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Basics of Neurotoxin Induced Animal Models for Comprehending Neurodegenerative Diseases Embracing Alzheimer's Disease and Parkinson's Disease

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Abstract: With the aging of the global population in the decades ahead, neurodegenerative diseases such as Parkinson's (PD) and Alzheimer's diseases (AD) are expected to afflict larger numbers of people worldwide. One of the main causes of neurodegenerative illnesses is neurotoxins. The identification of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as a potential contributor to Parkinson's disease symptoms in the 1980s prompted additional studies on neurotoxins. Neurotoxins can cause harm to the nervous system by upregulating proteins linked to cell death, increasing oxidative stress, decreasing mitochondrial activity, and causing neuroinflammation. Animal models have aided in gaining more insight into the pathological processes underlying neurodegenerative diseases. These models replicate several features of a particular disease, together with the primary symptoms and histological abnormalities. To create disease-modifying medications that can stop the onset of these disorders or at least reduce their course, it is essential to have a precise understanding of the etiopathogenic mechanisms underlying them. By consolidating information on diverse neurotoxic animal models and emphasizing the pathogenic pathways associated with these neurotoxins, this review focus to assist researchers in making informed choices and propelling the progress of PD and AD modelling.

Keywords: Animal models, Murine, Neurodegenerative, Neurotoxins, Parkinson's disease, Alzheimer's disease

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Introduction

Neurodegeneration involves the deterioration of a neuron's structure and function, encompassing conditions like Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease

(HD), Lewy body disease, and amyotrophic lateral sclerosis (ALS) (Nabi and Tabassum, 2022). These disorders are marked by protein buildup, synaptic loss, neuronal malfunction, mitochondrial dysfunction, and cell death (Memon *et al.*, 2020). While the world's demographic aging, there is a projected upsurge in diagnosis of neurodegenerative conditions. The development of these disorders involves genetics and exposure to both endogenous and exogenous neurotoxins (Cao *et al.*, 2021). Neurotransmitter transmissions can be disrupted by internal neurotoxins, causing the inhibition of ion channels such as potassium (K^+), calcium (Ca^{2+}), and sodium (Na^+), as well as neurotransmitter receptors like acetylcholine receptors, as well as the inhibition of enzymatic activity (such as tyrosine hydroxylase (TH)). Most of endogenous neurotoxin's methods involve suppressing mitochondrial function, raising levels of oxidative damage and neurological inflammation, and raising apoptotic proteins (Silva *et al.*, 2006). According to research by Shaw's group (Shaw and Höglinger, 2008), there is a suggested link between neurotoxins and the development of neurodegenerative disorders. For instance, the Guadeloupe population, who consume a hazardous chemical called "annonaceae", show an increased susceptibility to developing atypical Parkinsonism. Similarly, on Guam Island in the Western Pacific, where people frequently consume flour containing water-soluble neurotoxic agents, there is a prevalence of ALS-Parkinsonism Cognitive Comple. There is also consideration of an endogenous plausible neurotoxic component linked to Alzheimer's disease i.e. ammonia (Kosenko *et al.*, 2014).

Exposure to neurotoxicants like heavy metals, biotoxins, microbiological neurotoxins, and chemical toxins can increase the production of Amyloid-β (Aβ) peptide and hyperphosphorylation of tau (τ), leading to neuronal death (Lee *et al.*, 2019). Neurotoxicants have been shown to enhance Aβ levels and genes associated with Aβ synthesis, such as the PSEN1 gene (Huang *et al.*, 2017). These pathways are closely linked to abnormal protein accumulation, altered neuronal metabolism, behavioural deficits, disrupted calcium signalling, and impaired mitochondrial function (Pereira *et al.*, 2005). Additionally, neurotoxicants can trigger excessive microglial activation, leading to the overproduction of pro-inflammatory cytokines, reduced immune function, and neuroinflammation, all contributing to Alzheimer's disease (Harry and Kraft, 2008). Pesticide exposure has been significantly associated with tremors in Parkinson's disease, with specific pesticide combinations like PQ and Ziram linked to a higher risk of PD development. It is unclear whether a single factor or a combination of these elements accelerates the neurodegenerative process as illustrated in Figure 1 (Carrillo *et al.*, 2014). Understanding the role of neurotoxins in neurological conditions is crucial for further research and intervention.

The research has focused on studying the impact of neurotoxic agents on Parkinson's disease (PD) and Alzheimer's disease (AD), exploring how these chemicals disrupt metabolic processes and signalling networks, leading to cellular and molecular damage. Animal models have been utilized to better understand how neurotoxins contribute to brain damage in the onset of AD and PD (Nisa *et al.*, 2021). Creating animal models that mimic PD is crucial for testing new treatments and neuroprotective strategies (Tieu, 2011). PD is characterized by the loss of dopaminergic neurons in the substantia nigra, reduced dopaminergic innervation of the striatum, and decreased dopamine levels. Neurotoxic models in animals replicate damage to the nigrostriatal pathway caused by various substances. Similarly, neurotoxic animal models have been essential in studying AD, providing insights into the impact of neurotoxicity on its progression. Using appropriate animal models is vital for developing innovative treatments for AD (Song, 2018). This review will focus on murine models that exhibit neurotoxicity-induced neurodegenerative disorders.

Murine models used to study Parkinson's Disease induced by neurotoxins:

(i) *Effect of MPTP neurotoxin exposure on animals in inducing PD*:

During the 1980s, numerous drug users taking it via intravenous method from California were hospitalized with drasatic symptoms resembling Parkinson's disease (PD) (Davis *et al.*, 1979). These cases helped uncover the link to 1-methyl-4- phenyl- 1, 2, 3, 6-tetrahydropyridine (MPTP), which was found as a contaminant in synthetically produced meperidine taken by these individuals. Additional studies revealed that these individuals had self-administered MPTP- contaminated synthetic meperidine (Langston *et al.*, 1983). The challenges with motor functions experienced by individuals with these conditions' patients exhibited positive responses to L-dopa treatment, a key PD treatment, indicating that their underlying neuropathological and metabolic characteristics are similar with those of PD patients. Nigrostriatal structures were lost in these patients, according to postmortem studies (Davis *et al.*, 1979; Langston *et al.*, 1983). Deep brain stimulation therapy recently demonstrated a considerable clinical improvement in one of the survivors, further demonstrating that MPTP-induced basal ganglia impairment is as same as to that seen in PD in human. Pinpointing potential causal factors and elucidating mechanisms of cells dying in Parkinson's disease have proven to be significantly influenced by the discovery of MPTP. Research stemming from this model has generated theories highlighting mitochondrial dysfunction as an alternative pathogenic mechanism and neurotoxic substances as potential contributors to sporadic Parkinson's disease. Additionally, the MPTP model of Parkinson's disease has contributed to the development of some current therapies for the disease (Fox and Brotchie, 2010).

It has been extensively investigated and characterised how MPTP poisoning works (Dauer and Przedborski, 2003; Rappold and Tieu, 2010). Being lipophilic in nature, MPTP can easily pass the BBB. Monoamine oxidase-B is responsible for the breakdown of MPTP within astrocytes. Subsequently, MPTP is converted into the harmful cation known as 1-methyl-4-phenylpyridinium (MPP). MPP is released from astrocytes in the striatum and substantia nigra through organic cation transporter 3 into the extracellular space (Cui *et al.*, 2009). Dopaminergic neurons and terminals then selectively take up MPP via the dopamine transporter. Inside dopaminergic neurons, MPP accumulates and induces neurotoxicity primarily by inhibiting complex I of the mitochondrial electron transport chain. This inhibition leads to ATP depletion and increases oxidative stress, as depicted in Figure 2 (Nicklas *et al.*, 1985). Multiple mammalian species, such as, cats, dogs, guinea pigs, mice, monkey, rats, and sheep have been used to model Parkinson's disease (PD) experimentally through administration of the neurotoxin MPTP. Among these models, the MPTP-induced monkey model is the favoured approach for evaluating potential treatments for Parkinson's disease in preclinical studies. The mouse, however, continues to be a popular choice for many researchers because the monkey model lack resources and lacking in skilled staff. Additionally, researchers can evaluate the effects of specific genetic alterations in responses to MPTP neurotoxicity because of the availability of genetic mouse models. However, more studies should verify the validity of this therapy strategy to ensure its dependability. The motor dysfunctions brought on by the MPTP have been well studied in both mice and monkeys from a behavioural perspective. The drug L-dopa or an agonist of dopamine can reverse these peculiar behaviours, highlighting the connection between these signs and nigrostriatal system impairment (Ogawa *et al.*, 1985, Rozas *et al.*, 1998). MPTP has been shown to alter colon motility in mice and to decrease dopaminergic neurons in the intestinal nervous system, in addition to having an impact on the nigrostriatal pathway (Anderson *et al.*, 2007). More research is required to establish whether this observation relates to the frequent gastrointestinal problems seen in Parkinson's disease patients. In summary, MPTP will continue to play an important role in Parkinson's disease (PD) research due to its ability to selectively

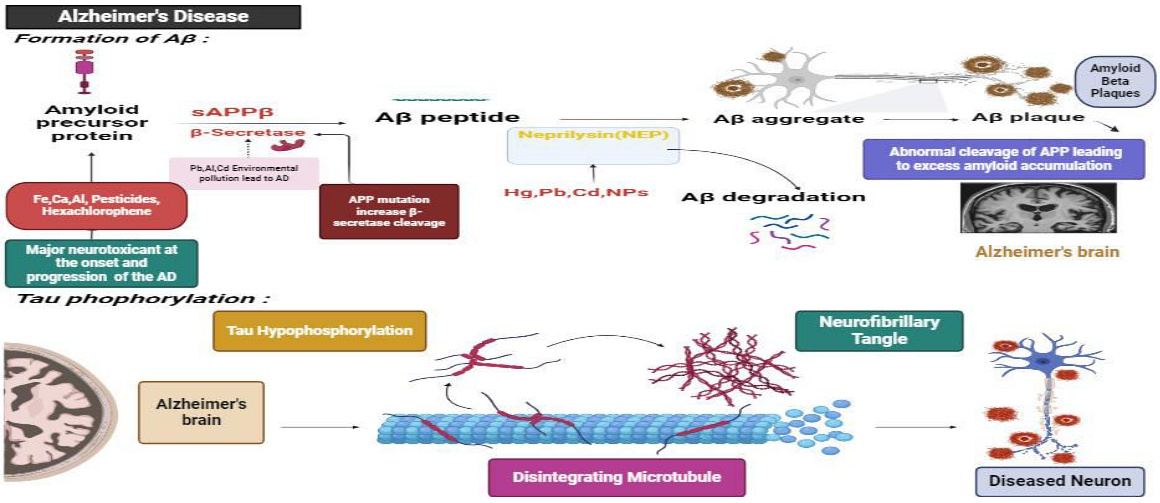

Fig. 1: Neurotoxins contributing to the projection of Alzheimer's disease. The early stages of Alzheimer's disease are marked by signature pathological changes, including the buildup of abnormal amyloid-beta protein into amyloid plaques and hyperphosphorylation of tau protein, leading to neurofibrillary tangles within and surrounding neurons. It has been discovered that a variety of neurotoxicants, including metals, insecticides, and nanoparticles, promote the development of Aβ aggregation and NFTs through a variety of pathways. These neurotoxins cause oxidative stress in neurons, which leads to A-peptide production and tau hyperphosphorylation. Neurotoxic substances control the expression of APP and the enzymatic functions of β-secretase. On the other hand, these neurotoxicants interfere with the function of antioxidant enzymes, proteins involved in amyloid-beta degradation, and certain receptors, contributing to the formation of amyloid plaques. Tau binds to neurotoxic substance, which separate the attached microtubules and allows them to become more hyperphosphorylated. Some neurotoxicants suppress enzymes that normally degrade tau protein, resulting in tau aggregation into neurofibrillary tangles.

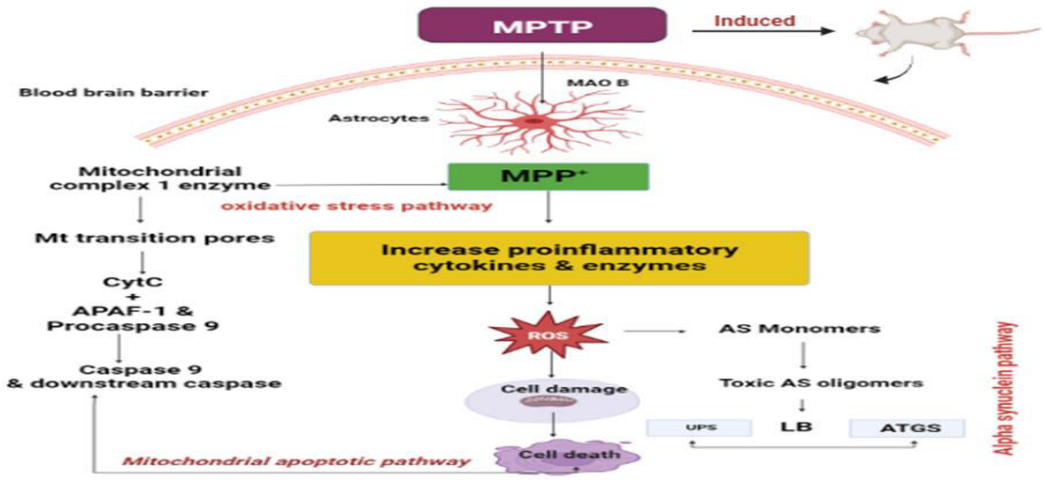

Fig. 2: Molecular mechanism of MPTP induced neurotoxicity. The mitochondrial apoptotic pathway, which is triggered by MPP+, results in COMPLEX-1 inhibition in the mitochondria, which opens transitional pores and releases cytochrome C, which sets off a chain reaction that damages cells. ROS levels rise because of COMPLEX 1 inhibition, which damages cells and ultimately results in the death of cells (oxidative stress pathway). Overproduction of reactive oxygen species (ROS) results to the generation of AS monomers. Then the monomers unite to create harmful oligomers, which hinder the ubiquitin-proteasome system (UPS) and autophagy-lysosome pathways (ATGS), ultimately resulting in cell death. (Alpha synuclein pathway). AS–Alpha-Synuclein, ATGS–Autophagy System; LB– Lewy body, MPP+–1-methyl-4-phenylpyridine, MPTP–1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine, Mt–mitochondria, ROS–reactive oxygen species, UPS–ubiquitin-proteasome system (Mustapha and Taib, 2011).

damage the nigrostriatal system, its consistent reaction to is well established that L-dopa can cause symptoms like Parkinson's disease in both non-human primates and people. Despite the acute hazards related to its neurotoxic qualities, its easy administration by normal intraperitoneal (i.p.) administration is noteworthy.

(ii) *Effect of 6-Hydroxydopamine (6-OHDA) exposure on animals in inducing PD*:

The discovery of 6-hydroxydopamine (6-OHDA), the hydroxylated counterpart of dopamine, dates back over five decades (Senoh and Witkop, 1959). The reduction of noradrenaline in a mouse's heart caused by 6-OHDA was first discovered by Porter *et al.* (1963). Later, it was discovered that 6-OHDA could cause sympathetic adrenergic nerve endings to selectively degenerate (Tranzer and Thoenen, 1968). This discovery popularized the novel idea of "chemical denervation" around the area of neurobiology by using a neurotoxic substance to affect a certain cell group only (Jonsson, 1980). 6-OHDA is used in many of the Parkinson's disease models used today to harm the nigrostriatal dopaminergic pathway. This idea is further supported by the data showing that this molecule can be detected internally in the brains of humans (Curtius *et al.*, 1974) and urine samples (Andrew *et al.*, 1993). 6-OHDA has the ability to cause deterioration of both dopamine and noradrenergic neurons inside brain (Ungerstedt, 1968). Because the dopamine transporter and the noradrenergic transporter, which are found on these types of neurons' plasma membranes, have high affinities for 6-OHDA, these neurons are particularly susceptible to it (Luthman *et al.*, 1989). As soon as it enters neurons, 6-OHDA builds up in neurons as soon as it reaches them, where it is rapidly oxidized, producing reactive oxygen species to be produced and ultimately oxidative stress-related apoptosis. Administering a modest dose of 6-OHDA (0.017 mg/kg) bilaterally into the substantia nigra pars compacta (SNc) significantly damages its dopamine neurons without inducing motor deficits. 6-OHDA is a popular unilateral model because of its appealing feature of allowing each animal to operate as its controller as it has an unlesioned hemisphere. In rat models, the substantia nigra pars compacta (SNc) are often unilaterally injected with a high dose of 6-OHDA (0.032 mg/kg). This causes significant impairment of mitochondrial complex I function, leading to dopaminergic neuron degeneration through oxidative mechanisms like excessive reactive oxygen species (ROS) production and decreased ATP synthesis (Zeng *et al.,*2018). The injection site significantly impacts the pattern and severity of 6-OHDA-induced neurodegeneration (Przedborsk *et al.*, 1995).

6-OHDA is typically administered unilaterally to both the striatum and substantia nigra. When introduced into the nigra, particularly through the medial forebrain bundle, it induces widespread and swift damage in the nigrostriatal pathway. Nerve terminals tend to be more vulnerable to the neurotoxic effects of 6-OHDA compared to axons and cell bodies (Malmfors and Sachs, 1968). When 6-OHDA is injected into the substantia nigra, degeneration of dopaminergic neuron cell bodies begins within 12 h, while significant loss of their striatal terminals typically becomes evident 2-3 days later (Faull and Laverty, 1969). But 6-OHDA causes striatal terminal degeneration when inserted within medial forebrain bundle, that precedes loss of dopaminergic cells (Sarre *et al.*, 2004). Unlike the nigra as well as the medial forebrain bundle, 6-OHDA causes the nigrostriatal structure to degenerate in a retrogressive manner gradually, progressively, and significantly for duration of up to 21 days when delivered to the striatum (Sauer and Oertel, 1994). The latter method of administration has the following three benefits: The increasing and less severe lesion is first and foremost more related to PD. Second, it has been observed that this course of treatment causes symptoms that are not motor such as gastrointestinal, mental, including cognitive problems (Branchi *et al.*, 2008). Thirdly, the ease of stereotactic injection into a sizable structure in mice, the striatum increases the chances of an effective administration. However, the acute neurodegeneration induced by 6-OHDA, and other

toxin models differs from the gradual, age-related disease progression in Parkinson's disease. Also, Lewy bodies, a key pathological feature in Parkinson's disease, are absent in the 6-OHDA model.

(iii) *Effect of rotenone neurotoxin exposure on animals in inducing PD*:

A pesticide called rotenone is frequently applied to lakes to eradicate invasive insects and fish. Plants that are a part of the Leguminosae family naturally contain this chemical. This substance has also been utilized in organic farming because it is natural. Rotenone has a high lipophilicity, which enables it to penetrate all cells without requiring a particular transporter and easily pass the blood-brain barrier. Rotenone's strong complex I inhibition is the main mediator of its hazardous effects.

There is a growing interest in utilizing rotenone as a Parkinson's disease model. This interest stems from the observation that MPP+ acts as a complex I antagonist, and diminished activity in this mitochondrial subunit has been noted in individuals with Parkinson's disease (Parker *et al*., 1989). The first *in vivo* rotenone effects in two distinct strains of rats were described by researchers in 2000 (Betarbet *et al*., 2000). In this study, rats that were two months old were involved given rotenone in varying doses by a subcutaneous mini pump that delivered the drug continuously and slowly into the jugular vein. Higher doses (>7 mg/kg/day) showed systemic and CVS virulence along death; nevertheless, 2-3 mg/kg/day for 7-35 days was determined to be the most effective dose for producing selective dopaminergic neurotoxic. According to the study's findings, an acute exposure caused tissue rotenone amount of 30 nm. blocked mitochondrial complex I (MC I) in the liver but not the brain. In the presence of elevated glutamate levels, acute rotenone therapy has been demonstrated to boost dopamine release without significant neuro-toxicity (Leng *et al*., 2003). On the other hand, from day 7 to day 36, rats subjected to a daily rotenone dosage of 2.5-2.75 mg/kg exhibited a progressive decline in both striatal terminals and dopaminergic neurons in nigra. Furthermore, systemic administration of rotenone resulted in an unusual buildup of cytoplasmic synuclein, characterized by positive inclusions within the dopamine neurons of the nigra a significant pathological trait correlated with Parkinson's disease (PD). The fact that there was no negative impact on postsynaptic striatal neurons suggests that rotenone preferentially targets dopaminergic neurons. This discovery sparked a lot of attention in the field as first, it indicated that, despite extensive complex I blockade in the brain, the nigrostriatal pathway experiences selective neurodegeneration. Firstly, it supports the idea that dopaminergic neurons in the substantia nigra are intrinsically more susceptible. It also confirms the link between complex I inhibition and the cell death pathways involved in Parkinson's disease pathogenesis. Thirdly, it suggests that generation of Lewy bodies may necessitate a prolonged, low-dose neurotoxic regimen. This hypothesis was subsequently applied to the chronic MPTP model, demonstrating that the continuous administration of MPTP over 30 days through osmotic minipumps results in the aggregation of α-synuclein (Sherer *et al*., 2003). However, other laboratories have not yet reported on the repeatability of this observation. Lastly, the rotenone model supports the idea that environmental factors could have an impact on the pathogenesis of PD. Table 1 outlines various neurotoxins that induce Parkinson's Disease along with their respective mechanisms of action for inducing neurotoxicity in rodent models.

Murine models used to study Alzheimer's disease induced by neurotoxins:

(a) *Effect of β-Amyloid protein-induced AD in animals*:

Alzheimer's disease (AD) is characterized by pathological features in the brain, including aggregates of neurofibrillary tangles and deposits known as senile plaques, coupled with progressive loss of synapses and neurons. The central component of these senile plaques consists of beta-amyloid protein aggregated together with

Table 1: Details of different neurotoxins induced PD in murine models

S. No.	Neurotoxins	Species	Routes and Dose	MOA	Pathology	Motor Behaviours	Reference
1.	MPTP	Mice	i.p 46 mg/kg/day	The transformation of MPTP into the polar MPP+ in the brain is enabled by the enzyme MAO-B. Subsequently, MPP+ is absorbed by dopaminergic neurons via the dopamine transporter (DAT), causing the targeted destruction of these neurons, notably in the substantia nigra pars compacta (SNpc) and striatum.	Nigrostriatal damage, Loss of dopaminergic neuron and DA in SNc, formation of Lewy bodies.	Impaired Movement	(Fox and Brotchie, 2010; Jiang *et al.,* 2013)
2.	6-OHDA	Rat	i.c 0.032 mg/kg/day	6-OHDA causes degeneration and eventual death of dopaminergic neurons due to its toxicity.	Damage DA neurons, striatal DA	Impaired: postural asymmetry	(Zeng *et al.,* 2018)
3.	Rotenone	Rat	s.c infusion 30 nm/kg	Inhibition of Mitochondrial Complex I lead to decrease ATP production	Dopamine neurons deterioration, LB formation (long term Exposure)	Hypokinesia and rigidity is impaired	(Parker *et al.,*1989; Leng *et al.,* 2003)

various additional types of proteins (Abraham *et al*., 1988). Correlations have been found between the amount of β-amyloid protein accumulation and the severity of memory loss, cognitive decline, and neuronal injury (Mann *et al*., 1985).

The studies utilized male Kbl Wistar rats initially weighing 280-320 g. Rats were pair- or triplet-housed under controlled conditions including light/dark cycles (12 h on from 9 AM) and stable temperature (23℃). Food and water were provided *ad libitum* throughout the experiments. Synthetic beta-amyloid protein was sourced commercially, dissolved in 35% acetonitrile with 0.1% trifluoroacetic acid (TFA), and administered via an implanted mini-osmotic pump connected to indwelling cannulas. Pumps continuously delivered set doses of beta-amyloid protein (0, 3, 30, or 300 picomoles per day) for duration of two weeks. There were seven rats in each group. On the first day after surgery, cannula was introduced into the ventricles on left side. (A -0.3, L 1.1, V 3.6). On days 9 to 13 after the infusion began, the water maze challenge was completed. Four rats from each group were sacrificed after the behavioural tests to measure ChAT activity. For the histochemical analysis, three rats were employed. Tukey's test and repeated-measure analysis of variance were employed to analyse the maze task data. The data on ChAT activity was analysed utilizing one-way analysis of variance followed by Tukey's multiple comparisons post hoc testing. Following a 14-day injection of β-amyloid protein, the cerebral cortex and hippocampus displayed an accumulation of β-amyloid protein as detected by immunohisto-chemical labelling. The investigation's main

findings are that learning deficit corresponds with β-amyloid accumulation of proteins inside brain and death of cholinergic neurons. The cholinergic neurons in the rats treated with β-amyloid protein degenerated, which negatively affected their performance in the water maze challenge. According to a prior study, mice performing a foot shock proactive avoiding task are negatively affected by just a single dose of the fragment of the β-amyloid protein. It is primitive evidence that a spatial memorial task causes amnesia when β-amyloid protein is continuously permeated into ventricles. The β-amyloid protein deposition process in AD patients and the settings of our experiment using the continual infusion technique for β-amyloid protein are extremely comparable. Additionally, in the frontal cortex and hippocampus of groups that had been given β-amyloid protein, there was a decline in ChAT activity, usually a marker for cholinergic neuronal dysfunctions. There have been conflicting results in attempts to show the neurotoxic effects of amyloid protein and associated peptides. *In vitro*, trophic impacts on rat hippocampal neurons have been seen with a synthetic peptide which matches the primitive 28 amino acids of the beta-amyloid protein (Whitson *et al*., 1989). Using the whole β-amyloid proteins in a comparable setting have shown cultured mouse cortical neurons *in vitro*, factors that promote neuronal dendrite branching differentiation can offset the neurotoxic effects induced by glutamate exposure (Koh *et al.*, 1990). According to report of Yankner *et al.* (1990), adding β-amyloid protein to hippocampus cultures at low levels results in the earliest neurotrophic effect but not neurotoxicity. To summarize, the data reveal an association between gradual buildup of beta-amyloid in the rodent brain, development of cognitive deficits on learning tasks, and loss of cholinergic neuronal integrity. These combined observations suggest that the continuous beta-amyloid administration paradigm in rats may provide a viable animal model recapitulating core pathological and functional hallmarks analogous to progression of Alzheimer's disease (AD) in humans.

(b) *Effect of streptozotocin induced AD in animals*:

Streptozotocin (STZ) is a chemical compound that is synthesized from *Streptomyces achromogenes*, a bacterium found in soil. The structure of streptozotocin contains a nitrosourea group coupled to a glucosamine (Wiley, 1981). Structurally Streptozotocin functions as an alkylating, with similarities to nitrosoureas, a class of anticancer drugs used for treating pancreatic cancer. In mice, ICV infusion of streptozotocin at the sub-diabetogenic level (3 mg/kg), twice spaced by 48 h, causes a gradual memory loss that is strikingly similar to AD's symptoms (Salkovic *et al*., 2014). Memory problems caused by ICV STZ are not influenced by its hyperglycaemic impact (Mayer *et al*., 1990). STZ is responsible for learning and memory deficiencies by altering the LTP-like development of plasticity of synapses in the hippocampus and other cerebral regions (Biessels *et al*., 1996). Intracerebroventricular injection of streptozotocin (STZ) is now understood to elicit neuronal damage through oxidative stress pathways, including generation of reactive oxygen species, formation of reactive nitrogen species (Zhou *et al*., 2013), elevated levels of the lipid peroxidation marker malondialdehyde, buildup of amyloid β peptides in brain, hyperphosphorylation of tau protein, and impaired expression of insulin signalling-associated genes like IGF-1 receptors. Through these mechanisms, centrally administered STZ adversely affects neurons as depicted in Figure 3 (Dalla *et al*., 2010). The insulin receptor system in the hippocampus has been proposed as integral for memory regulation. However, STZ-provoked memory impairment appears contingent on activity along the insulin receptor/insulin receptor substrate-1/Akt signalling cascade localized specifically to the CA3 hippocampal subregion (Agrawal *et al*., 2011). Liver-X receptors have been linked to the pathogenesis of dementia brought on by STZ (Sodhi and Singh, 2014). According to evidence, the cerebral cortex and hippocampus's ability to function as key glycolytic enzymes is impaired by ICV injection of STZ, which lowers the concentrations of ATP along with creatine

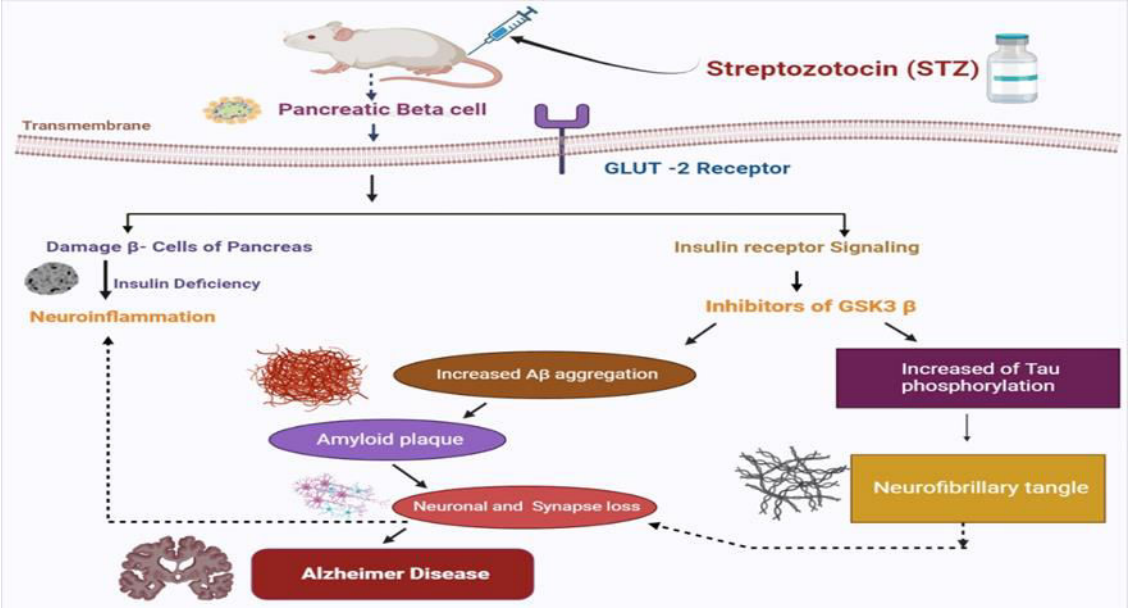

Fig. 3: Schematic expression of STZ induced memory impairment in animals. Glycogen synthase kinase, however, is altered by STZ, which also increases low insulin levels and damages insulin receptors in the brain. Thus, these modifications trigger elevated tau phosphorylation, the development of neurofibrillary tangles, and ultimately implicate neuronal and synaptic dysfunction leading to pathology simulating Alzheimer's disease. GSK3β: glycogen synthesis kinase β; Aβ: amyloid beta; GLUT 2: glucose transporter 2.

phosphate (Hoyer and Lannert, 2008). Reduced acetyl coenzyme-A production and disrupted cholinergic transmission are results of impaired metabolism of energy in the brain. Particularly, it has consistently been found that rats exposed to STZ had less ChAT activity in the hippocampus (Salkovic *et al*., 2014).

Rats administered intracerebroventricular doses of streptozotocin (STZ) demonstrate reduced expression of the acetylcholine (ACh)-synthesizing enzyme choline acetyltransferase (ChAT), which has been tied to ACh deficiencies found in these animals (Ahmed *et al*., 2013). Further exacerbating cholinergic disruption, elevated acetylcholinesterase activity has also been detected in the brains of STZ-treated rodents. This may reflect increased breakdown of already depleted ACh supplies. Beyond cholinergic impact, downstream variations in total and phosphorylated forms of glycogen synthase kinase-3β have been reported following intravenous STZ injection. These changes may relate to the development of amyloid beta aggregates that parallel those in Alzheimer's disease (Yang *et al*., 2013).The primary strength of the AF64A model lies in its resemblance to key pathological hallmarks found in humans with sporadic Alzheimer's disease (Mansouri *et al*., 2013). However, the model also presents limitations due to its demanding animal requirements and high mortality rate (Sodhi *et al*., 2014). Still, multiple therapeutic strategies first tested in this model have shown similar efficacy in Alzheimer's clinical trials, supporting the utility of this approach (Salkovic *et al*., 2013).

(c) *Effect of ethylcholine aziridinium induced AD in animals*:

The neurotoxic choline derivative Ethylcholine mustard aziridinium ion, abbreviated as AF64A, provokes enduring impairments in rodent cholinergic nerve terminal function without affecting monoaminergic systems. Administration of AF64A causes selective reductions in markers of cholinergic presynaptic function, such as high-affinity choline uptake and the activity of the enzyme choline acetyltransferase (Fisher *et al*.,

Table 2: Details of different neurotoxins induced AD in murine models

S. No.	Neurotoxins	Species	Routes and Dose	MOA	Pathology	Motor Behaviours	References
1.	β-Amyloid protein (Aβ)	Rat	i.p (0, 3, 30, 300) p mol/ day	Aβ produced through APP cleavage by enzymes like γ-secretase forms aggregates under pathological conditions. These Aβ oligomers are implicated in initiating Alzheimer's disease	Cholinergic neuronal loss and β-amyloid protein buildup in the brain	Learning deficits	(Tanokashia *et al.,* 2017)
2.	Streptozotocin	Rat	i.v (3 mg/kg) nm/kg/48hr	Hyperphosphorylated tau may impair synaptic plasticity and long-term potentiation in the hippocampus and other brain regions. Disrupting these processes could promote neurological disease.	Brain atrophy, less ChAT Activity increase Ach breakdown	Dementia	(Salkovic *et al.,* 2014)
3.	Ethylcholine Aziridinium	Rat	icv 6 nM	Modifying the action of choline acetyltransferase and altering acetylcholine concentrations in cortical and hippocampal areas of the brain.	Cholinergic deficits, astrogliosis	Cognitive deficit	(Yamada *et al.,* 2010)

1982). The primary processes that regulate the production of ACh are the focus of AF64A. The cholinergic nerve terminals (HAChT) are where all the choline is physiologically absorbed back by high affinity choline transporters. The chemical Ethylcholine resembles choline enough that it can enter cholinergic nerve terminals via the high affinity choline transporter (HAChT). However, Ethylcholine also contains a highly reactive aziridinium ring, making it cytotoxic. This neurotoxic choline derivative, termed AF64A, provokes cholinergic dysfunction once inside the nerve terminal by alkylating and damaging enzymes that depend on choline, like choline kinase, acetylcholinesterase, choline acety-ltransferase, and choline dehydrogenase. The alkylation occurs at the catalytic sites of these enzymes. AF64A administration ultimately triggers cell death, though the precise mechanisms leading to neuronal demise remain under investigation.

A recent experiment showed that injecting 6 nM of the neurotoxin AF64A into the cerebroventricular system of rats caused deficits in learning and memory retention when the rats were tested using the Morris water maze, along with histological evidence of cholinergic dysfunction, 20 days post-injection (Yamada *et al*., 2010). This mirrors previous research showing AF64A can induce pathology resembling Alzheimer's disease. Various neurotoxins with their pathological mechanisms for inducing Alzheimer's disease in rodent models has been included in Table 2.

Conclusion

Understanding the mechanism of memory impairments and Parkinson's disease has advanced significantly during the past few

decades. Natural or synthetic toxins have served as useful research aids examining the morphological, bio-chemical, and molecular underpinnings of brain function. The fundamental neurobiology of cells in the brain and the concepts of synaptic transmission have been demonstrated using toxins that disrupt certain neurons, axoplasmic transport mechanisms, ion channels, as well as neurotransmitter systems. For decades, researchers have used neurotoxins to: (i) investigate the processes associated with the production, retention, and emission of neuro-transmitters; (ii) elucidating how neurotrans-mitters bind to specific receptors and ion channels to exert effects; (iii) characterizing neural plasticity and the brain's ability to reorganize itself after injury; (iv) correlating changes in neurotransmitters to behavioural outcomes; and (v) developing animal models that accurately replicate the behavioural, biochemical, cognitive, and histopathological abnormalities seen in neurological illnesses marked by cognitive detriment which are not accurately simulated by any model that is now available. The main goal of enhancing the pathophysiology and phenotypes of PD and AD in existing models is to increase the usefulness of these type of models for finding treatments. These types of models collectively have influenced the creation for certain modern PD and AD medications. The behavioural, biochemical, cognitive and histopathological abnormalities seen in neurological illnesses marked by cognitive detriment are not accurately simulated by any model that is now available. The majority of these traits can, however, be mimicked by utilizing particular poisons. The transgenic animal model, on the other hand, is a popular secondary choice because it offers to closely replicate the disease pathogenesis. These models' high development and maintenance costs prevent them from being used on a bigger scale. Hence, more effective research is required to create more appropriate and human disease replicating animal models for neurodegenerative diseases.

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