Section I

Chapter 1: Introduction

1. Introduction

Human central nervous system (CNS) is the most complex system in the body. A CNS disorder can affect either spinal cord (myelopathy) or brain (encephalopathy), both of which are part of the CNS. Neurodegenerative diseases, cancer and infections of the brain are becoming more prevalent in the society as the population gets older. These diseases are posing a major medical challenge in a very short span of time. Hence, neuroprotective therapeutics offer the potential to play an important role in managing the global burden of neurological disorders requiring a long term care.[1]

The nature of these brain disorders changes across different stages of human lifespan. The young ones display higher incidents of psychiatric disorders, including depression, anxiety, schizophrenia and substance abuse. Whereas, the elderly suffer mostly from neurodegenerative conditions such as dementia or stroke.[2] Many research studies claim that more number of elderly people suffer from neurodegenerative disorders, like Parkinson's disease (PD) and Alzheimer's disease (AD), which are proliferating in the ageing population.[3]

1.1 Neurodegenerative disorders

Neurodegeneration can be found in many different levels of neuronal circuitry, ranging from molecular to systemic. Neurons are metabolically active cells with high energy demands at locations distant from the cell body. Neurodegeneration is the umbrella term for the progressive loss of structure or functions of neurons, including their death. Many neurodegenerative diseases including Parkinson's, Alzheimer's, and Huntington's occur as a result of neurodegenerative processes. As research progresses, many similarities appear which relate these diseases to one another on a sub-cellular level. Discovering these similarities offer a hope for therapeutic advances for achieving improvement in many such diseased conditions simultaneously. The most supposed cause for different neurodegenerative disorders include atypical protein assembling as well as induced cell death.[4,5]

Many neurodegenerative diseases are caused by genetic mutations, most of which are located in completely unrelated genes. But a majority of them progress by intracellular mechanisms. Axonal swelling and spheroids have been observed in many different neurodegenerative diseases. This suggests that not only the defective axons are present in

diseased neurons, but they also may cause certain pathological insult due to accumulation of certain organelles. Parkinson's disease and Huntington's disease are both late-onset in nature and associated with the accumulation of intracellular toxic proteins. Diseases caused by the aggregation of proteins are known as proteinopathies, and they are primarily caused by aggregates in the following structues:

- cytosol, e.g. Parkinson's & Huntington's
- nucleus, e.g. Spinocerebellar ataxia type 1 (SCA1)
- endoplasmic reticulum (ER) (as seen with neuroserpin mutations that cause familial encephalopathy with neuroserpin inclusion bodies)
- extracellularly excreted proteins, amyloid-β in Alzheimer's disease

There are two main avenues that eukaryotic cells use to remove troublesome proteins or organelles:

(1) Ubiquitin–proteasome: The protein ubiquitin along with enzymes is the key for the degradation of many proteins that cause proteinopathies including polyQ expansions and alpha-synucleins. Research indicates that proteasome enzymes may not be able to correctly cleave these irregular proteins which could possibly result in more toxic species. This is the primary route that the cells use to degrade proteins.[4]

(2) Autophagy–lysosome pathways: Autophagy is a form of programmed cell death (PCD), and becomes the favorable route when a protein is aggregate-prone (poor substrate for proteasome). Autophagy can be split into two forms: **macroautophagy** and **chaperone-mediated autophagy (CMA)**.[4]

PCD is death of a cell in any form, mediated by an intracellular program.[6] There are, however, situations in which these mediated pathways get artificially stimulated due to injury or disease.[5] Current research, often in transgenic animal models, implicates both apoptotic and non-apoptotic pathways in neurodegeneration. Different diseases may make the cells enter these pathways at different points, but once triggered these pathways can lead to interdependent pathways of cell death.[5]

Generally, cell death in neurodegeneration is due to apoptosis and most commonly through the intrinsic mitochondrial pathway.[7] This pathway controls the activation of caspase-9 by regulating the release of cytochrome c from the mitochondrial intermembrane space (IMS). Reactive oxygen species (ROS) are normal byproducts of

mitochondrial respiratory chain activity. ROS concentration is mediated by mitochondrial antioxidants such as manganese superoxide dismutase (SOD2) and glutathione peroxidase. Overproduction of ROS (in oxidative stress) is a central feature of all neurodegenerative disorders. In addition to the generation of ROS, mitochondria are also involved with life-sustaining functions including calcium homeostasis, PCD, mitochondrial fission and fusion, maintaining lipid concentration of the mitochondrial membranes, and the mitochondrial permeability transition. A mitochondrial disease leading to neurodegeneration likely, at least on some level, involves all of these functions.[7] There is a strong evidence indicating that mitochondrial dysfunction and oxidative stress play causal roles in neurodegenerative disease pathogenesis, including four of the well known diseases Alzheimer's, Parkinson's, Huntington's, and Amyotrophic lateral sclerosis.[8]

1.2 Alzheimer's disease

Alzheimer's is a type of dementia that causes problems with memory, thinking and behavior. Symptoms usually develop slowly and get worse over a period of time, becoming severe enough to interfere with daily tasks. Alzheimer's is the most common form of dementia, a general term for memory loss that affects other intellectual abilities serious enough to disrupt daily routine. Alzheimer's disease accounts for 60 to 80 percent of dementia cases. Alzheimer's is not a normal part of aging, although the greatest known risk factor is advancing of age, and a majority of people with Alzheimer's are 65 yrs of age and older. But Alzheimer's is not just a disease of old age. Up to 5 percent of people with the disease have early onset Alzheimer's (also known as younger-onset), which often appears when someone is in their 40s or 50s.

Alzheimer's is a progressive disease, where dementia symptoms gradually worsen over the years. In its early stages, memory loss is mild, but with late-stage Alzheimer's, individuals lose the ability to carry on a conversation and fail to respond to their environment. Alzheimer's is the sixth leading cause of death in the United States. Those with Alzheimer's live an average of eight years after their symptoms become noticeable to others, but survival can range from four to 20 years, depending on the age and other health conditions of the person.

Although current Alzheimer's treatments cannot stop Alzheimer's from progressing, they can temporarily slow the worsening of dementia symptoms and improve

the quality of life for the patients and their caregivers. Today, worldwide efforts are under way to find better ways to treat the disease, delay its onset, and prevent it from developing.

The most common early symptom of Alzheimer's is difficulty in remembering newly learned information. Just like the rest of our body, our brain changes as we age. Most of the people eventually notice some slowed thinking and occasional problems with remembering certain things with age. But, serious memory loss, confusion and other major changes in the way our minds work may be a sign that the brain cells are failing.

The most common early symptom of Alzheimer's is difficulty in remembering newly learnt information because Alzheimer's changes typically begin in the part of the brain that affects learning. As Alzheimer's advances through the brain it leads to increasingly severe symptoms, including disorientation, mood and behavior changes; deepening confusion about events, time and place; unfounded suspicions about family, friends and professional caregivers; more serious memory loss and behavior changes and difficulty in speaking, swallowing and walking. People with memory loss or other possible signs of Alzheimer’s may find it hard to recognize that they have a problem. Signs of dementia may be more obvious to family members or friends. Anyone experiencing dementia-like symptoms should see a doctor as soon as possible. Early diagnosis and intervention methods are improving dramatically, and treatment options and sources of support can improve the quality of life of the suffering person. Microscopic changes in the brain begin long before the first signs of memory loss.

The brain has 100 billion nerve cells (neurons). Each nerve cell connects with many others to form communication networks. Groups of nerve cells have special jobs. Some are involved in thinking, learning and remembering. Others help us see, hear and smell. To do their work, brain cells operate like tiny factories. They receive supplies, generate energy, construct equipment and get rid of waste. Cells also process and store information and communicate with other cells. Keeping everything running requires coordination as well as large amounts of fuel and oxygen.

Scientists believe that Alzheimer's disease prevents parts of a cell's factory from running well. They are not sure about the source from where the trouble starts. But just like a real factory, breakups and breakdowns in one system cause problems in other areas. As damage spreads, cells lose their ability to do their jobs and eventually die, causing irreversible changes in the brain.

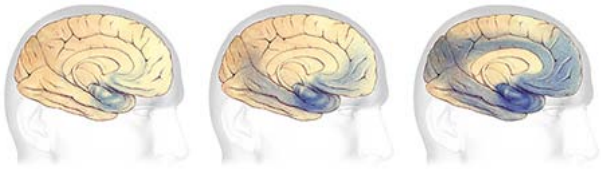

Fig. 1.1: Progressive illustrations of brain degeneration caused by Alzheimer's disease (Plaques and tangles shown in the blue-shaded areas tend to spread through the cortex in a predictable pattern as Alzheimer's disease progresses)

Two abnormal structures called plaques and tangles are prime suspects in damaging and killing nerve cells. Plaques and tangles tend to spread through the cortex as the Alzheimer's progresses (Fig. 1.1). Plaques are deposits of a protein fragment called beta-amyloid that builds up in the spaces between nerve cells. Tangles are twisted fibers of another protein called tau that builds up inside the cells.

Though most people develop some plaques and tangles as they age, those with Alzheimer's tend to develop far more. They also tend to develop them in a predictable pattern, beginning in areas important for memory before spreading to other regions. The exact role of plaques and tangles in Alzheimer's disease is still not certain. Most experts believe they somehow play a critical role in blocking communication among the nerve cells disrupting the processes that the cells need to survive. It's the destruction and death of nerve cells that causes memory failure, personality changes, problems carrying out daily activities and other symptoms of Alzheimer's disease.

Researchers are working to uncover as many aspects of Alzheimer's disease and related dementias as possible. Ninety percent of what we know about Alzheimer's has been discovered in the last 15 years only. Some of the most remarkable progress has shed light on how Alzheimer's affects the brain. The hope is that a better understanding will lead to new treatments. Many potential approaches are currently under investigation worldwide for the treatment of the disease.

1.3 Therapeutic targets

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder with two hallmarks: β-amyloid plaques and neurofibrillary tangles. No effective drugs and therapies have been developed, while mechanism-based explorations of therapeutic approaches have been intensively investigated. Outcomes of clinical trials suggested several pitfalls in the choice of biomarkers, development of drug candidates, and interaction of drug-target

molecules; however, they also aroused concerns on the potential deficiency in our understanding of pathogenesis of AD, and ultimately stimulated the advent of tests for novel drug targets. The increase in the number of AD patients in few decades makes development of better therapy an urgent issue.

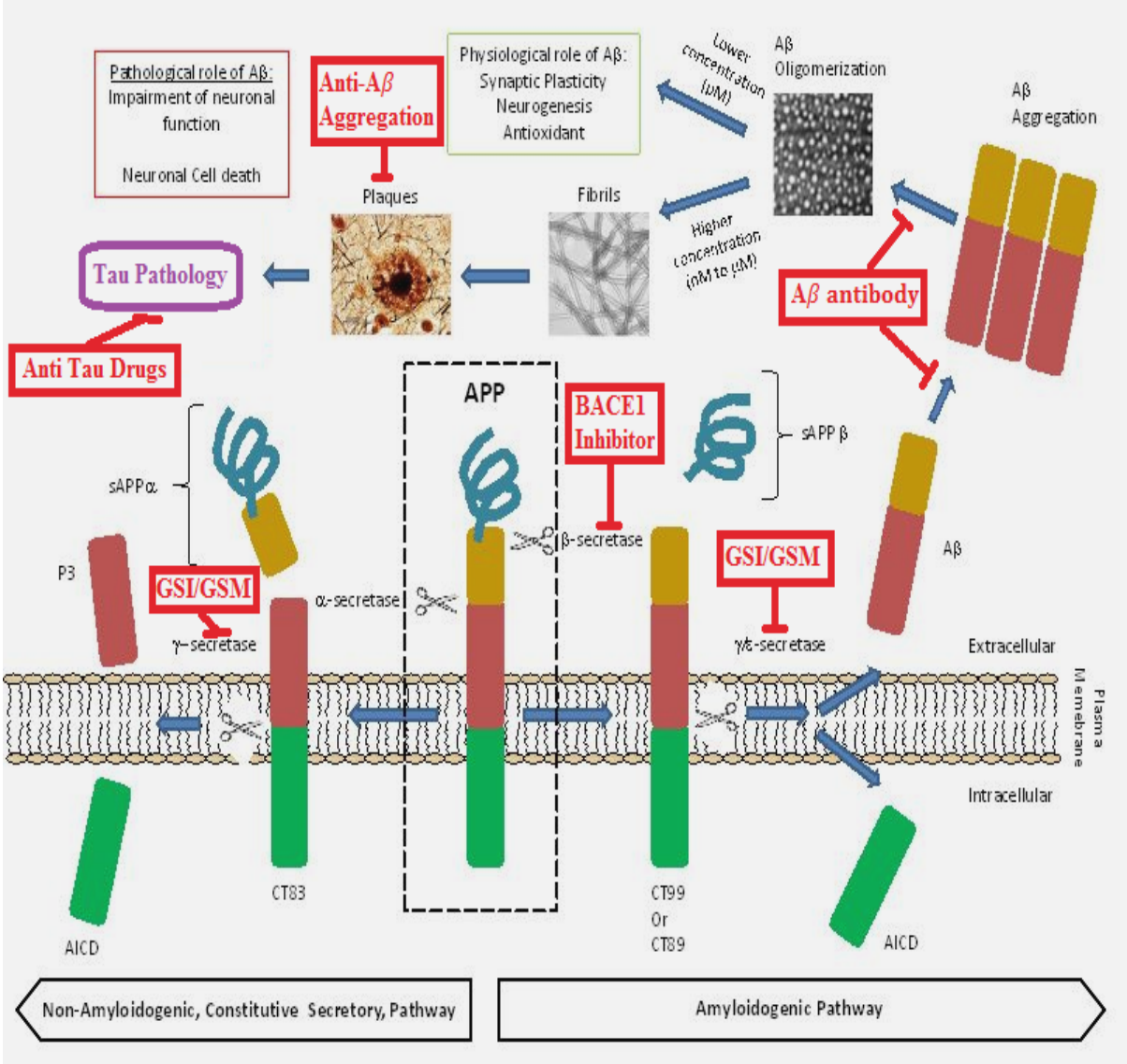

Fig. 1.2: Therapeutic targets for Alzheimer's disease

Currently, Food and Drug Administration (FDA) approved AD drugs are still limited within two categories: cholinesterase inhibitors and memantine (an NMDA receptor antagonist).[9-11] Unfortunately, the effects and benefits of these drugs are marginal and they only alleviate the symptoms.[12-14] However, in recent years, fundamental research focusing on the pathogenesis of AD paved the way for the development of new treatments targeting the radical source of Alzheimer's disease.[15] Numerous trials have been

conducted and currently a number of compounds in different stages of development are in line for the treatment of AD.

1.3.1 Therapeutic Targets Focusing on Aβ Cascade Hypothesis

Inhibition of Aβ Production

Amyloid precursor protein (APP) experiences sequential cleavage by β-secretase and γ-secretase giving rise to the culprit of dementia, β amyloid (Aβ) which is thought to initiate formation of soluble oligomers, insoluble fibrils, and accumulated plaques (Fig. 1.2). APP can be alternatively processed by α-secretase within the Aβ region and generates a longer C-terminal fragment under the first cleavage. To inhibit the production of Aβ, the three crucial APP processing enzymes become therapeutic targets in the development of new drugs i.e. inhibition of β- or γ-secretase or promoting α-secretase activity.

β-Secretase (BACE1) Inhibitors: Beta-site APP-cleaving enzyme 1 (BACE1) is a protease responsible for the initial cleavage of APP, initiating the production of neurotoxic suspect Aβ.[16,17] Mounting evidence corroborates the positive impact of BACE1 inhibition. BACE1 knock-out mice indicated a close correlation between the BACE1 inhibition and the Aβ decline.[18,19] It is reported that BACE1 inhibition improved memory deficits[20] and rescued Aβ-driven cholinergic dysfunction[21] in APP transgenic mice. Although the BACE1-deficient animal model presented a relatively benign phenotype with high viability, suggesting that the possibility of targeting β-secretase would be a safe therapeutic approach. Further testing indicated that the drastic inhibition would result in hypomyelination and behavioural abnormalities such as seizures.[22-25]

γ-Secretase Inhibitors (GSI) and Modulators (GSM): γ-Secretase is a transmembrane protease responsible for the eventual cleavage of amyloid precursor protein (APP) to generate Aβ (Fig. 1.2). Thus it is considered as a principal therapeutic target in Alzheimer's disease.[26,27] This enzyme complex consists of four components: Aph1, Pen2, glycosylated nicastrin, and endoproteolyzed presenilin as the catalytic core,[28] and it is involved in myriads of physiological processes.

α-Secretase Activation: APP can be cleaved by an alternative α-secretase rather than β-secretase in the first step to circumvent the generation of pathological Aβ peptide. Hence,

increasing the chance of α-cleavage could be an effective approach to decrease the Aβ formation and promote soluble APP production to protect neurons.[29]

Anti-β-Amyloid Aggregation

The pathological Aβ peptides, prone to assembly into aggregates as neuro-/synaptic toxic products spurred the idea of inhibition of Aβ aggregation or destabilization of the Aβ oligomer species. However, Aβ aggregations are characterized with a high stability resistance to disaggregation[30] that remain insoluble even with heat or SDS (sodium dodecyl sulfate).[31] The fact that amyloid fibrils have an extremely low energy state[30] and the lack of thorough understanding of Aβ aggregation process have complicated the issue. Besides, another challenge would be to access the compounds with high CNS bioavailability and low immunogenicity and toxicity. It is generally believed that there are three strategies that block Aβ aggregation: antiaggregate compounds, metal complexing agents and immunization.

Nonpeptidic Antiaggregates: The second generation of nonpeptidic antiaggregates were expected to cover drawbacks of the older nonpeptidic antiaggregates, tramiprosate regarding the safety, tolerance, penetration and the weak potency. Scyllo-inositol is thought to effectively impede Aβ aggregation, promote misfolding modulation, and accelerate aggregates' disassociation.[32]

Metal Complexing Agents: After Aβ peptides were produced and released into extracellular fluids, metals like Zn and Cu can motivate oligomerization into fibrils.[33] So metal chelators or metal complexing agents that can interfere with reaction of metal ions with Aβ are likely to be potential therapeutics.

Active Immunization: It is conventionally thought that clearance of CNS Aβ requires a BBB permeability property, confining the therapeutic targets in a very narrow realm: medicinal chemistry-driven and small molecules. Nonetheless, incredible work done by Schenk *et al*. revealed that immunization of PDAPP transgenic mice markedly mitigated amyloid plaque burden, improved neuritic dystrophy, and even reduced existing Aβ plaques.[34]

Passive Immunization: Another strategy to avoid immune response is direct administration of antibodies. This passive immunization has an approximate potency to remove amyloid

plaques and rescue neuritic and glial pathology,[35] reduce early tau hyperphosphorylation[36] and cytopathology,[37] and reverse abnormal hippocampus synaptic plasticity.[38]

1.3.2 Therapeutic Targets Focusing on Tau Hypothesis

According to Aβ hypothesis, intracellular neurofibrillary tangles (NFTs) induced by altered phosphatase/kinase activity is a downstream event of aggregation of β-amyloid (Fig. 1.2), and NFTs as catalyst will aggravate the oxidation and further result in neuronal dysfunction, cell death, and transmitter deficits. Tau is normally a highly soluble protein in cytoplasm binding to microtubules as a stabilizer. Formation of NFTs as a result of hyperphosphorylated and misfolded tau protein aggregation is toxic to neurons. The pathological tau proteins lose the capability to aid microtubules in transporting neuronal substances, leading to neuronal dysfunction and apoptosis.[39,40]

Inhibition of Tau Aggregation

Protein kinase, a group of critical enzymes responsible for tau overphosphorylation, is a prerequisite for the tau-induced toxicity. The first class of tau inhibitors aims to modulate tau phosphorylation via decreasing the activity of related kinases since imbalanced interaction between glycogen synthase kinase 3 beta (GSK3β) and protein phosphate 2 (PP2A) enhances tau hyperphosphorylation and NFT formation.[41] Another scenario to interfere with tau-induced NFT is to inhibit tau aggregation or promote tau assembly disassociation. Rember (methylene blue) is such a tau antiaggregant.[42] Preclinical data revealed a learning deficit reversing property, and a completed phase 2 trial proved that this agent can slow down AD progression with a good bioavailability.[43,44]

1.3.3 Therapeutic Targets Focusing on Glutamatergic Hypothesis

Neurotransmitters' depletion (basically referring to acetylcholine, ACh) and synaptic dysfunction are two classical features of AD.[45] Thus, two hypotheses have been established−cholinergic hypothesis[46] and glutamatergic hypothesis,[47] based on which FDA approved therapies- AchE inhibitors and NMDA receptor antagonists were developed to mitigate AD symptoms.

(*S*)-Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS). It operates through ionotropic (iGlu) and metabotropic glutamate receptors

(mGlu).[48] *N*-Methyl-*D*-aspartate (NMDA), kainate and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors represent the three major subtypes of ionotropic glutamate receptors found in the mammalian central nervous system.[49] NMDA receptor plays a central role in physiological processes, such as neuronal development, synaptic plasticity, learning, and memory. Overactivation of the NMDA receptor caused by excessive glutamate release leads to an unphysiologically high intracellular Ca^{2+} concentration, which results in uncontrolled activation of various Ca^{2+}-dependent enzymes, neuronal damage and cell death at the end. This process of excitotoxicity occurs during many acute adverse events (e.g., traumatic brain injury and stroke) and chronic neurodegenerative disorders (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease). Due to its central role in these processes the NMDA receptor represents an interesting target for the development of novel drugs.[50-52]

1.3.4 Therapeutic Targets Focusing on Cholinergic Hypothesis

Cholinergic neurons' impairment accompanies the early progression of dementia. In animal and human studies, administration of cholinesterase inhibitors stimulated memory and learning process.[53] Besides, a marked correlation between loss of cholinergic neurons and deterioration of defected memory was proved in animal models later.[54,55] Therefore, improvement of cholinergic system, including potentiating effects of acetylcholine (Ach) and inhibiting activity of cholinesterase is a potential therapeutic goal.

Ach is a ligand for nicotine receptors and exerts an excitatory effect on the postsynaptic neurons, an essential event for long-term potentiation (LTP) and memory formation. Several nicotinic receptor agonists to reinforce this event are being tested in clinical trials. EVP-6124, a selective agonist of the α-7 nicotinic acetylcholine receptor, has finished a phase 1/2 trial showing safe and well tolerated results and entered into phase 3 trials in 2013 to test the cognitive benefits. Quite a few other clinical trials involving nicotinic agonists are ongoing (ladostigil hemitartrate, phase 2; ispronicline, phase 1), completed (RO5313534), or terminated (ABT-089).

A transmitter that indirectly modulates neuron degeneration and memory deficits is serotonin (5-HT). Growing evidence indicated that inhibition of 5-HT_6 could facilitate Ach release and elevated cholinergic transmission whereby memory and learning defects were likely to be ameliorated. 5-HT_6 antagonists were widely reported in many studies to rescue anticholinergic drugs-induced amnesia.[56]

1.3.5 Potential Findings of Therapeutics for Alzheimer's Disease from Other Perspectives

In addition to the two hallmarks and neurotransmitter system impairment, there are several other features found in Alzheimer's disease, including inflammation, oxidative stress, mitochondrial dysfunction, neurotrophin deficiency, and so forth. These aspects are not systematically and thoroughly summarized and are likely to be neglected though; they do provide new perspectives in developing AD treatments.

Anti-Inflammation and Antioxidants: Chronic inflammation is an essential feature of AD and contributes to its pathogenesis in numerous ways. Microglia are brain's resident macrophages that monitor brain activity and play a contributing role in removal of redundant and apoptotic neurons,[57,58] remodeling of normal synapse,[59] and protection of CNS from pathogens and detritus.[60] However, they can shift to another phenotype to secrete series of inflammatory factors, exerting detrimental effects on bystander neurons and processes they are involved in. Aggregated Aβ appears to be a robust agent driving this alteration, since markers of activated microglia were densely colocalized within the deposits.[61,62] Microglia seem incapable of degrading Aβ that they intake,[63,64] leading to a frustrated phagocytosis instead. As clinical trials have been a major disappointment, agents that drive microglia to a phenotype that favors attack on pathogens rather than bystander neurons may hold therapeutic potential.

Mitochondrial Dysfunction: Mitochondrial dysfunction in early AD enhances synaptic damages and neuron apoptosis, so it is considered a causal factor of neurodegeneration.[65] APP and Aβ are transported into mitochondrion reacting with mitochondrial components, leading to an impaired ATP processing and increased oxidative stress level.[65,66] ApoE4, a risk factor for sporadic AD, harms mitochondrial trafficking and function and promotes mitochondrial apoptosis.[67,68] Replacing mitochondrial DNA (mtDNA) from one cell line with mtDNA from AD patients supported a mitochondrion cascade hypothesis,[69] offering new therapeutic targets.

ApoE (Apolipoprotein) and Aβ Export: ApoE is a powerful genetic factor[70,71] for sporadic AD beyond APP, PS1, and PS2 genes. The isoform ApoE4 substantially promotes the risk of AD and decreases the age of onset.[72] ApoE is generally thought to regulate Aβ clearance and thus influences fibrillogenesis. In CNS, ApoE, responsible for transportation of cholesterol to neurons, is primarily produced in astrocytes.[73] Aβ aggregation and

clearance are differently affected in an isoform (ε2, ε3, and ε4) dependent manner; frequency of AD and mean age at clinical onset are 91% and 68 years of age in ε4 homozygote, 47% and 76 years of age in ε4 heterozygote, and 20% and 84 years in ε4 noncarriers.[72-74] ApoE was found colocalized with amyloid plaques[75] and this coexistence is more abundant in ApoE4 carriers.[76] Additionally, ApoE4 is associated with cognition decline before appearance of clinically apparent syndromes.[77,78] ApoE4, as described previously, can work synergically with other risk factors, like insulin resistance and peripheral vascular diseases,[79,80] thus exerting a confounding effect on AD and triggering inflammatory cascade.

Neurotrophin: Nerve growth factor (NGF) as a neurotrophin plays a critical role promoting survival and maintaining the function of cholinergic neurons.[81,82] In AD patients, transcription and translation levels of NGF were changed,[83,84] suggesting that NGF supplementation probably is a treatment approach for Alzheimer's disease.

1.4 Excitotoxicity and NMDA receptor

Glutamate is a single powerful excitatory neurotransmitter that is responsible for rapidly conveying sensory information and complex motor commands from one part of the body to another, and to form thoughts and memories. There are other excitatory neurotransmitters in the brain, but glutamate is the most common and widely distributed one. The concentration of glutamate in most neurons (and also glia) gets high (~10 mM) after sequestration inside synaptic vesicles. Glutamate is released in milliseconds to communicate with other neurons via synaptic endings. Because glutamate is so powerful, its presence in excessive amounts or for excessive periods of time can literally excite cells to death. This phenomenon was first documented when Lucas and Newhouse observed that subcutaneously injected glutamate selectively damaged the inner layer of the retina (representing primarily the retinal ganglion cells).[85] Later on the term "excitotoxicity" was coined to describe this phenomenon by John Olney.[86]

Excessive release of glutamate is responsible for excitotoxic cell death within the nervous system. When the nervous system suffers a severe mechanical insult, as in head or spinal cord injury, large amounts of glutamate are released from injured cells to wash over thousands of nearby cells that had survived the original trauma, causing them to depolarize, swell, lyse, and die by necrosis. The lysed cells release more glutamate, leading to a cascade of autodestructive events and progressive cell death which continue

for hours or even days. In stroke also; the ischemic event deprives many neurons of the energy they need to maintain ionic homeostasis, causing them to depolarize, lyse, die, and propagate the same type of autodestructive events that are seen in traumatic injury.[87,88]

A slower form of excitotoxicity is implicated in a variety of neurodegenerative disorders as well as in stroke damage.[89] In disorders such as Huntington's disease, Parkinson's disease, Alzheimer's disease, multiple sclerosis, HIV-associated dementia, amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease), and glaucoma, it is hypothesized that chronic exposure to moderately elevated glutamate concentrations or glutamate receptor hyperactivity for longer periods of time than that occurs during normal neurotransmission, trigger cellular processes in neurons, eventually leading to apoptotic-like cell death, a form of cell death related to the programmed cell death that occurs during normal development.[90-96]

Elevations in extracellular glutamate are not necessary to invoke an excitotoxic mechanism as excitotoxicity can occur even with normal levels of glutamate if NMDA receptor activity is increased, e.g., when neurons are injured and thus get depolarized (more positively charged). In this condition there is a normal block of the ion channel by Mg^{2+} which abnormally increases NMDA receptor activity.[97] Increased activity of the enzyme nitric oxide synthase (NOS) is also associated with excitotoxic cell death which is physically tethered to the NMDA receptor and activated by Ca^{2+} influx via the receptor-associated ion channel. Increased levels of nitric oxide (NO) have been detected in animal models of stroke and neurodegenerative diseases.

1.5 Pathophysiology of excitotoxicity

Excessive stimulation of the NMDA subtype of glutamate receptors is responsible for apoptotic-like excitotoxicity which afterward leads to cell death (Fig. 1.3). When activated, the NMDA receptor opens a channel that allows Ca^{2+} (and other cations) to move into the cell as this activity is important for long-term potentiation (LTP), learning and memory formation. Under normal conditions of synaptic transmission, the NMDA receptor channel is blocked by Mg^{2+} ion resting in the channel and it gets activated only for brief periods of time. Under pathological conditions, overactivation of the receptor causes an excessive amount of Ca^{2+} influx into the nerve cell, which then triggers a variety of processes that can lead to apoptosis.[98-105]

NMDA is an important endogenous substance required for learning and memory functions. NMDA receptors consist of two major subunits (NR_1 and NR_{2A-D}) and (NR_{3A} or NR_{3B}) as minor subunits. NMDA receptor consists of binding sites for glutamate, the endogenous agonist, and glycine, which is required as a co-agonist for receptor activation[106] (Fig. 1.4). When glutamate and glycine bind to their respective sites and the cell is depolarized to remove Mg^{2+} block, the NMDA receptor channel opens with consequent influx of Ca^{2+} and Na^{+} into the cell. The two modulatory sites are the magnesium (Mg^{2+}) site within the ion channel and an S-nitrosylation site located toward the N terminus (and hence extracellular region) of the receptor. S-Nitrosylation reactions represent transfer of NO to a thiol or sulfhydryl group (-SH) of a critical cysteine residue to modulate protein function.

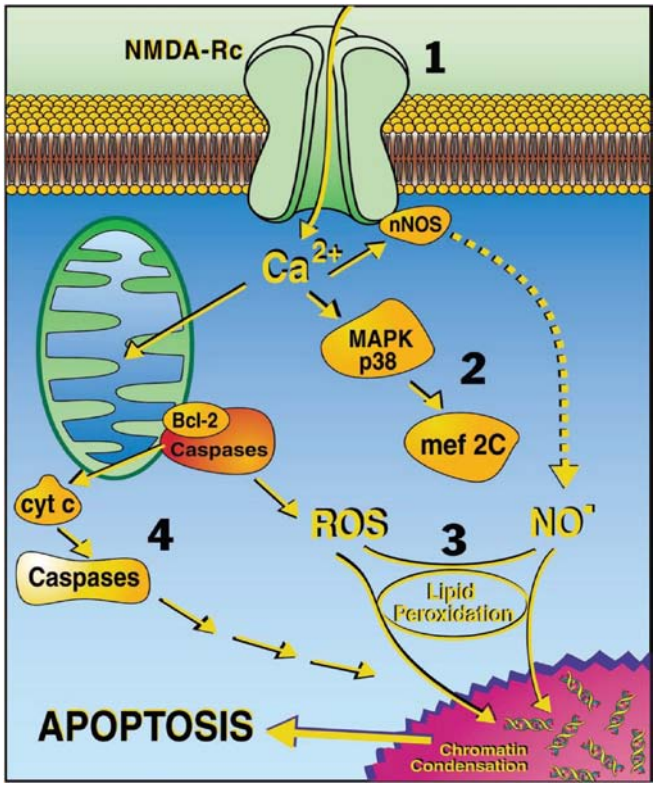

Fig. 1.3: Schematic illustration of apoptotic pathways triggered by excessive NMDA receptor activity. Step 1: NMDA receptor hyperactivation, Step 2: activation of the p38 MAPK-MEF2C (transcription factor) pathway (MEF2 is subsequently cleaved by caspases to form an endogenous dominant-interfering form that contributes to neuronal cell death), Step 3: toxic effects of free radicals such as NO and reactive oxygen species (ROS), Step 4: activation of apoptosis-inducing enzymes including caspases

Moreover, other modulatory sites also exist on the NMDA receptors and hence, each of these sites can be considered as targets for therapeutic intervention to block

excitotoxicity. These include binding sites for Zn^{2+}, polyamines, a *p*H-sensitive site (e.g., proton)and the binding site of the drug ifenprodil (the endogenous ligand remains unknown).[107,108] Clinically acceptable therapy must have blocking effects on excessive activation of the NMDA receptor while sparing the normal functions so that the side effects can be avoided. Drugs that simply compete with glutamate or glycine at the agonist binding sites block normal function and therefore do not meet this requirement, and thus have failed in clinical trials to date because of side effects (drowsiness, hallucinations, and even coma).[109-115]

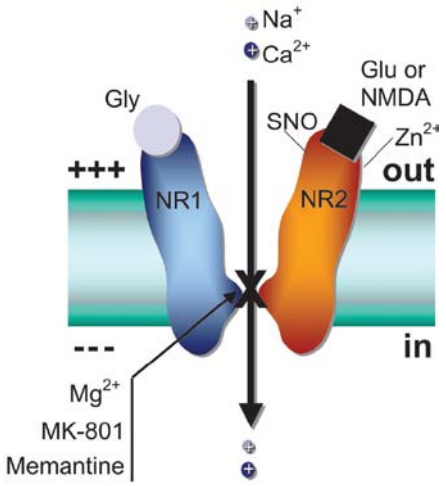

Fig. 1.4: NMDA receptor model illustrating important binding and modulatory sites.

Competitive antagonists for glutamate or glycine site will block healthy areas of the brain before they can affect pathological areas. A blocker that acts at the Mg^{2+} site within the channel does not effectively block excessive Ca^{2+} influx to the degree needed to prevent neurotoxicity and if it is strong enough then it hampers normal excitation hence clinically unacceptable. This is the case with MK-801; it is a very good excitotoxicity blocker, but because "dwell time" in the ion channel is so long due to high affinity for the Mg^{2+} site that MK-801 accumulates in the channels and therefore blocks critical normal functions. A human taking a neuroprotective dose of MK-801 not only becomes drowsy, but lapses into coma. A clinically tolerated NMDA receptor antagonist should not make a patient drowsy, hallucinate, or comatose, and in fact should spare normal neurotransmission while blocking the ravages of excessive NMDA receptor activation. In fact, one type of drug that could do this and block preferentially higher (pathological) levels of glutamate over normal (physiological) levels is an "uncompetitive" antagonist. An uncompetitive antagonist is distinct from a noncompetitive antagonist as it is an

inhibitor whose action depends upon prior activation of the receptor by the agonist. Hence, the same amount of antagonist blocks the higher concentrations of the agonist better than the lower concentrations of the agonist. This uncompetitive mechanism of action could yield a drug that blocks NMDA receptor-operated channels only when they are excessively open while relatively sparing the normal neurotransmission. Evidence suggests that memantine is such a drug which resembles the structure of benzazepine (cyclic amine). Hence, there is a need for the discovery of newer entities with good kinetics of the drug in the NMDA receptor-associated ion channel.[116-118]

It was found that the dwell time in the channel is the major determinant of clinical tolerability of an open-channel blocker because excessive dwell time causes the drug to accumulate in the channels, interfere with normal neurotransmission and produce unacceptable adverse effects (as is the case with MK-801). In contrast, too short a dwell time yields a relatively ineffectual blockade, especially with membrane depolarization, which relieves the block of positively charged molecules (as seen with Mg^{2+}).[116] The memantine class of drugs represents a relatively low-affinity, open-channel blocker, i.e., these drugs only enter the channel when it is opened by agonist. In case of memantine, at concentrations administered to patients the drug appears to enter the channel preferentially when it is (pathologically) activated for long periods of time, i.e., under conditions of excessive glutamate exposure. Memantine was found to have favourable kinetics in the channel to provide neuroprotection while displaying minimal adverse effects (occasional restlessness or, in rare cases, slight dizziness at higher dosages).[109,110,116]