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Cholinergic muscarinic M_1 and M_4 receptors as therapeutic targets for cognitive, behavioural, and psychological symptoms in psychiatric and neurological disorders

Daniel Erskine[1], John-Paul Taylor[1], Geor Bakker[2], Alastair J.H. Brown[2], Tim Tasker[2] and Pradeep J. Nathan[2,3,4]

[1] Institute of Neuroscience, Newcastle University, Newcastle, UK
[2] Sosei Heptares, Cambridge, UK
[3] Department of Psychiatry, University of Cambridge, Cambridge, UK
[4] School of Psychological Sciences, Monash University, Melbourne, VIC, Australia

Cholinergic dysfunction is involved in a range of neurological and psychiatric disorders, including schizophrenia, dementia and Lewy body disease (LBD), leading to widespread use of cholinergic therapies. However, such drugs have focused on increasing the availability of acetylcholine (ACh) generally, with relatively little work done on the muscarinic system and specific muscarinic receptor subtypes. In this review, we provide an overview of the major cholinergic pathways and cholinergic muscarinic receptors in the human brain and evidence for their dysfunction in several neurological and psychiatric disorders. We discuss how the selectivity of cholinergic system dysfunction suggests that targeted cholinergic therapeutics to the muscarinic receptor subtypes will be vital in treating several disorders associated with cognitive dysfunction and behavioural and psychological symptoms.

Introduction and general overview of topic

ACh was initially described by Henry Dale and Otto Loewi, making it among the first described neurotransmitters, and is known to have a crucial role in both the central and peripheral nervous systems, with its wide distribution meaning almost all areas of the brain are innervated by cholinergic efferents (Fig. 1). Given such widespread distribution, it is perhaps unsurprising that the cholinergic system has vital roles in cognitive and behavioural/psychological function, many of which are impaired in neurological and psychiatric disorders, meaning that the cholinergic system has been the focus of sustained interest in the development of novel therapeutics for such disorders. ACh is produced in the cytoplasm of cholinergic neurons, where the enzyme choline acetyltransferase (ChAT) synthesises ACh from choline and acetyl-coenzyme A (acetyl-CoA) before transport by the vesicular ACh transporter (VAChT) into synaptic vesicles. Upon depolarisation of a cholinergic neuron, ACh is released from synaptic vesicles, where it can activate both nicotinic and muscarinic subtypes, and is inactivated by the enzyme acetylcholinesterase, which is secreted by cholinergic neurons into the synaptic cleft.

Anatomy of the cholinergic system

The major sources of cholinergic projections were initially named Ch1–Ch8 by Mesulam from studies in rats. These refer to: the medial septal nucleus (Ch1), the vertical limb of the diagonal band of Broca (Ch2), the horizontal limb of the diagonal band of Broca (Ch3), the nucleus basalis of Meynert/(NBM) (Ch4), the pedunculopontine nucleus (PPN) (Ch5), the laterodorsal tegmental nucleus (Ch6), the medial habenula (Ch7), and parabigeminal nucleus (Ch8) [1]. Furthermore, cholinergic cell bodies are also found in the striatum and some thalamic nuclei, although these are localised to these systems.

Ch1–Ch4 nuclei are found within the basal forebrain and are implicated in attention, learning, and memory [2] as well as behavioural symptoms, including psychosis [3]. Most cortical cholinergic input to the neocortex originates from the Ch4/NBM [4]. Cholinergic cells of the medial septal (Ch2) and diagonal

Corresponding authors: Erskine, D. (Daniel.erskine@ncl.ac.uk), Nathan, P.J. (Pradeep.nathan@soseiheptares.com)

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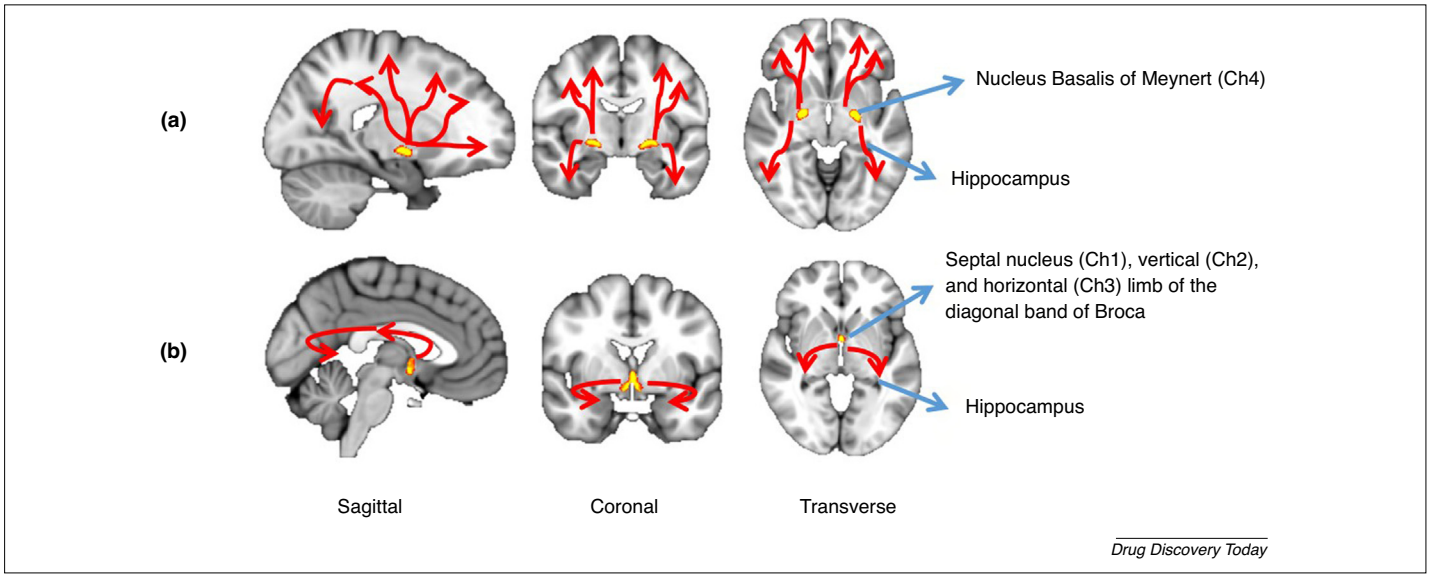

FIGURE 1

Overview of brain cholinergic circuitry. **(a)** Nucleus basalis of Meynert (CH4) cortical circuitry. Most of the cholinergic innervation (indicated by red arrows) to the cortex originates from the cholinergic neurons in the nucleus basalis of Meynert (CH4), with widespread projections throughout the cortex. **(b)** Septal-hippocampal circuitry. Cholinergic neurons originating in CH1 (septal nucleus) and CH2 (vertical limb of the diagonal band of Broca) project to the hippocampus and hypothalamus and have important roles in learning and memory.

band nuclei (Ch3) predominantly project to hippocampus and entorhinal cortex through the septohippocampal pathway, which is thought to modulate hippocampal memory processes [5] (Fig. 1).

The PPN is situated between the midbrain and pons and projects to diverse targets, including the striatum, globus pallidus, substantia nigra, ventral tegmentum, various thalamic nuclei, hypothalamus, NBM, superior and inferior colliculi, medulla, and various parts of the brainstem reticular formation [6]. The caudal pedunculopontine is predominantly cholinergic and appears to be involved in modulating attentional and awareness states, and probably contributes to gait and balance through nigral and brainstem locomotor regions [6].

The medial habenula is situated on the superior aspect of the posterior thalamus and, despite being understudied, is thought to be involved in adaption to new environments, impulsive behaviour, decision-making, spatial memory, and learning flexibility [7].

Cholinergic receptor subtypes

Cholinergic receptors comprise two broad classes; ionotropic nicotinic receptors and metabotropic muscarinic receptors. Here, we focus on the muscarinic receptors.

Muscarinic receptors

Muscarinic receptors are a group of Class I G-protein-coupled receptors (GPCRs) comprising five distinct subtypes, termed M_1, M_2, M_3, M_4, and M_5 [8]. The M_1, M_3, and M_5 receptor subtypes are excitatory and couple to Gq/G11, leading to activation of phospholipase C and inositol phosphates, whereas the M_2 and M_4 receptor subtypes are inhibitory and are coupled to Gi/Go, resulting in an inhibition of adenylyl cyclase and reduction in cAMP. Muscarinic receptors are expressed across cortical, limbic, and midbrain structures, typically on neurons rather than glia. The M_1 receptor is the most abundant muscarinic subtype in the neocortex, comprising 35–60% of all muscarinic receptors, with M_4 comprising only 20% [9]. All subtypes are present in the cortex, with M_1 found on almost all pyramidal neuron cell bodies in cortical layers 2/3 and 6 [10]; M_2 found presynaptically on nonpyramidal neurons in layer 4; M_3 found postsynaptically on neurons in layers 2/3; and M_4 found presynaptically as autoreceptors in glutamatergic synapses on neurons; M_5 is thought to have lower cortical expression [9].

In the hippocampus, a region thought to have a crucial role in the pathogenesis of many psychiatric disorders, the M_1 receptor is typically present on postsynapses of pyramidal neurons in the deep layer of the dentate gyrus and the pyramidal cell layer of CA3, CA1, and throughout Ammon's horn in the hippocampus, whereas the M_2 and M_4 receptors are largely located presynaptically, modulating glutamate release in Schaffer collaterals in CA1 and transmitter release at septal cholinergic and GABAergic terminals [9,11,12]. In the striatum, the M_4 receptor is particularly enriched presynaptically on autoreceptors present on medium spiny neurons. In the midbrain and brainstem, M_2 receptors are enriched on the presynapses of the colliculi, pedunculopontine, and laterodorsal tegmental nuclei; and the M_4 subtype is enriched in presynapses within the substantia nigra [13].

Across the five muscarinic receptor subtypes, the orthosteric ACh-binding pocket is highly conserved, making it difficult to develop subtype-selective agonists [14]. However, structure-based drug discovery has enabled the development of orthosteric agonists that are highly selective for muscarinic receptor subtypes, including the M_1 and M_4 subtypes [15], which could facilitate further research probing the roles of selective M_1 and M_4 receptor modulation on cognition and behaviour, with application to several neurological and psychiatric disorders.

Evidence for cholinergic dysfunction in disease

Cholinergic system and muscarinic receptor dysfunction in schizophrenia

The pathophysiology of schizophrenia has been linked to dysregulation in the glutamatergic and dopaminergic systems, and these abnormalities have been largely linked to the positive symptoms of the disorder. However, increasing evidence suggests cholinergic dysfunction in schizophrenia, which could, in part, contribute directly or indirectly to symptoms through modulation of dopaminergic or glutamatergic systems in regions such as the cortex, striatum, and hippocampus [16,17].

Early histopathological studies evaluating postmortem brain tissue from patients with schizophrenia reported preserved neuronal populations in the NBM and PPN [18]. Postmortem studies using [3 H]pirenzepine, a radioligand that binds to both M_1 and M_4 receptors, showed region-specific reductions in M_1 and M_4 receptor binding in the prefrontal cortex, anterior cingulate, striatum, superior temporal gyrus, and hippocampus [19–22]. Later studies using receptor-specific antibodies and levels of mRNA expression using *in situ* hybridisation demonstrated that the reductions in muscarinic receptors measured by [3H]pirenzepine binding were specific to the loss of M_1 receptor expression in the prefrontal cortex [22] and M_4 receptor expression in the hippocampus [19]. Furthermore, in a larger sample of postmortem schizophrenia brain tissues, large reductions (60–80%) in $M_{1/4}$ receptor binding as measured by [3H]pirenzepine) in the cortex were found in a subgroup comprising ~25% of patients who were defined as having a muscarinic receptor-deficit schizophrenia (MRDS) [23], but these findings are yet to be confirmed *in vivo*.

Postmortem studies have been supported *in vivo* in humans by a single photon emission computed tomography (SPECT) study using a non-selective M_1/M_4 receptor ligand, [I-123]iodoquinuclidinyl benzilate ([123I]IQNB), which showed a 20–33% reduction in muscarinic receptors in drug-naïve patients with schizophrenia in the cortex and striatum. In addition, muscarinic receptor availability in the frontal cortex and striatum were negatively associated with positive symptoms [24]. A more recent SPECT study using 123I-iododexetimide (123I-IDEX), a $M1/M_4$ selective ligand, also demonstrated a negative association between M_1/M_4 receptor expression in the prefrontal cortex and hippocampus and verbal learning and memory, as well as a negative relationship between M_1/M_4 receptor expression in the prefrontal cortex and negative symptoms in medication-free patients with psychotic disorders [19]. This cohort included both subjects in the first phase of the disorder, commonly termed 'first episode psychosis', and those with a diagnosis of schizophrenia. The association between M_1/M_4 receptor expression and clinical symptoms is further supported by the finding that the M_1/M_4 agonist xanomeline showed improvements in psychiatric symptoms, including positive and negative symptoms, as well as verbal learning and short-term memory, in patients with schizophrenia [25].

Summary

Comprehensive studies of the major cholinergic nuclei in schizophrenia are relatively lacking. Studies of cholinergic receptors suggest some evidence for M_1-specific reductions in the dorsolateral prefrontal cortex and M_4-related changes in the hippocampus, with relative sparing of M_2 and M_3 receptors. However, many of the studies have been limited by patients taking medication that might alter muscarinic receptor function and/or expression. Furthermore, many postmortem studies do not relate changes to clinical data obtained during life, limiting their ability to relate observed changes to clinical outcome. Nevertheless, *in vivo* molecular neuroimaging in medication-free patients with early psychosis or schizophrenia have shown some evidence linking changes in M_1/M_4 receptors and cognitive and positive or negative symptoms severity, providing preliminary *in vivo* support for a disbalance in M_1/M_4 receptor expression in schizophrenia that might directly impact clinical outcome. The preliminary evidence that the M_1/M_4 agonist xanomeline showed positive effects on both psychiatric and cognitive symptoms provide further supports that targeting M_1 and/or M_4 receptors could have therapeutic benefits in patients with schizophrenia and related psychotic disorders.

Cholinergic system dysfunction in AD

Alzheimer's disease (AD) is neuropathologically characterised by extracellular deposits of the amyloid-β peptide and intracellular accumulations of hyperphosphorylated tau protein [26]. Cholinergic neurons of the NBM are among the first neurons outside of the entorhinal cortex in which neurofibrillary tangles are observed and occurs concomitant with cognitive impairment, because of reduced cortical cholinergic activity [27]. Such is the severity of cholinergic alterations in AD that cholinergic silver stains were initially used at Newcastle General Hospital, UK, during the late 1970s to help hasten the identification of AD cases post mortem (Robert Perry, personal communication, 2019). As a result of the profound changes to the cholinergic system in AD, drugs that ameliorate cholinergic dysfunction have been frontline symptomatic treatments for the past three decades.

The basal forebrain nuclei are subject to neuronal loss and atrophy in AD, which correlates with the degree of cognitive impairment [28]. Loss of structural integrity of the basal forebrain nuclei is already present before clinical presentation of symptoms and precedes the spread of atrophy to the entorhinal cortex seen in AD [29]. The pedunculopontine, despite being less intensively studied, has also been reported to manifest neuronal loss and neurofibrillary tangle pathology [30]. The cholinergic enzymes choline acetyltransferase and acetylcholinesterase are reduced in the amygdala, hippocampus, and cortical regions with high burdens of neurofibrillary tangles in AD [31].

Muscarinic receptor changes in AD

Studies of muscarinic receptors initially used radio-labelled ligands to evaluate receptors before the development of modern approaches determining mRNA levels with PCR and antibody-based protein expression. Initial autoradiographical studies reported no significant changes to muscarinic receptor labelling in the hippocampus, frontal, temporal, or cingulate cortices [32]. However, studies evaluating particular subtypes of muscarinic receptors identified subtype-specific changes that appear to be region dependent. Such studies have frequently used autoradiographical methods on postmortem brain tissue, but were initially limited by the inability to distinguish between specific subtypes, instead using radioligands that bound several subtypes but not others.

M_1 receptors have arguably been the best characterised in AD. This subject has been the subject of a previous in-depth review [33] and prominent reductions have been reported in phenotypically

important regions, such as the CA1 sector of the hippocampus [34], whereas it is preserved in regions with less obvious associations to the AD clinical phenotype, such as the corpus striatum [35]. However, changes in receptor levels might simply indicate cell loss rather than selective loss and attention is increasingly focusing on M_1 receptor dysfunction rather than on alterations to absolute levels. The observation that increased levels of M_1 receptors are not accompanied by increased activity but rather with decreased ability to form high-affinity agonist states [36] suggests that there is a large pool of inactive or underactive M_1 receptors in AD, with important implications for therapeutic targeting.

Postmortem studies have been useful for identifying changes to the muscarinic system in end-stage cases, but give little information on changes over the disease course. *In vivo* SPECT imaging with [123I]IQNB in patients with moderate AD demonstrated reductions in M_1/M_4 receptors in bilateral frontal rectal gyrus, right parahippocampal gyrus, left hippocampus, left fusiform gyrus, and left superior temporal gyrus [37].

Fewer studies have specifically evaluated other muscarinic receptors (i.e., non-M_1 receptors) in AD. However, a postmortem autoradiography study hippocampal brain sections with the M_4-selective radioligand [(125)I]muscarinic toxin 4 demonstrated decreased binding in the dentate gyrus and CA4 region in patients with severe AD, in contrast to no changes in M_1 labelled by [(125)I] muscarinic toxin-1 and M_2 labelled nonselectively with [(3)H] AFDX-384 [38]. Consistent with the latter study, a recent *in vivo* PET imaging study using a new M_4 selective ligand, [^{11}C]MK-6884, showed 20–50% reductions in M_4 receptors in the frontal and temporal cortex in patients with moderate– severe AD [39]. Postmortem studies reported that M_2 receptors labelled with [3H]oxo-tremorine were decreased in hippocampus, but markedly increased in the striatum [40]. M_3 receptors have been indirectly studied in AD using the general muscarinic radioligand N-methyl-[3H]-scopolamine, followed by measuring displacement with the M_3 antagonist 1, 1-dimethyl-4-diphenylacetoxypiperidium iodide [40]. Using this approach, M_3 receptors were observed to be decreased in the hippocampus and visual cortex but increased in frontal cortex.

Summary

There is compelling evidence for cholinergic system degeneration and dysfunction in AD. Neuropathological studies have demonstrated profound degenerative changes to the basal forebrain nuclei during early prodromal stages of the disease. The cholinergic motor areas of the PPN and striatum exhibit less severe neurodegenerative changes, consistent with the AD phenotype of cognitive rather than motor decline. At the level of specific receptor subtypes, there is considerable evidence that distinct muscarinic subtypes have different brain circuit-specific susceptibilities to degenerative changes in AD, with both M_1 and M_4 receptors showing reductions in moderate–severe stages of AD. Muscarinic receptors might be preserved during early prodromal and mild stages of the disease, suggesting them as better targets for drug development for AD.

Cholinergic system changes in LBD

LBD is a collective term for Parkinson's disease (PD), Parkinson's disease dementia (PDD), and dementia with Lewy bodies (DLB), because all disorders are neuropathologically characterised by the aggregation of the protein α-synuclein into intracellular deposits termed 'Lewy bodies'. Initial studies of the cholinergic system in PD and/or PDD preceded the formal identification of DLB as a distinct disorder and were motivated, at least in part, by the observation that patients with PD often develop cognitive impairment. Furthermore, Lewy bodies were first identified by Friedreich Lewy in the NBM, a cholinergic region linked to AD cognitive impairment. Subsequent studies identified that choline acetyltransferase is present within Lewy bodies in cholinergic neurons, perhaps indicating that cholinergic functioning is impaired in surviving Lewy body-bearing cholinergic neurons because of its sequestration into protein aggregates [41].

The basal forebrain nuclei, including the NBM, medial septum, and diagonal band of Broca, are vulnerable to α-synuclein pathology in PD, PDD, and DLB. Neuronal loss has also been reported in the NBM, prominently affecting the large cholinergic neurons of the structure, in PD, PDD, and DLB [42]. Structural neuroimaging confirmed neuropathological studies during life, demonstrating a reduced volume in the basal forebrain in PDD, DLB, and AD [43]. However, of DLB and AD, only DLB basal forebrain loss was significantly related to memory impairment and severity of cognitive fluctuations [43]. The PPN is also subject to degeneration of cholinergic neurons in PD and/or PDD to a greater extent than DLB [44], and might contribute to behavioural and psychological symptoms in LBD through PPN cholinergic projections to the striatum and modulation of dopaminergic burst spike firing [45]. No studies have yet studied the habenula or parabigeminal nucleus in LBD.

Muscarinic receptor changes in LBD

Most studies evaluating receptor-specific changes in LBD have used autoradiographical analysis of autoptic brain tissue. Given the relatively later identification of DLB compared with PD, most studies have focussed on cholinergic receptor abnormalities in PD and/or PDD. Studies of cholinergic receptor expression in PD have largely focussed on the striatum because it receives input from the substantia nigra, a region prone to neurodegeneration of dopaminergic neurons and a major contributor to parkinsonian motor features.

Muscarinic receptors are arguably of greater interest in evaluating neurochemical substrates of behavioural and psychological symptoms in LBD, because there are phenotypic similarities to those observed following administration of antimuscarinic drugs, such as visual hallucinations and delirium. Specific changes in muscarinic receptors have been reported in PD, with reduced frontal AF-DX-384 binding, labelling M_1, M_2, and M_4 receptors, and decreased striatal and frontal cyclohexyl-(4-fluorophenyl)-hydroxy-(3-piperidin-1-ylpropyl)silane binding, which selectively recognises the M_3 receptor [46]. In DLB, fewer studies have evaluated the striatum, although one study reported loss of M_1 binding on [3H]pirenzepine autoradiography that exceeds that observed in PD [35].

In the neocortex in PD, total muscarinic binding on autoradiography with [3H]quinuclidinyl benzilate was increased in frontal cortex, with specific increases in [3H]pirenzepine indicating increased numbers of M_1 receptors [47,48]. Increased temporal [3H] pirenzepine binding has been associated with delusions, and combined increases in AF-DX 384 cortical binding have been associated with visual hallucinations, suggesting a role for M_1

receptors in delusions and M_2/M_4 receptors in visual hallucinations in DLB [49,50]. Postmortem studies are relatively consistent with *in vivo* molecular imaging, reporting reduced muscarinic binding as determined by 123I-iodo-quinuclidinyl-benzilate in frontal, temporal, and occipital binding in PDD, with only occipital reductions observed in DLB [51].

Summary

LBD is associated with significant neurodegeneration and adaptive changes in the cholinergic system. There is considerable evidence that the major cholinergic nuclei are subject to neurodegeneration in PD, PDD, and DLB, and that there are corresponding adaptive changes to cholinergic muscarinic receptors throughout the brain. At the level of individual receptor expression, there is compelling evidence to indicate that there is not a global reduction or increase in particular cholinergic receptors, but rather subtype-specific changes that vary across brain regions. Furthermore, there is evidence that cholinergic muscarinic receptor changes are associated with particular behavioural symptoms, such as hallucinations, delusions, and altered attention, which might be related, in part, to cholinergic modulation of dopaminergic pathways in the striatum.

Therapeutic strategies targeting muscarinic M_1 and/or M_4 receptor subtypes for cognitive, behavioural, and psychological symptoms

The cognitive, behavioural, and psychological symptoms of schizophrenia, AD, and DLB are difficult to treat, yet have a profound impact on patient wellbeing. Although acetylcholinesterase inhibitors (AChEIs) have demonstrated some efficacy in treating negative-symptom schizophrenia, AD, and DLB, they are often associated with dose-limiting adverse effects, as are dopaminergic agents for treating psychosis in schizophrenia. Such adverse effects with cholinergic medications likely arise because of their action of increasing ACh functioning systemically, yet a consistent feature of all diseases discussed is that cholinergic changes do not reflect uniform reductions in cholinergic activity. Importantly, selective changes to particular subclasses of cholinergic receptors have been specifically related to particular symptoms, highlighting the potential for targeted therapeutics to ameliorate particularly troubling symptoms as part of a personalised medicine package.

Despite the relatively small cognitive benefit of AChEI use, which might not translate to clinically meaningful functional improvement, clinically significant adverse effects can occur, in part because of the ubiquity of cholinergic activity across the central and peripheral nervous system [52]. Allosteric modulators hold particular promise for the treatment of neuropsychiatric disorders because they enable targeting of particular muscarinic receptor subtypes known to be perturbed in specific disorders, enabling symptom attenuation without the risks and adverse effects associated with globally increasing ACh activity. Recent structure-based drug discovery platforms have also enabled the development of orthosteric agonists that are highly selective for muscarinic receptor subtypes, with good safety and tolerability profiles. However, the relative efficacy and safety of orthosteric versus allosteric agonists are yet to be fully characterised in patients with AD, PD, PDD, DLB, or schizophrenia. It is likely that the efficacy and safety profiles will vary depending on the extent of cholinergic neurodegeneration and autonomic function status.

Targeting M_1 and/or M_4 muscarinic receptors in schizophrenia

M_1 and M_4 receptors are promising targets for cognitive dysfunction and neuropsychiatric symptoms in schizophrenia. Preclinical studies have shown that selective M_1 agonists have procognitive effects [53,54] In support, a clinical study in humans showed that the selective M_1 bitopic agonist GSK1034702 improved episodic memory (i.e., verbal learning) in a nicotine abstinence cognitive impairment model (Fig. 2) [55]. A clinical study in patients with schizophrenia with the $M_1/_4$ agonist xanomeline also showed improvements in verbal learning and memory [25]. There has also been increasing interest in M_4 receptors as an antipsychotic target, given the efficacy of xanomeline in improving positive and negative symptom domains in schizophrenia [25].

Although the procognitive effects of M_1 agonists might relate to the modulation of cortical and hippocampal M_1 receptors, the modulation of behavioural and/or neuropsychiatric effects, including psychosis, are hypothesised to be mediated by M_4 receptors in the striatum and hippocampus. M_4 receptors are co-expressed exclusively in a subpopulation of D_1 receptor-expressing medium spiny projection neurons, and modulate striatal dopamine release and dopamine-related behaviours [56]. Furthermore, M_4 receptor agonists, including the M_4 PAM VU0152100, showed efficacy on multiple behavioural tasks that predict antipsychotic-like effects [57–59] and modulated dopamine release [59], suggesting that the antipsychotic-like activity is mediated, in part, through modulation of dopamine function in striatal circuits.

In addition to potential direct effects on the striatum, M_4 modulation might also indirectly modulate dopamine via the hippocampus. Several studies have demonstrated hyperactivity or hyperexcitability in the hippocampus (secondary to GABAergic parvalbumin interneuron loss) in animal models treated prenatally with methylazoxymethanol acetate [57]. Consistent with studies in preclinical models of schizophrenia phonotypes, increased hippocampal perfusion in schizophrenia has been reported using various *in vivo* imaging methodologies, with increases also shown to be associated with psychosis symptoms [58–60]. Hippocampal hyperactivity can increase phasic dopamine release in the striatum [61] and M_4 receptor activation selectively decreases the activity of hippocampal excitatory synapses by inhibiting glutamate release [62,63]. Therefore, it is tempting to speculate that reductions of striatal dopamine release secondary to M_4 receptor agonism could result from inhibition of hippocampal hyperexcitability. Furthermore, M_4 receptors might also modulate glutamate projections to the striatum that regulate striatal dopaminergic release [64].

A subset of patients with schizophrenia (i.e., those with MRDS) defined by marked (60–80%) reduction in cortical M_1 binding measured with [3 H]-pirenzepine showed reduced muscarinic agonist potency to stimulate Gαq/11-[^{35}S]-GTPγS binding, with lower binding observed with the allosteric agonist AC-42 compared with the full agonist oxotremorine-M [65]. A further study in this muscarinic receptor-deficient population indicated that the ability of the allosteric agonist BQCA to modulate the displacement of [^{3}H]n-methylscopolamine ([^{3}H]NMS) by ACh was reduced in MRDS, reflecting a decrease in cortical M_1 in these subjects [66]. Together, these data suggest that, in MRDS, muscarinic M_1 receptors are too few to allow a full pharmacodynamic response to M_1 allosteric modulators. Therefore, although positive allosteric modulators of M_1 receptors hold considerable promise in the

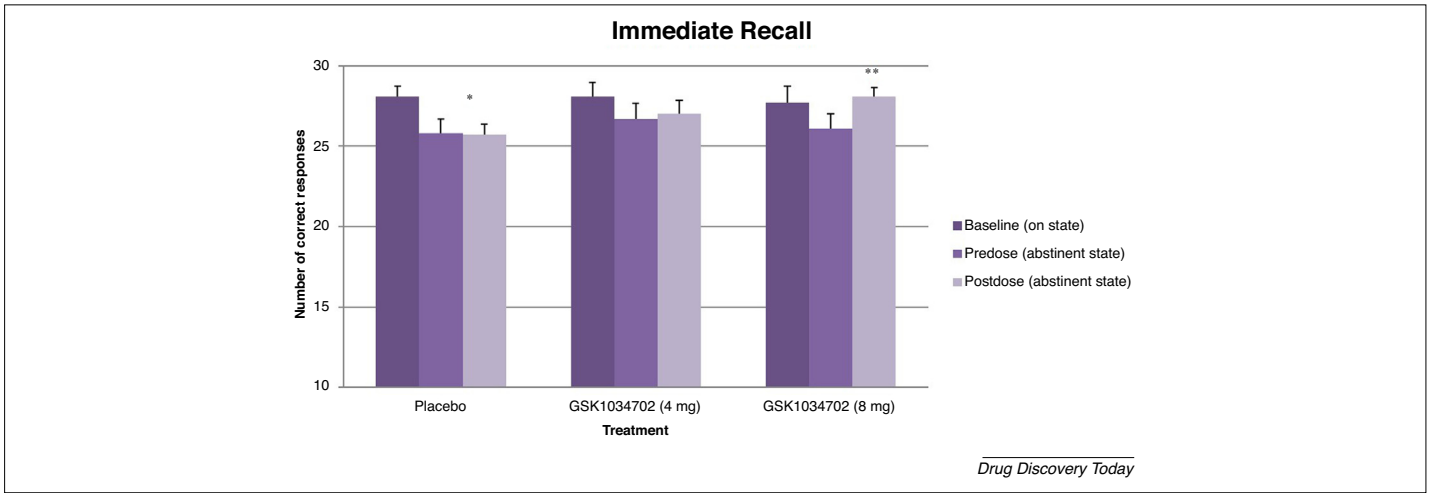

FIGURE 2

International shopping list task. The figure shows impairment in immediate recall after nicotine abstinence (i.e., nicotine abstinence model of cognitive impairment) and reversal of this impairment by GSK1034702 (8 mg). *$P < 0.05$ for difference between nicotine 'on-state' and nicotine 'abstinent state'. **$P < 0.05$ for treatment difference in the nicotine 'abstinent state'. Data are expressed as means ± S.E.M. and are from [55].

therapeutic management of schizophrenia, further understanding of clinical and pathological subgroups and patient stratification will be important in future trials.

Targeting M_1 and/or M_4 receptors in AD

Muscarinic receptors, particularly M_1, have been evaluated at the level of their relationship to symptomatic and potentially disease-modifying potential in AD. Stimulation of muscarinic M_1 and M_3 receptors with AF267B in rodent models of AD has demonstrated improved cognition and reduced burden of amyloid-β pathology by increasing α-secretase activity and reduced tau phosphorylation [67]. M_1 and M_3 receptors are binding partners of tau protein, which constitutes neurofibrillary tangle pathology, and might be permissive to its proposed spread throughout the nervous system in AD [68]. Knockout of M_4 receptors from the hippocampus alters cholinergic activity in the hippocampus and elicits cognitive dysfunction reminiscent of AD, suggesting that reduced M_4 activation is crucial for hippocampal deficits thought to underlie the amnestic features that characterise AD [69]. However, the M_4 receptor might also be important in modulating behavioural symptoms of AD, including agitation, delusions, and hallucinations, because these symptoms reduced following administration of the M_1/M_4 agonist xanomeline [70]. Specific targeting of several individual muscarinic receptors, particularly M_1 and M_4, might have important implications for symptomatic treatment without the dose-limiting adverse effects of global cholinergic agents. Furthermore, such treatments might also have disease-modifying effects when administered during the prodromal stages of AD by attenuating pathological progression by modulating amyloid-β/tau deposition, and disease-modifying effects have already been reported with acetylcholinesterase inhibitors [71].

However, the potential efficacy of any M_1 and/or M_4 agonist in AD might depend whether the drug is an orthosteric or allosteric modulator and the severity of disease. Given that cholinergic neurons degenerate with disease progression in AD, including reductions in ACh synthesis, allosteric modulators might not be the optimal pharmacological strategy because their efficacy is linked to endogenous ACh availability. A recent study in patients with AD treated with the M_1 positive allosteric modulator MK-7622 showed no improvements in cognition or clinical outcome [72]. An alternative and potentially more effective strategy is to target postsynaptic M_1 and M_4 receptors that are relatively preserved in AD with orthosteric agonists or partial agonists. Several such compounds are currently in development [55] (Clinical Trials.gov #NCT02291783 and #NCT03244228).

Targeting M_1 and/or M_4 receptors in LBD

The cholinergic system is a promising potential target in LBD because there is more widespread cholinergic losses in LBD compared with AD, in which cholinergic therapies have been successful. Based on this premise, AChEIs have been demonstrated in randomised placebo-controlled trials to improve neuropsychiatric symptoms, such as visual hallucinations and delusions, while also improving attentional deficits [73]. A recent study also suggested that the basal forebrain cholinergic neuron integrity is a predictor of future psychosis in LBD (i.e., PD) [3], providing further support for targeting the cholinergic system for neuropsychiatric symptoms.

Unlike many other disorders, the neuropsychiatric features of LBD are typically transient in nature, such as visual hallucinations and fluctuating changes in consciousness [73]. Such changes likely reflect altered activity patterns possibly resulting from changes in neural activity resulting from an imbalance between excitatory and inhibitory mechanisms. In experimental models, selective activation of M_1 and M_4 receptors was demonstrated to alter excitatory and inhibitory activity to normalise neural network activity, and networks based on M_1/M_4 receptors showed a response to AChEIs [74]. The importance of such modulation cannot be overstated because restoration of cortical activity in mouse models was demonstrated to not only attenuate abnormal network activity, with presumed amelioration of resulting behavioural deficits, but also to reduce neuropathological lesion severity [75]. Although demonstrated in an AD model, such findings are of arguably more relevance to LBD given the well-described

abnormal cortical activity giving rise to cognitive features, coupled with neural activity patterns governing the exocytosis of α-synuclein necessary for its spread through the brain [76]. Although purely speculative, restoration of normal cortical rhythm might not only attenuate symptoms, but also reduce exocytosis of α-synuclein, which accumulates extracellularly in an activity-dependent manner [76], raising the potential to inhibit its spread. Reduction of amyloid-β and tau pathology through selective muscarinic targeting was discussed earlier in the context of AD; both pathologies are often present in LBD, where they contribute to worse outcomes for patients [73]. Although we have focused here on neuropsychiatric features of neurodegenerative disorders, M_4 agonism might also reduce motor features characteristic of LBD [77]. Therefore, it will be important to evaluate the therapeutic potential of selective M_1 and M_4 agonism with the aim of controlling the abnormal cortical activity thought to underlie some of the most distressing and treatment-resistant aspects of LBD.

Concluding remarks

Here, we have summarised the considerable literature implicating cholinergic dysfunction, particularly of muscarinic receptors, in cognitive and neuropsychiatric features of psychiatric and neurodegenerative disorders. Whereas previous therapies focussed on broadly increasing levels of ACh in neurodegenerative disorders or reducing dopamine in schizophrenia, there is compelling evidence that off-target effects of this approach limit dose and treatment compliance. Therefore, there is a pressing need to develop targeted therapeutics with the aim of specifically modulating activity in cellular populations of particular phenotypic relevance.

There are theoretical reasons to believe that targeting M_1 and/or M_4 receptors would ameliorate cognitive and neuropsychiatric symptoms. Evidence from postmortem studies, increasingly supported by molecular neuroimaging, has demonstrated alterations to both types of receptor subtype that correlate with phenotypic severity in both psychiatric and neurodegenerative disorders. Furthermore, preclinical studies have increasingly demonstrated that M_1/M_4 agonism can attenuate several of the psychotic features of the aforementioned disorders. Allosteric modulators and selective orthosteric partial agonists hold particular promise because of their increased specificity, with important implications for safety and tolerability, given the adverse events associated with off-target activation, particularly in the peripheral nervous system. Several clinical studies are ongoing and are expected to report data in the next 2–3 years.

Conflict of interest

P.J.N., A.B., G.B., and T.T. are employees of Sosei Heptares and involved in the development of muscarinic receptor agonists. P.J.N. is on the editorial board of *Drug Discovery Today* but was not involved in the review of this paper. D.E. is funded by an Alzheimer's Research UK Fellowship, UK (ARUK-RF2018C-005). J.P.T. is supported by National Institute for Health Research (NIHR) Newcastle Biomedical Research Centre based at Newcastle upon Tyne Hospitals NHS Foundation Trust and Newcastle University.

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