Hypocholinergic Stress and Neuronal Pruning in Alzheimer's Disease

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Abstract

Alzheimer's disease is a neurodegenerative disorder characterized by the loss of memory and cognitive decline. Although the underlying mechanisms that cause Alzheimer's disease remain unknown, research suggests that dysregulation of processes related to neuronal growth and development may play a critical role. This dysregulation leads to a phenomenon called neuronal pruning, a developmental apoptotic process that shapes neural pathways by removing neurons that fail to form viable synaptic connections. While the accumulation of β-amyloid plaques and neurofibrillary tangles contributes to the progression of the disease, the processes that produce these disease markers, specifically the overproduction of amyloid precursor protein and microtubule growth through hyperphosphorylation of tau, also suggest a developmental basis for the disease. This developmental link also coincides with the dual role of acetylcholine, which acts as both a neurotransmitter and a regulator of growth and development. Therefore, it is postulated that risk factors for Alzheimer’s disease contribute to a hypocholinergic status, upsetting neuronal homeostasis, and ultimately resulting in a cascade of programmed neural death similar to developmental neuronal pruning. This theory has significant implications for understanding the initial stages of Alzheimer's disease and may provide a basis for improving preventative and therapeutic interventions.

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Development of cholinergic complexity

Alzheimer's disease is a neurodegenerative disorder that primarily affects the cholinergic nervous system. Therefore, the molecular mechanisms underlying Alzheimer's disease might be linked to the development of acetylcholine as a neurotransmitter. As an adaptation, the evolution of cholinergic neurons represents a major change in the biology of acetylcholine. Acetylcholine is ubiquitously found throughout the tree of life and is known to regulate growth and development (Venter et al., 1988; Wessler & Kirkpatrick, 2008; Spieker et al., 2020). As such, acetylcholine release provides a proximity signal between cells, regulating proliferation and differentiation, or as a chemotaxis trail for cellular migration or axon growth (Wessler & Kirkpatrick, 2008). For cholinergic synapses to evolve, concurrent mechanisms for regulating acetylcholine concentration and distribution had to be established (Burkhardt & Sprecher, 2017). This was partially achieved through the differentiation of cholinergic receptors and cholinesterases (Venter et al., 1988; Johnson & Moore, 2012).

Generally, cholinergic neurons propagate neuronal impulses via ion channel receptors that have a low affinity for acetylcholine (Gilson et al., 2016). These receptors, known as nicotinic receptors, are mainly located in the synaptic cleft (Paterson & Nordberg, 2000). To produce the millimolar concentrations of acetylcholine required to activate nicotinic receptors, acetylcholine breakdown must be highly controlled. To achieve this, acetylcholinesterase, the enzyme mainly associated with synaptic acetylcholine hydrolysis, evolved to be inhibited by acetylcholine at high concentrations (Shafferman et al., 1992). That is, in the initial stages of synaptic acetylcholine release, the enzyme responsible for its breakdown is partially inhibited. This allows acetylcholine to diffuse across the synapse and activate the postsynaptic nicotinic receptors. As the concentration of acetylcholine is reduced through diffusion and hydrolysis, acetylcholinesterase becomes more catalytically active, clearing the synapse for the subsequent release of acetylcholine.

Outside the synapse, acetylcholine must be regulated differently, as most of the body reacts to acetylcholine through muscarinic receptors that can be responsive to nanomolar concentrations of acetylcholine (Gilson et al., 2016). Muscarinic receptors are coupled to G-protein cascades that control a multitude of cellular functions such as growth, differentiation, and apoptosis (Caulfield, 1993; Neves, 2002; Eglen, 2006). Owing to their physiological role, the escape of high concentrations of acetylcholine from synapses could be detrimental to processes mediated by muscarinic receptors. To deal with potential synaptic seepage, the other major cholinesterase found systemically in vertebrates (Johnson & Moore 2012; Petrov et al., 2014), commonly known as butyrylcholinesterase, has evolved as a substrate-activated enzyme, where at higher concentrations its catalytic rate increases (Masson et al., 2001; Chen et al., 2012). The main function of butyrylcholinesterase seems to be the maintenance of basal background levels of acetylcholine, allowing more sensitive muscarinic receptors to respond to acetylcholine concentrations in the micromolar to nanomolar range (Gilson et al., 2015).

This regional separation of effective acetylcholine concentration, which can vary by up to a hundred thousand-fold when comparing muscarinic and nicotinic receptor affinities, allowed acetylcholine to take on the role of a neurotransmitter while maintaining its role in growth and development (Resende & Adhikari, 2009). This biological change in cholinesterase activity also permitted further diversification of the physiological role of acetylcholine into functions such as the regulation of epithelial cells in the respiratory system (Kummer et al., 2008), insulin release (Gautam et al., 2006), and immune responses throughout the body (Cox et al., 2019; Cox et al., 2020; Wessler & Kirkpatrick, 2020).

From a simplified view of growth regulation, acetylcholine can be considered as a signal that prevents cellular propagation by alerting cells to the proximity of their neighbors. This phenomenon has been observed throughout the tree of life (Venter et al., 1988; Wessler & Kirkpatrick, 2008; Spieker et al., 2020); however, in higher organisms, the presence of a nervous system introduces another degree of complexity to the organization of cellular growth.

Neural networks are produced through competition to establish viable synaptic connections between neurons that grow along the network pathways. These competitions start with more potential neurons than necessary, resulting in the preservation of neurons that form successful synaptic connections and the apoptotic pruning of those that do not (Haanen & Vermes, 1996; Hughes 1961; Blaschke et al., 1998). Synaptic connectivity and activity are major positive reinforcements required for neuronal viability (Resende & Adhikari, 2009). As a result, acetylcholine is a key regulator of the growth, maturation, and stabilization of cholinergic neurons and neural networks.

Alzheimer’s disease and developmental processes

In Alzheimer’s disease, neuronal death has been associated with the accumulation of amyloid plaques and neurofibrillary tangles. However, in late-onset Alzheimer’s disease, the accumulation of plaques and tangles is preceded by neuronal atrophy and mild cognitive impairment (Edmonds et al. 2016; Thomas et al. 2020). The significance of this neurodegeneration is recognized by its inclusion alongside amyloid deposition and neurofibrillary tangles as a biomarker for Alzheimer’s disease by the National Institute on Aging and the Alzheimer’s Association in their recent research framework for diagnosing Alzheimer’s disease (Jack et al., 2018). This initial neurodegeneration suggests that the progression of late-onset Alzheimer’s disease is distinct from familial forms of the disorder, which are genetically linked to amyloid plaque formation. Familial forms of Alzheimer’s disease generally result from mutations that encourage the formation of β-amyloid, indicating a direct link between neurodegeneration and β-amyloid production (Andrade-Guerrero et al., 2023). However, the presence of neurodegeneration before or in the complete absence of β-amyloid plaques, combined with the discovery of normal individuals with a heavy plaque burden, suggests a weaker association between plaque formation and the initiation of late-onset Alzheimer’s disease (Kim et al., 2022; Kok et al., 2022).

Studies on the potential mechanisms underlying the observed neuronal atrophy suggest that the same muscarinic receptor-mediated immunological processes involved in developmental neuronal pruning are involved in Alzheimer’s disease neuronal apoptosis (Resende and Adhikari, 2009; Kole et al., 2013; Luchena et al., 2018). A role for developmental processes in Alzheimer’s disease is also supported by changes in the neurogenic processes of patients,

observed as altered hippocampal neurotrophic factor production (Sampaio et al., 2017) and decreased adult neurogenesis by hippocampal stem cells (Choi & Tanzi, 2019). The regulatory similarity between neurodegeneration and developmental processes suggests that acetylcholine's role as a regulator of cholinergic neuronal development (Resende and Adhikari, 2009) may be central to the presentation of Alzheimer's disease.

Developmental processes in Alzheimer's disease may also provide a rationale for the accumulation of both neurofibrillary tangles and β-amyloid in late-onset Alzheimer's disease. During development, neuronal growth requires microtubule assembly to support axon elongation (Arendt et al., 2016). This is associated with high phosphorylation of the Tau protein, which regulates microtubule assembly (Goode and Feinstein, 1994; Brion et al., 1993). Tau hyperphosphorylation early in Alzheimer's disease mimics developmental microtubule assembly in growing axons, but when present in mature neurons, it results in the aggregation of neurofibrillary tangles (Brion et al., 1993; Arendt et al., 2016). Stimulation of M1 muscarinic receptors, the most abundant muscarinic receptors in the central nervous system, reduces tau phosphorylation (Forlenza et al., 2000; Dwomoh, Tejeda, & Tobin, 2022), suggesting that acetylcholine signaling stabilizes microtubules, while low hypocholinergic levels of acetylcholine, common during development, promote growth.

Similarly, neuronal expression and processing of the amyloid precursor protein is strongly linked to developmental processes through its regulation by nerve growth factor (Roßner et al., 1998) and through its modulation by the M1 muscarinic receptor (Fisher 2012; Dwomoh, Tejeda & Tobin 2022). This matches the observed physiological role of the amyloid precursor protein as a cell adhesion molecule involved in neural growth and synaptogenesis (Roßner et al., 1998; Müller, Deller & Korte, 2017). Animal models have shown that stimulation of the M1 receptor reduces the formation of neurofibrillary tangles and amyloid plaques, while M1 knockout models or inhibition of the M1 receptor promotes neurodegenerative processes (Dwomoh, Tejeda & Tobin 2022). These observations suggest that high expression of the amyloid precursor protein results from hypocholinergic conditions, such as those present during the development of the nervous system.

Hypocholinergic status and neuronal pruning

Hypocholinergic states, such as those found during development, stimulate neuronal growth, but without eventual positive feedback through successful synaptogenesis, result in apoptosis. Similarly, prolonged hypocholinergic stress likely reduces positive feedback in mature neural networks, thereby promoting growth and apoptosis (Russell, 1996). Russell et al. (1992) demonstrated that rat models of hypocholinergic stress produced long-lasting cognitive deficits, suggesting that chronic hypocholinergic states could lead to dementia (Russell, 1996). Similarly, patients prescribed drugs known to produce hypocholinergic states, termed anticholinergic burdens, have an increased risk of all forms of dementia, including Alzheimer's disease (Pfistermeister et al., 2017; Zheng et al., 2021; Meng et al., 2022; Russo et al., 2022). Barrett et al. (2021) have suggested that deprescribing anticholinergic drugs or limiting anticholinergic exposures may be warranted to reduce the future risk of dementia in Parkinson disease patients who are commonly prescribed anticholinergic drugs.

The effects of anticholinergic burden, combined with the evidence that the best treatment for Alzheimer's disease,

although considered symptomatic, has consistently been the inhibition of cholinesterases (Kim et al., 2022; Moss & Perez, 2021), suggest that a hypocholinergic state may be the cause of the disease. The minimal effectiveness of cholinesterase inhibitors as a therapeutic intervention for halting or reversing Alzheimer's disease may be more of a reflection of their inability to reverse the long-term accumulation of damage caused by hypocholinergic stress (Russel, 1996), rather than a failure to address the mechanism of the disease. This is supported by evidence that cholinesterase inhibition reduces atrophy and cognitive decline in patients with mild cognitive impairment, delaying the progression of Alzheimer's disease (Moss & Perez, 2021; Xu et al., 2021).

A hypocholinergic state in the nervous system likely destabilizes neuronal integrity by activating developmental processes and promoting axonal growth and synaptogenesis. This encourages the production of neurofibrillary tangles as the axons are stimulated to grow and overexpression of amyloid precursor protein as the neurons are triggered to form new synapses. Without proper acetylcholine feedback reinforcement to stabilize neuronal connectivity, neurons undergo apoptosis (Knorr et al., 2023), mimicking developmental neuronal pruning (Kole et al., 2013). Additionally, overproduction of amyloid precursor protein increases its cleavage into β-amyloid, which aggregates into amyloid plaques, further damaging the surrounding tissue.

While it might be expected that acetylcholine production decreases as the cholinergic neurons producing it die off, this does not directly suggest an explanation as to how hypocholinergic stress might trigger the neurodegeneration that results in late-onset Alzheimer's disease.

Cholinesterase biology potentially explains how the central nervous system may be pushed towards a hypocholinergic state. As previously mentioned, acetylcholinesterase and butyrylcholinesterase are subject to substrate modulation by acetylcholine (Ordentlich et al., 1993; Tormos et al., 2005). This makes them susceptible to compounds that mimic substrate modulation. Butyrylcholinesterase, which is activated by acetylcholine, has also been found to be activated by the metabolic side product homocysteine thiolactone (Darvesh et al. 2007, Walsh et al., 2007). This stimulation of cholinesterase activity, through substrate activation mimicry, provides a potential link between many genetic, bacterial, and nutritional risk factors associated with late-onset Alzheimer's disease. Hypocholinergic stress produced by butyrylcholinesterase stimulation may directly promote neurodegeneration, which commonly proceeds amyloidosis. This may likewise explain the neuroprotective effects of cholinesterase inhibitors, reducing amyloid and neurofibrillary tangles (Moss & Perez 2021; Moreira et al., 2022), which mirrors the neuroprotective effects produced by stimulating the M1 muscarinic receptor (Dwomoh, Tejeda & Tobin 2022).

Risk factors linking hypocholinergic stress to Alzheimer's disease

Metabolic and genetic risk factors support the involvement of hypocholinergic stress in the progression of Alzheimer's disease. Genetic risk factors and nutritional deficiencies that affect the build-up of homocysteine have been recognized in the one-carbon theory, which suggests that homocysteine build-up may be central to the development of Alzheimer's (Clare et al., 2019). Similarly, Alzheimer's has been associated with bacterial gut floral infections that produce increased

homocysteine levels (Kountouras et al., 2023). Risk factors related to increased homocysteine levels have also been associated with the production of homocysteine thiolactone (Jakubowski 2023). This homocysteine thiolactone production, in turn, links risk factors that affect its breakdown to Alzheimer's disease (Jakubowski 2023), further suggesting a link to hypocholinergic stress through homocysteine thiolactone stimulation of butyrylcholinesterase (Darvesh et al. 2007).

Many mutations that reduce the activity of butyrylcholinesterase have been described; however, the K variant, which reduces butyrylcholinesterase activity by 33%, has been directly implicated in the risk of Alzheimer's disease (Lehmann et al., 1997; McIlroy et al., 2000). However, the link between butyrylcholinesterase activity and Alzheimer's disease is controversial, as some studies suggest that the K variant is only a risk factor when associated with the APOE e4 allele (Lehmann et al., 1997; Lane et al., 2008; Vijayaraghavan et al., 2018), whereas other studies have suggested that the K variant is protective (Holmes et al., 2005; Vijayaraghavan et al., 2018) and fortifies against mild cognitive impairment (Pongthanaracht et al. 2017). Complementing the view that reduced cholinesterase activity may be protective are studies suggesting the wild-type form of butyrylcholinesterase may increase the risk for Alzheimer's disease (Ferris et al., 2009; Mueller and Adler 2015).

Additionally, decreased butyrylcholinesterase plasma activity has been proposed as a diagnostic indicator for the amnestic-mild cognitive impairment preceding Alzheimer's disease (Kozlova et al., 2022). This decrease in activity may correspond to miRNA feedback control, which is known to downregulate butyrylcholinesterase while favoring acetylcholine release in response to hypocholinergic stress (Soreq, 2015). This decreased cholinesterase activity was shown to be alleviated by the administration of Citicoline (Kozlova et al., 2022), a cholinergic stimulant, further suggesting that the downregulation of butyrylcholinesterase may correspond to hypocholinergic stress. The same group also reported an even greater (38%) decrease in butyrylcholinesterase activity with the onset of dementia (Zhuravin et al., 2015). This contradicts a study in which no change in butyrylcholinesterase activity was observed and decreased butyrylcholinesterase activity was linked to Dementia with Lewy bodies (Josviak et al., 2017). However, in the latter study, Alzheimer's patients were using the acetylcholinesterase inhibitor donepezil, which is also known to inhibit butyrylcholinesterase (Darvesh et al., 2003). Thus, the inhibition may have induced an increase in cholinesterase production in response.

As previously stated, butyrylcholinesterase activity is stimulated by homocysteine thiolactone, in a manner similar to substrate stimulation (Darvesh et al., 2007). Homocysteine thiolactone is a cyclized form of homocysteine, a non-genetically encoded amino acid (Jakubowski, 2019). Elevated homocysteine levels, known as hyperhomocysteinemia, are a risk factor for Alzheimer's disease (Seshadri et al., 2002). Mild hyperhomocysteinemia, which occurs in 5%–7% of the population (Son and Lewis, 2021), has also been identified as a risk factor for the development of Alzheimer's disease (Hu et al., 2016). Hyperhomocysteinemia is associated with mutations that affect homocysteine catabolism and folic acid deficiencies, which also decrease homocysteine breakdown (Jakubowski, 2019).

Although primarily associated with folic acid deficiencies, hyperhomocysteinemia can also result from insufficient levels of B12 (Ubbink et al., 1994). Both folic acid and vitamin B12, members of the folate vitamins, are essential in the conversion of homocysteine to methionine by methionine synthase, where vitamin B12 is used as a cofactor in catalysis and folic acid

is used to regenerate B12 (Lauer et al., 2022).

While there seems to be a clear link between Alzheimer's disease and folate deficiencies (Snowdon et al., 2000; Wang et al., 2001; Smith and Refsum, 2016), the link between Alzheimer's disease and mutations affecting homocysteine catabolism is more tenuous (Roostaei et al., 2018; Lehmann and Cortina-Borja, 2019), potentially due to their lethality and co-association with developmental problems such as Down syndrome and neural tube defects (Clare et al., 2019). However, the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism, which phenotypically presents as a folate deficiency increasing homocysteine levels, has consistently been associated with increased susceptibility to Alzheimer's disease (Rai, 2017). Elevated homocysteine levels have been shown to result in the metabolic byproduct homocysteine thiolactone through a cyclization catalyzed by methionyl-tRNA synthetase (Jakubowski, 2019). Conversely, the breakdown of homocysteine thiolactone has been linked to three enzymes, paraoxonase 1, bleomycin hydrolase, and valacyclovir hydrolase (Jakubowski, 2019). Paraoxonase 1 is found extracellularly, whereas bleomycin hydrolase is expressed in the cytoplasm and valacyclovir hydrolase is produced in the mitochondria (Jakubowski, 2019). The extracellular localization of paraoxonase 1 has a primary influence on extracellular homocysteine thiolactone levels, where mutations such as Q192R, which decrease its activity, result in higher serum concentrations (Perła-Kaján et al., 2018). Studies linking Q192R to Alzheimer's have been contested, as meta-analysis suggests no linkage (Nie et al., 2017). However, studies on mutations affecting the expression of paraoxonase 1 (Brophy et al., 2001) have shown a link between lower production of the enzyme and susceptibility to Alzheimer's disease and, conversely, high production and protective effects (Nie et al., 2017). This is supported by studies that have found lower paraoxonase 1 activity in the serum and cerebrospinal fluid of Alzheimer's patients (Romani et al., 2020). Similarly, the I443V mutation, which reduces the activity of bleomycin hydrolase (O'Farrell et al., 1999), has been associated with an increased risk of Alzheimer's disease (Papassotiropoulos et al., 2000). This is supported by studies relating decreased bleomycin hydrolase activity in the brain tissue of Alzheimer's patients to the build-up of N-linked protein homocysteine (Suszynska et al., 2010), a sign of homocysteine thiolactone production and another detrimental consequence of homocysteine thiolactone accumulation.

The APOE e4 allele of apolipoprotein is believed to be the greatest risk factor for late-onset Alzheimer's (Dumurgier and Tzourio 2020). However, apolipoprotein E plays a central role in lipid transport throughout the body, making it a significant regulator of growth and metabolic homeostasis. As such, disruption of lipid transport produced by APOE variants is not specific to Alzheimer's disease but produces significant widespread effects that enhance the degenerative effects of a host of disorders (Martínez-Martínez et al., 2020; Miao et al., 2023).

Alzheimer's disease has also been associated with intestinal disorders and bacterial infections (Fu, Gao & Yung, 2019). One of the most striking associations is related to *Helicobacter pylori* infections, which are believed to infect up to half of the global population and has been linked to hyperhomocysteinemia-related brain cortical thinning (Kountouras et al., 2023). *Helicobacter pylori* infections contribute to vitamin B12 and folate deficiencies by reducing their absorption (Kountouras et al., 2007).

Hyperhomocysteinemia-related cortical thinning related to *Helicobacter pylori* infections has been found to occur up to a decade before the onset of cognitive impairment in asymptomatic elderly populations (Tan et al., 2018; Kountouras et al.,

2023). Most carriers are infected during childhood, with the infection persisting their entire life if untreated (Kountouras et al., 2023).

Additionally, Alzheimer's disease is associated with a reduction in gut microbiota diversity (Wu et al., 2021). This reduction has been correlated with a decrease in bacteria that produce short-chain fatty acids, specifically butyrate (Wu et al., 2021). Butyrate has been shown to reduce neuroinflammation, promote neuronal maturation, promote cellular growth, and has been suggested to have a protective role in other neurodegenerative diseases, such as Parkinson's disease (Cook & Sellin, 1998; Ahmed et al., 2019; Mohamed Elfadil et al., 2023). Butyrate can be produced at levels as high as 10mM in the gut (Cook & Sellin, 1998), and there is some indication that a limited amount of butyrate is converted to butyrylcholine, potentially modulating acetylcholine receptors in the colon (Moreno et al., 2016). Butyrate easily passes through the blood-brain barrier (Oldendorf 1973) and has been shown to increase acetylcholinesterase activity up to 10-fold in mouse neuroblastoma models (Prasad & Vernadakis, 1972), and 6-fold in human cholinergic neuroblastoma cell lines (Casper & Davies, 1989). It is unknown whether butyrate affects the activity of butyrylcholinesterase; however, butyrylcholinesterase is less restrictive in its substrate specificity than acetylcholinesterase (Brestkin et al., 1983). Butyrylcholinesterase is known to hydrolyze a wide range of acylcholine substrates (Kinchen et al., 2021) and has a higher affinity for butyrylcholine than for acetylcholine. This preference gives the enzyme its current name, having previously been known as pseudocholinesterase. The affinity of butyrylcholinesterase for butyrylcholine suggests a natural affinity for butyrate, which is supported by the use of butyrate to stabilize the enzyme when the crystal structure was determined (Nicolet et al., 2003; Tormos et al., 2005). Whether butyrate affects the enzymatic activity of butyrylcholinesterase is unknown; however, as a product formed by the hydrolysis of butyrylcholine, butyrate may inhibit butyrylcholinesterase activity through product inhibition, reducing its ability to hydrolyze acetylcholine and thus reducing hypocholinergic stress.

It is also interesting to speculate on the potential role quorum signal molecules, specifically the role acyl-homoserine lactones, may play in bacterial-mediated susceptibility to Alzheimer's disease. Acyl-homoserine lactone signaling regulates the establishment and severity of bacterial infections (Kanojiya, Banerji & Saroj, 2022). Acylation of the homoserine lactone ring can vary from 4 to 14 carbon atoms in length (Kanojiya, Banerji & Saroj, 2022). Similar to homocysteine thiolactone, the nonacylated homoserine lactone molecule stimulates human butyrylcholinesterase (Darvesh, Walsh & Martin, 2007), and similar to homocysteine thiolactone, acylhomoserine lactones are hydrolyzed by the paraoxonase family of enzymes (Manco, Porzio & Carusone, 2021; Jeelani, Tabassum & Rashid, 2020). Hydrolysis by paraoxonases destroys the lactone ring, resulting in an acyl-functionalized serine.

Currently, an amidase capable of releasing homoserine lactone has not been identified in humans; however, cholinesterases are known to have amidase activity (Moore & Hess, 1975; Darvesh et al., 2006), suggesting that cholinesterases or other enzymes that are catalytically promiscuous may be able to release homoserine lactones from acyl-homoserine lactones.

Conclusions

Many theories have been proposed to explain Alzheimer's disease; however, none of them provides a cohesive logical explanation of the progression and complexity of the disorder (Liu et al., 2019). The failure of these theories to provide beneficial therapeutic impacts has been attributed to a lack of understanding of the underlying pathophysiology of Alzheimer's disease (Kim et al., 2022). The hypothesis that Alzheimer's disease results from neuronal pruning induced by chronic hypocholinergic stress provides a novel framework for rationalizing clinical and basic scientific observations. Chronic hypocholinergic stress potentially explains why cholinesterase inhibition, primarily considered a symptomatic treatment, has remained the primary therapeutic choice for this disease and has been observed to modify neurodegenerative processes at work in Alzheimer's disease. Specifically, cholinesterase inhibitors appear to have a pronounced effect in patients with mild cognitive impairment, effectively delaying the onset of Alzheimer's disease (Petersen et al., 2005; Shanks et al., 2009), while also reducing cortical atrophy in the white matter of patients with Alzheimer's disease (Venneri & Lane, 2009). Hypocholinergic neuronal pruning also provides a basic logical mechanism that can be expanded into a comprehensive explanation for the complexity of Alzheimer's disease.

Stimulation of butyrylcholinesterase by homocysteine thiolactone provides the basis for chronic hypocholinergic stress. This disrupts neuronal homeostasis by removing acetylcholine-based positive feedback, altering neurotropic factor expression, and favouring neuronal pruning. Consequently, these altered growth signals increase amyloid precursor protein expression and increase Tau phosphorylation, resulting in the neuropathic debris of plaques and tangles (Figure 1).

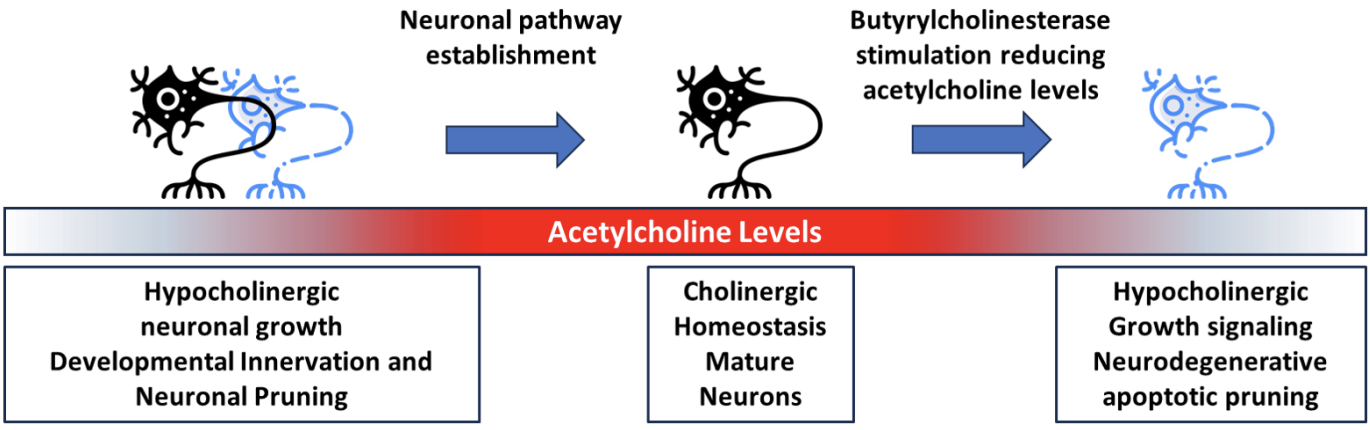

Figure 1. Hypocholinergic status results in neuronal growth signaling, which, during the generation of Alzheimer's disease, is related to increased cholinesterase activity through the stimulation of butyrylcholinesterase by homocysteine thiolactone.

In conclusion, the theory of neuronal pruning through hypocholinergic stress suggests that cholinesterase inhibition is more than just a symptomatic treatment but may be of less use when damage has progressed as far as the formation of neurofibrillary tangles and amyloid plaques. It also links the susceptibility of late-onset Alzheimer's disease to nutritional and environmental influences, suggesting that early therapeutic intervention and preventive measures should prioritize nutritional and probiotic approaches. However, it will be interesting to see if earlier therapeutic interventions based on the use of cholinesterase inhibitors in individuals with mild cognitive impairment or cortical thinning will improve outcomes with

Alzheimer's disease and other neurodegenerative disorders. It will also be interesting to determine if the modulation of cholinesterase activity can be adapted to neuronal regenerative approaches.

References

- Ahmed S, Busetti A, Fotiadou P, Vincy Jose N, Reid S, Georgieva M, Brown S, Dunbar H, Beurket-Ascencio G, Delday MI, Ettorre A. In vitro characterization of gut microbiota-derived bacterial strains with neuroprotective properties. Frontiers in cellular neuroscience. 2019 Sep 20;13:402.
- Andrade-Guerrero J, Santiago-Balmaseda A, Jeronimo-Aguilar P, Vargas-Rodríguez I,
- Cadena-Suárez AR, Sánchez-Garibay C, Pozo-Molina G, Méndez-Catalá CF,
- Cardenas-Aguayo MD, Diaz-Cintra S, Pacheco-Herrero M. Alzheimer's Disease: An
- Updated Overview of Its Genetics. International Journal of Molecular Sciences.
- 2023 Feb 13;24(4):3754.
- Arendt T, Stieler JT, Holzer M. Tau and tauopathies. Brain research bulletin. 2016 Sep
- 1;126:238-92.
- Barrett MJ, Sargent L, Nawaz H, Weintraub D, Price ET, Willis AW. Antimuscarinic anticholinergic medications in Parkinson disease: to prescribe or deprescribe?.
- Movement Disorders Clinical Practice. 2021 Nov;8(8):1181-8.
- Blaschke AJ, Weiner JA, Chun J. 1998. Programmed cell death is a universal feature of embryonic and postnatal neuroproliferative regions throughout the central nervous system. J Comp Neurol. 1998 Jun 22;396(1):39-50
- Brestkin AP, Rozengart EV, Abduvakhabov AA, Sadykov AA. Carboxylic acid esters as substrates of cholinesterases. Russian Chemical Reviews. 1983 Oct 31;52(10):931.
- Brion, J.P., Smith, C., Couck, A.M., Gallo, J.M., Anderton, B.H., 1993. Developmental changes in τ phosphorylation: fetal τ is transiently phosphorylated in a manner similar to paired helical filament- τ characteristic of Alzheimer's disease. J.
- Neurochem. 61 (6), 2071–2080. http://dx.doi.org/10.1111/j.1471-
- 4159.1993.tb07444.x.
- Brophy VH, Jampsa RL, Clendenning JB, McKinstry LA, Jarvik GP, Furlong CE. Effects of 5′ regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. The American journal of human genetics. 2001 Jun 1;68(6):1428-36.
- Burkhardt P, Sprecher SG. Evolutionary origin of synapses and neurons–Bridging the gap. BioEssays. 2017 Oct;39(10):1700024.
- Casper D, Davies P. Stimulation of choline acetyltransferase activity by retinoic acid and sodium butyrate in a cultured human neuroblastoma. Brain research. 1989 Jan
- 23;478(1):74-84.
- Caulfield MP. Muscarinic receptors—characterization, coupling and function.
- Pharmacology & therapeutics. 1993 Jan 1;58(3):319-79.

- Chen X, Fang L, Liu J, Zhan CG. Reaction pathway and free energy profiles for butyrylcholinesterase-catalyzed hydrolysis of acetylthiocholine. Biochemistry. 2012 Feb 14;51(6):1297-305.
- Choi SH, Tanzi RE. Is Alzheimer's disease a neurogenesis disorder?. Cell stem cell. 2019 Jul 3;25(1):7-8.
- Clare CE, Brassington AH, Kwong WY, Sinclair KD. One-carbon metabolism: linking nutritional biochemistry to epigenetic programming of long-term development.
- Annual review of animal biosciences. 2019 Feb 15;7:263-87.
- Cook SI, Sellin JH. Short chain fatty acids in health and disease. Alimentary pharmacology & therapeutics. 1998 Jun 1;12(6):499-507.
- Cox MA, Bassi C, Saunders ME, Nechanitzky R, Morgado-Palacin I, Zheng C, Mak TW. Beyond neurotransmission: acetylcholine in immunity and inflammation. Journal of internal medicine. 2020 Feb;287(2):120-33.
- Cox MA, Duncan GS, Lin GH, Steinberg BE, Lisa XY, Brenner D, Buckler LN, Elia AJ, Wakeham AC, Nieman B, Dominguez-Brauer C. Choline acetyltransferase–expressing T cells are required to control chronic viral infection. Science. 2019 Feb 8;363(6427):639-44.
- Darvesh S, McDonald RS, Darvesh KV, Mataija D, Mothana S, Cook H, Carneiro KM, Richard N, Walsh R, Martin E. On the active site for hydrolysis of aryl amides and choline esters by human cholinesterases. Bioorganic & medicinal chemistry. 2006 Jul 1;14(13):4586-99.
- Darvesh S, Walsh R, Kumar R, Caines A, Roberts S, Magee D, Rockwood K, Martin E.
- Inhibition of human cholinesterases by drugs used to treat Alzheimer disease.
- Alzheimer Disease & Associated Disorders. 2003 Apr 1;17(2):117-26.
- Darvesh S, Walsh R, Martin E. Homocysteine thiolactone and human cholinesterases.
- Cellular and Molecular Neurobiology. 2007 Jan 1;27(1):33-48.
- Dumurgier J, Tzourio C. Epidemiology of neurological diseases in older adults. Revue neurologique. 2020 Nov 1;176(9):642-8.
- Dwomoh L, Tejeda GS, Tobin AB. Targeting the M1 muscarinic acetylcholine receptor in Alzheimer's disease. Neuronal Signaling. 2022 Apr;6(1):NS20210004.
- Edmonds EC, Eppig J, Bondi MW, Leyden KM, Goodwin B, Delano-Wood L, McDonald
- CR, Alzheimer's Disease Neuroimaging Initiative. Heterogeneous cortical atrophy patterns in MCI not captured by conventional diagnostic criteria. Neurology. 2016 Nov 15;87(20):2108-16.
- Eglen RM. Muscarinic receptor subtypes in neuronal and non-neuronal cholinergic function. Autonomic and Autacoid Pharmacology. 2006 Jul;26(3):219-33.
- Ferris S, Nordberg A, Soininen H, Darreh-Shori T, Lane R. Progression from Mild Cognitive Impairment to Alzheimer's disease: effects of gender, butyrylcholinesterase genotype and rivastigmine treatment. Pharmacogenetics and genomics. 2009 Aug;19(8):635.
- Fisher A. Cholinergic modulation of amyloid precursor protein processing with emphasis on M1 muscarinic receptor: perspectives and challenges in treatment of Alzheimer's disease. Journal of neurochemistry. 2012 Jan;120:22-33.
- Forlenza OV, Spink JM, Dayanandan R, Anderton BH, Olesen OF, Lovestone S. Muscarinic agonists reduce tau phosphorylation in non-neuronal cells via GSK-3β inhibition and in neurons. Journal of neural transmission. 2000

Oct;107:1201-12.

- Fu P, Gao M, Yung KK. Association of intestinal disorders with Parkinson's disease and
- Alzheimer's disease: a systematic review and meta-analysis. ACS Chemical Neuroscience. 2019 Dec 26;11(3):395-405.
- Gautam D, Han SJ, Hamdan FF, Jeon J, Li B, Li JH, Cui Y, Mears D, Lu H, Deng C,
- Heard T. A critical role for β cell M3 muscarinic acetylcholine receptors in regulating insulin release and blood glucose homeostasis in vivo. Cell metabolism. 2006 Jun
- 1;3(6):449-61.
- Gilson MK, Liu T, Baitaluk M, Nicola G, Hwang L, Chong J. BindingDB in 2015: a public database for medicinal chemistry, computational chemistry and systems pharmacology. Nucleic acids research. 2016 Jan 4;44(D1):D1045-53.
- Goode, B.L., Feinstein, S.C., 1994. Identification of a novel microtubule binding and assembly domain in the developmentally regulated inter-repeat region of tau. J. Cell Biol. 124, 769–782. http://dx.doi.org/10.1083/jcb.124.5.769.
- Haanen C, Vermes I. Apoptosis: programmed cell death in fetal development. 1996. Eur
- J Obstet Gynecol Reprod Biol. 1996 Jan;64(1):129-33
- Holmes C, Ballard C, Lehmann D, Smith AD, Beaumont H, Day IN, Khan MN, Lovestone S, McCulley M, Morris CM, Munoz DG. Rate of progression of cognitive decline in Alzheimer's disease: effect of butyrylcholinesterase K gene variation.
- Journal of Neurology, Neurosurgery & Psychiatry. 2005 May 1;76(5):640-3.
- Hu Q, Teng W, Li J, Hao F, Wang N. Homocysteine and Alzheimer's disease: evidence for a causal link from Mendelian randomization. Journal of Alzheimer's Disease. 2016 Jan 1;52(2):747-56.
- Hughes A. Cell degeneration in the larval ventral horn of Xenopus laevis (Daudin). J
- Embryol Exp Morphol. 1961 Jun;9:269-84
- Jack Jr CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E. NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimer's & Dementia. 2018 Apr 1;14(4):535-62.
- Jakubowski H. Homocysteine modification in protein structure/function and human disease. Physiological reviews. 2019 Jan 1;99(1):555-604.
- Jakubowski H. Proteomic exploration of paraoxonase 1 function in health and disease.
- International journal of molecular sciences. 2023 Apr 24;24(9):7764.
- Jeelani H, Tabassum N, Rashid F. Association of paraoxonase1 enzyme and its genetic single nucleotide polymorphisms with cardio-metabolic and neurodegenerative diseases. Gene Reports. 2020 Sep 1;20:100775.
- Johnson G, Moore SW. Why has butyrylcholinesterase been retained? Structural and functional diversification in a duplicated gene. Neurochemistry international. 2012 Oct 1;61(5):783-97.
- Josviak ND, Batistela MS, Souza RK, Wegner NR, Bono GF, Sulzbach CD, Simão-Silva
- DP, Piovezan MR, Souza RL, Furtado-Alle L. Plasma butyrylcholinesterase activity: a possible biomarker for differential diagnosis between Alzheimer's disease and dementia with Lewy bodies?. International Journal of Neuroscience. 2017 Dec 2;127(12):1082-6.

- Kanojiya P, Banerji R, Saroj SD. Acyl Homoserine Lactone in Interspecies Bacterial
- Signaling. Microbiology and Biotechnology Letters. 2022;50(1):1-4.
- Kim CK, Lee YR, Ong L, Gold M, Kalali A, Sarkar J. Alzheimer's disease: Key insights from two decades of clinical trial failures. Journal of Alzheimer's Disease. 2022 Jan
- 1;87(1):83-100.
- Kinchen JM, Mohney RP, Pappan KL. Long-chain acylcholines link butyrylcholinesterase to regulation of non-neuronal cholinergic signaling. Journal of proteome research. 2021 Nov 11;21(3):599-611.
- Knorr DY, Demirbas D, Heinrich R. Multifaceted promotion of apoptosis by acetylcholinesterase. Frontiers in Cell Death. 2023.
- Kok FK, van Leerdam SL, de Lange E. Potential mechanisms underlying resistance to dementia in non-demented individuals with Alzheimer's disease neuropathology.
- Journal of Alzheimer's Disease. 2022 Jan 1(Preprint):1-31.
- Kole AJ, Annis RP, Deshmukh M. Mature neurons: equipped for survival. Cell death & disease. 2013 Jun;4(6):e689.
- Kountouras J, Doulberis M, Papaefthymiou A, Polyzos SA, Zavos C, Kazakos E,
- Arapoglou S, Kyrailidi F, Mouratidou MC, Boziki M, Vardaka E. Controlling the
- Impact of Helicobacter pylori-Related Hyperhomocysteinemia on
- Neurodegeneration. Medicina. 2023 Mar 4;59(3):504.
- Kountouras J, Gavalas E, Boziki M, Zavos C. Helicobacter pylori may be involved in cognitive impairment and dementia development through induction of atrophic gastritis, vitamin B-12–folate deficiency, and hyperhomocysteinemia sequence. The
- American journal of clinical nutrition. 2007 Sep 1;86(3):805-6.
- Kozlova DI, Khizha VV, Anosova LV, Korolkova AA, Vasilev DS, Rybakov AV,
- Pakhomov KV, Shishkin AB, Sumina SV, Ballyzek MF. A New Diagnostic Index Based on the Activity of Butyrylcholinesterase Isoforms for Laboratory Confirmation of Mild Cognitive Impairment Diagnosis. Journal of Evolutionary Biochemistry and
- Physiology. 2022 May;58(3):885-93.
- Kummer W, Lips KS, Pfeil U. The epithelial cholinergic system of the airways.
- Histochemistry and cell biology. 2008 Aug 1;130(2):219.
- Lane R, Feldman HH, Meyer J, He Y, Ferris SH, Nordberg A, Darreh-Shori T, Soininen
- H, Pirttilä T, Farlow MR, Sfikas N. Synergistic effect of apolipoprotein E ε4 and butyrylcholinesterase K-variant on progression from mild cognitive impairment to
- Alzheimer's disease. Pharmacogenetics and genomics. 2008 Apr 1;18(4):289-98.
- Lauer AA, Grimm HS, Apel B, Golobrodska N, Kruse L, Ratanski E, Schulten N, Schwarze L, Slawik T, Sperlich S, Vohla A. Mechanistic link between vitamin b12 and Alzheimer's disease. Biomolecules. 2022 Jan;12(1):129.
- Lehmann DJ, Cortina-Borja M. Genetic influence of plasma homocysteine on
- Alzheimer's disease. Neurobiology of aging. 2019 Apr;76:217-8.
- Lehmann DJ, Johnston C, Smith AD. Synergy between the genes for butyrylcholinesterase K variant and apolipoprotein

E4 in late-onset confirmed
- Alzheimer's disease. Human molecular genetics. 1997 Oct 1;6(11):1933-6.
- Liu PP, Xie Y, Meng XY, Kang JS. History and progress of hypotheses and clinical trials for Alzheimer's disease. Signal transduction and targeted therapy. 2019 Aug 23;4(1):29.
- Luchena C, Zuazo-Ibarra J, Alberdi E, Matute C, Capetillo-Zarate E. Contribution of neurons and glial cells to complement-mediated synapse removal during development, aging and in Alzheimer's disease. Mediators of Inflammation. 2018 Nov 11;2018.
- Manco G, Porzio E, Carusone TM. Human paraoxonase-2 (PON2): protein functions and modulation. Antioxidants. 2021 Feb 7;10(2):256.
- Martínez-Martínez AB, Torres-Perez E, Devanney N, Del Moral R, Johnson LA,
- Arbones-Mainar JM. Beyond the CNS: the many peripheral roles of APOE.
- Neurobiology of disease. 2020 May 1;138:104809.
- Masson P, Xie W, Froment MT, Lockridge O. Effects of mutations of active site residues and amino acids interacting with the Ω loop on substrate activation of butyrylcholinesterase. Biochimica et Biophysica Acta (BBA)-Protein Structure and
- Molecular Enzymology. 2001 Jan 12;1544(1-2):166-76.
- McIlroy SP, Crawford VL, Dynan KB, McGleenon BM, Vahidassr MD, Lawson JT, Passmore AP. Butyrylcholinesterase K variant is genetically associated with late onset Alzheimer's disease in Northern Ireland. Journal of medical genetics. 2000 Mar 1;37(3):182-5.
- Meng D, Mohammadi-Nejad AR, Sotiropoulos SN, Auer DP, Alzheimer's Disease Neuroimaging Initiative. Anticholinergic drugs and forebrain magnetic resonance imaging changes in cognitively normal people and those with mild cognitive impairment. European Journal of Neurology. 2022 May;29(5):1344-53.
- Miao G, Zhuo D, Han X, Yao W, Liu C, Liu H, Cao H, Sun Y, Chen Z, Feng T. From degenerative disease to malignant tumors: Insight to the function of ApoE.
- Biomedicine & Pharmacotherapy. 2023 Feb 1;158:114127.
- Mohamed Elfadil O, Mundi MS, Abdelmagid MG, Patel A, Patel N, Martindale R. Butyrate: More Than a Short Chain Fatty Acid. Current Nutrition Reports. 2023 Feb
- 10:1-8.
- Moore DE, Hess GP. Acetylcholinesterase-catalyzed hydrolysis of an amide.
- Biochemistry. 1975 Jun 1;14(11):2386-9.
- Moreira NC, de Freitas Lima JE, Marchiori MF, Carvalho I, Sakamoto-Hojo ET.
- Neuroprotective Effects of Cholinesterase Inhibitors: Current Scenario in Therapies
- for Alzheimer's Disease and Future Perspectives. Journal of Alzheimer's disease reports. 2022 Apr 18;6(1):177-93.
- Moreno S, Gerbig S, Schulz S, Spengler B, Diener M, Bader S. Epithelial propionyl-and butyrylcholine as novel regulators of colonic ion transport. British Journal of
- Pharmacology. 2016 Sep;173(18):2766-79.
- Moss DE, Perez RG. Anti-Neurodegenerative Benefits of Acetylcholinesterase Inhibitors in Alzheimer's Disease: Nexus

of Cholinergic and Nerve Growth Factor Dysfunction.

- Current Alzheimer research. 2021;18(13):1010.
- Mueller B, Adler G. Prevalence of wild-type butyrylcholinesterase genotype in patients with Alzheimer's dementia. World Journal of Neuroscience. 2015;5(03):175.
- Müller UC, Deller T, Korte M. Not just amyloid: physiological functions of the amyloid precursor protein family. Nature Reviews Neuroscience. 2017 May;18(5):281-98.
- Neves SR, Ram PT, Iyengar R. G protein pathways. Science. 2002 May
- 31;296(5573):1636-9.
- Nicolet Y, Lockridge O, Masson P, Fontecilla-Camps JC, Nachon F. Crystal structure of human butyrylcholinesterase and of its complexes with substrate and products.
- Journal of Biological Chemistry. 2003 Oct 17;278(42):41141-7.
- Nie Y, Luo D, Yang M, Wang Y, Xiong L, Gao L, Liu Y, Liu H. A meta-analysis on the relationship of the PON genes and Alzheimer disease. Journal of Geriatric
- Psychiatry and Neurology. 2017 Nov;30(6):303-10.
- O'Farrell PA, Gonzalez F, Zheng W, Johnston SA, Joshua-Tor L. Crystal structure of human bleomycin hydrolase, a self-compartmentalizing cysteine protease.
- Structure. 1999 Jun 15;7(6):619-27.
- Oldendorf WH. Carrier-mediated blood-brain barrier transport of short-chain monocarboxylic organic acids. American Journal of Physiology-Legacy Content.
- 1973 Jun 1;224(6):1450-3.
- Ordentlich A, Barak D, Kronman C, Flashner Y, Leitner M, Segall Y, Ariel N, Cohen S, Velan B, Shafferman A. Dissection of the human acetylcholinesterase active center determinants of substrate specificity. Identification of residues constituting the anionic site, the hydrophobic site, and the acyl pocket. Journal of Biological Chemistry. 1993 Aug 15;268(23):17083-95.
- Papassotiropoulos A, Bagli M, Jessen F, Frahnert C, Rao ML, Maier W, Heun R. Confirmation of the association between bleomycin hydrolase genotype and
- Alzheimer's disease. Molecular psychiatry. 2000 Mar;5(2):213-5.
- Paterson D, Nordberg A. Neuronal nicotinic receptors in the human brain. Progress in neurobiology. 2000 May 1;61(1):75-111.
- Perła-Kaján J, Borowczyk K, Głowacki R, Nygård O, Jakubowski H. Paraoxonase 1 Q192R genotype and activity affect homocysteine thiolactone levels in humans. The FASEB Journal. 2018 Nov;32(11):6019-24.
- Petersen RC, Thomas RG, Grundman M, Bennett D, Doody R, Ferris S, Galasko D, Jin S, Kaye J, Levey A, Pfeiffer E. Vitamin E and donepezil for the treatment of mild cognitive impairment. New England Journal of Medicine. 2005 Jun 9;352(23):2379-
- 88.
- Petrov KA, Girard E, Nikitashina AD, Colasante C, Bernard V, Nurullin L, Leroy J,
- Samigullin D, Colak O, Nikolsky E, Plaud B. Schwann cells sense and control acetylcholine spillover at the

neuromuscular junction by α7 nicotinic receptors and butyrylcholinesterase. Journal of Neuroscience. 2014 Sep 3;34(36):11870-83.

- Pfistermeister B, Tümena T, Gaßmann KG, Maas R, Fromm MF. Anticholinergic burden and cognitive function in a large German cohort of hospitalized geriatric patients.
- PloS one. 2017 Feb 10;12(2):e0171353.
- Prasad KN, Vernadakis A. Morphological and biochemical study in x-ray-and dibutyryl cyclic AMP-induced differentiated neuroblastoma cells. Experimental Cell Research.
- 1972 Jan 1;70(1):27-32.
- Pongthanaracht N, Yanarojana S, Pinthong D, Unchern S, Thithapandha A, Assantachai P, Supavilai P. Association between butyrylcholinesterase K variant and mild cognitive impairment in the Thai community-dwelling patients. Clinical Interventions in Aging. 2017;12:897.
- Rai V. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and Alzheimer disease risk: a meta-analysis. Molecular neurobiology. 2017 Mar 1;54(2):1173-86.
- Resende RR, Adhikari A. Cholinergic receptor pathways involved in apoptosis, cell proliferation and neuronal differentiation. Cell Communication and Signaling. 2009 Dec 1;7(1):20.
- Romani A, Trentini A, van der Flier WM, Bellini T, Zuliani G, Cervellati C, Teunissen CE. Arylesterase activity of paraoxonase-1 in serum and cerebrospinal fluid of patients with Alzheimer's disease and vascular dementia. Antioxidants. 2020 May 25;9(5):456.
- Roostaei T, Felsky D, Nazeri A, De Jager PL, Schneider JA, Bennett DA, Voineskos AN, Alzheimer's Disease Neuroimaging Initiative. Genetic influence of plasma homocysteine on Alzheimer's disease. Neurobiology of aging. 2018 Feb 1;62:243e7.
- Roßner S, Ueberham U, Schliebs R, Perez-Polo JR, Bigl V. The regulation of amyloid precursor protein metabolism by cholinergic mechanisms and neurotrophin receptor signaling. Progress in neurobiology. 1998 Dec 1;56(5):541-69.
- Russell RW. Continuing the search for cholinergic factors in cognitive dysfunction. Life sciences. 1996 Apr 26;58(22):1965-70.
- Russell RW, Booth RA, Jenden DJ, Chang AS, Rice KM, Roch M, Lauretz SD. Incomplete reversibility of an experimentally induced hypocholinergic state: biochemical and physiological, but not behavioral, recovery. Pharmacology Biochemistry and Behavior. 1992 Feb 1;41(2):433-44.
- Russo M, De Rosa MA, Calisi D, Consoli S, Evangelista G, Dono F, Santilli M, Granzotto A, Onofrj M, Sensi SL. Migraine pharmacological treatment and cognitive impairment: risks and benefits. International Journal of Molecular Sciences. 2022 Sep 27;23(19):11418.
- Sampaio TB, Savall AS, Gutierrez ME, Pinton S. Neurotrophic factors in Alzheimer's and Parkinson's diseases: implications for pathogenesis and therapy. Neural regeneration research. 2017 Apr;12(4):549.
- Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PW, Wolf PA. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. New England Journal of Medicine. 2002 Feb 14;346(7):476-83.
- Shafferman A, Velan B, Ordentlich A, Kronman C, Grosfeld H, Leitner M, Flashner Y, Cohen S, Barak D, Ariel N.

Substrate inhibition of acetylcholinesterase: residues affecting signal transduction from the surface to the catalytic center. The EMBO journal. 1992 Oct;11(10):3561-8.

- Shanks M, Kivipelto M, Bullock R, Lane R. Cholinesterase inhibition: is there evidence for disease-modifying effects?. Current Medical Research and Opinion. 2009 Oct 1;25(10):2439-46.
- Smith AD, Refsum H. Homocysteine, B vitamins, and cognitive impairment. Annu Rev
- Nutr. 2016 Jul 17;36(1):211-39.
- Snowdon DA, Tully CL, Smith CD, Riley KP, Markesbery WR. Serum folate and the severity of atrophy of the neocortex in Alzheimer disease: findings from the Nun study. The American journal of clinical nutrition. 2000 Apr 1;71(4):993-8.
- Son P, Lewis L. Hyperhomocysteinemia. InStatPearls [Internet] 2021 May 12.
- StatPearls Publishing.
- Soreq H. Checks and balances on cholinergic signaling in brain and body function.
- Trends in neurosciences. 2015 Jul 1;38(7):448-58.
- Spieker J, Frieß JL, Sperling L, Thangaraj G, Vogel-Höpker A, Layer PG. Cholinergic control of bone development and beyond. International Immunopharmacology. 2020 Jun 1;83:106405.
- Suszynska J, Tisonczyk J, Lee HG, Smith MA, Jakubowski H. Reduced homocysteinethiolactonase activity in Alzheimer's disease. Journal of Alzheimer's Disease. 2010
- Jan 1;19(4):1177-83.
- Tan B, Venketasubramanian N, Vrooman H, Cheng CY, Wong TY, Ikram MK, Chen C, Hilal S. Homocysteine and cerebral atrophy: the epidemiology of dementia in Singapore study. Journal of Alzheimer's Disease. 2018 Jan 1;62(2):877-85.
- Thomas KR, Bangen KJ, Weigand AJ, Edmonds EC, Wong CG, Cooper S, DelanoWood L, Bondi MW, Alzheimer's Disease Neuroimaging Initiative. Objective subtle cognitive difficulties predict future amyloid accumulation and neurodegeneration.
- Neurology. 2020 Jan 28;94(4):e397-406.
- Tormos JR, Wiley KL, Seravalli J, Nachon F, Masson P, Nicolet Y, Quinn DM. The reactant state for substrate-activated turnover of acetylthiocholine by butyrylcholinesterase is a tetrahedral intermediate. Journal of the American Chemical Society. 2005 Oct 26;127(42):14538-9.
- Ubbink JB, Vermaak WH, van der Merwe A, Becker PJ, Delport R, Potgieter HC. Vitamin requirements for the treatment of hyperhomocysteinemia in humans. The Journal of nutrition. 1994 Oct 1;124(10):1927-33.
- Venneri A, Lane R. Effects of cholinesterase inhibition on brain white matter volume in
- Alzheimer’s disease. Neuroreport 2009;20:285-8
- Venter JC, Di Porzio U, Robinson DA, Shreeve SM, Lai J, Kerlavage AR, Fracek Jr SP, Lentes KU, Fraser CM. Evolution of neurotransmitter receptor systems. Progress in neurobiology. 1988 Jan 1;30(2-3):105-69.
- Vijayaraghavan S, Darreh-Shori T, Rongve A, Berge G, Sando SB, White LR, Auestad BH, Witoelar A, Andreassen OA, Ulstein ID, Aarsland D. Association of butyrylcholinesterase-K allele and apolipoprotein E ε4 allele with cognitive decline in dementia with Lewy bodies and Alzheimer’s disease. Journal of Alzheimer's Disease. 2016 Jan 1;50(2):567-

76.

- Walsh R, Martin E, Darvesh S. A versatile equation to describe reversible enzyme inhibition and activation kinetics: Modeling β-galactosidase and butyrylcholinesterase. Biochimica et Biophysica Acta (BBA)-General Subjects. 2007
- May 1;1770(5):733-46
- Wang HX, Wahlin Å, Basun H, Fastbom J, Winblad B, Fratiglioni L. Vitamin B12 and folate in relation to the development of Alzheimer's disease. Neurology. 2001 May 8;56(9):1188-94.
- Wessler I, Kirkpatrick CJ. Acetylcholine beyond neurons: the non-neuronal cholinergic system in humans. British journal of pharmacology. 2008 Aug;154(8):1558-71.
- Wessler I, Kirkpatrick CJ. Cholinergic signaling controls immune functions and promotes homeostasis. International Immunopharmacology. 2020 Jun 1;83:106345.
- Wu S, Liu X, Jiang R, Yan X, Ling Z. Roles and mechanisms of gut microbiota in patients with Alzheimer's disease. Frontiers in Aging Neuroscience. 2021 May 28;13:650047.
- Xu H, Garcia-Ptacek S, Jönsson L, Wimo A, Nordström P, Eriksdotter M. Long-term effects of cholinesterase inhibitors on cognitive decline and mortality. Neurology.
- 2021 Apr 27;96(17):e2220-30.
- Zheng YB, Shi L, Zhu XM, Bao YP, Bai LJ, Li JQ, Liu JJ, Han Y, Shi J, Lu L. Anticholinergic drugs and the risk of dementia: A systematic review and metaanalysis. Neuroscience & Biobehavioral Reviews. 2021 Aug 1;127:296-306.
- Zhuravin IA, Nalivaeva NN, Kozlova DI, Kochkina EG, Fedorova YB, Gavrilova SI. The activity of blood serum cholinesterases and neprilysin as potential biomarkers of mild-cognitive impairment and Alzheimer's disease. Zhurnal nevrologii i psikhiatrii imeni SS Korsakova. 2015 Jan 1;115(12):110-7.