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# Cholinergic Neurons of The Rat Nervous System

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**Received Date: March 05, 2024**; **Accepted Date: March 25, 2024; Published Date: March 31, 2025**

**Citation:** Bon E.I., Maksimovich N.Ye., Otlivanchik N.I., Yurchenko P.A., Martysyuk A.A., (2025), Cholinergic Neurons of The Rat Nervous System, *Clinical Trials and Clinical Research*,4(2); **DOI:**10.31579/2834-5126/095



**Abstract**

Neurons that produce acetylcholine are widely distributed and widely projected throughout the central nervous system. In mammals, the nuclei that form the cholinergic system are generally divided into seven distinct complexes, which include: cortical neurons; striatal interneurons; basal neurons of the forebrain; diencephalic/hypothalamic neurons; pontomesencephalic neurons; medullary tegmental neurons; and motor neurons of the nuclei of the cranial nerves and spinal cord.

**Keywords:** cholinergic neurons; rat; nervous system

## Introduction

Neurons that produce acetylcholine are widely distributed and widely projected throughout the central nervous system. In mammals, the nuclei that form the cholinergic system are generally divided into seven distinct complexes, which include: cortical neurons; striatal interneurons; basal neurons of the forebrain; diencephalic/hypothalamic neurons; pontomesencephalic neurons; medullary tegmental neurons; and motor neurons of the nuclei of the cranial nerves and spinal cord. [1]. Cholinergic synapses are ubiquitous in the human central nervous system. Their high density in the thalamus, striatum, limbic system, and neocortex suggests that cholinergic transmission is likely critical for memory, learning, attention, and other higher brain functions. Several lines of research suggest an additional role for cholinergic systems in overall brain homeostasis and plasticity. [2]. The cholinergic system powerfully modulates physiology and behavior in normal health and disease. It is the most widely used pharmacological target to improve the progression of Alzheimer's disease, which is the leading cause of aging worldwide. However, the comprehensive genetic and schema-based structure of how the cholinergic system is organized to engage its diverse behaviors is still elucidated. [3]. Aminoacids play an important role in the metabolism and functioning of the brain. This is explained not only by their exceptional role as sources of synthesis of a large number of biologically important compounds (proteins, mediators, lipids, biologically active amines). Amino acids and their derivatives are involved in synaptic transmission as neurotransmitters and neuromodulators (glutamate, aspartate, glycine, GABA, taurine), and some amino acids are involved in the formation of neurotransmitters of the nervous system: methionine – acetylcholine, DOPA, dopamine; tyrosine – catecholamines; serine and cysteine – taurine; tryptophan – serotonin; L-arginine – NO; glutamic acid – glutamate [20]. The habenula is a complex nucleus composed of lateral and medial subnuclei, which connect between the limbic forebrain and midbrain. Recently, the medial habenula cholinergic system has attracted more attention because of its potential to provide therapeutic targets for the treatment of nicotine withdrawal symptoms, drug addiction, and various mood disorders [8]. Approximately one-third of individuals diagnosed with major depressive disorder also have a substance use disorder (excluding nicotine dependency), with almost half of all patients with depression having a family history of substance use disorders. Accordingly, both addiction to multiple drugs of abuse and mood-associated conditions, and the medial habenula-interpeduncular nucleus circuit may represent a junction at which signaling underlying both sets of conditions occurs [10]. Interneurons of the striatum are critical to basal ganglia function and have emerged as a locus of pathology in a number of neuropsychiatric conditions [13]. Striatal cholinergic interneurons (ChIs) are central for the processing and reinforcement of reward-related behaviors that are negatively affected in states of altered dopamine transmission, such as in Parkinson's disease or drug addiction [11]. Cholinergic projection neurons of the basal forebrain (usually known as basal forebrain cholinergic neurons (BFCNs)) span the entire rostrocaudal extent of the telencephalon but loosely cluster in several broad subregions (defined on the basis of anatomical landmarks), including the medial septum, the vertical and horizontal subdivisions of the diagonal band, the ventral pallidum, the substantia innominata and nucleus basalis. However, increasing evidence now points to a functional organization that extends beyond these anatomical boundaries and suggests that this, together with their subregional heterogeneity, enables them to coordinate diverse actions across the brain [14]. BF cholinergic neurons are located on the base of the brain and can be found in clusters from the olfactory tubercle to the rostral end of the lateral geniculate bodies. At the anterior pole of the BF are cholinergic neurons in the medial septum and diagonal band of Broca (MS/DBB). These cholinergic neurons project to the Hipp and midline cortical structures such as the posterior anterior cingulate cortex,

retrosplenial cortex (RSC), and mPFC, including the infralimbic cortex (IL) [15]. Targeted depletion of basal forebrain cholinergic neurons results in significant impairments in training on the rotarod task of coordinated movement. Cholinergic neuromodulation is required during training sessions as chemogenetic inactivation of cholinergic neurons also impairs task acquisition [16]. In recent years there has been a renewed interest in the basal forebrain cholinergic system as a target for the treatment of cognitive impairments in patients with Parkinson's disease, due in part to the need to explore novel approaches to treat the cognitive symptoms of the disease and in part to the development of more refined imaging tools that have made it possible to monitor the progressive changes in the structure and function of the basal forebrain system as they evolve over time [19].

## Results and discussion

Acetylcholine is the main neurotransmitter in the brain, exerting activity throughout the cerebral cortex, basal ganglia, and basal forebrain. Choline is a critical substrate for the synthesis of acetylcholine. Acetyl coenzyme A (Ac CoA), which is formed as a result of the breakdown of glucose (carbohydrate) via glycolysis (Krebs cycle), together with the enzyme choline acetyltransferase (ChAT), are crucial for the synthesis of acetylcholine (Ach). Once the neurotransmitter acetylcholine is released into the synapse, it binds to (activates) the postsynaptic receptor (M1), thereby transmitting a signal from one neuron to another. Excess neurotransmitter in the synaptic cleft is broken down by the enzyme acetylcholinesterase (AChE) into choline and acetate, which are returned by the uptake mechanism for recycling to acetylcoenzyme A.[2] Neural networks are regulated by three-dimensional spatial and structural properties. Despite strong evidence of a functional effect on cognitive modulation, little is known about the three-dimensional internal organization of cholinergic networks in the forebrain. Cholinergic networks in the forebrain mainly originate in the subcortical nuclei, namely the septum, the basal nucleus, the globus pallidus, the nucleus accumbens, and the caudate putamen. Thus, the present study analyzed the three-dimensional spatial organization of 14,000 cholinergic neurons expressing choline acetyltransferase (ChAT) in these subcortical nuclei of the mouse forebrain. The theory of point processes and graph signal processing methods have defined three topological principles of organization. First, cholinergic interneuronal distance is uneven in different brain regions. Specifically, in the septum, globus pallidus, nucleus accumbens, and caudate putamen, cholinergic neurons were grouped compared to a uniform random distribution. In contrast, in the basal nucleus, cholinergic neurons had a spatial distribution with greater regularity than a uniform random distribution. Second, a quarter of the caudate putamen consists of axonal bundles, however, the spatial distribution of cholinergic neurons remained clustered when axonal bundles were taken into account. However, a comparison with the inhomogeneous Poisson distribution showed that the results of basalis nucleus and caudate putamen can be explained by density gradients in these structures. Third, the number of cholinergic neurons varies depending on the volume of a particular area of the brain, but the volume of the cell body is constant in different areas. [7]The cholinergic projection system of the basal forebrain has historically been divided into groups called Ch1, Ch2, Ch3, and Ch4 based on the arrangement of their bodies in cells and projection patterns, where Ch1 and 2 (medial septum and vertical diagonal band nucleus) project into the hippocampus, Ch3 (horizontal diagonal nucleus) projects onto the olfactory bulb, and Ch4 (basal nucleus) projects onto the cortex and However, in the decades since this classification, it has become clear that there is an additional organization within each of these groups that results in cells with different projection fields. Similarly, while striatum cholinergic interneurons can be anatomically divided into two primary populations located in the nucleus accumbens and the caudate of the putamen, respectively, it is possible that additional functionally significant subgroups may be further distinguished. Cholinergic interneurons show considerable diversity in their physiology, morphology, and connections, suggesting that such subgroups probably exist. [4] Cholinergic fibers originate primarily from the basal forebrain, including the medial septum (MS), which contains ~10% acetylcholine-producing neurons (Ach) and is the predominant source of cholinergic innervation in hippocampal formation. neurons showed divergent outcomes, including learning and memory rewards, plasticity, attention, sleep-wake cycles, and even arousal. While different behavioral assays and lesion techniques can contribute to this diversity of results, they can also be derived from considering cholinergic neurons in the brain as a homogeneous population. Recent studies using electrophysiology and synaptic tracing techniques have shown the heterogeneity of cholinergic neurons in the basal forebrain, similar to the dopaminergic and noradrenergic systems in the brain. However, the nature of cholinergic heterogeneity and its potential role in shaping the behavior of individual cholinergic neurons and/or types of neurons remain unknown. Choline acetyltransferase (ChAT) is an enzyme for the synthesis of the neurotransmitter Ach and is widely used as a cellular marker for cholinergic neurons. In the basal forebrain, cholinergic neurons also express a number of other proteins from human tissues, including the low-affinity p75 nerve growth factor receptor and calbindin-D28K (D28K). D28K acts as aCa2+ buffer and sensor in mammalian cells. However, the molecular, physiological, and functional features of D28K expression (D28K) versus D28K-absence (D28K)+—) of cholinergic neurons have yet to be studied, mainly due to technical limitations and fundamental methodological difficulties. [3] Cholinergic neurons in the brain are primarily projection neurons connecting different areas of the CNS, with motor neurons and some autonomic neurons interacting between the CNS and the peripheral nervous system. The striatum has the highest density of cholinergic markers in the brain, highlighting the crucial but poorly understood role of cholinergic interneurons in the Functions of the striatum. 12 The most extensive system of cholinergic projection in the brain is the basal complex of the forebrain, which also contains populations of GABAergic and glutamatergic neurons. Cholinergic neurons in the forebrain project into the cortical mantle and are associated with attention, memory, and learning.At the same time, studies have revealed clusters with specific links with cortical targets. Neurons in the dorsal motor nucleus of the vagus nerve are cholinergic in nature and provide parasympathetic innervation to several organs, including from the lower esophagus to the proximal colon in the gastrointestinal tract. The distal colon and other organs receive parasympathetic innervation from sacral cholinergic preganglionic neurons [5] Cholinergic neurons, as an important part of the central nervous system, play an important role in regulating nerve excitability, influencing synaptic transmission, inducing synaptic plasticity, and coordinating the excitation of neuronal populations, and can respond to internal and external stimuli by altering the state of neural networks in the brain. [6] Currently, there are two main types of cholinergic neurons in the brain, one is a projection neuron innervating the end region of the fiber, and the other is local interneurons scattered between target cells. Among them, projective neurons are distributed in many brain nuclei, such as the pontine nucleus of the foot and the lateral tegmental nucleus, the medial nucleus, and the basal complex of the forebrain, including the medial septal nucleus, which widely projects and innervates neural activity throughout the central nervous system. A large number of studies have shown that cholinergic processes such as cognition, learning, and memory, as well as synaptic plasticity, are widely involved in physiological processes such as cognition, learning, and memory, as well as synaptic plasticity, while cholinergic processes can regulate neuronal activity in structural regions of the temporal lobe, such as the acticholinergic

release from the basal forebrain by light in the CA1 region of the hippocampus, which can activate VIP/IS inhibitory neurons, which selectively innervate other inhibitory neurons. Thus, the disinhibition effect is enhanced. In addition, light-activated cholinergic projection from the nucleus to the hippocampus suppresses the excitation of sharp waves in the hippocampus and promotes theta rhythm.[ 6]An increase in glutamate levels while maintaining glutamine and GABA levels may be associated with an increase in glutaminase activity and/or transamination/restorative amination in neurons. At the same time, changes in the levels of AA with the properties of excitatory neurotransmitters (aspartate and glutamate) were multidirectional: a tendency to increase the level of glutamate and to decrease aspartate. The decrease in aspartate, in contrast to glutamate, can be explained by its increased utilization as glycogenic AA in oxidation reactions with the formation of energy[21]. Previous morphological studies in rats in the dynamics of subtotal cerebral ischemia revealed a decrease in the size of neuronal perikarions, an aggravation of their elongation, a decrease in the number of normochromic and hyperchromic neurons, and an increase in the proportion of hyperchromic shrunken neurons and cells with pericellular edema. At the ultrastructural level, mitochondria swelled with a decrease in the number and length of their cristae, vacuolization of the granular endoplasmic reticulum, and a predominance of free ribosomes over bound ribosomes were noted. These morphological changes were a consequence of pronounced disturbances in energy metabolism, especially when used as a substrate of succinate in vitro studies, indicating the most severe damage to the succinate dehydrogenase complex of the electron transport chain and accompanied by a decrease in the content of ATP synthase, an enzyme that reacts to form ATP from ADP[20].

In one-hour subtotal cerebral ischemia, there was a tendency to increase the content of the inhibitory neurotransmitter glycine in both studied regions, while the level of amino acids with excitatory neurotransmitter properties (aspartate and glutamate), on the contrary, tended to decrease.In SIGM, there was a tendency to decrease the level of one of the aromatic amino acids in the parietal lobe – tryptophan (a source of serotonin), while changes in the content of other aromatic amino acids (tyrosine, phenylalanine) were not observed in both the parietal lobe and the hippocampus ($p>0.05$). This may be the result of increased serotonin synthesis or a decrease in the transport of tryptophan to the brain. In this regard, it can be assumed that there are disorders in the formation of catecholamines in SIGM[20].Thus, the one-hour CHIGM is characterized by the following changes in the AA pool: an increase in glutamate and GABA without changes in the ratio of excitatory and inhibitory amino acids-transmitters, a decrease in the content of essential AAs with an increase in the "Non-essential/Essential" AA ratio as a reflection of the increased utilization of essential AAs. There were no changes in sulfur-containing AAs, except for a decrease in the content of methionine in the parietal lobe, which indicates insignificant disturbances in the prooxidant-oxidant balance in this model of IGM. There was a decrease in the content of branched-chain amino acids and a tendency to a decrease in the level of aromatic AAs (tyrosine, tryptophan, phenylalanine) with a decrease in their ratio as a reflection of a more pronounced utilization of ARUCs in comparison with aromatic AAs[21]. The cholinergic hypothesis of Alzheimer's disease is based on the progressive loss of limbic and neocortical cholinergic innervation. Neurofibrillary degeneration in the basal forebrain is thought to be the primary cause of dysfunction and death of cholinergic neurons in the forebrain, resulting in widespread presynaptic cholinergic denergic denervation. Cholinesterase inhibitors increase the availability of acetylcholine at brain synapses and are among the few drugs that have proven clinical efficacy in the treatment of dementia in Alzheimer's disease, thereby confirming that the cholinergic system is an important therapeutic target in the disease. This review includes a review of the role of the cholinergic system in cognitive ability and an updated understanding of how cholinergic deficits in Alzheimer's disease interact with other aspects of the disease's pathophysiology, including plaques made up of amyloid β proteins. This review also documents the benefits of cholinergic therapy at various stages of Alzheimer's disease and during long-term follow-up, as visualized in new imaging studies. The body of evidence supports the continued value of cholinergic drugs as a standard, cornerstone pharmacological approach to treating Alzheimer's disease, especially as we plan future combination therapies that address symptoms as well as disease progression. [2] The cholinergic hypothesis has revolutionized the field of Alzheimer's disease research, moving it from the field of descriptive neuropathology to the modern concept of synaptic neurotransmission. It is based on three main stages: the discovery of depleted presynaptic cholinergic markers in the cerebral cortex; the discovery that the basal Meinert nucleus (NBM) in the basal The forebrain region is the source of cortical cholinergic innervation, which undergoes severe neurodegeneration in Alzheimer's disease and demonstrates that cholinergic antagonists impair memory, while agonists have the opposite effect. The hypothesis was strongly supported when cholinesterase inhibitor therapy was shown to cause significant symptomatic improvement in patients with Alzheimer's disease.[2] The habenula is a small, bilateral brain structure located at the dorsal end of the diencephalon. This structure sends projections to the dopaminergic striatum and receives inputs from the limbic forebrain, making the habenula a unique modulator of cross-talk between these brain regions. The habenular complex on the dorsal diencephalon is surrounded by the third ventricle and includes the medial habenula (MHb) and lateral habenula (LHb)[9]. Studies have shown that neuronal activity in the lateral habenula (LHb) is regulated by factors in the external environment, such as rewards or aversive stimuli, and this altered LHb neuronal activity is associated with the onset of depression. The medial habenula (MHb) is divided into two subnuclei on the basis of cell type. Cholinergic neurons are located in the ventral two-thirds of the MHb (MHbV), and substance P-ergic neurons are located exclusively in the dorsal part of the MHb (MHbD) which mediates exercise motivation, regulates the hedonic state, and supports primary reinforcement[8]. The MHb projects to the Interpeduncular Nucleus (IPN) via the internal portion of the fasciculus retroflexus (FR) while the external portion of the FR axon bundle connects the LHb to the rostromedial tegmental nucleus[9]. The MHbV receives neural input from the triangular septum (TS) and projects to the central part of the IPN[8]. The MHb projections to the IPN are particularly noteworthy because they contain three major output neurotransmitters: acetylcholine(ACh), Substance P, and Glu. Unlike the LHb, which diffusely expresses neurotransmitters across sub-nuclei, neurotransmitter expression in the MHb is highly localized. The superior MHb is glutamatergic and also expresses Interleukin-18; the dorsal-central MHb is both glutamatergic and substance P-ergic; and the inferior, ventral-center and lateral MHb are both cholinergic and glutamatergic[9]. Since choline acetyltransferase (ChAT) is the only enzyme responsible for the biosynthesis of ACh, it is frequently used as a marker of cholinergic neurons. Immunohistochemistry with an anti-ChAT antibody and in situ hybridization against ChAT mRNA both show that habenula cholinergic neurons are restricted to the MHbV[8] Abstinence from chronic use of addictive drugs triggers an aversive withdrawal syndrome that compels relapse and deters abstinence. The dorsal diencephalic conduction system, and MHb and MHb-IPN in particular, have been identified as critical to the emergence of aversive states that arise both as a result, and independently, of addiction to opioids and alcohol. When considering another addictive drug, nicotine, individuals diagnosed with mental illnesses are approximately twice as likely to smoke tobacco, and studies have shown that 81.8% of individuals diagnosed with bipolar disorder, and 76.8% diagnosed with generalized anxiety disorder have

smoked daily for at least a month[10]. Genome-wide association studies suggest that specific single-nucleotide polymorphisms associated with an increased risk of nicotine dependence and nicotine addiction are located within a specific gene cluster on human chromosome 15 that encodes the α5 subunit[8]. The α5 subunit is expressed in the MHb and high doses of nicotine are shown to stimulate the MHb-IPN tract and inhibit nicotine consumption. However, mice lacking the nAChR α5 subunit have exhibited decreased MHb to IPN input and lack nicotine-induced inhibition of the brain's reward system[9]. The role of LHb in regulating dopaminergic activity in the ventral tegmental area via the rostromedial tegmental nucleus has been established, and represents an important mechanism by which aversion and addiction are modulated[10]. The habenula has been linked to drug addiction more generally through a series of rodent experiments and human genome-wide association studies. Habenula cholinergic neurons regulate the self-administration of drugs such as cocaine and methamphetamine, as well as the reinstatement of drug-seeking behaviors. Chemogenetic activation of habenula cholinergic neurons by a cre-dependent DREADD (Designer Receptors Exclusively Activated by Designer Drugs) system in ChAT-cre mice induces behaviors that mimic drug-seeking behavior, such as the reinstatement of a cocaine-induced conditioned place preference[8]. The MHb play an important role in anxiety and fear. Severing the parallel pathways between the MHb and the TS and bed nucleus of the anterior commissure in mice results in reduced anxiety and amplified fear responses. In zebrafish, nitroreductase lesions of the MHb increased fear-induced freezing behavior when electric shocks were paired with a red light compared to non-lesioned control fish. Similarly, when light chain tetanus toxin expressing larval zebrafish were trained to swim away from a red light that predicted shock, fish with tetanus toxin-induced MHb lesions developed freezing behavior gradually while control fish did not develop freezing behavior[9]. While the size of the habenular complex renders current neuroimaging technology incapable of distinguishing the LHb from the MHb in human studies, some studies indicate a role played by the habenular complex in the pathophysiology of bipolar disorder (BD). In a study using high-resolution magnetic resonance imaging (MRI), it was found that patients diagnosed with BD who had either never been medicated, or had been un-medicated for at least two months, exhibited smaller habenular volumes than healthy controls[3].

The striatum is a large subcortical nucleus involved in motor co-ordination and cognition, as well as disorders such as Parkinson's disease, Tourette's syndrome, Huntington's disease, schizophrenia, and drug addiction[12]. The striatum which is commonly divided into the dorsal and ventral striatum is the main entryway through which extrinsic afferents can influence functionally diverse basal ganglia circuits to generate context-dependent, goal-directed, and habitual behaviors. The dorsal striatum consists of the caudate nucleus and putamen, divided from each other by the internal capsule, whereas in rodents, it is a single mass of gray matter often referred to as the caudate–putamen complex. The ventral striatum consists of the nucleus accumbens and the striatal portion of the olfactory tubercle, along with the ventromedial extension of the caudate nucleus and putamen[11]. The principal neurons of the striatum are the medium spiny neurons (MSNs) which constitute ~90% of the striatal neuron population and form the striatal output. The remaining striatal neurons are comprised of at least three types of interneuron, including the large, aspiny, tonically active ChIs[12]. ChIs are the largest cells in the striatum that are recognized for their key regulatory roles of striatal and basal ganglia function in normal and diseased states which regulate aversive, attentional, motivational, and reward-related behaviors, as well as synaptic plasticity, conditioned learning, and action selection in the striatum. ChIs modulate the activity of striatal neurons through direct and indirect mechanisms via a wide range of pre- and postsynaptic cholinergic receptors[11]. Striatal ACh acts at two classes of cholinergic receptors, nicotinic acetylcholine receptors (nAChRs), and muscarinic acetylcholine receptors (mAChRs). Nicotinic receptors are ligand-gated ion channels composed of five subunits arranged symmetrically around a central pore. Muscarinic receptors are seven transmembrane domain, G-protein-coupled receptors[12]. The G protein–coupled muscarinic cholinergic receptors (mAChRs) and ionotropic nicotinic cholinergic receptors (nAChRs) are located on the surface of striatal neurons and their various synaptic afferents. Five types of mAChRs have been genetically identified (M1–M5) and categorized into two groups on the basis of their distinct pharmacological properties upon activation: the Gq/11-coupled M1-like receptors (M1, M3, and M5) that enhance internal calcium release through stimulation of phospholipases, and the Gi/o-coupled M2-like receptors (M2 and M4) that block calcium channel activity by reducing cyclic AMP formation through the inhibition of adenylyl cyclase. The abnormal activity of multiple neurotransmitter systems, including the cholinergic systems, precedes or coincides with dopaminergic dysfunction in parkinsonism and drug addiction, suggesting that a striatal dopamine–acetylcholine imbalance underlies some aspects of the pathophysiology of parkinsonism and substance abuse[11].Dysfunction of the cortico-basal circuitry – including its primary input nucleus, the striatum – contributes to neuropsychiatric disorders, including autism and Tourette Syndrome. These conditions show marked sex differences, occurring more often in males than in females. Regulatory interneurons, including cholinergic interneurons (CINs) and parvalbumin-expressing GABAergic fast spiking interneurons, are implicated in human neuropsychiatric disorders, and ablation of these interneurons produces relevant behavioral pathology in male mice, but not in females. The mechanisms underlying these differential patterns of interneuron density and distribution remain to be elucidated but are likely to involve sexually dimorphic modulation of developmental processes[13]. Many conditions affect males and females differentially; for example, TS is diagnosed approximately twice as often in males as in females. Whether striatal interneuron pathology is similarly sexually dimorphic is unknown, but preclinical evidence is beginning to suggest that it may be. The striatal circuitry can be impacted by the estrous cycle; both GABAergic and cholinergic interneurons express estrogen receptors. Furthermore, depletion of CINs during development (Cadeddu et al, 2023), or conjoint depletion of both CINs and FSIs in adults, produces dysregulated striatal activity and behavioral abnormalities of potential relevance to TS and related conditions – repetitive behavioral pathology, anxiety, and social deficits – in male mice, but not in females. This suggests an underlying sex difference in striatal interneurons, their regulation of local microcircuits, and their role in the modulation of striatum-dependent behaviors[13]. In the BF, cholinergic neurons are codistributed with several other cell populations, including GABAergic and various neurons containing calcium binding protein for example calbindin, calretinin or parvalbumin. These neurons project to all areas and layers of the cortex. The cholinergic projections modulate the response of pyramidal cells to other cortical-glutamatergic inputs, facilitating the bottom-up sensory information processing within the cortex. Furthermore, the long radiating dendrites of the cholinergic BF neurons receive inputs from all the brainstem and hypothalamic arousal systems, for example cholinergic ponto-mesencephalic neurons, noradrenergic LC neurons, dopaminergic ventral-mesencephalic neurons, histaminergic tubero-mammillary neurons and orexinergic perifornical neurons[17]. BFCNs are derived from progenitors in the ventricular zone of the ventral telencephalon (also known as the subpallium), which comprises various subregions: the ganglionic eminences (with medial, caudal and lateral subdivisions known as the medial ganglionic eminence, caudal ganglionic eminence and lateral ganglionic eminence, respectively), the anterior

entopeduncular area, the preoptic area and the septum. BFCNs are born predominantly in the medial ganglionic eminence, anterior entopeduncular area, preoptic area and septum. In addition, the ventral pallium — located between the lateral ganglionic eminence and the lateral pallium — gives rise to cholinergic neurons that will eventually reside within the subpallium[14]. Cholinergic neurons signal through mAChRs and nAChRs. Activation of mAChRs lead to stimulation of phosphoinositol synthesis and or inhibition of cAMP synthesis and changes in the permeability of the neuronal membrane to K+, Ca2+, and Cl- channels. NAChRs are pentameric ion channels comprised of α and β subunits in different configurations[15]. BFCNs are likely also capable of co-release and/or co-transmission, although evidence for this is limited. Markers of BFCNs (CHAT and/or VACHT) colocalize with vesicular transporters for glutamate (VGLUT1, VGLUT2 and VGLUT3), GABA (VGAT) and, to a lesser degree, monoamines (VMAT2) and zinc (ZNT3). The co-expression of different combinations of neurotransmitter markers in BFCNs is also evident from immunohistochemistry, in situ hybridization and more recently (and in a broader dataset) immunogold electron microscopy and single-cell transcriptomics studies[14]. Cholinergic neurons have extremely long and complex processes with a single human neuron having an estimated arborization length of >100 m. BFCNs express several neurotransmitter receptors that include adrenergic, glutamatergic, GABAergic, estrogen receptors, and endocannabinoids. Their neuronal projections extend to the cerebral cortex, hippocampus, and amygdala and are the primary source of innervations to the cortex. Unlike primary sensory cortical neurons, cholinergic neurons remodel their axonal arborizations and synapses continually through the lifespan[15]. Cholinergic projections arising from basal forebrain subregions innervate distinct targets, modulating functions varying from motor control, sensory and perceptual coding, attention, memory, to anxiety. Neuromodulation requires temporal precision to modulate target area dynamics and synaptic plasticity, as well as to reinforce cognitive and reward behaviors. ACh release rapidly modulates neuronal excitability, circuit dynamics, and cortical coding; all processes required for processing complex sensory information, cognition, and attention[16]. Also, the basal forebrain (BF) cholinergic system has an important role in attentive functions. The cholinergic system can be activated by different inputs, and in particular, by orexin neurons, whose cell bodies are located within the postero-lateral hypothalamus. Recently the orexin-producing neurons have been proved to promote arousal and attention through their projections to the BF. Attention may be defined as the behavioral and cognitive process that allows us to select the information present in our environment on the basis of their relevance along with the ability to ignore irrelevant stimuli. Different in vitro studies have tried to understand how orexin neurons activate the BF focusing primarily on the effects of orexins on medial septum (MS) neurons that project to the hippocampus and to the cortically projecting neurons of the BF. In the MS, orexins directly excite septo-hippocampal cholinergic neurons through the activation of the sodium, calcium exchanger and inhibition of potassium channels, presumably an inward rectifier, increasing hippocampal acetylcholine release and promoting arousal. Orx-A excites BF cholinergic neurons inducing cortical release of acetylcholine and increasing attention. Cortical acetylcholine levels increase even more under demanding attention tasks and orexin neurons increase firing to promote arousal and during exploratory behaviors in response to salient external stimuli[17]. The interest in the basal forebrain (BF) cholinergic system and its role in the development of cognitive impairments in Parkinson's and Alzheimer's diseases (PD and AD), goes back to discoveries made in the 1970s. At this time it was shown that administration of cholinergic antagonists cause memory impairments and that presynaptic cholinergic markers, choline acetyltransferase (ChAT) in particular, are reduced in the hippocampus and cortex in AD patients[19]. Individuals with Parkinson's disease (PD) frequently experience cognitive decline, with up to 80% developing dementia. This decline is driven by dysfunction in multiple neurotransmitter networks including the frontostriatal dopamine network, the mesocortical dopamine network, the norandrogenic network and the cortical cholinergic network. Recent studies have shown that atrophy in the CBF correlates with cognition and predicts cognitive decline in PD. In addition, prior research has shown an association between CBF volume and presynaptic integrity of the cholinergic system[18]. The cholinergic system has a long history of therapeutic manipulation in PD that predates the use of dopaminergic agents. these agents have continued to be used on the basis that there is excessive cholinergic activation in the striatal complex secondary to the dopaminergic loss, and that this may contribute to some of the motor features in PD, most notably the tremor. However, it is also clear that anti-cholinergic agents cause problems of memory and are associated with an increased risk of dementia, and that dopaminergic drugs are very potent in their own right, including controlling the tremor in many patients. There are also recent data suggesting that anti-cholinergic drugs, used to treat other non-motor aspects of PD, such as bladder irritability with antimuscarinic agents, can drive cognitive decline in PD and even the pathology of dementia. As such, anti-cholinergic agents are rarely used in PD except in younger tremor dominant patients who are cognitively intact[19]. The cholinergic system could also be targeted with neuromodulatory interventions. Non-invasive vagal nerve stimulation (n-VNS) may activate regions that receive dense connections from the CBF, including dorsolateral prefrontal cortex (DLPFC). Transcranial direct current stimulation (tDCS) and TMS have also been used in a number of cognitive interventions in PD, although outcomes vary. This inconsistency may be in part due to an almost exclusive targeting of frontal regions, with a particular focus on DLPFC. This approach is reasonable given DLFPC involvement in frontal dopaminergic corticostriatal circuits and its role in multiple functional brain networks. However, cholinergic denervation also occurs in posterior cortical regions, most notably posterior parietal cortex. This region is a hub of the frontoparietal network that is key to attentional control, and therefore could serve as an additional target region in neuromodulatory studies. Future studies should compare the effect of targeting anterior and posterior hubs of this network, as well as stimulating both hubs simultaneously, to determine whether the benefits of these approaches differ[18].By infusing excitotoxins in the NBM that preferentially destroy BF amygdalopetal cholinergic neurons, a previous study has shown that BF amygdalopetal cholinergic lesions disrupt IA. Furthermore, memory deficits induced by BF amygdalopetal cholinergic lesions are attenuated by mAChR agonism in the BLA immediately after IA, which suggests BF amygdalopetal cholinergic neurons are critical for contextual fear memory consolidation in IA. BF amygdalopetal cholinergic lesions also disrupt Pavlovian contextual fear conditioning and mAChR agonism in the BLA enhances consolidation of Pavlovian contextual fear conditioning. Systemic administration of histaminergic agents that decrease ACh levels in the BLA disrupt Pavlovian contextual fear conditioning. The complementary effects that BF amygdalopetal excitotoxic lesions, direct BLA mAChR manipulation, and decreasing BLA ACh levels have on IA and Pavlovian contextual fear conditioning suggest that BF amygdalopetal cholinergic input to mAChRs in the BLA are critical for acquisition and consolidation of contextual fear memory[15].

## Conclusion

While progress has been made in uncovering the intrinsic and external factors that influence the overall development of cholinergic neurons in the forebrain, how subpopulations with different projection patterns, connections, and physiological properties are generated is not ymet well

understood. Based on previous studies that have examined the mechanisms by which neural diversity is generated in other cell types, It seems likely that cholinergic neurons acquire their specific mature properties through a combination of internal decisions about the fate of cells programmed to develop, local environmental signals, and afferent/efferent communication. Unraveling the interplay of these factors and the aspects of cholinergic development they affect will improve our understanding of the cholinergic system of the forebrain as a whole, its diversity, and the circuits in which it is involved. [4]

Despite their different morphological and functional properties, cholinergic interneurons and projection neurons appear to evolve through very similar transcriptional programs. While we have some understanding of what defines cholinergic identity in general, little is currently known about what distinguishes interneurons from projection neurons during their respective developmental trajectories. In this review, we discuss what is currently known about the specification of forebrain cholinergic neurons, in particular the factors that distinguish them from other cell types originating from the same progenitor region (primarily cortical and striatal GABAergic interneurons and other types of subcortical projection neurons). [4]

Changes in the parietal lobe and hippocampus in SIGM were of a similar nature, except for the absence of a drop in cysteate and tryptophan levels in the hippocampus, which is a reflection of a disruption of the metabolic pathway of serotonin formation from tryptophan in the hippocampus and a higher sensitivity of the parietal lobe to oxygen deficiency compared to the hippocampus.

The consequences of cholinergic degeneration cannot be considered in isolation but in the context of the severe dopaminergic losses and other pathological changes in Parkinson's disease. Topographically distinct cortical and subcortical changes in the cholinergic system in Parkinson's disease reflect differential vulnerabilities associated with clinical features. The neural circuits involve distinct neurotransmitters. For example, cholinergic neurons in the pedunculopontine nucleus project to GABAergic and glutamatergic neurons in the mesencephalic locomotor area, medulla oblongata (reticulospinal tract), medial vestibular nuclei, midbrain dopaminergic neurons (substantia nigra and ventral tegmental area), and thalamus. 7,8 Targeted neurostimulation of brain regions with impaired cholinergic systems will produce cholinergic and non-cholinergic effects that may improve the function of neural circuits associated with dementia, as well as with postural instability and walking difficulties - the hypocholinergic subtype of Parkinson's disease [20].

The advancing field of optogenetics which exploits genetics to incorporate light activated ion channels (rhodopsins) into genetically defined populations of neurons to allow activation or inhibition of these neurons with high temporal precision, will undoubtedly facilitate future studies of the complex interplay of a multitude of neurotransmitters and neuromodulators within the highly heterogenous striatum [12]. Estrogen receptors are expressed on both cholinergic and GABAergic interneurons, providing a potential mechanism for sex hormones to regulate interneuron function. These findings represent a starting point for future work analyzing the impact of these differences on sex dependent outcomes in both normal basal ganglia function and in the pathophysiology of a range of neuropsychiatric conditions [13]. BFCNs send extensive, highly branched projections to many cortical and subcortical regions which are metabolically demanding to maintain. It is possible that ageing contributes to a loss of cholinergic projections, which alters cholinergic tone and leads to a cascade of pathology. It is important to investigate why this occurs earlier in some regions than in others, and in some subsets of cholinergic neurons before others. Understanding the factors that contribute to the differential resilience and vulnerability of cholinergic circuits in pathological ageing will therefore be a critical next step towards the development and deployment of next-generation therapeutics [14].

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