Review Article
Synaptic Secretion and Beyond: Targeting Synapse and Neurotransmitters to Treat Neurodegenerative Diseases

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The nervous system is important, because it regulates the physiological function of the body. Neurons are the most basic structural and functional unit of the nervous system. The synapse is an asymmetric structure that is important for neuronal function. The chemical transmission mode of the synapse is realized through neurotransmitters and electrical processes. Based on vesicle transport, the abnormal information transmission process in the synapse can lead to a series of neurorelated diseases. Numerous proteins and complexes that regulate the process of vesicle transport, such as SNARE proteins, Munc18-1, and Synaptotagmin-1, have been identified. Their regulation of synaptic vesicle secretion is complicated and delicate, and their defects can lead to a series of neurodegenerative diseases. This review will discuss the structure and functions of vesicle-based synapses and their roles in neurons. Furthermore, we will analyze neurotransmitter and synaptic functions in neurodegenerative diseases and discuss the potential of using related drugs in their treatment.

1. Background

The nervous system plays an important role in regulating the physiological function of the body [1, 2]; neurons are the most basic structural and functional units of this system [1, 2]. Billions of neurons exist in the nervous system, most of which are distributed in the central nervous system (CNS) of the brain [3, 4]. Neurons can contact each other and transmit information; they use synapse as the site of information exchange, which then determines the function of the nervous system [5]. The synapse is an asymmetric structure composed of presynaptic membrane, postsynaptic membrane, and synaptic cleft between two membranes [6, 7]. Synapse formation involves many extracellular factors, cell adhesion molecules, and intracellular signaling or structural proteins [7]. After the establishment of synaptic connections, synapses undergo structural or functional changes, known as synaptic plasticity [8], which is mediated by neuronal activity and a variety of secreted factors [8].

There is a highly specialized site at the presynaptic nerve terminal, known as the active zone, which is exquisitely designed to facilitate the fusion of synaptic vesicles with the plasma membrane [9, 10]. A high-density region also exists in the postsynaptic membrane [11], which is a protein-rich collection, and is composed of large scaffold proteins, some neurotransmitter receptor proteins, and
related elements regulating synaptic activity to form postsynaptic density (PSD) [12, 13], which is the structural basis of postsynaptic signal transduction and integration [14, 15].

The chemical transmission mode of the synapse is realized through neurotransmitters and electrical processes [16, 17]. When the electrical signal transmitted from the cell body reaches the axon terminal, it causes the depolarization of the presynaptic membrane, activates the voltage sensitive calcium channel on the presynaptic membrane, leads to the influx of extracellular Ca\(^{2+}\), and subsequently triggers the fusion of synaptic vesicles and presynaptic membrane [18]; then, it releases neurotransmitters into the synaptic cleft [16]. Neurotransmitters in the synaptic cleft bind to specific receptors on the postsynaptic membrane, causing the next neuron or effector cell to complete the signal transmission of the nervous system [19]. According to the differences between chemical transmitters and specific receptors, postsynaptic potentials can be classified into two types [20–23], as follows: excitatory postsynaptic potential (EPSP), which depolarizes the postsynaptic membrane and manifests as the excitation of postsynaptic neurons [23], and inhibitory postsynaptic potential (IPSP), which hyperpolarizes the postsynaptic membrane and manifests as the inhibition of postsynaptic membrane excitability [22]. Neurotransmitters can only be released into the synaptic cleft through vesicles in the presynaptic membrane to act on the postsynaptic membrane [16, 17]. The unidirectionality of chemical synapses [16, 17], the specificity of postsynaptic receptors [24], and the plasticity of chemical synapses ensure that the postsynaptic membrane selectively receives and transmits the information in an orderly manner from the presynaptic membrane [25, 26], based on the fact that synapse is a functional unit of the brain, whose dysfunction can lead to a series of neuroredrelated diseases [27–29].

2. Basic Process of Synaptic Secretion

Synapses communicate with one another by releasing neurotransmitters and other chemicals from presynaptic vesicles [30, 31]. Vesicles are widely reported as among the important functional structural components of the endomembrane system that are directly transported to different membrane structures [32, 33]. According to the different morphologies and contents, two kinds of vesicles were involved in exocytosis, namely, small clear vesicles (SCVs) and dense core vesicles (DCVs) [34, 35]. SCVs become synaptic vesicles (SVs) at the end of neurons [36]. In mammals, the diameter of SVs is generally less than 50 nm, and the vesicle contents are small molecular neurotransmitters, such as acetylcholine [35]. DCVs are distributed in the axons and dendrites of neurons and have diameters in the range of 70–200 nm [37]. The DCVs’ contents include neuromodulators, nerve growth factor, monoamine, and other neuromodulatory substances [35, 37]. Although morphological structure and function differ between the two kinds of vesicles, the exocytosis processes of vesicles are the same, including tethering, docking, priming, and fusion [38] (Figure 1). Neurotransmitter secretion is the fusion process of synaptic vesicle and presynaptic membrane and is a calcium-dependent process (Figure 1) [19, 39]. The increase of intracellular calcium concentration triggers the fusion between synaptic vesicles and presynaptic membrane, resulting in the release of neurotransmitters [39–41]. During the fusion, a hydrophilic pore called fusion pore is formed [42–44]. Chemicals in vesicles need to be released through fusion pores [42–44]. Vesicle fusion is an energy-consuming process, and the zipper assembly of Soluble N-ethylmaleimide-Sensitive factor Attachment protein REceptors (SNAREs) complex can provide energy for membrane fusion [45, 46].

The contents of vesicles are believed to be released through two main modes [43, 47, 48]. One mode is the incomplete fusion and rapid closure (kiss-and-run) that limits the release of substances in vesicles [49–51]. This mode only allows catecholamines and other small molecules to be released through a narrow fusion pore [49–51]. The other mode is the irreversible expansion of the vesicle membrane until it flattens (full collapse) to promote the complete fusion of transmitter release [52–54]. Studies have found that both full collapse and kiss-and-run modes exist simultaneously in the CNS, and the two modes can be interchanged to better complete the vesicle recycling cycle [52, 55]. In the intimal fusion system, besides the transporting of neurotransmitters and other substances to the plasma membrane through vesicles and releasing them to the synaptic cleft through membrane fusion in exocytosis [56–58], endocytosis is also required to recover extracellular molecules into the cell to supplement raw materials [59], such as lipids or proteins, for the next round of intracellular activities [60, 61]. This series of complex biological reactions constitutes a dynamic and efficient membrane fusion system [57, 58, 62–64]. Numerous proteins and complexes that are widely reported to regulate these processes have been identified, and their regulation of synaptic vesicle secretion is complicated and delicate [57, 62].

3. Regulatory Proteins and Mechanisms in Synaptic Secretion

Three decades of researches and many major discoveries have been reported, providing important insights into synaptic secretion and generating a functional model of Ca\(^{2+}\)-triggered neurotransmitter release mechanisms mediated by protein-protein interaction cascades with SNARE complex as the core [65].

3.1. SNARE Proteins. Soluble N-ethylmaleimide-Sensitive factor Attachment protein REceptors (SNAREs) are a molecular machine that mediate such membrane fusion [45, 46]. SNAREs have been identified and elucidated in Saccharomyces cerevisiae over the past few decades [66]. In fungi, more than twenty subtypes of SNARE proteins exist and function in different organelles or cellular regions [66, 67]. In multicellular organisms, the number of SNARE subtypes varies from 30 to 50 [68, 69]. Notably, in the nervous system, SNARE complexes are composed of three proteins [45, 70]; the canonical and most well-defined SNAREs are as follows: syntaxin-1 and SNAP-25 (synaptosome-associated protein of 25kDa) located in the presynaptic membrane,
which belong to t-SNARE (target-SNARE) [45]; VAMP-2/Synaptobrevin-2 (vesicle-associated membrane protein) located in the membrane of synaptic vesicles, which belongs to v-SNARE (vesicle-SNARE) [71]. Syntaxin-1 and VAMP-2/Synaptobrevin-2 are anchored to the presynaptic membrane and synaptic vesicle membrane via the C-terminal transmembrane region, respectively [66], whereas SNAP-25 has no transmembrane region and is anchored to the presynaptic membrane via the fatty acyl group of four cysteine residues in the mesenchymal region [72].

SNARE complexes are formed by binding to each other through SNARE motifs [73–75]. Although SNARE proteins differ in amino acid length and structure, the SNARE motifs with a length of about 65 amino acids are highly conserved [76, 77]. When SNARE proteins exist alone, their SNARE motifs are mostly random curls; when these regions are combined together, they fold to form tight SNARE core complexes [78, 79]. The crystal structure of the core complex consists of parallel four helical bundles with an overall length of 12 nm [79]. Among the helical bundles, both syntaxin-1 and VAMP-2/Synaptobrevin-2 provide one α-helix, and SNAP-25 provides two α-spirals [80]. The core of the helix bundle consists of 15 layers of hydrophobic amino acid residues, except for the layer called “0” in the center of the helix bundle [81], which is a hydrophilic layer containing one arginine residue and three glutamine residues that form hydrogen bonds within the hydrophobic core [79]. Arginine residues come from VAMP-2/Synaptobrevin-2 and are called R-SNARE proteins [82]. Three glutamine residues come from syntaxin-1 and SNAP-25 and are named Q-SNARE proteins [82]. Among them, syntaxin-1 is called Qa, and the N-terminal and C-terminal of SNAP-25 protein are called Qb and Qc, respectively [82]. Biochemical experiments showed that the SNARE core complex has high thermal stability [46, 83, 84]. These characteristics show that the formation of the complex is very favorable in terms of energy, which is a key feature of current membrane fusion models [85, 86].

In the process of vesicle fusion, the assembly of SNARE complex is ordered from N- to C-terminal, also known as N-terminal nucleation [87–90]. The assembly energy of each layer of SNARE complex differs. Macroscopically, the energy...
released by N-terminal (-7 layers to -1 layer) assembly is higher than that of C-terminal (+1 to +8 layers) and also more stable after installation [81, 91]. The C-terminal assembly is reversible, because the N-terminal contains more hydrophobic amino acids with larger side chain volume compared with the carbon end, thereby providing more binding energy and making the hydrophobic core closer [85, 90]. Considering that the N-terminal and C-terminal of SNARE complex have different thermodynamic properties [88], the assembly of SNARE complex is a stepwise process; that is, the N-terminal is responsible for nucleation effect and stable assembly state, whereas the C-terminal connects the assembly of the SNARE complex with membrane fusion process [88, 89, 92]. Although this theory is deeply supported by theory and experiment, several studies demonstrated that the assembly of the SNARE complex in vitro is continuous [92].

According to previous studies, the energy released by the assembly of SNARE complexes is close to 35 k_BT, and this energy is enough to overcome the barrier and lead to fusion [90, 93], which means that only one group of SNARE complexes can complete the membrane fusion process [94]. In fact, the conclusion is based on the continuous assembly of SNARE complex [94]. However, there are multiple proteins and complexes regulating the assembly of SNARE complex under physiological conditions [95]. The assembly of SNARE complex is unlikely to meet the conditions of continuous assembly under precise regulation [96, 97]. Therefore, five to six groups of SNARE complexes are needed to meet the formation of fusion pores between synaptic vesicles and presynaptic membranes [96, 97].

3.2. Munc18-1. Munc18-1 is a member of the Sec1/Munc18 (SM) protein family [98–100], which is expressed in neurons and neuroendocrine cells and plays an important role in the release of neurotransmitters [101–104]. Multiple experimental evidences show that Munc18-1 is involved in the process of synaptic vesicle anchoring, priming, and fusion [105–107]. These functions are related to the interaction between Munc18-1 and SNARE proteins [108], the most significant of which is syntaxin-1 [104, 105, 108, 109]. The interaction surface between Munc18-1 and syntaxin-1 is complicated, and the binding modes are diverse, which is why the affinity between Munc18-1 and syntaxin-1 is high [105, 110].

The binding of Munc18-1 to syntaxin-1 is important for the regulation of synaptic vesicle secretion [107, 111]. The kinetic data show that free syntaxin-1 exists in a mixture of at least two different conformations [112]. When syntaxin-1 combines with Munc18-1, Munc18-1 can make syntaxin-1 in a stable closed conformation [38, 109, 113]. In addition, Munc18-1, as a molecular chaperone, contributes to the correct transport and localization of syntaxin-1 [102, 109, 114]. Munc18-1/syntaxin-1 complex can prevent syntaxin-1 from forming a heterodimer with SNAP-25, affect the formation of normal SNARE complex, and protect syntaxin-1 before the arrival of the signal [113, 115].

Although the combination of Munc18-1 and syntaxin-1 is also important for the fusion of vesicles, the results of SNARE complex recombination experiment in vitro show that when syntaxin-1 exists as Munc18-1/syntaxin-1 complex, the SNARE motif H3 of syntaxin-1 is locked and cannot participate in the formation of SNARE complex, resulting in the incomplete vesicle fusion [105, 113]. The results in vitro seem to contradict the physiological results in vivo; however, the contradiction is resolved with the analysis of the function of the regulatory factor Munc13-1 [116, 117]. The recombination experiment in vitro showed that Munc13-1 could change Munc18-1/syntaxin-1 complex from "closed" state to "open" state, thereby forming the SNARE complex [117–119]. Therefore, Munc18-1 initiates the assembly of SNARE complex and ultimately achieves the fusion of vesicles [106, 107, 120].

3.3. Synaptotagmin-1. The speed of information transmission by the nervous system can be accounted by millisecond and depends on calcium signals [57]. In the presynaptic membrane region, a calcium receptor that can respond to calcium signal called Synaptotagmin-1 is present [121–123]. Synaptotagmin-1 is anchored to synaptic vesicles by its N-terminal transmembrane domain [124]. The cytoplasmic region of Synaptotagmin-1 contains two C_2 domains, which are called C_2A and C_2B [123, 124]. C_2A binds three calcium ions, whereas C_2B binds two calcium ions [122, 125, 126].

In response to calcium ions, the two C_2 domains of Synaptotagmin-1 bind to negatively charged biofilms and shorten the distance between synaptic vesicles and the presynaptic membrane [124, 125]. Thus, they reduce the energy barrier to be overcome and ultimately mediate the fusion of synaptic vesicles and the release of neurotransmitters by presynaptic membrane [57]. The C_2B domain of Synaptotagmin-1 has two specialized regions that are rich in basic amino acids [127, 128]. One region is called the polybasic stretch, which consists of two amino acid sites, namely, K326 and K327 [129]. The other region is called R398–399 [130], which consists of two positively charged amino acids, namely, R398 and R399. These two regions bind phosphatidylinositol-4, 5-diphosphate (PIP2) and SNARE complexes enriched in the presynaptic membrane, respectively, which are particularly important for the function of Synaptotagmin-1 [129, 130]. They work together to close the distance between vesicles and the presynaptic membrane, stabilize vesicles anchoring or initiating in the presynaptic active region, and prevent the further assembly of SNARE complexes [128, 131].

At this point, Ca^{2+} in the C_2 domain binds to the pocket’s negatively charged amino acid residues and targets the membrane to generate a same-charge repulsion, thus inhibiting the fusion process of synaptic secretion [131]. After Ca^{2+} influx, Ca^{2+} binds the pocket of the C_2A domain and C_2B domain and thereby shields the negative charge and results in a net positive charge [125, 132]. This positive charge and the positive charge of the highly conserved amino acid residues on each pocket act like an instantaneous electrostatic switch, pulling vesicles closer to the negatively charged presynaptic membrane [133]. Meanwhile, the insertion of pocket hydrophobic amino acid residues in the C_2 domain causes lipid disorder, changes the membrane curvature, and deforms the membrane [134], which is conducive to the transformation of the trans-SNARE complex to the
cis-SNARE complex and ultimately promotes membrane fusion and neurotransmitter release [135–138].

3.4. Munc13-1. Munc13-1 contains three C2 domains, namely, C2A, C2B, and C2C domains [139]. The C2A domain can interact with the upstream of the Rab3-interacting molecules (RIMs) [140]. RIMs are a class of Rab3 effectors with high molecular weight and exist as scaffold proteins of the active zone in the presynaptic membrane [135, 141]. The C2B domain is the only one of the three C2 domains in Munc13-1 that can bind Ca2+ and PIP2 [142]. The C2B domain of Munc13-1 can be used as a potential calcium receptor. A C1 domain, which can bind diacylglycerol (DAG), is present at the N-terminal of the C2B domain [143–145]. The combination of C1 and C2B domains enables Munc13-1 to bind phospholipid molecules in the presynaptic membrane [146]. The C2C domain at the C-terminal does not bind Ca2+ and negatively charged phospholipid molecules in the presence of Ca2+ [144]. However, it can bind to the fatty acid chain inside the phospholipid bilayer due to the existence of hydrophobic amino acids in its periphery so that the C2C domain can nonselectively have affinities to the membrane [147]. A calmodulin-binding motif (CaMb) also exists near the N-terminal of the C1 domain [148, 149], which is believed to be strongly correlated with the function of Munc13-1 in calcium-regulated neurotransmitter secretion [150–154].

The most important core domain of Munc13-1 protein is the central MUN domain [155]. The MUN domain, as a key functional element of Munc13, plays an important role in synaptic secretion [155]. MUN domain is also present in BAP3, CAPS, and other proteins in most eukaryotes [156]; it is structurally similar to other CATCHR family members that play roles in different transport steps [157, 158]. These CATCHR proteins form a series of aligned α-helical bundles with flexible hinge regions that bind vesicles to the fusion sites, suggesting that Munc13 can participate in the process of vesicle binding through the MUN domain [158, 159]. In addition, the MUN domain of Munc13-1 interacts weakly with SNARE complexes, Munc18-1, and SNAP-25 function of syntaxin-1, which are essential for Munc13-1 function [116, 155, 160–163].

Munc13-1 is also involved in the opening of the syntaxin-1 protein closed by Munc18-1 [117, 119] and can significantly accelerate the transformation of syntaxin-1 from Munc18-1-syntaxin-1 complex to SNARE complex depending on the “NF” pocket catalytic active center in MUN domain [118]. Recent studies showed the interaction between VAMP2/Synaptobrevin-2 and Munc13-1 MUN domain and analyzed the crystal structure of this complex [164]. This quaternary complex cooperates to start the assembly and membrane fusion process of the SNARE complex [161, 165, 166]. These studies revealed the function and molecular mechanism of Munc13-1 in SNARE complex assembly and synaptic vesicle priming, thereby providing a strong theoretical basis for understanding the molecular mechanism of neural signal transduction [165, 167].

3.5. CAPS-1. Mammals express two CAPS isoforms, namely, CAPS-1 and CAPS-2, which are in neurons and endocrine cells [168]. CAPS is a multidomain protein that contains the following: the C2 domain, which is involved in CAPS dimerization [169, 170]; pleckstrin homologous (PH) domain, which is characterized as a PIP2-binding domain to mediate CAPS interaction with the plasma membrane [171–176]; DAMH domain, which exhibits sequence homology to the Munc13 MUN domain and is required for CAPS binding to SNAREs [156, 158, 171, 177–179]; and dense core vesicle binding domain (DCVBD), which appears to be important for CAPS’ association with DCVs [172, 180].

Both CAPS-1 and Munc13-1 contain key regions that bind to PIP2 clusters on the plasma membrane, but unlike Munc13-1, CAPS-1 binds PIP2 through the PH domain in a calcium-independent manner [171, 173]. CAPS and Munc13 are both the promoters of DCVs and SVs exocytosis, and their functions are nonredundant [181–184], whereas the molecular mechanism underlying the regulation of exocytosis secretion in time and space has not been clarified.

Interestingly, a study showed that natural CAPS-2 splicing isomer, which has C2-PH domains and misses DAMH and DCV binding domains, can rescue the exocytosis of chromaffin cells and neurons lacking CAPS-1 and CAPS-2 [185]. This activity increases the possibility that the initiation of DCVs in the early development stage of chromatin cells can be realized through the C2-PH domain, whereas the initiation function of CAPS needs the participation of other domains in more mature cells [185]. A subsequent study about the successful crystal structure analysis of the DAMH domain offers the possibility of further understanding the function of CAPS-1, thereby revealing the dual role of CAPS-1 in SNARE complex formation [186], as follows: (1) CAPS-1 DAMH domain interacts with Munc13-1 MUN, and the interaction hinders Munc13-1 activity to open Munc18-1/SNARE, which further leads to the assembly of the SNARE complex failure. (2) After syntaxin-1 is activated, CAPS-1 stabilizes the active state of syntaxin-1 through the interaction between the DAMH domain with the syntaxin-1/SNAP25 complex, thereby accelerating the assembly of the SNARE complex and finally promoting synaptic exocytosis [178].

Therefore, based on these studies, a model in which CAPS and Munc13 jointly regulate vesicle secretion was proposed (Figure 2) [186]: in the resting state, CAPS-1 is first located on the cytoplasmic membrane through the calcium-independent interaction between PH and PIP2. Munc13-1 cannot bind to Munc18-1/syntaxin-1 complex due to the interaction of PH–PIP2 and DAMH–MUN [173, 186]. Thus, the anchored DCVs and SVs cannot enter the vesicle priming stage. Under the action of intracellular calcium level, CAPS-1 and Munc13-1 can promote vesicle recruitment to the PIP2-rich cytoplasmic membrane in a calcium-dependent manner. At this time, some Munc13-1 successfully escape the binding and inhibition of CAPS-1 protein; then, Munc13-1 can bind to Munc18-1/syntaxin-1 complex and catalyze the opening of syntaxin-1. When syntaxin-1 protein is open and SNAP-25 exists, CAPS-1 binds to syntaxin-1/SNAP-25 complex to further stabilize the open state of syntaxin-1 and promotes binding with Synaptobrevin-2 to form the SNARE complex [178]. With
the influx of extracellular calcium, the increase of intracellular calcium level will activate phospholipase PLC\_\eta that leads to PIP\_2 hydrolysis and DAG formation [181]. Subsequently, the hydrolysis of PIP\_2 will lead to the decrease of CAPS-1 activity, and the increase of DAG level will stabilize the function of Munc13-1 protein [181, 182]. Some key fusion proteins, including complexin-1 and Synaptotagmin-1, may also promote the formation of the SNARE complex together with CAPS-1 and Munc13-1 so that vesicle membrane fusion can occur quickly and effectively [97, 187]. Although this model needs to be further improved and clarified, it demonstrates a strong sequence and coordination between CAPS-1 and Munc13-1 in the formation of SNARE complexes; it also shows that the calcium-dependent spatial distribution of PIP\_2 and DAG changes the distribution of CAPS-1 and Munc13-1 in the presynaptic membrane and modulates their activity [186].

In addition to the proteins described above, there are a large number of Ca\^{2+} channels in presynaptic nerve terminals to regulate the concentration of Ca\^{2+} in neurons ([Ca\^{2+}]_i), which play important roles in the release of neurotransmitters [188–190]. There are many types of Ca\^{2+} channels with different molecular compositions and properties [188], which are mainly P/Q-type and N-type channels (referred to as Ca\_2,1 and Ca\_2,2) responsible for initiating synaptic transmission at fast conventional synapses [189, 191, 192]. These Ca\^{2+} channels coexist in the same presynaptic nerve terminals and have a synergistic relationship to promote transmitter release [193]. The inhibition of the activity of any type of Ca\^{2+} channel will reduce the release of presynaptic transmitter. The plasma membrane SNARE proteins (syntaxin-1 and SNAP-25) and synaptotagmin-1 can specifically interact with the channels in a Ca\^{2+}-dependent manner by binding to the synaptic protein interaction (synprint) sites of Ca\_2,1 and Ca\_2,2 channels [194–196]. This interaction regulates channel function and thus controls synaptic transmission [197].

4. Neurotransmitters and Synaptic Function in Neurodegenerative Diseases

Synapses are the functional part of the connection between neurons and the key part of the physiological function of neurons [8, 198]. They are not in a static state in the body and undergo relatively lasting dynamic changes called synaptic plasticity under the stimulation of neuronal activity or other factors [8, 31, 199]. Changes in synaptic plasticity are the main mechanisms of the CNS growth, development, learning, and memory [8, 199]. Degenerative alterations include loss of synapses, branch atrophy, and cell death in different types of cells, such as cholinergic, glutamatergic, noradrenergic, and inhibitory neurons [200]. In clinical patients or animal models, structural degeneration, such as reduction in neurons, generally does not appear until the middle-late stage, and cognitive impairment in the early stage of the disease is more likely to be caused by abnormal synaptic function in specific brain regions (prefrontal cortex and hippocampus) [201].
In fact, many studies showed that the oligomeric Aβ protein, a characteristic pathological marker of Alzheimer’s disease (AD) [202], has strong synaptic toxicity, which specifically reduces synaptic density, damages long-term synaptic enhancement, facilitates long-term synaptic weakening, and suppresses brain learning and memory function [203–205]. The generation or disturbance of neural activity is largely determined by the state of excitation-inhibition balance, which is closely related to the release and circulation of neurotransmitters in the neural circuit [206].

Therefore, exploring changes in the neurotransmitter system is critical to the elucidation of the biochemical mechanisms of normal aging and age-related neurological/psychiatric disorders such as Parkinson’s disease (PD), AD, presenile deafness, and depression (Tables 1 and 2). Several types of transmitters are released from the presynaptic neuron in the CNS, such as glutamate, GABA, and dopamine [207], whereas the neurotransmitters released from neuromuscular connectors are acetylcholine [208, 209]. Synaptic changes in the CNS are often the main manifestations and thus turn into important targets in the clinical therapy of neurodegenerative diseases. Presently, pharmacological interventions of cholinergic and glutamatergic neurotransmission, including cholinesterase inhibitors and N-Methyl-D-Aspartate (NMDA) receptors antagonist, are the only FDA-approved medications for AD but are unable to significantly improve cognitive dysfunction [210]. Similarly, treatments of PD are symptomatic, and levodopa is the typical pharmacologic approach, but with limited modifying effects as well [211]. As a consequence, it is of great theoretical and practical value to study the synaptogenesis and pathological changes in the CNS to further elucidate neurodegenerative diseases [212].

4.1. Glutamate. Glutamate (Glu) is the most important excitatory neurotransmitter in mammalian CNS [213]. Glu serves multiple functions in the brain, and such functions are mediated by Glu receptors [214, 215]. The activation of Glu receptors is involved in rapid excitatory synaptic transmission and regulates neurotransmitter release, synaptic plasticity, long-term synaptic enhancement, long-term synaptic inhibition, and other normal physiological functions in the CNS [213, 216]. However, high Glu concentration in the intercellular space can produce toxicity to neurons and lead to neuronal degeneration, senescence, and death [217].

The excitatory toxicity of glutamate is closely related to the occurrence and development of many neurodegenerative diseases and is the important mechanism of the death of nerve cells in neurodegenerative diseases [217, 218]. Glu receptors play two main roles in neurodegenerative diseases [214, 215, 218, 219]. One role is to participate in normal synaptic transmission and serve a neuroprotective function when synaptic activity is enhanced [214, 215]. Another role is the excitatory toxicity mediated by ionic Glu receptors [217, 218].

Excitatory toxicity refers to the neurotoxic effects of the overdose of excitatory amino acids (EAA) and involves two mechanisms [220]. One mechanism is mediated by the overexcitation of NMDA receptors, which can occur over hours to days and is characterized by sustained Ca2+ influx and delayed injury of nerve cells [221]. Mitochondrial function can therefore be lost due to large influx of Ca2+ and the rapid accumulation of Ca2+ in mitochondria [222]. The activity of nitric oxide synthase can also increase, so that NO synthase can increase the toxicity of nerve cells [223]. In most pathological cases, delayed injury of nerve cells caused by Ca2+ influx and mediated by NMDA receptor overexcitation dominates excitatory toxicity [205, 221]. The other mechanism is mediated by hyperactivation of α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid (AMPA) and KA receptors [224], which can occur within hours and are characterized by Na+ influx, passive influx of Cl− and water, and acute osmotic swelling of nerve cells [225]. The normal structure of the glutamatergic system and the function of Glu transporters and reuptake of Glu were altered in the brain tissues of AD patients [226]. In addition, β amyloid precursor protein (APP) and tau protein can inhibit extracellular Glu uptake, which leads to increased extracellular Glu levels, resulting in excitotoxic effects [227–229].

In PD patients and experimental animal models, there is a large increase in Glu neurons projecting from the dorsal subthalamic nucleus to the substantia nigra striatum [230, 231]. These studies confirm that the overactivation of Glu receptors on dopamine neurons is one of the causes of excitatory toxic cell death [232, 233]. Meanwhile, Glu uptake disorder also aggravates Glu receptor hyperactivation that leads to excessive calcium influx, which ultimately further leads to nerve cell death and a series of acute or chronic neurodegenerative diseases (such as stroke and AD) [234].

Several drugs are developed for diseases caused by Glu, such as ginsenoside Rh3, which can reduce the increase of Ca2+ in neurons possibly by inhibiting calcium influx induced by NMDA receptors and alleviating calcium overload, thereby preventing hypoxic injury caused by cerebral ischemia [235–237]. Huperzine A can inhibit the NMDA-induced toxicity of the cerebral cortex and synaptic plasma membrane [238–240]. In addition, memantine is an antagonist of NMDA receptors and antagonizes excitatory amino acid toxicity to neurons [241–243].

4.2. GABA. γ-Aminobutyric acid (GABA) is the most widely distributed inhibitory neurotransmitter in the CNS [244]. It is formed by the removal of carboxyl group of Glu in the brain under the action of glutamic acid decarboxylase (GAD) [245]. GABA participates in a variety of metabolic activities and has high physiological activity [244]. Immunological studies show that the highest concentration of GABA is found in the substantia nigra, and at least 70% of the afferents to substantia nigra dopaminergic neurons are GABAergic [246]. The cognitive impairment caused by nervous system diseases, such as severe depression and epilepsy, is directly related to the increase or decrease of GABA transmission [246, 247]. Changes in brain GABA content and receptor function are crucial for many factors of learning and memory [244, 247]. On the one hand, when the content of GABA in the brain is reduced or the receptor function is impaired, it can induce neurological diseases related to cognitive impairment, and appropriate supplementation and repair of GABA function can improve the cognitive
On the other hand, if the excitatory neurons are overexcited, then they will produce excitatory toxicity, which will eventually lead to abnormal activity of the neural network and lead to cognitive deficits [220]. When GABA is activated, it can inhibit the neurotoxic effect caused by Glu abnormal excitation and improve the learning and memory function decline caused by neural abnormalities [207, 248].

GABA receptors are divided into three types, namely, GABA<sub>A</sub> receptors, GABA<sub>B</sub> receptors, and GABA<sub>C</sub> receptors [247]. Different types of GABA receptors distributed in different brain regions have different mechanisms underlying learning and memory [249]; however, they all have inhibitory effects [247]. Their receptor antagonists can improve the inhibitory effect of learning and memory, which may be due to the promotion of the release of excitatory

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**Table 1:** A list of neurotransmitters types [307, 308]. The types of neurotransmitters, including choline, monoamines, and amino acids and their distribution and functions were shown. PD: Parkinson’s disease; AD: Alzheimer’s disease; HD: Huntington’s disease; ALS: amyotrophic lateral sclerosis; FAD: frontotemporal dementia; VaD: vascular dementia.

| Types          | Distribution                          | Function                                           | Diseases                      |
|----------------|---------------------------------------|----------------------------------------------------|-------------------------------|
| Choline        | Acetylcholine (ACh)                   | Tertiary neurons emitted by thalamic afterload, brain stem reticular ascending exciter system | PD, AD, HD, ALS, FTD, and others |
| Dopamine (DA)  | The substantia-striatum, limbic system, and nodal-fundal part | An important transmitter of the vertical exoskeleton | PD, AD, HD, ALS, FTD, and others |
| Monoamines     | Norepinephrine (NE)                   | Mainly located in the lower brain stem | PD, AD, and others |
| Neurotransmitters | Serotonin (5-HT)                    | Concentrated in the raphe nucleus | AD, VaD, and others |
|                | y-Aminobutyric acid (GABA)            | Superficial layer of the cortex, cerebellar cortex | PD, AD, HD, ALS, FTD, and others |
| Amino acids    | Glycine                               | Spinal inhibitory neurons | PD, AD, FAD, and others |
|                | Glutamate                             | Sensory afferent nerve and cerebral cortex | PD, AD, HD, FAD, and others |
| Others         | Opioids, brain-gut peptides, NO, and CO can all serve as central neurotransmitters or modulators. | | PD, AD, HD, ALS, FTD, and others |

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**Table 2:** A list of neurotransmitter release processes [19]. The neurotransmitters in the release process, including tethering and docking, priming, and fusion and their definition and functions have been summarized in this table.

| Release processes | Definition                                                                 | Regulatory proteins |
|-------------------|---------------------------------------------------------------------------|---------------------|
| Tethering and docking | The process of vesicle localization on the target membrane. Generally, the distance between the vesicle membrane and the target membrane is about 75–150 nm in tethered state and 5–10 nm in docked state. | SNAREs GTP-binding protein |
| Priming           | The process of transforming synaptic vesicles into a state with the ability to fuse with the presynaptic membrane of the active zone, which is a rate limiting step in Ca<sup>2+</sup>-dependent exocytosis. | SNAREs, Munc13, Rim, Munc18, CAPS, Snapin, Complexin, Rab3a, Doc2, Syntaphilin Tomosyn, SV2, NSF, SNAPs |
| Fusion            | Vesicle membrane fuse with presynaptic membrane and release neurotransmitters to synaptic cleft triggered by Ca<sup>2+</sup> in milliseconds. | SNAREs, Synaptotagmins |
neurotransmitters in the synapses [250]. The neurotransmitters reach a coordinated and balanced state. GABA works in the adult brain primarily by acting on GABA_A and GABA_B receptors [251].

GABA_A receptors can be activated by a high concentration of GABA and are a kind of ligand-gated Cl channel receptor that induces synaptic inhibitory response [252]. They affect the rhythmic activity generated in the neural network. The application of GABA_A receptor antagonist Bicuculline (Bic) can improve the spatial learning and memory [261]. CGP35348, a GABA_B receptor antagonist, can improve this treatment of clinical cognitive disorders, the specific cognition has achieved certain success and has guided the application of GABA A receptor antagonist in multiple brain functions, such as recognition, learning, memory, and anxiety [253, 254]. Experimental studies showed that baclofen, a GABA_B receptor agonist, could affect the acquisition and consolidation of learning and memory [255–257]. CGP35348, a GABA_B receptor antagonist, can improve this situation, because CGP35348 inhibits the inhibitory postsynaptic electrical potential (IPSP) and enhances the activation of GABA receptors [248], thereby promoting memory formation [258]. The GABA_C receptors are similar to the GABA_A receptors but are insensitive to Bic and baclofen [259, 260]. The GABA_C receptor antagonist TPMPA can block the inhibitory effect of GABA at lower doses on learning and memory [261].

Although the research on the influence of GABA on cognition has achieved certain success and has guided the treatment of clinical cognitive disorders, the specific mechanism underlying the influence of GABA signal on learning and memory has not been fully elucidated and needs further discussion.

4.3. Dopamine. Another neurotransmitter associated with disease is dopamine (DA) [262]. DA regulates various physiological functions of the CNS [263, 264]. The dysregulation of DA system affects the progression of PD, schizophrenia, Tourette syndrome, attention deficit hyperactivity syndrome, and pituitary tumor [265]. PD is a slow progressive neurodegenerative disease that affects middle-aged and elderly population [266], and the main pathological change is the progressive death of dopaminergic neurons in the substantia nigra (SN), which eventually leads to the severe loss of DA in the striatum [267, 268]. The formation of Lewy body is one of the main pathological changes of PD [269]. A close connection exists between the DA system and α-synuclein, which is the main component of Lewy body [227, 269].

In the process of DA metabolism, the activity of DA-induced intermediates can be inhibited by combining with α-synuclein that selectively induces the formation of α-synuclein fibrils and increases fibrillar aggregation [270]. Similarly, the abnormal aggregation of α-synuclein leads to the imbalance of normal anabolism of DA, the increase of intracellular toxic-free DA, and the blocking of the vesicle transport of DA [270]. This vicious cycle is formed, thereby intensifying the occurrence of cell death and disease.

Molecules involved in maintaining DA homeostasis have successively become drug targets due to the central role of DA in the pathogenesis of PD. The metabolism of DA in vivo is carried out by monoamine oxidase-B (MAO-B) and catechol-O-methyltransferase (COMT) [271–273]. The inhibitors of these enzymes can reduce the degradation of DA and thus play roles in PD treatment.

In detail, the MAO-B inhibitor selegiline has become one of the main drugs in the treatment of PD and is currently approved for use in treatment in China [274]. Recently, rasagiline, a new MAO-B inhibitor, has been approved by the Advisory Committee of the European Medicines Evaluation Agency [274]. DA receptor agonists can bypass the denaturing neurons, directly stimulate the postsynaptic DA receptors, slow down the synthesis of DA, reduce the generation of free radicals, and protect the remaining substantia nigra neurons [275]. Currently, PD treatment is still limited to symptomatic treatment, and the drug target is mostly the production of DA, such as L-DOPA, DA receptor agonists, and the DA-related metabolism enzymes mentioned above [276]. In recent years, both traditional Chinese medicine and acupuncture have achieved good results in the treatment of PD in animal models [277]. They can relieve the motor symptoms of animals with PD and reduce the loss of DAergic neurons in the substantia nigra [277]. These treatments may provide a new therapeutic strategy for PD patients [277, 278].

4.4. Acetylcholine. Cholinergic synapses are ubiquitous in the human CNS [279]. Their high density in the thalamus, striatum, limbic system, and neocortex suggests that cholinergic transmission may be critical for memory, learning, attention, and other higher brain functions [208]. The cholinergic system plays an important role in global brain homeostasis and plasticity [280]. Acetylcholine (ACh), the first neurotransmitter to be identified [281], is used by all cholinergic neurons and has a critical important role in the peripheral and CNS [282]. ACh is synthesized from choline and acetyl-coenzyme A (acetyl-CoA) via the enzyme choline acetyltransferase (ChAT) and then transferred by vesicular acetylcholine transporter (VACHT) [283, 284]. When cholinergic neurons depolarize, ACh is released from synaptic vesicles into the synaptic cleft, where it can activate nicotinic receptors (N-receptors) and muscarinic receptors (M-receptors) [208]. ACh in the synaptic clefts is rapidly inactivated by acetylcholinesterase (ACHE), thereby releasing choline and acetate [285]. Stimulation of N-receptors present on the membranes of presynaptic neurons in CNS increases the concentration of presynaptic Ca^{2+} [286, 287], which may promote the release of many neurotransmitters, such as ACh, Glu, GABA, DA, serotonin, and norepinephrine [287, 288]. Thus, ACh can influence the strength and fidelity of various synapses and modulate overall CNS neurotransmission [288].

In addition, the cholinergic and glutamatergic systems seem to be interrelated, because the role of ACh in learning and memory seems to be related to the regulation of glutamatergic neurotransmission [221, 289]. Many N-receptor agonists were found, such as nicotine, DMPP (1,1-dimethyl-4-phenylpiperazinium), and cyanate [289, 290]. Agonist sensitivity is highly influenced by N-receptor subunit composition [290]. Additionally, curare is the best known antagonist for N-receptors that cannot block CNS nicotinic receptors [291]. M-receptors are widely present in the parasympathetic...
postganglion fiber-dominated effecter cells [208, 292]. When ACh binds to such receptors, it produces a series of parasympathetic terminal excitatory effects [292]. These receptors can also bind to muscarine to produce a similar effect [293, 294]. Atropine, a blocker of these M-receptors, can compete with ACh for M-receptors in the postsynaptic membrane of parasympathetic nerve postganglionic fibers [295], thereby antagonizing muscarinic symptoms and the CNS caused by the excessive acetylcholine stimulation of the postsynaptic membrane. Cholinergic neurotransmission has been implicated in a number of disease states [280, 282]. Defects in cholinergic transmission may affect all aspects of cognition and behavior, including cortical and hippocampal processing of information [296], which was found not only in AD but also in PD, Down syndrome, and ALS [297, 298]. In addition, Huntington’s disease seems to be related to the decrease of ChAT activity [297, 298]. Selective injury of cholinergic neurons in the basal forebrains of AD rodent models is reportedly related to increased deposition of Aβ and levels of hyperphosphorylated tau in the hippocampus and cortex [299]. The animal experiments showed that cholinergic depletion promoted Aβ deposition and tau pathology, therefore leading to cognitive impairment [300]. The main therapeutic strategy for AD is to restore cholinergic function through the use of compounds that block the enzymes that break down ACh [301, 302]. Cholinesterase inhibitors (ChEI) are generally considered as the symptomatic treatments for AD [303]. They are a class of drugs that can bind with ChE and inhibit ChE activity [285]; they are also known as anticholinesterase drugs [285, 303]. Their role is to release the ACh accumulated by cholinergic nerve terminals, thereby showing enhanced M-like and N-like effects and activating cholinergic receptors [304]; they are the so-called quasicholinergic drugs [303]. In addition, rivastigmine, donepezil, and galantamine are currently available FDA-approved ChEI drugs used for AD treatment [305]. These drugs have positive effects for only a short period of time (about 1 year to 3 years) and cannot alter disease progression [306].

5. Outlook

The synapse is the key structure of the connection among neurons in a neural network and has multiple important physiological functions [6, 7]. Synaptic secretion is involved in several important cellular activities, such as neurotransmitter release, hormone secretion, and natural immunity [8, 309]. The molecular basis of synaptic secretion has fascinated scientists for decades. There are hundreds of proteins involved in regulation, and new ones are still being discovered [57, 310]. Neural communication relies on the tight regulation of synaptic vesicle fusion at nerve endings, which results in neurotransmitter release with strict time and quantum precision [16, 17]. In the resting state, synaptic vesicle
fusion is inhibited [19, 311]. When action potentials mediate Ca^{2+} influx to nerve endings, vesicle fusion is induced following the rapid release of neurotransmitters at the millisecond level [311]. These processes are subject to strict regulatory controls that prevent excessive neurotransmitter release and ensure high-fidelity neuronal communication that otherwise leads to disruption of neurotransmission [19, 57, 138, 312].

The coordination of these precise events requires a series of presynaptic proteins [19, 313]. SNARE proteins provide the core fusion mechanism for the energy required for synaptic vesicles to fuse with the plasma membrane [57, 91, 310]. Other biological molecules, such as Synaptotagmin, Munc18, Munc13, CAPS, RIM, Rab, and Complexin, are involved in the regulation of synaptic vesicle secretion in physiological environments [111, 120, 158, 187, 314]. Given this complexity, defects in this mechanism expectedly lead to a range of neurological disorders [2]. There are various neurotransmitters in information communication, including excitatory neurotransmitters and inhibitory neurotransmitters, which play unique roles and jointly regulate neuronal growth and development, synaptogenesis, and synaptic signal transmission [244, 315].

Whether due to genetics, drug abuse, aging process, viral infection, or other reasons, the abnormal communication

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**Table 3: A summary of FDA approved-drugs related to neurotransmitter transmission [256, 262, 276, 285, 302]. The drug name, action mechanisms, application in diseases, and the approval year by the FDA are listed.**

| Drug name       | Mechanism                                      | Application                                      | FDA approval year |
|-----------------|------------------------------------------------|-------------------------------------------------|-------------------|
| Glutamate       |                                                |                                                 |                   |
| Memantine       | NMDA receptor antagonist                       | AD                                              | 2003              |
| Acamprosate     | NMDA receptor agonist                          | The treatment of alcohol dependence             | 2004              |
| Perampanel      | AMPA receptor antagonist                        | Epilepsy                                        | 2012              |
| GABA            |                                                |                                                 |                   |
| Propofol (Pro)  | GABA\(_A\) receptor agonist                   | Induction and maintenance of general anesthesia | 1989              |
| Baclofen        | GABA\(_A\) receptor agonist                   | Treats muscle spasms caused by certain conditions (such as multiple sclerosis, spinal cord injury/disease) | 2010              |
| Gabapentin      | Modulates the action of GAD                     | Epilepsy                                        | 1993              |
| Topiramate      | GABA\(_A\) receptor agonist                   | Epilepsy                                        | 2009              |
| Dopamine        |                                                |                                                 |                   |
| Selegiline      | MAO-B inhibitor                                | PD                                              | 2006              |
| Rasagiline      | MAO-B inhibitor                                | PD                                              | 2006              |
| Quetiapine      | Dopamine receptor antagonist                   | Obesity                                         | 2014              |
| Naltrexon/ bupropion | Opioid receptor antagonist, dopamine agonist, and NE reuptake inhibitor |                                                 |                   |
| Clozapine       | Dopamine receptor/5-HT2A receptor antagonist    | Antipsychotic drugs, mainly for acute and chronic schizophrenia | 1990              |
| Risperidone     | Dopamine receptor/5-HT2A receptor antagonist    | Schizophrenia                                   | 2009              |
| Olanzapine      | Dopamine receptor/5-HT2A receptor antagonist    | Schizophrenia                                   | 2009              |
| Aripiprazole    | Dopamine receptor/5-HT1A receptor antagonist    | Schizophrenia and bipolar disorder              | 2015              |
| Ziprasidone     | Dopamine receptor/5-HT receptor antagonist      | Schizophrenia                                   | 2001              |
| Rotigotine      | Dopamine receptor/5-HT receptor/ adrenergic receptor agonist | PD                                              | 2007              |
| Acetylcholine   |                                                |                                                 |                   |
| Rivastigmine    | AChE inhibitor                                 | AD                                              | 2000              |
| Huperzine A     | AChE inhibitor                                 | AD                                              | 1999              |
| Donepezil       | AChE inhibitor                                 | AD                                              | 1996              |
| Galantamine     | AChE inhibitor                                 | AD and age-associated memory impairment (AAMI)   | 2001              |
| Neostigmine     | AChE inhibitor                                 | Myasthenia gravis (MG)                          | 2003              |
| Mestinon        | AChE inhibitor                                 | MG, obesity, dementia, epilepsy                 | 1955              |
| Atropine        | M-receptor antagonist                          | Antispasmodic agents                            | 2018              |
| Nicotine        | N-receptor agonist                             | Reduces appetite, improves mood, and has some antidepressant properties | 1997              |
between neurons may be common to several neuropsychiatric diseases (such as schizophrenia, PD, autism, AD, HD, and depression) [316–318]. Recent studies showed that synaptogenesis disorders can lead to neurological dysfunction [20, 36]; the important pathological changes in several neurodegenerative diseases are the structural changes, the reduction of the number of synapses, and the synaptic dysfunction [36, 203]. It is important to study and elucidate the mechanisms of neurotransmitter release at the molecular level, because understanding these basic mechanisms can better clarify the etiology of neuropsychiatric diseases, which is the key to further understanding the release effect of drugs for disease treatment [318].

According to the successfully developed drugs for disease treatment, multiple drugs affecting neurotransmitter transmission act on neurotransmitter receptors, especially presynaptic neurotransmitter receptors (Figure 3 and Table 3) [291, 292]. Some proteins with transport or enzyme functions can also be used as drug targets [291, 303, 319]. Neurotransmitter release mechanisms appear to be relatively poor drug targets, because SNARE proteins, Munc18, Synaptotagmin, and others modulate neurotransmitter release through protein–protein interactions that are difficult to influence with small molecules [320, 321].

Nevertheless, synaptic vesicle protein 2A (SV2A), which is involved in the regulation of neurotransmitter release and vesicle circulation [322], is the action site of the new antiepileptic drug levetiracetam [323, 324]. Currently, 15 anti-PD drugs targeting α-synuclein are in the preclinical stage [325]. Therefore, exploring the function and release mechanism of neurotransmitters is of great significance in understanding the role of current drugs and stimulating the development of new drugs.

6. Conclusion

Synapses transmit information through synaptic secretion to realize cellular communication. The exocytosis process of vesicles includes tethering, docking, priming, and fusion and mediates the release of transmitters. Damage to any of these steps can lead to functional disorders, further leading to neurodegenerative diseases as well as neurodevelopmental and psychiatric disorders. Important advances have been made in functional models of Ca^{2+}-triggered neurotransmitter release mechanisms coregulated by SNARE proteins and other regulatory factors. An in-depth understanding of proteins and their regulatory mechanisms will contribute to a better understanding of neuronal plasticity, as well as diseases caused by cellular communication defects, and have important strategic implications for the prevention and treatment of related diseases and the development of new drugs.

Data Availability

All data generated or analyzed in this study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors’ Contributions

Ziqing Wei, Mingze Wei, Xiaoyu Yang, Yuming Xu, and Kaidi Ren conceptualized and wrote the manuscript and created figures. Ziqing Wei, Siqi Gao, and Kaidi Ren contributed to the writing of the manuscript. Ziqing Wei, Mingze Wei, Yuming Xu, and Kaidi Ren reviewed and modified the manuscript. All authors approved the final version of the manuscript.

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References

[1] F. Núñez-Batalla, J. P. Díaz-Molina, M. Costales-Marcos, C. M. Galindo, and C. Suárez-Nieto, “Neuroaryngology,” Acta Otorrinolaringológica Española, vol. 63, no. 2, pp. 132–140, 2012.
[2] S. H. Frey, L. Fogassi, S. Grafton et al., “Neurological principles and rehabilitation of action disorders: computation, anatomy, and physiology (CAP) model,” Neurorehabilitation and Neural Repair, vol. 25, 5_Supplement, pp. 65–208, 2011.
[3] J. B. Furness, “Types of neurons in the enteric nervous system,” Journal of the Autonomic Nervous System, vol. 81, no. 1-3, pp. 87–96, 2000.
[4] T. Horie, M. Nakagawa, Y. Sasakura, and T. G. Kusakabe, “Cell type and function of neurons in the ascidian nervous system,” Development, Growth & Differentiation, vol. 51, no. 3, pp. 207–220, 2009.
[5] T. C. Südhof, “The presynaptic active zone,” Neuron, vol. 75, no. 1, pp. 11–25, 2012.
[6] Z. Cheng, C. Rios, W. H. P. Pernice, C. D. Wright, and H. Bhaskaran, “On-chip photonic synapse,” Science Advances, vol. 3, 9, article e1700160, 2017.
[7] Z. Luo, “Synapse formation and remodeling,” Science China. Life Sciences, vol. 53, no. 3, pp. 315–321, 2010.
[8] A. Citri and R. C. Malenka, “Synaptic plasticity: multiple forms, functions, and mechanisms,” Neuropsychopharmacology, vol. 33, no. 1, pp. 18–41, 2008.
[9] K. Michel, J. A. Müller, A. M. Oprejoreanu, and S. Schoch, “The presynaptic active zone: a dynamic scaffold that regulates synaptic efficacy,” Experimental Cell Research, vol. 335, no. 2, pp. 157–164, 2015.
[10] R. Couteaux and M. Pécot-Dechavassine, “Synaptic vesicles and pouches at the level of “active zones” of the neuromuscular junction,” Comptes Rendus Hebdomadaires des Séances de l’Académie des Sciences. Série D: Sciences Naturelles, vol. 271, no. 25, pp. 2346–2349, 1970.
[11] P. Siekevitz, “The postsynaptic density: a possible role in long-lasting effects in the central nervous system,” Proceedings of the National Academy of Sciences of the United States of America, vol. 82, no. 10, pp. 3494–3498, 1985.
and fusion pores of secretory vesicles in human beta-cells," Pflügers Archiv, vol. 457, no. 6, pp. 1343–1350, 2009.

[50] E. Alés, L. Tabares, J. M. Payot, V. Valero, M. Lindau, and G. A. de Toledo, "High calcium concentrations shift the mode of exocytosis to the kiss-and-run mechanism," Nature Cell Biology, vol. 1, no. 1, pp. 40–44, 1999.

[51] S. W. Schneider, "Kiss and run mechanism in exocytosis," The Journal of Membrane Biology, vol. 181, no. 2, pp. 67–76, 2001.

[52] T. C. Südhof, "Endocytosis in neurons: new players and new mechanisms," Annual Review of Cell Biology, vol. 13, no. 1, pp. 225–257, 1999.

[53] M. C. Harata, A. C. Aravanis, and R. W. Tsien, "Kiss-and-run and full-collapse fusion as modes of exo-endocytosis in neurosecretion," Journal of Neurochemistry, vol. 97, no. 6, pp. 1546–1570, 2006.

[54] W. Shin, L. Ge, G. Arpino et al., "Visualization of membrane pore in live cells reveals a dynamic-pore theory governing fusion and endocytosis," Cell, vol. 173, no. 4, pp. 934–945.e12, 2018.

[55] J. Rizo and J. Xu, "The synaptic vesicle release machinery," Annual Review of Biophysics, vol. 44, no. 1, pp. 339–367, 2015.

[56] A. M. Cárdenas and F. D. Marengo, "Rapid endocytosis and vesicle recycling in neuroendocrine cells," Cellular and Molecular Neurobiology, vol. 30, no. 8, pp. 1365–1370, 2010.

[57] M. C. Willingham and I. Pastan, "Endocytosis and Vesicle Exocytosis: Current Concepts of Traffic in Animal Cells," International Review of Cytology, vol. 92, pp. 51–92, 1984.

[58] L. G. Wu, T. A. Ryan, and L. Lagnado, "Modes of vesicle retrieval at ribbon synapses, calyx-type synapses, and small central synapses," The Journal of Neuroscience, vol. 27, no. 44, pp. 11793–11802, 2007.

[59] Y. Saheki and P. de Camilli, "Synaptic vesicle endocytosis," Cold Spring Harbor Perspectives in Biology, vol. 4, no. 9, article a005645, 2012.

[60] T. C. Südhof, "The synaptic vesicle cycle: a cascade of protein-protein interactions," Nature, vol. 375, no. 6533, pp. 645–653, 1995.

[61] T. C. Südhof, "The synaptic vesicle cycle," Annual Review of Neuroscience, vol. 27, no. 1, pp. 509–547, 2004.

[62] S. Houy, P. Croisé, O. Guhar et al., "Exocytosis and endocytosis in neuroendocrine cells: inseparable membranes!," Frontiers in Endocrinology, vol. 4, p. 135, 2013.

[63] S. Mochida, "Protein-protein interactions in neurotransmitter release," Neuroscience Research, vol. 36, no. 3, pp. 175–182, 2000.

[64] L. Burri and T. Lithgow, "A complete set of SNAREs in yeast," Traffic, vol. 5, no. 1, pp. 45–52, 2004.

[65] G. D. Gupta and I. Brent Heath, "Predicting the distribution, conservation, and functions of SNAREs and related proteins in fungi," Fungal Genetics and Biology, vol. 36, no. 1, pp. 1–21, 2002.

[66] Y. A. Chen, S. J. Scales, S. M. Patel, Y. C. Doung, and R. H. Scheller, "SNARE complex formation is triggered by Ca2+ and drives membrane fusion," Cell, vol. 97, no. 2, pp. 165–174, 1999.

[67] A. Sanderfoot, "Increases in the number of SNARE genes parallels the rise of multicellularity among the green plants," Plant Physiology, vol. 144, no. 1, pp. 6–17, 2007.

[68] M. K. Bennett and R. H. Scheller, "The molecular machinery for secretion is conserved from yeast to neurons," Proceedings of the National Academy of Sciences of the United States of America, vol. 90, no. 7, pp. 2559–2563, 1993.

[69] W. S. Trimb, D. M. Cowan, and R. H. Scheller, "VAMP-1: a synaptic vesicle-associated integral membrane protein," Proceedings of the National Academy of Sciences of the United States of America, vol. 85, no. 12, pp. 4538–4542, 1988.

[70] M. A. Poirier, J. C. Hao, P. N. Malkus et al., "Protease Resistance of Syntaxin SNAP-25 VAMP Complexes: implications for assembly and structure," The Journal of Biological Chemistry, vol. 273, no. 18, pp. 11370–11377, 1998.

[71] T. Weimbs, S. H. Low, J. E. Chapin, K. E. Mostov, P. Bucher, and K. Hofmann, "A conserved domain is present in different families of vesicular fusion proteins: a new superfamily," Proceedings of the National Academy of Sciences of the United States of America, vol. 94, no. 7, pp. 3046–3051, 1997.

[72] D. M. Terriam and M. K. White, "Phylogenetic analysis of membrane trafficking proteins: a family reunion and secondary structure predictions," European Journal of Cell Biology, vol. 73, no. 3, pp. 198–204, 1997.

[73] T. Weimbs, K. Mostov, S. Hui Low, and K. Hofmann, "A model for structural similarity between different SNARE complexes based on sequence relationships," Trends in Cell Biology, vol. 8, no. 7, pp. 260–262, 1998.

[74] H. R. Pelham, "SNAREs and the Secretory Pathway—Lessons from Yeast," Experimental Cell Research, vol. 247, no. 1, pp. 1–8, 1999.

[75] R. Jahn and T. C. Südhof, "Membrane fusion and exocytosis," Annual Review of Biochemistry, vol. 68, no. 1, pp. 863–911, 1999.

[76] R. C. Lin and R. H. Scheller, "Structural organization of the synaptic exocytosis core complex," Neuron, vol. 19, no. 5, pp. 1087–1094, 1997.

[77] M. A. Poirier, W. Xiao, J. C. Macosko, C. Chan, Y. K. Shin, and M. K. Bennett, "The synaptic SNARE complex is a parallel four-stranded helical bundle," Nature Structural Biology, vol. 5, no. 9, pp. 765–769, 1998.

[78] D. Fasshauer, W. K. Eliason, A. T. Brünger, and R. Jahn, "Identification of a minimal core of the synaptic SNARE complex sufficient for reversible assembly and disassembly," Biochemistry, vol. 37, no. 29, pp. 10354–10362, 1998.

[79] R. B. Sutton, D. Fasshauer, R. Jahn, and A. T. Brünger, "Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 Å resolution," Nature, vol. 395, no. 6700, pp. 347–353, 1998.

[80] D. Fasshauer, R. B. Sutton, A. T. Brünger, and R. Jahn, "Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q- and R-SNAREs," Proceedings of the National Academy of Sciences of the United States of America, vol. 95, no. 26, pp. 15781–15786, 1998.
[83] T. Hayashi, S. Yamasaki, S. Nauenburg, T. Binz, and H. Niemann, “Disassembly of the reconstituted synaptic vesicle membrane fusion complex in vitro,” *The EMBO Journal*, vol. 14, no. 10, pp. 2317–2325, 1995.

[84] D. Fasshauer, H. Otto, W. K. Eliasson, R. Jahn, and A. T. Brünger, “Structural Changes Are Associated with Soluble N-Ethylmaleimide-sensitive Fusion Protein Attachment Protein Receptor Complex Formation,” *The Journal of Biological Chemistry*, vol. 272, no. 44, pp. 28036–28041, 1997.

[85] F. Li, F. Pincet, E. Perez et al., “Energetics and dynamics of SNAREpin folding across lipid bilayers,” *Nature Structural & Molecular Biology*, vol. 14, no. 10, pp. 890–896, 2007.

[86] M. Rüttger, A. Kádková, A. Scheutzow, J. Malsam, T. H. Söllner, and J. B. Sørensen, “An electrostatic energy barrier for SNARE-dependent spontaneous and evoked synaptic transmission,” *Cell Reports*, vol. 26, no. 9, pp. 2340–2352.e5, 2019.

[87] N. C. Harata, S. Choi, J. L. Pyle, A. M. Aravanis, and R. W. Tsien, “Frequency-dependent kinetics and prevalence of kiss-and-run and reuse at hippocampal synapses studied with novel quenching methods,” *Neuron*, vol. 49, no. 2, pp. 243–256, 2006.

[88] A. V. Pobbati, A. Stein, and D. Fasshauer, “N- to C-terminal SNARE complex assembly promotes rapid membrane fusion,” *Science*, vol. 313, no. 5787, pp. 673–676, 2006.

[89] J. B. Sørensen, K. Wiederhold, E. M. Müller et al., “Sequential N- to C-terminal SNARE complex assembly drives priming and fusion of secretory vesicles,” *The EMBO Journal*, vol. 25, no. 5, pp. 955–966, 2006.

[90] F. Li, N. Tiwari, J. E. Rothman, and F. Pincet, “Kinetic barriers to SNAREpin assembly in the regulation of membrane docking/priming and fusion,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 113, no. 38, pp. 10536–10541, 2016.

[91] R. Jahn and R. H. Scheller, "SNAREs – engines for membrane fusion," *Nature Reviews Molecular Cell Biology*, vol. 7, no. 9, pp. 631–643, 2006.

[92] Y. A. Chen, S. J. Scales, and R. H. Scheller, "Sequential SNARE assembly underlies priming and triggering of exocytosis," *Neuron*, vol. 30, no. 1, pp. 161–170, 2001.

[93] T. Weber, B. V. Zemelman, J. A. McNew et al., "SNAREpins: minimal machinery for membrane fusion," *Cell*, vol. 92, no. 6, pp. 759–772, 1998.

[94] G. van den Bogaart, M. G. Holt, G. Bunt, D. Riedel, F. S. Wouters, and R. Jahn, "One SNARE complex is sufficient for membrane fusion," *Nature Structural & Molecular Biology*, vol. 17, no. 3, pp. 358–364, 2010.

[95] H. Bao, D. Das, N. A. Courtney et al., "Dynamics and number of _trans_-SNARE complexes determine nascent fusion pore properties," *Nature*, vol. 554, no. 7691, pp. 260–263, 2018.

[96] H. Bao, M. Goldsch-c-Ohm, P. Jeggle, B. Chanda, J. M. Edwardson, and E. R. Chapman, "Exocytotic fusion pores are composed of both lipids and proteins," *Nature Structural & Molecular Biology*, vol. 23, no. 1, pp. 67–73, 2016.

[97] A. J. B. Kreutzberger, V. Kiesling, B. Liang et al., "Reconstitution of calcium-mediated exocytosis of dense-core vesicles," *Science Advances*, vol. 3, no. 7, article e1603208, 2017.

[98] R. Jahn, "Sec1/Munc18 Proteins: Mediators of Membrane Fusion Moving to Center Stage," *Neuron*, vol. 27, no. 2, pp. 201–204, 2000.

[99] Y. Hata, C. A. Slaughter, and T. C. Südhof, "Sympathetic vesicle fusion complex contains unc-18 homologue bound to syntaxin," *Nature*, vol. 366, no. 6453, pp. 347–351, 1993.

[100] P. Novick, C. Field, and R. Schekman, "Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway," *Cell*, vol. 21, no. 1, pp. 205–215, 1980.

[101] R. F. Toonen and M. Verhage, "Munc18-1 in secretion: lonely Munc joins SNARE team and takes control," *Trends in Neurosciences*, vol. 30, no. 11, pp. 564–572, 2007.

[102] T. Voets, R. F. Toonen, E. C. Brian et al., "Munc18-1 Promotes Large Dense-Core Vesicle Docking," *Neuron*, vol. 31, no. 4, pp. 581–592, 2001.

[103] N. Korteweg, A. S. Maia, B. Thompson, E. W. Roubos, J. P. H. Burbach, and M. Verhage, “The role of Munc18-1 in docking and exocytosis of peptide hormone vesicles in the anterior pituitary,” *Biology of the Cell*, vol. 97, no. 6, pp. 445–455, 2005.

[104] Y. Hata and T. C. Südhof, "A Novel Ubiquitous Form of Munc-18 Interacts with Multiple Syntaxins: use of the yeast two-hybrid system to study interactions between proteins involved in membrane traffic," *The Journal of Biological Chemistry*, vol. 270, no. 22, pp. 13022–13028, 1995.

[105] M. Khvotchev, I. Dulubova, J. Sun, H. Dai, J. Rizo, and T. C. Südhof, "Dual modes of Munc18-1/SNARE interactions are coupled by functionally critical binding to syntaxin-1 N terminus," *The Journal of Neuroscience*, vol. 27, no. 45, pp. 12147–12155, 2007.

[106] T. C. Südhof and J. E. Rothman, "Membrane fusion: grappling with SNARE and SM proteins," *Science*, vol. 323, no. 5913, pp. 474–477, 2009.

[107] F. Deák, Y. Xu, W. P. Chang et al., "Munc18-1 binding to the neuronal SNARE complex controls synaptic vesicle priming," *The Journal of Cell Biology*, vol. 184, no. 5, pp. 751–764, 2009.

[108] I. Dulubova, M. Khvotchev, S. Liu, I. Huryeva, T. C. Südhof, and J. Rizo, "Munc18-1 binds directly to the neuronal SNARE complex," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 8, pp. 2697–2702, 2007.

[109] J. Rizo and T. C. Südhof, "The membrane fusion enigma: SNAREs, Sec1/Munc 18 proteins, and their accomplices—guilty as charged?", *Annual Review of Cell and Developmental Biology*, vol. 28, no. 1, pp. 279–308, 2012.

[110] B. Yang, M. Steegmaier, L. C. Gonzalez Jr., and R. H. Scheller, "nSec1 binds a closed conformation of Syntaxin1a," *The Journal of Cell Biology*, vol. 148, no. 2, pp. 247–252, 2000.

[111] C. M. Carr, E. Grote, M. Munson, F. M. Hughson, and P. J. Novick, "Sec1p binds to SNARE complexes and concentrates at sites of secretion," *The Journal of Cell Biology*, vol. 146, no. 2, pp. 333–344, 1999.

[112] M. Margittai, J. Widengren, E. Schweinberger et al., "Single-molecule fluorescence resonance energy transfer reveals a dynamic equilibrium between closed and open conformations of syntaxin 1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 26, pp. 15516–15521, 2003.

[113] I. Dulubova, S. Sugita, S. Hill et al., "A conformational switch in syntaxin during exocytosis: role of munc 18," *The EMBO Journal*, vol. 18, no. 16, pp. 4372–4382, 1999.

[114] J. M. McEwen and J. M. Kaplan, "UNC-18 promotes both the anterograde trafficking and synaptic function of syntaxin," *Molecular Biology of the Cell*, vol. 19, no. 9, pp. 3836–3846, 2008.
Conformational switch of syntaxin-1 controls synaptic vesicle fusion, "Science, vol. 321, no. 5895, pp. 1507–1510, 2008.

Identification of the Minimal Protein Domain Required for Priming Activity of Munc13-1, "Current Biology, vol. 15, no. 24, pp. 2243–2248, 2005.

Munc13 mediates the transition from the closed syntaxin-Munc18 complex to the SNARE complex, "Nature Structural & Molecular Biology, vol. 18, no. 5, pp. 542–549, 2011.

Syntxin opening by the MUN domain underlies the function of Munc13 in synaptic-vesicle priming, "Nature Structural & Molecular Biology, vol. 22, no. 7, pp. 547–554, 2015.

An open form of syntaxin bypasses the requirement for UNC-13 in vesicle priming, "Nature, vol. 412, no. 6844, pp. 338–341, 2001.

"Snare and munc18 in synaptic vesicle fusion, "Nature Reviews. Neuroscience, vol. 3, no. 8, pp. 641–653, 2002.

"Synaptotagmins are trafficked to distinct subcellular domains including the postsynaptic compartment, "The Journal of Cell Biology, vol. 166, no. 2, pp. 249–260, 2004.

Synaptotagmin: mechanisms of an electrostatic switch, "Neuroscience Letters, vol. 722, p. 134834, 2020.

Synaptotagmin: a calcium sensor on the synaptic vesicle surface, "Science, vol. 256, no. 5059, pp. 1021–1025, 1992.

"Synaptotagmin-syntxin interaction: the C 2 domain as a Ca2+-dependent electrostatic switch, "Neuron, vol. 18, no. 1, pp. 133–142, 1997.

"Ca2+ binding to syntaptotagmin: how many Ca2+ ions bind to the tip of a C2A-domain?, "The EMBO Journal, vol. 17, no. 14, pp. 3921–3930, 1998.

"Synaptotagmin I functions as a calcium regulator of release probability, "Nature, vol. 410, no. 6824, pp. 41–49, 2001.

"Synaptotagmin-1 C2B domain interacts simultaneously with SNAREs and membranes to promote membrane fusion, "Elife, vol. 5, 2016.

"Dynamic binding mode of a Syntaxin1/1-SNARE complex in solution, "Nature Structural & Molecular Biology, vol. 22, no. 7, pp. 555–564, 2015.

Architecture of the syntaptotagmin-SNARE machinery for neuronal exocytosis, "Nature, vol. 525, no. 7567, pp. 62–67, 2015.

"Dual roles of the C1B domain of syntaptotagmin I in synchronizing Ca2+-dependent neurotransmitter release, "The Journal of Neuroscience, vol. 24, no. 39, pp. 8542–8550, 2004.

Calcium binding by syntaptotagmin’s C1A domain is an essential element of the electrostatic switch that triggers synchronous synaptic transmission, "The Journal of Neuroscience, vol. 32, no. 4, pp. 1253–1260, 2012.

"Syntaptotagmin-mediated bending of the target membrane is a critical step in Ca2+-regulated fusion, "Cell, vol. 138, no. 4, pp. 709–721, 2009.

PIP2 increases the speed of response of syntaptotagmin and steers its membrane-penetration activity toward the plasma membrane, "Nature Structural & Molecular Biology, vol. 11, no. 1, pp. 36–44, 2004.

"RIM, Munc13, and Rab3A interplay in acrosomal exocytosis, "Experimental Cell Research, vol. 318, no. 5, pp. 478–482, 2012.

"The importance of an asymmetric distribution of acidic lipids for syntaptotagmin 1 function as a Ca2+ sensor, "The Biochemical Journal, vol. 443, no. 1, pp. 223–229, 2012.

"Mammalian homologues of Caenorhabditis elegans unc-13 gene define novel family of C1-domain proteins, "The Journal of Biological Chemistry, vol. 270, no. 42, pp. 25273–25280, 1995.

"RIM proteins activate vesicle priming by reversing autoinhibitory homodimerization of Munc 13, "Neuron, vol. 69, no. 2, pp. 317–331, 2011.

"The RIM/NIM family of neuronal C1 domain proteins. Interactions with Rab 3 and a new class of Src homology 3 domain proteins, "The Journal of Biological Chemistry, vol. 275, no. 26, pp. 20033–20044, 2000.

"Ca2+ regulator of synaptic exocytosis, "Nature Structural & Molecular Biology, vol. 17, no. 3, pp. 280–288, 2010.

"Pre-synaptic phorbol ester receptor that enhances neurotransmitter release, "Neuron, vol. 21, no. 1, pp. 123–136, 1998.

"Beta phorbol ester- and diacylglycerol-induced augmentation of transmitter release is mediated by Munc 13s and not by PKCαs, "Cell, vol. 108, no. 1, pp. 121–133, 2002.

"Intramolecular occlusion of the diacylglycerol-binding site in the C1 domain of munc 13-1, "Biochemistry, vol. 44, no. 4, pp. 1089–1096, 2005.

"A C1-C2 module in Munc13 inhibits calcium-dependent neurotransmitter release, "Neuron, vol. 95, no. 3, pp. 577–590, 2017.

"Functional synergy between the Munc13 C-terminal C1 and C2 domains, "Elife, vol. 5, 2016.
[148] I. Augustin, C. Rosenmund, T. C. Südhof, and N. Brose, “Munc13-1 is essential for fusion competence of glutamatergic synaptic vesicles,” Nature, vol. 400, no. 6743, pp. 457–461, 1999.

[149] C. Rosenmund, A. Sigler, I. Augustin, K. Reim, N. Brose, and J. S. Rhee, “Differential control of vesicle priming and short-term plasticity by Munc13 isoforms,” Neuron, vol. 33, no. 3, pp. 411–424, 2002.

[150] H. J. Junge, J. S. Rhee, O. Jahn et al., “Calmodulin and Munc13 form a Ca2+ sensor effector complex that controls short-term synaptic plasticity,” Cell, vol. 118, no. 3, pp. 389–401, 2004.

[151] K. Dimova, H. Kawabe, A. Betz, N. Brose, and O. Jahn, “Characterization of the Munc13-calmodulin interaction by photoaffinity labeling,” Biochimica et Biophysica Acta, vol. 1763, no. 11, pp. 1256–1265, 2006.

[152] N. Lipstein, T. Sakaba, B. H. Cooper et al., “Dynamic control of synaptic vesicle replenishment and short-term plasticity by Ca2+-calmodulin-Munc13-1 signaling,” Neuron, vol. 79, no. 1, pp. 82–96, 2013.

[153] F. Rodríguez-Castañeda, M. Maestre-Martínez, N. Coudévert et al., “Modular architecture of Munc13-calmodulin complexes: dual regulation by Ca2+ and possible function in short-term synaptic plasticity,” The EMBO Journal, vol. 29, no. 3, pp. 680–691, 2010.

[154] S. Herbst, N. Lipstein, O. Jahn, and A. Sinz, “Structural insights into calmodulin/Munc13 interaction,” Biological Chemistry, vol. 395, no. 7–8, pp. 763–768, 2014.

[155] J. Basu, N. Shen, I. Dulubova et al., “A minimal domain responsible for Munc13 activity,” Nature Structural & Molecular Biology, vol. 12, no. 11, pp. 1017–1018, 2005.

[156] H. Koch, K. Hofmann, and N. Brose, “Definition of Munc13-homology domains and characterization of a novel ubiquitously expressed Munc13 isoform,” The Biochemical Journal, vol. 349, Part 1, pp. 247–253, 2000.

[157] K. Weninger, M. E. Bowen, U. B. Choi, S. Chu, and A. T. Brungger, “Accessory proteins stabilize the acceptor complex for synaptobrevin, the 1: 1 syntaxin/SNAP-25 complex,” Structure, vol. 16, no. 2, pp. 308–320, 2008.

[158] D. J. James and T. F. Martin, “CAPS and Munc13: CATCHRs that SNARE vesicles,” Frontiers in Endocrinology, vol. 4, p. 187, 2013.

[159] R. Guan, H. Dai, and J. Rizo, “Binding of the Munc13-1 MUN domain to membrane-anchored SNARE complexes,” Biochemistry, vol. 47, no. 6, pp. 1474–1481, 2008.

[160] E. He, K. Wierda, R. Van Westen et al., “Munc13-1 and Munc18-1 together prevent NSF-dependent de-priming of synaptic vesicles,” Nature Communications, vol. 8, article 15915, 2017.

[161] T. Shu, H. Jin, J. E. Rothman, and Y. Zhang, “Munc13-1 MUN domain and Munc18-1 cooperatively chaperone SNARE assembly through a tetrameric complex,” Proceedings of the National Academy of Sciences of the United States of America, vol. 117, no. 2, pp. 1036–1041, 2020.

[162] K. Zhou, T. M. Stawicki, A. Goncharov, and Y. Jin, “Position of UNC-13 in the active zone regulates synaptic vesicle release probability and release kinetics,” Elife, vol. 2, article e01180, 2013.

[163] J. M. Madison, S. Nurrish, and J. M. Kaplan, “UNC-13 interaction with syntaxin is required for synaptic transmission,” Current Biology, vol. 15, no. 24, pp. 2236–2242, 2005.
Required for Vesicle Fusion*, The Journal of Biological Chemistry, vol. 285, no. 46, pp. 35320–35329, 2010.

[178] D. J. James, J. Kowalchyk, N. Daly, M. Petrie, and T. F. J. Martin, “CAPS drives trans-SNARE complex formation and membrane fusion through syntaxin interactions,” Proceedings of the National Academy of Sciences of the United States of America, vol. 106, no. 41, pp. 17308–17313, 2009.

[179] C. Khodhong, G. Kabachinski, D. J. James, and T. F. J. Martin, “Munc13 homology domain-1 in CAPS/UNC31 mediates SNARE binding required for priming vesicle exocytosis,” Cell Metabolism, vol. 14, no. 2, pp. 254–263, 2011.

[180] G. Kabachinski, D. M. Kielar-Grevstad, X. Zhang, D. J. James, and T. F. J. Martin, “Resident CAPS on dense-core vesicles docks and primes vesicles for fusion,” Molecular Biology of the Cell, vol. 27, no. 4, pp. 654–668, 2016.

[181] G. Kabachinski, M. Yamaga, D. M. Kielar-Grevstad, S. Bruinsma, and T. F. J. Martin, “CAPS and Munc13 utilize distinct PIP2-linked mechanisms to promote vesicle exocytosis,” Molecular Biology of the Cell, vol. 25, no. 4, pp. 508–521, 2014.

[182] W. J. Jockusch, D. Speidel, A. Sigler et al., “CAPS-1 and CAPS-2 are essential synaptic vesicle priming proteins,” Cell, vol. 131, no. 4, pp. 796–808, 2007.

[183] C. Imig, S. W. Min, S. Krinner et al., “The morphological and molecular nature of synaptic vesicle priming at presynaptic active zones,” Neuron, vol. 84, no. 2, pp. 416–431, 2014.

[184] Y. Liu, C. Schirra, L. Edelmann et al., “Two distinct secretory vesicle-priming steps in adrenal chromaffin cells,” The Journal of Cell Biology, vol. 190, no. 6, pp. 1067–1077, 2010.

[185] C. Q. Nguyen Truong, D. Nestvogel, O. Ratai et al., “Secretory vesicle priming by CAPS is independent of its SNARE-binding MUN domain,” Cell Reports, vol. 9, no. 3, pp. 902–909, 2014.

[186] H. Zhou, Z. Wei, S. Wang, D. Yao, R. Zhang, and C. Ma, “Structural and functional analysis of the CAPS SNARE-binding domain required for SNARE complex formation and exocytosis,” Cell Reports, vol. 26, no. 12, pp. 3347–3359.e6, 2019.

[187] Y. Lai, U. B. Choi, J. Leitz et al., “Molecular mechanisms of synaptic vesicle priming by Munc13 and Munc18,” Neuron, vol. 95, no. 3, pp. 591–607.e10, 2017.

[188] R. W. Tsien, D. Lipscombe, D. V. Madison, K. R. Bley, and A. P. Fox, “Multiple types of neuronal calcium channels and their selective modulation,” Trends in Neurosciences, vol. 11, no. 10, pp. 431–438, 1988.

[189] K. Dunlap, J. I. Luebke, and T. J. Turner, “Exocytotic Ca2+ channels in mammalian central neurons,” Trends in Neurosciences, vol. 18, no. 2, pp. 89–98, 1995.

[190] W. A. Catterall and A. P. Few, “Calcium channel regulation and presynaptic plasticity,” Neuron, vol. 59, no. 6, pp. 882–901, 2008.

[191] M. C. Nowycky, A. P. Fox, and R. W. Tsien, “Three types of neuronal calcium channel with different calcium agonist sensitivity,” Nature, vol. 316, no. 6027, pp. 440–443, 1985.

[192] W. A. Catterall, “Structure and regulation of voltage-gated Ca2+ channels,” Annual Review of Cell and Developmental Biology, vol. 16, pp. 521–555, 2000.

[193] L. Lando and R. S. Zucker, “Ca2+ cooperativity in neurosecretion measured using photolabile Ca2+ chelators,” Journal of Neurophysiology, vol. 72, no. 2, pp. 825–830, 1994.

[194] O. Wiser, M. K. Bennett, and D. Atlas, “Functional interaction of syntaxin and SNAP-25 with voltage-sensitive L- and N-type Ca2+ channels,” The EMBO Journal, vol. 15, no. 16, pp. 4100–4110, 1996.

[195] Z. H. Sheng, C. T. Yokoyama, and W. A. Catterall, “Interaction of the synprint site of N-type Ca2+ channels with the C2B domain of synaptotagmin I,” Proceedings of the National Academy of Sciences of the United States of America, vol. 94, no. 10, pp. 5405–5410, 1997.

[196] H. Zhong, C. T. Yokoyama, T. Scheuer, and W. A. Catterall, “Reciprocal regulation of P/Q-type Ca2+ channels by SNAP-25, syntaxin and synaptotagmin,” Nature Neuroscience, vol. 2, no. 11, pp. 939–941, 1999.

[197] R. Felix, “Molecular regulation of voltage-gated Ca2+ channels,” Journal of Receptor and Signal Transduction Research, vol. 25, no. 2, pp. 57–71, 2005.

[198] M. Mayford, S. A. Siegelbaum, and E. R. Kandel, “Synapses and memory storage,” Cold Spring Harbor Perspectives in Biology, vol. 4, no. 6, 2012.

[199] S. J. Martin, P. D. Grimwood, and R. G. Morris, “Synaptic plasticity and memory: an evaluation of the hypothesis,” Annual Review of Neuroscience, vol. 23, pp. 649–711, 2000.

[200] A. B. Borisov, E. I. Dedkov, and B. M. Carlson, “Interrelations of myogenic response, progressive atrophy of muscle fibers, and cell death in denervated skeletal muscle,” The Anatomical Record, vol. 264, no. 2, pp. 203–218, 2001.

[201] L. A. Raymond, V. M. Andre, C. Cepeda, C. M. Gladding, A. J. Milnerwood, and M. S. Levine, “Pathophysiology of Huntington’s disease: time-dependent alterations in synaptic and receptor function,” Neuroscience, vol. 198, pp. 252–273, 2011.

[202] S. Tu, S. Okamoto, S. A. Lipton, and H. Xu, “Oligomeric Aβ-induced synaptic dysfunction in Alzheimer’s disease,” Molecular Neurodegeneration, vol. 9, no. 1, p. 48, 2014.

[203] D. J. Selkoe, “Alzheimer’s disease is a synaptic failure,” Science, vol. 298, no. 5594, pp. 789–791, 2002.

[204] K. Ghosal, S. L. Vogt, M. Liang, Y. Shen, B. T. Lamb, and S. W. Pimplikar, “Alzheimer’s disease-like pathological features in transgenic mice expressing the APP intracellular domain,” Proceedings of the National Academy of Sciences of the United States of America, vol. 106, no. 43, pp. 18367–18372, 2009.

[205] M. Talantova, S. Sanz-Blasco, X. Zhang et al., “Abeta induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss,” Proceedings of the National Academy of Sciences of the United States of America, vol. 110, no. 27, pp. E2518–E2527, 2013.

[206] S. Zhou and Y. Yu, “Synaptic excitatory-inhibitory balance underlying efficient neural coding,” Advances in Neurobiology, vol. 21, pp. 85–100, 2018.

[207] R. M. Ryan, S. L. Ingram, and A. Scimemi, “Regulation of glutamate, GABA and dopamine transporter uptake, surface mobility and expression,” Frontiers in Cellular Neuroscience, vol. 15, article 670346, 2021.

[208] W. D. Phillips, “Acetylcholine receptors and the cytoskeletal connection,” Clinical and Experimental Pharmacology & Physiology, vol. 22, no. 12, pp. 961–965, 1995.

[209] C. H. Fry, M. Hussain, C. McCarthy, Y. Ikeda, G. P. Sui, and W. A. Catterall, “Recent advances in detrusor muscle function,” Scandinavian Journal of Urology and Nephrology. Supplementum, vol. 215, pp. 20–25, 2004.
M. Blaschke, B. U. Keller, R. Rivosecchi, M. Hollmann, A. Almeida, S. J. Heales, J. P. Bolaños, and J. M. Medina, B. U. Keller, M. Hollmann, S. Heinemann, and A. Konnerth, M. J. Armstrong and M. S. Okun, M. R. Duchen, J. T. Brosnan and M. E. Brosnan, D. Bleakman, A. Alt, and E. S. Nisenbaum, L. Iovino, M. E. Tremblay, and L. Civiero, N. Scheefhals and H. D. Macgillavry, F. M. Ribeiro, L. B. Vieira, R. G. Pires, R. P. Olmo, and S. S. G. Fi, channels, mediated mitochondrial dysfunction and glutathione depletion, vol. 90, no. 14, pp. 6528–6520, 2020.

Y. Chen, J. Xu, and Y. Chen, "Regulation of neurotransmitters by the gut microbiota and effects on cognition in neurological disorders," *Nutrients*, vol. 13, no. 6, 2021.

J. T. Brosnan and M. E. Brosnan, "Glutamate: a truly functional amino acid," *Amino Acids*, vol. 45, no. 3, pp. 413–418, 2013.

S. F. Traynelis, L. P. Wollmuth, C. J. McBain et al., "Glutamate receptor ion channels: structure, regulation, and function," *Pharmacological Reviews*, vol. 62, no. 3, pp. 405–496, 2010.

D. Bleakman, A. Alt, and E. S. Nisenbaum, "Glutamate receptors and pain," *Seminars in Cell & Developmental Biology*, vol. 17, no. 5, pp. 592–604, 2006.

Y. Zhou and N. C. Danbolt, "Glutamate as a neurotransmitter in the healthy brain," *Journal of Neural Transmission (Vienna)*, vol. 121, no. 8, pp. 799–817, 2014.

L. Iovino, M. E. Tremblay, and L. Civiero, "Glutamate-induced excitotoxicity in Parkinson's disease: the role of glial cells," *Journal of Pharmacological Sciences*, vol. 144, no. 3, pp. 151–164, 2020.

F. M. Ribeiro, L. B. Vieira, R. G. Pires, R. P. Olmo, and S. S. G. Ferguson, "Metabotropic glutamate receptors and neurodegenerative diseases," *Pharmacological Research*, vol. 115, pp. 179–191, 2017.

N. Scheefhals and H. D. Macgillavry, "Functional organization of postsynaptic glutamate receptors," *Molecular and Cellular Neurosciences*, vol. 91, pp. 82–94, 2018.

V. Bruno, U. Scapagnini, and P. L. Canonico, "Excitatory amino acids and neurotoxicity," *Functional Neurology*, vol. 8, no. 4, pp. 279–292, 1993.

F. A. Dajas-Bailador, P. A. Lima, and S. Wonnacott, "The alpha 7 nicotinic acetylcholine receptor subtype mediates nicotine protection against NMDA excitotoxicity in primary hippocampal cultures through a Ca\textsuperscript{2+} dependent mechanism," *Neuropharmacology*, vol. 39, no. 13, pp. 2799–2807, 2000.

M. R. Duchen, "Mitochondria and calcium: from cell signaling to cell death," *The Journal of Physiology*, vol. 529, Part 1, pp. 57–68, 2000.

A. Almeida, S. J. Heales, J. P. Bolaños, and J. M. Medina, "Glutamate neurotoxicity is associated with nitric oxide-mediated mitochondrial dysfunction and glutathione depletion," *Brain Research*, vol. 790, no. 1-2, pp. 209–216, 1998.

M. Blaschke, B. U. Keller, R. Rivosecchi, M. Hollmann, S. Heinemann, and A. Konnerth, "A single amino acid determines the subunit-specific spider toxin block of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate receptor channels," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 14, pp. 6528–6532, 1993.

B. U. Keller, M. Hollmann, S. Heinemann, and A. Konnerth, "Calcium influx through subunits GluR1/GluR3 of kainate/AMPA receptor channels is regulated by cAMP dependent protein kinase," *The EMBO Journal*, vol. 11, no. 3, pp. 891–896, 1992.

M. E. Conway, "Alzheimer's disease: targeting the glutamatergic system," *Biogerontology*, vol. 21, no. 3, pp. 257–274, 2020.

M. Goedert, "Alzheimer’s and Parkinson’s diseases: the prion concept in relation to assembled Abeta, tau, and alpha-synuclein," *Science*, vol. 349, no. 6248, article 1255555, 2015.

G. Gallardo and D. M. Holtzman, "Amyloid-beta and Tau at the crossroads of Alzheimer's disease," *Advances in Experimental Medicine and Biology*, vol. 1184, pp. 187–203, 2019.

J. X. Song, S. Malampati, Y. Zeng et al., "A small molecule transcription factor EB activator ameliorates beta-amyloid precursor protein and Tau pathology in Alzheimer's disease models," *Aging Cell*, vol. 19, no. 2, article e13069, 2020.

T. Foltynie, C. Brayne, and R. A. Barker, "The heterogeneity of idiopathic Parkinson's disease," *Journal of Neurology*, vol. 249, no. 2, pp. 138–145, 2002.

G. E. Alexander, "Biology of Parkinson's disease: pathogenesis and pathophysiology of a multisystem neurodegenerative disorder," *Dialogues in Clinical Neuroscience*, vol. 6, no. 3, pp. 259–280, 2004.

G. Mereu, E. Costa, D. M. Armstrong, and S. Vicini, "Glutamate receptor subtypes mediate excitatory synaptic currents of dopamine neurons in midbrain slices," *The Journal of Neuroscience*, vol. 11, no. 5, pp. 1359–1366, 1991.

C. Rosin, T. E. Bates, and S. D. Skaper, "Excitatory amino acid induced oligodendrocyte cell death in vitro: receptor-dependent and -independent mechanisms," *Journal of Neurochemistry*, vol. 90, no. 5, pp. 1173–1185, 2004.

A. S. Hazell, "Excitotoxic mechanisms in stroke: an update of concepts and treatment strategies," *Neurochemistry International*, vol. 50, no. 7-8, pp. 941–953, 2007.

Q. T. Bu, W. Y. Zhang, Q. C. Chen et al., "Anti-diabetic effect of ginsenoside Rb3 (3) in alloxan-induced diabetic mice," *Medicinal Chemistry*, vol. 8, no. 5, pp. 934–941, 2012.

J. J. Xing, J. G. Hou, Z. N. Ma et al., "Ginsenoside Rb3 provides protective effects against cisplatin-induced nephrotoxicity via regulation of AMPK/mTOR-mediated autophagy and inhibition of apoptosis in vitro and in vivo," *Cell Proliferation*, vol. 52, no. 4, article e12627, 2019.

M. Sun, Y. Ji, Z. Li et al., "Ginsenoside Rb3 inhibits pro-inflammatory cytokines via MAPK/akt/NF-kappa B pathways and attenuates rat alveolar bone resorption in response to Porphyromonas gingivalis LPS," *Molecules*, vol. 25, no. 20, 2020.

U. Damar, R. Gersner, J. T. Johnstone, S. Schachter, and A. Rotenberg, "Huperzine A as a neuroprotective and antiepileptic drug: a review of preclinical research," *Expert Review of Neurotherapeutics*, vol. 16, no. 6, pp. 671–680, 2016.

S. J. Tsai, "Huperzine-A, a versatile herb, for the treatment of Alzheimer's disease," *Journal of the Chinese Medical Association*, vol. 82, no. 10, pp. 750–751, 2019.

G. Yang, Y. Wang, J. Tian, and J. P. Liu, "Huperzine A for Alzheimer's disease: a systematic review and meta-analysis of randomized clinical trials," *PLoS One*, vol. 8, no. 9, article e74916, 2013.

T. Kikuchi, "Is Memantine Effective as an NMDA Receptor Antagonist in Adjunctive Therapy for Schizophrenia?*, *Bio-molecules*, vol. 10, no. 8, 2020.

T. Kishi, S. Matsunaga, K. Oya, I. Nomura, T. Ikuta, and N. Iwata, "Memantine for Alzheimer's disease: an updated systematic review and meta-analysis," *Journal of Alzheimer's Disease*, vol. 60, no. 2, pp. 401–425, 2017.
[243] S. Matsunaga, T. Kishi, I. Nomura et al., “The efficacy and safety of memantine for the treatment of Alzheimer’s disease,” Expert Opinion on Drug Safety, vol. 17, no. 10, pp. 1053–1061, 2018.

[244] E. Cherubini, J. L. Gaiarsa, and Y. Ben-Ari, “GABA: an excitatory transmitter in early postnatal life,” Trends in Neurosciences, vol. 14, no. 5, pp. 251–259, 1991.

[245] I. B. Yogeswara, S. Maneerat, and D. Haltrich, “Glutamate decarboxylase from lactic acid bacteria—a key enzyme in GABA synthesis,” Microorganisms, vol. 8, no. 12, 2020.

[246] J. M. Tepper and C. R. Lee, “GABAergic control of substantia nigra dopaminergic neurons,” Progress in Brain Research, vol. 160, pp. 189–208, 2007.

[247] M. Watanabe, K. Maemura, K. Kanbara, T. Tamayama, and J. M. Tepper and C. R. Lee, “GABAergic control of substantia nigra dopaminergic neurons,” Progress in Brain Research, vol. 160, pp. 189–208, 2007.

[248] J. Bormann, “Electrophysiology of GABAA and GABAB receptor subtypes,” Trends in Neurosciences, vol. 11, no. 3, pp. 112–116, 1988.

[249] R. L. Macdonald and R. W. Olsen, “GABAA receptor channels,” Annual Review of Neuroscience, vol. 17, pp. 569–602, 1994.

[250] F. H. Marshall and S. M. Foord, “Heterodimerization of the GABAB receptor–implications for GPCR signaling and drug discovery,” Advances in Pharmacology, vol. 58, pp. 63–91, 2010.

[251] C. L. Padgett and P. A. Slesinger, “GABAB receptor coupling to G-proteins and ion channels,” Advances in Pharmacology, vol. 58, pp. 123–147, 2010.

[252] B. Cohen, M. Dai, S. B. Yakushin, and T. Raphan, “Baclofen, motion sickness susceptibility and the neural basis for velocity storage,” Progress in Brain Research, vol. 171, pp. 543–553, 2008.

[253] T. Wang, W. Huang, and F. Chen, “Baclofen, a GABAB receptor agonist, inhibits human hepatocellular carcinoma cell growth in vitro and in vivo,” Life Sciences, vol. 82, no. 9-10, pp. 536–541, 2008.

[254] M. Dai, T. Raphan, and B. Cohen, “Effects of baclofen on the angular vestibulo-ocular reflex,” Experimental Brain Research, vol. 171, no. 2, pp. 262–271, 2006.

[255] B. E. Albrecht, U. Breitenbach, T. Stühmer, R. J. Harvey, and M. G. Darlison, “In situ hybridization and reverse transcription–polymerase chain reaction studies on the expression of the GABA (C) receptor rho 1- and rho 2-subunit genes in avian and rat brain,” The European Journal of Neuroscience, vol. 9, no. 11, pp. 2414–2422, 1997.

[256] W. Sieghart, “Multiplicity of GABAA–benzodiazepine receptors,” Trends in Pharmacological Sciences, vol. 10, no. 10, pp. 407–411, 1989.

[257] C. G. Ting Wong, T. Bottiglieri, and O. C. Sneed III, “GABA, gamma-hydroxybutyric acid, and neurological disease,” Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society, vol. 54, Supplement 6, pp. S3–S12, 2003.

[258] E. Mohammadi, A. Shamisizadeh, E. Salari, I. Fatemi, M. Allah tavakoli, and A. Roohbakhsh, “Effect of TIPMA (GABAC receptor antagonist) on neuronal responses properties in rat barrel cortex,” Somatosensory & Motor Research, vol. 34, no. 2, pp. 108–115, 2017.

[259] M. O. Klein, D. S. Battagello, A. R. Cardoso, D. N. Hauser, J. C. Bittencourt, and R. G. Correa, “Dopamine: functions, signaling, and association with neurological diseases,” Cellular and Molecular Neurobiology, vol. 39, no. 1, pp. 31–59, 2019.

[260] K. A. Kempadoo, E. V. Mosharov, S. J. Choi, D. Sulzer, and E. R. Kandel, “Dopamine release from the locus coeruleus to the dorsal hippocampus promotes spatial learning and memory,” Proceedings of the National Academy of Sciences of the United States of America, vol. 113, no. 51, pp. 14835–14840, 2016.

[261] C. Liu, P. Goel, and P. S. Kaeser, “Spatial and temporal scales of dopamine transmission,” Nature Reviews. Neuroscience, vol. 22, no. 6, pp. 345–358, 2021.

[262] D. Vallone, R. Picetti, and E. Borrelli, “Structure and function of dopamine receptors,” Neuroscience and Biobehavioral Reviews, vol. 24, no. 1, pp. 125–132, 2000.

[263] T. R. Mhyre, J. T. Boyd, R. W. Hamill, and K. A. Maquire-Zeiss, “Parkinson’s disease,” Sub-Cellular Biochemistry, vol. 65, pp. 389–455, 2012.

[264] E. Esposito, V. Di Matteo, and G. Di Giovanni, “Death in the substantia nigra: a motor tragedy,” Expert Review of Neurotherapeutics, vol. 7, no. 6, pp. 677–697, 2007.

[265] D. J. Surmeier, J. N. Guzman, J. Sanchez-Padilla, and J. A. Goldberg, “What causes the death of dopaminergic neurons in Parkinson’s disease?,” Progress in Brain Research, vol. 183, pp. 59–77, 2010.

[266] K. Albert, M. H. Voutilainen, A. Domanskyi, and M. Airavaara, “AAV vector-mediated gene delivery to substantia nigra dopamine neurons: implications for gene therapy and disease models,” Genes (Basel), vol. 8, no. 2, 2017.

[267] A. Rekas, R. B. Knott, A. Sokolova et al., “The structure of dopamine induced alpha-synuclein oligomers,” European Biophysics Journal, vol. 39, no. 10, pp. 1407–1419, 2010.

[268] A. Torkaman-Boutorabi, G. Ali Shahidi, S. Choopani, and M. Reza Zarrindast, “Association of monoamine oxidase B and catechol-O-methyltransferase polymorphisms with sporadic Parkinson’s disease in an Iranian population,” Valtia Neurupathologica, vol. 50, no. 4, pp. 382–389, 2012.

[269] A. Torkaman-Boutorabi, G. Ali Shahidi, S. Choopani, and M. Reza Zarrindast, “The catechol-O-methyltransferase and monoamine oxidase B polymorphisms and levodopa therapy in the Iranian patients with sporadic Parkinson’s disease,” Acta Neurobiologiae Experimentalis (Wars), vol. 72, no. 3, pp. 272–282, 2012.

[270] Y. Yin, Y. Liu, M. Xu, X. Zhang, and C. Li, “Association of COMT rs 4680 and MAO-B rs 1799836 polymorphisms with levodopa-induced dyskinesia in Parkinson’s disease—a meta-analysis,” Neurological Sciences, vol. 42, no. 10, pp. 4085–4094, 2021.

[271] E. Cereda, R. Cilia, M. Canesi et al., “Efficacy of rasagiline and selegiline in Parkinson’s disease: a head-to-head 3-year retrospective case-control study,” Journal of Neurology, vol. 264, no. 6, pp. 1254–1263, 2017.
[275] K. Radcliﬀe, G. Gille, and W. D. Rausch, "Short review on dopamine agonists: insight into clinical and research studies relevant to Parkinson’s disease," Pharmacological Reports, vol. 57, no. 6, pp. 701–712, 2005.

[276] S. N. Rai, P. Singh, R. Varshney et al., "Promising drug targets and associated therapeutic interventions in Parkinson’s disease," Neural Regeneration Research, vol. 16, no. 9, pp. 1730–1739, 2021.

[277] Q. J. Zhang, Y. Y. Zhang, and W. Y. Huang, "Traditional Chinese medicine in treatment of Parkinson’s disease," Zhong Xi Yi Jie He Xue Bao: Journal of Chinese Integrative Medicine, vol. 2, no. 1, pp. 75–77, 2004.

[278] J. L. Yang, J. S. Chen, Y. F. Yang et al., "Neuroprotection effects of retained acupuncture in neurotoxin-induced Parkinson’s disease mice," Brain, Behavior, and Immunity, vol. 25, no. 7, pp. 1452–1459, 2011.

[279] C. R. Houser, "Cholinergic synapses in the central nervous system: studies of the immunocytochemical localization of choline acetyltransferase," Journal of Electron Microscopy Technique, vol. 15, no. 1, pp. 2–19, 1990.

[280] R. T. Bartus, R. L. Dean III, B. Beer, and A. S. Lippa, "Nicotine-induced modulation of glutamate and GABA synaptic transmission in Parkinson’s disease mice," Brain, Behavior, and Immunity, vol. 25, no. 7, pp. 1654–1659, 2011.

[281] A. J. Ewins, "Acetylcholine, a new active principle of ergot," The Biochemical Journal, vol. 8, no. 1, pp. 44–49, 1914.

[282] T. H. Ferreire-Vieira, I. M. Guimaraes, F. R. Silva, and M. Ribeiro, "Alzheimer’s disease: targeting the cholinergic system," Current Neuropharmacology, vol. 14, no. 1, pp. 101–115, 2016.

[283] L. T. Potter, "Synthesis, storage and release of [14C] acetylcholine in isolated rat diaphragm muscles," The Journal of Physiology, vol. 206, no. 1, pp. 145–166, 1970.

[284] S. H. Zeisel, K. A. Da Costa, P. D. Franklin et al., "Choline, an essential nutrient for humans," The FASEB Journal, vol. 5, no. 7, pp. 2093–2098, 1991.

[285] S. F. Michardy, H. L. Wang, S. V. McCown, and M. C. Valdez, “Recent advances in acetylcholinesterase inhibitors and reactivators: an update on the patent literature (2012-2015)," Expert Opinion on Therapeutic Patents, vol. 27, no. 4, pp. 455–476, 2017.

[286] K. A. Radcliffe, J. L. Fisher, R. Gray, and J. A. Dani, "Nicotinic modulation of glutamate and GABA synaptic transmission of hippocampal neurones," Annals of the New York Academy of Sciences, vol. 868, pp. 591–610, 1999.

[287] X. Yang, H. E. Criswell, and G. R. Breese, "Nicotine-induced inhibition in medial septum involves activation of presynaptic nicotinic cholinergic receptors on gamma-aminobutyric acid-containing neurons," The Journal of Pharmacology and Experimental Therapeutics, vol. 276, no. 2, pp. 482–489, 1996.

[288] H. R. Arias, K. M. Targowska-Duda, J. García-Colunga, and M. O. Ortega, "Is the antidepressant activity of selective serotonin reuptake inhibitors mediated by nicotinic acetylcholine receptors?," Molecules, vol. 26, no. 8, 2021.

[289] M. J. O’Neill, T. K. Murray, V. Lakics, N. P. Visanji, and S. Duty, "The role of neuronal nicotinic acetylcholine receptors in acute and chronic neurodegeneration," Current Drug Targets, CNS and Neurological Disorders, vol. 1, no. 4, pp. 399–411, 2002.

[290] C. W. Luetje and J. Patrick, "Both alpha- and beta-subunits contribute to the agonist sensitivity of neuronal nicotinic acetylcholine receptors," The Journal of Neuroscience, vol. 11, no. 3, pp. 837–845, 1991.

[291] C. J. Lingle and J. H. Steinbach, "Neuromuscular blocking agents," International Anesthesiology Clinics, vol. 26, no. 4, pp. 288–301, 1988.

[292] P. A. Van Zwieten and H. N. Doods, "Muscarinic receptors and drugs in cardiovascular medicine," Cardiovascular Drugs and Therapy, vol. 9, no. 1, pp. 159–167, 1995.

[293] P. Wang, G. R. Luthin, and M. R. Ruggieri, "Muscarinic acetylcholine receptor subtypes mediating urinary bladder contractility and coupling to GTP binding proteins," The Journal of Pharmacology and Experimental Therapeutics, vol. 273, no. 2, pp. 959–966, 1995.

[294] E. Woldemussie, B. J. Feldmann, and J. Chen, "Characterization of muscarinic receptors in cultured human iris sphincter and ciliary smooth muscle cells," Experimental Eye Research, vol. 56, no. 4, pp. 385–392, 1993.

[295] G. Lauriti, V. Cascini, P. L. Chiesa, A. Pierro, and A. Zani, "Atropine treatment for hypertrophic pyloric stenosis: a systematic review and meta-analysis," European Journal of Pediatric Surgery, vol. 28, no. 5, pp. 393–399, 2018.

[296] R. T. Bartus, "On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis," Experimental Neurology, vol. 163, no. 2, pp. 495–529, 2000.

[297] V. Fodale, F. Magrì, V. Caminiti, and G. Grasso, "The cholinergic system in Down’s syndrome," Journal of Intellectual Disabilities, vol. 10, no. 3, pp. 261–274, 2006.

[298] E. Bukharoeva, V. Khuzakhmetova, S. Dmitrieva, and A. Tsentsentsievsky, "Adrenoceptors modulate cholinergic synaptic transmission at the neuromuscular junction," International Journal of Molecular Sciences, vol. 22, no. 9, 2021.

[299] J. C. Vickers, T. C. Dickson, P. A. Adlard, H. L. Saunders, C. E. King, and G. McCormack, "The cause of neuronal degeneration in Alzheimer’s disease," Progress in Neurobiology, vol. 60, no. 2, pp. 139–165, 2000.

[300] J. J. Ramos-Rodriguez, M. Pacheco-Herrero, D. Thyssen et al., "Rapid beta-amyloid deposition and cognitive impairment after cholinergic denervation in APP/PS1 mice," Journal of Neuropathology and Experimental Neurology, vol. 72, no. 4, pp. 272–285, 2013.

[301] S. Lovestone and R. Howard, “Alzheimer’s disease: a treatment in sight?,” Journal of Neurology, Neurosurgery, and Psychiatry, vol. 59, no. 6, pp. 566–567, 1995.

[302] F. Massoud and S. Gauthier, "Update on the pharmacological treatment of Alzheimer’s disease," Current Neuropharmacology, vol. 8, no. 1, pp. 69–80, 2010.

[303] J. Birks, "Cholinesterase inhibitors for Alzheimer’s disease," Cochrane Database of Systematic Reviews, vol. 1, article CD005593, 2006.

[304] P. T. Carroll, "Evidence to suggest that extracellular acetate is accumulated by rat hippocampal cholinergic nerve terminals for acetylcholine formation and release," Brain Research, vol. 753, no. 1, pp. 47–55, 1997.

[305] R. A. Hansen, G. Gartlehner, A. P. Webb, L. C. Morgan, C. G. Moore, and D. E. Jonas, "Efficacy and safety of donepezil, galantamine, and rivastigmine for the treatment of Alzheimer’s disease: a systematic review and meta-analysis," Clinical Interventions in Aging, vol. 3, no. 2, pp. 211–225, 2008.
