHCs, outer hair cells; ERBB2, receptor tyrosine-protein kinase erbB-2; TMIE, transmembrane inner ear; IL-1β, interleukine 1β; PAMs, positive allosteric modulators, TM, transmembrane region.

☆ The multifaceted activities of nervous and non-nervous neuronal nicotinic acetylcholine receptors in physiology and pathology. Eds: Dr Cecilia Gotti, Prof Francesco Clementi, Prof Michele ZOli.

* Correspondence to: Vuelta de Obligado 2490, 1438 Buenos Aires, Argentina.
E-mail addresses: abelgoyhen@gmail.com, elgoyhen@dna.uba.ar.

https://doi.org/10.1016/j.phrs.2023.106735
Received 10 January 2023; Received in revised form 6 March 2023; Accepted 14 March 2023
Available online 15 March 2023

[16] and subsequently from a human cochlear cDNA library [18]. Similar to the α9 subunit, early studies localized this subunit to cochlear and vestibular hair cells [16,24,25], pars tuberalis of the pituitary and keratinocytes [18]. Further studies have described the expression of α9 and/or α10 subunits in several non-neuronal, mostly epithelial cells, such us the adrenal medulla [26], immune cells [27–33], keratinocytes [34], urothelium [35], placenta [36], bronchial epithelium [37], to name a few expression sites. Several studies have reported the expression of α9 subunits in the retina [38–40]. Since retinal vascular endothelial cells express the α9 subunit mRNA [40], caution should be taken when arriving at conclusions of neuronal expression when performing RT-PCR experiments from retinal tissue. Further experiments are needed in order to determine a putative neuronal function of α9* in the retina. Although some studies have claimed the presence of α9 and α10 subunits in dorsal root ganglia [41], these results have not been replicated. Thus, in a meta-analysis of single-cell gene expression data from mouse dorsal root ganglia neurons, α9 expression was not detected and α10 only at very low levels in a few cell subtypes or not found at all [42–44], an observation in line with that reported by quantitative PCR and functional assays [45]. This has been further confirmed with RNAscope in situ hybridization [46]. However, this same study has shown the presence of α9 (α10 at very low levels) mRNA in 25% of human dorsal root ganglia neurons [46]. Whether this peculiar differential expression of α9 subunits in humans is extended to other sites, is unknown at present. In addition, although α9 and α10 neuronal and brain expression has also been proposed [47–49], a wealth of experimental data indicates that these nAChR subunits are not expressed in the central nervous system (for review see [50]). Thus, taken into account their known main expression pattern, α9 and α10 subunits should not be included within the neuronal subdivision of nAChRs.

3. Physyology of α9* and α10* nAChRs

3.1. The inner ear hair cell nicotinic receptor

The first characterized, and best known function of α9α10 nAChRs, is that in inner ear mechanosensory hair cells [16,17]. Moreover, it is only in hair cells that the α9α10 nAChR has been unequivocally described to serve a canonical ion channel function in vivo. Thus, the proposal to name α9 and α10 as the hair cell subunit branch of nAChRs [42]. An ancestral feature of vertebrate inner ear hair cells is the presence of efferent innervation from (mainly) cholinergic neurons located in the ventral hindbrain that project all the way to the peripheral sensory system [51]. In adult mammals medial olivocochlear (MOC) neurons make direct synaptic contacts with the base of outer hair cells (OHCs), the cochlear amplifier, and inhibit somatic electromotility and thus sound amplification [51]. During a critical pre-hearing period, MOC neurons transiently contact inner hair cell, eliciting α9α10 mediated responses [52,53]. Inhibition is brought about by the release of acetylcholine (ACh) from efferent terminals, the activation of very high calcium permeable α9α10 nAChRs [54–56] of OHCs, coupled to the subsequent activation of Ca^{2+} activated SK2 K^{+} channels and hair cell hyperpolarization [57,58] (Fig. 1). This gives rise to the biphasic nature of postsynaptic responses to ACh and is the only example of a nicotinic receptor serving an overall inhibitory synaptic transmission [53,59–62]. A similar biphasic response mediated by α9* receptors coupled to K^{+} channels mediates efferent inhibition of Type II vestibular hair cells [63]. While α9 subunits can assemble into functional homomeric receptors when expressed in heterologous systems [16,18], these do not play a major role in inner ear hair cells in vivo, as seen in *Chrna10* gene knockout mice [14].

A detailed analysis of the synaptic properties of the MOC-OHC synapse performed in cochlear explants, has shown that a correct operation of this feedback requires matching of acoustic input with the strength of cochlear inhibition [64–66]. This is driven by the rate of MOC activity, by short-term facilitation mechanisms present at the MOC-OHC synapse

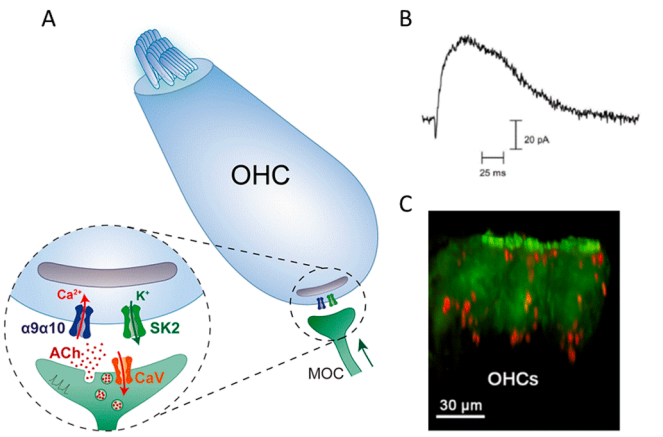

Fig. 1. α9α10 nAChRs mediate transmission at the MOC efferent-hair cells synapses of the cochlea. A. Schematic diagram of the efferent synapse between a medial olivocochlear fiber and an outer hair cell, highlighting the entry of calcium through the α9α10 nAChR and the coupling the SK2 channel. B. Representative spontaneous synaptic current recorded in a P10 OHC from a mouse apical cochlear coil, voltage-clamped at -60mV. A rapid inward current, mediated by the α9α10 nAChR is followed by a larger and longer-lasting outward current, mediated by the SK2 potassium channel. C. Cy3-RgIA-5727 labelling (red) of α9α10 nAChRs in OHCs.
A. (Reproduced from Lipovsek et al., 2021, under the Creative Commons license (http://creativecommons.org/licenses/by/4.0/). B. (Reproduced from Katz and Elgoyhen, 2014, under the Creative Commons license (http://creativecommons.org/licenses/by/4.0/). C. (Reproduced from Fisher etal., 2021, under the Creative Commons license (http://creativecommons.org/licenses/by/4.0/).

[61,67], and by a tight compartmentalization of calcium ions at the base of hair cells [68,69]. In this regard, α9 and α10 knockout mice lack OHCs ACh-evoked currents, and therefore efferent inhibition of OHC function [14,15]. On the other hand, mice carrying a threonine for leucine substitution at position 9´(L9 T) of transmembrane region (TM) 2 of the α9 subunit, lead to hair cell ACh-evoked responses with increased agonist apparent affinity and decreased desensitization kinetics [70] (Fig. 2). This greatly elongates hair cell-evoked inhibitory post-synaptic currents and Ca^{2+} signals, leading to enhanced and sustained synaptic responses (Fig. 2) and hair cell hyperpolarization upon high-frequency stimulation of MOC terminals and a longer time course of efferent MOC suppression [70,71]. Since the kinetics of this synapse sets the function of the efferent MOC system, the development of positive allosteric modulators (PAMs) of α9α10 nAChRs have been suggested as possible candidates to treat inner ear disorders [72,73].

The generation of α9 and α10 knockout mice [14,15,74] and the L9 T knockin [70] have helped elucidate the function of the efferent system in inner ear physiology. Thus, experiments performed with these genetically modified mice have supported the participation of the efferent system in the development of the entire auditory circuitry [71,75–80], in sound localization [81], in the suppression of auditory distractors during selective attention to visual stimuli [82,83], in the protection of the cochlea from the damage produced by the exposure to loud sounds [70,84], and in age-related hearing loss [85]. The use of these genetically modified mice are beginning to disentangle the effect of the efferent innervation to the vestibular system [86].

3.2. Non-auditory functions of α9* and α10* nAChRs

The non-auditory functions of α9* and/or α10* nAChRs are less clear and are mainly the result of the expression of α9 and α10 subunits in epithelial cells outside the inner ear. Of particular importance is the role of ACh as a regulator of the immune system with pro- and anti-inflammatory potential [87,88]. In this regard, the presence of α9 and

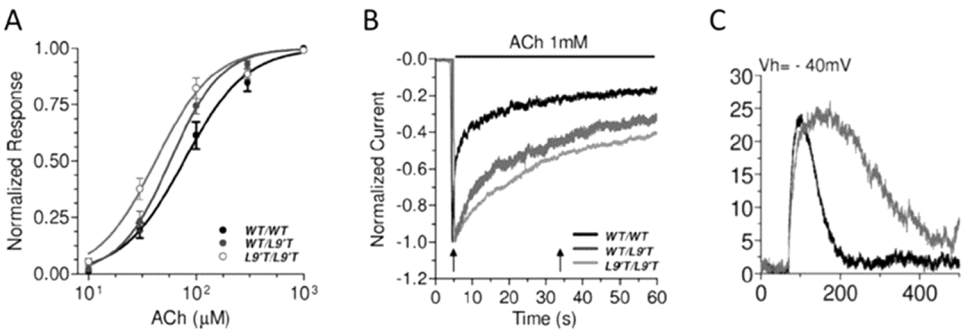

Fig. 2. Responses in α9 L9 T knockin mice. A. Concentration-response curves to ACh performed in P9–P10 IHCs. α9 L9 T hair cells are hypersensitive to ACh. B. Wave-form of responses to 1 mM ACh during 1 min ACh application in P6–P10 IHCs voltage-clamped at − 90 mV. α9(L9 T)α10 receptors exhibit slower desensitization kinetics. C. Spontaneous synaptic currents in inner hair cells voltage clamped at − 40 mV, showing prolonged responses in α9 L9 T knockin mice.
Modified from Taranda etal., 2009, under the Creative Commons license (http://creativecommons.org/licenses/by/4.0/).

α10 nAChRs in immune cells leads to their participation in several immune and inflammatory responses. For example, brain activity directly enhances the adaptive immune responses in lymphoid organs in a manner that requires B-cell responsiveness to ACh mediated by α9* nAChRs [32]. In addition, α9* nAChRs are involved in endogenous pro-inflammatory mechanisms required for disease initiation and evolution of experimental autoimmune encephalomyelitis, a mouse model for multiple sclerosis, likely via the endogenous agonist acetylcholine [89–91]. Moreover, several antagonists of α9* nAChR have been shown to be analgesic and anti-inflammatory in animal models of neuropathic pain [33,92]. In contrast, α9* nAChRs mediate anti-inflammatory effects during the acute phase response of systemic inflammation caused by trauma or infection [93].

Signaling through α9* nAChR is also important for keratinocyte adhesion and completion of the very early stages of epithelialization [94,95] and form part of the skin non-neuronal cholinergic system which comprises several nicotinic and muscarinic receptors [96]. Moreover, the human α9* nAChR is targeted in Pemphigus vulgaris autoimmunity, suggesting its participation in skin pathology [34]. In addition, a role for α9* nAChRs in stress responses has been suggested by comparing the stress- and affect-related phenotypes of wild-type and α9 knockout mice [97]. Thus, α9 knockout mice show decreased stress-induced arousal and increased anxiety-like behavior after a sub-chronic restraint stress. This might be related to the expression of α9 nAChR subunits in the adrenal medulla [26]. Finally, α9* nAChR have been increasingly involved in the formation, progression, and metastatic spread of various tumors, including lung, breast, glioblastoma, and melanoma cancers (for review see [98,99]).

Recent evidence supports the existence of non-canonical signaling pathways used by ligand-gated ion channels [100]. In particular, nAChRs expressed in numerous non-neuronal cell types serve functions that do not require canonical ion channel function, but rather affect intracellular signal transduction pathways and regulate gene expression [101]. The pharmacology for such non-canonical signaling through nAChRs is different from channel activation [101]. The metabotropic mode of action of nAChRs was first shown for the α7* nAChR in leukocytes [102]. In T cells, activation of α7* nAChRs induces metabotropic signaling that results in an increase of intracellular Ca^{2+} concentrations, independent of obvious ionotropic receptor functions [102]. It seems likely that the effects of nAChRs in immune cells is always mediated by non-canonical metabotropic pathways, since no ion-currents have been detected in response to nAChR agonists [28,102–105].

In human and murine monocites, ACh acting as a paracrine or autocrine regulator elicits a metabotropic response to reduce ionotropic P2×7 mediated ATP-induced intracellular calcium signals that lead to inflammasome activation, by limiting the release of the pro-inflammatory interleukine 1β (IL-1β) into the circulation. Moreover, phosphocholine modifies macromolecules produced by pathogens and canonical nicotinic agonists like nicotine and choline inhibit IL-1β release and trigger anti-inflammatory mechanisms via α7 and α9α10 nAChRs [104,105]. Since phosphocholine and nicotine do not evoke ion current responses in α9 and α10 cRNA injected *Xenopus laevis* oocytes, α9α10 nAChR-mediated immuno-modulatory effect is not due to a canonical ionotropic receptor function [105]. Moreover, choline-gated currents in *Xenopus* oocytes expressing human α9α10 nAChR are reversibly inhibited by phosphocholine, resembling a silent agonist or antagonist function [105]. Furthermore, metabolites of phosphatidylcholines such as lysophosphatidylcholine and glycerophosphocholine also trigger α9α10 nAChR metabotropic activity in monocytes, but do not elicit ion currents in α9α10-expressing oocytes [106]. The mechanism downstream of nAChR activation controlling monocyte ATP ion-channel function is for the most part unknown and currently under investigation [107].

Another example of non-canonical signaling of α9* and α10* nAChRs is that in cancer cells (for review see [99,108]). In a recent work, several α9 nAChR interacting and downstream proteins were identified from a resource of cancer membrane protein-regulated networks (CaMPNets), containing 63,746 high confidence protein–protein interactions for 1962 membrane proteins, using expression profiles from 5922 tumors across 15 human cancers [98]. These were related to cell growth and communication in more than six cancer types, thus suggesting that α9 plays a role in the formation, progression, and metastatic spread of various tumors [98]. These interactions were further verified by immunoprecipitation and FRET analysis and include proto-oncogene tyrosine-protein kinase, stratifin, receptor tyrosine-protein kinase erbB-2 (ERBB2), receptor tyrosine-protein kinase erbB-3, tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Gamm, and casein kinase I isoform delta. Moreover, this work shows dissociation of the α9/ERBB2 complex in the presence of nicotine, indicating that the α9 interactome in cancer cells might be a pharmacotherapeutic target [98].

4. Structure-function of α9* and α10* nAChRs

Early on classical biochemical and mutagenesis analysis [109,110], together with the low resolution of the Torpedo muscle-type nAChR [111,112], delineated the structure of nAChRs. These studies have been recently refined by the crystal structures of the molluscan ACh binding proteins (AChBPs) [113,114], the high resolution monomeric extracellular domains of the α1, α9 and β3 [115–117,118] and homopentameric α2 nAChRs [119], the α7-AChBP chimera [119], the near-intact α4β2 [120], the cryoEM structures of α4β2 [121] and of the near-intact α7 nAChR [122,123].

nAChRs are pentameric assemblies of homologous subunits. Each has a similar structure with a large extracellular N-terminal region, four transmembrane helices (TM1–TM4), and an intracellular domain between TM3 and TM4 [112,113,124]. The orthosteric agonist binding site lies at the interface of the extracellular domains of adjacent subunits and is formed by six noncontiguous regions (loops A–F). Each binding site is composed of a principal component or (+) face provided by loops A-C of one subunit and a complementary component (-) comprising loops D-F

of the adjacent subunit [112,113,124]. Moreover, nAChRs can be assembled from either homomeric or heteromeric subunit combinations [125]. In the case of homomeric receptors, such as α7, the ACh binding sites are composed from the identical principal and complementary components and are therefore equivalent. In contrast to homomeric nAChRs, heteromeric receptors can have nonequivalent ACh binding sites provided by different subunit interfaces due to the combination of α and β subunits, in the case of neuronal receptors [125].

So far only functional heteromeric assemblies of α9 and α10 subunits have been reported, lacking convincing evidence of assembly with other nAChR subunits [17,42,126]. Taking into account its tissue distribution, sequence identity to other nAChR subunits and pharmacology and evolution, α9 and α10 subunits are atypical members of the nAChR family. Different to muscle and neuronal nAChRs, when combined into a heteromeric assembly they form a receptor composed only of α subunits [16,18]. A $(\alpha9)_2(\alpha10)_3$ stoichiometry has been determined for the rat recombinant receptor [127], although expression of a 10-fold excess of α9 in *Xenopus* oocytes can lead to an additional receptor isoform with a $(\alpha9)_3(\alpha10)2$ stoichiometry [128]. The arrangement of subunits in the pentamer and the relative contribution of each subunit to the binding pocket of the heteromeric α9α10 receptor is unknown, since only monomers of the α9 human extracellular domain have been crystalized [115,116]. Since murine α9 subunits can form functional homomeric α9 receptors [17], the α9 subunit is capable of providing principal and complementary components to functional agonist binding sites. In contrast, initial experiments indicated that rat and human α10 subunits do not form functional channels when heterologously expressed in *Xenopus laevis* oocytes [16,18], leading to the proposal that α10 might only provide complementary components to agonist binding and serve as a structural subunit, similar to the β subunits of heteromeric nAChRs [58]. However, this is not the case, since site-directed mutagenesis coupled to molecular docking analysis indicate that murine α10 subunits contribute to the principal component of the binding site [129]. In addition, these studies have shown that the contribution of α9 and α10 to the complementary component of the binding site is nonequivalent, since mutagenesis studies have indicated that the binding site does not equally involve residue W55 of loop D (which is key for ACh binding and gating of nAChRs [10,130]) when either provided by α9 or α10 [129]. Moreover, docking analysis show that for rat α9α10 nAChRs the most frequent conformations with ACh bound in the correct orientation are observed at the interface in which α10 contributes to the principal and α9 to the complementary component [129]. The non-equivalent contribution of murine α9 and α10 subunits to the complementary component of the binding site might be due to differences in the subunit amino acid sequence. Thus, residues on a radial distribution of 5 Å from the ACh binding site are the same in α9 and α10 for the principal components (Y93, S148, W149, Y190, C192, and Y197), whereas an excess of positively charged residues are present in the complementary component of α10 compared to α9 [124,129]. This might produce a repulsion of positively charged compounds and less favorable contribution of α10 to the (-) face. In addition, recent experiments show that ACh-evoked currents can be obtained in *Xenopus* oocytes that express human α10 homomers, by exposing the oocytes to the alkaloids strychnine, brucine, or methyllycaconitine, further indicating that this subunit can contribute to principal and complementary components of the binding site [131]. Finally, the crystal structure of the potent α9α10 receptor antagonist α-CTx RgIA bound to the monomeric extracellular domain of α9, followed by molecular dynamics experiments [115], in addition to mutagenesis analysis [132] have shown that this conotoxin interacts at the α9(+)α9(-), α10(+)α9(-) rather than the α9(+)α10(-) nAChR subunit interface, indicating the participation of α10 subunits mainly to the principal component of the binding site.

One distinct feature of the recombinant and native hair cell α9α10 nAChR is its pharmacological profile. Although the α9α10 nAChR is activated by ACh, most classical nicotinic receptor agonists, including nicotine, do not elicit responses and block ACh-evoked currents [16,18,133]. Based on the α9 extracellular domain crystal structures [115,116] and molecular dynamics simulations [132], it has been suggested that the mode of engulfment of a ligand by loop C may differ significantly in α9α10 nAChRs compared with other nAChR subtypes [124], due to an excess accumulation of positively charged residues in (-) sides of α9 and α10 subunits. This could explain the conversion of classical agonists to antagonists in the case of α9-containing nAChRs [124]. In addition, the canonical metabotropic muscarinic receptor agonist muscarine also blocks α9α10 nAChRs [16]. Moreover, rat and chicken α9α10 nAChRs are blocked by the classical nicotinic and muscarinic antagonists curare and atropine, respectively, thus exhibiting a mixed nicotinic and muscarinic pharmacological profile [16,134]. Most strikingly, α9α10 nAChRs are blocked by the glycinergic antagonist strychnine, the GABAergic antagonist bicuculline and the serotonin type 3 receptor antagonists ICS 205–930 and ondansetron, thus sharing pharmacological profiles with other members of the Cys-loop family of pentameric ligand-gated ion channels [16,135,136]. Compared to muscle and α7 which are irreversibly blocked by α-bungarotoxin, α9α10 nAChRs are reversible blocked by this compound [16,18], further indicating differential structural determinants and kinetics of ligand binding. Several highly potent and selective peptides that block α9α10 nAChR have been isolated from the venom of Conus marine snails [137–141]. These open up new opportunities for developing novel peptide therapeutics and molecular probes to study the structure and function of α9α10 nAChR [92,138,142].

Recombinant α9α10 nAChR were only classically expressed in *Xenopus* oocytes [16,18], due to their inefficient expression in cell lines [143], with the exception of mammalian GH4C1 cells [54]. However, using genome-wide complementary DNA screening to identify functional partners for α9α10, choline acetyltransferase and transmembrane inner ear (TMIE) were isolated and both elicit α9α10 nAChR expression in HEK cells [144]. Since expression is also increased by pre-incubation of cells with either ACh or the potent α9α10 antagonist methyllycaconitine, the isolation of choline acetyltransferase most likely indicates that expression is enhanced by ligand binding [144]. On the other hand, although co-immunoprecipitation experiments suggest that TMIE might be an auxiliary component of the α9α10 receptor [144], its mechanism of action is less clear. TMIE is mainly implicated in mechanotransduction near the tips of hair cell stereocilia [145] and mutations in this gene leads to human deafness [146], a phenotype not seen in α9 and α10 knockout mice [14,15,80]. Moreover, TMIE knockout mice do not have a clear lack of efferent function phenotype [144]. Single channel analysis will be required in order to decipher the effect of TMIE in α9α10 nAChR function.

5. Evolution of α9 and α10 nAChRs subunits

The cloning of the α9 nAChR subunit and the initial comparison of its amino acid sequence with that of other members of the nAChR family, indicated that whereas neuronal nAChR α subunits and the muscle α1 subunit share aminoacid sequence identities that range from 48% to 70%, the sequence identity between α9 and all known nAChR subunits is less than 39%. This, taken together with the fact that the intron-exon structure of the gene encoding the α9 subunit differs from that of other known nAChR subunit genes, indicate that α9 is a distinct and distant member of the nAChR family [17]. This has been proved in recent years, with the evolutionary analysis of the increasing amount of known nAChR receptor subunit sequences from different species and *in silico* molecular evolutionary tools, coupled to mutagenesis analysis [9,11,42,55,147].

Several phylogenetic analyses of the nAChR family of subunits have been reported in the literature [11,42,55,147–155]. Some of them pre-date the high throughput sequencing era, include few sequences and therefore are less informative. The ancestral subunit from which the entire family of extant nAChR subunits radiated, is unknown. The presence of both a neuronal/muscle-like subunit and a α7- like subunit

in the last common ancestor of Bilateria is supported by the identification of corresponding groups of subunits in primitive invertebrates by several groups [11,151,153,156–159]. In contrast, current evidence is weak in order to clearly establish the presence of an α9-like subunit in the last common ancestor of Bilateria. As sequences become available, further comprehensive analysis of Eumetazoan nAChR subunits will be required in order to claim an ancient and early split of the α9 nAChR [9], as suggested early on [135,148–150,160] and from then onwards paraphrased in the literature.

The evolution of nAChR subunits in the vertebrate lineage has been extensively studied and the results obtained replicated and reliable. The last common ancestor of vertebrates had a repertoire of 10 genes coding for nAChR subunits [11]. The extant complement of vertebrate subunits most likely resulted from the first and second rounds of tetraploidization which took place in the stem vertebrate branch, gene losses and occasional de novo duplications and, in the teleost lineage, a third whole genome duplication. Thus, the different extant subunits are encoded by paralogous genes, most likely derived from five paralogons [11].

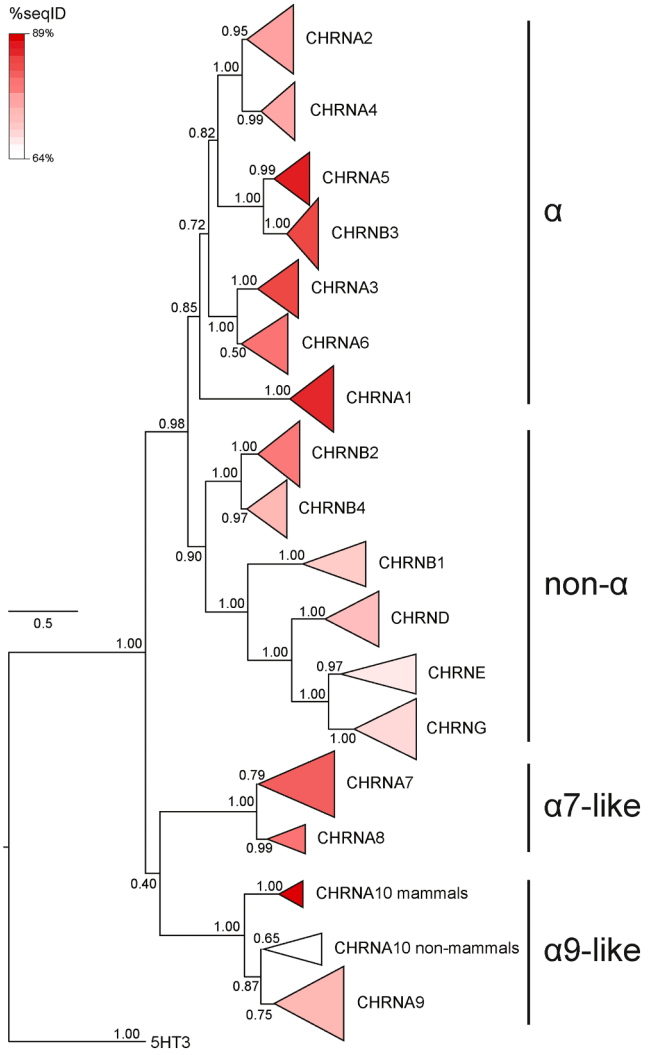

Fig. 3. Phylogenetic tree of vertebrate nicotinic subunits. The branches corresponding to the same subunits of different species were collapsed to their respective node. The length of each triangle indicates the sequence divergence from the corresponding node. Triangle colors show the average sequence identity within the branch. Bootstrap values are indicated by the numbers in each branch. The scale bar indicates the number of amino acid substitutions per site.

Modified from Marcovich et al., 2020 and Lipovsek et al., 2021, under the Creative Commons license (http://creativecommons.org/licenses/by/4.0/).

The latest phylogenetic study of the nAChR family includes 392 amino acid sequences from 17 nAChR subunits of 29 vertebrate species [42]. The family of nAChR subunits can be divided into four groups: α, non-α, α7-like, and α9-like (Fig. 3). Due to the lowest degree of sequence identity when compared against all other nAChR subunits, α9 and α10 subunits form their own group and the outermost branch on the tree. It should be once again noted that, since all phylogenetic trees have been built based only on sequence identity, this position of the α9/α10 group cannot be used as evidence for an ancestral origin of these subunits [9].

One important and peculiar feature of the tree is that, due to their high degree of coding sequence conservation, all sequences for each one of the nAChR subunits fall within their own respective branch, except α10. These latter subunits are unique in presenting a segregated grouping of orthologs. Thus, non-mammalian α10 subunits form a sister group to all α9 subunits, while mammalian α10 subunits are an outgroup to the α9/non-mammalian α10 branch [42]. This differential grouping of the α9-like branch of subunits has been systematically reported in several studies, which have included different number of sequences from different species [55,147,153]. The grouping of α10 orthologs into differential branches results from a low percentage of amino acid sequence identity between vertebrate α10 subunits, together with a high conservation of α10 subunit sequences within the mammalian lineage. The variability in primary sequence of non-mammalian α10 subunits has resulted from a high rate of acquisition of non-synonymous amino acid substitutions in the coding region of the protein [11,42,55,147], that were subjected to Darwinian positive selection during the course of evolution. Thus, signatures of positive selection acting on CHRNA10 have been determined by several *in silico* molecular evolutionary analysis [55,147]. Moreover, the *in silico* search for site-specific shifts in the amino acid biochemical state between clades, indicates functionally significant amino acid changes when comparing α9 and α10 mammalian subunits with their sauropsid counterparts, and no changes in the case of neuronal nAChR subunits [42].

The distinct evolutionary trajectories of α9 and α10 compared to that of neuronal nAChR subunits has prompted functional and mutagenesis analysis in order to understand their physiological consequences. Thus, a comprehensive analysis of functional and biophysical properties (ACh sensitivity, calcium permeation and modulation, desensitization rate, voltage-current relationship) of α9α10 receptors from three representative tetrapod species (rat, chicken, and frog) [42] and zebrafish [13], have shown striking differences across them, following the functional divergence analysis at the amino acid sequence. This is in strike contrast to the high degree of functional conservation observed for rat, chicken and frog neuronal α4β2 and α7 nAChRs [42].

Positively selected sites identified in the α10 subunit reside mainly within the conserved ligand-binding and gating regions of the protein [55,147]. Thus, the asymmetric contribution of α9 and α10 subunits to the (-) face of the ACh binding site described above might result from the adaptive evolution that occurred in mammalian CHRNA10 genes. For example, residue 117 in Loop E of the complementary component of the binding site, is a positively charged arginine in mammalian α10 subunits and a non-charged threonine or methionine in α9 and non-mammalian α10 subunits [147]. Homology models of the extracellular domain with ACh docked in the binding site show that the positively charged R117 in rat α10 is located ~8–9 Å from the ACh amino group and can thus produce repulsion of the agonist in mammalian, but not in non-mammalian subunits [129]. This seems to be the case, since contrary to that observed for rat subunits, in chicken α9α10 nAChRs both α9 and α10 equally contribute to the (-) face of the intersubunit interface when expressed in *Xenopus* oocytes [129]. Moreover, when rat α10 R117 is replaced by a methionine, mimicking that seen in non-mammalian α10 and all α9 subunits, R117M rat mutant α10 subunits can readily contribute to the complementary component of the binding site of α9α10R117M receptors [129]. In addition, the evolutionary changes acquired in the mammalian α10 (-) face of the binding site rendered a rat α9α10 nAChR in which choline acts as a partial agonist, when compared

to its full efficacy in chicken receptors [161].

Clade-specific functionally significant amino acid changes also occurred during the evolution of the α9 subunits, albeit with a lower prevalence when compared to α10 [162]. Using molecular dynamics simulations and an evolutionary-based mutagenesis strategy that included ancestral sequence reconstructions, three specific amino acid substitutions in the α9 subunit that rendered a high calcium permeable mammalian (but not chicken) α9α10 nAChR were identified, stemming from a low calcium permeable amniote ancestor. The α9 identified sites are located at the extracellular vestibule (D110 and S127) and at the exit of the channel pore (4′A) [162].

Taken together, the inter-clade sequence divergence and the positive selection of non-synonymous substitutions in mammalian α9 and/or α10 subunits, indicate that contrary to that observed for neuronal nAChR subunits, the evolution of the former subunits has been dominated by functionally significant changes in the coding sequences. This might relate to the fact that hair cells, where the α9α10 nAChR plays a major sensory synaptic role as a canonical ion channel, do not express other nAChR subunits [19,20]. Thus, innovation of receptor function across species during evolution could only occur through changes in functional properties of the α9α10, based on changes at the protein coding sequence and not through toggling between alternative expressed subunits, as observed for neuronal nAChRs [9,42]. The evolutionary trajectory of the α9α10 nAChR is in line with what has been described for an increasing number of inner ear expressed genes, pinpointing adaptive molecular evolution of protein sequences as a major player in the emergence of morphological and functional innovations in the mammalian inner ear [163,164].

6. Targeting α9α10 nAChRs in pharmacotherapeutics

6.1. Inner ear disorders

Hearing loss is one of the most prevalent sensory disabilities and a major public health problem. It impairs language development and education, communication, mental health, employment, with impact in interpersonal relationships and/or psychosocial well-being [165,166]. Moreover, recent studies indicate that hearing loss accelerates cognitive decline and dementia risk in older adults [167–169]. The World Report on Hearing from the World Health Organization [170] and the Global Burden of Disease Study 2019 [171], estimate that 1.5 billion people worldwide will have some hearing decline during their life and that at least 430 million will require intervention, and this will escalate in the years to come. Hearing loss is sometimes accompanied with phantom sound perception, also known as tinnitus, which can be a debilitating condition on its own [172–174]. Within the lifestyle and environmental factors that can be prevented and contribute the most to hearing loss and tinnitus is the exposure to elevated noise (occupational, recreational or environmental) [175–177]. Thus, hearing loss and tinnitus require an urgent call for global attention.

Although a wide variety of drugs have been investigated and/or used in the treatment of noise-induced hearing loss and tinnitus, there is no effective drug on the market [73,178–180]. The fact that the efferent system protects the inner ear from damage produced by exposure to overly loud sounds [70,84,181,182], the enhancement of the efferent feedback to the inner year by activating α9α10 nAChRs, appears as an attractive approach. The phenotype of α9 L9T knockin mice, which are resistant to permanent noise-induced and hidden hearing loss and have a delayed presbycusis [70,84,85], resembles the effect of type II PAMs described for other nAChRs [183–185], since efferent synaptic responses are prolonged, ACh responses are potentiated and have a slower desensitization kinetics [70,71] (Fig. 2). PAMs which reinforce endogenous cholinergic neurotransmission without directly stimulating the target receptors, do not lead to receptor permanent desensitization, have less side effects and have attracted attention to target neuronal nAChRs in CNS disorders [186]. Thus, the development of PAMs that target α9α10 nAChRs appear as attractive molecules to treat/prevent noise-induced hearing loss. The observation that the store active compound ryanodine [187] and the serotonin type 3 receptor agonist 1-(*m*-chlorophenyl)-biguanid [136], are not agonists but potentiate α9α10-mediated ACh-responses, indicates that as for other nAChRs, PAMs that target α9α10 can be developed. The recent successful expression of α9α10 receptors in HEK cells coupled to FLIPR calcium assay [144], opens the possibility of high throughput screening for α9α10 nAChR selective lead compounds. In addition, the availability of the crystal structure of the α9 subunit extracellular domain [116], coupled to radioligand binding assays of compounds to purified extracellular α9 protein fragments and mutagenesis structure-function analysis, will aid to decipher the orthosteric or allosteric interaction of novel α9α10 nAChR ligands [188].

6.2. Non-auditory α9α10 nAChR pharmacotherapeutics

The fact that the α9α10 nAChR has a restricted expression pattern, has no CNS expression [17,19,50,189] and has a differential pharmacological profile, makes this receptor a suitable pharmacotherapeutic target for the design of drugs for inner ear disorders with less side effects, specially of central origin. However, the expression of α9 and α10 subunits in epithelial cells outside the inner ear serving non-canonical cholinergic effects should be considered, both as potential targets of side effects when using compounds as otoprotectants and as targets for pharmacotherapeutics. Side effects of drugs used for inner ear disorders can be circumvented by local delivery using gels and nanoparticles [190,191].

The pharmacotherapeutic activity of α9 and/or α9α10 nAChR antagonists like conotoxins have been investigated in several types of pain in animal models [92,192–194]. The analgesic effect is most likely due the participation of α9* nAChR which are present in immune cells involved in inflammatory processes (for an extended review see [33]). Moreover, α9 and/or α10 nAChR subunit blockers have been also suggested to be of use in animal models of immune diseases such as experimental autoimmune encephalomyelitis [89,90]. In addition, pCF3-N,N-diethyl-N´-phenyl-piperazine (pCF3-diEPP), in part due to its non-canonical effect on α9* nAChRs, has been suggested as useful in the prevention of life-threatening trauma-induced inflammation, either as an emergency treatment for accident victims or in the context of major surgery [195]. The non-competitive α9α10 nAChR antagonist alphaO-Conotoxin GeXIVA and the competitive antagonist RgIA4, result in antitumor effects [196–198]. Thus, the non-auditory functions of α9* and α10* nAChRs open a wide variety of uses of potential drugs that target the non-canonical metabotropic signalling of these receptors.

7. Conclusions and forward

Much has been advanced since the first cloning of α9 [17] and α10 [16], the last nAChR subunits to be identified. For the auditory field, the puzzle concerning the structure of the hair cell nAChR and the decades long lasting debate between a nicotinic versus a muscarinic ACh receptor [134,199–201], was solved. This enabled a precise characterization of the pharmacology, biophysical and structure function analysis of the α9α10 nAChR (for review see [9,57,58,67]) and the crystallization of the α9 monomeric extracellular domain [115,116]. Moreover, the kinetics of *ex vivo* hair cells α9α10 nAChR-mediated currents and the properties of efferent synaptic transmission have been extensively analyzed and results replicated by several laboratories (for review see [67]). In addition, the generation of *Chrna9* and *Chrna10* genetically modified mice [14,15,70,74,202], has helped elucidate the function of the inner ear efferent system in audition. It should be noted that it is only in hair cells where a clear ionotropic function of α9α10 nAChR has been reproducibly reported. Only one report has proposed that synaptic currents in chromaffin cells are due to α9*, in addition to α7* and α3* nAChRs [26]. This is based on the observation that the frequency

and amplitude of evoked post-synaptic currents are blocked by the α9* antagonist α-RgIA and on the fact that chromaffin cells express the α9 subunit [26]. These results should be expanded using additional compounds in order to perform an entire characterization of the pharmacological profile of responses, in addition to experiments in *Chrna9*, *Chrna10*, *Chrna3 and Chrna7* knockout mice, to unequivocally ascertain the molecular nature of the receptors mediating ACh ion channel responses in chromaffin cells.

From the pharmacological point of view, the cloning of the α9 and α10 nAChRs added "odd cousins to an old family" of nicotinic pentameric ligand channels [9]. *In vivo*, ionotropic responses require the assembly of both subunits into pentameric receptors [14,16,18] (with the exception of the zebrafish lateral line hair cell receptor which appears to be a α9 homomeric nAChR [13]). No clear evidence of co-assembly with other nAChRs has been reported [17,126]. α9 and α10 form a distinct and distant clade within the nAChR phylogeny and have a distinct evolutionary trajectory when compared to neuronal and muscle nAChR subunits [42,55,147]. Moreover, they have a distinct, mainly non-neuronal expression pattern [9,17,19,42,50,203]. In addition, α9 and α9α10 nAChRs have peculiar pharmacological and biophysical properties [16–18,56,133,135,136,204,205]. Therefore, the α9 and α10 subunits are functionally isolated from the remainder nicotinic subunits. Although within the dichotomic subdivision of nAChR subunits into muscle and neuronal α9 and α10 have been classically and repeatedly included within the neuronal subgroup [10], based on their evolutionary trajectory, expression pattern, biophysical and pharmacological properties, they are clearly non-neuronal. Thus, as previously proposed [9], α9 and α10 subunits could be re-classified as ‘‘hair cell’’ nAChR subunits, based on their known canonical ion channel function mediating efferent olivocochlear inhibition of inner ear hair cells.

Finally, the increasing knowledge of the canonical and non-canonical functions of α9* and α10* nAChRs paves the way to new pharmacotherapeutical applications and urges the discovery of new selective and safe lead molecules that target these receptors.

CRediT author statement

The review was completely written by Ana Belén Elgoyhen.

Declarations of interest

none.

Data Availability

No data was used for the research described in the article.

Acknowledgements

This work was supported by Agencia Nacional de Promoción Científica y Técnica, Argentina, the Scientific Grand Prize of the Fondation Pour l′Audition and National Institutes of Health Grant R01 DC001508.

References

[1] P.J. Corringer, et al., Structure and pharmacology of pentameric receptor channels: from bacteria to brain, Structure 20 (6) (2012) 941–956.
[2] M. Jaiteh, A. Taly, J. Hénin, Evolution of pentameric ligand-gated ion channels: pro-loop receptors, PLoS One 11 (3) (2016), e0151934.
[3] N. Bocquet, et al., A prokaryotic proton-gated ion channel from the nicotinic acetylcholine receptor family, Nature 445 (7123) (2007) 116–119.
[4] A. Tasneem, et al., Identification of the prokaryotic ligand-gated ion channels and their implications for the mechanisms and origins of animal Cys-loop ion channels, Genome Biol. 6 (1) (2005) R4.
[5] J. Changeux, J. Giraudat, M. Dennis, The nicotinic acetylcholine receptor: molecular architecture of a ligand-regulated ion channel, TIPS 8 (1987) 459–465.
[6] T.G. Smart, F.A. Stephenson, A half century of γ-aminobutyric acid, Brain Neurosci. Adv. 3 (2019), p. 2398212819858249.
[7] A.V. Maricq, et al., Primary structure and functional expression of the 5HT3 receptor, a serotonin-gated ion channel, Science 254 (5030) (1991) 432–437.
[8] A. Karlin, M. Akabas, Toward a structural basis for the function of nicotinic acetylcholine receptors and their cousins, Neuron 15 (1995) 1231–1244.
[9] M. Lipovsek, I. Marcovich, A. Elgoyhen, The hair cell α9α10 nicotinic acetylcholine receptor: odd cousin in an old family, Front. Cell. Neurosci. (2021) (in press).
[10] A. Karlin, Ion channel structure: emerging structure of the nicotinic acetylcholine receptors, Nat. Rev. Neurosci. 3 (2002) 102–114.
[11] J.E. Pedersen, C.A. Bergqvist, D. Larhammar, Evolution of vertebrate nicotinic acetylcholine receptors, BMC Evol. Biol. 19 (1) (2019) 38.
[12] P.V. Plazas, A.B. Elgoyhen, The cholinergic lateral line efferent synapse: structural, functional and molecular similarities with those of the cochlea, Front Cell Neurosci. 15 (2021), 765083.
[13] A.E. Carpaneto Freixas, et al., Unraveling the molecular players at the cholinergic efferent synapse of the zebrafish lateral line, J. Neurosci. 41 (1) (2021) 47–60.
[14] D.E. Vetter, et al., The alpha10 nicotinic acetylcholine receptor subunit is required for normal synaptic function and integrity of the olivocochlear system, Proc. Natl. Acad. Sci. USA 104 (51) (2007) 20594–20599.
[15] D.E. Vetter, et al., Role of alpha9 nicotinic ACh receptor subunits in the development and function of cochlear efferent innervation, Neuron 23 (1) (1999) 93–103.
[16] A.B. Elgoyhen, et al., alpha10: a determinant of nicotinic cholinergic receptor function in mammalian vestibular and cochlear mechanosensory hair cells, Proc. Natl. Acad. Sci. USA 98 (6) (2001) 3501–3506.
[17] A.B. Elgoyhen, et al., a9: an acetylcholine receptor with novel pharmacological properties expressed in rat cochlear hair cells, Cell 79 (1994) 705–715.
[18] F. Sgard, et al., A novel human nicotinic receptor subunit, α10, that confers functionality to the α9-subunit, Mol. Pharm. 61 (2002) 150–159.
[19] B. Morley, et al., Identification of the subunits of the nicotinic cholinergic receptors in the rat cochlea using RT-PCR and in situ hybridization, Mol. Brain Res. 53 (1998) 78–87.
[20] H. Hiel, et al., Expression of nicotinic acetylcholine receptor mRNA in the adult rat peripheral vestibular system, Brain Res. 738 (1996) 347–352.
[21] D.D. Simmons, B.J. Morley, Differential expression of the alpha 9 nicotinic acetylcholine receptor subunit in neonatal and adult cochlear hair cells, Brain Res Mol. Brain Res. 56 (1–2) (1998) 287–292.
[22] L. Lustig, H. Hiel, P. Fuchs, Vestibular hair cells of the chick express the nicotinic acetylcholine receptor subunit alpha9, J. Vestib. Res 9 (1999) 359–367.
[23] L. Luo, et al., Developmental expression of alpha 9 acetylcholine receptor mRNA in the rat cochlea and vestibular inner ear, J. Comp. Neurol. 393 (1998) 320–331.
[24] B.J. Morley, D.D. Simmons, Developmental mRNA expression of the alpha10 nicotinic acetylcholine receptor subunit in the rat cochlea, Brain Res Dev. Brain Res 139 (1) (2002) 87–96.
[25] D.D. Simmons, B.J. Morley, Spatial and temporal expression patterns of nicotinic acetylcholine α9 and α10 subunits in the embryonic and early postnatal inner ear, Neuroscience 194 (2011) 326–336.
[26] C. Colomer, et al., Functional characterization of alpha9-containing cholinergic nicotinic receptors in the rat adrenal medulla: implication in stress-induced functional plasticity, J. Neurosci. 30 (19) (2010) 6732–6742.
[27] L.R. Lustig, et al., Molecular cloning and mapping of the human nicotinic acetylcholine receptor α10 (CHRNA10), Genomics 73 (2001) 272–283.
[28] H. Peng, et al., Characterization of the human nicotinic acetylcholine receptor subunit alpha (alpha) 9 (CHRNA9) and alpha (alpha) 10 (CHRNA10) in lymphocytes, Life Sci. 76 (3) (2004) 263–280.
[29] G. Galvis, K.S. Lips, W. Kummer, Expression of nicotinic acetylcholine receptors on murine alveolar macrophages, J. Mol. Neurosci. 30 (1–2) (2006) 107–108.
[30] A.I. Chernyavsky, et al., Auto/paracrine control of inflammatory cytokines by acetylcholine in macrophage-like U937 cells through nicotinic receptors, Int Immunopharmacol. 10 (3) (2010) 308–315.
[31] L. Koval, et al., Differential involvement of α4β2, α7 and α9α10 nicotinic acetylcholine receptors in B lymphocyte activation in vitro, Int J. Biochem Cell Biol. 43 (4) (2011) 516–524.
[32] X. Zhang, et al., Brain control of humoral immune responses amenable to behavioural modulation, Nature 581 (7807) (2020) 204–208.
[33] A.J. Hone, J.M. McIntosh, Nicotinic acetylcholine receptors: therapeutic targets for novel ligands to treat pain and inflammation, Pharm. Res 190 (2023), 106715.
[34] V.T. Nguyen, A. Ndoye, S.A. Grando, Novel human α9 acetylcholine receptor regulating keratinocyte aghesion is targeted by Penphigus vulgaris autoimmunity, Am. J. Pathol. 157 (2000) 1377–1391.
[35] T. Bschleipfer, et al., Expression and distribution of cholinergic receptors in the human urothelium, Life Sci. 80 (24–25) (2007) 2303–2307.
[36] K.S. Lips, et al., Nicotinic acetylcholine receptors in rat and human placenta, Placenta 26 (10) (2005) 735–746.
[37] X.W. Fu, J. Lindstrom, E.R. Spindel, Nicotine activates and up-regulates nicotinic acetylcholine receptors in bronchial epithelial cells, Am. J. Respir. Cell Mol. Biol. 41 (1) (2009) 93–99.
[38] M.L. Smith, et al., Acetylcholine receptors in the retinas of the α7 nicotinic acetylcholine receptor knockout mouse, Mol. Vis. 20 (2014) 1328–1356.
[39] J. Liu, et al., Nicotinic acetylcholine receptor subunits in rhesus monkey retina, Invest Ophthalmol. Vis. Sci. 50 (3) (2009) 1408–1415.
[40] S.F. Hackett, et al., The nicotinic cholinergic pathway contributes to retinal neovascularization in a mouse model of retinopathy of prematurity, Invest Ophthalmol. Vis. Sci. 58 (2) (2017) 1296–1303.

[41] K.S. Lips, U. Pfeil, W. Kummer, Coexpression of alpha 9 and alpha 10 nicotinic acetylcholine receptors in rat dorsal root ganglion neurons, Neuroscience 115 (1) (2002) 1–5.
[42] I. Marcovich, et al., Distinct evolutionary trajectories of neuronal and hair cell nicotinic acetylcholine receptors, Mol. Biol. Evol. 37 (4) (2020) 1070–1089.
[43] D. Usoskin, et al., Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing, Nat. Neurosci. 18 (1) (2015) 145–153.
[44] N. Sharma, et al., The emergence of transcriptional identity in somatosensory neurons, Nature 577 (7790) (2020) 392–398.
[45] A.J. Hone, et al., Nicotinic acetylcholine receptors in dorsal root ganglion neurons include the α6β4* subtype, Faseb J. 26 (2) (2012) 917–926.
[46] S. Shiers, R.M. Klein, T.J. Price, Quantitative differences in neuronal subpopulations between mouse and human dorsal root ganglia demonstrated with RNAscope in situ hybridization, Pain 161 (10) (2020) 2410–2424.
[47] O. Lykhmus, et al., Nicotinic acetylcholine receptor α9 and α10 subunits are expressed in the brain of mice, Front Cell Neurosci. 11 (2017) 282.
[48] C.J. Keiger, J.C. Walker, Individual variation in the expression profiles of nicotinic receptors in the olfactory bulb and trigeminal ganglion and identification of alpha2, alpha6, alpha9, and beta3 transcripts, Biochem Pharm. 59 (3) (2000) 233–240.
[49] A. Vivekanandarajah, K.A. Waters, R. Machaalani, Postnatal nicotine effects on the expression of nicotinic acetylcholine receptors in the developing piglet hippocampus and brainstem, Int J. Dev. Neurosci. 47 (Pt B) (2015) 183–191.
[50] B.J. Morley, P. Whiteaker, A.B. Elgoyhen, Commentary: nicotinic acetylcholine receptor α9 and α10 subunits are expressed in the brain of mice, Front Cell Neurosci. 12 (2018) 104.
[51] J.J. Guinan, Physiology of olivocochlear efferents, in: Popper, Fay (Eds.), The Cochlea, Dallos, Springer-Verlag, New York, 1996, pp. 435–502.
[52] E. Katz, et al., Developmental regulation of nicotinic synapses on cochlear inner hair cells, J. Neurosci. 24 (36) (2004) 7814–7820.
[53] E. Glowatzki, P.A. Fuchs, Cholinergic synaptic inhibition of inner hair cells in the neonatal mammalian cochlea, Science 288 (5475) (2000) 2366–2368.
[54] S. Fucile, A. Sucapane, F. Eusebi, Ca2+ permeability through rat cloned alpha9-containing nicotinic acetylcholine receptors, Cell Calcium 39 (4) (2006) 349–355.
[55] M. Lipovsek, et al., Phylogenetic differences in calcium permeability of the auditory hair cell cholinergic nicotinic receptor, Proc. Natl. Acad. Sci. USA 109 (11) (2012) 4308–4313.
[56] N. Weisstaub, et al., The alpha9alpha10 nicotinic acetylcholine receptor is permeable to and is modulated by divalent cations, Hear Res 167 (1–2) (2002) 122–135.
[57] E. Katz, A.B. Elgoyhen, P. Fuchs, Cholinergic Inhibition of hair Cells, in: D. K. Ryugo, R.R. Fay, A.N. Popper (Eds.), Auditory and Vestibular Efferents, Springer, New York, 2011, pp. 103–133.
[58] A.B. Elgoyhen, E. Katz, The efferent medial olivocochlear-hair cell synapse, J. Physiol. Paris 106 (1–2) (2012) 47–56.
[59] P.A. Fuchs, B.W. Murrow, Cholinergic inhibition of short (outer) hair cells of the chick's cochlea, J. Neurosci. 12 (3) (1992) 800–809.
[60] J.D. Goutman, P.A. Fuchs, E. Glowatzki, Facilitating efferent inhibition of inner hair cells in the cochlea of the neonatal rat, J. Physiol. 566 (Pt 1) (2005) 49–59.
[61] J. Ballestero, et al., Short-term synaptic plasticity regulates the level of olivocochlear inhibition to auditory hair cells, J. Neurosci. 31 (41) (2011) 14763–14774.
[62] D. Oliver, et al., Gating of Ca2+-activated K+ channels controls fast inhibitory synaptic transmission at auditory outer hair cells, Neuron 26 (2000) 595–601.
[63] Z. Yu, et al., Efferent synaptic transmission at the vestibular type II hair cell synapse, J. Neurophysiol. 124 (2) (2020) 360–374.
[64] R. Galambos, Suppression of auditory nerve activity by stimulation of efferent fibers to the cochlea, J. Neurophysiol. 19 (1956) 424–437.
[65] M.L. Wiederhold, N.Y. Kiang, Effects of electric stimulation of the crossed olivocochlear bundle on single auditory-nerve fibers in the cat, J. Acoust. Soc. Am. 48 (4) (1970) 950–965.
[66] M.L. Gifford, J.J. Guinan Jr., Effects of electrical stimulation of medial olivocochlear neurons on ipsilateral and contralateral cochlear responses, Hear Res 29 (2–3) (1987) 179–194.
[67] E. Katz, A.B. Elgoyhen, Short-term plasticity and modulation of synaptic transmission at mammalian inhibitory cholinergic olivocochlear synapses, Front Syst. Neurosci. 8 (2014) 224.
[68] M.J. Moglie, et al., Compartmentalization of antagonistic Ca(2+) signals in developing cochlear hair cells, Proc. Natl. Acad. Sci. USA 115 (9) (2018) E2095–e2104.
[69] M.J. Moglie, et al., Synaptic contributions to cochlear outer hair cell Ca(2+) dynamics, J. Neurosci. (2021).
[70] J. Taranda, et al., A point mutation in the hair cell nicotinic cholinergic receptor prolongs cochlear inhibition and enhances noise protection, PLoS Biol. 7 (1) (2009), e18.
[71] C. Wedemeyer, et al., A gain-of-function mutation in the α9 nicotinic acetylcholine receptor alters medial olivocochlear efferent short-term synaptic plasticity, J. Neurosci. 38 (16) (2018) 3939–3954.
[72] A.B. Elgoyhen, E. Katz, P.A. Fuchs, The nicotinic receptor of cochlear hair cells: a possible pharmacotherapeutic target? Biochem Pharm. 78 (7) (2009) 712–719.
[73] A.B. Elgoyhen, The α9α10 nicotinic acetylcholine receptor: a compelling drug target for hearing loss? Expert Opin. Ther. Targets 26 (3) (2022) 291–302.
[74] B.J. Morley, et al., Generation and characterization of α9 and α10 nicotinic acetylcholine receptor subunit knockout mice on a C57BL/6J background, Front Neurosci. 11 (2017) 516.
[75] S.L. Johnson, et al., Cholinergic efferent synaptic transmission regulates the maturation of auditory hair cell ribbon synapses, Open Biol. 3 (11) (2013), 130163.
[76] M.N. Di Guilmi, et al., Strengthening of the efferent olivocochlear system leads to synaptic dysfunction and tonotopy disruption of a central auditory nucleus, J. Neurosci. 39 (36) (2019) 7037–7048.
[77] T.A. Babola, et al., Purinergic signaling controls spontaneous activity in the auditory system throughout early development, J. Neurosci. 41 (4) (2021) 594–612.
[78] A. Clause, et al., The precise temporal pattern of prehearing spontaneous activity is necessary for tonotopic map refinement, Neuron 82 (4) (2014) 822–835.
[79] V. Murthy, et al., SK2 channels are required for function and long-term survival of efferent synapses on mammalian outer hair cells, Mol. Cell Neurosci. 40 (1) (2009) 39–49.
[80] Y. Wang, et al., Efferent feedback controls bilateral auditory spontaneous activity, Nat. Commun. 12 (1) (2021) 2449.
[81] A. Clause, A.M. Lauer, K. Kandler, Mice lacking the Alpha9 subunit of the nicotinic acetylcholine receptor exhibit deficits in frequency difference limens and sound localization, Front Cell Neurosci. 11 (2017) 167.
[82] G. Terreros, et al., Selective attention to visual stimuli using auditory distractors is altered in alpha-9 nicotinic receptor subunit knock-out mice, J. Neurosci. 36 (27) (2016) 7198–7209.
[83] P. Jorratt, et al., Difference in perseverative errors during a visual attention task with auditory distractors in alpha-9 nicotinic receptor subunit wild type and knock-out mice, Front Cell Neurosci. 11 (2017) 357.
[84] L.E. Boero, et al., Enhancement of the medial olivocochlear system prevents hidden hearing loss, J. Neurosci. 38 (34) (2018) 7440–7451.
[85] L.E. Boero, et al., Preventing presbycusis in mice with enhanced medial olivocochlear feedback, Proc. Natl. Acad. Sci. USA 117 (21) (2020) 11811–11819.
[86] H.H.V. Chang, B.J. Morley, K.E. Cullen, Loss of α-9 nicotinic acetylcholine receptor subunit predominantly results in impaired postural stability rather than gaze stability, Front Cell Neurosci. 15 (2021), 799752.
[87] P.S. Olofsson, et al., Rethinking inflammation: neural circuits in the regulation of immunity, Immunol. Rev. 248 (1) (2012) 188–204.
[88] L.V. Borovikova, et al., Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin, Nature 405 (6785) (2000) 458–462.
[89] A.R. Simard, et al., Differential modulation of EAE by α9*- and β2*-nicotinic acetylcholine receptors, Immunol. Cell Biol. 91 (3) (2013) 195–200.
[90] Q. Liu, et al., Attenuation in nicotinic acetylcholine receptor α9 and α10 subunit double knock-out mice of experimental autoimmune encephalomyelitis, Biomolecules 9 (12) (2019).
[91] Q. Liu, et al., Distinctive roles for α7*- and α9*-nicotinic acetylcholine receptors in inflammatory and autoimmune responses in the murine experimental autoimmune encephalomyelitis model of multiple sclerosis, Front Cell Neurosci. 11 (2017) 287.
[92] A.J. Hone, J.M. McIntosh, Nicotinic acetylcholine receptors in neuropathic and inflammatory pain, FEBS Lett. 592 (7) (2018) 1045–1062.
[93] K. Richter, et al., Negative regulation of ATP-induced inflammasome activation and cytokine secretion by acute-phase proteins: a mini review, Front Pharm. 13 (2022), 981276.
[94] V.T. Nguyen, et al., Synergistic control of keratinocyte adhesion through muscarinic and nicotinic acetylcholine receptor subtypes, Exp. Cell Res 294 (2) (2004) 534–549.
[95] A.I. Chernyavsky, et al., Central role of alpha9 acetylcholine receptor in coordinating keratinocyte adhesion and motility at the initiation of epithelialization, Exp. Cell Res 313 (16) (2007) 3542–3555.
[96] H. Kurzen, et al., The non-neuronal cholinergic system of human skin, Horm. Metab. Res 39 (2) (2007) 125–135.
[97] S.A. Mohammadi, T.J. Burton, M.J. Christie, α9-nAChR knockout mice exhibit dysregulation of stress responses, affect and reward-related behaviour, Behav. Brain Res. 328 (2017) 105–114.
[98] C.Y. Lin, et al., Membrane protein-regulated networks across human cancers, Nat. Commun. 10 (1) (2019) 3131.
[99] S. Pucci, et al., α9-containing nicotinic receptors in cancer, Front Cell Neurosci. 15 (2021), 805123.
[100] S. Valbuena, J. Lerma, Non-canonical signaling, the hidden life of ligand-gated ion channels, Neuron 92 (2) (2016) 316–329.
[101] R.L. Papke, J.M. Lindstrom, Nicotinic acetylcholine receptors: conventional and unconventional ligands and signaling, Neuropharmacology 168 (2020), 108021.
[102] S. Razani-Boroujerdi, et al., T cells express alpha7-nicotinic acetylcholine receptor subunits that require a functional TCR and leukocyte-specific protein tyrosine kinase for nicotine-induced Ca2+ response, J. Immunol. 179 (5) (2007) 2889–2898.
[103] W.J. de Jonge, L. Ulloa, The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation, Br. J. Pharm. 151 (7) (2007) 915–929.
[104] A. Hecker, et al., Phosphocholine-modified macromolecules and canonical nicotinic agonists inhibit ATP-induced IL-1β release, J. Immunol. 195 (5) (2015) 2325–2334.
[105] K. Richter, et al., Phosphocholine - an agonist of metabotropic but not of ionotropic functions of α9-containing nicotinic acetylcholine receptors, Sci. Rep. 6 (2016) 28660.
[106] A. Zakrzewicz, et al., Canonical and novel non-canonical cholinergic agonists inhibit ATP-induced release of monocytic interleukin-1β via different combinations of nicotinic acetylcholine receptor subunits α7, α9 and α10, Front Cell Neurosci. 11 (2017) 189.

[107] V. Grau, et al., Conopeptides [V11L;V16D]ArIB and RgIA4: powerful tools for the identification of novel nicotinic acetylcholine receptors in monocytes, Front Pharm. 9 (2018) 1499.
[108] S.A. Grando, Connections of nicotine to cancer, Nat. Rev. Cancer 14 (6) (2014) 419–429.
[109] J.P. Changeux, Discovery of the first neurotransmitter receptor: the acetylcholine nicotinic receptor, Biomolecules 10 (4) (2020).
[110] J.P. Changeux, Structural identification of the nicotinic receptor ion channel, Trends Neurosci. 41 (2) (2018) 67–70.
[111] N. Unwin, Acetylcholine receptor channel imaged in the open state, Nature 373 (1995) 37–43.
[112] N. Unwin, Refined structure of the nicotinic acetylcholine receptor at 4A resolution, J. Mol. Biol. 346 (4) (2005) 967–989.
[113] K. Brejc, et al., Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors, Nature 411 (6835) (2001) 269–276.
[114] P.H. Celie, et al., Nicotine and carbamylcholine binding to nicotinic acetylcholine receptors as studied in AChBP crystal structures, Neuron 41 (6) (2004) 907–914.
[115] M. Zouridakis, et al., Crystal structure of the monomeric extracellular domain of α9 nicotinic receptor subunit in complex with α-conotoxin RgIA: molecular dynamics insights Into RgIA binding to α9α10 nicotinic receptors, Front Pharm. 10 (2019) 474.
[116] M. Zouridakis, et al., Crystal structures of free and antagonist-bound states of human α9 nicotinic receptor extracellular domain, Nat. Struct. Mol. Biol. 21 (11) (2014) 976–980.
[117] P. Giastas, et al., Structural insights into the role of β3 nAChR subunit in the activation of nicotinic receptors, Molecules 27 (14) (2022).
[118] C.D. Dellisanti, et al., Crystal structure of the extracellular domain of nAChR alpha1 bound to alpha-bungarotoxin at 1.94 A resolution, Nat. Neurosci. 10 (8) (2007) 953–962.
[119] N. Kouvatsos, et al., Crystal structure of a human neuronal nAChR extracellular domain in pentameric assembly: Ligand-bound α2 homopentamer, Proc. Natl. Acad. Sci. USA 113 (34) (2016) 9635–9640.
[120] C.L. Morales-Perez, C.M. Noviello, R.E. Hibbs, X-ray structure of the human α4β2 nicotinic receptor, Nature 538 (7625) (2016) 411–415.
[121] R.M. Walsh Jr., et al., Structural principles of distinct assemblies of the human α4β2 nicotinic receptor, Nature 557 (7704) (2018) 261–265.
[122] Y. Zhao, et al., Structural basis of human α7 nicotinic acetylcholine receptor activation, Cell Res 31 (6) (2021) 713–716.
[123] C.M. Noviello, et al., Structure and gating mechanism of the α7 nicotinic acetylcholine receptor, Cell 184 (8) (2021), p. 2121-2134.e13.
[124] P. Giastas, M. Zouridakis, S.J. Tzartos, Understanding structure-function relationships of the human neuronal acetylcholine receptor: insights from the first crystal structures of neuronal subunits, Br. J. Pharm. 175 (11) (2018) 1880–1891.
[125] N.S. Millar, C. Gotti, Diversity of vertebrate nicotinic acetylcholine receptors, Neuropharmacology 56 (1) (2009) 237–246.
[126] D. Scheffer, et al., The α1 subunit of nicotinic acetylcholine receptors in the inner ear: transcriptional regulation by ATOH1 and co-expression with the γ subunit in hair cells, J. Neurochem 103 (6) (2007) 2651–2664.
[127] P.V. Plazas, et al., Stoichiometry of the alpha9alpha10 nicotinic cholinergic receptor, J. Neurosci. 25 (47) (2005) 10905–10912.
[128] D.C. Indurthi, et al., Presence of multiple binding sites on α9α10 nAChR receptors alludes to stoichiometric-dependent action of the α-conotoxin, Vc1.1, Biochem Pharm. 89 (1) (2014) 131–140.
[129] J.C. Boffi, et al., Differential contribution of subunit interfaces to α9α10 nicotinic acetylcholine receptor function, Mol. Pharm. 91 (3) (2017) 250–262.
[130] S.B. Hansen, et al., Structural and ligand recognition characteristics of an acetylcholine-binding protein from Aplysia californica, J. Biol. Chem. 279 (23) (2004) 24197–24202.
[131] A.J. Hone, J.M. McIntosh, Alkaloid ligands enable function of homomeric human α10 nicotinic acetylcholine receptors, Front Pharm. 13 (2022), 981760.
[132] L. Azam, et al., Molecular interaction of α-conotoxin RgIA with the rat α9α10 nicotinic acetylcholine receptor, Mol. Pharm. 87 (5) (2015) 855–864.
[133] M. Verbitsky, et al., Mixed nicotinic-muscarinic properties of the a9 nicotinic cholinergic receptor, Neuropharmacology 39 (2000) 2515–2524.
[134] P.A. Fuchs, B.W. Murrow, A novel cholinergic receptor mediates inhibition of chick cochlear hair cells, Proc. Biol. Sci. 248 (1321) (1992) 35–40.
[135] C.V. Rothlin, et al., The alpha9 nicotinic acetylcholine receptor shares pharmacological properties with type A gamma-aminobutyric acid, glycine, and type 3 serotonin receptors, Mol. Pharm. 55 (2) (1999) 248–254.
[136] C.V. Rothlin, et al., Direct interaction of serotonin type 3 receptor ligands with recombinant and native alpha 9 alpha 10-containing nicotinic cholinergic receptors, Mol. Pharm. 63 (5) (2003) 1067–1074.
[137] M. Ellison, et al., Alpha-RgIA: a novel conotoxin that specifically and potently blocks the alpha9alpha10 nAChR, Biochemistry 45 (5) (2006) 1511–1517.
[138] F. Fisher, et al., Cy3-RgIA-5727 Labels and Inhibits α9-Containing nAChRs of Cochlear Hair Cells, Front Cell Neurosci. 15 (2021), 697560.
[139] J.M. McIntosh, et al., A novel alpha-conotoxin, PeIA, cloned from Conus pergrandis, discriminates between rat alpha9alpha10 and alpha7 nicotinic cholinergic receptors, J. Biol. Chem. 280 (34) (2005) 30107–30112.
[140] S.B. Christensen, et al., αS-conotoxin GVIIIB potently and selectively blocks α9α10 nicotinic acetylcholine receptors, Biochem Pharm. 96 (4) (2015) 349–356.
[141] J. Liang, et al., Mechanism of action and structure-activity relationship of α-Conotoxin Mr1.1 at the human α9α10 nicotinic acetylcholine receptor, J. Med Chem. 65 (24) (2022) 16204–16217.
[142] X. Li, et al., Medicinal chemistry, pharmacology, and therapeutic potential of α-conotoxins antagonizing the α9α10 nicotinic acetylcholine receptor, Pharm. Ther. 222 (2021), 107792.
[143] E.R. Baker, et al., Pharmacological properties of alpha 9 alpha 10 nicotinic acetylcholine receptors revealed by heterologous expression of subunit chimeras, Mol. Pharm. 65 (2) (2004) 453–460.
[144] S. Gu, et al., Hair cell α9α10 nicotinic acetylcholine receptor functional expression regulated by ligand binding and deafness gene products, Proc. Natl. Acad. Sci. USA 117 (39) (2020) 24534–24544.
[145] C.L. Cunningham, et al., TMIE defines pore and gating properties of the mechanotransduction channel of mammalian cochlear hair cells, Neuron 107 (1) (2020), p. 126-143.e8.
[146] S. Naz, et al., Mutations in a novel gene, TMIE, are associated with hearing loss linked to the DFNB6 locus, Am. J. Hum. Genet 71 (3) (2002) 632–636.
[147] L.F. Franchini, A.B. Elgoyhen, Adaptive evolution in mammalian proteins involved in cochlear outer hair cell electromotility, Mol. Phylogenet Evol. 41 (3) (2006) 622–635.
[148] M. Ortells, G. Lunt, Evolutionary history of the ligand-gated ion-channel superfamily of receptors, TINS 18 (1995) 121–127.
[149] N. Le Novere, J. Changeux, Molecular evolution of the nicotinic acetylcholine receptor: an example of multigene family in excitable cells, J. Molec. Evol. 40 (1995) 155–172.
[150] K. Tsunoyama, T. Gojobori, Evolution of nicotinic acetylcholine receptor subunits, Mol. Biol. Evol. 15 (5) (1998) 518–527.
[151] J.A. Dent, Evidence for a diverse Cys-loop ligand-gated ion channel superfamily in early bilateria, J. Mol. Evol. 62 (5) (2006) 523–535.
[152] M. Li, et al., in: M. Li (Ed.), Evolutionary Relationship of Nicotinic Acetylcholine Receptor Subunits in Both Vertebrate and Invertebrate Species, in Nicotinic Acetylcholine Receptor Technologies. Neuromethods, Humana Press, New York, NY, 2016.
[153] D.Z. Faltine-Gonzalez, M.J. Layden, Characterization of nAChRs in Nematostella vectensis supports neuronal and non-neuronal roles in the cnidarian-bilaterian common ancestor, Evodevo 10 (2019) 27.
[154] Y. Jiao, et al., Massive expansion and diversity of nicotinic acetylcholine receptors in lophotrochozoans, BMC Genom. 20 (1) (2019) 937.
[155] A.K. Jones, et al., The cys-loop ligand-gated ion channel gene superfamilies of the cockroaches Blattella germanica and Periplaneta americana, Pest Manag Sci. 77 (8) (2021) 3787–3799.
[156] A.K. Jones, D.B. Sattelle, Diversity of insect nicotinic acetylcholine receptor subunits, Adv. Exp. Med Biol. 683 (2010) 25–43.
[157] A.K. Jones, D.B. Sattelle, Functional genomics of the nicotinic acetylcholine receptor gene family of the nematode, Caenorhabditis elegans, Bioessays 26 (1) (2004) 39–49.
[158] J.A. Dent, The evolution of pentameric ligand-gated ion channels, Adv. Exp. Med Biol. 683 (2010) 11–23.
[159] L. Holden-Dye, et al., Nicotinic acetylcholine receptors: a comparison of the nAChRs of Caenorhabditis elegans and parasitic nematodes, Parasitol. Int 62 (6) (2013) 606–615.
[160] N. Le Novere, P.J. Corringer, J.P. Changeux, The diversity of subunit composition in nAChRs: evolutionary origins, physiologic and pharmacologic consequences, J. Neurobiol. 53 (4) (2002) 447–456.
[161] M.J. Moglie, et al., Loss of choline agonism in the inner ear hair cell nicotinic acetylcholine receptor linked to the α10 subunit, Front Mol. Neurosci. 14 (2021), 639720.
[162] M. Lipovsek, et al., Tracking the molecular evolution of calcium permeability in a nicotinic acetylcholine receptor, Mol. Biol. Evol. (2014) (p. in press).
[163] F. Pisciottano, et al., Inner ear genes underwent positive selection and adaptation in the mammalian lineage, Mol. Biol. Evol. 36 (8) (2019) 1653–1670.
[164] M. Lipovsek, A.B. Elgoyhen, The evolutionary tuning of hearing, Trends Neurosci. (2023).
[165] B.O. Olusanya, K.J. Neumann, J.E. Saunders, The global burden of disabling hearing impairment: a call to action, Bull. World Health Organ 92 (5) (2014) 367–373.
[166] Ø. Nordvik, et al., Generic quality of life in persons with hearing loss: a systematic literature review, BMC Ear Nose Throat Disord. 18 (2018) 1.
[167] J.A. Deal, et al., Hearing treatment for reducing cognitive decline: design and methods of the aging and cognitive health evaluation in elders randomized controlled trial, Alzheimers Dement. 4 (2018) 499–507.
[168] D.G. Loughrey, et al., Association of age-related hearing loss with cognitive function, cognitive impairment, and dementia: a systematic review and meta-analysis, JAMA Otolaryngol. Head. Neck Surg. 144 (2) (2018) 115–126.
[169] G. Livingston, et al., Dementia prevention, intervention, and care, Lancet 390 (10113) (2017) 2673–2734.
[170] WHO, World report on hearing. ⟨https://www.who.int/publications/i/item/world-report-on-hearing⟩, 2021.
[171] L.M. Haile, G.H.L. Collaborators, Hearing loss prevalence and years lived with disability, 1990-2019: findings from the global burden of disease study 2019, Lancet 397 (10278) (2021) 996–1009.
[172] A. McCormack, et al., A systematic review of the reporting of tinnitus prevalence and severity, Hear Res 337 (2016) 70–79.
[173] D. De Ridder, et al., Tinnitus and tinnitus disorder: Theoretical and operational definitions (an international multidisciplinary proposal), Prog. Brain Res 260 (2021) 1–25.
[174] A.B. Elgoyhen, et al., Tinnitus: network pathophysiology-network pharmacology, Front Syst. Neurosci. 6 (2012) 1.

[175] M. Concha-Barrientos, D. Campbell-Lendrum, K. Steenland, Occupational Noise: Assessing the Burden of Disease from Work-related Hearing Impairment at National and Local Levels, World Health Organization (WHO Environmental Burden of Disease Series, Geneva, 2004.
[176] A. Lie, et al., Occupational noise exposure and hearing: a systematic review, Int Arch. Occup. Environ. Health 89 (3) (2016) 351–372.
[177] M. Śliwińska-Kowalska, K. Zaborowski, WHO environmental noise guidelines for the european region: a systematic review on environmental noise and permanent hearing loss and tinnitus, Int J. Environ. Res Public Health 14 (10) (2017).
[178] B. Langguth, A.B. Elgoyhen, C.R. Cederroth, Therapeutic Approaches to the Treatment of Tinnitus, Annu Rev. Pharm. Toxicol. 59 (2019) 291–313.
[179] B. Langguth, A.B. Elgoyhen, Emerging pharmacotherapy of tinnitus, Expert Opin. Emerg. Drugs 16 (4) (2011) 603–606.
[180] A.B. Elgoyhen, B. Langguth, Pharmacological approaches to the treatment of tinnitus, Drug Disco Today 15 (7–8) (2010) 300–305.
[181] M.C. Liberman, W.Y. Gao, Chronic cochlear de-efferentation and susceptibility to permanent acoustic injury, Hear Res 90 (1–2) (1995) 158–168.
[182] S.F. Maison, H. Usubuchi, M.C. Liberman, Efferent feedback minimizes cochlear neuropathy from moderate noise exposure, J. Neurosci. 33 (13) (2013) 5542–5552.
[183] J.H. Grønlien, et al., Distinct profiles of alpha7 nAChR positive allosteric modulation revealed by structurally diverse chemotypes, Mol. Pharm. 72 (3) (2007) 715–724.
[184] T. Collins, G.T. Young, N.S. Millar, Competitive binding at a nicotinic receptor transmembrane site of two α7-selective positive allosteric modulators with differing effects on agonist-evoked desensitization, Neuropharmacology 61 (8) (2011) 1306–1313.
[185] J. Wang, et al., A Novel α2/α4 Subtype-selective Positive Allosteric Modulator of Nicotinic Acetylcholine Receptors Acting from the C-tail of an α Subunit, J. Biol. Chem. 290 (48) (2015) 28834–28846.
[186] J. Wang, J. Lindstrom, Orthosteric and allosteric potentiation of heteromeric neuronal nicotinic acetylcholine receptors, Br. J. Pharm. 175 (11) (2018) 1805–1821.
[187] J.C. Boffi, et al., Positive modulation of the alpha9alpha10 nicotinic cholinergic receptor by ascorbic acid, Br. J. Pharm. 168 (4) (2013) 954–965.
[188] E.V. Kryukova, et al., Orthosteric and/or allosteric binding of α-conotoxins to nicotinic acetylcholine receptors and their models, Mar. Drugs 16 (12) (2018).
[189] J. Zuo, et al., Visualization of alpha9 acetylcholine receptor expression in hair cells of transgenic mice containing a modified bacterial artificial chromosome, Proc. Natl. Acad. Sci. USA 96 (24) (1999) 14100–14105.
[190] A.H. Liao, et al., Development of thermosensitive poloxamer 407-based microbubble gel with ultrasound mediation for inner ear drug delivery, Drug Deliv. 28 (1) (2021) 1256–1271.
[191] D.H. Kim, et al., Local drug delivery using poly(lactic-co-glycolic acid) nanoparticles in thermosensitive gels for inner ear disease treatment, Drug Deliv. 28 (1) (2021) 2268–2277.
[192] A.J. Hone, D. Servent, J.M. McIntosh, α9-containing nicotinic acetylcholine receptors and the modulation of pain, Br. J. Pharm. 175 (11) (2018) 1915–1927.
[193] H.K. Romero, et al., Inhibition of α9α10 nicotinic acetylcholine receptors prevents chemotherapy-induced neuropathic pain, Proc. Natl. Acad. Sci. USA 114 (10) (2017) E1825–e1832.
[194] S.B. Christensen, et al., RgIA4 potently blocks mouse α9α10 nAChRs and provides long lasting protection against oxaliplatin-induced cold allodynia, Front Cell Neurosci. 11 (2017) 219.
[195] K. Richter, et al., Comparison of the anti-inflammatory properties of two nicotinic acetylcholine receptor ligands, phosphocholine and pCF3-diEPP, Front Cell Neurosci. 16 (2022), 779081.
[196] Z. Sun, et al., Differential expression of nicotine acetylcholine receptors associates with human breast cancer and mediates antitumor activity of αO-Conotoxin GeXIVA, Mar. Drugs 18 (1) (2020).
[197] S. Luo, et al., Cloning, synthesis, and characterization of αO-conotoxin GeXIVA, a potent α9α10 nicotinic acetylcholine receptor antagonist, Proc. Natl. Acad. Sci. USA 112 (30) (2015) E4026–E4035.
[198] S. Pucci, et al., Choline and nicotine increase glioblastoma cell proliferation by binding and activating α7- and α9- containing nicotinic receptors, Pharm. Res 163 (2021), 105336.
[199] G.D. Housley, J.F. Ashmore, Direct measurement of the action of acetylcholine on isolated outer hair cells of the guinea pig cochlea, Proc. R. Soc. Lond. B 244 (1991) 161–167.
[200] T. Shigemoto, H. Ohmori, Muscarinic receptor hyperpolarizes cochlear hair cells of chick by activating Ca(2+)-activated K+ channels, J. Physiol. 442 (1991) 669–690.
[201] S. Kakehata, et al., Cellular mechanism of acetylcholine-induced response in dissociated outer hair cells of guinea-pig cochlea, J. Physiol. (Lond.) 463 (1993) 227–244.
[202] J. Taranda, et al., Constitutive expression of the alpha10 nicotinic acetylcholine receptor subunit fails to maintain cholinergic responses in inner hair cells after the onset of hearing, J. Assoc. Res Otolaryngol. 10 (3) (2009) 397–406.
[203] Allen Institute for Brain Science, Allen Mouse Brain Atlas [dataset]. Available from mouse.brain-map.org. 2011.
[204] M.E. Gomez-Casati, et al., Biophysical and pharmacological characterization of nicotinic cholinergic receptors in rat cochlear inner hair cells, J. Physiol. 566 (Pt 1) (2005) 103–118.
[205] E. Katz, et al., High calcium permeability and calcium block of the α9 nicotinic acetylcholine receptor, Hear. Res 141 (2000) 117–128.