



Review

Muscarinic Receptors and Alzheimer's Disease: New Perspectives and Mechanisms

Martina Monaco ¹, Hanna Trebesova ¹ and Massimo Grilli ^{1,2,*}

¹ Department of Pharmacy, University of Genoa, Viale Cembrano 4, 16148 Genoa, Italy; martina.monaco@edu.unige.it (M.M.); hanna.trebesova@unige.it (H.T.)

² Inter-University Center for the Promotion of the 3Rs Principles in Teaching & Research (Centro 3R), 16148 Genoa, Italy

* Correspondence: massimo.grilli@unige.it

Abstract: Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases on a global scale. Historically, this pathology has been linked to cholinergic transmission, and despite the scarcity of effective therapies, numerous alternative processes and targets have been proposed as potential avenues for comprehending this complex illness. Nevertheless, the fundamental pathophysiological mechanisms underpinning AD remain largely enigmatic, with a growing body of evidence advocating for the significance of muscarinic receptors in modulating the brain's capacity to adapt and generate new memories. This review summarizes the current state of the art in the field of muscarinic receptors' involvement in AD. A specific key factor was the relationship between comorbidity and the emergence of new mechanisms.

Keywords: Alzheimer's disease; muscarinic receptors; cholinergic transmission; neuroinflammation; innovative therapy



Citation: Monaco, M.; Trebesova, H.; Grilli, M. Muscarinic Receptors and Alzheimer's Disease: New Perspectives and Mechanisms. *Curr. Issues Mol. Biol.* **2024**, *46*, 6820–6835. <https://doi.org/10.3390/cimb46070407>

Academic Editor: Rafael Franco

Received: 4 June 2024

Revised: 28 June 2024

Accepted: 29 June 2024

Published: 2 July 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

1.1. Alzheimer's Disease Impact

In 2022, according to the American Journal of Managed Care, the total cost of Alzheimer's disease (AD) and associated dementia treatment was USD 321 billion and is expected to increase to reach USD 1 trillion in 2050. Almost half of these costs are due to medical care, but take into account medical assistance and care communities [1]. These numbers come from the USA, but then, if considered globally, WHO reports that AD is the most common form of dementia, contributing to 60–70% of cases. The worldwide impact of dementia was already USD 1.3 trillion in 2019 (World Health Organization, WHO, Dementia (who.int)).

1.2. Alzheimer's Disease Pathophysiology

AD is a progressive neurodegenerative disease that, to date, does not have a specific cure. Multiple pathological mechanisms have been proposed: amyloid beta (A β) accumulation, neurofibrillary tangles formation, and tau hyperphosphorylation [2]. Although the amyloid cascade hypothesis is currently the most studied theory for the development of AD, there are some more recent hypotheses, such as neuroinflammation, oxidative stress, and mitochondrial dysfunction [3]. Mechanisms, such as the gut–brain axis involvement, vascular dysfunction, and autophagy, also seem to be implicated in AD [4]. The overall picture gives us a devastating, probably multifactorial pathology that we intercept too late.

It is known that neuroinflammation plays a crucial role in the development and progression of Alzheimer's disease [5,6]. For example, the accumulation of A β and hyperphosphorylated tau protein aggregates in AD triggers the activation of astrocytes and microglia, releasing pro-inflammatory cytokines and reactive oxygen and nitrogen species, which contribute to neuroinflammation [6]. This inflammatory response can have a dual function, with both protective and detrimental effects on AD progression [7]. Indeed,

inflammatory cytokines, such as interleukin 1- β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α), are increased in AD and mild cognitive impairment (MCI) patients. High levels of IL-6 have been associated with an increased risk of cognitive decline [8]. Exaggerated release of reactive oxygen species within the brain may lead to a neuroinflammatory type of AD. Activation of microglia through these reactive compounds can induce the release of multiple pro-inflammatory cascades and general brain atrophy [9]. Dysbiosis of the gut microbiota can prime neuroinflammation and the deposition of A β plaques, characteristic of this pathology [10]. AD has been linked to gut microbiota alterations, with studies showing the presence of gut dysbiosis in AD patients [11]. The gut microbiota can affect the brain and behavior of AD patients, including their cognitive function [12]. From a mechanistic point of view, a reduction in short-chain fatty acid-producing strains may be involved in the pathology of AD [13,14]. Accordingly, dietary supplementation with prebiotics or fatty acids improved both histopathological and cognitive aspects in animal models of AD [15].

Historically, a cholinergic hypofunction and a degeneration of cholinergic neurons seem to be associated with this pathology [16]. The loss of cholinergic elements in the brain, a characteristic feature of AD, may be due to the toxic interaction of protein tau with muscarinic acetylcholine receptors (mAChRs) [17]. Among the receptors of the cholinergic system, the mAChRs participate in acetylcholine (ACh)-induced neurotransmission and have been implicated in AD [18]. Nevertheless, nicotinic receptors are also involved in AD [19–23]. It is of interest to note that these receptors can interact with mAChRs, giving rise to functional crosstalk phenomena that can be influenced by the action of A β [24,25]. In addition, it is well known that a disruption in cholinergic neurotransmission leads to cognitive impairments and behavioral alterations [26,27].

1.3. Early Hallmarks of AD

Converging hypotheses sustain that a delayed diagnosis could limit the discovery of effective therapies [28–30]. In agreement, it has become critical to identify early symptoms [31,32]. Before the evidence of clinical symptoms, several neurodegenerative diseases, including AD, could be anticipated by sleep alterations [33,34]. Hence, maintaining sleep might be effective in slowing AD appearance or progression. One potential therapeutic target for improving sleep is fatty acid amide hydrolase, as suggested in a study by Martin et al. [34]. In their study, an acute inhibition with PF3845, a selective inhibitor of fatty acid amide hydrolase, improved sleep behaviors in male and female transgenic Tau P301S mice (model of Tauopathy and AD [35]). Unfortunately, under chronic conditions, the treatment was not able to counteract progressive sleep loss, neuroinflammation, and cognitive decline [34]. However, sleep disruption and circadian rhythm alteration are also present in patients with frontotemporal dementia [33]. In a study conducted by Filardi, patients underwent actigraphy to monitor their sleep. Although the sleep duration increased in these patients, the quality of sleep decreased. Furthermore, the study has found that sleep duration is associated with reduced cortical thickness in frontal regions, as calculated by magnetic resonance imaging [33]. AD patients present diffuse brain atrophy regions, but some regions show an increase in size. Additionally, recent work has suggested neural facilitation in areas that are anticorrelated with atrophied regions in frontotemporal dementia [36].

Recent investigations have demonstrated that AD plays a pivotal role in the disruption of lipid metabolism, which is a significant contributing factor in the development of the disease [37]. The overexpression of the familial amyloid precursor protein (APP) calls for particular consideration, particularly in light of the advances in translational molecular imaging. These developments have made it feasible to probe cholesterol metabolism in the living human brain using positron emission tomography. This presents an appealing prerequisite for future clinical trials aimed at targeting the brain cholesterol machinery in patients with AD [38].

1.4. AD and Diabetes

The difficulty in finding an effective therapy for AD led us to evaluate its relationship with other diseases [39,40]. In particular, the association with diabetes seemed rational [41,42]. Therefore, understanding the link between AD and diabetes could lead to new strategies for treating both diseases together. AD and diabetes share several metabolic similarities. These include mitochondrial dysfunction and cellular lipotoxicity. Mitochondria produce less energy when they are dysfunctional, leading to oxidative stress and inflammation—factors that contribute to both diseases. Cellular lipotoxicity, the accumulation of harmful lipids in cells, can damage mitochondria, disrupt insulin signaling and promote inflammation. This increases the risk of AD and type 2 diabetes. Insulin has been shown to both clear A β in the brain and impact tau protein phosphorylation while also improving synaptic activity and neuron plasticity in both human and animal studies [43,44]. These metabolic connections between AD and diabetes open new therapeutic possibilities: some antidiabetic drugs, such as metformin, may have beneficial effects on cognition and reduce the risk of AD in diabetic patients [45]. Recent studies have provided promising evidence for using glucagon-like peptide-1 (GLP-1) receptor agonists to treat both diabetes and AD. These findings suggest that it may be possible to reduce the risk of developing dementia by using GLP-1 receptor agonists to target the underlying pathophysiological mechanisms associated with both diseases. This provides a novel approach that could potentially result in the development of a common therapy for both diabetes and AD, with significant implications for managing both conditions.

1.5. Treating Alzheimer's Disease: Managing Symptoms and Exploring New Targets

The current state of the art in AD therapies involves the use of acetylcholinesterase (AChE) inhibitors to enhance cholinergic neurotransmission and improve cognitive and behavioral symptoms [46]. These inhibitors, such as donepezil and galantamine, have shown moderate clinical benefits in treating AD [21]. The “cholinergic hypothesis of AD” assumes that memory and learning deficits result from the loss of cholinergic innervation to the cortex and hippocampus from nuclei in the basal forebrain. It was demonstrated that reduced ACh synthesis and degeneration of cholinergic innervation are principal contributors to AD [47]. Thus, one of the most pursued therapeutic strategies is the inhibition of AChE to increase ACh levels or aimed at directly stimulating cholinergic receptors. However, these treatments manage only a part of the symptoms and are not devoid of side effects, especially gastrointestinal [48]. Additionally, the use of N-methyl-D-aspartate (NMDA) receptor antagonists, like memantine, has been found to have a clinical effect on behavioral symptoms and is often combined with AChE inhibitors to enhance their efficacy [17]. ACh is assumed to be active in memory formation by potentiating NMDA receptor currents by M₁ mAChRs [49]. Accordingly, mAChRs have been identified as potential targets for new drugs in AD therapy [50]. The primary mAChRs, M₁R, and the α 7 nicotinic ACh receptor subtype have been the focus of recent experimental targets for mAChR agonist strategies [51]. The development of more selective M₁R compounds, including positive allosteric modulators (PAMs), is being explored to improve cognitive function and potentially modify the progression of AD [52,53]. PAMs for the M₁R mAChRs have shown promise in preclinical models for the treatment of AD [48]. These PAMs can enhance the binding and efficacy of ACh, improving impaired cholinergic transmission associated with AD [54]. A significant advance in the treatment of AD is the development of monoclonal anti-A β antibodies, although their real effectiveness, established in clinical trials, is still controversial [55–57]. It is established that even those compounds were unable to block the disease, let alone reverse the cognitive decline of AD patients. Their main function appears to be to slow down the rate of decline in cognitive and everyday functions. Interestingly, a recent randomized clinical trial, DIAN-TU-001, reports the beneficial impact of fibrillar A β reduction on fluid markers of synaptic dysfunction and neuroinflammation induced by gantenerumab and solanezumab [58]. Therefore, these anti-A β antibodies

could be incorporated into AD drug therapy in conjunction with other drugs, lifestyle modifications, and supportive care for the patient and family members [58,59].

2. Muscarinic Receptors' Involvement in Pathological Conditions

Dysfunctions of mAChRs are present in AD, Parkinson's disease, and schizophrenia [60]. The muscarinic receptor consists of the M₁-M₅ muscarinic receptors. Each subtype has defined functions in the nervous system and mediates the actions of ACh. All mAChRs are G-protein coupled receptors (GPCRs) [61]. M₁Rs are widely distributed in the cerebral cortex, hippocampus, and basal ganglia. The M₁R subtype is considered an important potential therapeutic target for AD [16]. M₂R regulates neurotransmitter release and ACh itself. M₃Rs are poorly represented in the brain, while M₄Rs are found in the striatum, hippocampus, and cortex. They are implicated in the regulation of dopamine release and have been associated with conditions such as schizophrenia and Parkinson's disease. M₅R is also involved in regulating dopamine release and is distributed in the same brain regions [61]. Historically, the involvement of mAChRs in AD has been proposed. However, it is now crucial to evaluate the involvement of these receptors in the pathological mechanisms that have been associated with AD (Figure 1, Table 1).

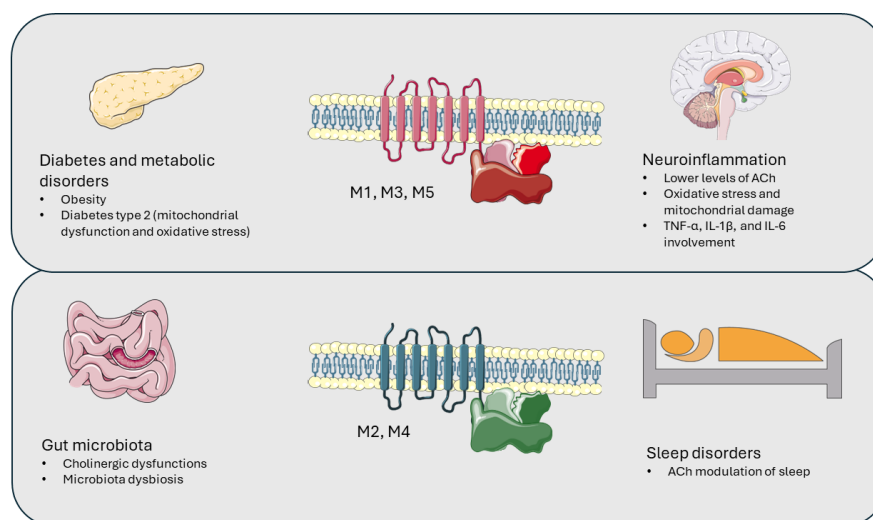


Figure 1. Contribution and involvement of muscarinic receptors in some pathological conditions. The figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license (<https://smart.servier.com/>, accessed on 27 May 2024).

Table 1. The dysregulation and potential positive effects of action on mAChRs.

Subtype	Negative Effects	Reference	Positive Effects	Reference
M ₁	Cognitive dysfunction	[62]	Improvement in object location in memory tasks	[63]
	mAChRs dysregulations	[64]	σ1 chaperone collaboration, Ca ²⁺ homeostasis (ANAVEX2-73)	[35,65,66]
	Mitochondrial function alterations	[67]	Modulation of APP metabolism (ANAVEX3-71)	[68–70]
M ₂	Interfere with APP processing	[71]	Biparmacophoric inhibition of cholinesterase (10-C10)	[72]
	Hallucination and behavioral symptoms	[73]	Enhancement of cognition by facilitating ACh release (SCH577790)	[74]

Table 1. Cont.

Subtype	Negative Effects	Reference	Positive Effects	Reference
M ₃	Mood disorders	[75]	Cognitive and behavioral improvement (LU 25-109)	[76,77]
	Sleep dysfunctions	[78]	PAM activity (N-pyrimidyl/pyridyl-2-thiazolamine)	[79]
	Reduced excitatory synaptic drive onto CA3 pyramidal neurons	[80,81]		
M ₄	Locomotor alterations	[82]	Cognitive and behavioral modulation (compound-110)	[82,83]
			Treatment of movement disorders	[84]
			mAChR agonist (Xanomeline)	[85]
			Reduction in glutamate release from CA1 and CA3 pyramidal neurons (PT-3763)	[86]
M ₅	Reduced blood flow in the hippocampus results in neuronal atrophy and memory impairments	[87]	M ₅ activation (VU0357017, VU0152100, VU0238429)	[88]

2.1. Muscarinic Receptors and Neuroinflammation

Neuroinflammation is a complex process characterized by the activation of the brain's immune cells in response to numerous factors such as injury, infection, or neurodegenerative conditions. This immune response can contribute to the progression of neurodegenerative diseases. The scientific evidence indicates that mAChRs are also involved in neuroinflammation and may regulate phenomena associated with neurodegenerative diseases [89]. Gatta et al. state that there is evidence that the levels of ACh are lower in multiple sclerosis patients: this suggests that a deficiency in the cholinergic system may be correlated with neuroinflammation, typical of neurodegenerative diseases [90]. According to research conducted on blood, serum, and cerebrospinal fluid from multiple sclerosis (MS) patients as well as those conducted on animal models of the disease, cholinergic changes may be a factor in the dysregulated inflammatory processes associated with multiple sclerosis [91]. Interestingly, Oxotremorine, a non-selective mAChRs agonist, prevents DNA fragmentation caused by the A β ₁₋₄₂ peptide and improves cell survival in SH-SY5Y neuroblastoma cells [92]. The same treatment inhibited oxidative stress and mitochondrial morphological/functional damage linked to exposure to A β ₁₋₄₂ cells. Another study performed on AD patients also determined that decreased levels of ACh and increased neuroinflammation were associated with cognitive decline, suggesting that the use of anticholinesterase drugs could lead to an improvement in the clinical condition of these patients, especially if an early diagnosis can be made [93]. Furthermore, mAChRs are also present in the microglia. Both endogenous and external inflammatory signals are detected by microglia, which then coordinates the ensuing neuroinflammation [94].

2.2. Muscarinic Receptors and Gut Microbiota

In 2020, Teratani and colleagues published a groundbreaking article revealing the presence of a liver-brain-gut neural arc that fine-tunes immune responses in the intestine. This pathway involves mAChRs and predisposes the gut to inflammation, suggesting a potential role in the development of neural diseases [95]. A study by Wu et al. explored the potential of pyridostigmine (PYR), a cholinesterase inhibitor that boosts vagal activity, to regulate the disrupted gut microbiota in diabetic mice. Their findings revealed that PYR effectively alleviated gut microbiota dysbiosis [96], suggesting a connection between the cholinergic system and the gut microbiota. Since dysbiosis of the gut microbiota seems to

be a prodrome to the onset of AD, as well as cholinergic dysfunction, it could be extremely useful to use drugs that act on the cholinergic system to treat intestinal disorders as well.

2.3. Muscarinic Receptors and Sleep Disorders

ACh plays a crucial role in the regulation of arousal and sleep, and the vigilance state is correlated with considerable changes in its release in different regions of the brain [97,98]. M₂Rs and M₄Rs seem to be involved in the modulation of sleep during homeostatic challenge. Accordingly, M₂Rs are reduced in rats who are sleep-deprived [99]. It has long been clear that anticholinesterase drugs, such as donepezil, already used in AD patients, can also contribute to the treatment of sleep disorders. This happens because by increasing the neurotransmitter ACh in the brain, the latter, by binding to mAChRs, increases rapid eye movement (REM) sleep [100,101].

2.4. Muscarinic Receptors and Metabolic Disorders

The significance of muscarinic receptors in the context of metabolic disorders such as obesity and subsequent diabetes is of considerable importance. The research highlights the significance of mAChRs in regulating energy homeostasis and metabolic health, suggesting their potential as therapeutic targets for managing obesity and type 2 diabetes [102]. The effect of cevimeline, an M₃R agonist, on olanzapine-induced metabolic disorders in rats has been investigated. Co-treatment of cevimeline with olanzapine significantly attenuated olanzapine-induced weight gain, dyslipidemia, and impaired glucose metabolism by modulating the hepatic M₃R-AMPK α pathway and improving AKT-GSK3 β signaling. This highlights the importance of targeting M₃R-related pathways to manage metabolic disturbances [103].

3. Muscarinic Receptors as a Pharmacological Target

3.1. M₁: Conceivable AD Target

Activation of the M₁Rs has shown potential as a disease-modifying target in several neurodegenerative diseases. In particular, M₁ mAChRs have been shown to play a role in cognitive dysfunctions associated with AD [62]. A β has been found to dysregulate mAChRs activity [64]. A β oligomers, which accumulate in the brains of the patients, disrupt synaptic plasticity and dendritic loss through interactions with NMDA receptors (NMDAR) and metabotropic glutamate receptor 5 (mGluR5) [18]. The dysregulation of mAChRs by A β -mediated activation of mGluR5 negatively affects mAChRs function [104]. M₁Rs deletion in cortical neurons affects mitochondrial function and ultrastructure; this produces mitochondrial pathophysiological deficits in AD [67]. In the context of M₁Rs as a therapeutic target, a recent study on C57BL/6 mice by Huff and colleagues suggests that M₁R activation promotes the updating of object location in memory tasks [63]. Mice were evaluated in object-location and object-updated-location tasks with or without scopolamine, non-selective mAChR antagonist, and MK-801, non-selective NMDAR antagonist. The study concluded that scopolamine was able to prevent novelty-induced destabilization and memory update in localization tasks. Likewise, MK-801 impairs object location memory reconsolidation in mice [63]. MK-801 seems to provoke deficits in social memory alongside the prairie voles model of schizophrenia [105]. This finding strengthens the potential of M₁R agonists as therapeutic agents for AD, as they may not only improve cognitive function but also enhance the brain's ability to adapt and form new memories. Recently, an ultrastructural observation consistent with electrophysiological studies has demonstrated M₁Rs and NMDARs co-localization in hippocampal dendrites [49], and activation of M₁Rs has been shown to play a crucial role in mnemonic functions in the anterior basolateral nucleus of the amygdala. Consequently, targeting M₁Rs by PAMs could ameliorate memory impairments in neurodegenerative disorders such as AD [49,106]. On the other hand, even sensory stimuli could be implicated in memory formation. Mishra and colleagues have investigated the primary somatosensory cortex in mice and its influence on the animal's sensitivity to detect vibrotactile stimuli [26].

Among new therapeutic strategies, broad neuroprotection rather than direct A β or hyperphosphorylated tau targeting seems to be encouraging. In particular, a mixed action on M₁ and σ_1 chaperone proteins of the endoplasmic reticulum (ER) has been investigated in recent years [65]. In the presence of chronic ER stress, σ_1 chaperone protein activates the Ca²⁺-dependent intracellular cascade, increasing or modifying σ_1 protein activation, using agonist, therefore exerting a pharmacological action on Ca²⁺ homeostasis and simultaneously activating neuroprotective pathways [65]. This double effect was found in ligands of the ANAVEX series [35,65,66]. One of the promising compounds, ANAVEX2-73, also known as blarcamesine, was able to block A β_{25-35} -induced Recognition Memory Deficits to prevent tau hyperphosphorylation and A β_{1-42} seeding in A β_{25-35} -injected mice [65]. ANAVEX2-73 has shown good results in a mouse model of Rett syndrome, a severe neurodevelopmental disease that is often associated with mutations in the transcriptional regulator *MECP2*, improving motor coordination and balance, sensory, acoustic and visual responses, showing a good safety profile due to a low dose (10 μ g/kg) that minimizes the possibility of peripheral cholinergic side effects [66]. ANAVEX2-73 has passed the phase 3 study for AD and Rett syndrome; additional studies for the treatment of PD and related dementia are planned for this compound.

Another interesting candidate is ANAVEX3-71, known as AF107B, which is more focused on AD and frontotemporal dementia; at the moment, this compound has finished phase 1, and phases 2 and 3 are planned [35,68]. This compound was tested on 3xTg-AD mice and McGill-R-Thy1-APP, attenuating cognitive deficits and mediating APP metabolism; no side effects were observed at a maximum dose of 50 mg/kg. Clinical studies have evaluated the pharmacokinetic profile (PK) of ANAVEX3-71 and its active metabolite M8 in health candidates. The studies concluded that the PK was linear, dose-proportional, and time-invariant. Additionally, food does not affect the PK of both compounds and their metabolites [69]. Assessing the effect of both on electrocardiogram (ECG) parameters in healthy participants did not result in clinically relevant alterations to the cardio-dynamic profile [70].

3.2. M₂: Complex Non-Selective Target

The M₂R is moderately abundant throughout the brain, and it is present in particular on cholinergic neurons and nonpyramidal neurons in the cortex and hippocampus. Like all mAChRs, it plays a role in modulating neurotransmitter release, synaptic transmission, and cognitive function [71]. A study highlighted that when specific subtypes of M₁R and M₂R are activated in tissue slices, it affects the processing of APP [71]. The activation of M₂R suppresses the non-amyloidogenic processing pathway of APP, while the activated M₁R leads to an increase in the expression of BACE1 (beta-site APP cleaving enzyme 1), an enzyme involved in amyloid plaque formation. These findings suggest that the cholinergic system plays a significant role in APP processing and in the development of AD. In this context, bi-pharmacophoric inhibitors of the cholinesterase with affinity to the M₁R and M₂R were tested, and one of the compounds resulted in a good candidate for the development of an anti-AD drug [72]. Notably, there is a selective decrease in M₂R in AD-affected brain regions containing senile plaques [107]. Another study highlighted the reduction in M₂R density detected in specific brain regions of AD patients [107]. Subgroup analyses revealed differential alterations in neurochemical variables among AD patients exhibiting various behavioral symptoms, with an increase in M₂R density observed in select symptom groups. Notably, delusions predicted variability in M₂R density in one brain region, while hallucinations were positively associated with M₂R density in another region [73]. Although recent studies have focused more on other mAChR subtypes, several compounds selective for M₂R have been highlighted in the past. Early compounds targeting M₂R lacked the desired combination of M₂R affinity, subtype selectivity, and central nervous system (CNS) activity. SCH57790 showed promising M₂R binding affinity and selectivity, leading to further development of other compounds. Because M₂R activation inhibits ACh release in brain regions that are critical for learning and memory processes, M₂R antagonists

have been proposed as a potential treatment for AD, as they can enhance cognition by facilitating ACh release in the brain [74].

Both sporadic and familial AD display cholinergic and lipid dysregulations [108]. In postmortem brain of AD patients, the distribution of phospholipids/sphingolipids and the activity of cannabinoid 1 (CB1), sphingosine 1-phosphate 1 (S1P1), and muscarinic M₂/M₄ receptors in the frontal cortex were investigated through MALDI-mass spectrometry imaging [109,110]. Compared to patients with MCI and not cognitively impaired subjects, AD patients have presented a downregulation of phosphatidylinositol levels in white matter compared to controls. On the other hand, M₂/M₄Rs activation was decreased in AD patients [110].

In the case of AChE inhibitors used in patients with mild to moderate dementia, it is necessary to consider possible side effects. In particular, peripheral M₂Rs could cause bradycardia, conduction abnormalities, and hypotension [111].

3.3. M₃: Involvement in Mood Disorders and Sleep Functions

Similarly to M₁R and M₅R, the M₃R is coupled to Gq-11, stimulating phospholipase C and inositol phosphate, thereby mediating an excitatory effect through intracellular calcium influx [112]. M₃Rs are poorly expressed in the CNS [113]. To date, only a limited number of studies have explored changes in M₃R expression in the frontal cortex in the context of mood disorders, which could also be linked to AD [75]. An analogous consideration should be made for M₂R and M₃R in terms of side effects [114]. Lu 25-109 has failed to produce a significant difference in cognitive or behavioral symptoms of AD [76], probably because it also acts as an M₂/M₃Rs antagonist [77].

A recent study has shown that M₁R and M₃R encoded by CHRM₁ and CHRM₃ genes, respectively, are involved in sleep functions [78]. Sanfilippo and colleagues have demonstrated that the expression levels of CHRM₁ and CHRM₃ were significantly reduced in AD brains, compared with those of age and sex-matched non-demented healthy control brains. Moreover, these associations were found to be modulated by sex. Accordingly, males expressed higher levels of CHRM₁ and CHRM₃ than females in the temporal and occipital regions of healthy subjects. In AD patients, males exhibited higher levels of CHRM₁ and CHRM₃ in the temporal and frontal regions, respectively, than females [78].

Furthermore, another study has investigated the role of M₁R and M₃R in learning and plasticity at the hippocampus level [80]. In particular, the study has focused on the mossy fibers (MF)–CA3 pyramidal cell synapse obtained from hippocampal slices of double knockout mice. Interestingly, it was found that long-term plasticity was affected by reduced excitatory synaptic drive onto CA3 pyramidal cells rather than short-term plasticity. Previous studies have demonstrated similar effects on behavioral flexibility, working memory, and hippocampal plasticity in M₂R-knockout mice [81]. Brain TNF- α , IL- β , and IL-6 mRNA expression were induced by acute M₃ activation. Nevertheless, this “pro-inflammatory” effect was eliminated upon repeated muscarinic activation, showing that repeated activation of M₃Rs in microglia contributes to the development of microglial tolerance. As the researchers themselves point out, further studies are needed, but this finding gives hope for the development of new selective therapies.

3.4. M₄: Promising AD Target

Nevertheless, the M₁R is well documented; in the literature, several studies have investigated the effects of M₁/M₄R-acting compounds. Conversely, M₄R modulation has been less extensively studied [60,85]. The M₄R is primarily expressed in the brain, specifically in regions such as the striatum and the hippocampus. M₄R is present in the cortex, expressed and found presynaptically as autoreceptors in glutamatergic synapses on neurons, and is particularly enriched presynaptically within the substantia nigra [82]. Moreover, structure-based drug discovery enables the development of orthosteric agonists that are highly selective for mAChRs, including the M₁ and M₄ subtypes, which play a pivotal role in cognitive and behavioral modulation [82]. In the study conducted by

Wang and colleagues, several ligands were investigated for their activity on M₄Rs [83]. A promising compound-110 has demonstrated allosteric agonist binding with M₄, resulting in antipsychotic activity in a schizophrenia mouse model. Additionally, it has been observed to reverse MK-801-induced hyperlocomotion without inducing catalepsy in mice [83]. Given that cholinergic neurons degenerate with the progression of the disease, it may be more advantageous to target postsynaptic M₄Rs, which are relatively preserved in AD, with orthosteric agonists [82].

This receptor is co-expressed in a subpopulation of dopamine-1 receptor (D1R)-expressing medium spiny projection neurons in the striatum, where it plays a role in modulating striatal dopamine release and dopamine-related behaviors [82]. Anomalies in dopaminergic transmission can affect synaptic plasticity, motor activities, and cognitive behavior, which are essential aspects of AD. Moreover, the existing evidence strongly supports the occurrence of functional interactions between M₄Rs and DR at different levels [115–117]. M₄Rs exert inhibitory control over D1R-mediated locomotor stimulation, likely at the level of striatal projection neurons where both receptors are highly co-expressed [115]. M₄R is a promising therapeutic target for the treatment of movement disorders and additionally acts as a disease modifier due to its cholinergic activity [84]. The role of M₄Rs in dopaminergic signaling, which is involved in movement, is relevant in the context of both AD and Parkinson's disease [60,84].

M₄R agonists have demonstrated positive effects on psychiatric and cognitive symptoms in patients with schizophrenia and related psychotic disorders, while reductions in M₄R activation have been associated with hippocampal deficits thought to underlie the amnesic features of AD [82]. Xanomeline, a multitargeting drug with muscarinic and serotonergic activities [118], has the potential to enhance wakefulness and arousal in aging, MCI, and AD patient populations. This is suggested by its ability to reverse wake fragmentation and disruptions in arousal in nonpathologically aged mice [85]. The activation of M₄R in rodents' brains using a compound called PT-3763 reduced the amount of input received by CA1 and CA3 pyramidal neurons in a dose-dependent manner when assessed *ex vivo*. Furthermore, the administration of this compound throughout the body or directly into specific brain areas (*in vivo*) reduced the activity of CA1 pyramidal neurons in a dose-dependent manner. When PT-3763 was systemically administered, it resulted in a reduction in the release of the neurotransmitter glutamate from CA3 pyramidal neurons. Treatment with this compound also improved the performance of rodents in the Morris water maze [86]. Novel selective M₄R PAMs have been developed, demonstrating efficacy in preclinical models predictive of antipsychotic and pro-cognitive effects. Selective M₄R PAMs have shown potential for managing neuropsychiatric symptoms associated with AD, such as agitation and aggression, with improved therapeutic margins for cholinergic adverse effects compared to less selective muscarinic receptor agonists [119]. To quantify M₄R engagement in the brain, positron emission tomography (PET) tracers such as [¹¹C]MK-6884 and [¹¹C]PF06885190 have been developed, which have aided in the development of new drugs and the conduct of clinical trials [119,120]. The PET ligand [¹¹C]MK-6884 has demonstrated selectivity for the muscarinic M₄R subtype over other subtypes and exhibits high brain uptake. It can indicate changes in M₄R density and may provide crucial insights into the diagnosis of neurodegenerative diseases, including AD. A study evaluated [¹¹C]MK-6884 in AD, MCI, and normal healthy volunteer samples. A reduction in muscarinic M₄R density was observed in AD tissues in comparison to normal healthy volunteer samples [121].

3.5. M₅: Relevant Role in Memory Impairment in AD

The M₅R is a receptor that is coupled to Gq/11 proteins. It has been successfully cloned in humans and exhibits structural similarities with the M₃R. This mAChR is expressed in various brain regions, including the hippocampus, hypothalamus, substantia nigra, and ventral tegmental area [122]. Araya and colleagues generated M₅R knockout mice to elucidate the receptor's role in cerebral ischemia. These rodents exhibited reduced

cerebral blood flow in the cortex, hippocampus, basal ganglia, and thalamus. In vivo experiments were conducted, and magnetic resonance angiography revealed that the decrease in blood flow to hippocampal pyramidal neurons resulted in significant neuronal atrophy and memory impairment in the Novel Object Recognition protocol. This suggests the potential use of M₅R as a target to be reached for the treatment of memory impairment in AD patients [87]. Wieronska and colleagues have found that activators of M₅R, as well as M₁ and M₄ mAChRs, effectively counteract several aspects of MK-801-induced memory impairment in the Morris Water Maze test. This highlights the significance of these receptors in a multitude of cognitive functions and cyclic guanosine monophosphate (cGMP)-dependent processes [88]. The expression of M₅Rs in these cells was confirmed via reverse transcription polymerase chain reaction and immunoblotting. The use of short interfering RNA (siRNA) silencing demonstrated that the downregulation of M₅Rs resulted in a significant reduction in the Ca²⁺ response to ACh, thereby confirming the role of this receptor in mediating ACh-induced Ca²⁺ signals in hCMEC/D3 cells [123].

4. Supporting Data

The review is supported by the literature that was gathered using the PubMed, Scopus, and Google Scholar databases, as well as from papers cited within the initial articles retrieved. Search terms included combinations of the following words: Alzheimer's disease, neuroinflammation, metabolic disorders and AD, gut-brain axis, microbiota and AD, muscarinic receptors and sleep disorders, new pharmacological targets for muscarinic receptors, and similar combinations.

5. Conclusions

In conclusion, the hypothesis that mAChRs play a role in AD remains a recurrent topic of interest (Table 1). The notion that this theory has spanned decades of research and resurfaces periodically without foundation seems implausible. The muscarinic component appears to be most involved in aspects related to memory and the cognitive sphere. It can be postulated that alterations in these receptors may also contribute to the relationship between AD and certain emerging comorbidities. It should be noted that this review has some limitations, such as the lack of precise analysis of side effects. However, this aspect is partially due to the need for additional studies to clarify the effectiveness of new therapeutic approaches. Accordingly, we have indicated side effects for compounds already tested in clinical trials. Finally, this review is part of a comprehensive existing literature that presents a multitude of high-level works with the objective of summarising the latest evidence in the context of comorbidity with AD.

Author Contributions: Conceptualization, M.G. and H.T.; writing—original draft preparation, H.T. and M.M.; writing—review and editing, M.G.; supervision, M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work is supported by #NEXTGENERATIONEU (NGEU) and funded by the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006)—A Multiscale integrated approach to the study of the nervous system in health and disease (DN. 1553 11.10.2022).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created; all information was gathered from already published papers.

Acknowledgments: The graphical abstract was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license (<https://smart.servier.com/>), accessed on 30 May 2024).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Skaria, A.P. The Economic and Societal Burden of Alzheimer Disease: Managed Care Considerations. *Am. J. Manag. Care* **2022**, *28*, S188–S196. [[CrossRef](#)] [[PubMed](#)]
2. Dwomoh, L.; Tejada, G.S.; Tobin, A.B. Targeting the M1 Muscarinic Acetylcholine Receptor in Alzheimer's Disease. *Neuronal Signal*. **2022**, *6*, 20210004. [[CrossRef](#)] [[PubMed](#)]
3. Nasb, M.; Tao, W.; Chen, N. Alzheimer's Disease Puzzle: Delving into Pathogenesis Hypotheses. *Ageing Dis.* **2024**, *15*, 43–73. [[CrossRef](#)] [[PubMed](#)]
4. Theerasri, A.; Janpajit, S.; Tencomnao, T.; Prasansuklab, A. Beyond the Classical Amyloid Hypothesis in Alzheimer's Disease: Molecular Insights into Current Concepts of Pathogenesis, Therapeutic Targets, and Study Models. *WIREs Mech. Dis.* **2023**, *15*, e1591. [[CrossRef](#)] [[PubMed](#)]
5. Si, Z.Z.; Zou, C.J.; Mei, X.; Li, X.F.; Luo, H.; Shen, Y.; Hu, J.; Li, X.X.; Wu, L.; Liu, Y. Targeting Neuroinflammation in Alzheimer's Disease: From Mechanisms to Clinical Applications. *Neural Regen. Res.* **2023**, *18*, 708–715. [[CrossRef](#)] [[PubMed](#)]
6. Novoa, C.; Salazar, P.; Cisternas, P.; Gherardelli, C.; Vera-Salazar, R.; Zolezzi, J.M.; Inestrosa, N.C. Inflammation Context in Alzheimer's Disease, a Relationship Intricate to Define. *Biol. Res.* **2022**, *55*, 39. [[CrossRef](#)] [[PubMed](#)]
7. Kaiyrylkyzy, A.; Zholdasbekova, G.; Alzhanova, D.; Olzhayev, F.; Baibulatova, A.; Tsoy, A.; Askarova, S. The Expression Levels of Pro-inflammatory and Anti-inflammatory Cytokines and Chemokines in Blood Serum of Alzheimer's Dementia Patients: Report from a Pilot Study in Kazakhstan. *Alzheimer's Dement.* **2022**, *18*, 2–3. [[CrossRef](#)]
8. Leonardo, S.; Fregni, F. Association of Inflammation and Cognition in the Elderly: A Systematic Review and Meta-Analysis. *Front. Aging Neurosci.* **2023**, *15*, 1069439. [[CrossRef](#)]
9. Anwar, M.M. Oxidative Stress-A Direct Bridge to Central Nervous System Homeostatic Dysfunction and Alzheimer's Disease. *Cell Biochem. Funct.* **2022**, *40*, 17–27. [[CrossRef](#)]
10. Cammann, D.; Lu, Y.; Cummings, M.J.; Zhang, M.L.; Cue, J.M.; Do, J.; Ebersole, J.; Chen, X.; Oh, E.C.; Cummings, J.L.; et al. Genetic Correlations between Alzheimer's Disease and Gut Microbiome Genera. *Sci. Rep.* **2023**, *13*, 5258. [[CrossRef](#)]
11. Bostancikhoğlu, M. Connectivity between Gut Microbiota and Terminal Awakenings in Alzheimer's Disease. *Curr. Alzheimer Res.* **2023**, *20*, 3–10. [[CrossRef](#)] [[PubMed](#)]
12. Zhu, J.; Liu, S.; Zhang, H.; Zhao, W.; Ding, J.; Dai, R.; Xu, K.; He, C.; Liu, J.; Yang, L.; et al. Dynamic Distribution of Gut Microbiota during Alzheimer's Disease Progression in a Mice Model. *APMIS* **2023**, *131*, 480–490. [[CrossRef](#)]
13. Krishaa, L.; Ng, T.K.S.; Wee, H.N.; Ching, J. Gut-Brain Axis through the Lens of Gut Microbiota and Their Relationships with Alzheimer's Disease Pathology: Review and Recommendations. *Mech. Ageing Dev.* **2023**, *211*, 111787. [[CrossRef](#)]
14. Murray, E.R.; Kemp, M.; Nguyen, T.T. The Microbiota-Gut-Brain Axis in Alzheimer's Disease: A Review of Taxonomic Alterations and Potential Avenues for Interventions. *Arch. Clin. Neuropsychol.* **2022**, *37*, 595–607. [[CrossRef](#)]
15. Ameen, A.O.; Freude, K.; Aldana, B.I. Fats, Friends or Foes: Investigating the Role of Short- and Medium-Chain Fatty Acids in Alzheimer's Disease. *Biomedicines* **2022**, *10*, 2778. [[CrossRef](#)]
16. Jiang, S.; Li, Y.; Zhang, C.; Zhao, Y.; Bu, G.; Xu, H.; Zhang, Y.W. M1 Muscarinic Acetylcholine Receptor in Alzheimer's Disease. *Neurosci. Bull.* **2014**, *30*, 295–307. [[CrossRef](#)]
17. Wysocka, A.; Palasz, E.; Steczkowska, M.; Niewiadomska, G. Dangerous Liaisons: Tau Interaction with Muscarinic Receptors. *Curr. Alzheimer Res.* **2020**, *17*, 224–237. [[CrossRef](#)]
18. Lombardero, L.; Llorente-Ovejero, A.; Manuel, I.; Rodríguez-Puertas, R. Neurotransmitter Receptors in Alzheimer's Disease: From Glutamatergic to Cholinergic Receptors. In *Genetics, Neurology, Behavior, and Diet in Dementia: The Neuroscience of Dementia*; Academic Press: Cambridge, MA, USA, 2020; Volume 2, pp. 441–456. [[CrossRef](#)]
19. Ahmad, F.; Liu, P. Synaptosome as a Tool in Alzheimer's Disease Research. *Brain Res.* **2020**, *1746*, 147009. [[CrossRef](#)]
20. Takata, K.; Kimura, H.; Yanagisawa, D.; Harada, K.; Nishimura, K.; Kitamura, Y.; Shimohama, S.; Tooyama, I. Nicotinic Acetylcholine Receptors and Microglia as Therapeutic and Imaging Targets in Alzheimer's Disease. *Molecules* **2022**, *27*, 2780. [[CrossRef](#)]
21. Verma, S.; Kumar, A.; Tripathi, T.; Kumar, A. Muscarinic and Nicotinic Acetylcholine Receptor Agonists: Current Scenario in Alzheimer's Disease Therapy. *J. Pharm. Pharmacol.* **2018**, *70*, 985–993. [[CrossRef](#)]
22. Sharma, M.; Pal, P.; Gupta, S.K. The Neurotransmitter Puzzle of Alzheimer's: Dissecting Mechanisms and Exploring Therapeutic Horizons. *Brain Res.* **2024**, *1829*, 148797. [[CrossRef](#)] [[PubMed](#)]
23. Roberts, C.F.; Cao, Y.; Im, W.; Nichols, R.A.; Lukas, R.J.; George, A.A. Neuroprotective Amyloid β N-Terminal Peptides Differentially Alter Human A7- and A7 β 2-Nicotinic Acetylcholine (NACH) Receptor Single-Channel Properties. *Br. J. Pharmacol.* **2024**, 1–20. [[CrossRef](#)] [[PubMed](#)]
24. Zappettini, S.; Grilli, M.; Olivero, G.; Mura, E.; Preda, S.; Govoni, S.; Salamone, A.; Marchi, M. Beta Amyloid Differently Modulate Nicotinic and Muscarinic Receptor Subtypes Which Stimulate in Vitro and in Vivo the Release of Glycine in the Rat Hippocampus. *Front. Pharmacol.* **2012**, *3*, 146. [[CrossRef](#)] [[PubMed](#)]
25. Govoni, S.; Mura, E.; Preda, S.; Racchi, M.; Lanni, C.; Grilli, M.; Zappettini, S.; Salamone, A.; Olivero, G.; Pittaluga, A.; et al. Dangerous Liaisons between Beta-Amyloid and Cholinergic Neurotransmission. *Curr. Pharm. Des.* **2014**, *20*, 2525–2538. [[CrossRef](#)] [[PubMed](#)]
26. Mishra, W.; Kheradpezhoh, E.; Arabzadeh, E. Activation of M1 Cholinergic Receptors in Mouse Somatosensory Cortex Enhances Information Processing and Detection Behaviour. *Commun. Biol.* **2024**, *7*, 3. [[CrossRef](#)]

27. Cantone, A.F.; Burgaletto, C.; Di Benedetto, G.; Pannaccione, A.; Secondo, A.; Bellanca, C.M.; Augello, E.; Munafò, A.; Tarro, P.; Bernardini, R.; et al. Taming Microglia in Alzheimer's Disease: Exploring Potential Implications of Choline Alfoscerate via A7 NACHR Modulation. *Cells* **2024**, *13*, 309. [[CrossRef](#)]
28. Graff-Radford, J.; Yong, K.X.X.; Apostolova, L.G.; Bouwman, F.H.; Carrillo, M.; Dickerson, B.C.; Rabinovici, G.D.; Schott, J.M.; Jones, D.T.; Murray, M.E. New Insights into Atypical Alzheimer's Disease in the Era of Biomarkers. *Lancet Neurol.* **2021**, *20*, 222–234. [[CrossRef](#)]
29. Trejo-Lopez, J.A.; Yachnis, A.T.; Prokop, S. Neuropathology of Alzheimer's Disease. *Neurotherapeutics* **2022**, *19*, 173–185. [[CrossRef](#)] [[PubMed](#)]
30. Nelson, P.T.; Schneider, J.A.; Jicha, G.A.; Duong, M.T.; Wolk, D.A. When Alzheimer's Is LATE: Why Does It Matter? *Ann. Neurol.* **2023**, *94*, 211–222. [[CrossRef](#)]
31. Farvadi, F.; Hashemi, F.; Amini, A.; Alsadat Vakilinezhad, M.; Raee, M.J. Early Diagnosis of Alzheimer's Disease with Blood Test: Tempting but Challenging. *Int. J. Mol. Cell Med.* **2023**, *12*, 172–210. [[CrossRef](#)]
32. Smirnov, D.S.; Ashton, N.J.; Blennow, K.; Zetterberg, H.; Simrén, J.; Lantero-Rodriguez, J.; Karikari, T.K.; Hiniker, A.; Rissman, R.A.; Salmon, D.P.; et al. Plasma Biomarkers for Alzheimer's Disease in Relation to Neuropathology and Cognitive Change. *Acta Neuropathol.* **2022**, *143*, 487–503. [[CrossRef](#)]
33. Filardi, M.; Gnoni, V.; Tamburrino, L.; Nigro, S.; Urso, D.; Vilella, D.; Tafuri, B.; Giugno, A.; De Blasi, R.; Zoccolella, S.; et al. Sleep and Circadian Rhythm Disruptions in Behavioral Variant Frontotemporal Dementia. *Alzheimer's Dement.* **2024**, *20*, 1966–1977. [[CrossRef](#)] [[PubMed](#)]
34. Martin, S.C.; Joyce, K.K.; Harper, K.M.; Harp, S.J.; Cohen, T.J.; Martin, S.C.; Joyce, K.K.; Harper, K.M.; Harp, S.J.; Cohen, T.J.; et al. Evaluating Fatty Acid Amide Hydrolase as a Suitable Target for Sleep Promotion in a Transgenic TauP301S Mouse Model of Neurodegeneration. *Pharmaceuticals* **2024**, *17*, 319. [[CrossRef](#)] [[PubMed](#)]
35. Cuello, A.C.; Hall, H.; Do Carmo, S. Experimental Pharmacology in Transgenic Rodent Models of Alzheimer's Disease. *Front. Pharmacol.* **2019**, *10*, 189. [[CrossRef](#)] [[PubMed](#)]
36. Phan, T.X.; Baratono, S.; Drew, W.; Tetreault, A.M.; Fox, M.D.; Darby, R.R. Increased Cortical Thickness in Alzheimer's Disease. *Ann. Neurol.* **2024**, *95*, 929–940. [[CrossRef](#)] [[PubMed](#)]
37. Bornstein, S.R.; Voit-Bak, K.; Rosenthal, P.; Tselmin, S.; Julius, U.; Schatz, U.; Boehm, B.O.; Thuret, S.; Kempermann, G.; Reichmann, H.; et al. Extracorporeal Apheresis Therapy for Alzheimer Disease—Targeting Lipids, Stress, and Inflammation. *Mol. Psychiatry* **2019**, *25*, 275–282. [[CrossRef](#)]
38. Zhang, X.; Chen, C.; Liu, Y. Navigating the Metabolic Maze: Anomalies in Fatty Acid and Cholesterol Processes in Alzheimer's Astrocytes. *Alzheimer's Res. Ther.* **2024**, *16*, 63. [[CrossRef](#)] [[PubMed](#)]
39. Maciejewska, K.; Czarnecka, K.; Szymański, P. A Review of the Mechanisms Underlying Selected Comorbidities in Alzheimer's Disease. *Pharmacol. Rep.* **2021**, *73*, 1565–1581. [[CrossRef](#)] [[PubMed](#)]
40. Nday, C.M.; Eleftheriadou, D.; Jackson, G. Shared Pathological Pathways of Alzheimer's Disease with Specific Comorbidities: Current Perspectives and Interventions. *J. Neurochem.* **2018**, *144*, 360–389. [[CrossRef](#)]
41. Ahmed, S.; Mahmood, Z.; Zahid, S. Linking Insulin with Alzheimer's Disease: Emergence as Type III Diabetes. *Neurol. Sci.* **2015**, *36*, 1763–1769. [[CrossRef](#)]
42. Schubert, M. The Correlation between Diabetes Mellitus and Neurodegenerative Diseases. *Klin. Monatsblätter Augenheilkd.* **2023**, *240*, 130–135. [[CrossRef](#)]
43. Dubey, S.K.; Lakshmi, K.K.; Krishna, K.V.; Agrawal, M.; Singhvi, G.; Saha, R.N.; Saraf, S.; Saraf, S.; Shukla, R.; Alexander, A. Insulin Mediated Novel Therapies for the Treatment of Alzheimer's Disease. *Life Sci.* **2020**, *249*, 117540. [[CrossRef](#)] [[PubMed](#)]
44. Du, H.; Meng, X.; Yao, Y.; Xu, J. The Mechanism and Efficacy of GLP-1 Receptor Agonists in the Treatment of Alzheimer's Disease. *Front. Endocrinol.* **2022**, *13*, 1033479. [[CrossRef](#)] [[PubMed](#)]
45. Goodarzi, G.; Tehrani, S.S.; Fana, S.E.; Moradi-Sardareh, H.; Panahi, G.; Maniati, M.; Meshkani, R. Crosstalk between Alzheimer's Disease and Diabetes: A Focus on Anti-Diabetic Drugs. *Metab. Brain Dis.* **2023**, *38*, 1769–1800. [[CrossRef](#)] [[PubMed](#)]
46. Giacobini, E.; Cuello, A.C.; Fisher, A. Reimagining Cholinergic Therapy for Alzheimer's Disease. *Brain* **2022**, *145*, 2250–2275. [[CrossRef](#)]
47. Gallrein, C.; Williams, A.B.; Meyer, D.H.; Messling, J.E.; Garcia, A.; Schumacher, B. Baz-2 Enhances Systemic Proteostasis in Vivo by Regulating Acetylcholine Metabolism. *Cell Rep.* **2023**, *42*, 113577. [[CrossRef](#)]
48. Nguyen, H.T.M.; van der Westhuizen, E.T.; Langmead, C.J.; Tobin, A.B.; Sexton, P.M.; Christopoulos, A.; Valant, C. Opportunities and Challenges for the Development of M1 Muscarinic Receptor Positive Allosteric Modulators in the Treatment for Neurocognitive Deficits. *Br. J. Pharmacol.* **2022**, *181*, 2114–2142. [[CrossRef](#)]
49. McDonald, A.J. Colocalization of M1 Muscarinic Cholinergic Receptors and NMDA Receptors in Dendrites and Spines of Pyramidal Neurons of the Mouse Basolateral Amygdala: An Ultrastructural Analysis. *Neurosci. Lett.* **2022**, *779*, 136624. [[CrossRef](#)]
50. Scarpa, M.; Hesse, S.; Bradley, S.J. M1 Muscarinic Acetylcholine Receptors: A Therapeutic Strategy for Symptomatic and Disease-Modifying Effects in Alzheimer's Disease? *Adv. Pharmacol.* **2020**, *88*, 277–310. [[CrossRef](#)]
51. Vecchio, I.; Sorrentino, L.; Paoletti, A.; Marra, R.; Arbitrio, M. The State of The Art on Acetylcholinesterase Inhibitors in the Treatment of Alzheimer's Disease. *J. Cent. Nerv. Syst. Dis.* **2021**, *13*, 11795735211029113. [[CrossRef](#)]

52. Abd-Elrahman, K.; Colson, T.-L.L.; Ferguson, S.S. A Positive Allosteric Modulator of M1 Acetylcholine Receptors Improves Cognitive Deficits in Male and Female APP^{swe}/PSEN1 Δ E9 Mice via Divergent Mechanisms. *J. Pharmacol. Exp. Ther.* **2023**, *385*, 479. [[CrossRef](#)]
53. Rook, J.; Xiang, Z.; Dickerson, J.; Dogra, S.; Jensen, K.; Kim, I.; Ohler, M.; Weiss, K.; Conn, P.J. M1 Positive Allosteric Modulators Enhance Domains of Cognitive Function by Activation of SsT-Expressing Interneurons in the Prefrontal Cortex. *J. Pharmacol. Exp. Ther.* **2023**, *385*, 407. [[CrossRef](#)]
54. Nguyen, H.T.M.; van der Westhuizen, E.T.; Khajehali, E.; Valant, C.; Christopoulos, A. Investigating Drivers for M1 Muscarinic Acetylcholine Receptor-Mediated Adverse Events by M1 Positive Allosteric Modulators. *FASEB J.* **2022**, *36*. [[CrossRef](#)]
55. van Dyck, C.H.; Swanson, C.J.; Aisen, P.; Bateman, R.J.; Chen, C.; Gee, M.; Kanekiyo, M.; Li, D.; Reyderman, L.; Cohen, S.; et al. Lecanemab in Early Alzheimer's Disease. *N. Engl. J. Med.* **2023**, *388*, 9–21. [[CrossRef](#)]
56. Budd Haeberlein, S.; Aisen, P.S.; Barkhof, F.; Chalkias, S.; Chen, T.; Cohen, S.; Dent, G.; Hansson, O.; Harrison, K.; von Hehn, C.; et al. Two Randomized Phase 3 Studies of Aducanumab in Early Alzheimer's Disease. *J. Prev. Alzheimers Dis.* **2022**, *9*, 197–210. [[CrossRef](#)] [[PubMed](#)]
57. Jucker, M.; Walker, L.C. Alzheimer's Disease: From Immunotherapy to Immunoprevention. *Cell* **2023**, *186*, 4260–4270. [[CrossRef](#)]
58. Wagemann, O.; Liu, H.; Wang, G.; Shi, X.; Bittner, T.; Scelsi, M.A.; Farlow, M.R.; Clifford, D.B.; Supnet-Bell, C.; Santacruz, A.M.; et al. Downstream Biomarker Effects of Gantenerumab or Solanezumab in Dominantly Inherited Alzheimer Disease the DIAN-TU-001 Randomized Clinical Trial. *JAMA Neurol.* **2024**, *81*, 582–593. [[CrossRef](#)]
59. Perneczky, R.; Dom, G.; Chan, A.; Falkai, P.; Bassetti, C. Anti-Amyloid Antibody Treatments for Alzheimer's Disease. *Eur. J. Neurol.* **2024**, *31*, e16049. [[CrossRef](#)] [[PubMed](#)]
60. Avilés-Rosas, V.H.; Rendón-Ochoa, E.A.; Hernández-Flores, T.; Flores-León, M.; Arias, C.; Galarraga, E.; Bargas, J. Role of M4-Receptor Cholinergic Signaling in Direct Pathway Striatal Projection Neurons during Dopamine Depletion. *Synapse* **2024**, *78*, e22287. [[CrossRef](#)]
61. Brandl, K.; Laiken, N. Chapter 12—Acetylcholine and Muscarinic Receptors. In *Primer on the Autonomic Nervous System*, 4th ed.; Biaggioni, I., Browning, K., Fink, G., Jordan, J., Low, P.A., Paton, J.F.R., Eds.; Academic Press: Cambridge, MA, USA, 2023; pp. 73–76. ISBN 978-0-323-85492-4.
62. Benarroch, E.E. Glutamatergic Synaptic Plasticity and Dysfunction in Alzheimer Disease: Emerging Mechanisms. *Neurology* **2018**, *91*, 125–132. [[CrossRef](#)]
63. Huff, A.E.; O'Neill, O.S.; Messer, W.S.; Winters, B.D. Muscarinic Receptor Activation Promotes Destabilization and Updating of Object Location Memories in Mice. *Behav. Brain Res.* **2024**, *461*, 114847. [[CrossRef](#)] [[PubMed](#)]
64. Zhao, L.X.; Ge, Y.H.; Li, J.B.; Xiong, C.H.; Law, P.Y.; Xu, J.R.; Qiu, Y.; Chen, H.Z. M1 Muscarinic Receptors Regulate the Phosphorylation of AMPA Receptor Subunit GluA1 via a Signaling Pathway Linking CAMP-PKA and PI3K-Akt. *FASEB J.* **2019**, *33*, 6622–6631. [[CrossRef](#)] [[PubMed](#)]
65. Lahmy, V.; Meunier, J.; Malmström, S.; Naert, G.; Givalois, L.; Kim, S.H.; Villard, V.; Vamvakides, A.; Maurice, T. Blockade of Tau Hyperphosphorylation and A β 1–42 Generation by the Aminotetrahydrofuran Derivative ANAVEX2-73, a Mixed Muscarinic and Σ 1 Receptor Agonist, in a Nontransgenic Mouse Model of Alzheimer's Disease. *Neuropsychopharmacology* **2013**, *38*, 1706–1723. [[CrossRef](#)] [[PubMed](#)]
66. Kaufmann, W.E.; Sprouse, J.; Rebowe, N.; Hanania, T.; Klamer, D.; Missling, C.U. ANAVEX $\text{\textcircled{R}}$ 2-73 (Blarcamesine), a Sigma-1 Receptor Agonist, Ameliorates Neurologic Impairments in a Mouse Model of Rett Syndrome. *Pharmacol. Biochem. Behav.* **2019**, *187*, 172796. [[CrossRef](#)] [[PubMed](#)]
67. Sabbir, M.G.; Swanson, M.; Albeni, B.C. Loss of Cholinergic Receptor Muscarinic 1 Impairs Cortical Mitochondrial Structure and Function: Implications in Alzheimer's Disease. *Front. Cell Dev. Biol.* **2023**, *11*, 1158604. [[CrossRef](#)] [[PubMed](#)]
68. Hall, H.; Iulita, M.F.; Gubert, P.; Flores Aguilar, L.; Ducatenzeiler, A.; Fisher, A.; Cuello, A.C. AF710B, an M1/Sigma-1 Receptor Agonist with Long-Lasting Disease-Modifying Properties in a Transgenic Rat Model of Alzheimer's Disease. *Alzheimer's Dement.* **2018**, *14*, 811–823. [[CrossRef](#)] [[PubMed](#)]
69. Fadiran, E.O.; Hammond, E.; Tran, J.; Missling, C.U.; Ette, E. Population-Based Characterization of the Pharmacokinetics and Food Effect of ANAVEX3-71, a Novel Sigma-1 Receptor and Allosteric M1 Muscarinic Receptor Agonist in Development for Treatment of Frontotemporal Dementia, Schizophrenia, and Alzheimer Disease. *Orig. Artic. Clin. Pharmacol. Drug Dev.* **2023**, *2024*, 21–31. [[CrossRef](#)] [[PubMed](#)]
70. Fadiran, E.O.; Hammond, E.; Tran, J.; Xue, H.; Chen, J.; Kaufmann, W.E.; Missling, C.U.; Darpo, B. Concentration–QTc Relationship from a Single Ascending Dose Study of ANAVEX3-71, a Novel Sigma-1 Receptor and Allosteric M1 Muscarinic Receptor Agonist in Development for the Treatment of Frontotemporal Dementia, Schizophrenia, and Alzheimer's Disease. *Clin. Pharmacol. Drug Dev.* **2023**, *12*, 888–901. [[CrossRef](#)] [[PubMed](#)]
71. Levey, A.I. Muscarinic Acetylcholine Receptor Expression in Memory Circuits: Implications for Treatment of Alzheimer Disease. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 13541–13546. [[CrossRef](#)]
72. Messerer, R.; Dallanocce, C.; Matera, C.; Wehle, S.; Flammini, L.; Chirinda, B.; Bock, A.; Irmen, M.; Tränkle, C.; Barocelli, E.; et al. Novel Bipharmacophoric Inhibitors of the Cholinesterases with Affinity to the Muscarinic Receptors M1 and M2. *Medchemcomm* **2017**, *8*, 1346–1359. [[CrossRef](#)]
73. Lai, M.K.P.; Lai, O.F.; Keene, J.; Esiri, M.M.; Francis, P.T.; Hope, T.; Chen, C.P.L.H. Psychosis of Alzheimer's Disease Is Associated with Elevated Muscarinic M2 Binding in the Cortex. *Neurology* **2001**, *57*, 805–811. [[CrossRef](#)] [[PubMed](#)]

74. Boyle, C.D.; Lachowicz, J.E. Orally Active and Selective Benzylidene Ketal M2 Muscarinic Receptor Antagonists for the Treatment of Alzheimer's Disease. *Drug Dev. Res.* **2002**, *56*, 310–320. [[CrossRef](#)]
75. Gibbons, A.S.; Scarr, E.; McLean, C.; Sundram, S.; Dean, B. Decreased Muscarinic Receptor Binding in the Frontal Cortex of Bipolar Disorder and Major Depressive Disorder Subjects. *J. Affect. Disord.* **2009**, *116*, 184–191. [[CrossRef](#)] [[PubMed](#)]
76. Thal, L.J.; Forrest, M.; Loft, H.; Mengel, H. Lu 25-109, a Muscarinic Agonist, Fails to Improve Cognition in Alzheimer's Disease. Lu25-109 Study Group. *Neurology* **2000**, *54*, 421–426. [[CrossRef](#)] [[PubMed](#)]
77. Sánchez, C.; Arnt, J.; Didriksen, M.; Dragsted, N.; Moltzen Lenz, S.; Matz, J. In Vivo Muscarinic Cholinergic Mediated Effects of Lu 25-109, a M1 Agonist and M2/M3 Antagonist in Vitro. *Psychopharmacology* **1998**, *137*, 233–240. [[CrossRef](#)] [[PubMed](#)]
78. Sanfilippo, C.; Giuliano, L.; Castrogiovanni, P.; Imbesi, R.; Ulivieri, M.; Fazio, F.; Blennow, K.; Zetterberg, H.; Di Rosa, M. Sex, Age, and Regional Differences in CHRM1 and CHRM3 Genes Expression Levels in the Human Brain Biopsies: Potential Targets for Alzheimer's Disease-Related Sleep Disturbances. *Curr. Neuropharmacol.* **2022**, *21*, 740–760. [[CrossRef](#)] [[PubMed](#)]
79. Tanaka, H.; Akaiwa, M.; Negoro, K.; Kawaminami, E.; Mihara, H.; Fujii, H.; Okimoto, R.; Ino, K.; Ishizu, K.; Takahashi, T. Design, Synthesis, and Structure–Activity Relationships Study of N-Pyrimidyl/Pyridyl-2-Thiazolamine Analogues as Novel Positive Allosteric Modulators of M3 Muscarinic Acetylcholine Receptor. *Chem. Pharm. Bull.* **2021**, *69*, 360–373. [[CrossRef](#)] [[PubMed](#)]
80. Zheng, F.; Wess, J.; Alzheimer, C. Long-Term-But Not Short-Term-Plasticity at the Mossy Fiber-CA3 Pyramidal Cell Synapse in Hippocampus Is Altered in M1/M3 Muscarinic Acetylcholine Receptor Double Knockout Mice. *Cells* **2023**, *12*, 1890. [[CrossRef](#)] [[PubMed](#)]
81. Seeger, T.; Fedorova, I.; Zheng, F.; Miyakawa, T.; Koustova, E.; Gomez, J.; Basile, A.S.; Alzheimer, C.; Wess, J. M2 Muscarinic Acetylcholine Receptor Knock-out Mice Show Deficits in Behavioral Flexibility, Working Memory, and Hippocampal Plasticity. *J. Neurosci.* **2004**, *24*, 10117–10127. [[CrossRef](#)]
82. Erskine, D.; Taylor, J.-P.; Bakker, G.; Brown, A.J.H.; Tasker, T.; Nathan, P.J. Cholinergic Muscarinic M1 and M4 Receptors as Therapeutic Targets for Cognitive, Behavioural, and Psychological Symptoms in Psychiatric and Neurological Disorders. *Drug Discov. Today* **2019**, *24*, 2307–2314. [[CrossRef](#)]
83. Wang, J.; Wu, M.; Chen, Z.; Wu, L.; Wang, T.; Cao, D.; Wang, H.; Liu, S.; Xu, Y.; Li, F.; et al. The Unconventional Activation of the Muscarinic Acetylcholine Receptor M4R by Diverse Ligands. *Nat. Commun.* **2022**, *13*, 2855. [[CrossRef](#)] [[PubMed](#)]
84. Chambers, N.E.; Millett, M.; Moehle, M.S. The Muscarinic M4 Acetylcholine Receptor Exacerbates Symptoms of Movement Disorders. *Biochem. Soc. Trans.* **2023**, *51*, 691–702. [[CrossRef](#)] [[PubMed](#)]
85. Russell, J.K.; Ingram, S.M.; Teal, L.B.; Lindsley, C.W.; Jones, C.K. M1/M4-Preferring Muscarinic Cholinergic Receptor Agonist Xanomeline Reverses Wake and Arousal Deficits in Nonpathologically Aged Mice. *ACS Chem. Neurosci.* **2023**, *14*, 435–457. [[CrossRef](#)] [[PubMed](#)]
86. Popiolek, M.; Mandelblat-Cerf, Y.; Young, D.; Garst-Orozco, J.; Lotarski, S.M.; Stark, E.; Kramer, M.; Butler, C.R.; Kozak, R. In Vivo Modulation of Hippocampal Excitability by M4 Muscarinic Acetylcholine Receptor Activator: Implications for Treatment of Alzheimer's Disease and Schizophrenic Patients. *ACS Chem. Neurosci.* **2019**, *10*, 1091–1098. [[CrossRef](#)] [[PubMed](#)]
87. Araya, R.; Noguchi, T.; Yuhki, M.; Kitamura, N.; Higuchi, M.; Saido, T.C.; Seki, K.; Itohara, S.; Kawano, M.; Tanemura, K.; et al. Loss of M5 Muscarinic Acetylcholine Receptors Leads to Cerebrovascular and Neuronal Abnormalities and Cognitive Deficits in Mice. *Neurobiol. Dis.* **2006**, *24*, 334–344. [[CrossRef](#)] [[PubMed](#)]
88. Wierońska, J.M.; Cieślak, P.; Burnat, G.; Kalinowski, L. Activation of Metabotropic Glutamate Receptor (mGlu2) and Muscarinic Receptors (M1, M4, and M5), Alone or in Combination, and Its Impact on the Acquisition and Retention of Learning in the Morris Water Maze, NMDA Expression and cGMP Synthesis. *Biomolecules* **2023**, *13*, 1064. [[CrossRef](#)] [[PubMed](#)]
89. Dwomoh, L.; Rossi, M.; Scarpa, M.; Khajehali, E.; Molloy, C.; Herzyk, P.; Mistry, S.N.; Bottrill, A.R.; Sexton, P.M.; Christopoulos, A.; et al. M1 Muscarinic Receptor Activation Reduces the Molecular Pathology and Slows the Progression of Prion-Mediated Neurodegenerative Disease. *Sci. Signal.* **2022**, *15*, eabm3720. [[CrossRef](#)]
90. Gatta, V.; Mengod, G.; Reale, M.; Tata, A.M. Possible Correlation between Cholinergic System Alterations and Neuro/Inflammation in Multiple Sclerosis. *Biomedicines* **2020**, *8*, 153. [[CrossRef](#)] [[PubMed](#)]
91. Di Bari, M.; Di Pinto, G.; Reale, M.; Mengod, G.; Tata, A.M. Cholinergic System and Neuroinflammation: Implication in Multiple Sclerosis. *Cent. Nerv. Syst. Agents Med. Chem.* **2016**, *17*, 109–115. [[CrossRef](#)] [[PubMed](#)]
92. Nuzzo, D.; Frinchi, M.; Giardina, C.; Scordino, M.; Zuccarini, M.; De Simone, C.; Di Carlo, M.; Belluardo, N.; Mudò, G.; Di Liberto, V. Neuroprotective and Antioxidant Role of Oxotremorine-M, a Non-Selective Muscarinic Acetylcholine Receptors Agonist, in a Cellular Model of Alzheimer Disease. *Cell Mol. Neurobiol.* **2023**, *43*, 1941–1956. [[CrossRef](#)]
93. Mufson, E.J.; Counts, S.E.; Perez, S.E.; Ginsberg, S.D. Cholinergic System during the Progression of Alzheimer's Disease: Therapeutic Implications. *Expert. Rev. Neurother.* **2008**, *8*, 1703–1718. [[CrossRef](#)]
94. Binning, W.; Hogan-Cann, A.E.; Yae Sakae, D.; Maksoud, M.; Ostapchenko, V.; Al-Onaizi, M.; Matovic, S.; Lu, W.-Y.; Prado, M.A.M.; Inoue, W.; et al. Chronic HM3Dq Signaling in Microglia Ameliorates Neuroinflammation in Male Mice. *Brain Behav. Immun.* **2020**, *88*, 791–801. [[CrossRef](#)] [[PubMed](#)]
95. Teratani, T.; Mikami, Y.; Nakamoto, N.; Suzuki, T.; Harada, Y.; Okabayashi, K.; Hagihara, Y.; Taniki, N.; Kohno, K.; Shibata, S.; et al. The Liver–Brain–Gut Neural Arc Maintains the Treg Cell Niche in the Gut. *Nature* **2020**, *585*, 591–596. [[CrossRef](#)]
96. Wu, Q.; Zhao, M.; Li, D.; He, X.; Zang, W. Cholinergic Drugs Reduce Metabolic Inflammation and Diabetic Myocardial Injury by Regulating the Gut Bacterial Component Lipopolysaccharide-Induced ERK/Egr-1 Pathway. *FASEB J.* **2023**, *37*, 1–21. [[CrossRef](#)] [[PubMed](#)]

97. Sarter, M.; Bruno, J.P. Cortical Cholinergic Inputs Mediating Arousal, Attentional Processing and Dreaming: Differential Afferent Regulation of the Basal Forebrain by Telencephalic and Brainstem Afferents. *Neuroscience* **1999**, *95*, 933–952. [[CrossRef](#)] [[PubMed](#)]
98. Turner, J.; Hughes, L.F.; Toth, L.A. Sleep, Activity, Temperature and Arousal Responses of Mice Deficient for Muscarinic Receptor M2 or M4. *Life Sci.* **2010**, *86*, 158–169. [[CrossRef](#)] [[PubMed](#)]
99. Salín-Pascual, R.J.; Díaz-Muñoz, M.; Rivera-Valerdi, L.; Ortiz-López, L.; Blanco-Centurión, C. Decrease in Muscarinic M2 Receptors from Synaptosomes in the Pons and Hippocampus after REM Sleep Deprivation in Rats. *Sleep. Res. Online* **1998**, *1*, 19–23.
100. Pooladgar, P.; Sakhabakhsh, M.; Taghva, A.; Soleiman-Meigooni, S. Donepezil Beyond Alzheimer’s Disease? A Narrative Review of Therapeutic Potentials of Donepezil in Different Diseases. *Iran. J. Pharm. Res.* **2022**, *21*, e128408. [[CrossRef](#)]
101. Yamada, R.G.; Ueda, H.R. Molecular Mechanisms of REM Sleep. *Front. Neurosci.* **2020**, *13*, 1402. [[CrossRef](#)] [[PubMed](#)]
102. Falk, S.; Lund, C.; Clemmensen, C. Muscarinic Receptors in Energy Homeostasis: Physiology and Pharmacology. *Basic. Clin. Pharmacol. Toxicol.* **2020**, *126*, 66–76. [[CrossRef](#)]
103. Han, M.; Lian, J.; Su, Y.; Deng, C. Cevimeline Co-Treatment Attenuates Olanzapine-Induced Metabolic Disorders via Modulating Hepatic M3 Muscarinic Receptor: AMPK α Signalling Pathway in Female Rats. *J. Psychopharmacol.* **2022**, *36*, 202–213. [[CrossRef](#)] [[PubMed](#)]
104. Yi, J.H.; Whitcomb, D.J.; Park, S.J.; Martinez-Perez, C.; Barbati, S.A.; Mitchell, S.J.; Cho, K. M1 Muscarinic Acetylcholine Receptor Dysfunction in Moderate Alzheimer’s Disease Pathology. *Brain Commun.* **2020**, *2*, fcaa058. [[CrossRef](#)]
105. Basurto, E.; González-Flores, O.; Hoffman, K. Chronic MK-801 Administration Provokes Persistent Deficits in Social Memory in the Prairie Vole (*Microtus Ochrogaster*): A Potential Animal Model for Social Deficits of Schizophrenia. *Behav. Brain Res.* **2024**, *465*, 114948. [[CrossRef](#)]
106. McDonald, A.J. Functional Neuroanatomy of Basal Forebrain Projections to the Basolateral Amygdala: Transmitters, Receptors, and Neuronal Subpopulations. *J. Neurosci. Res.* **2024**, *102*, 25318. [[CrossRef](#)]
107. Zuchner, T.; Schliebs, R.; Perez-Polo, J.R. Down-Regulation of Muscarinic Acetylcholine Receptor M2 Adversely Affects the Expression of Alzheimer’s Disease-Relevant Genes and Proteins. *J. Neurochem.* **2005**, *95*, 20–32. [[CrossRef](#)]
108. Wang, L.; Qu, F.; Yu, X.; Yang, S.; Zhao, B.; Chen, Y.; Li, P.; Zhang, Z.; Zhang, J.; Han, X.; et al. Cortical Lipid Metabolic Pathway Alteration of Early Alzheimer’s Disease and Candidate Drugs Screen. *Eur. J. Med. Res.* **2024**, *29*, 199. [[CrossRef](#)]
109. Jha, D.; Blennow, K.; Zetterberg, H.; Savas, J.N.; Hanrieder, J. Spatial Neurolipidomics-MALDI Mass Spectrometry Imaging of Lipids in Brain Pathologies. *J. Mass. Spectrom.* **2024**, *59*, e5008. [[CrossRef](#)] [[PubMed](#)]
110. Moreno-Rodriguez, M.; Perez, S.E.; Martinez-Gardeazabal, J.; Manuel, I.; Malek-Ahmadi, M.; Rodriguez-Puertas, R.; Mufson, E.J. Frontal Cortex Lipid Alterations During the Onset of Alzheimer’s Disease. *J. Alzheimers Dis.* **2024**, *98*, 1515–1532. [[CrossRef](#)]
111. Rampa, L.; Santangelo, R.; Gaspardone, C.; Cerutti, A.; Magnani, G.; Piscazzi, F.; Sgherzi, G.; Fiore, G.; Filippi, M.; Agosta, F.; et al. Potential Cardiologic Protective Effects of Acetylcholinesterase Inhibitors in Patients With Mild to Moderate Dementia. *Am. J. Cardiol.* **2023**, *200*, 162–170. [[CrossRef](#)] [[PubMed](#)]
112. Vaidya, S.; Guerin, A.A.; Walker, L.C.; Lawrence, A.J. *Clinical Effectiveness of Muscarinic Receptor-Targeted Interventions in Neuropsychiatric Disorders: A Systematic Review*; Springer International Publishing: Berlin/Heidelberg, Germany, 2022; Volume 36, ISBN 0123456789.
113. Levey, A.I.; Edmunds, S.M.; Heilman, C.J.; Desmond, T.J.; Frey, K.A. Localization of Muscarinic M3 Receptor Protein and M3 Receptor Binding in Rat Brain. *Neuroscience* **1994**, *63*, 207–221. [[CrossRef](#)]
114. Scarr, E. Muscarinic Receptors in Psychiatric Disorders—Can We Mimic “Health”? *Neurosignals* **2009**, *17*, 298–310. [[CrossRef](#)]
115. Gomeza, J.; Zhang, L.U.; Kostenis, E.; Felder, C.; Bymaster, F.; Brodtkin, J.; Shannon, H.; Xia, B.; Deng, C.-X.; Wess, J. Enhancement of D1 Dopamine Receptor-Mediated Locomotor Stimulation in M4 Muscarinic Acetylcholine Receptor Knockout Mice. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 10483–10488. [[CrossRef](#)]
116. Myslivecek, J. Two Players in the Field: Hierarchical Model of Interaction between the Dopamine and Acetylcholine Signaling Systems in the Striatum. *Biomedicines* **2021**, *9*, 25. [[CrossRef](#)]
117. Jeon, J.; Dencker, D.; Wörtwein, G.; Woldbye, D.P.D.; Cui, Y.; Davis, A.A.; Levey, A.I.; Schütz, G.; Sager, T.N.; Mørk, A.; et al. A Subpopulation of Neuronal M4 Muscarinic Acetylcholine Receptors Plays a Critical Role in Modulating Dopamine-Dependent Behaviors. *J. Neurosci.* **2010**, *30*, 2396–2405. [[CrossRef](#)]
118. Jakubík, J.; Tuček, S.; El-Fakahany, E.E. Role of Receptor Protein and Membrane Lipids in Xanomeline Wash-Resistant Binding to Muscarinic M₁ Receptors. *J. Pharmacol. Exp. Ther.* **2004**, *308*, 105–110. [[CrossRef](#)]
119. Li, W.; Basile, A.S.; Tong, L.; Van Laere, K.; Masdeu, J.C.; Hostetler, E.D.; Marshall, F.; Uslander, J.M. The Novel M4 PAM PET Tracer [11C] MK-6884: A Novel Biomarker for Measuring Target Engagement of Muscarinic M4 Positive Allosteric Modulators (PAMs) as Well as Cholinergic Tone in Patients with Alzheimer’s Disease. *Alzheimer’s Dement.* **2022**, *18*, e067249. [[CrossRef](#)]
120. Lange, H.S.; Vardigan, J.D.; Cannon, C.E.; Puri, V.; Henze, D.A.; Uslander, J.M. Effects of a Novel M4 Muscarinic Positive Allosteric Modulator on Behavior and Cognitive Deficits Relevant to Alzheimer’s Disease and Schizophrenia in Rhesus Monkey. *Neuropharmacology* **2021**, *197*, 108754. [[CrossRef](#)]
121. d’Orchymont, F.; Narvaez, A.; Raymond, R.; Sachdev, P.; Charil, A.; Krause, S.; Vasdev, N. In Vitro Evaluation of PET Radiotracers for Imaging Synaptic Density, the Acetylcholine Transporter, AMPA-Tarp-I8 and Muscarinic M4 Receptors in Alzheimer’s Disease. *Am. J. Nucl. Med. Mol. Imaging* **2024**, *14*, 1. [[CrossRef](#)]

122. Bender, A.M.; Garrison, A.T.; Lindsley, C.W. The Muscarinic Acetylcholine Receptor M5: Therapeutic Implications and Allosteric Modulation. *ACS Chem. Neurosci.* **2019**, *10*, 1025–1034. [[CrossRef](#)]
123. Zuccolo, E.; Laforenza, U.; Negri, S.; Botta, L.; Berra-Romani, R.; Faris, P.; Scarpellino, G.; Forcaia, G.; Pellavio, G.; Sancini, G.; et al. Muscarinic M5 Receptors Trigger Acetylcholine-Induced Ca²⁺ Signals and Nitric Oxide Release in Human Brain Microvascular Endothelial Cells. *J. Cell Physiol.* **2019**, *234*, 4540–4562. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.