Chapter

Central Nicotinic and Muscarinic Receptors in Health and Disease

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Abstract

Without acetylcholine (ACh) no skeletal muscle contraction, no preganglionic sympathetic or parasympathetic activity can be obtained. This can result in dysregulation of cardiac, respiratory, gastrointestinal, and renal functions as well as disruption of fluid secretion from various glands such as tears, saliva, digestive juices, sweat, and milk. Importantly, ACh deficiency in the brain can have severe cognitive consequences. The action of ACh is mediated by two distinct classes of receptors, namely the muscarinic (mAChRs), which are G-protein coupled (metabotropic) receptors and nicotinic receptors (nAChRs), which are ligand-gated ion channels (ionotropic receptors). The focus of this chapter is on interaction of these two distinct receptor classes and its implication in health and disease. Thus, following a brief description of ACh actions and its central circuitry, an update on mAChRs and nAChRs and how their interaction may impact neuropsychiatric/neurodegenerative diseases will be provided. Moreover, potential novel therapeutic intervention based on these interactions, particularly in relationship to Alzheimer's and Parkinson's diseases will be touched upon.

Keywords: acetylcholine, nicotinic receptors, muscarinic receptors, glial cells, neuroinflammation

1. Introduction

It is now 100 years since acetylcholine (ACh), described as "vagus stuff" by Otto Loewi has been recognized as the first discovered neurotransmitter. This discovery was based on an ingenious experiment where the exposure of a second heart to the media obtained from the electrical stimulation of the vagus nerve of the first heart, resulted in slowing of the second heart, similar to the effect observed by vagal stimulation of the first heart. ACh is now recognized as a critical neurotransmitter at various vital sites such as neuromuscular junction, autonomic ganglia and the brain or the central nervous system (CNS).

Without ACh no skeletal muscle contraction, no preganglionic sympathetic or parasympathetic activity can be obtained. This can result in dysregulation of cardiac, respiratory, gastrointestinal, and renal functions as well as disruption of secretion from various glands such as tears, salvia, digestive juices, sweat and milk. Moreover, since ACh is also the neurotransmitter at the adrenal medulla, its absence at this

site would prevent the release of adrenaline, an essential hormone in regulating the fight-fright response. Importantly, lack of ACh in the brain can have severe cognitive consequences.

Acetylcholine, as the name implies, is made up from two substances, an acetyl group (derived from glucose) and choline, a nutrient derived from foods such as egg yolks, soy and legumes. Choline is also synthesized by the liver. ACh synthesis is catalyzed by choline acetyltransferase (ChAT), the presence of which in a neuron implies that ACh is used as a neurotransmitter by that neuron. A distinguished feature of ACh in comparison with other neurotransmitters is that its action in the synapse is readily terminated by the enzyme acetylcholinesterase (AChE), in contrast to the reuptake mechanism prevalent with other neurotransmitters. Upon the action of AChE, ACh is broken down into acetate and choline, where the latter is taken up for re-use by the nerve. Inhibition of AChE by insecticides or nerve gases can result in accumulation of ACh. Excess ACh at the neuromuscular junction would cause depolarization of the post-synaptic cell and paralysis. Death from the nerve gas is primarily due to excess secretion and respiratory paralysis. On the other hand, some AChE inhibitors (AChEIs) can be used as therapeutic agents in diseases where ACh transmission is inadequate. This includes myasthenia gravis where AChEIs raise the level of ACh in the neuromuscular junction, and improve muscle activation, contraction, and strength, or in neurological disease such as Alzheimer's disease (AD).

The action of ACh is mediated by two distinct classes of receptors, namely the muscarinic (mAChRs) and nicotinic receptors (nAChRs). The focus of this chapter is on interaction of these 2 distinct receptor classes and its implication in health and disease. Thus, following a brief description of ACh actions and its central circuitry, an update on mAChRs and nAChRs and how their interaction may impact neuropsychiatric/neurodegenerative diseases will be provided. Moreover, potential novel therapeutic intervention based on these interactions will be touched upon.

2. Brain ACh pathways and their significance

An extensive local interneuron network in brain areas involved in motor, cognitive and reward activities such as the striatum, nucleus accumbens, and neocortex utilize ACh as a neurotransmitter. In addition, cholinergic pathways connecting the basal forebrain, a complex of 4 cholinergic nuclei that project to: cerebral cortex, hippocampus, amygdala and the olfactory bulb, are critical in regulating cognition, motivation, hedonic state and reinforcement. Cholinergic input to the substantia nigra pars compacta (SNpc), ventral tegmental area (VTA), thalamus and hypothalamus, areas critical in regulating motor, reward and endocrine systems are provided by the pedunculopontine nucleus (PPN) and laterodorsal tegmental nucleus (LDTN) [1]. Moreover, lateral habenula (LatH), part of a complex nucleus which connects the midbrain to the limbic forebrain and uses ACh, has received considerable attention because of its potential role in cognition and in the pathogenesis of various psychiatric disorders. The medial habenula (MedH), which can be further subdivided into a dorsal region containing non-cholinergic excitatory neurons such as tachykinin and substance P and a ventral region containing dense cholinergic neurons, has recently been investigated more thoroughly. It is now believed that cholinergic projections of MedH is involved in mood regulation as well as drug addiction and that manipulation of this system may be therapeutically exploited [2].

Thus, extensive trajectories throughout the brain, such as cortical connections mediating decision making and planning; projections to the hippocampus and amygdala influencing attention, memory, fear, and stress responses; mesolimbic pathways affecting response to reward; hypothalamic system controlling homeostatic responses such as thermoregulation, food intake, and sleep, all utilize ACh as a neurotransmitter. Furthermore, cholinergic system plays an important role in facilitating synaptic plasticity and neuronal development [1]. For these reasons, the cholinergic systems, particularly, the basal forebrain complex, PPN and LDTN have been extensively studied in relation to age-related progressive neurodegenerative diseases such as Alzheimer's disease (AD) and PD [1].

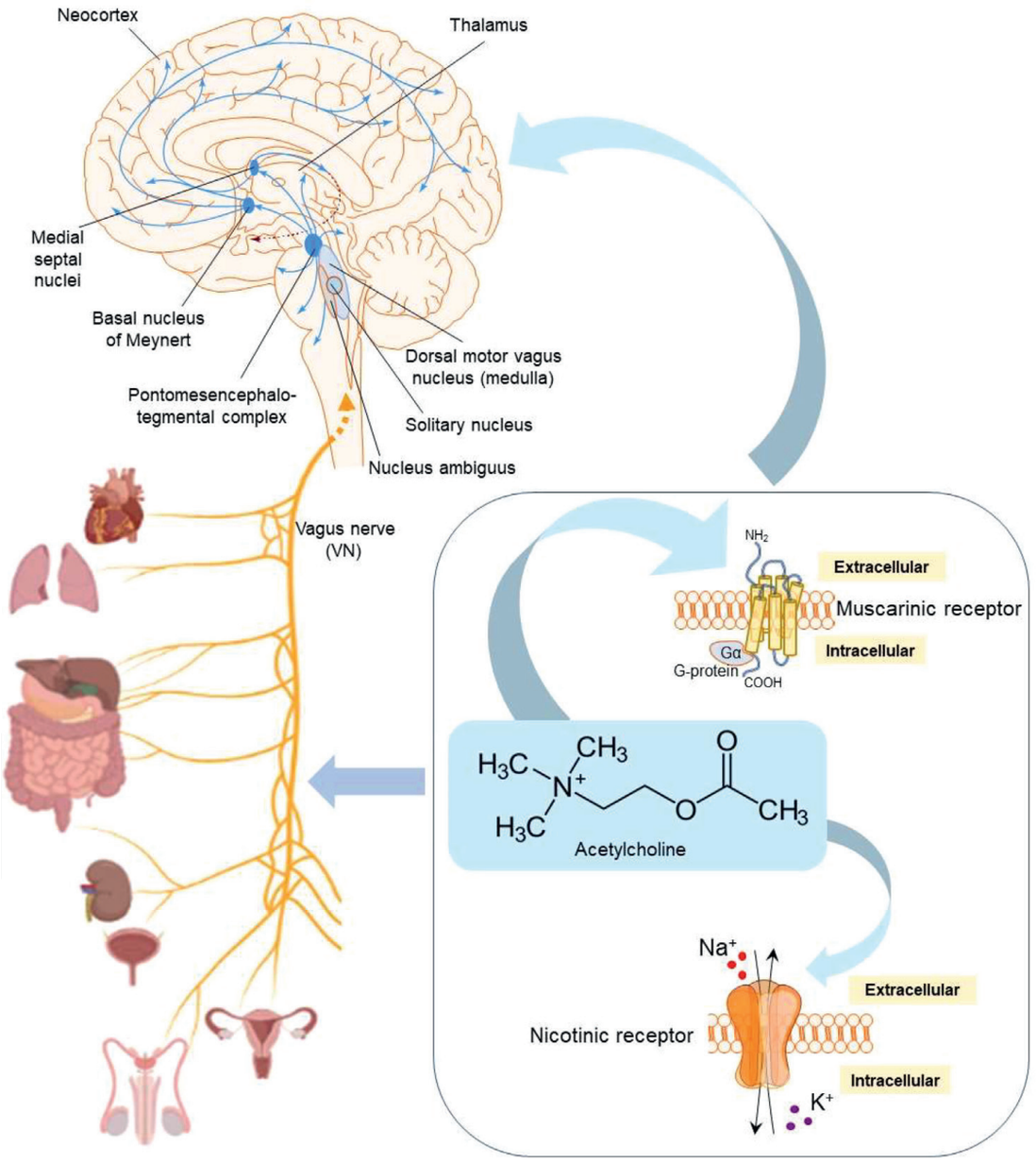

Figure 1.
Simplified schematic diagram depicting central cholinergic system as well as its interaction with the peripheral organs. The afferent and efferent connections of the vagus nerve with the heart, lung, gastrointestinal (GI) tract, kidney and the gonadal system are highlighted. Moreover, the mediators of acetylcholine (ACh) actions (i.e., muscarinic and nicotinic receptors) and their structural composition (i.e., G-protein coupled receptor vs. ligand-gated ion channels) are distinctly shown in the insert.

It is also of relevance to note that the cholinergic projections, in general, have a modulatory, rather than strictly excitatory or inhibitory effects on other neuronal systems. As mentioned earlier, ACh action is mediated by both nicotinic and muscarinic receptors, which interact at both pre- and post-synaptic junctions. A major emphasis of this chapter is to provide an up-to-date understanding of this complex interaction (**Figure 1**).

3. ACh receptors

As mentioned above, the cholinergic system is involved in a wide variety of functions in peripheral as well as in CNS. ACh is the neurotransmitter widely distributed in CNS, used by motor neurons at the neuromuscular junction, and by sympathetic and parasympathetic preganglionic neurons in the autonomic nervous system (ANS), by the parasympathetic innervated organs and select sympathetic-innerved organs including sweat glands, the piloerector muscle (responsible for skin hair to stand up), and other smooth muscles such as irises, which control the diameter of the pupils. In all these, ACh effect is mediated by activating two distinct types of receptors: muscarinic acetylcholine receptors (mAChRs) and nicotinic acetylcholine receptors (nAChR) that differ in both structure and function but share common neuronal circuits. Moreover, these two receptors may co-localized in the same or in different cells, where they can interact with each other. For example, nAChRs in ganglionic cells may modulate the functions of mAChRs in target organs such as smooth muscle, cardiomyocytes, epithelium, and exocrine cells. Below, following brief descriptions of the two classes of receptors, their role in relation to the neurodegenerative diseases will be the focus.

3.1 Muscarinic acetylcholine receptors (mAChRs)

There are five subtypes of mAChRs: M1, M2, M3, M4 and M5 which are G-protein coupled receptors (GPCRs) responsive to the agonist muscarine with equal affinity [3]. Depending on the subtype of mAChR stimulated, distinct signaling pathways are activated. For example, stimulation of the M2 and M4 subtypes, leads to activation of inhibitory G-protein (Gi) which results in inhibition of cyclic AMP (cAMP) and consequent effects downstream. In contrast, activation of M1, M3 and M5 subtypes is generally coupled to a (Gq) which activates phospholipase C that leads to the formation of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP3 causes mobilization of Ca^{2+} from intracellular stores, while DAG activates protein kinase C (PKC) isozymes [4], which can phosphorylate a multitude of downstream molecules that exert tissue-specific functions [5]. In addition, activation of mAChRs can modulate several types of ion channels and currents. For example, Ca^{2+} currents can be suppressed by M1 and M2 receptors in the mouse and M1 and M4 receptors in the rat, whereas M1 receptors can suppress M-type K^{+} current in the rat [6]. Furthermore, MAP kinases and small GTPases, such as Rho and Rac proteins, are also activated by mAChRs [4]. It is worth mentioning that Gq and Gs are designation for stimulatory, whereas Gi signifies inhibitory effect on second messenger such as cAMP.

3.2 Brain distribution of mAChRs

The predominant mAChR subtypes expressed in the cortex of adult brain are: M1(40%), M2 (37%) and M4 (15%). In the hippocampus, however, 36%, 33% and

27% represent M1, M2 and M4, respectively. As mentioned earlier, M1 receptors are found throughout the brain with highest concentrations in cortical regions and the hippocampus. Cortical M1 receptors, most dominant in cortical layers III and V/VI in pyramidal neurons are primarily located post-synaptically and are associated with excitatory synapses. M2 receptors, on the other hand, are highly localized in the nucleus basalis and occipital cortex, and with lesser density in the hippocampus, caudate putamen, and other cortical regions. M2 receptors are located both pre- and post-synaptically in the cortex, where the latter is present in a subset of glutamatergic synapses and GABAergic interneurons. The presynaptic M2 receptors are located on the axons of symmetric synapses and function as autoreceptors. An autoreceptor is a type of receptor located in the nerve membrane and serves as part of a negative feedback loop in signal transduction and is only sensitive to the neurotransmitters released by the neuron on which it is located. M3 receptors have similar distribution to that of M1 but with a much lower level of expression. Like M1, M3 receptors also are present in cortical pyramidal neurons and glial cells. In contrast, M4 receptors are highest in the caudate putamen and are often associated with dopaminergic neurotransmission. M5 receptors are present in very low levels in the hippocampus, substantia nigra, and ventral tegmental area.

3.3 Function of mAChRs

mAChRs shape neuronal and local network processing abilities, and depending on the network involved, affect cognitive functions including learning and memory. For example, AD related-cognitive impairment is associated with reduced muscarinic cholinergic activity, although ascribing specific contribution of individual mAChR subtypes to a specific cognitive performance is not tenable due to heterogeneous distribution of mAChR subtypes within the brain [7]. Knockout studies show that mAChRs functions are subtype dependent. For instance, in paradigms requiring hippocampal processing, M1−/− animals appear to have normal learning and memory [8, 9], suggesting other AChRs may be at play. However, in paradigms thought to require interactions between the hippocampus and cortex, M1−/− animals show deficits [10]. These deficits are thought to be analogous to working memory impairment which may require communication between the two regions and possibly through recruitment of M1 receptors. Thus, mAChRs, with their potential to modulate cognition, have stimulated a high degree of interest as a therapeutic target.

3.4 Nicotinic acetylcholine receptors (nAChRs)

Advances in this area have identified various subtypes of nAChRs with distinct anatomical, physiological, and pharmacological characteristics. These ionotropic classified receptors act by directly regulating the opening of a cation channel in the neuronal membrane. It is important to note that nAChRs present at the neuromuscular junction differ from those in autonomic ganglia which are also different from those occurring in CNS, as each has its distinct subunit structure. Neuronal nicotinic receptors are primarily alph4-beta2 or homomeric alpha7 subtypes. Extensive research on these receptors has led to the suggestion of therapeutic potential for selective nicotinic receptor agonists in various neuropsychiatric and neurodegenerative disorders, including PD, AD, schizophrenia, depression, pain as well as smoking cessation [11–13].

3.5 Brain distribution of nAChRs

The nAChRs are distributed in various brain regions including the ventral tegmental area, hippocampus, prefrontal cortex, amygdala, and nucleus accumbens. The two most abundant nAChRs, α7 nAChRs (α-bungarotoxin sensitive) and α4β2 (α-bungarotoxin insensitive), are localized in the brainstem, cerebellum, mesencephalic structures, limbic system and cortex. The α4β2, the first to be pharmacologically characterized, constitutes the principal nAChR subtype in the cortex, striatum, superior colliculus, lateral geniculate nucleus and cerebellum. The second abundant nAChR subtype, α7 subunit-containing receptors, have high expression in the cortex, hippocampus and subcortical limbic regions, and are expressed at low levels in the thalamic regions and basal ganglia. Although α7 nAChRs anatomical distribution differ markedly from α4β2, the two co-localize in some areas such as in the superficial layer of the superior colliculus. nAChRs are located in pre- and postsynaptic regions well as in extra-synaptic locations. Specially, receptors containing the β2 subunit are located diffusely throughout the membrane of the neuron.

3.6 Function of nAChRs

Studies from receptor subunit knockout mice have shown that brain nAChRs are not essential for survival or for the execution of basic behaviors. They are, however, critical for control of several complex behaviors and maintenance of mental health. Modulation of presynaptic nAChRs and, less frequently, postsynaptic nAChRs is responsible for large number of behaviors and brain functions including locomotion, nociception, anxiety, learning and memory, as well as behaviors associated with drug abuse and mental illness. Moreover, stimulation of presynaptic nAChRs receptors promotes neurotransmitter release including dopamine (DA), norepinephrine (NE), serotonin (5HT), glutamate, GABA and ACh.

4. AChRs and inflammation

4.1 Role of nicotinic receptors

It is now recognized that there exists a cholinergic anti-inflammatory pathway that acts primarily but not exclusively, through nicotinic acetylcholine receptors. In particular, such pathway has been well characterized in peripheral organs such as spleen where splenic nerve stimulation leads to release of norepinephrine, which in turn, causes release of ACh, where an anti-inflammatory effect is produced [14]. This is because abundant nicotinic (α7 nAChR) are expressed in variety of immune cells including B cells, T cells and macrophages. Activation of these receptors can suppress production of pro-inflammatory cytokines such as TNF- α, IL-1, IL-6 without affecting the anti-inflammatory cytokines such as IL-10. Indeed, several animal models such as sepsis, ischemia–reperfusion, and pancreatitis, which are associated with elevated levels of pro-inflammatory cytokines, show improvement by vagal stimulation. It is believed that this improvement is mediated via activation of α7 nAChRs on macrophages [14, 15]. This contention is further supported by the finding that α7 nAChRs deficient mice show increased endotoxin-induced TNF-α production, which do not respond to electrical vagal stimulation. Because nicotine is a potent activator of

nAChRs, including α7 subtype, it was proposed as a potential intervention in control of cytokine storm associated with COVID-19 [11].

4.2 Role of muscarinic receptors

A role for mAChRs in inflammatory response is also evident. However, it appears that the direction of effect, at least in some tissues such as airway smooth muscle, is opposite to that of nicotinic receptor stimulation, in that mAChRs stimulation, leads to pro-inflammatory, rather than anti-inflammatory consequence [16]. But, in intestinal epithelial cells mAChRs play an important role in the maintenance of homeostasis. Notedly, these cells in addition to absorbing essential nutrients, also prevent the entry of foreign antigens (micro-organisms and undigested food) through mucus secretion and epithelial barrier formation. Since disruption of the intestinal epithelial homeostasis exacerbates inflammation, mAChR agonists may be of therapeutic potential in diseases associated with such disruptions such as inflammatory bowel disease [17]. However, it is important to note that unselective activation of mAChRs may have deleterious effects on intestinal epithelial barrier function. This is because subtypes of mAChRs may have distinct and in some cases opposing effects [17]. Additionally, a comprehensive understanding of the ACh network throughout the intestinal tissue, including the relationship between muscarinic and nicotinic receptors, is yet to be elucidated. In the following section, we concentrate of central interactions between these 2 distinct classes of receptors.

5. Nicotinic-muscarinic receptor interactions

5.1 Central Co-localization of nicotinic and muscarinic receptors

The overlapping distributions of nAChRs and mAChRs in the brain is well characterized. Up to 90% of central cholinergic neurons express both types of receptors as seen in several thalamic nuclei, the interpeduncular nucleus, the superior colliculus, and the cerebral cortex. These overlaps signify important interactions. For example, in striatal DA system, nAChRs and mAChRs counteract each other's effects, in that DA efflux is stimulated by nAChRs activation or in contrast, by inhibition of mAChRs [18]. Similarly, in corpus striatum, ACh released from cholinergic interneurons can activate α4β2 nAChRs mediating release of GABA. This evoked release, on the other hand, can be negatively modulated by M4 mAChRs co-expressed on the same GABAergic terminals [19]. Therefore, it appears that some of the counteractive effects of nAChRs and mAChRs observed in peripheral organs (described above, see 4.2) also extend to the brain. However, exploitation of such interactions is yet to be fully explored.

5.2 Desensitization of nAChRs and effect on mAChRs

Chronic nicotine exposure is associated with a long-lasting desensitization of nAChRs, which is both time- and concentration-dependent. In desensitization state, an intrinsic property of brain nAChRs, the receptor does not respond to nicotine or ACh. Although desensitization may lead to upregulation of nAChRs (an increase in the number of receptors), the overall response is diminished. This desensitization of nAChRs results in hypersensitization of mAChRs, stimulation of which by a muscarinic agonist can lead to electroencephalogram seizures, behavioral convulsions,

tremors and inhibition of spontaneous locomotor activity [20]. Moreover, hypersensitivity of mAChRs does not occur after nAChR recovery from desensitization. In addition to affecting mAChRs, desensitization of nAChRs also affects the activities of other systems. For example, desensitized nAChRs reduce GABA release from interneurons leading to disinhibition of pyramidal cells in hippocampus and cerebral cortex [21]. Conversely, activation of interneuron nAChRs enhances GABA release which inhibits pyramidal cells in these areas.

Interestingly, chronic administration of a mAChR antagonist such as scopolamine results in upregulation of cortical nAChRs [22]. Thus, it appears that inhibition of one receptor class such as nAChRs via desensitization, or inhibition of the other receptor class such as mAChRs via administration of an antagonist, results in upregulation or hypersensitization of the second receptor class, possibly as a compensatory mechanism. This fits with the findings that show administration of nAChRs and mAChRs antagonists together significantly decreases the development of kindled seizures in the amygdala, whereas either drug alone is ineffective [23].

6. Excess ACh

Overall, excess ACh in synaptic cleft results in overstimulation of nicotinic or muscarinic receptors, which in turn, result in activation of the glutamatergic system and the development of seizures [24]. Other central effects include cognitive impairments (discussed below), as well as motivational, arousal and attentional problems. Indeed, this is the mechanism of toxicity induced by organophosphorus compounds, including nerve gases, whereby inhibition of AChE causes accumulation of ACh in the synapse. Aside from central site, peripheral accumulation of ACh may lead to dysregulation in heart contraction, blood pressure, decrease heart rate, increase glandular secretion including saliva, tear, sweat and digestive juices, increase in urination frequency, visual disturbance and importantly, inhibition of muscle contraction due to nicotinic receptor desensitization. Generation of reactive oxygen species (ROS), neuroinflammation are other causes of neuropathies.

Atropine, a mAChR antagonist, is the primary antidote used to counter organophosphate poisoning. However, specific cases of inhibition of nAChRs by atropine have also been reported [25]. In order to overcome the effect of organophosphates on nicotinic receptors, which can result in muscle weakness, fasciculation and paralysis, pralidoxime (2-PAM) also should be given, as 2-PAM tends to reactivate AChE. Interestingly, 2-PAM may also have some muscarinic inhibition, although such effect is not clinically significant and hence necessity of co-administration with atropine [25].

In addition, patients with seizures are given benzodiazepine (BZ), which stimulate GABAAR. Since BZs are of limited efficacy in overall organophosphate toxicity, it is suggested that antagonizing the hyperactivity of the glutamatergic system could provide an even more efficacious approach in protecting the brain from permanent damage. This may be further helped by adding an anticholinergic agent [24].

7. Nicotinic-muscarinic interaction in memory and cognition

Cholinergic system is implicated in memory and cognition functions. ACh is diffusely released throughout the cortex during periods of high attentional demand which could act on nAChRs and mAChRs, both of which are critically important

in cognitive processes such as learning, memory, attention, and other higher brain functions. Manipulation of these receptors exert distinguishable effects on different cognitive functions. For instance, working memory is required for remembering information that varies unpredictably in time and/or in content. It refers to the cognitive system that holds information temporarily and is important for reasoning and decision-making. In contrast, reference memory is a long-term memory that deals with the recall of the content and place of an event.

Both mAChRs and nAChRs modulate not only working and reference memory, but other indices of cognitive functions such as attention and learning. For example, muscarinic M1 receptors in prefrontal cortex [26, 27], and nicotinic receptors, particularly α7 and α4β2 receptors, modulate firing of dorsolateral prefrontal cortex excitatory networks that underlie working memory function [28, 29]. Moreover, muscarinic M1 receptors, may also interact with glutamatergic NMDA receptors in regulation of working memory [30].

Several pharmacological studies have attempted to disentangle the role of nAChRs and mAChRs in different cognitive domains. Blocking mAChRs by scopolamine causes impairment of different aspects of memory processing such as acquisition of new information, consolidation of memory, sustained attention, reaction time, as well as visual discrimination [31, 32]. Antagonism of nAChRs, on the other hand, affects declarative memory (immediate and delayed word recall and delayed recognition), attention and psychomotor function (reaction time), suggesting that attention and psychomotor (reaction time) may be mediated by both mAChRs and nAChRs. This contention is further supported by the findings that blockade of both mAChRs and nAChRs impair working memory (spatial and non-spatial), short-term memory, declarative memory, sustained visual attention, and psychomotor function far more than each antagonist alone [33]. Similar observations were also seen in object and spatial n-back working memory performance where simultaneous antagonism of mAChRs and nAChRs produced greater effect than each antagonist alone [34].

In another cognitive domain, inspection time (IT), a measure of early visual information processing speed, it was determined that the efficiency of visuospatial attention is sensitive to manipulation by both nAChRs and mAChRs. Simultaneous antagonism of both mAChRs and nAChRs induced larger impairments in early information processing (in an inspection time task) than antagonism of either receptor alone [31]. Curiously, impairments in early information processing, a hallmark feature of diverse neuropsychiatric disorders including schizophrenia and AD, may contribute to impairments in other cognitive domains including attention and memory. It is of relevance to note that age-related alterations in mAChR and nAChR interactions also occur [35]. Hence, several lines of evidence implicate a dysfunction of the cholinergic system in cognitive dysfunctions including in AD. This topic is further discussed below, mainly in relationship to AD and PD.

8. Glial cells

There are greater number of glial cells than neurons (between five and ten times more) in CNS. Different types of cells comprise glia. For example, astrocytes, radial glia, and oligodendroglia are of neural origin, whereas microglia are differentiated blood monocytes during ontogeny, where neurons develop first, and glial cells develop later. Glial cells exert a profound effect on neuronal development by providing trophic support essential for neuronal survival and are involved in neuronal

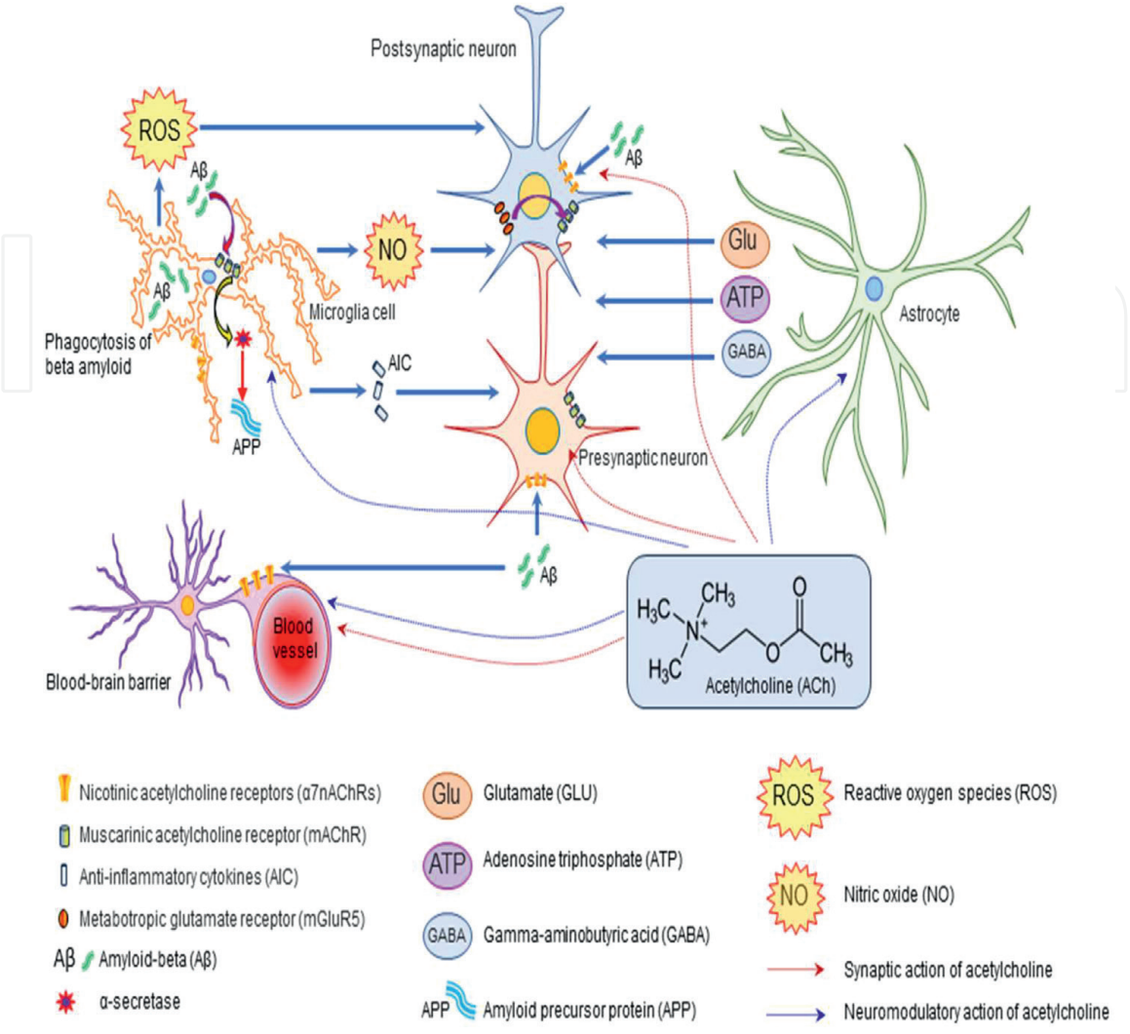

Figure 2.
Schematic diagram depicting the influence of ACh as well as the roles of mAChRs and nAChRs (primarily alpha7 subtype) in microglia and astroglia modulation of pre- and post-synaptic neurons. Note the production of ROS and NO by microglia as well as interaction of Aβ with both nicotinic and muscarinic receptors.

migration, axon, and dendrite outgrowth, and in synaptogenesis (**Figure 2**) [36]. Below, the role of microglia and astrocytes in AD pathology and the influence of cholinergic system on them is discussed in more detail.

8.1 Alzheimer's disease (AD): microglia

AD, the most common age-related neurodegenerative disease, is characterized by cognitive decline in people over 65 years old. Pathologically, AD is presented with amyloid-β protein (Aβ) deposition (plaques), abnormal phosphorylation aggregation of the microtubule-associated protein tau (tangles), neuroinflammation, oxidative stress, and synaptic dysfunction. Neuroinflammation is underscored by microglial reaction and increased cytokine production. Microglia are also major sources of free radicals such as superoxide and nitric oxide in the brain. Microglia are considered the innate immune cells of the CNS and act as brain macrophages. They are mainly found in the subventricular and subgranular zone, where under physiological conditions self-renew over an organism's entire lifespan. Microglia are not uniformly distributed throughout the brain. A large number is present in the hippocampal dentate gyrus,

substantia nigra, and parts of the basal ganglia. Interestingly, olfactory telencephalon in mice has the largest microglial population.

Microglia differ in size and ramification patterns within and between different histological layers of the cerebellar cortex. Substantia nigra contains the largest proportion of microglia (about 12%) compared to 5% in the cortex and corpus callosum. This regional heterogeneity is attributed to the residential environment, especially interactions with neurons or neural progenitor cells, as well as intrinsic mechanisms. Microglia are critical for regulation of the neuronal network as they support the development, maintenance, homeostasis, and repair of the brain by wiping out cell debris and phagocytizing viruses and bacteria. There are several stages in microglia morphology and function. For example, during the resting state, microglia are sensitive to environmental stimuli such as stress that can activate aberrant microglia functioning and lead to neurodegenerative and psychiatric disorders. Thus, it is critical to recognize microglial heterogeneity in identifying microglia-selectively therapies and uncover the underlying mechanisms that activate the reparative and regenerative functions of microglia [37, 38].

Pro-inflammatory microglia (M1-activated state) secrete proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and inducible nitric oxide synthase (iNOS), which typically lead to dysfunction following chronic activation. In contrast, neuroprotective microglia (M2 state) phagocytose cell debris and misfolded proteins, promote tissue repair and reconstruction of the extracellular matrix and support neuron survival mediated by neurotrophic factors [39].

AD is also associated with changes in neurovascular unit NVU, a structural and functional complex that maintains microenvironmental homeostasis and metabolic balance in CNS. Microglia are one of the most important components of the NVU. In AD, microglia may also cause blood–brain barrier (BBB) breakdown due to loss of pericytes. Pericytes are cells present at intervals along the walls of capillaries and are important for blood vessel formation, maintenance of BBB, regulation of immune cell entry into CNS and control of brain blood flow. Thus, BBB breakdown can lead to infiltration of peripheral white blood cells into CNS, abnormal contraction of cerebral vessels and neurovascular uncoupling (**Figure 2**) [40].

Family history is the second strongest risk factor following advanced age. Twin and family studies indicate that genetic factors are estimated to play a role in at least 80% of AD [41]. Moreover, autosomal dominant and late onset sporadic AD share a common pathophysiology [42]. Numerous sporadic AD risk genes including apolipoprotein E (ApoE) and complement receptor 1 (CR1), are highly expressed in microglia and affect microglial phagocytosis of amyloid-beta peptides. Actually, during the early stages of AD, microglia may provide protection against amyloid accumulation but in advanced AD stage they promote neuropathology. Indeed, approximately two-thirds of AD patients risk single nucleotide polymorphisms that are exclusively or dominantly expressed in microglia. Furthermore, AD is associated with increased microglial proliferation. It is noteworthy that microglia exert a "double-edged sword" effect involving neuroprotective or neurotoxic functions dependent on contextual factors as well as disease stage. Thus, at the early stage of AD, microglia are involved in clearance of Aβ and tau proteins from the brain, whereas in the later stages of AD, sustained microglial activation leads to chronic pro-inflammatory state, associated with increase production of pro-inflammatory cytokines, reactive oxygen species (ROS), and dysfunctional lysosomal deposits, all of which adversely affect neuronal survival by promoting protein aggregation and hence causing neuronal damage. Altogether, the findings suggest that further characterization of microglia and its detailed role in neurodegeneration, can lead to novel therapeutic targets for AD [38, 39, 43].

8.2 AD: astrocytes

It is believed that astrocytes are involved in many vital cognitive functions, including learning and memory. Moreover, astrocytes through production of antioxidant and anti-inflammatory proteins are involved in CNS protection. They also clean the extracellular environment and facilitate neuronal communication and help in maintenance of homeostasis. However, full exploitation of glial system as potential development of novel drugs and techniques to reverse oxidative stress and/or excess of inflammation that occurs in many CNS diseases, remains to be investigated (**Figure 2**) [44].

In contrast to microglia, astrocytes are brain cells that mainly control metabolic and redox homeostasis. Due to their swift response to brain pathology in the initial stages of the disease, their activation and differentiation are implicated in the pathogenesis of multiple neurodegenerative diseases, including AD [45]. Astrocytes play an important role in synaptic function, K+ buffering, BBB maintenance and neuronal metabolism. For example, BBB disruption occurs in the early stages of AD, which is associated with cognitive decline and might accelerate the disease progression. Reactive astrocytes denote astrocytes undergoing morphological, molecular and functional remodeling in response to pathological stimuli. During AD progression, reactive astrogliosis occur. For this reason, it has been suggested that manipulation of astrocytes and/or astrocytic biomarkers could be developed in diagnosis and/or treatment of AD (**Figure 2**) [46, 47].

Blood biomarkers have been investigated for the diagnosis, prognosis, and monitoring of AD. Although Aβ and tau are primarily blood biomarkers, recent studies have identified other reliable candidates such as glial fibrillary acidic protein (GFAP), an astrocytic cytoskeletal protein that can be detected in blood samples. Indeed, it has been suggested that GFAP levels can be used to detect early-stage AD. This is based on observations where GFAP level in the blood was higher in the Aβ-positive group than in the negative groups, and in individuals with AD or mild cognitive impairment (MCI) compared to the healthy controls [47]. Thus, astrocyte activation, accompanied by high levels of GFAP is often observed in AD patients. This elevated GFAP occurs around Aβ plaque, indicative of elevated phagocytosis. Structural alterations in AD astrocytes including swollen endfeet and soma shrinkage contribute to disruption in vascular integrity at capillary and arterioles levels. Astrocyte endfeet enwrap the entire vascular tree within CNS where they perform important functions in regulating BBB, cerebral blood flow, nutrient uptake, and waste clearance [48]. Like microglia in AD, astrocytes also are skewed into proinflammatory and oxidative profiles with increased secretions of vasoactive mediators inducing endothelial junction disruption and immune cell infiltration [49]. Regarding biomarkers, astrocytic α7nAChR levels or activity, was recently proposed as a marker since this receptor subtype is implicated in instigation and potentiation of early Aβ pathology. The same receptors could provide a target for therapeutic intervention in AD [46].

Recently, it has been proposed that the term "type III diabetes (T3DM)" be used in conjunction with AD as both conditions share similar molecular and cellular features. For example, T3DM is associated with insulin resistance and cognitive decline (memory deficits) in elderly individuals. Since astrocytes are involved in brain metabolism (e.g., glucose metabolism, lipid metabolism), neurovascular coupling, synapses, and synaptic plasticity, targeting them might be promising in alleviating neurodegeneration in these patients [50].

8.3 ACh-AChRs: microglia

As mentioned above, neuroinflammation linked to glial function has been demonstrated to participate in the pathogenesis of AD (**Figure 2**). Moreover, anti-inflammatory and neuroprotective properties of ACh in several neurodegenerative disorders was also alluded to. More recently, specific influence of ACh on neuroinflammation and neurodegeneration in AD was investigated. It was reported that microglia played a key role in lipopolysaccharide (LPS)-induced hippocampal neuronal toxicity and that ACh, via activation of α7nAChR provided anti-inflammatory and neuroprotective effects. Furthermore, in neuron–microglia co-cultures, LPS increased the expression of pro-inflammatory factors, including iNOS, interleukin-1α, and tumor necrosis factor-α, and decreased expression of neurotrophic factors such as insulin-like growth factor-1, and neuronal apoptosis. However, ACh, via the action of α7nAChR on microglia, inhibited LPS-induced inflammatory response and provided neuroprotection, which was further enhanced by promoting microglial neurotrophic factor production [51]. Targeting microglia in age-related cognitive decline and AD, and bearing in mind the heterogeneity of microglia in these conditions and how pharmacological agents could target specific microglial states, has been recently reviewed [52]. Infiltration of immune cells into the brain might play a role in detrimental effects of activated microglia as this can lead to T-cell infiltration, which can induce tauopathy, another marker of AD neuropathology. Interestingly, drugs or antibodies that can result in death of microglia, have shown protection against brain atrophy in mice [53].

Microglia may contain both nAChRs and mAChRs. It is believed that a subpopulation of microglia that express functional mAChRs play a role in stroke and AD. These microglia tend to expand in these conditions, which are sensitive to blockers of protein synthesis and correlate with an upregulation of the M3 receptor subtype. Thus, carbachol, a mAChR agonist acts as a chemoattractant for microglia and reduces their phagocytic activity [54]. In addition to M3 receptor upregulation, there is an increased expression of major histocompatibility complex (MHC)-I and MHC-II. MHC molecules plays an important role in alerting the immune system to virally infected cells [55].

As mentioned above, nAChRs presence in microglia and consisting primarily of α7nAChR provide anti-inflammatory and neuroprotective effects. Hence, manipulation of microglial nAChRs and mAChRs may offer a new therapeutic strategy in neurodegenerative diseases in general, and AD, in particular.

8.4 ACh-AChRs: astrocytes

ACh and AChRs are present in the brain before synaptogenesis occurs and are believed to be involved in neuronal maturation. Astrocytes express mAChRs whose activation stimulates a robust intracellular signaling that regulate neurite outgrowth in hippocampal neurons, a system intimately involved in cognitive function. In fact, stimulation of astrocytes induces the release of permissive factors that accelerate neuronal development [36]. Moreover, it was recently demonstrated that M1 muscarinic receptors in astrocytes mediate cholinergic regulation of adult hippocampal neurogenesis [56].

In CNS, as mentioned earlier, ACh is mainly present in interneurons. However, at least two important cholinergic pathways have also been identified. One is the cholinergic projection from the nucleus basalis of Meynert (in the basal forebrain) to the forebrain neocortex and associated limbic structures, degeneration of which is one of

the pathologies associated with AD. The other is a projection from the medial septal and diagonal band region to limbic structures, commonly referred to as the septo-hippocampal pathway that is also involved in memory formation [57]. In both cases, both nAChRs and mAChRs mediate the effects of ACh, where nicotinic agonists including nicotine, have been shown to improve working memory, whereas muscarinic agonists may be more relevant to improvement of reference memory [58, 59].

AD patients have a substantial reduction in nAChRs in the cortex and hippocampus. Recently, using local cholinergic lesions it was possible to manipulate the cholinergic system more finely to determine the role of AChRs as well as nicotinic-muscarinic receptor interactions that can either synergize or antagonize the behavioral outcomes. Therefore, potential utility of combining selective nAChR subtypes as well selective mAChRs on memory and cognition warrants further investigation [60, 61]. It is noteworthy that along such selective agonists, manipulation of vesicular ACh transporter should also be considered [62].

That astrocytes express nAChRs was mentioned above. These receptors, predominantly α7nAChR and regulating calcium signaling, are likely mediators of nicotine's effects on morphological and functional changes of the astrocytes [63]. Interestingly, nicotine does not induce reactive astrocytosis even at high concentrations (10 μM) as determined by cytokine release and GFAP expression in-vitro. In vivo also, nicotine induces a change in the volume of astrocytes in the prefrontal cortex, CA1 of the hippocampus, and the substantia nigra. These and other findings indicate potential use of nicotine in neurodegenerative diseases including AD [10, 64, 65]. However, mode of nicotine administration appears to be an important factor in its therapeutic application. It is argued that pulsatile (e.g., via inhalation or nasal spray), rather than continuous administration of nicotine (e.g., via patch) would likely be effective for providing neuroprotection in any neurodegenerative disease [66].

Muscarinic M1 and M4 ACh receptors are also highly pursued drug targets for neurological diseases including AD. However, due to high sequence homology in M1-M5 mAChRs, selective targeting of any subtype through endogenous ligand binding site has been difficult to achieve. Recent discovery of highly subtype selective mAChR positive allosteric modulators has provided a new frontier in novel drug development. However, due to side effects, where M1 mAChR over-activation can have detrimental consequences, a drug candidate may need to exhibit a biased signaling profile. In this regard, recent studies in mice suggest that allosteric modulators for the M1 mAChR that bias signaling toward specific pathways may be therapeutically important [67].

9. Parkinson's disease (PD)

PD, the second most common progressive neurodegenerative disorder, is associated with loss of dopaminergic neurons in SNpc that leads to striatal DA deficiency. This loss of dopaminergic neurons results in motor deficits characterized by akinesia, rigidity, resting tremor, and postural instability as well as non-motor symptoms that might also involve other neurotransmitter systems. The non-motor symptoms may involve emotional changes such as apathy, anxiety and depression, mild or severe cognitive impairment, sleep disturbance (either insomnia or hypersomnia), autonomic dysfunction affecting bladder (frequent and urgent need to urinate), blood pressure (orthostatic hypotension), sweat glands (excessive sweating), sensory dysfunction (feeling of pain, loss of acuity in vision and olfaction), gastrointestinal disturbance (constipation and/

or nausea) as well as "social symptoms" such as inability to recognize other's verbal and nonverbal cues or produce facial expression.

The neuronal degeneration in PD likely involves several cellular and molecular events including accumulation of misfolded proteins aggregates, failure of protein clearance pathways, mitochondrial damage, oxidative stress, neuroinflammation, immune dysregulation, apoptosis, excitotoxicity, Ca++ dysregulation, autophagy and dysbiosis. Implicated in neuronal degeneration are also mutations in genes such as Parkin RBR E3 ubiquitin protein ligase (PARK2), Leucine-rich repeat kinase 2 (LRRK2), PTEN-induced putative kinase 1(PINK1), Parkinson disease protein 7 (PARK7), and Synuclein Alpha (SNCA) as well as polymorphism in DRD2 gene Taq1A (DRD2Taq1A) and DA receptor D2 (DRD2).

PD is believed to be a multifactorial disease, where both genes and environmental factors play a crucial role. Old age, starting at 60 years is considered the primary risk factor for PD. This risk increases with advanced age. In addition, it is postulated that exposure to environmental toxicants such as pesticides, herbicides, and heavy metals may increase the risk of PD.

Serendipitously, it was discovered in the early 1980's that administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), an underground laboratory preparation, could result in motor symptom typical of PD. This discovery was the impetus to use MPTP, a potent analog of meperidine, an opioid analgesic, as a pharmacological model of PD. MPTP is metabolized into the neurotoxin MPP^{+} (1-methyl-4-phenylpyridinium), which is not only substrate for DA transporter (DAT) but is also a potent mitochondrial complex-I inhibitor. Because MPP^{++} selectively damages dopaminergic cells in SNpc, it is commonly used to investigate the mechanism of neurotoxicity and/or development of novel therapeutics. Similarly, rotenone, a pesticide that selectively inhibits mitochondrial complex I, is used to generate animal models of PD. Finally, exposure to heavy metals such as manganese or iron have also been implicated in PD etiology. Interestingly, reduction of iron content was associated with a remarkable improvement of the motor and non-motor deficits in an MPTP-induced monkey model of PD [12, 13].

Oxidative stress has also been linked with the onset and/or progression of several neurodegenerative diseases including PD. In fact, overproduction of reactive oxygen species correlates with AD, Huntington's disease, amyotrophic lateral sclerosis, multiple sclerosis (MS) and PD [68]. It is noteworthy that oxidative stress and neuroinflammation are linked and affect one another [69].

9.1 Treatment modalities for PD

Despite tremendous effort in understanding the causes and/or treatment of PD, no cure is yet available. Current medications are geared toward replenishing central DA transmission, which can only offer symptomatic relief without dealing with the neurodegenerative aspect of the disease. In this case, DA replacement or use of DA agonists to directly stimulate DA receptors are the mainstay of therapy. Unfortunately, these therapies lose efficacy after few years and in some cases such as with L-Dopa treatment, the side effects, commonly referred to L-Dopa-induce dyskinesia can be just as bad if not worse than PD symptoms. Thus, to prolong the efficacy of L-Dopa, it is combined with carbidopa. Alternatively, DA agonists such as pramipexole or ropinirole are used first. Other options include use of monoamine oxidase inhibitors, such as selegiline or rasagiline, or catechol-O-methyltransferase (COMT) inhibitors, such as entacapone and tolcapone. Combination therapy using multiple

drugs with various mechanism of action may also be applied. Non-pharmacological interventions may include physical and occupational therapy, repetitive transcranial magnetic stimulation (rTMS), and in specific circumstances neurosurgery (i.e., deep brain stimulation), which is reserved for those who meet well-defined criteria. Moreover, significant effort is devoted in developing regenerative treatments in the form of autologous or stem cell-derived grafts as well as viral gene therapies designed to replace the function of the neurons that have been lost. Thus, as our knowledge of contributing factors and their mechanisms become more clear, potential development of novel therapies also become a reality [12, 13].

9.2 ACh: PD

The cholinergic neurons of the mesopontine tegmental area and the basal forebrain send projections throughout the brain, regulating many discrete functions. Along with dopaminergic loss, cholinergic dysfunction also plays a substantial role in many PD symptoms such as cognitive impairment, gait problems, freezing of gait, falls, REM sleep behavior disorder (RBD), depression, visual hallucination, psychosis, and olfactory impairment. Thus, cholinergic dysfunction in PD could be contributing to a specific phenotype. This contention is further supported by the finding that a combination of RBD and a history of falls was able to predict combined thalamic and cortical cholinergic deficits. Nonetheless, further elucidation of cholinergic dysfunction, particularly in early stages of the disease is warranted [1]. Below, an update of our current knowledge regarding cholinergic receptors and PD is provided.

9.3 mAChRs: PD

It is now well-accepted that the striatum is the primary input structure of the basal ganglia, which participates in motivational and goal-directed behaviors. Basal ganglia output is controlled by local cholinergic interneurons (ChIs) and dopaminergic afferents. In general, the release of the neurotransmitters DA and ACh, acting through their respective receptors, elicits opposite effects on medial spiny neurons (MSNs). MSNs constitute 90–95% of all striatal neurons, while the remaining population consists of local ChIs and GABAergic interneurons in the striatum. Interestingly, a novel receptor-receptor interaction (i.e., heteromerization) between DA D2 receptor (D2R) and the muscarinic acetylcholine M1 receptor (M1R) was observed. This D2R-M1R complex coordinates a sophisticated interplay between the dopaminergic and cholinergic neurotransmission systems. Based on the existence of this heteromer within the striatum the use of anticholinergics drugs in the treatment of PD was suggested. Indeed, it was demonstrated that an M1R-selective antagonist could potentiate the antiparkinsonian-like efficacy of an ineffective D2R-selective agonist in a rodent model of experimental parkinsonism. Overall, the novel D2R-M1R heteromer could serve as a specific drug target to alleviate motor deficits in PD but with less side effects compared to other drugs [70].

Although giant, aspiny ChIs only represent 1–3% of striatal neurons, they are responsible for the highest concentration of ACh in the brain and interact with DA inputs to regulate motor function. ChIs possess an intrinsic firing activity referred to as autonomous pacemakers which modulate the activities of neuronal afferents. ChIs effects are mediated by both mAChRs and nAChRs. As mentioned earlier, the excitatory M1-like receptors (M1R, M3 R and M5R) transduce their signals through Gq/11proteins, whereas the inhibitory M2-like receptors (M2 R and M4R) are coupled

to Gi/o proteins. The complexity of the striatal circuitry is underscored by the variety of DARs, mAChRs and nAChRs, their subcellular location in ChIs and MSNs as well as their interaction [70].

Due to the development of improved pharmacological agents targeting specific mAChR subtypes, the interest to modulate striatal function by anticholinergic drugs has been renewed in recent years. This is due to the findings that pharmacological blockade of mAChR subtypes, specifically M1R and M4R, can significantly add to other antiparkinsonian treatments. On the other hand, wild-type mice treated with M1R-selective agonist (i.e., telenzepine) had reduced anxiety-like behaviors. Moreover, mice deficient in M1R- exhibit an increased locomotor activity as well as elevated extracellular striatal DA levels. These mice, however, do not exhibit impairment in contextual fear condition, a test of hippocampal-dependent learning. Thus, M1R antagonist can be of benefit in motor impairments, whereas M1R agonist can have anxiolytic and, in some cases, (see above) cognitive enhancement effects. Moreover, M1R antagonists may suppress D2R-MSNs more efficiently than D1R-MSNs, through their interaction with potassium channels. Interestingly, striatal D2R-M1R formation might result in further differentiation of M1R signalization between the striato-pallidal and striato-nigral neurons. Additional support for reciprocal interaction between D2Rs and M1Rs is provided by the findings that systemic administration of scopolamine (i.e., non-selective mAChR antagonist) and benztropine (i.e., moderate M1R-selective antagonist) reduce the affinity of raclopride and spiperone (both D2R antagonists) for D2R in monkey brains [70].

Thus, it may be suggested that the dopaminergic-cholinergic imbalance, which is seen in most movement disorders, may be normalized by a combination of selective D2R agonist and M1R antagonist [70].

9.4 nAChRs: PD

The cholinergic system, particularly nAChRs are essential in modulating the striatal cells regulating cognitive and motor functions. Thus, nAChRs stimulation reduces neuroinflammation and facilitates neuronal survival, neurotransmitter release, and synaptic plasticity. PD is associated with loss of striatal nAChRs, which may aggravate the loss of dopaminergic neurons in this area, leading to pathological consequences. Additionally, nAChRs activation may also stimulate other brain cells supporting cognitive and motor functions [71].

Furthermore, the impairments in DA release observed in various animal models of PD (e.g., 6-OHDA lesioned rodents), appear to be exacerbated by loss of nAChRs activation. This suggests that DAergic imbalance may be ameliorated by nicotinic agonists and hence, nicotinic receptors may offer therapeutic targets for PD. In this regard, several in-vitro and in-vivo studies including primates and genetically modified mice have shown protective effects of nicotine against neuronal damage and/or neurotoxicity induced by 6-OHDA, MPTP, rotenone, methamphetamine, glutamate and β-amyloid. These effects are mediated via selective nAChR subtypes containing β2 and α7 subunits. Protective effects of nicotine against endogenous substances such as salsolinol and aminochrome that selectively damage dopaminergic cells have also been observed. More recently, protective effects of nicotine against toxicity induced by iron and manganese were also observed in cell culture. Interestingly, nicotinic cholinergic system may also play a role in L-Dopa-induced dyskinesias. Finally. an inverse relationship between PD incidence and any form of nicotine intake such as cigarette smoking, smokeless tobacco, exposure to environmental tobacco smoke or even from a dietary

source such as peppers, also suggest a therapeutic potential for nicotine in PD. Hence, targeting nicotinic cholinergic receptors could be a novel intervention in PD. Nicotine's effects are likely to involve suppression of pro-inflammatory cytokines and stimulation of neurotrophic factors as well as suppression of oxidative stress [12, 13].

10. Importance of mode of nicotine administration

Several human studies have assessed the effects of nicotine gum or patch in PD, most of which have not yielded positive results. The negative finding in these trials is likely due to the mode of administration of nicotine. Thus, it is very important to consider the route of nicotine administration, where its subdermal administration via patch may not achieve the desirable nAChR stimulation that is obtained via pulsatile nicotine administration (e.g., via inhalation). The very complex dynamic interaction of nicotine with its receptors, where initial stimulation can be followed by rapid and differential desensitization of receptor subtypes, must be critically considered in experimental paradigms so that maximal therapeutic outcome may be obtained. It may be concluded therefore, that pulsatile stimulation of specific nAChRs in selective brain regions such as the nigrostriatal pathway is critical for the therapeutic efficacy of nicotine or nicotinic agonists in PD. Pulsatile stimulation of central nicotinic receptors may be achieved by currently available nicotine inhaler of nicotine nasal spray. The necessity of pulsatile stimulation may explain the negative outcome of nicotine patch in PD trials. A recent clinical study using oral administration of nicotine (pulsatile), reported positive effects of nicotine on falls and freezing gait in PD. Furthermore, pulsatile nicotine preparations in forms of inhalers or nasal spray are available and approved by FDA for smoking cessation and could be re-purposed for PD pending evaluation of their effectiveness in clinical trials. In addition, pulsatile nicotine administration may also be helpful in improving non-motor symptoms (e.g., depression or cognitive decline) that are commonly associated with PD [12, 13].

11. AD-PD-MS (multiple sclerosis)

In addition to the co-morbid manifestation of neuropsychiatric disorders with neurodegenerative diseases, potential co-morbid occurrence of neurodegenerative diseases such as AD and PD also exists [72]. This might not be very surprising given that the neurobiological substrates of one neurogenerative disease such as mitochondrial dysfunction, oxidative stress and inflammation may be very similar to another disease, except that the anomalies may be confined to specific brain areas and/or circuitries in different diseases. However, when there is an overlap or cross-over of circuitries, similar phenotypes could be manifested. For example, correlation between cholinergic system alterations, oxidative stress-and neuroinflammation in MS has been noted. MS is an autoimmune and demyelinating disease of the central nervous system, characterized by leucocytes infiltration, demyelination, axonal degeneration and neuronal death. As a result of disrupted central communication, different symptoms including vision loss, pain, fatigue, and impaired coordination may be manifested.

The role played by ACh in MS has been recently investigated. Cholinergic alterations may contribute to the dysregulated inflammatory processes of MS [73, 74]. Whether muscarinic or nicotinic receptor manipulation may be of therapeutic potential in MS also, needs to be further investigated.

12. Conclusion

In summary, ACh has maintained its importance in etiology and progression of various neurodegenerative diseases. Although the relevance of mAChRs and their exploitation was the predominant mode of cholinergic intervention in various disorders, including neurodegenerative diseases, the advent of nicotinic receptors and their prominent role in many important CNS functions, including cognitive behaviors such as learning, and memory has opened novel therapeutic potentials. This is not only applicable to neurodegenerative diseases such as AD and PD but also to neuropsychiatric disorders such as depression and schizophrenia. Moreover, significant interaction between these 2 distinct classes of receptors occurs. For example, as depicted in **Figure 2**, Aβ, a culprit protein in AD interacts with both receptors albeit at different locations and different cells (e.g., neuronal vs. glial). Despite notable advancements in our knowledge of these receptors, the complexity of cholinergic system in general, and nicotinic system in particular, requires further investigation on specific role of receptor subtypes in health and disease. Notedly, it is argued that pulsatile administration of nicotine or nicotinic agonists-modulators should be considered in any neurodegenerative and/or neuropsychiatric disease. This is due to the complex pharmacokinetic and pharmacodynamic interactions of nicotine with its receptors, where continuous exposure to nicotine (e.g., via patch) may lead to receptor desensitization, whereas pulsatile administration allows functional recovery of the receptor and hence further stimulation. As our understanding of cholinergic system evolves, more therapeutic targets for neurodegenerative and/or neuropsychiatric diseases are anticipated.

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Conflict of interest

The authors declare no conflict of interest.

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