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REVIEW ARTICLE

The cholinergic system in the pathophysiology and treatment of Alzheimer's disease

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Cholinergic synapses are ubiquitous in the human central nervous system. Their high density in the thalamus, striatum, limbic system, and neocortex suggest that cholinergic transmission is likely to be critically important for memory, learning, attention and other higher brain functions. Several lines of research suggest additional roles for cholinergic systems in overall brain homeostasis and plasticity. As such, the brain's cholinergic system occupies a central role in ongoing research related to normal cognition and age-related cognitive decline, including dementias such as Alzheimer's disease. The cholinergic hypothesis of Alzheimer's disease centres on the progressive loss of limbic and neocortical cholinergic innervation. Neurofibrillary degeneration in the basal forebrain is believed to be the primary cause for the dysfunction and death of forebrain cholinergic neurons, giving rise to a widespread presynaptic cholinergic denervation. Cholinesterase inhibitors increase the availability of acetylcholine at synapses in the brain and are one of the few drug therapies that have been proven clinically useful in the treatment of Alzheimer's disease dementia, thus validating the cholinergic system as an important therapeutic target in the disease. This review includes an overview of the role of the cholinergic system in cognition and an updated understanding of how cholinergic deficits in Alzheimer's disease interact with other aspects of disease pathophysiology, including plaques composed of amyloid-β proteins. This review also documents the benefits of cholinergic therapies at various stages of Alzheimer's disease and during long-term follow-up as visualized in novel imaging studies. The weight of the evidence supports the continued value of cholinergic drugs as a standard, cornerstone pharmacological approach in Alzheimer's disease, particularly as we look ahead to future combination therapies that address symptoms as well as disease progression.

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Abbreviations: MCI = mild cognitive impairment; NBM = nucleus basalis of Meynert

Introduction

Late-onset Alzheimer's disease dementia, the most prevalent age-related neurodegenerative disease, is clinically characterized by a progressive loss of memory and other cognitive functions. In contrast to early-onset autosomal dominant forms of Alzheimer's disease, which are directly linked to abnormalities of amyloid-β, the cascade of pathophysiological events that leads to late-onset Alzheimer's disease is not yet fully understood. Contemporary evidence suggests that late-onset Alzheimer's disease is a complex polygenic disease that involves aberrant interaction among several molecular pathways. By definition, age is the strongest risk factor (Hebert *et al.*, 1995) followed by the ε4 allele of apolipoprotein E (*APOE* ε4) (Liu *et al.*, 2013; Shi *et al.*, 2017), and probably also cardiovascular and lifestyle risk factors (de Bruijn and Ikram, 2014). The neuropathological features of Alzheimer's disease include the accumulation of several abnormal proteins such as amyloid-β in plaques and hyperphosphorylated-tau in neurofibrillary tangles, leading to massive loss of synapses, dendrites, and eventually neurons. Clinical expression of the disease reflects the dysfunction and eventual failure of both neurochemical and structural neural networks, including the 'cholinergic system'. Although the pivotal events in the pathogenesis of Alzheimer's disease are not fully understood, several competing theories on the underlying biology of the neurodegeneration have guided research into interventions to modify, arrest, or delay the progression of the disease and its clinical manifestations. In recent years, however, failure of clinical trials in Alzheimer's disease has been the rule rather than the exception, and no new drugs for Alzheimer's disease have been approved by the US Food and Drug Administration (FDA) since 2003. The multifaceted, heterogeneous, progressive, and interactive pathophysiology of Alzheimer's disease also suggests a likely need for individualized combination treatments that may need to be varied from one stage of the disease to another, and perhaps also from one patient to another.

The cholinergic hypothesis revolutionized the field of Alzheimer's disease research by transporting it from the realm of descriptive neuropathology to the modern concept of synaptic neurotransmission. It is based on three milestones: the discovery of depleted presynaptic cholinergic markers in the cerebral cortex (Bowen *et al.*, 1976; Davies and Maloney, 1976); the discovery that the nucleus basalis of Meynert (NBM) in the basal forebrain is the source of cortical cholinergic innervation that undergoes severe neurodegeneration in Alzheimer's disease (Mesulam, 1976; Whitehouse *et al.*, 1981); and the demonstration that cholinergic antagonists impair memory whereas agonists have the opposite effect (Drachman and Leavitt, 1974). The hypothesis received compelling validation when cholinesterase inhibitor therapies were shown to induce significant symptomatic improvement in patients with Alzheimer's disease (Summers *et al.*, 1986). Although other relevant pathophysiological mechanisms have received more research attention in recent years, treatments that improve cholinergic function remain critical in the management of patients with Alzheimer's disease. The goal of this review is to characterize the nature of the cholinergic lesion in Alzheimer's disease, its potential interactions with other components of the pathology, and its relevance to treatment. We do not aim to provide a comprehensive review of Alzheimer's disease pathogenesis or to rank order the impact of the cholinergic lesion among all other components of this disease. Furthermore, our comments will be limited to late-onset Alzheimer's disease in patients who do not have disease-causing dominant mutations. We should also point out that the brain contains several cholinergic pathways, each with its unique receptor signature, postsynaptic targets and disease vulnerabilities. Unless noted otherwise, our comments in this review will address the forebrain pathway that originates in the basal forebrain and that innervates the neocortex and limbic system. This review also provides a comprehensive evaluation of the known benefits of cholinergic therapies throughout the various stages of Alzheimer's disease.

We aim to demonstrate the enduring value of cholinergic drugs in the pharmacological therapy of Alzheimer's disease, especially in the context of future combination therapies that may affect both symptoms and disease progression.

Nature and impact of the cholinergic lesion

Acetylcholine is a major neurotransmitter in the brain, with activity throughout the cortex, basal ganglia, and basal forebrain (Mesulam, 2013). Figure 1 illustrates the key steps in the synthesis, release, and reuptake of the neurotransmitter acetylcholine.

Human studies assessing the neuropathological diagnosis of Alzheimer's disease have shown that the cholinergic lesion, emerging as early as asymptomatic or prodromal stages of the disease, is mainly presynaptic rather than postsynaptic. In other words, the cholinergic loss is based on the degeneration of NBM cholinergic neurons and of the axons they project to the cerebral cortex. As part of the cholinergic lesion, nicotinic (ionotropic) receptors and muscarinic (metabotropic) receptors of the cerebral cortex also undergo changes. Most studies show a loss of nicotinic receptors in the cerebral cortex. For example, there is a decrease of postsynaptic nicotinic receptors on cortical neurons (Nordberg and Winblad, 1986; Schroder *et al.*, 1991). However, there may also be an equally important presynaptic component based on the loss of nicotinic receptors located on the degenerating cholinergic axons coming from the NBM. With respect to muscarinic receptors of the cerebral cortex, it is interesting that the muscarinic (M)1 receptors (mostly postsynaptic) are not decreased whereas the M2 receptors (mostly presynaptic) are decreased (Mash *et al.*, 1985). However, there is evidence that the remaining postsynaptic M1 receptors of the cerebral cortex may be dysfunctional (Jiang *et al.*, 2014). Thus, a progressive loss of basal cholinergic neurons represents a key neurochemical event with a subsequent anterograde cortical cholinergic deafferentation, of the cerebral cortex, hippocampus and amygdala (Sassin *et al.*, 2000). The alternative possibility of an initial degeneration of cortical cholinergic endings that lead to a retrograde degeneration of NBM neurons cannot be ruled out but is unlikely.

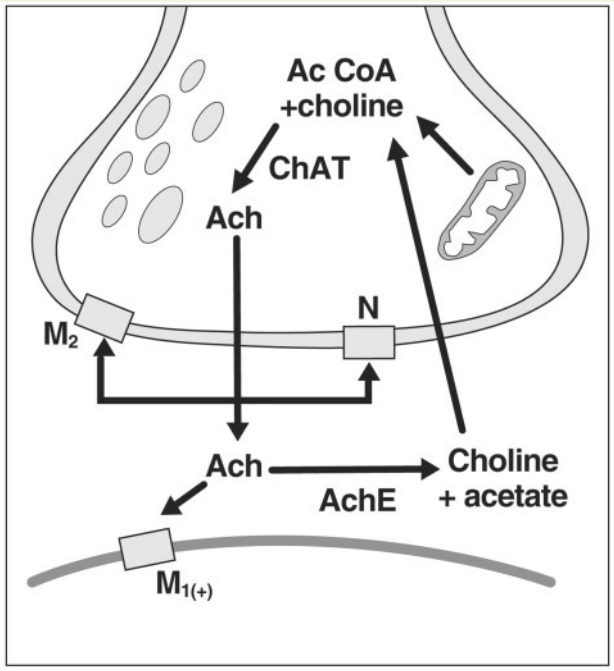

Figure 1 Physiology of the cholinergic synapse. Choline is the critical substrate for the synthesis of acetylcholine. Acetyl coenzyme A (Ac CoA), which is produced by the breakdown of glucose (carbohydrate) through glycolysis (Krebs cycle), along with the enzyme choline acetyltransferase (ChAT) are critical for the synthesis of acetylcholine (Ach). Once the neurotransmitter acetylcholine is released into the synapse, it binds (activates) postsynaptic receptor (M_1), thus transmitting a signal from one neuron to the other. The excess neurotransmitter in the synaptic cleft is broken down by the enzyme acetyl cholinesterase (AChE) into choline and acetate, which are returned by an uptake mechanism for recycling into acetyl coenzyme A.

As noted above, in contrast to M1 receptors, which are mostly preserved, there is a loss of cortical nicotinic receptors. Postsynaptic $\alpha 7$ nicotinic receptor enhances the neuronal firing rates contributing to the hippocampal long-term potentiation, a neuronal-level component of learning and memory (Francis *et al.*, 2010). The application of cholinergic agonists and antagonists to rat hippocampal slices has clarified the role for acetylcholine in long-term potentiation (Blitzer *et al.*, 1990; Auerbach and Segal, 1996). Therefore, altered patterns of nicotinic and muscarinic receptor distribution in Alzheimer's disease are likely to influence many functions of the cerebral cortex and limbic areas through perturbations of synaptic physiology. An upregulation of cortical choline acetyltransferase neuronal expression has been shown in prodromal Alzheimer's disease patients, suggesting that such neurochemical events may compensate for the depletion of basal cholinergic neurons (Ikonomovic *et al.*, 2007). Moreover, it has been shown that Alzheimer's disease patients have higher levels of $\alpha 7$ nicotinic gene expression compared to healthy controls. The influence of these dynamic changes upon Alzheimer's disease pathogenesis remains to be elucidated.

There is also evidence implicating acetylcholine in a variety of essential functions that promote experience-induced neuroplasticity, the synchronization of neuronal activity, and network connectivity. For instance, variable stimulation of the rat NBM, an acetylcholine-rich area of the basal forebrain with wide projections to the cortex, has been shown to produce extensive cortical remodelling and to modulate cortical sensory maps (Kilgard and Merzenich, 1998). Through intrinsic (NBM) and extrinsic (perivascular postganglionic sympathetic nerve) innervation, the cholinergic system has also been shown to promote cerebral

vasodilation and perfusion (Claassen and Jansen, 2006; Van Beek and Claassen, 2011). In mice, electrical and chemical stimulation of cholinergic neurons in the NBM results in a significant increase in cerebral blood flow in several cortical areas (Lacombe *et al.*, 1989; Sato and Sato, 1990; Barbelivien *et al.*, 1995; Lacombe *et al.*, 1997; Vaucher *et al.*, 1997). In addition to disrupting synaptic transmission in cortex and limbic areas, the cholinergic lesion of Alzheimer's disease may therefore also interfere with multiple aspects of neuroplasticity and with cerebral haemodynamic processes.

Anticholinergic agents and cholinergic therapies

The negative pharmacological effects of anticholinergic drugs on human memory and learning have been reported since at least the 1970s (Drachman and Leavitt, 1974; Petersen, 1977; Mewaldt and Ghoneim, 1979; Izquierdo, 1989), and more recent data support these observations. The use of anticholinergic medications in non-demented older adults has been associated with significantly slower reaction times on a measure of rapid information processing and lower cognitive test scores (Stroop test) (Uusvaara *et al.*, 2009; Sittironnarit *et al.*, 2011). Moreover, the increased use of anticholinergic medications was correlated with reduced cognitive function in a systematic review of 33 studies performed in older adults (Fox *et al.*, 2014). The cumulative effect of anticholinergic drugs has also been associated with poorer cognitive abilities, as well as poorer functional outcomes (i.e. activities of daily living) in cohort studies of older populations (Salahudeen *et al.*, 2015). Furthermore, a recent meta-analysis demonstrated that the exposure of older adults with cardiovascular disease to anticholinergic drugs was associated with an increased risk of cognitive impairment (Ruxton *et al.*, 2015). In that study, a greater burden of anticholinergic exposure was shown to more than double the odds of all-cause mortality.

Recent data also suggest that the negative cognitive effects of cumulative anticholinergic drugs in older adults may not be transient. Among cognitively healthy individuals in the ADNI (Alzheimer Disease Neuroimaging Initiative) and Indiana Memory and Aging Study, the 52 participants who had been regularly taking one or more medications with medium or high anticholinergic activity prior to study entry demonstrated worse immediate recall and executive function than the 350 participants who were not actively using anticholinergic medications at study entry (Risacher *et al.*, 2016). Strikingly, cognitively normal adults taking anticholinergic medication were observed to have reduced total cortex volume, increased bilateral lateral ventricle volume, and increased inferior lateral ventricle volume. In addition, across both groups of participants, there was a significant longitudinal association between anticholinergic use and later progression to mild cognitive impairment (MCI) or Alzheimer's disease dementia ($P = 0.01$; hazard ratio, 2.47). Concordantly, in a prospective population-based cohort study of 3434 participants $\geqslant 65$ years with no dementia at study entry, greater cumulative use of anticholinergic drugs over 10 years (based on computerized pharmacy dispensing data) was linked to a statistically increased risk for incident dementia and for Alzheimer's disease specifically. Thus, higher estimates of cumulative exposure to anticholinergic therapies were associated with a greater risk for incident dementia or Alzheimer's disease dementia than were lower levels of cumulative anticholinergic exposure (Gray *et al.*, 2015). In addition to these findings, doses of anticholinergic medication appear to unmask signs of impending dementia in individuals with preclinical Alzheimer's disease. In a study of healthy older adults at risk for Alzheimer's disease, single-dose administration of the anticholinergic drug scopolamine unmasked cognitive deficits and poorer cognitive performance more often in patients with higher brain amyloid-β burden on PET images (Lim *et al.*, 2015). More recently, impaired performance in response to a low-dose scopolamine challenge test among cognitively unimpaired adults at risk for Alzheimer's disease predicted both amyloid-β positivity on PET images and a decline in episodic memory at 27 months (Snyder *et al.*, 2017).

Treatment that promotes cholinergic function in individuals with, or at risk for, Alzheimer's disease may also have more durable beneficial biological effects on the brain than a temporary augmentation of cognitive function. The French Hippocampus Study Group found, in a placebo-controlled trial in people with suspected prodromal Alzheimer's disease, that use of the cholinesterase inhibitor donepezil was associated with substantially less regional cortical thinning and basal forebrain atrophy over time (Cavedo *et al.*, 2016, 2017). A placebo-controlled study on the same population found a 45% reduction in the rate of hippocampal atrophy after 1 year of treatment with donepezil (Dubois *et al.*, 2015), a finding previously reported by another research group investigating patients with fully expressed dementia (Hashimoto *et al.*, 2005). Although these results have not yet been linked to a specific biological mechanism, they raise the possibility of substantial brain structural protective effects of cholinergic treatment during various stages of Alzheimer's disease. Several studies have also explored the role of cholinesterase inhibitors on cerebrovascular perfusion in Alzheimer's disease and other dementias (Geaney *et al.*, 1990; Ebmeier *et al.*, 1992; Arahata *et al.*, 2001; Venneri *et al.*, 2002; Lojkowska *et al.*, 2003; Ceravolo *et al.*, 2006). Patients with Alzheimer's disease dementia receiving a single dose of cholinesterase inhibitor treatment showed an increase (Geaney *et al.*, 1990; Ebmeier *et al.*, 1992) or a stabilization of cerebral blood flow (Venneri *et al.*, 2002; Van Beek and Claassen, 2011) in the posterior parieto-temporal and superior frontal regions. A recent study showed decreased regional cerebral blood flow in the parietal cortex, and an increase in the frontal and the limbic cortices after 18

months of treatment with donepezil or galantamine (Shirayama *et al.*, 2017). Case reports and investigations with small sample sizes have reported increased cerebral blood flow after treatment with cholinesterase inhibitors in patients with vascular dementia, dementia with Lewy bodies, and dementia of Parkinson's disease (Arahata *et al.*, 2001; Mori, 2002; Lojkowska *et al.*, 2003; Ceravolo *et al.*, 2006). The clinical impact of these haemodynamic events has not been clarified.

Interactions between the cholinergic system and the other pathophysiological hallmarks of Alzheimer's disease

The main pathological hallmarks of Alzheimer's disease include not only amyloid-β plaques and neurofibrillary tangles but also neuroinflammation, altered insulin resistance, oxidative stress and cerebrovascular abnormalities. These pathological hallmarks have complex reciprocal interactions with the cholinergic lesion. Previous post-mortem studies have shown that the loss of cortical cholinergic innervation is associated with and probably caused by the neurofibrillary tangles in the NBM (Geula and Mesulam, 1994; Braak and Del Tredici, 2013; Mesulam, 2013). The basal forebrain cholinergic neurons are among the cell bodies most susceptible to neurofibrillary degeneration and neurofibrillary tangle formation (Mesulam, 2013). There exists a long-established relationship between cholinergic abnormalities and amyloid-β pathology. Perry *et al.* (1978) correlated diminishing activity of the acetylcholine-synthesizing enzyme choline acetyltransferase with increasing numbers of neuritic plaques in the post-mortem brains of patients with Alzheimer's disease (Perry *et al.*, 1978). This correlation was also shown in cognitively unimpaired persons whose brains at autopsy revealed amyloid-β plaques. More recently, an inverse correlation was found between choline acetyltransferase activity and amyloid-β deposition in the inferior temporal gyrus of persons, at autopsy, who had had normal cognitive function (Beach *et al.*, 2000). Moreover, presynaptic and postsynaptic markers of cholinergic activity were significantly reduced in non-demented individuals whose brains demonstrated neuritic plaques at autopsy—an effect that was even more pronounced in demented individuals with pathologically confirmed Alzheimer's disease (Potter *et al.*, 2011). Studies investigating regional correlations between the loss of cholinergic axons and the density of amyloid-β deposits in Alzheimer's disease-affected human brains have also shown conflicting results. Although the correlation between cholinergic loss and neurofibrillary tangle (both presynaptically in the NBM and postsynaptically in the cortex) is more robust, this correlation is not uniform throughout the brain—specifically in the cingulate cortex (Geula *et al.*, 1998; Potter *et al.*, 2011).

Animal experiments have suggested that the cholinergic depletion promotes amyloid-β deposition and tau pathology in ways that contribute to the cognitive impairment (Ramos-Rodriguez *et al.*, 2013). For example, selective lesions of cholinergic neurons in the basal forebrains of Alzheimer's disease rodent models have been reported to be associated with increased deposition of amyloid-β and levels of hyperphosphorylated tau in the hippocampus and cortex. These types of effects have been reported in the past but have been difficult to replicate. Cholinergic deficits in rat brains have also been shown to interact with acute proinflammatory mechanisms to produce or exacerbate cognitive impairment (Field *et al.*, 2012).

Stimulation of α7 nicotinic receptors may have a neuroprotective effect against amyloid-β-induced toxicity trough activation of the PI3K-Ak axis, the anti-apoptotic factor bcl2 and downregulation of glycogen synthase kinase-3 (GSK3) (Beaulieu, 2012). GSK3 over activation is correlated with high levels of toxic amyloid-β oligomers, hyperphosphorylated tau strains and neurofibrillary tangles (Jaworski *et al.*, 2011; Chu *et al.*, 2017), activation of the α7 nicotinic receptor is associated with anti-inflammatory pathways also through downregulation of NFκB via Jak2 (Kalkman and Feuerbach, 2016).

Nitsch *et al.* (1992) and Mori *et al.* (1995) demonstrated that the stimulation of cholinergic receptors either by muscarinic agonists or by cholinesterase inhibitor treatment shifted the processing of amyloid precursor protein (APP) towards non-amiloidogenic pathways.

Additional evidence has shown that muscarinic agonists, mainly M1 and less so M3, can downregulate amyloidogenic and tau-generating pathways. The mechanisms are not fully understood yet. However, it has been shown that M1 agonist may act as functional activators of protein kinase C (PKC) signalling which, in turn, promotes a metabolic shift towards α-secretase activity via upregulating ADAM17 [also known as tumour necrosis factor-α-converting enzyme (TACE)]. In support of this hypothesis, animal studies have demonstrated that orthosteric M1-selective agonists are associated with increased levels of APPs cleaved by alpha secretase (Cisse *et al.*, 2011; Welt *et al.*, 2015). Conceivably, α7 nicotinic and coupling of M1 to PKC may lead to a downregulation of detrimental cell processes occurring in Alzheimer's disease such as GSK3-mediated tau hyperphosphorylation (Espada *et al.*, 2009).

The loss of acetylcholine-mediated vasomotor control of the blood–brain barrier could also potentially lead to an aberrant diffusion and transportation of metabolites between the interstitial fluid and the CSF. One possible consequence for this is the impairment of the clearance of amyloid-β from brain (Hunter *et al.*, 2012). As shown by Weller and colleagues, cholinergic deafferentation may alter the blood–brain barrier and the dynamics of arterial and

perivascular lymphatic drainage of amyloid-β (Engelhardt *et al.*, 2016).

These observations illustrate the highly complex interactions that are likely to exist between cholinergic denervation and other pathological features of Alzheimer's disease (Ramos-Rodriguez *et al.*, 2013; Szutowicz *et al.*, 2013; Hartig *et al.*, 2014; Kolisnyk *et al.*, 2017). In addition, important neurophysiological relationships with other major neurotransmitter (serotonergic, dopaminergic, GABAergic) and neurohormonal (renin-angiotensin) systems that are also likely to take place remain to be elucidated (Bodiga and Bodiga, 2013).

Complex interactions among different neurotransmitter systems are essential for adaptive responses and compensatory mechanisms both in physiological and pathophysiological conditions. For example, the activity of presynaptic α7 nicotinic receptor may facilitate glutamate release, while activation of muscarinic receptors may decrease both the release and the concentration of glutamate in the synaptic cleft (Higley *et al.*, 2009). Although changes of neurotransmitters other than acetylcholine have been demonstrated in Alzheimer's disease (Limon *et al.*, 2012; Chalermpalanupap *et al.*, 2013; McNamara *et al.*, 2014) it should be underlined than no drugs selectively acting on noradrenergic, serotoninergic or GABAergic systems have been approved. Supplementary Table 1 provides an overview of the available evidence regarding the involvement of different neurotransmitter in Alzheimer's disease, as well as the main molecular mechanisms associated with each receptor activity and their interplay with acetylcholine.

The cholinergic system and *APOE* genetic risk factor

The *APOE* ε4 allele is the strongest genetic risk factor for sporadic/late onset Alzheimer's disease. The presence of two *APOE* ε4 alleles has been linked to disruptions of amyloid-β and tau proteostasis (Liu *et al.*, 2013; Shi *et al.*, 2017), impaired clearance, aberrant post-translational modifications (i.e. hyperphosphorylation), mitochondrial dysfunction, and neuroinflammatory processes in ageing and Alzheimer's disease. The *APOE* ε4 allele is strongly correlated with faster cognitive and functional decline (Whitehair *et al.*, 2010). It is still unclear whether the presence of *APOE* ε4 allele affects the NBM neuronal functioning, and if it does whether this happens indirectly through amyloid-β and tau accumulation in the basal forebrain. To date, only two human retrospective post-mortem studies have shown that both healthy older individuals and mild Alzheimer's disease patients, carrying the ε4 allele, had reduced neuronal metabolic activity in the NBM as measured by the size of the Golgi apparatus (Salehi *et al.*, 1998; Dubelaar *et al.*, 2004). Previous studies showed that *APOE* genotype does not significantly influence the magnitude of the cholinesterase inhibitor response in mild-to-moderate Alzheimer's disease (Miranda *et al.*, 2015; Waring *et al.*, 2015). These studies suffer from methodological limitations that might have remarkably biased their results. In particular, several potentially confounding factors have not been taken into account i.e. stage of pathophysiological processes, pharmacogenomic background, and comorbidities. Interestingly, it has been recently shown that *APOE* genotype may influence cholinergic compensatory mechanisms. In particular, the *APOE* ε4 allele is associated with deficits in the cholinergic hippocampal compensatory sprouting and remodelling in response to cholinergic deafferentation (Bott *et al.*, 2016). Based on these considerations, further work needs to be performed to investigate whether the *APOE* ε4 status influences the response to cholinomimetic therapy.

Anatomy, selectivity and specificity of the cholinergic deficit in Alzheimer's disease

The cholinergic loss is one of the most prominent components of the neuropathology of Alzheimer's disease. In the mid-1970s in the UK, investigators autopsied the brains of people with Alzheimer's disease and reported a selective and statistically significant reduction in the activity of choline acetyltransferase in the limbic system and cerebral cortex (Bowen *et al.*, 1976; Davies and Maloney, 1976; Perry *et al.*, 1977*a*, *b*). At the time, the origin of this cholinergic innervation was unknown. In 1976, axonal transport studies, combined with cholinergic histochemistry, revealed the NBM as the source of cholinergic innervation in the cerebral cortex of the primate brain (Mesulam, 1976). These studies led to the investigation of the NBM in Alzheimer's disease and to the post-mortem data from Whitehouse *et al.* (1981, 1982), which demonstrated a profound loss of cholinergic neurons in the basal forebrain, specifically the NBM, of patients with Alzheimer's disease. The NBM can be considered a rostral extension of the brainstem reticular formation. It innervates the entire cerebral cortex and limbic system, including the hippocampus, and the entorhinal cortex. It has been well established that cholinergic deficits play a key role in the neuropathology of Alzheimer's disease, not only in late disease, but in preclinical and early stages as well. Accumulated abnormal phosphorylated tau, in the form of neurofibrillary tangles and pretangles, has been found specifically in the cholinergic neurons of the basal forebrain in cognitively normal elderly subjects and patients with MCI and to correlate significantly with performance in memory tasks (Mesulam *et al.*, 2004). A progression of abnormalities has been observed in the cholinergic neurons of the basal forebrain of non-demented younger adults, non-demented elderly people, and people with mild or severe Alzheimer's disease (Geula *et al.*, 2008). Thickened cholinergic nerve fibres and ballooned terminals, demonstrated in middle-aged adults, have been shown to increase with age, suggesting that cholinergic loss in established Alzheimer's disease is preceded

by this cholinergic pathology (Geula *et al.*, 2008). Cholinergic function outside of the NBM—namely, in the caudate, putamen, and thalamus—appears relatively spared in this process. There is, therefore, no generalized 'cholinergic vulnerability' in Alzheimer's disease but, instead, a preferential vulnerability of the NBM. The underlying mechanism may be the location of the NBM within the corticoid-limbic belt of the forebrain, which includes other limbic structures such as the hippocampus, amygdala, and entorhinal cortex, areas that are collectively the most vulnerable to neurofibrillary degeneration and neurofibrillary tangle formation in the ageing–MCI–Alzheimer's disease continuum (Mesulam, 2013). With the use of longitudinal MRIs and amyloid-β biomarkers, it has been shown that volume loss in the NBM precedes and predicts memory impairment and degeneration of the entorhinal cortex (Schmitz *et al.*, 2016). This observed relationship strengthens the conclusion that the loss of NBM neurons is an early and perhaps also clinically relevant event in Alzheimer's disease.

Unlike the cholinergic neurons and synaptic terminations of the caudate, putamen, and thalamus, the NBM and medial septum cholinergic neurons are fully dependent on the retrograde transport of nerve growth factor (NGF) for the maintenance of their anatomic and biochemical characteristics and their terminal synapses in the cerebral cortex and hippocampus (Cuello *et al.*, 2007, 2010; Cuello, 2013). It is well accepted that the interactions of NGF with the forebrain cholinergic system is of significance in Alzheimer's disease (Mufson *et al.*, 2008; Schliebs and Arendt, 2011; Cattaneo and Calissano, 2012; Triaca and Calissano, 2016; Turnbull *et al.*, 2018). There is evidence that cholinergic neurons in the NBM may well be deprived of trophic support even before clinical manifestations of Alzheimer's disease. While the biosynthesis of NGF in the cerebral cortex is not affected in Alzheimer's disease, experimental animal data and human post-mortem brain material would indicate that trophic support of the NGF-dependent cholinergic neurons in the NBM may be compromised by defective retrograde transport of NGF or the diminished conversion of pre-NGF to mature NGF (neuroguidin) (Cuello *et al.*, 2007, 2010; Iulita and Cuello, 2014; Iulita *et al.*, 2017). In individuals with Down syndrome, who are at high risk for early-onset Alzheimer's disease by amyloid-β-mediated mechanisms, rising plasma levels of amyloid-β and inflammatory markers have been associated with biomarker evidence of NGF dysregulation (Iulita *et al.*, 2016*a*, *b*). These data suggest that NGF dysregulation may be precipitated by the accumulation of amyloid-β and amyloid-β-driven inflammation, the end result of which is cholinergic loss in the NBM. The potential downstream effects of amyloid-β on cholinergic neurons in the NBM, by way of dysregulated NGF, deserve further exploration. Therefore, the NGF metabolic pathway remains a potential pharmacological target in the effort to slow the loss of critical cholinergic function in Alzheimer's disease, especially at preclinical stages (McDade and Bateman, 2017). However, intracerebrally- and exogenously-applied NGF has so far shown to be unsuccessful. It is important to keep in mind that exogenous NGF may reach undesirable ectopic targets producing undesirable effects (pain, anorexia, other). On the other hand, the pharmacological normalization of the NGF metabolic pathway, if attainable at early Alzheimer's disease pathology stages, could potentially halt the NBM degeneration by selectively boosting the trophic influence of NGF with greater physiological selectivity.

Pathology of the NBM is not unique to Alzheimer's disease. Synucleinopathies such as Parkinson's disease and especially Lewy body dementia are also associated with NBM degeneration and the resultant cortical cholinergic denervation. In Lewy body dementia this effect may be even more severe than in Alzheimer's disease. In contrast to Alzheimer's disease where the NBM degeneration is based on neurofibrillary tangle formation, in Lewy body dementia the degeneration is associated with intracellular Lewy bodies. It is interesting that cholinesterase inhibitors can improve cognition also in Parkinson's disease and Lewy body dementia (Graff-Radford *et al.*, 2012).

The role of cholinergic therapy for Alzheimer's disease

The prevailing therapeutic strategy in the management of Alzheimer's disease is based on the restoration of cholinergic function through the use of compounds that block the enzymes that break down acetylcholine (Lovestone and Howard, 1995; Massoud and Gauthier, 2010). Cholinesterase inhibitors are designed to inhibit the breakdown of acetylcholine and sustain its activity at cholinergic synapses. Currently available FDA-approved cholinesterase inhibitors for the treatment of Alzheimer's disease are donepezil, rivastigmine, and galantamine (Table 1). These drugs have been shown to statistically significantly improve cognition, daily and global function, and some behavioural manifestations of Alzheimer's disease, compared with placebo treatment (Massoud and Gauthier, 2010). As such, cholinesterase inhibitors are generally considered symptomatic treatments for Alzheimer's disease. For the purpose of the discussion on therapy, we will use the term 'Alzheimer's disease' to mean 'Alzheimer disease dementia' rather than 'Alzheimer disease pathology.' This distinction is important because Alzheimer's pathology emerges many years before symptom onset and there are currently no approved guidelines concerning cholinergic therapy during preclinical stages of the disease.

A meta-analysis of 26 studies of donepezil, rivastigmine, and galantamine showed a modest but clinically meaningful overall benefit of these drugs for stabilizing cognition, function, behaviour, and global clinical change (Hansen *et al.*, 2008). Results from the few existing head-to-head comparisons of cholinesterase inhibitors have been mixed; however, an adjusted analysis of placebo-controlled data suggested

Table 1 FDA-approved cholinesterase inhibitors for Alzheimer's disease[a]

Cholinesterase inhibitors	First FDA Approval	Indication(s)	Dosages
Donepezil tablets (or oral solution) (Aricept, 2016)	1996	Mild–moderate Alzheimer's disease Moderate–severe Alzheimer's disease	5 or 10 mg daily 10 or 23 mg daily
Rivastigmine transdermal system (Exelon Patch, 2016)	2000	Mild–moderate Alzheimer's disease Severe Alzheimer's disease	4.6, 9.5 or 13.3 mg/24 h 13.3 mg/24 h
Rivastigmine capsules (Elexon, 2016)[b]	2000	Mild–moderate Alzheimer's disease	3, 4.5, or 6 mg twice daily
Galantamine extended-release capsules, tablets, or oral solution (Razadyne ER/Razadyne, 2013)	2001	Mild–moderate Alzheimer's disease	8 or 12 mg twice daily (tablets, oral solution) 16 or 24 mg once daily (extended-release)

[a]Minimum effective dosages are provided.
[b]Rivastigmine is also available as an oral solution, at a concentration of 2 mg/ml.
FDA = US Food and Drug Administration.

that donepezil might have a slight advantage over rivastigmine and galantamine in efficacy and tolerability (Hansen *et al.*, 2008). These results did not include the rivastigmine transdermal delivery system, which has fewer side effects than the oral formulation of rivastigmine. In a systematic review of seven studies that examined the economics of cholinesterase inhibitors, treatment of Alzheimer's disease with cholinesterase inhibitors appeared to be a cost-effective, if not a cost-saving, strategy—although a considerable number of variables, such as the length of treatment and medication discounts, contributed to general uncertainty as to their benefits (Pouryamout *et al.*, 2012). A large Medicare beneficiary study concluded that each additional month of cholinesterase inhibitors treatment is associated with a 1% reduction in total all-cause healthcare costs (Mucha *et al.*, 2008).

Long-term data indicate that the use of a cholinesterase inhibitor in Alzheimer's disease reduces the risk for nursing home placement by ~30% for each year of treatment (Feldman *et al.*, 2009). In addition, patients with Alzheimer's disease who are treated with a higher mean dose of cholinesterase inhibitors compared with patients receiving a lower mean dose have been shown to experience delayed nursing home placement (Wattmo *et al.*, 2011). These data are supported by a *post hoc* analysis of the DOMINO-AD trial, in which the nursing home placement of community-dwelling patients with moderate-severe Alzheimer's disease was assessed (Howard *et al.*, 2015). Patients who were randomized to discontinue donepezil therapy (10 mg/day) were twice as likely to enter a nursing home after 1 year as were individuals who continued treatment with cholinesterase inhibitors; however, this effect lost statistical significance after 3 years. Finally, cholinesterase inhibitors have also been shown to reduce the burden experienced by caregivers of patients with Alzheimer's disease, by reducing caregiver time devoted to the patient, caregiver stress, and some of the behavioural symptoms (Feldman *et al.*, 2003; Hashimoto *et al.*, 2009; Schoenmakers *et al.*, 2009; Adler *et al.*, 2014).

A recent meta-analysis carried out on 142 randomized controlled trials (RCTs), quasi-RCTs, and non-randomized studies in individuals with Alzheimer's disease treated with cholinesterase inhibitors, only patients treated with galantamine showed a decreased odds-ratio of mortality when compared with placebo (Tricco *et al.*, 2018). It has been reported that cholinesterase inhibitors delay the need for nursing home placement and institutionalization (Jelic and Winblad, 2016). This interesting finding has been linked also to a potential effect of such drugs on behavioural and psychological symptoms of dementia (BPSD) (Cumbo and Ligori, 2014). It is demonstrated that BPSD are positively associated with a faster decline in global functioning and higher caregiver burden (Lyketsos *et al.*, 2011; Collins *et al.*, 2016). Loss of cerebral dopaminergic tone has been likened to apathetic syndrome, which is one of the most frequent and persistent BPSD in Alzheimer's disease. The impaired dopamine release in the brain reward system has been hypothesized as a potential trigger of apathy in Alzheimer's disease. Despite this interesting rationale, RCTs investigating the potential cholinomimetic influence on dopamine release effects have not been performed so far (Lanctot *et al.*, 2017). It is generally believed that cholinesterase inhibitors are a part of the standard of care for management of Alzheimer's disease, and the foundation of Alzheimer's disease pharmacotherapy (Hort *et al.*, 2010; O'Brien *et al.*, 2011; Segal-Gidan *et al.*, 2011; Moore *et al.*, 2014). In mild–moderate Alzheimer's disease, the expected treatment benefit of cholinesterase inhibitors is a mean of 3 to 4 points on the cognitive subscale of the ADAS-Cog (Alzheimer's Disease Assessment Scale), when placebo treatment is the reference standard. This score difference corresponds roughly to the expected cognitive decline in people with mild–moderate Alzheimer's disease over 6 months if the disease is left untreated at these stages (Hort *et al.*, 2010).

Additional data from both laboratory-based investigations and clinical trials have suggested that cholinesterase inhibitors may have a broader mechanism of action than enhancing cholinergic activity and that these drugs are associated with a stabilizing effect on the course of Alzheimer's disease dementia that may be greater than expected by cholinesterase inhibition alone (Giacobini, 1997,

2002). Prospective long-term observational studies suggest that cholinesterase inhibitors offer a benefit over the long-term course of Alzheimer's disease (Atri *et al.*, 2008). Cognitive decline has been observed to occur significantly more slowly with cholinesterase inhibitors compared with no treatment, suggesting a delay relative to typical clinical course (Giacobini, 2001). These observations are supported by other long-term data showing declines in cognitive and global functioning were slower with the persistent use of donepezil over a mean follow-up period of 3 years (Wallin *et al.*, 2007). At least two other prospective observational Alzheimer's disease studies offer similar results, demonstrating slower cognitive and functional deterioration with the persistent and continued use of cholinesterase inhibitors (Rountree *et al.*, 2009; Gillette-Guyonnet *et al.*, 2011).

Suboptimal use of cholinesterase inhibitors is common

Despite clinical data and guideline recommendations supporting the use of cholinesterase inhibitors throughout all stages of Alzheimer's disease, these drugs are often inappropriately regarded as ineffective in Alzheimer's disease and therefore are underused. According to a US survey of 25 561 outpatient visits for Alzheimer's disease specifically or dementia more generally, fewer than half (46%) of patients were prescribed cholinesterase inhibitors, with donepezil being the most frequently prescribed (68%) (Maneno *et al.*, 2006). Of note, psychiatrists and neurologists were significantly more likely to prescribe cholinesterase inhibitors than were other physicians (odds ratios 5.5 and 2.6, respectively). In a Canadian survey of 803 physicians, doctors reported that they would be more likely to prescribe a cholinesterase inhibitor if it enabled a person with mild Alzheimer's disease to remain clinically stable for 15 months and a person with moderate Alzheimer's disease to remain clinically stable for 11 months (Oremus *et al.*, 2007). Survey responses also suggested that a cholinesterase inhibitor prescription was more likely if a physician had less stringent requirements for clinical efficacy. In another survey, 40 US primary care physicians held mostly ambivalent (51%) or negative (31%) views about cholinesterase inhibitor treatment for dementia (Franz *et al.*, 2007). Potential barriers to the use of cholinesterase inhibitors were physicians' lack of knowledge and experience with cholinesterase inhibitor treatment, although these primary care clinicians often yielded to pressure from family members to prescribe it.

Overall treatment persistence with cholinesterase inhibitors is suboptimal. In a large Medicare beneficiary study of more than 3000 patients with Alzheimer's disease treated between 2001 and 2003, treatment persistence at 1 year among patients with Alzheimer's disease who initially received cholinesterase inhibitors ranged from 64% to 68% (Mucha *et al.*, 2008). Persistence of cholinesterase inhibitors therapy was even lower in a large Irish study, drawing on data from 2006 to 2010 (Brewer *et al.*, 2013). Among elderly patients with Alzheimer's disease who received cognition-enhancing drugs, rates of non-persistence (a prescription gap exceeding 63 days) were 30% at 6 months and 44% at 12 months; although rates of impersistence were lower in the more recent cohort and in patients taking multiple anti-dementia medications. European and Australian studies suggest that the reasons for not prescribing cholinesterase inhibitors and the impersistence of Alzheimer's disease therapy are complex and highly variable across clinical settings (Pariente *et al.*, 2008; Robinson *et al.*, 2009; Hollingworth and Byrne, 2011; van den Bussche *et al.*, 2011; Tifratene *et al.*, 2012; Ray and Prettyman, 2013; Zilkens *et al.*, 2014).

Despite physician ambivalence about the efficacy of cholinesterase inhibitors in Alzheimer's disease and their inconsistent and limited use, data support the prescription of cholinesterase inhibitors throughout all stages of the disease. In an analysis of four placebo-controlled studies of people with severe Alzheimer's disease, statistically significant cognitive improvement, and in some cases improvement in global functioning, was observed at 24 or 26 weeks with donepezil treatment at a dosage of 10 mg daily (Deardorff and Grossberg, 2016). In a pooled analysis of these trials, relative improvement was observed across all levels of cognitive score, including patients with the most severe cognitive impairment (Cummings *et al.*, 2010). In an expansive compendium of cholinesterase inhibitor trials in patients with more advanced Alzheimer's disease, including patients in a nursing home setting, less decline in daily and global function was consistently documented with donepezil or rivastigmine treatment, although clinical evidence supporting rivastigmine use was less extensive (Kerwin and Claus, 2011). Although choline acetyltransferase activity in the neocortex, as a marker of cholinergic function, keeps declining, some residual choline acetyltransferase activity can be detected in advanced dementia (Bierer *et al.*, 1995; Davis *et al.*, 1999). This suggests that residual cholinergic input may be present in severe Alzheimer's disease and thus provides a biological target for cholinesterase inhibitor therapy in this late stage. Other studies, however, have shown near total destruction of cholinergic axons in the cerebral cortex of patients with advanced Alzheimer's disease, suggesting that the effect of cholinesterase inhibitors at these stages may be mediated through spared cholinergic pathways of the thalamus and basal ganglia rather than cerebral cortex and limbic regions (Mesulam, 2013).

Dosing cholinesterase inhibitors to achieve maximum benefits

Incremental increases in cholinesterase inhibitor dosages have shown further benefit in Alzheimer's disease, specifically in more advanced disease. In a phase 3 24-week study of patients with moderate–severe Alzheimer's disease who were taking a stable dose of 10 mg donepezil per day, a

dosage increase to 23 mg per day was associated with statistically significant increases in cognitive scores (Farlow *et al.*, 2010). A *post hoc* analysis of individuals with more severe cognitive dysfunction in this study revealed significantly improved cognitive and global function scores for individuals who received the higher dosage (Sabbagh and Cummings, 2011). In both assessments, the magnitude of score change was considered clinically meaningful. Although treatment-emergent adverse events—nausea (6.1% versus 1.9%), vomiting (5.0% versus 0.8%), and diarrhoea (3.2% versus 1.5%)—were higher with the increased cholinesterase inhibitors dosage, these adverse events were reportedly infrequent after 1 month of therapy. Similar clinical data support the use of high-dose rivastigmine in severe Alzheimer's disease for improvements in cognitive function and activities of daily living at 16 and 24 weeks (Farlow *et al.*, 2013). When considering the value of pharmacological management of Alzheimer's disease, it is essential to consider the natural progression of untreated disease (Geldmacher *et al.*, 2006). The initial stabilization of—or even improvement in—cognition and daily functioning with the use of currently approved anti-dementia drugs cannot be sustained indefinitely. Yet, with consistent treatment, a less precipitous decline can be expected over the long term, relative to the known, progressive manifestations of untreated disease.

It is also interesting to highlight that acetylcholine is one of the core neuromodulators involved in the regulation of the sleep-wake cycle, the preservation of which has been reported to be essential for many cognitive functions related to memory processes (Aston-Jones *et al.*, 2001; Power, 2004). There is a circadian rhythm in central cholinergic transmission with a shift to low levels of acetylcholine release during slow-wave sleep compared with wakefulness. In addition, circadian fluctuations have been reported for cholinergic enzyme activity and cholinergic receptor regulation, raising the possibility that therapeutic strategies may need to consider the diurnal timing of administration and the half-life of the agent. Age-related alterations of this circadian rhythm occur in Alzheimer's disease in tandem with the progression of clinical features (Mitsushima *et al.*, 1996). Whether cholinesterase inhibitors influence these altered circadian rhythms in Alzheimer's disease remains to be determined.

How early to treat with cholinesterase inhibitors?

In patients with early-stage Alzheimer's disease specifically, an initial lapse in cholinesterase inhibitors therapy may lead to the irretrievable loss of potential treatment benefits. For example, in placebo-controlled studies of rivastigmine, an initial 26-week phase was followed by a 26-week open-label extension in which all patients received rivastigmine (Farlow *et al.*, 2000; Doraiswamy *et al.*, 2002). For the first 26 weeks, rivastigmine provided statistically significant symptomatic benefits to patients with mild–moderate Alzheimer's disease or more severe disease compared with patients on placebo. However, when patients initially treated with placebo received rivastigmine for the second 26 weeks, they failed to 'catch up' to individuals who received the drug for the full year. In a similar trial of galantamine in people with mild–moderate Alzheimer's disease, patients originally assigned to placebo for the first phase of the trial did not attain a similar level of cognitive benefit at the end of the open-label phase of the study as did patients originally taking galantamine (Raskind *et al.*, 2000).

Clinical data to support the use of cholinesterase inhibitors earlier in the trajectory, specifically in patients with MCI who are at risk for Alzheimer's disease, are mixed (Russ and Morling, 2012; Petersen *et al.*, 2018; Richter *et al.*, 2018). Donepezil, at a dosage of 10 mg daily, showed either marginal or no cognitive benefits, relative to placebo, in two well-controlled studies (Salloway *et al.*, 2004; Doody *et al.*, 2009). Similar disappointing results were reported with rivastigmine and galantamine (Feldman *et al.*, 2007; Winblad *et al.*, 2008). Investigators cautioned, however, that cognitive changes during this prodromal phase of Alzheimer's disease are subtler and therefore harder to measure (Doody *et al.*, 2009). In a 3-year study, donepezil appeared to reduce the risk for conversion of MCI to Alzheimer's disease, but only during the first year of treatment (Petersen *et al.*, 2005). Nevertheless, individuals at higher genetic risk for Alzheimer's disease (with $\geqslant 1$ *APOE* ε4 alleles) experienced greater benefit with donepezil treatment for the entire duration of the study. A recent practice guideline update could find no Level A evidence that cholinesterase inhibitors offer symptomatic improvement at the MCI stage (Petersen *et al.*, 2018). Some of these negative results may be attributed to the heterogeneity of MCI. In the future, when MCI trials are based exclusively on patients with biomarker evidence of Alzheimer's disease pathology, results may become more encouraging.

Cholinesterase inhibitors may also provide pathological and anatomical benefits, particularly before the emergence of clinical symptoms of Alzheimer's disease. As noted earlier, the effects of donepezil (10 mg/day) on brain structure were recently demonstrated in a placebo-controlled longitudinal study of community-based adults with prodromal Alzheimer's disease (Dubois *et al.*, 2015; Cavedo *et al.*, 2016, 2017). Over the course of 4 years, patients who received donepezil demonstrated a statistically significant lessening in the annual rate of hippocampal atrophy on MRI. During the first year of treatment specifically, the rate of hippocampal atrophy was reduced by 45% in donepezil-treated subjects in comparison with untreated patients with Alzheimer's disease (Dubois *et al.*, 2015).

In the future, indications for cholinomimetic therapies, including cholinesterase inhibitors, may become limited to

patients with biomarker confirmation. This more rational approach may show that cholinergic therapies are even more effective than heretofore suspected when applied to a more homogeneous patient population with cholinergic dysfunction as a known component of dementia pathology. Novel technologies such as quantitative magneto- and electro-encephalogram may also allow the detection of subtle neurophysiological alterations induced by cholinesterase inhibitors and other cholinergic drugs that may not be detected at the clinical level. Thus, besides 'classical' clinical outcomes, even electrophysiological outcome measures could be introduced into clinical trials, hopefully helping to identify more effective novel therapies.

Integrating complex disease-related processes: future paradigms and implications

The neuropathological and clinical data summarized above make it very likely that cholinesterase inhibitors or other cholinomimetic interventions will remain essential components of therapy for Alzheimer's disease. The demonstration of early involvement (Schmitz *et al.*, 2016; Richter *et al.*, 2018) of the cholinergic system starting at preclinical stages of the disease, suggests that cholinomimetics, along with anti-amyloid and anti-tau interventions, may each have a distinct role in disease prevention. Future research and clinical paradigms related to Alzheimer's disease may rely more heavily upon the 'systems biology' approach to the disease, stressing the interaction of factors such as genetic predisposition, oxidative stress, mitochondrial dysfunction, inflammatory mechanisms, vascular insufficiency, the accumulation of amyloid-β, neurofibrillary degeneration, cholinergic deficits, and other neurotransmitter abnormalities. A systems biology approach explicitly recognizes the multifactorial, dynamic nature of diseases like Alzheimer's disease and helps clinicians customize therapeutic regimens that are targeted at multiple levels of pathology over the course of the disease.

At its most basic level, the pharmacological management of Alzheimer's disease will likely incorporate tailored combination therapies—by using, for example, currently available and novel cognition-enhancing treatments [e.g. cholinesterase inhibitors, NMDA (*N*-methyl-D-aspartate) receptor antagonists, and other therapies in development] with medications that are potentially disease-modifying (e.g. anti-amyloid-β or anti-tau therapies). As our understanding of Alzheimer's disease pathophysiology expands and we identify additional clinically useful risk factors and biomarkers, the therapeutic approach to Alzheimer's disease will likely parallel the way in which physicians currently manage other complex, variable, and highly idiosyncratic diseases.

An extension of tailored therapy for complex diseases lies at the core of precision medicine, which should guide future strategies for preventing or treating Alzheimer's disease. The ultimate goal of precision medicine is to be able to administer a personalized therapy that specifically targets an individual's known disease risks and disease process at the molecular level (Reitz, 2016). Given the complexity and heterogeneity of Alzheimer's disease pathophysiology, precision medicine may involve the determination of genetic risk profiles, the use of brain imaging, and the detection of biomarkers in plasma or CSF to fashion a specific preventive or therapeutic regimen for a particular individual at risk for or with Alzheimer's disease. To this end, ongoing trials, such as DIAN (Dominantly Inherited Alzheimer Network trial), the Alzheimer's Prevention Initiative, and the A4 (Anti-Amyloid Treatment in Asymptomatic Alzheimer Disease) trial, are studying people at high risk for Alzheimer's disease and tracking biomarkers to identify individuals who might be most responsive to specific, targeted, disease-modifying interventions (Reiman *et al.*, 2011; Mills *et al.*, 2013; Sperling *et al.*, 2014). In the meantime, extensive clinical investigations into cholinesterase inhibitors have already been conducted in broad and largely heterogeneous populations, with success seen across multiple patient 'types' defined by severity and other important characteristics. These developments consolidate the role of cholinomimetic agents as essential elements of the combined pharmacologic treatments for Alzheimer's disease that will be developed in the future.

Summary

The cholinergic system is important for neuronal function in memory, learning, and other essential aspects of cognition and plays a wider role in the promotion of neuronal plasticity. Multidisciplinary investigations are revealing how dysfunction in cholinergic networks arising from the basal forebrain, interact with other important pathophysiologic aspects of Alzheimer's disease—including amyloid-β plaques, neurofibrillary tangles, inflammation, oxidative stress, and vascular insufficiency to undermine cognition. A wealth of clinical literature supports the benefit of promoting cholinergic activity in Alzheimer's disease through the use of cholinesterase inhibitors. Moreover, new data based on MRI are showing evidence of hippocampal protection and, perhaps, disease course alterations in individuals who receive cholinesterase inhibitors for long periods of time. Interest remains high in understanding the temporal sequence and cascade of these complex interactions and their synergistic feedback mechanisms over the course of Alzheimer's disease. It is anticipated that optimal Alzheimer's disease management will integrate a systems biology approach based on precision medicine to help tailor combinatorial therapeutic regimens for different stages of Alzheimer's disease on the basis of genetic risks, brain imaging, and biomarkers. As we anticipate major developments in the treatment strategies of Alzheimer's disease, cholinergic interventions are likely to maintain their critical roles in the therapeutic armamentarium.

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Conflicts of interest

Axovant supported the travel and lodging expenses of the authors for two meetings of the CWG in New York City. The authors were also paid as consultants at customary rates for the time spent at the two meetings of the CWG; there were no other honoraria provided to the authors. A science writer, paid by Axovant help to compile the contributions of the authors into a coherent document. However, the various sections of the paper were prepared exclusively by the authors who were not paid in any way or the time spent in writing-editing the manuscript. All proceedings of the CWG were independent from Axovant. The support for the meetings was accepted by the CWG with the stipulation that Axovant would have no input to the deliberations of the Workgroup and/or influence in anyway the final conclusions/recommendations of the WG. Thus, no member of the company participated in development, discussion or drafting of this manuscript. Axovant has had under development a compound RVT-101 (aka Intepirdine) an antagonist of the serotonin receptor 6 (5-HT6) (which was found to lack efficacy for the treatment of Alzheimer's disease), as well as RVT-104 (combination of glycopyrrolate and high-dose rivastigmine) a compound that targets the cholinergic mechanism

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Supplementary material

Supplementary material is available at *Brain* online.

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