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REVIEW ARTICLE

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Regulators of cholinergic signaling in disorders of the central nervous system

Katarzyna Winek[1,2] | Hermona Soreq[1,2] | Andreas Meisel[3]

[1]The Edmond and Lily Safra Center for Brain Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel

[2]The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel

[3]Department of Neurology with Experimental Neurology, Center for Stroke Research Berlin, NeuroCure Clinical Research Center, Charité-Universitätsmedizin Berlin, Berlin, Germany

Correspondence
Katarzyna Winek, The Hebrew University of Jerusalem, Edmond J. Safra Campus Givat Ram, 91904 Jerusalem, Israel.
Email: katarzyna.winek@mail.huji.ac.il

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Abstract

Cholinergic signaling is crucial in cognitive processes, and degenerating cholinergic projections are a pathological hallmark in dementia. Use of cholinesterase inhibitors is currently the main treatment option to alleviate symptoms of Alzheimer's disease and has been postulated as a therapeutic strategy in acute brain damage (stroke and traumatic brain injury). However, the benefits of this treatment are still not clear. Importantly, cholinergic receptors are expressed both by neurons and by astrocytes and microglia, and binding of acetylcholine to the α7 nicotinic receptor in glial cells results in anti-inflammatory response. Similarly, the brain fine-tunes the peripheral immune response over the cholinergic anti-inflammatory axis. All of these processes are of importance for the outcome of acute and chronic neurological disease. Here, we summarize the main findings about the role of cholinergic signaling in brain disorders and provide insights into the complexity of molecular regulators of cholinergic responses, such as microRNAs and transfer RNA fragments, both of which may fine-tune the orchestra of cholinergic mRNAs. The available data suggest that these small noncoding RNA regulators may include promising biomarkers for predicting disease course and assessing treatment responses and might also serve as drug targets to attenuate signaling cascades during overwhelming inflammation and to ameliorate regenerative capacities of neuroinflammation.

KEYWORDS

Acetylcholine, inflammation, microRNA, neurology, transfer RNA fragments

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; AChE-R, the readthrough variant of acetylcholinesterase; AChE-S, the synaptic (classical variant) of acetylcholinesterase; AD, Alzheimer's disease; BBB, blood-brain barrier; BChE, butyrylcholinesterase; BDNF, brain-derived neurotrophic factor; BMI, body-mass index; ChAT, choline acetyltransferase; CIDS, central nervous system injury–induced immunodepression syndrome; CNS, central nervous system; COPD, chronic obstructive pulmonary disease; FDA, Food and Drug Administration; HPA, hypothalamus – pituitary – adrenal glands axis; ICH, intracerebral hemorrhage; IL, interleukin; lncRNA, long non-coding RNA; LPS, lipopolysaccharide; mAChR, muscarinic acetylcholine receptor; MCAo, middle cerebral artery occlusion; miRNA, microRNA; nAChR, nicotinic acetylcholine receptor; NMB, nucleus basalis Meynert; OCD, obsessive-compulsive disorder; PD, Parkinson's disease; PTSD, post-traumatic stress disorder; TBI, traumatic brain injury; TGFβ, transforming growth factor beta; tRF, transfer RNA fragment; VAChT, vesicular acetylcholine transporter; VNS, vagus nerve stimulation.

> "Although what follows is self-explanatory, I still think it desirable to state it expressly: in all cases in which the neurochemical mechanism occurs, the nerves only control function to the extent of the release of the substance: the place where this occurs is in the effector organ of the nerve. From then onwards, the released substance exerts control: the functioning organ is, therefore, its effector organ exclusively. And now we must consider in which directions our knowledge of the physiological process has been extended, beyond what we have already said, by the discovery of the neurochemical mechanism."
>
> *Otto Loewi, Nobel Lecture 1936* (Loewi, 1936)

1 | INTRODUCTION

Cholinergic signaling is widely used by many organisms across the tree of life. Even bacteria are capable of synthesizing acetylcholine (ACh; Roshchina, 2010), such that this neurotransmitter may serve as an interkingdom signaling molecule, sending messages across the human body. A growing body of evidence indicates that ACh together with catecholamines are important communication molecules between human microbial commensals (microbiota) and the immune system (Islas Weinstein et al. 2015). This example is certainly not the function of ACh that Otto Loewi had in mind when talking about *"the directions our knowledge (…) has been extended (…) by the discovery of the neurochemical mechanism."* However, it illustrates the fact that although ACh has been acknowledged over more than eight decades as one of the main neurotransmitters in the body, recent discoveries keep unraveling new and versatile functions of this signaling molecule in health and disease.

ACh is synthesized by interaction of the acetyl group from acetyl coenzyme A with choline, catalyzed by choline acetyltransferase (ChAT). Further, ACh is packed in vesicles by the vesicular ACh transporter (VAChT) and degraded by acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Receptors for ACh include nicotinic (nAChR) and muscarinic (mAChR) cholinergic receptors, named after their naturally occurring alkaloid agonists. The ionotropic nAChR receptors are ion channels opening upon binding of ACh and permeable to sodium, potassium, and in some cases, calcium ions. They consist of different combinations of five subunits (α2-α10 and β2-β4 in case of the neuronal type and α1, β1, γ, δ, ε in muscle cells) (Changeux & Taly, 2008; Kalamida et al. 2007). Conversely, mAChRs belong to the family of metabotropic receptors, using G proteins in signal transduction. Muscarinic receptors can be divided to five subtypes, M1–M5 which display different distribution across cell and tissue types (Saternos et al. 2018). The cholinergic system is regulated at many levels, including ACh synthesis, transport and breakdown, and the expression levels of its receptors. All of these levels of regulating ACh's actions have been extensively investigated and are overall well understood (Madrer & Soreq, 2020; Soreq, 2015). In the first part of this mini-review, we provide an overview of the role of cholinergic signaling in the healthy and diseased brain. In the second part, we summarize recent findings on the role of cholinergic regulators in brain recovery from disease states and briefly introduce novel research concepts in this area, focusing on the molecular regulators of the cholinergic pathway.

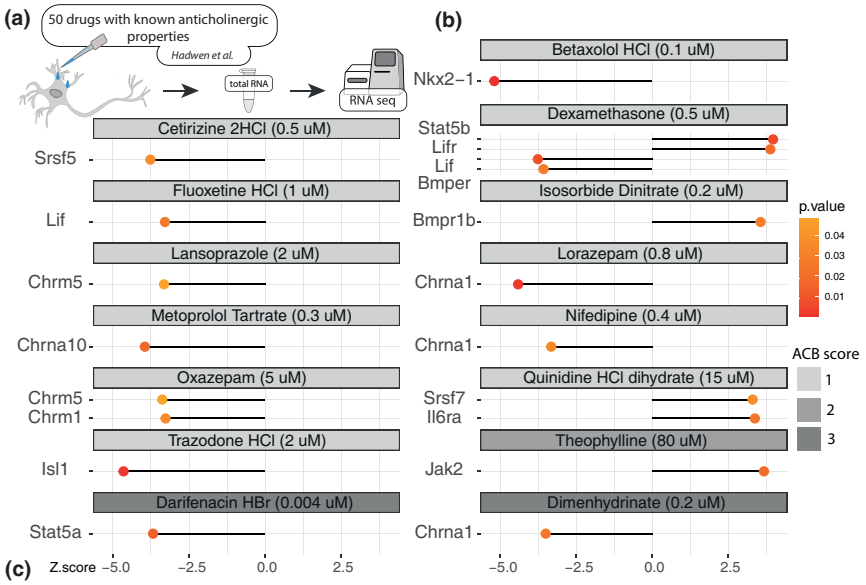
2 | BRAIN DISORDERS WITH AFFECTED CHOLINERGIC SIGNALING

The late 19th century brought the description of neurons and glia as cells of the nervous system, but for many years, the transfer of stimuli between nerve cells was considered a purely electrical process. The seminal discoveries of Sir Henry Dale and Otto Loewi then identified a chemical mode of communication between neurons or between neurons and cells of other types in the body. The *Vagusstoff* (vagus substance) described by Loewi and identified later as the chemical ACh compound that was discovered by Dale, became the first characterized neurotransmitter (McCoy & Tan 2014). Ever since then and until these days, the role of ACh in the brain, the peripheral nervous system, and non-neuronal cells became a subject of intensive research providing exciting findings.

In the peripheral nervous system, ACh is mostly found at neuromuscular junctions, in preganglionic neurons of the sympathetic nervous system and in the parasympathetic nervous system. In the central nervous system (CNS), cholinergic neurons are primarily located in four areas: (1) the brainstem, (2) a group of thalamic nuclei, (3) the striatum (as interneurons), and (4) the nuclei of the basal forebrain (Ballinger et al. 2016). Importantly, the basal forebrain plays a crucial role in cortical activation, attention, motivation, and memory (Záborszky et al. 2018). Disturbances in the CNS cholinergic system are implicated in many chronic disorders, including neurodegenerative conditions such as Alzheimer's and Parkinson's disease (AD, PD), schizophrenia, stress, and depression (Higley & Picciotto, 2014; Záborszky et al. 2018). Correspondingly, recent discoveries highlight the role of ACh in acute brain diseases, such as traumatic brain injury (TBI) (Shin & Dixon, 2015), stroke, or intracerebral hemorrhage (ICH) (Martín et al. 2018).

2.1 | Cholinergic origins of cognitive impairments

Several findings led to the cholinergic hypothesis in AD's etiology. First of all, reduced activity of ChAT and AChE was observed in brains of AD patients; further, it was shown that cholinergic projections of the nucleus basalis Meynert (NMB) undergo degeneration in AD brains, and lastly, an association of cholinergic antagonists with cognitive impairment was found, contrasting the procognition impact of AChE inhibitors used for symptomatic treatment of AD patients (Hampel et al. 2018). Moreover, compelling evidence suggests that in aged patients, long-term treatment with anticholinergic agents may be linked to increased risk of cognitive



drug	examples of clinical use
Betaxolol	hypertension, glaucoma
Cetirizine	allergy
Darifenacin	overactive bladder
Dexamethasone	anti-inflammatory; used i.a. in allergology, neurology, rheumatology
Dimenhydrinate	nausea, vomiting, motion sickness
Fluoxetine	depression, OCD, bulimia
Isosorbide Dinitrate	heart failure, angina pectoris

drug	examples of clinical use
Lansoprazole	peptic ulcer, gastrointestinal reflux
Lorazepam	anxiety, epileptic seizures
Metoprolol	hypertension, arrhythmia, coronary artery disease
Nifedipine	hypertensive emergency, angina pectoris, vasospastic angina
Oxazepam	anxiety, insomnia
Quinidine	arrhythmia
Theophylline	asthma, COPD
Trazodone	depression

FIGURE 1 Drugs with anticholinergic properties impact the expression of cholinergic genes. (a) We analyzed RNA-sequencing data from mouse neuronal cell cultures treated with over 200 drugs (Hadwen et al. 2018). (b) Seeking differentially expressed cholinergic genes under drug treatment compared to vehicle-treated cells involved a published list of cholinergic genes (Winek et al. 2020) and only considered genes whose expression was changed with $p < .05$. Shown are results of analyses of 50 drugs identified as medications with anticholinergic properties used in Germany, whose anticholinergic burden (ACB) score was 1–3 (weak to moderate anticholinergic effects) (Kiesel et al. 2018). (c) This table lists drugs with anticholinergic properties which were identified in our analysis as significantly altering the expression levels of cholinergic genes. It also shows that examples of conditions and accompanying symptoms these drugs are aimed to relieve. Notably, those drugs are used in clinical routine, sometimes as long-term treatment

impairment and dementia (Figure 1) (Coupland et al. 2019; Gray et al. 2015; Lechevallier-Michel et al. 2005; Pieper et al. 2020; Weigand et al. 2020). Classical anticholinergic drugs are predominantly used in urology (in treatment of urinary incontinence or overactive bladder) and pulmonology—mostly for treating chronic obstructive pulmonary disease (COPD), as mydriatics in ophthalmology and antispasmodics in gastroenterology. Additionally, a number of drugs that are frequently used in long-term therapies have anticholinergic effects, such as antihistamines, antipsychotics, and cyclic antidepressants (Durán et al. 2013). However, whether these drugs affect upstream levels in the process of cholinergic gene expression has not yet been studied systematically. To enable an initial insight into the influence of anticholinergics on the pathway of cholinergic gene expression, we have analyzed a transcriptomic dataset of cultured mouse cortical neurons subjected to over 200 drugs (GSE110256, accessed on http://bigbear.med.uottawa.ca:1000/) (Hadwen et al. 2018). According to recent analysis (Kiesel et al. 2018), 50 of these drugs have anticholinergic properties (Figure 1a). Importantly, we found that treating cultured cortical neurons with several of these drugs induced significant changes in cholinergic mRNA transcripts, most of which were downregulated, reflecting impaired cholinergic signaling (Figure 1b). Notably, many of those drugs identified as significant suppressors of cholinergic gene expression are widely used in clinical practice (Figure 1c). This analysis indicates that the elevated risk of dementia in patients under prolonged anticholinergic medications may reflect, among other processes, modified expression of cholinergic-related genes, whose expression was affected. Further investigations should verify if those changes are reflected at the protein level.

Clinical data about the causes of cognitive impairments linked to anticholinergic medications are largely derived from observation studies. Therefore, there is an urgent need for further in-depth investigations (Pieper et al. 2020) and full characterization of the cognitive risk associated with using agents with anticholinergic properties. Specific examples include positron emission tomography brain mapping of AD patients, which feature down-regulation nAChRs (Nordberg, 2006). Also, a growing body of brain mapping evidence indicates that the degenerative processes in the basal forebrain precede the entorhinal and neocortical degeneration in AD (Fernández-Cabello et al. 2020; Schmitz & Nathan Spreng 2016). Likewise, recent reports show that the reduced NMB

volume precedes cognitive impairment in PD patients and that its volume measurements may help to estimate the risk of dementia (Pereira et al. 2020; Ray et al. 2018). Moreover, cholinergic disturbances (dopamine/ACh imbalance and impaired functioning of the striatal cholinergic interneurons) are postulated as being involved in the motor symptoms of PD (Calabresi et al. 2006; Perez-Lloret & Barrantes, 2016). It is well known that striatal cholinergic and dopaminergic systems are interconnected: presynaptic nAChRs control dopamine release (Exley & Cragg, 2008), damage in substantia nigra correlates with loss of nAChRs in the striatum (Quik et al. 2008), and recent studies show that deficiency in dopamine decreases the activity of the striatal cholinergic interneurons (McKinley et al. 2019). Additionally, nicotine may act as a neuroprotective agent, protecting the nigrostriatal system from neuronal loss (Quik et al. 2008). Correspondingly, results from the recent and controversially discussed (Cheng & Wang, 2020) British Doctors cohort study indicate that current tobacco smoking is associated with lowering of the risk of PD and that this effect might be related to nicotine (Mappin-Kasirer et al. 2020).

Apart from being risk factors for cognitive decline (Weigand et al. 2020), cholinergic imbalances have been postulated to accompany disease-free aging. Specifically, healthy aging is associated with progressive loss of cholinergic dendritic and axonal structures; this occurs, however, in a manner which differs from the total cellular loss of cholinergic neurons in pathological processes (Schliebs & Arendt, 2011). Conversely, medications facilitating cholinergic signal transmission, such as cholinesterase inhibitors, besides memantine are the only FDA-approved treatment to relieve symptoms in AD (Sabbagh et al. 2019; H. Ferreira-Vieira et al. 2016). Increasing ACh levels in the synaptic cleft improves signaling in the cholinergic pathways of neuronal networks, upregulates the expression of neuronal nicotinic receptors, and additionally improves signaling in other neurotransmitter systems (Parsons et al. 2013). Pharmaceutics increasing ACh levels in treating cognitive impairment have also been tested in patients after TBI. Brain injury induces an initial increase in ACh levels, followed by a subsequent decrease in cholinergic function that ultimately leads to cholinergic hypoactivity associated both with low ACh levels and low AChE activity in the neocortex (Shin & Dixon, 2015). Further, post-TBI volume loss of frontal cholinergic brain regions correlates with worsened cognitive outcome (Östberg et al. 2020). While the cholinesterase inhibitors donepezil and rivastigmine have been proposed as possible therapeutics, several studies in patients after TBI (Masanic et al. 2001; Silver et al. 2006) resulted in conflicting results (Bengtsson & Godbolt, 2016; Noble & Hauser, 2007). Likewise, the cholinesterase inhibitor and nicotinic receptor interacting agent galantamine revealed promising results in TBI rat models; animals treated with galantamine had reduced BBB permeability and neuronal loss as well as improved hippocampal functions (Zhao et al. 2018). However, data from large clinical trials are missing, and a small clinical study in patients with posttraumatic stress disorder (PTSD) and TBI revealed no benefits of galantamine (McAllister et al. 2016).

Intriguingly, dementia patients treated with AChE inhibitors, which potentiate cholinergic signaling showed decreased risk of ischemic stroke (Lin et al. 2016; Tan et al. 2018). In the first study, analyzing $n = 10.364$ patients, the group using AChE inhibitors was 49.2% less likely to develop a stroke (Lin, Wu, et al., 2016). In the second one, including a larger cohort of $n = 44.288$ subjects, patients treated with AChE inhibitors were 15% less likely to suffer a stroke (Tan et al. 2018). This could be related to the anti-inflammatory actions of ACh and/or its effects supporting endothelial function (Lin, Wu, et al., 2016). It is important to note here that several AChE inhibitors used in clinical practice encompass agents with peripheral action, not crossing the BBB (e.g., pyridostigmine used in treatment of myasthenia gravis); and medications with CNS effects, like rivastigmine, galantamine, and donepezil (Pohanka, 2014). Studies discussed before refer to the centrally active AChE inhibitors, but it should be mentioned that even peripherally active pharmaceutics may affect CNS functions when used in very high dosages or in conditions when BBB is compromised (Friedman et al. 1996).

Further, the peripheral levels of cholinesterases were shown to differ between acute stroke patients and age-, sex-, and BMI-matched controls, and extremely low levels of AChE were linked to an increased mortality (Ben Assayag et al. 2010) (see also "Peripheral cholinergic links of brain disorders"). Several experimental and clinical studies focused on vagus nerve stimulation (VNS) as possible intervention improving motor recovery in stroke (Engineer et al. 2019). As shown in the rat models, VNS is postulated to increase plasticity in motor pathways (Meyers et al. 2018) having neuroprotective, anti-inflammatory and anti-apoptotic actions (Jiang et al. 2014). Use of cholinesterase inhibitors for treatment of cognitive impairment has also been postulated as a promising option in ischemic stroke, but the results of clinical studies are limited (Barfejani et al. 2020). A recent meta-analysis showed that treatment with cholinesterase inhibitors may improve cognitive functions in patients after stroke and vascular dementia (Kim et al. 2020). Correspondingly, patients with stroke affecting cholinergic pathways had worse functional long-term outcome (Qu et al. 2018). Importantly, cholinergic signaling in cases of brain injury is of major importance not only for neuronal cells but also for the inflammatory responses of glial as well as peripheral immune cells to the lesion (Norris & Kipnis, 2019).

2.2 | The anti-inflammatory actions of ACh in acute brain damage

In ischemic stroke, the sudden drop in perfusion of brain tissues triggers excitotoxicity (due to the excess of glutamate and overactivation of its receptors), peri-infarct depolarizations, cell necrosis and apoptosis, and inflammatory response (Dirnagl et al. 1999). In TBI, the primary injury leads to immediate lesions in brain parenchyma, while the secondary effects involve delayed ischemic and/or hemorrhagic damage triggering excitotoxicity, axonal degeneration, oxidative stress, mitochondrial dysfunction, and neuroinflammation which offer a potential therapeutic window (Ng & Lee, 2019). Both TBI and ischemic stroke induce specific response patterns of pathways with distinct dynamics, resulting in tissue damage. Also, the

immune response plays an important role in the acute and chronic phase of the disease in both conditions (Iadecola & Anrather, 2011; Jassam et al. 2017; McKee & Lukens, 2016). Neuroinflammatory response in brain injury underlies cholinergic surveillance, as the cholinergic system regulates astrocytic and microglial responses to brain damage (Patel et al. 2017).

Microglial cells express several nicotinic and muscarinic ACh receptors (nAChRs α3, α5, α6, α7 and β4) and mAChRs (Liu et al. 2016; Stolero & Frenkel, 2020). Microglia are crucial in the early inflammatory response to brain damage, secreting proinflammatory cytokines (TNFα, IL-1β, IL-6), reactive oxygen species, and prostaglandins, but they also participate in subsequent tissue repair processes producing, for example, BDNF, TGF-β, and IL-10 (Michell-Robinson et al. 2015). Rodent studies and cell culture experiments show that binding of ACh to the α7nAChR on microglia leads to decreased production of proinflammatory cytokines (see also "Peripheral cholinergic links of brain disorders") (Morioka et al. 2018; Katsuki & Matsumoto, 2018). Additionally, it has been postulated that stimulation of this receptor may influence the expression of microglial glutamate transporters, in turn reducing harmful excitotoxicity after brain injury and leading to neuroprotective effects. This hypothesis, however, remains to be challenged in experimental settings (Morioka et al. 2018). Recent studies in experimental mouse models demonstrated that microglia and brain macrophages upregulate the expression of M3 mAChR after cerebral ischemia, resulting in beneficial effects on the outcome measured by infarct size and behavioral tests (Costa et al. 2020; Pannell et al. 2016).

Astrocytes express mAChRs and α7nAChR (Guizzetti et al. 2008; Patel et al. 2017; Revathikumar et al. 2016). Although the role of astrocytes in neuroinflammatory responses is less well characterized than the function of microglia, it was shown that the anti-inflammatory pathway in astrocytes is mediated, similar to microglial cells by the α7nAChR. Stimulated cultured astrocytes decrease production of IL-1β, IL-6, and TNFα upon addition of nicotine, which has been attributed to actions over the prostaglandin pathway (Revathikumar et al. 2016). Also, mouse studies show that the anti-inflammatory actions in astrocytes are linked to inhibition of NF-κB and activation of Nrf2 signaling (Patel et al. 2017). Interestingly, glial cholinergic responses regulating inflammatory actions may be involved in the pathogenesis of delirium, a state of acute disturbance in consciousness and cognitive functions (Sfera et al. 2015), which is associated with cholinergic deficiency (Inouye, 2006).

The resident CNS cells are not the only ones involved in responses to brain damage. After brain injury, due to intense inflammatory signaling in the CNS and impaired BBB permeability, peripheral immune cells infiltrate the brain parenchyma and further shape the local inflammatory response. For example, in ischemic stroke, neutrophils and monocytes/macrophages are the first cells to arrive at the site of injury, followed by T- and later B lymphocytes (Iadecola & Anrather, 2011; Dirnagl et al. 1999; Doyle et al. 2015; Berchtold et al. 2020). Importantly, BBB function might also be influenced by signaling over α7nAChR (Dash et al. 2016). Peripheral immune cells express cholinergic receptors, ChAT and AChE (Fujii et al. 2017). Cholinergic signaling may therefore impact the responses to brain injury both for the brain-resident immune competent cells, as well as by regulating the function of infiltrating peripheral immune cells. Moreover, due to intense brain–body signaling, the cholinergic pathways will be activated systematically, affecting other organs and tissues, as briefly discussed below.

3 | PERIPHERAL CHOLINERGIC LINKS OF BRAIN DISORDERS

Brain injury substantially impacts the cholinergic-related systemic immune response. For example, ischemic stroke and TBI induce a local CNS and systemic proinflammatory boost, followed by a systemic suppression of immune function (CIDS: CNS-injury-induced immunodepression syndrome) (Hazeldine et al. 2015; Meisel et al. 2005; Saand et al. 2019), whether similar mechanisms take place in ICH and subarachnoid hemorrhage remains to be elucidated (Saand et al. 2019). Systemic non-neuronal cholinergic signaling and cholinergic actions of peripheral immune cells infiltrating the CNS also play an important role in the recovery process following brain injury. As ACh is an important mediator of communication between the brain and the immune system (Figure 2), the parasympathetic nervous system (mainly the vagus nerve) participates, together with the sympathetic nervous system and the HPA axis, in the systemic fine-tuning of the immune response after CNS injury. Further, many immune cells use intrinsic cholinergic signals, including dendritic and myeloid cells, T-, B- and NK lymphocytes (Fujii et al. 2017). These cells express all mAChRs and several nAChRs, including the α7 nAChR (Fujii et al. 2017). In particular, lymphocytes express enzymes participating in the synthesis (ChAT) and degradation of acetylcholine (AChE). AChE dimers are also covalently linked to the membrane of erythrocytes, and its activity decreases with age, is higher in females, and elevates during inflammation and under exposure to neurotoxic substances (Saldanha, 2017), including deliberate poisoning cases.

In lymphocytes, binding of ACh to muscarinic receptors enhances cytotoxicity and increases proliferation (Kawashima & Fujii, 2000), while stimulation of nAChR a7 in $CD4^+$ T cells may regulate cytokine production and Th17/Treg phenotypes (Mashimo et al. 2019). In monocytes/macrophages, α7nAChR signaling leads to suppressed production of proinflammatory cytokines, similarly to microglia and astrocytes. In the classical cholinergic anti-inflammatory pathway (Borovikova et al. 2000; Rosas-Ballina et al. 2008), activation of the efferent vagus nerve fibers decreases the release of TNFα from macrophages, involving signaling over the adrenergic splenic nerve and ACh-producing T lymphocytes (Rosas-Ballina et al. 2011). This pathway is crucial for dampening the systemic immune response after ischemic stroke, leading to increased susceptibility to infections, with pneumonia being primarily linked to increased mortality and worsened neurological outcome (Finlayson et al. 2011). Correspondingly, mice with α7nAChR deficiency show lower infection rates after cerebral ischemia (Engel et al. 2015), with no

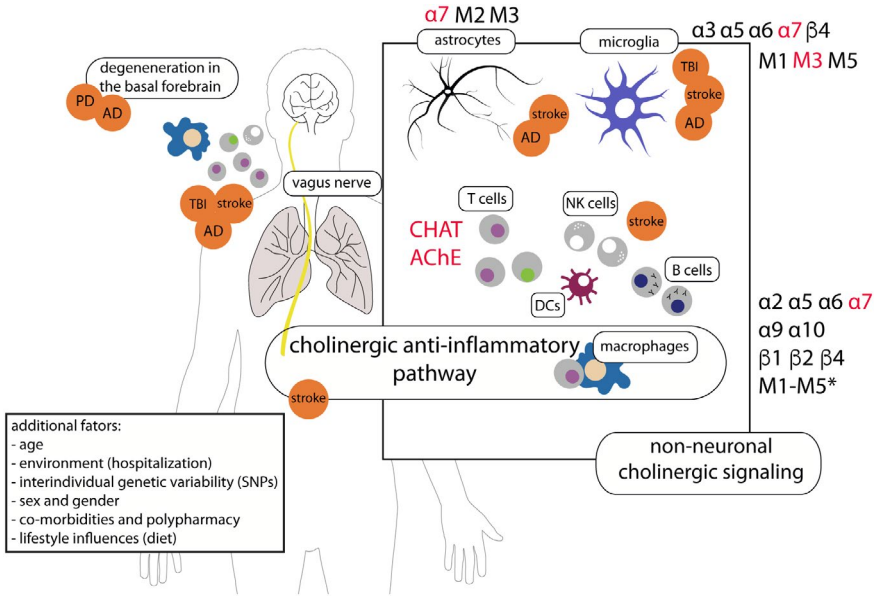


FIGURE 2 The diverse functions of cholinergic signaling in the human body. Cholinergic pathways in the central nervous system (CNS) are crucial in cognitive processes and degeneration of cholinergic projections in the basal forebrain leads to cognitive impairment. Further, non-neuronal cholinergic signaling participates in regulating immune responses of glial cells and immune cells in the CNS and the periphery. The cholinergic anti-inflammatory pathway provides a brain–body signaling route over the vagus nerve and regulates the inflammatory responses in macrophages. Fine-tuning of systemic immunity is of great importance in acute CNS injuries, which elicit strong neuroinflammatory response, leading to infiltration of peripheral immune cells to brain parenchyma. For more details about the nAChRs (α and β subunits) and mAChRs (M1-M5) in immune/glial cells, see Guizzetti et al. (2008), Stolero and Frenkel (2020), and Fujii et al. (2017); most relevant receptors, discussed in this manuscript, are highlighted in red. *The expression patterns differ between the immune cell subpopulations; see Fujii et al. (2017). AD, Alzheimer's disease; AChE, acetylcholinesterase; ChAT, choline acetyltransferase; PD, Parkinson's disease; TBI, traumatic brain injury

differences in initial infarct volumes. However, other subunits of nAChRs appear to have no roles in the suppression of lung immunity after stroke (Jagdmann et al. 2020). Another experimental murine stroke study showed that cholinergic signaling suppresses NK cells in the CNS (Liu et al. 2017) but not systematically; suggesting that NK cells in the periphery may be regulated by different mechanisms including catecholamines and the HPA axis (Liu et al. 2017). Considering the fact that brain injury elicits complex, orchestrated immune response, involving many cell populations with distinct roles, more studies characterizing the brain–immune interplay after CNS lesion and the distinct profiles of CNS-infiltrating and peripheral immune cells are urgently needed.

An active role of cholinergic signaling has also been postulated in multiple sclerosis (Gatta et al. 2020), with studies in mouse models linking ACh-producing NK cells with decreased infiltration of myeloid cells, underscoring their protective role in immunity (Jiang et al. 2017). The role of the peripheral immune system is yet more evident in neurodegenerative diseases, where recent studies show that CD8 lymphocytes patrol the CSF of AD patients (Gate et al. 2020) and can be found in the brain parenchyma in a mouse model of AD pathology (Sanchez et al. 2020). Moreover, genetically engineered AD mice subjected to antibody-based depletion of CD8 cells show altered hippocampal neuronal transcript profiles (Unger et al. 2020). However, the role played by cholinergic signaling in the regulation of immune responses in AD, including T cell responses, remains to be elucidated, as is evident in Figure 2 above.

4 | NOVEL AND ESTABLISHED MOLECULAR REGULATORS OF THE CHOLINERGIC PATHWAY

Molecular fine-tuners of cholinergic signaling have been studied in recent years both as potential disease biomarkers and as therapeutic targets in disorders involving cholinergic imbalance. We provide here a brief perspective on the complex and expanding landscape of the molecular regulators of cholinergic signaling.

One group of such molecules is the microRNAs (miRNAs), involved in the post-transcriptional regulation of gene expression. The main mechanism of action of these small (ca. 21–25 nt) RNAs involves their loading into the RNA-induced silencing complex (RISC), binding to complementary sequence motifs in the noncoding 3′-untranslated region (3′-UTRs) and translational arrest, often accompanied by cleavage of the mRNA (Bartel, 2009; Madrer & Soreq, 2020). One miRNA can have many targets, and one target can be regulated by many miRNAs; miRNAs can therefore operate at network levels. Currently, several miRNA-based interventions (leading to increased expression of a miRNA molecule under synthetic mimics or its diminished function under antagomiR suppressors) are tested as potential therapeutics in clinical trials (Hanna et al. 2019) and in studies investigating their utility as clinical biomarkers (Bonneau et al. 2019). Circulating miRNAs, including molecules targeting cholinergic transcripts, have been considered as markers of acute as well as chronic brain disease including stroke (Tiedt et al. 2017), TBI (Bhomia

et al. 2016; Polito et al. 2020), delirium (Fong et al. 2019), and neurodegenerative diseases (Sheinerman et al. 2017). Interestingly, systemic levels of certain miRNAs enriched in CNS may be correlated with age (Sheinerman et al. 2018), and aging is the main risk factor for many neurological disorders, especially neurodegenerative conditions (Hou et al. 2019). However, a causal relationship between altered cholinergic-targeted miRNA patterns in the elderly and neurodegenerative diseases remains to be proven.

Multiple miRNAs have targets in the cholinergic pathway, and the group of miRNAs will be further called the "CholinomiRs." Many of them are primate-specific and have been implicated in neurological disorders [for a comprehensive review see (Nadorp & Soreq, 2014)]. Here, we provide several examples of miRNA regulators of cholinergic pathways (focusing on those targeting the AChE mRNA transcripts), known or predicted to impact brain function and recovery via different mechanisms linked to their CNS effects or roles in the periphery.

A classic example of a CholinomiR targeting AChE is miR-132 (Shaked et al. 2008). AChE has three main splice variants—synaptic AChE-S being the main neuronal transcript encoding protein tetramers which are linked to the synaptic membrane via the Prima protein, AChE-R — the monomeric soluble readthrough variant, and the AChE-E dimers expressed mainly in erythrocytes and bound covalently to their membrane (Soreq & Seidman, 2001). Stress responses involve a rapid decline of AChE-S mRNA accompanied by accumulation of AChE-R mRNA, linked to long-lasting hypersensitivity of neuronal cells (Meshorer et al. 2002). Notably, miR-132 targets the AChE-S variant alone. Mouse hippocampal stress responses were shown to involve miR-132 increases, leading to decreased AChE-S levels and avoiding declined cognitive performance (Shaltiel et al. 2013). Correspondingly, TgR transgenic mice expressing AChE-R in excess show increased miR-132 and decreased AChE-S levels, accompanied by elevated anxiety and impaired locomotion and cognition (Moshitzky et al. 2020). Further, peripheral application of lipopolysaccharide (LPS) leads to increased cortical miR-132, decreased AChE-S, and elevated AChE-R levels, together with increased levels of brain TNFα, IL-6, IL-1β, and IL-18 reflecting intensified inflammatory response. Peripheral treatment with anti-miR-132 (AM-132) inversely leads to increased AChE-S and other miR-132 targets and downregulated proinflammatory cytokines. Since AM-132 cannot cross the intact BBB, the cortical effects of this treatment may result from the body–brain communication over vagal afferents (Mishra et al. 2017). Peripheral application of LPS also leads to elevated miR-132 levels in leukocytes, decreasing AChE production, increasing cholinergic signaling, and potentiating the anti-inflammatory response to balance the inflammatory stimuli (Shaked et al. 2009). Experiments involving targeting of this miRNA in the murine experimental stroke model (middle cerebral artery occlusion, MCAo) showed that *pre-stroke* intracerebroventricular administration of miR-132/212 protected the blood–brain barrier, decreased infarct volume, and improved neurological outcome (Zuo et al. 2019). In contrast, in vitro overexpression of the AChE-targeting miR-132/212 under hypoxic conditions was shown to damage blood–brain–barrier integrity (Burek et al. 2019).

Another CholinomiR, predicted to target both AChE-S and AChE-R (Nadorp & Soreq, 2014) and being involved in the cholinergic anti-inflammatory pathway, is miR-124, produced in macrophages upon ACh binding to the α7nAChR. Experiments in vitro and in mouse models demonstrated that miR-124 levels increase after LPS exposure combined with nicotine treatment and that miR-124 targets two transcripts in the inflammatory pathway: STAT3, leading to decreased production of IL-6 and TNFα converting enzyme (TACE) and downregulating the secretion of TNFα (Sun et al. 2013). Interestingly, miR-124 is highly expressed in the mammalian brain, where it also targets the brain's glucocorticoid receptor and is postulated to contribute to responses to stress, neurodegenerative processes, stroke and autoimmune disorders (Sun et al. 2015; Vreugdenhil et al. 2009). Further, miR-124 is downregulated in the hippocampus of stressed rats, together with upregulated miR-134 and miR-183 in amygdala, which target the SC35 splicing factor controlling the alternative splicing of AChE transcripts under exposure to stress (Meerson et al. 2010). Recent studies in a mouse model of repetitive TBI revealed that injury causes initial elevation and subsequent decrease of miR-124-3p, elevating the risk of neurodegenerative disease. Correspondingly, treatment with microglial exosomes loaded with miR-124-3p improved cognitive functions after repetitive TBI in mouse in a manner attributed to interaction of miR-124-3p with Rela, which is an inhibitory transcription factor of ApoE (Ge et al. 2020). In experimental spinal cord injury, a protective role of neuronal exosomal miR-124-3p was attributed to anti-inflammatory actions in microglia and astrocytes (Jiang et al. 2020).

AChE is also targeted by the primate-specific miR-608 (Hanin et al. 2014). Importantly, carriers of the minor allele of a single nucleotide polymorphism (SNP) in the 3′-UTR of AChE, defined as rs17228616, showed weakened interaction of miR-608 with AChE (Hanin et al. 2014), accompanied by preferential suppression of other targets, for example, CDC42 and IL-6 in cell culture tests and in the human amygdala (Lin, Simchovitz, et al., 2016). Human carriers of the rare allele of this SNP further have higher brain AChE levels, decreased cortisol, and elevated blood pressure (Hanin et al. 2014), which in turn might affect their risk of developing cerebrovascular disease like ischemic stroke. Other features of rs17228616 carriers include intensified amygdala activity and also compensatory increased prefrontal cortex reaction to stress, which may protect them from increased susceptibility to PTSD (Lin, Simchovitz, et al., 2016).

These three examples of CholinomiRs highlight the fact that miRNA regulation has multiple action levels related to the many possible targets of a given miRNA. Another perspective predicts that miRNAs involved in cholinergic signaling might need to compete with other small RNAs that mediate cholinergic effects, either by directly targeting components of the cholinergic pathway or via indirect actions. For instance, miR-210 was shown to mediate the beneficial responses to VNS in a rat model of brain ischemia, possibly via targeting apoptosis-related transcripts (Jiang et al. 2015). This miRNA has also been considered as a marker in AD (Swarbrick et al. 2019), so its further cholinergic links should be tested. The

examples above reflect the simplest miRNA actions, and much remains unknown about their full scope of activities.

Importantly, the nonexclusivity of the miRNAs and their targets indicates that their organismal responses are orchestrated processes that are quite complex to model and study. A recent integrative attempt combining cell culture tests with small RNA-sequencing and tissue sequencing of patient brain samples revealed differences in male/female miRNA-regulatory networks in psychiatric disorders, highlighting the importance of the sexually dimorphic miR-10 and miR-199 families in regulating cholinergic and neurokine pathways (Lobentanzer et al. 2019). One member of the miR-10 family is miR-125-5p, shown to be differentially expressed when comparing male to female neuroblastoma cell lines (LA-N-5 versus. LA-N-2 cells) and to target AChE (Lobentanzer et al. 2019). Future studies of miRNA functions should definitely consider that complexity of the miRNA responses rather than focusing on single interactions with a given target, as schematically presented in Figure 3.

Another layer of complexity while tuning cholinergic signaling involves miRNA-related surveillance by long noncoding RNAs (lncRNAs, ca. 200 nt), which add to the multilevel molecular regulation of the cholinergic pathway. LncRNAs may sponge the miRNAs or competitively bind to mRNA transcripts, ultimately leading to inhibition of miRNA actions (Madrer & Soreq, 2020). For example, lncRNA Gm21284 competes with miR-30e-3p, promoting the differentiation of cholinergic hippocampal neurons in a rat model (Cheng et al. 2019).

New small RNA discoveries add one more regulatory tool to this picture. Transfer-RNA fragments (tRFs) are a recently rediscovered group of regulatory molecules. Free fragments of tRNA were identified in sequencing data several years ago but have been considered a pure degradation product (Li et al. 2018). Yet more recently, multiple new reports highlight functional roles of those tRFs in health and disease. Interestingly, one mode of action of these tRFs may be similar to that of miRNAs, resulting in post-transcriptional suppression of gene expression (Maute et al. 2013). Others include displacement of RNA-binding proteins, leading to transcript destabilization (Goodarzi et al. 2015), increased translation of ribosomal proteins (Kim et al. 2017) or inhibiting translation (Ivanov et al. 2014), as recently reviewed (Kim et al. 2020). Aging rats show a dynamic and specific tRF profile, with an increase of fragments originating from the 3′-end of tRNA in older animals (Grigoriev & Karaiskos, 2016). At the mechanistic level, experimental and clinical studies showed that tRFs contribute to ischemic stroke responses (Li et al. 2016), PD progression (Magee et al. 2019), epilepsy (Hogg et al. 2019), and amyotrophic lateral sclerosis (Ivanov et al. 2014). In a rat model of spinal cord injury, Qin et al. reported an upregulation of a tRF derived from Gly-GCC tRNA that targets BDNF (Qin et al. 2020). In blood cells from patients 2 days after cerebral ischemia, we described a "changing of the guards" with decreased miRNA levels and increased levels of tRFs, both enriched in molecules predicted to target cholinergic transcripts. In-depth investigations suggested that the post-stroke

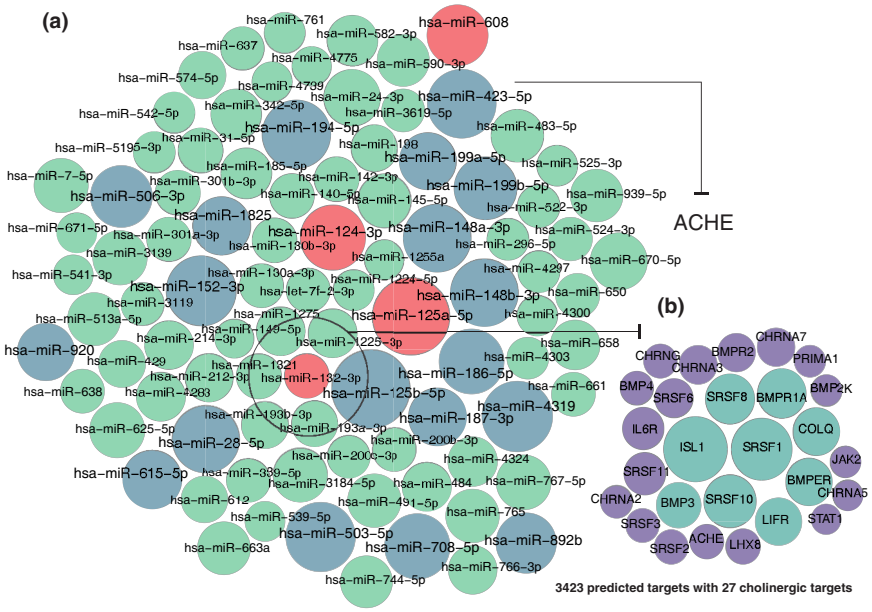


FIGURE 3 miRNAs are nonexclusive: One miRNA may target many transcripts and particular transcripts may be regulated by several miRNAs. (a) AChE is predictably targeted by at least 93 miRNAs. Shown is the outcome of bioinformatic target prediction derived from the mirDIP database (http://ophid.utoronto.ca/mirDIP/) considering only miRNAs with "very high" (blue circles) and "high" (green circles) prediction scores. mirDIP provides a resource collecting prediction data from 30 different databases. The miRNAs–target interactions are classified according to the integrative targeting score, which accounts for predictions derived from all resources. Top 1% of all predicted miRNA–target interactions according to the integrative score receive "very high," top 5%—"high" categories (Shirdel et al. 2011; Tokar et al. 2018). The circle sizes on the graph correspond to the integrative targeting. miRNAs discussed in detail in this manuscript are marked in red. (b) miR-132-3p predictably targets 3,423 transcripts with very high and high scores according to mirDIP, including 27 cholinergic targets [definition of the cholinergic genes based on (Winek et al. 2020)]. Circle diameters correspond to the integrative targeting score (Shirdel et al. 2011; Tokar et al. 2018)

modified tRFs may be essential for regulating $CD14^+$ monocyte responses to inflammatory events, which can be modulated by nicotine (Winek et al. 2020). Further tests to fully elucidate the orchestrated role of miRNAs and tRFs in different CNS pathologies with special focus on their involvement in regulation of cholinergic signaling in blood and brain are required.

5 | A BRIEF LOOK INTO THE FUTURE

Detailed characterization of ACh actions in the CNS and in brain's disease states of non-neuronal peripheral signaling is still lacking, especially in acute brain injuries like TBI or stroke. Additionally, new unconventional ACh pools may remain unidentified (e.g., in microbiota, although only 10–12 bacterial species, for example, *Lactobacillus plantarum* [a well-known probiotic species present in fermented foods] have been shown to synthesize ACh (Roshchina, 2010). Based on our current knowledge, future therapeutic concepts should consider personalized approaches tailored to the genetic makeup and lifestyle of patients. Importantly, SNPs in cholinergic target genes as well as miRNAs modulating the expression of transcripts carrying such SNPs may influence the complementarity of cholinergic-regulating miRNAs and their targets [see rs17228616 in AChE (Hanin et al. 2014) and rs4919510 in its molecular regulator—hsa-miR-608 (Lin, Simchovitz, et al., 2016)] and, in consequence, modify the effectiveness of gene expression surveillance based on small RNAs (Simchovitz et al. 2017). Additionally, future therapies should consider sex and gender differences impacting patients' responses to treatments, compliance, and interactions with other medications (Mauvais-Jarvis et al. 2020; Simchovitz-Gesher & Soreq, 2020). It was shown for instance that female AD patients, more often than male ones, suffer from being treated simultaneously with agents having opposing actions, including AChE inhibitors and anticholinergics (Mauvais-Jarvis et al. 2020). Ideally, such confounders should be taken into consideration when designing future preclinical and clinical research, accounting also for the complex multilayered landscape of molecular regulators of cholinergic functions. Combining already established techniques with modern "omics" technologies, and advanced bioinformatic tools, sampling from multiple tissues, biobanking, and strict adherence to scientific standards will all be required to allow a more holistic view of the pathophysiology of brain disorders and hopefully identify new therapeutic targets.

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CONFLICT OF INTEREST

HS is a co-editor of this special issue. The authors declare no other conflict of interest.

ORCID

Katarzyna Winek https://orcid.org/0000-0003-3085-9054
Hermona Soreq https://orcid.org/0000-0002-0955-526X
Andreas Meisel https://orcid.org/0000-0001-7233-5342

REFERENCES

Assayag, E. B., Shenhar-Tsarfaty, S., Ofek, K., Soreq, L., Bova, I., Shopin, L., Berg, R. M. G., Berliner, S., Shapira, I., Bornstein, N. M., & Soreq, H. (2010). Serum cholinesterase activities distinguish between stroke patients and controls and predict 12-month mortality. *Molecular Medicine*, *16*, 278–286. https://doi.org/10.2119/molmed.2010.00015.

Ballinger, E. C., Ananth, M., Talmage, D. A., & Role, L. W. (2016). Basal forebrain cholinergic circuits and signaling in cognition and cognitive decline. *Neuron*, *91*, 1199–1218. https://doi.org/10.1016/j.neuron.2016.09.006.

Barfejani, A. H., Jafarvand, M., Seyedsaadat, S. M., & Rasekhi, R. T. (2020). Donepezil in the treatment of ischemic stroke: Review and future perspective. *Life Sciences*, *263*, 118575.–https://doi.org/10.1016/j.lfs.2020.118575.

Bartel, D. P. (2009). MicroRNAs: Target recognition and regulatory functions. *Cell*, *136*, 215–233. https://doi.org/10.1016/j.cell.2009.01.002.

Bengtsson, M., & Godbolt, A. K. (2016). Effects of acetylcholinesterase inhibitors on cognitive function in patients with chronic traumatic brain injury: A systematic review. *Journal of Rehabilitation Medicine*, *48*, 1–5.

Berchtold, D., Priller, J., Meisel, C., & Meisel, A. (2020). Interaction of microglia with infiltrating immune cells in the different phases of stroke. *Brain Pathology*, *30*(6), 1208–1218. https://doi.org/10.1111/bpa.12911

Bhomia, M., Balakathiresan, N. S., Wang, K. K., Papa, L., & Maheshwari, R. K. (2016). A panel of serum MiRNA biomarkers for the diagnosis of severe to mild traumatic brain injury in humans. *Scientific Reports*, *6*, 1–12.

Bonneau, E., Neveu, B., Kostantin, E., Tsongalis, G. J., & De Guire, V. (2019). How close are miRNAs from clinical practice? A perspective on the diagnostic and therapeutic market. *The electronic Journal of the International Federation of Clinical Chemistry*, *30*, 114–127.

Borovikova, L. V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G. I., Watkins, L. R., Wang, H. H., Abumrad, N., Eaton, J. W., & Tracey, K. J. (2000). Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature*, *405*, 458–462. https://doi.org/10.1038/35013070.

Burek, M., König, A., Lang, M., Fiedler, J., Oerter, S., Roewer, N., Bohnert, M., Thal, S. C., Blecharz-Lang, K. G., Woitzik, J., Thum, T., & Förster, C. Y. (2019). Hypoxia-induced MicroRNA-212/132 alter blood-brain barrier integrity through inhibition of tight junction-associated proteins in human and mouse brain microvascular endothelial cells. *Transl. Stroke Res.*, *10*(6), 1–12. https://doi.org/10.1007/s12975-018-0683-2.

Calabresi, P., Picconi, B., Parnetti, L., Di Filippo, M. (2006). A convergent model for cognitive dysfunctions in Parkinson's disease: The critical dopamine-acetylcholine synaptic balance. *The Lancet Neurology*, *5*, 974–983. https://doi.org/10.1016/S1474-4422(06)70600-7.

Changeux, J. P., & Taly, A. (2008). Nicotinic receptors, allosteric proteins and medicine. *Trends in Molecular Medicine*, *14*, 93–102.

Cheng, X., Li, H., Zhao, H., Li, W., Qin, J., & Jin, G. (2019). Function and mechanism of long non-coding RNA Gm21284 in the development of hippocampal cholinergic neurons. *Cell & Bioscience*, *9*, 72. https://doi.org/10.1186/s13578-019-0336-5.

Cheng, Y., & Wang, Y.-J. (2020). Tobacco smoking and the reduced risk of Parkinson disease: A puzzle of 60 years. *Neurology*, *94*, 860–861. https://doi.org/10.1212/WNL.0000000000009431.

Costa, A., Haage, V., Yang, S., Wegner, S., Ersoy, B., Ugursu, B., Rex, A., Kronenberg, G., Gertz, K., Endres, M., Wolf, S. A., & Kettenmann, H.

(2020). Deletion of muscarinic acetylcholine receptor 3 in microglia impacts brain ischemic injury. *Brain, Behavior, and Immunity*, *91*, 89–104. https://doi.org/10.1016/j.bbi.2020.09.008

Coupland, C. A. C., Hill, T., Dening, T., Morriss, R., Moore, M., & Hippisley-Cox, J. (2019). Anticholinergic drug exposure and the risk of dementia: A nested case-control study. *JAMA Internal Medicine*, *179*, 1084–1093.

Dash, P. K., Zhao, J., Kobori, N., Redell, J. B., Hylin, M. J., Hood, K. N., & Moore, A. N. (2016). Activation of alpha 7 cholinergic nicotinic receptors reduce blood–brain barrier permeability following experimental traumatic brain injury. *Journal of Neuroscience*, *36*, 2809–2818. https://doi.org/10.1523/JNEUROSCI.3197-15.2016.

Dirnagl, U., Iadecola, C., & Moskowitz, M. A. (1999). Pathobiology of ischaemic stroke: An integrated view. *Trends in Neurosciences*, *22*, 391–397. https://doi.org/10.1016/S0166-2236(99)01401-0.

Doyle, K. P., Quach, L. N., Sole, M., Axtell, R. C., Nguyen, T.-v V., Soler-Llavina, G. J., Jurado, S., Han, J., Steinman, L., Longo, F. M., Schneider, J. A., Malenka, R. C., & Buckwalter, M. S. (2015). B-lymphocyte-mediated delayed cognitive impairment following stroke. *Journal of Neuroscience*, *35*, 2133–2145. https://doi.org/10.1523/JNEUROSCI.4098-14.2015.

Durán, C. E., Azermai, M., & Stichele, R. H. V. (2013). Systematic review of anticholinergic risk scales in older adults. *European Journal of Clinical Pharmacology*, *69*, 1485–1496.

Engel, O., Akyüz, L., da Costa Goncalves, A. C., Winek, K., Dames, C., Thielke, M., Herold, S., Böttcher, C., Priller, J., Volk, H. D., Dirnagl, U., Meisel, C., & Meisel, A. (2015). Cholinergic pathway suppresses pulmonary innate immunity facilitating pneumonia after stroke. *Stroke*, *46*, 3232–3240. https://doi.org/10.1161/STROKEAHA.115.008989.

Engineer, N. D., Kimberley, T. J., Prudente, C. N., Dawson, J., Tarver, W. B., & Hays, S. A. (2019). Targeted vagus nerve stimulation for rehabilitation after stroke. *Frontiers Media S.A.*, https://doi.org/10.3389/fnins.2019.00280.

Exley, R., & Cragg, S. J (2008). Presynaptic nicotinic receptors: a dynamic and diverse cholinergic filter of striatal dopamine neurotransmission. *British Journal of Pharmacology*, *153*(S1), S283–S297.

Fernández-Cabello, S., Kronbichler, M., Van Dijk, K. R. A., Goodman, J. A., Spreng, R. N., & Schmitz, T. W. (2020). Basal forebrain volume reliably predicts the cortical spread of Alzheimer's degeneration. *Brain*, *143*, 993–1009. https://doi.org/10.1093/brain/awaa012.

Finlayson, O., Kapral, M., Hall, R., Asllani, E., Selchen, D., & Saposnik, G., Canadian Stroke N., Stroke Outcome Research Canada Working G (2011). Risk factors, inpatient care, and outcomes of pneumonia after ischemic stroke. *Neurology*, *77*, 1338–1345. https://doi.org/10.1212/WNL.0b013e31823152b1.

Fong, T. G., Vasunilashorn, S. M., Libermann, T., Marcantonio, E. R., & Inouye, S. K. (2019). Delirium and Alzheimer disease: A proposed model for shared pathophysiology. *International Journal of Geriatric Psychiatry*, *34*, 781–789.

Friedman, A., Kaufer, D., Shemer, J., Hendler, I., Soreq, H., & Tur-Kaspa, I. (1996). Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response. *Nature Medicine*, *2*, 1382–1385.

Fujii, T., Mashimo, M., Moriwaki, Y., Misawa, H., Ono, S., Horiguchi, K., & Kawashima, K. (2017). Expression and function of the cholinergic system in immune cells. *Frontiers in Immunology*, *8*, 1085.

Gate, D., Saligrama, N., Leventhal, O., Yang, A. C., Unger, M. S., Middeldorp, J., Chen, K., Lehallier, B., Channappa, D., De Los Santos, M. B., McBride, A., Pluvinage, J., Elahi, F., Tam, G.-Y., Kim, Y., Greicius, M., Wagner, A. D., Aigner, L., Galasko, D. R., … Wyss-Coray, T. (2020). Clonally expanded CD8 T cells patrol the cerebrospinal fluid in Alzheimer's disease. *Nature*, *577*, 399–404. https://doi.org/10.1038/s41586-019-1895-7.

Gatta, V., Mengod, G., Reale, M., & Tata, A. M. (2020). Possible correlation between cholinergic system alterations and neuro/inflammation in multiple sclerosis. *Biomedicines*, *8*, 153.–https://doi.org/10.3390/biomedicines8060153.

Ge, X., Guo, M., Hu, T., Li, W., Huang, S., Yin, Z., Li, Y. et al (2020). Increased microglial Exosomal miR-124-3p alleviates neurodegeneration and improves cognitive outcome after rmTBI. *Molecular Therapy*, *28*, 503–522.

Goodarzi, H., Liu, X., Nguyen, H. C. B., Zhang, S., Fish, L., & Tavazoie, S. F. (2015). Endogenous tRNA-derived fragments suppress breast cancer progression via YBX1 displacement. *Cell*, *161*, 790–802. https://doi.org/10.1016/j.cell.2015.02.053.

Gray, S. L., Anderson, M. L., Dublin, S., Hanlon, J. T., Hubbard, R., Walker, R., Yu, O., Crane, P. K., & Larson, E. B. (2015). Cumulative use of strong anticholinergics and incident dementia: A prospective cohort study. *JAMA Internal Medicine*, *175*, 401–407.

Grigoriev, A., & Karaiskos, S. (2016). Dynamics of tRNA fragments and their targets in aging mammalian brain. *F1000Research*, *5*, 2758. https://doi.org/10.12688/f1000research.10116.1.

Guizzetti, M., Moore, N. H., Giordano, G., & Costa, L. G. (2008). Modulation of neuritogenesis by astrocyte muscarinic receptors. *Journal of Biological Chemistry*, *283*, 31884–31897.

H. Ferreira-Vieira, T., M. Guimaraes, I., R. Silva, F., & M. Ribeiro, F. (2016). Alzheimer's disease: Targeting the Cholinergic System. *Current Neuropharmacology*, *14*, 101–115. https://doi.org/10.2174/1570159X13666150716165726.

Hadwen, J., Schock, S., Mears, A., Yang, R., Charron, P., Zhang, L., Xi, H. S., & MacKenzie, A. (2018). Transcriptomic RNAseq drug screen in cerebrocortical cultures: Toward novel neurogenetic disease therapies. *Human Molecular Genetics*, *27*, 3206–3217. https://doi.org/10.1093/hmg/ddy221.

Hampel, H., Mesulam, M.-M., Cuello, A. C., Farlow, M. R., Giacobini, E., Grossberg, G. T., Khachaturian, A. S., Vergallo, A., Cavedo, E., Snyder, P. J., & Khachaturian, Z. S. (2018). The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain*, *141*, 1917–1933. https://doi.org/10.1093/brain/awy132.

Hanin, G., Shenhar-Tsarfaty, S., Yayon, N., Hoe, Y. Y., Bennett, E. R., Sklan, E. H., Rao, D. C. et al (2014). Competing targets of microRNA-608 affect anxiety and hypertension. *Human Molecular Genetics*, *23*, 4569–4580.

Hanna, J., Hossain, G. S., & Kocerha, J. (2019). The potential for microRNA therapeutics and clinical research. *Frontiers in Genetics*, *10*, 478.

Hazeldine, J., Lord, J. M., & Belli, A. (2015). Traumatic brain injury and peripheral immune suppression: Primer and prospectus. *Frontiers in Neurology*, *6*, 1. https://doi.org/10.3389/fneur.2015.00235.

Higley, M. J., & Picciotto, M. R. (2014). Neuromodulation by acetylcholine: Examples from schizophrenia and depression. *Current Opinion in Neurobiology*, *29*, 88–95.

Hogg, M. C., Raoof, R., Naggar, H., El, M. N., Delanty, N., O'Brien, D. F., Bauer, S., Rosenow, F., Henshall, D. C., & Prehn, J. H. M. (2019). Elevation of plasma tRNA fragments precedes seizures in human epilepsy. *Journal of Clinical Investigation*, *129*, 2946–2951. https://doi.org/10.1172/JCI126346.

Hou, Y., Dan, X., Babbar, M., Wei, Y., Hasselbalch, S. G., Croteau, D. L., & Bohr, V. A. (2019). Ageing as a risk factor for neurodegenerative disease. *Nature Reviews Neurology*, *15*, 565–581.

Iadecola, C., & Anrather, J. (2011). The immunology of stroke: From mechanisms to translation. *Nature Medicine*, *17*, 796–808. https://doi.org/10.1038/nm.2399.

Inouye, S. K. (2006). Delirium in older persons. *New England Journal of Medicine*, *354*, 1157–1165.

Islas, W. L., Revuelta, A., & Pando, R. H. (2015). Catecholamines and acetylcholine are key regulators of the interaction between microbes and the immune system. *Annals of the New York Academy of Sciences*, *1351*, 39–51.

Ivanov, P., O'Day, E., Emara, M. M., Wagner, G., Lieberman, J., & Anderson, P. (2014). G-quadruplex structures contribute to the

neuroprotective effects of angiogenin-induced tRNA fragments. *Proceedings of the National Academy of Sciences*, *111*, 18201–18206. https://doi.org/10.1073/pnas.1407361111.

Jagdmann, S., Dames, C., Berchtold, D., Winek, K., Weitbrecht, L., Meisel, A., & Meisel, C. (2020). Impact of key nicotinic achr subunits on post-stroke pneumococcal pneumonia. *Vaccines*, *8*, 253. https://doi.org/10.3390/vaccines8020253.

Jassam, Y. N., Izzy, S., Whalen, M., McGavern, D. B., El Khoury, J. (2017). Neuroimmunology of traumatic brain injury: Time for a paradigm shift. *Neuron*, *95*, 1246–1265. https://doi.org/10.1016/j.neuron.2017.07.010.

Jiang, D., Gong, F., Ge, X., Lv, C., Huang, C., Feng, S., Zhou, Z. et al (2020). Neuron-derived exosomes-transmitted miR-124-3p protect traumatically injured spinal cord by suppressing the activation of neurotoxic microglia and astrocytes. *Journal of Nanobiotechnology*, *18*, 1–20.

Jiang, W., Li, D., Han, R., Zhang, C., Jin, W. N., Wood, K., Liu, Q., Shi, F. D., & Hao, J. (2017). Acetylcholine-producing NK cells attenuate CNS inflammation via modulation of infiltrating monocytes/macrophages. *Proceedings of the National Academy of Sciences*, *114*, E6202–E6211. https://doi.org/10.1073/pnas.1705491114.

Jiang, Y., Li, L., Liu, B., Zhang, Y., Chen, Q., & Li, C. (2014). Vagus nerve stimulation attenuates cerebral ischemia and reperfusion injury via endogenous cholinergic pathway in rat. *PLoS One*, *9*, e102342.–https://doi.org/10.1371/journal.pone.0102342.

Jiang, Y., Li, L., Tan, X., Liu, B., Zhang, Y., & Li, C. (2015). MiR-210 mediates vagus nerve stimulation-induced antioxidant stress and anti-apoptosis reactions following cerebral ischemia/reperfusion injury in rats. *Journal of Neurochemistry*, *134*, 173–181. https://doi.org/10.1111/jnc.13097.

Kalamida, D., Poulas, K., Avramopoulou, V., Fostieri, E., Lagoumintzis, G., Lazaridis, K., Sideri, A., Zouridakis, M., & Tzartos, S. J. (2007). Muscle and neuronal nicotinic acetylcholine receptors: Structure, function and pathogenicity. *FEBS Journal*, *274*(15), 3799–3845. https://doi.org/10.1111/j.1742-4658.2007.05935.x.

Katsuki, H., & Matsumoto, K. (2018) Nicotinic acetylcholine receptors in regulation of pathology of cerebrovascular disorders. In A. Akaike, S. Shimohama, & Y. Misu (Eds.), *Nicotinic acetylcholine*, (pp. 113–136). Springer. https://doi.org/10.1007/978-981-10-8488-1_7.

Kawashima, K., & Fujii, T. (2000). Extraneuronal cholinergic system in lymphocytes. *Pharmacology & Therapeutics*, *86*, 29–48.

Kiesel, E. K., Hopf, Y. M., & Drey, M. (2018). An anticholinergic burden score for German prescribers: Score development. *BMC Geriatrics*, *18*, 239. https://doi.org/10.1186/s12877-018-0929-6.

Kim, H. K., Fuchs, G., Wang, S., Wei, W., Zhang, Y., Park, H., Roy-Chaudhuri, B., Li, P., Xu, J., Chu, K., Zhang, F., Chua, M.-S., So, S., Zhang, Q. C., Sarnow, P., & Kay, M. A. (2017). A transfer-RNA-derived small RNA regulates ribosome biogenesis. *Nature*, *552*, 57. https://doi.org/10.1038/nature25005.

Kim, H. K., Yeom, J. H., & Kay, M. A. (2020). Transfer RNA-derived small RNAs: Another layer of gene regulation and novel targets for disease therapeutics. *Molecular Therapy*, *28*, 2340–2357. https://doi.org/10.1016/j.ymthe.2020.09.013.

Kim, J. O., Lee, S. J., & Pyo, J. S. (2020). Effect of acetylcholinesterase inhibitors on post-stroke cognitive impairment and vascular dementia: A meta-analysis. *PLoS One*, *15*, e0227820. https://doi.org/10.1371/journal.pone.0227820.

Lechevallier-Michel, N., Molimard, M., Dartigues, J. F., Fabrigoule, C., & Fourrier-Reglat, A. (2005). Drugs with anticholinergic properties and cognitive performance in the elderly: Results from the PAQUID Study. *British Journal of Clinical Pharmacology*, *59*, 143–151. https://doi.org/10.1111/j.1365-2125.2004.02232.x.

Li, Q., Hu, B., Hu, G. W., Chen, C. Y., Niu, X., Liu, J., Zhou, S. M., Zhang, C. Q., Wang, Y., & Deng, Z. F. (2016). TRNA-derived small non-coding RNAs in response to ischemia inhibit angiogenesis. *Scientific Reports*, *6*, 20850.

Li, S., Xu, Z., & Sheng, J. (2018). tRNA-derived small RNA: A novel regulatory small non-coding RNA. *Genes (Basel)*, *9*, 246. https://doi.org/10.3390/genes9050246.

Lin, T., Simchovitz, A., Shenhar-Tsarfaty, S., Vaisvaser, S., Admon, R., Hanin, G., Hanan, M., Kliper, E., Bar-Haim, Y., Shomron, N., Fernandez, G., Lubin, G., Fruchter, E., Hendler, T., & Soreq, H. (2016). Intensified vmPFC surveillance over PTSS under perturbed microRNA-608/AChE interaction. *Translational Psychiatry*, *6*, e801. https://doi.org/10.1038/tp.2016.70.

Lin, Y.-T., Wu, P.-H., Chen, C.-S., Yang, Y.-H.- Y.-H., & Yang, Y.-H.- Y.-H. (2016). Association between acetylcholinesterase inhibitors and risk of stroke in patients with dementia. *Scientific Reports*, *6*, 29266. https://doi.org/10.1038/srep29266.

Liu, H., Leak, R. K., & Hu, X. (2016). Neurotransmitter receptors on microglia. *Stroke and Vascular Neurology*, *1*, 52–58.

Liu, Q., Jin, W.-N., Liu, Y., Shi, K., Sun, H., Zhang, F., Zhang, C., Gonzales, R. J., Sheth, K. N., La Cava, A., & Shi, F.-D. (2017). Brain ischemia suppresses immunity in the periphery and brain via different neurogenic innervations. *Immunity*, *46*, 474–487. https://doi.org/10.1016/j.immuni.2017.02.015.

Lobentanzer, S., Hanin, G., Klein, J., & Soreq, H. (2019). Integrative transcriptomics reveals sexually dimorphic control of the cholinergic/neurokine interface in schizophrenia and bipolar disorder. *Cell Reports*, *29*, 764–777.e5. https://doi.org/10.1016/j.celrep.2019.09.017.

Loewi, O. (1936) Nobel Lecture. *NobelPrize.org. Nobel Media AB 2020* 2020-11–22.

Madrer, N., & Soreq, H. (2020). Cholino-ncRNAs modulate sex-specific- and age-related acetylcholine signals. *FEBS Letters*, *594*, 2185–2198. https://doi.org/10.1002/1873-3468.13789.

Magee, R., Londin, E., & Rigoutsos, I. (2019). TRNA-derived fragments as sex-dependent circulating candidate biomarkers for Parkinson's disease. *Parkinsonism & Related Disorders*, *65*, 203–209.

Mappin-Kasirer, B., Pan, H., Lewington, S., Kizza, J., Gray, R., Clarke, R., & Peto, R. (2020). Tobacco smoking and the risk of Parkinson disease: A 65-year follow-up of 30,000 male British doctors. *Neurology*, *94*, e2132–e2138.

Martín, A., Domercq, M., & Matute, C. (2018). Inflammation in stroke: The role of cholinergic, purinergic and glutamatergic signaling. *Therapeutic Advances in Neurological Disorders*, *11*. https://doi.org/10.1177/1756286418774267.

Masanic, C. A., Bayley, M. T., VanReekum, R., & Simard, M. (2001). Open-label study of donepezil in traumatic brain injury. *Archives of Physical Medicine and Rehabilitation*, *82*, 896–901.

Mashimo, M., Komori, M., Matsui, Y. Y., Murase, M. X., Fujii, T., Takeshima, S., Okuyama, H., Ono, S., Moriwaki, Y., Misawa, H., & Kawashima, K. (2019). Distinct roles of α7 nAChRs in antigen-presenting cells and CD4+ T cells in the regulation of T cell differentiation. *Frontiers in Immunology*, *10*, 1102. https://doi.org/10.3389/fimmu.2019.01102.

Maute, R. L., Schneider, C., Sumazin, P., Holmes, A., Califano, A., Basso, K., & Dalla-Favera, R. (2013). tRNA-derived microRNA modulates proliferation and the DNA damage response and is down-regulated in B cell lymphoma. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 1404–1409.

Mauvais-Jarvis, F., Bairey Merz, N., Barnes, P. J., Brinton, R. D., Carrero, J.-J., DeMeo, D. L., De Vries, G. J., Epperson, C. N., Govindan, R., Klein, S. L., Lonardo, A., Maki, P. M., McCullough, L. D., Regitz-Zagrosek, V., Regensteiner, J. G., Rubin, J. B., Sandberg, K., & Suzuki, A. (2020). Sex and gender: Modifiers of health, disease, and medicine. *Lancet*, *396*, 565–582. https://doi.org/10.1016/S0140-6736(20)31561-0.

McAllister, T. W., Zafonte, R., Jain, S., Flashman, L. A., George, M. S., Grant, G. A., He, F., Lohr, J. B., Andaluz, N., Summerall, L., Paulus, M. P., Raman, R., & Stein, M. B. (2016). Randomized placebo-controlled trial of methylphenidate or Galantamine for persistent emotional and cognitive symptoms associated with PTSD and/or traumatic

brain injury. *Neuropsychopharmacology*, *41*, 1191–1198. https://doi.org/10.1038/npp.2015.282.

McCoy, A. N. & Tan, S. Y., & (2014). Otto loewi (1873–1961): Dreamer and nobel laureate. *Singapore Medical Journal*, *55*, 3–4.

McKee, C. A., & Lukens, J. R. (2016). Emerging roles for the immune system in traumatic brain injury. *Frontiers in Immunology*, *7*, 556.

McKinley, J. W., Shi, Z., Kawikova, I., Hur, M., Bamford, I. J., Sudarsana Devi, S. P., Vahedipour, A., Darvas, M., & Bamford, N. S. (2019). Dopamine deficiency reduces striatal cholinergic interneuron function in models of Parkinson's disease. *Neuron*, *103*, 1056–1072.e6. https://doi.org/10.1016/j.neuron.2019.06.013.

Meerson, A., Cacheaux, L., Goosens, K. A., Sapolsky, R. M., Soreq, H., & Kaufer, D. (2010). Changes in brain MicroRNAs contribute to cholinergic stress reactions. *Journal of Molecular Neuroscience*, *40*, 47–55.

Meisel, C., Schwab, J. M., Prass, K., Meisel, A., & Dirnagl, U. (2005). Central nervous system injury-induced immune deficiency syndrome. *Nature Reviews Neuroscience*, *6*, 775–786. https://doi.org/10.1038/nrn1765.

Meshorer, E., Erb, C., Gazit, R., Pavlovsky, L., Kaufer, D., Friedman, A., Glick, D., Ben-Arie, N., & Soreq, H. (2002). Alternative splicing and neuritic mRNA translocation under long-term neuronal hypersensitivity. *Science*, *80-.)*, *295*, 508–512.

Meyers, E. C., Solorzano, B. R., James, J., Ganzer, P. D., Lai, E. S., Rennaker, R. L., Kilgard, M. P., & Hays, S. A. (2018). Vagus nerve stimulation enhances stable plasticity and generalization of stroke recovery. *Stroke*, *49*, 710–717. https://doi.org/10.1161/STROKEAHA.117.019202.

Michell-Robinson, M. A., Touil, H., Healy, L. M., Owen, D. R., Durafourt, B. A., Bar-Or, A., Antel, J. P., & Moore, C. S. (2015). Roles of microglia in brain development, tissue maintenance and repair. *Brain*, *138*, 1138–1159. https://doi.org/10.1093/brain/awv066.

Mishra, N., Friedson, L., Hanin, G., Bekenstein, U., Volovich, M., Bennett, E. R., Greenberg, D. S., & Soreq, H. (2017). Antisense miR-132 blockade via the AChE-R splice variant mitigates cortical inflammation. *Scientific Reports*, *7*, 42755.

Morioka, N., Hisaoka-Nakashima, K., & Nakata, Y. (2018) Regulation by nicotinic acetylcholine receptors of microglial glutamate transporters: Role of microglia in neuroprotection. In A. Akaike S. Shimohama, & Y. Misu (Eds.), *Nicotinic acetylcholine receptor signaling in neuroprotection*, (pp. 73–82). Springer. https://doi.org/10.1007/978-981-10-8488-1_5.

Moshitzky, G., Shoham, S., Madrer, N., Husain, A. M., Greenberg, D. S., Yirmiya, R., Ben-Shaul, Y., & Soreq, H. (2020). Cholinergic stress signals accompany MicroRNA-associated stereotypic behavior and Glutamatergic Neuromodulation in the Prefrontal Cortex. *Biomolecules*, *10*, 848. https://doi.org/10.3390/biom10060848.

Nadorp, B., & Soreq, H. (2014). Predicted overlapping microRNA regulators of acetylcholine packaging and degradation in neuroinflammation-related disorders. *Frontiers in Molecular Neuroscience*, *7*, 9. https://doi.org/10.3389/fnmol.2014.00009.

Ng, S. Y., & Lee, A. Y. W. (2019). Traumatic brain injuries: Pathophysiology and potential therapeutic targets. *Frontiers in Cellular Neuroscience 13*, 528. https://doi.org/10.3389/fncel.2019.00528.

Noble, J. M., Hauser, W. A., Silver, J. M., & Harvey, P. D. (2007). Effects of rivastigmine on cognitive function in patients with traumatic brain injury. *Neurology*, *68*, 1749. https://doi.org/10.1212/01.wnl.0000266745.86958.ce.

Nordberg, A. (2006). Emerging biology of the cholinergic system across the spectrum of Alzheimer's disease. *International Psychogeriatrics*, *18*, S3–S16. https://doi.org/10.1017/S1041610206003991.

Norris, G. T., & Kipnis, J. (2019). Immune cells and CNS physiology: Microglia and beyond. *Journal of Experimental Medicine*, *216*, 60–70.

Östberg, A., Ledig, C., Katila, A., Maanpää, H.-R., Posti, J. P., Takala, R., Tallus, J., Glocker, B., Rueckert, D., & Tenovuo, O. (2020). Volume change in frontal cholinergic structures after traumatic brain injury and cognitive outcome. *Frontiers in Neurology*, *11*, 832.

Pannell, M., Meier, M. A., Szulzewsky, F., Matyash, V., Endres, M., Kronenberg, G., Prinz, V., Waiczies, S., Wolf, S. A., & Kettenmann, H. (2016). The subpopulation of microglia expressing functional muscarinic acetylcholine receptors expands in stroke and Alzheimer's disease. *Brain Structure and Function*, *221*, 1157–1172.

Parsons, C. G., Danysz, W., Dekundy, A., & Pulte, I. (2013). Memantine and cholinesterase inhibitors: complementary mechanisms in the treatment of Alzheimer's disease. *Neurotoxicity Research*, *24*(3), 358–369.

Patel, H., McIntire, J., Ryan, S., Dunah, A., & Loring, R. (2017). Anti-inflammatory effects of astroglial α7 nicotinic acetylcholine receptors are mediated by inhibition of the NF-KB pathway and activation of the Nrf2 pathway. *Journal of Neuroinflammation*, *14*(1), 192. https://doi.org/10.1186/s12974-017-0967-6.

Pereira, J. B., Hall, S., Jalakas, M., Grothe, M. J., Strandberg, O., Stomrud, E., Westman, E., vanWesten, D., & Hansson, O. (2020). Longitudinal degeneration of the basal forebrain predicts subsequent dementia in Parkinson's disease. *Neurobiology of Disease*, *139*, 104831

Perez-Lloret, S., & Barrantes, F. J. (2016). Deficits in cholinergic neurotransmission and their clinical correlates in Parkinson's disease. *NPJ Parkinson's Disease*, *2*, 1–12.

Pieper, N. T., Grossi, C. M., Chan, W.-Y., Loke, Y. K., Savva, G. M., Haroulis, C., Steel, N., Fox, C., Maidment, I. D., Arthur, A. J., Myint, P. K., Smith, T. O., Robinson, L., Matthews, F. E., Brayne, C., Richardson, K. (2020). Anticholinergic drugs and incident dementia, mild cognitive impairment and cognitive decline: A meta-analysis. *Age and Ageing*, *49* (6), 939–947. https://doi.org/10.1093/ageing/afaa090.

Pohanka, M. (2014). Inhibitors of acetylcholinesterase and butyrylcholinesterase meet immunity. *International Journal of Molecular Sciences*, *15*, 9809–9825.

Polito, F., Famà, F., Oteri, R., Raffa, G., Vita, G., Conti, A., Daniele, S. et al (2020). Circulating miRNAs expression as potential biomarkers of mild traumatic brain injury. *Molecular Biology Reports*, *47*, 2941–2949.

Qin, C., Feng, H., Zhang, C., Zhang, X., Liu, Y., Yang, D.-G., Du, L.-J. et al (2020). Differential expression profiles and functional prediction of tRNA-derived small RNAs in rats after traumatic spinal cord injury. *Frontiers in Molecular Neuroscience*, *12*, 326.

Qu, J. F., Chen, Y. K., Luo, G. P., Zhao, J. H., Zhong, H. H., & Yin, H. P. (2018). Severe lesions involving cortical cholinergic pathways predict poorer functional outcome in acute ischemic stroke. *Stroke*, *49*, 2983–2989. https://doi.org/10.1161/STROKEAHA.118.023196.

Quik, M., O'Leary, K., & Tanner, C. M. (2008). Nicotine and Parkinson's disease: Implications for therapy. *Movement Disorders*, *23*(12), 1641–1652.

Ray, N. J., Bradburn, S., Murgatroyd, C., Toseeb, U., Mir, P., Kountouriotis, G. K., Teipel, S. J., & Grothe, M. J. (2018). In vivo cholinergic basal forebrain atrophy predicts cognitive decline in de novo Parkinson's disease. *Brain*, *141*, 165–176. https://doi.org/10.1093/brain/awx310.

Revathikumar, P., Bergqvist, F., Gopalakrishnan, S., Korotkova, M., Jakobsson, P. J., Lampa, J., Maître, E., & Le Maître, E. (2016). Immunomodulatory effects of nicotine on interleukin 1β activated human astrocytes and the role of cyclooxygenase 2 in the underlying mechanism. *Journal of Neuroinflammation*, *13*(1), 256. https://doi.org/10.1186/s12974-016-0725-1.

Rosas-Ballina, M., Ochani, M., Parrish, W. R., Ochani, K., Harris, Y. T., Huston, J. M., Chavan, S., & Tracey, K. J. (2008). Splenic nerve is required for cholinergic antiinflammatory pathway control of TNF in endotoxemia. *Proceedings of the National Academy of Sciences*, *105*, 11008–11013. https://doi.org/10.1073/pnas.0803237105.

Rosas-Ballina, M., Olofsson, P. S., Ochani, M., Valdés-Ferrer, S. I., Levine, Y. A., Reardon, C., Tusche, M. W. et al (2011). Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. *Science*, *80-.)*, *334*, 98–101.

Roshchina, V. V. (2010). Evolutionary considerations of neurotransmitters in microbial, plant, and animal cells, in *Microb. Endocrinol. Interkingdom Signal. Infect. Dis. Heal.* (M. Lyte & P. P. Freestone eds), pp. 17. Springer Science and Business Media LLC.

Saand, A. R., Yu, F., Chen, J., & Chou, S. H. Y. (2019). Systemic inflammation in hemorrhagic strokes – A novel neurological sign and therapeutic target? *Journal of Cerebral Blood Flow and Metabolism*, *39*, 959–988. https://doi.org/10.1177/0271678X19841443.

Sabbagh, M. N., Hendrix, S., & Harrison, J. E. (2019). FDA position statement "Early Alzheimer's disease: Developing drugs for treatment, Guidance for Industry". *Alzheimer's & Dementia: Translational Research & Clinical Interventions*, *5*, 13–19.

Saldanha, C. (2017). Human erythrocyte acetylcholinesterase in health and disease. *Molecules*, *22*, 1499–https://doi.org/10.3390/molecules22091499.

Sanchez, J. R., Marsh, S., McIntyre, L., Davtyan, H., Walsh, C., & Blurton-Jones, M. (2020). Cytotoxic T cells infiltrate the brain and interact with microglia to reduce Alzheimer's Disease pathogenesis. *The Journal of Immunology*, *204*(Suppl 1), 64.4.

Saternos, H. C., Almarghalani, D. A., Gibson, H. M., Meqdad, M. A., Antypas, R. B., Lingireddy, A., & Aboualaiwi, W. A. (2018). Distribution and function of the muscarinic receptor subtypes in the cardiovascular system. *American Physiological Society*, *50*(1), 1–9. https://doi.org/10.1152/physiolgenomics.00062.2017.

Schliebs, R., & Arendt, T. (2011). The cholinergic system in aging and neuronal degeneration. *Behavioral Brain Research*, *221*, 555–563.

Schmitz, T. W., & Nathan, S. R. (2016). Basal forebrain degeneration precedes and predicts the cortical spread of Alzheimer's pathology. *Nature Communications*, *7*, 1–13.

Sfera, A., Osorio, C., Price, A. I., Gradini, R., & Cummings, M. (2015). Delirium from the gliocentric perspective. *Frontiers in Cellular Neuroscience*, *9*, 171. https://doi.org/10.3389/fncel.2015.00171

Shaked, I., Meerson, A., Wolf, Y., Avni, R., Greenberg, D., Gilboa-Geffen, A., & Soreq, H. (2009). MicroRNA-132 potentiates cholinergic anti-inflammatory signaling by targeting acetylcholinesterase. *Immunity*, *31*, 965–973. https://doi.org/10.1016/j.immuni.2009.09.019.

Shaked, I., Zimmerman, G., & Soreq, H. (2008). Stress-induced alternative splicing modulations in brain and periphery: Acetylcholinesterase as a case study. *Annals of the New York Academy of Sciences*, *1148*, 269–281. https://doi.org/10.1196/annals.1410.001.

Shaltiel, G., Hanan, M., Wolf, Y., Barbash, S., Kovalev, E., Shoham, S., & Soreq, H. (2013). Hippocampal microRNA-132 mediates stress-inducible cognitive deficits through its acetylcholinesterase target. *Brain Structure and Function*, *218*, 59–72.

Sheinerman, K. S., Toledo, J. B., Tsivinsky, V. G., Irwin, D., Grossman, M., Weintraub, D., Hurtig, H. I. et al (2017). Circulating brain-enriched microRNAs as novel biomarkers for detection and differentiation of neurodegenerative diseases. *Alzheimer's Research & Therapy*, *9*, 1–13.

Sheinerman, K., Tsivinsky, V., Mathur, A., Kessler, D., Shaz, B., & Umansky, S. (2018). Age- and sex-dependent changes in levels of circulating brain-enriched microRNAs during normal aging. *Aging (Albany., NY)*, *10*, 3017–3041.

Shin, S. S., & Dixon, C. E. (2015). Alterations in cholinergic pathways and therapeutic strategies targeting cholinergic system after traumatic brain injury. *Journal of Neurotrauma*, *32*, 1429–1440. https://doi.org/10.1089/neu.2014.3445.

Shirdel, E. A., Xie, W., Mak, T. W., & Jurisica, I. (2011). NAViGaTing the micronome—Using multiple microRNA prediction databases to identify signalling pathway-associated microRNAs. *PLoS One*, *6*, e17429. https://doi.org/10.1371/journal.pone.0017429.

Silver, J. M., Koumaras, B., Chen, M., Mirski, D., Potkin, S. G., Reyes, P., Warden, D., Harvey, P. D., Arciniegas, D., Katz, D. I., & Gunay, I. (2006). Effects of rivastigmine on cognitive function in patients with traumatic brain injury. *Neurology*, *67*, 748–755. https://doi.org/10.1212/01.wnl.0000234062.98062.e9.

Simchovitz, A., Heneka, M. T., & Soreq, H. (2017). Personalized genetics of the cholinergic blockade of neuroinflammation. *Journal of Neurochemistry*, *142*, 178–187. https://doi.org/10.1111/jnc.13928.

Simchovitz-Gesher, A., & Soreq, H. (2020). Pharmaceutical implications of sex-related RNA divergence in psychiatric disorders. *Trends in Pharmacological Sciences*, *41*(11), 840–850.

Soreq, H. (2015). Checks and balances on cholinergic signaling in brain and body function. *Trends in Neurosciences*, *38*, 448–458. https://doi.org/10.1016/j.tins.2015.05.007.

Soreq, H., & Seidman, S. (2001). Acetylcholinesterase–new roles for an old actor. *Nature Reviews Neuroscience*, *2*, 294–302. https://doi.org/10.1038/35067589.

Stolero, N., & Frenkel, D. (2020). The dialog between neurons and microglia in Alzheimer's disease: The neurotransmitters view. *Journal of Neurochemistry*, https://doi.org/10.1111/jnc.15262.

Sun, Y., Li, Q., Gui, H., Xu, D. P., Yang, Y. L., Su, D. F., & Liu, X. (2013). MicroRNA-124 mediates the cholinergic anti-inflammatory action through inhibiting the production of pro-inflammatory cytokines. *Cell Research*, *23*, 1270–1283. https://doi.org/10.1038/cr.2013.116.

Sun, Y., Luo, Z. M., Guo, X. M., Su, D. F., & Liu, X. (2015). An updated role of microRNA-124 in central nervous system disorders: A review. *Frontiers in Cellular Neuroscience*, *9*, 193.

Swarbrick, S., Wragg, N., Ghosh, S., & Stolzing, A. (2019). Systematic review of miRNA as biomarkers in Alzheimer's disease. *Molecular Neurobiology*, *56*, 6156–6167.

Tan, E. C. K., Johnell, K., Garcia-Ptacek, S., Haaksma, M. L., Fastbom, J., Bell, J. S., & Eriksdotter, M. (2018). Acetylcholinesterase inhibitors and risk of stroke and death in people with dementia. *Alzheimer's & Dementia*, *14*, 944–951. https://doi.org/10.1016/j.jalz.2018.02.011.

Tiedt, S., Prestel, M., Malik, R., Schieferdecker, N., Duering, M., Kautzky, V., Stoycheva, I., Böck, J., Northoff, B. H., Klein, M., Dorn, F., Krohn, K., Teupser, D., Liesz, A., Plesnila, N., Holdt, L. M., & Dichgans, M. (2017). RNA-seq identifies circulating miR-125a-5p, miR-125b-5p, and miR-143-3p as potential Biomarkers for acute ischemic stroke. *Circulation Research*, *121*, 970–980. https://doi.org/10.1161/CIRCRESAHA.117.311572.

Tokar, T., Pastrello, C., Rossos, A. E. M., Abovsky, M., Hauschild, A. C., Tsay, M., Lu, R., & Jurisica, I. (2018). MirDIP 4.1—Integrative database of human microRNA target predictions. *Nucleic Acids Research*, *46*, D360–D370.

Unger, M. S., Li, E., Scharnagl, L., Poupardin, R., Altendorfer, B., Mrowetz, H., Hutter-Paier, B., Weiger, T. M., Heneka, M. T., Attems, J., & Aigner, L. (2020). CD8+ T-cells infiltrate Alzheimer's disease brains and regulate neuronal- and synapse-related gene expression in APP-PS1 transgenic mice. *Brain, Behavior, and Immunity*, *89*, 67–86. https://doi.org/10.1016/j.bbi.2020.05.070.

Vreugdenhil, E., Verissimo, C. S. L., Mariman, R., Kamphorst, J. T., Barbosa, J. S., Zweers, T., Champagne, D. L., Schouten, T., Meijer, O. C., Ron de Kloet, E., & Fitzsimons, C. P. (2009). MicroRNA 18 and 124a down-regulate the glucocorticoid receptor: Implications for glucocorticoid responsiveness in the brain. *Endocrinology*, *150*(5), 2220–2228. https://doi.org/10.1210/en.2008-1335.

Weigand, A. J., Bondi, M. W., Thomas, K. R., Campbell, N. L., Galasko, D. R., Salmon, D. P., Sewell, D., Brewer, J. B., Feldman, H. H., & Delano-Wood, L. (2020). Association of anticholinergic medication and AD biomarkers with incidence of MCI among cognitively normal older adults. *Neurology*, *95*, https://doi.org/10.1212/WNL.0000000000010643

Winek, K., Lobentanzer, S., Nadorp, B., Dubnov, S., Dames, C., Jagdmann, S., Moshitzky, G. et al (2020). Transfer RNA fragments replace microRNA regulators of the cholinergic poststroke immune blockade. *Proceedings of the National Academy of Sciences of the United States of America*, *117*, 32606–32616.

Záborszky, L., Gombkoto, P., Varsanyi, P., Gielow, M. R., Poe, G., Role, L. W., Ananth, M., Rajebhosale, P., Talmage, D. A., Hasselmo, M.

E., Dannenberg, H., Minces, V. H., & Chiba, A. A. (2018). Specific basal forebrain–cortical cholinergic circuits coordinate cognitive operations. *Journal of Neuroscience*, *38*, 9446–9458. https://doi.org/10.1523/JNEUROSCI.1676-18.2018.

Zhao, J., Hylin, M. J., Kobori, N., Hood, K. N., Moore, A. N., & Dash, P. K. (2018). Post-injury administration of galantamine reduces traumatic brain injury pathology and improves outcome. *Journal of Neurotrauma*, *35*, 362–374. https://doi.org/10.1089/neu.2017.5102.

Zuo, X., Lu, J., Manaenko, A., Qi, X., Tang, J., Mei, Q., Xia, Y., & Hu, Q. (2019). MicroRNA-132 attenuates cerebral injury by protecting blood-brain-barrier in MCAO mice. *Experimental Neurology*, *316*, 12–19.

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