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Rodent Models for Alzheimer Disease: Experimental Induction, Pathophysiological Mechanisms, and Biomarker Profiling

Animal Models for AD: From Induction to Biomarker Validation

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Abstract:

- ***Background***

Alzheimer's disease (AD), the most common neurodegenerative disorder, is driven by amyloid-beta (Aβ) plaques, neurofibrillary tangles (NFTs), neuroinflammation, and oxidative stress. Rodent models are critical for studying its multifactorial etiology, combining genetic, environmental, and epigenetic factors. This review evaluates rodent AD models, including chemical induction (e.g., aluminum, scopolamine) and transgenic systems (e.g., 5xFAD, APP/PS1). Chemical models mimic sporadic AD triggers, while transgenics replicate genetic mutations. Combinatorial approaches (e.g., toxin-exposed transgenics) address limitations. Biomarkers such as Aβ/tau ratios, neuroinflammatory cytokines (TNF-α, IL-1β), and oxidative stress markers (MDA, SOD) validate pathology, measured via ELISA, PET imaging, and omics technologies.

Keywords: *Alzheimer Disease; Biomarkers, Animal Models, Pathophysiology, Neuroinflammation*

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I. INTRODUCTION

Alzheimer's disease (AD), the most prevalent neurodegenerative disorder and leading cause of dementia, accounts for 60-80% of dementia cases worldwide, with its prevalence rising sharply among aging populations-a trend poised to strain global healthcare systems as demographic shifts accelerate [1,2]. Clinically, AD manifests as progressive cognitive decline, marked by memory loss, impaired reasoning, behavioral disturbances, and functional disability, alongside systemic comorbidities such as hypertension, depression, and retinal neurodegeneration, the latter sharing pathophysiological overlaps with age-related macular degeneration (AMD) [3–5]. At its core, AD pathology is defined by two hallmark lesions: extracellular amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles (NFTs) of hyper phosphorylated tau protein. These lesions underpin the amyloid cascade and tau hypotheses, respectively, which posit Aβ aggregation as the initiating trigger of neurodegeneration and tau dysfunction as the driver of synaptic failure and neuronal death [6,7].

However, AD's complexity extends beyond Aβ and tau. Emerging paradigms emphasize the role

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of neuroinflammation (microglial activation, cytokine release), oxidative stress (ROS-mediated lipid peroxidation), mitochondrial dysfunction, and cholinergic deficits (reduced acetylcholine signaling) as synergistic contributors to disease progression [3,8]. Compounding this complexity are environmental risk factors, such as heavy metal exposure (aluminum, cadmium), dietary patterns, and chronic stress that interact with genetic susceptibility (e.g., APOE ε4, APP mutations) to modulate epigenetic changes (DNA methylation, histone modification) and accelerate pathological cascades [5,9].

This multifactorial etiology necessitates preclinical models that recapitulate AD's diverse mechanisms while enabling biomarker discovery for early diagnosis, disease monitoring, and therapeutic development [10,11]. Rodent models, ranging from toxin-induced (e.g., $AlCl_3$ for aluminum neurotoxicity, scopolamine for cholinergic blockade) to transgenic systems (e.g., 5xFAD for rapid Aβ deposition, tau-P301L mice for NFT formation), serve as indispensable tools for:

- **Validating pathogenic hypotheses** (e.g., Aβ immunotherapy in APP/PS1 mice),
- **Mapping biomarker trajectories** (e.g., CSF p-tau, plasma Aβ42/40 ratios, neurofilament light chain),
- **Evaluating therapeutics** (e.g., antioxidants for oxidative stress, BACE inhibitors for amyloidogenesis), and
- **Deciphering gene-environment interactions** (e.g., heavy metal-exposed transgenics).

Yet, no single model fully replicates human AD. Chemical models excel in mimicking sporadic AD's environmental triggers but often lack progressive Aβ/tau pathology, while transgenics recapitulate genetic mutations yet overlook sporadic AD's multifactorial nature. This gap underscores the need for combinatorial approaches—such as toxin-exposed transgenics or humanized models with iPSC-derived neurons—to bridge mechanistic and translational divides.

In this review, we critically assess the landscape of rodent models in AD research, their alignment with disease hypotheses, and the biomarkers used to quantify pathology. We explore how model selection, whether targeting mitochondrial dysfunction (sodium azide), neuroinflammation (LPS), or synaptic loss (Aβ oligomers), must be hypothesis-driven to address specific research questions. By synthesizing advances in model systems, biomarker technologies (e.g., PET imaging, ultrasensitive immunoassays), and translational frameworks, we aim to guide researchers in leveraging these tools to accelerate therapeutic innovation and refine diagnostic precision in AD.

II. CHEMICAL METHODS TO INDUCE AD-LIKE SYMPTOMS IN RODENTS

➢ *$AlCl_3$-Induced AD Models:*

Aluminum (Al), a recognized neurotoxicant, is implicated in AD pathogenesis due to its propensity to accumulate in the central nervous system (CNS) and exacerbate neurodegenerative processes. Human exposure occurs via dietary sources (e.g., Aluminum foil, cookware, packaged foods), cosmetics (antiperspirants), pharmaceuticals (antacids, vaccines), and water treatment (Aluminum sulphate in drinking water) [7,12]. Upon entering the bloodstream, Al crosses the blood-brain barrier via transferrin receptor-mediated transport [13], where it promotes AD-related pathology through multiple mechanisms.

- **Amyloidogenesis & Tauopathy:** Al enhances amyloid-beta (Aβ) fiber formation and tau hyperphosphorylation, driving plaque and tangle formation [3,14].
- **Oxidative Stress:** Al indirectly induces lipid peroxidation by interacting with iron, destabilizing neuronal membranes and depleting antioxidants (glutathione, SOD, catalase) while elevating malondialdehyde (MDA), a marker of oxidative damage [12,15].
- **Cholinergic Dysfunction:** Al binds acetylcholinesterase (AChE), altering its structure and amplifying activity, thereby degrading acetylcholine and impairing synaptic signaling [9,13].

In rodent models, chronic Al exposure recapitulates AD-like neurochemical changes, including memory deficits, reduced antioxidant defenses, and elevated AChE activity, validating its utility for studying environmental contributions to neurodegeneration [3,15].

Table 1 $AlCl_3$-Induced Alzheimer's Model with Biomarkers and Morphological Changes

Model and route of administration and dose	Biomarkers & histopathological changes	Behavioral changes	References
70mg/kg for 20 days Intraperitoneally	↑ AChE, BuChE, GSK-3β, LOX-5, Rho-II, Na+/K+ ATPase, TNF-α, COX-2, NO, MDA. ↑Vacuolar space and irregular cellular morphology in hippocampal and cortex region	Spatial and learning memory impairment	[3]
100mg/kg by route for 42 days orally	↓ AChE, ChAT, BDNF, DA, GABA, IL1β, IL6, NFKβ, TNF-α ↑ MDA inwhole brain	Spatial and learning memory impairment	(Kazmi et al. 2024)
100mg/kg by route for 21 days orally	↓CAT, GSH, GR, AChE ↑ MDA in Cortex and hippocampus	Spatial and learning memory impairment Fear memory loss Decreased muscle strength	[7]

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100 mg/kg for 60 days intraperitoneally	↑ Al conc., AChE, Amyloid β, APP, β secretase, γ- Secretase in hippocampus and cortex	Spatial memory impairment Suppressed locomotion	[13]
150 mg/kg for 90 days orally	↓ DA, NE ↑AChE, glutamic acid, Ab42, MDA, mRNA levels of IL-1 β, IL-6, TNF-a and MHC-II inhippocampus	Spatial memory loss Suppressed locomotion	[16]
100 mg/kg for 28 days intraperitonially	↑ iNOS, COX-2, NF-kB, IK β α, TNF-α, IL-6, IL-10, MDA, LDH, NO, AChE, Na^+/K^+ATPase ↓ SOD, CAT, GSH in hippocampus and cortex	Reduced locomotor activity Increased transfer latency in EPM Spatial memory loss Neuron shrinkages, hyperchromatic nuclei and vacuole spacing	[17]
175 mg/kg for 25 days orally	↑ Aβ 1-42, MDA, (IL)-1β, PPAR-c, p38MAPK, and NF-jB/p65 ↓ SOD and BDNF Degenerated cells in DG and CA, microglia cells, reduced cell densityin hippocampus	Slowed exploration Rearing and grooming frequency declined More droppings	[9]
175 mg/kg for 8 weeks orally	↑ MDA, BACE1, CHOP and Caspase-12,LRP1 ↓ NEP, SOD Brain endoplasmic reticulum stress analysis was donein hippocampus and cortex	Spatial memory loss Decreased discrimination index	[18]
100 mg/kg for 70 days orally	↑ AChE, hyper-phosphorylated tau in cortex Degeneration of pyramidal cells in the CA1, CA2 and CA3 in hippocampus	Spatial memory loss	[19]

➢ *Scopolamine Induced AD:*

Scopolamine, a tropane alkaloid and muscarinic receptor antagonist, is widely used to model Alzheimer's disease (AD) in rodents by inducing cholinergic dysfunction, a hallmark of AD pathology. By blocking central muscarinic acetylcholine receptors (mAChRs), scopolamine disrupts cholinergic signaling, leading to progressive learning and memory deficits—core features of AD [20,21]. Beyond acetylcholine depletion, scopolamine elevates acetylcholinesterase (AChE) activity, further degrading synaptic acetylcholine, while promoting amyloid-beta (Aβ) deposition, tau hyperphosphorylation, and oxidative stress through increased lipid peroxidation (increased malondialdehyde) and antioxidant depletion (decreased catalase, SOD) [21]. Chronic administration replicates AD-like synaptic disruption, inhibits hippocampal neurogenesis (notably in the dentate gyrus), and alters monoamine neurotransmission, elevating hippocampal dopamine and norepinephrine levels [4,22]. The model also upregulates AD-associated genes, providing insights into molecular pathways involved in neurodegeneration. As a pharmacodynamic tool, the scopolamine-induced AD model enables rapid screening of cognitive enhancers and disease-modifying therapies targeting cholinergic, amyloidogenic, and oxidative pathways.

Table 2 Scopolamine-Induced Alzheimer's Model with Biomarkers and Morphological Changes

Mode and Route of administration and dose	Biomarkers & histopathological changes	Behavioral changes	References
2 mg/kg for 28 days Intraperitoneally	↓ GSH, GST and GPx, ↑ AChE, brain cholesterol/phospholipids ratio, iNOS, Aβ-42, ↑ iNOS, G6PD, Tau, ADAM-17 mRNA Congestion in the blood capillaries, reactive gliosis, flame-shaped cells, vacuolation Observed in hippocampus and cortex	Spatial & learning memory impairment	[20]
2 mg/kg for 6 weeks Orally	↑ levels of Aβ, mRNA expression of APP, ptau, GSK-3β, Glutamate, AChE, TNF-α, IL-1β ↓ neprilysin, AChE, DA, GABA, NGF, BDNF, VEGF, IL-2, Nrf2, CREB, Seladin 1 in Hippocampus	Spatial and learning memory loss	[21]
1mg/kg for 7 days intraperitoneally	↓GSH, CAT ↑ AChE, MDAin whole brain	Spatial and learning memory impairment Decreased discrimination index	[23]

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		Decreased locomotion	
1mg/kg for 14 days Intraperitoneally	↓ mACh, R M1, BDNF, ↓ACH, M1, MPO, GSH. ↑ MDA, AChE m RNA in hippocampus, Prefrontal cortex and amygdala	Fear memory loss Long-term memory decline Short-term memory impaired object recognition memory	[24]
0.7 mg/kg 13 days intraperitoneally	↑ AChE, MDA, GSH ↓ GPx, CAT, SOD Observed in whole brain and blood serum	Spatial/learning memory impairment ↑ locomotion	[25]
1 mg/kg for 9 days intraperitoneally	↑ IL-6, TNF-α, APLP2 mRNA, APP mRNA, Corticosterone in hippocampus &prefrontal cortex and hypothalamic pituitary adrenal axis	↓ Discrimination index Increased anxiety	[26]

➢ *Lipopolysaccharide (LPS) Induced AD:*

Neuroinflammation, a critical driver of Alzheimer's disease (AD) pathophysiology, is effectively modeled using lipopolysaccharide (LPS), a component of gram-negative bacteria membranes. LPS activates microglia and astrocytes via Toll-like receptor 4 (TLR4), triggering NF-κB-mediated up regulation of pro-inflammatory cytokines (IL-1β, TNF-α), enzymes (iNOS, COX-2), and cytotoxic factors, which collectively impair cognition and promote amyloid-beta (Aβ) production [27,28]. Systemic exposure to *Porphyromonas gingivalis*-derived LPS exacerbates neuroinflammation and induces peripheral organ dysfunction (e.g., sarcopenia, cardiac injury), underscoring its role in multi-organ AD-related pathology [29]. LPS further amplifies oxidative stress by activating caspases and ROS generation, culminating in neuronal apoptosis and synaptic loss [27]. Emerging evidence highlights the gut-brain axis in modulating neuroinflammation; for instance, butyric acid production by gut microbiota may counteract LPS-induced inflammation [30].

- *Therapeutic Strategies Targeting LPS-driven AD Include:*

✓ **Anti-inflammatory agents**: Blocking TLR4/NF-κB signaling or cytokine activity [28].
✓ **Apoptosis inhibition**: Suppressing caspase activation and HMGB1/TLR4/RAGE pathways [31].
✓ **Antioxidant and autophagy enhancers**: Mitigating oxidative damage and improving protein clearance.

Neuropathological outcomes vary with LPS administration parameters (route, dose, serotype), necessitating standardized protocols to optimize translational relevance.

Table 3 Lipopolysaccharide-Induced Alzheimer's Model with Biomarkers and Morphological Changes

Model and route of administration and dose	**Biomarkers & histopathological changes**	**Behavioral changes**	**References**
500 µg/kg for 6th, 12th, 24th, and 48th h intraperitoneally	↑ IL- 17 A, TNF-α, Iba, IL-6 In hippocampus and blood serum	Fear memory observed Learning memory impairment	[32]
C57BL/6 N mice 250 µg/kg for 7 days intraperitoneally	↑ p-NFκβ, COX-2, TNF-α, BACE-1, APP, Aβ, PSAD_95, SNAP-23, SYP, p-tau ↓ AChEin hippocampus	Learning and Spatial memory impairment short-term memory impairment	[28]
250 µg/kg for 7 days Intraperitoneally	↑ MDA, Nrf2, TGF-β1, NLRP3, NF-κB, (IL)-1β, IL8, IL-18, MCP-1, Ki- PI3K/Akt/mTOR signaling, caspase 3 & 9 ↓ GSH, LC3-II, beclin-1, BCL-2 dysmorphic pyramidal cells, shrunken nuclei, mitochondrial swelling in hippocampus	Decline in the discrimination index Learning and Spatial memory impairment	[31]
5mg/kg intraperitoneally	↑ Total cholesterol, LDL & VLDL, LPO, $_{S}$AA, PTK, TNF-α ↓ HDL, TAOs, MPO, Observed in blood, liver and kidney LPS severely damaged the lungs		[33]
250µg/kg once daily for 7 days Intraperitoneal	Increased NF-KB, IL-1B, ROS, NOS, BDNF, CYP 2E1, GFAP, TNF- α in whole brain	Impaired in learning and acquisition	
250 µg/kg/day of LPS for 7 days	↑ Ab1-42, APP, BACE1, GFAP, Iba-1, IL-1b, IL-6, TNF- α, H2O2, MDA, Activation of NF-kB ↓ GSH/GSSG ratio	Learning and Spatial memory impairment	[34]

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	↑ iNOS, COX-2 in whole brain		
250µg/kg once daily for 7 days Intraperitoneally	↑ NF-KB, IL-1B, BDNF, CYP 2E1, GFAP, TNF-α	Impaired in learning and acquisition	[35]

➢ *Streptozotocin induced AD:*

Streptozotocin (STZ), a glucosamine-nitrosourea compound, selectively targets insulin-producing β-cells, inducing cytotoxicity (Sorial & El Sayed, 2017). When administered intracerebroventricularly (ICV) at low doses, STZ disrupts brain insulin signaling, triggering insulin resistance and mimicking Alzheimer's disease (AD) pathology [36]. This model replicates key AD features:

- **Metabolic Dysregulation:** Reduced cerebral glucose uptake, impaired ATP/acetyl-CoA synthesis, and energy deficits [37].
- **Pathological Hallmarks:** Amyloid-beta accumulation, tau hyperphosphorylation, and synaptic dysfunction [36].
- **Neuroinflammation:** Elevated TNF-α and NF-κBp65, with suppressed IκBα, driving inflammatory cascades [38].

Chronic ICV-STZ administration in rodents induces progressive memory decline and cognitive deficits, mirroring AD progression [39]. Unlike transgenic models, the ICV-STZ paradigm offers a non-genetic approach to study sporadic AD mechanisms, circumventing the need for genetic modifications [40].

- *Preparation and Administration of STZ:*

Streptozotocin is prepared in citrate buffer at pH 4.4 just prior to administration and injected twice intracerebroventricularly at a flow rate of 1 µl/min on alternate days through the cannula using Hamilton micro syringe in a volume of 2µl/min into each lateral cerebral ventricle. For the administering process the rats is anesthetized and the head is placed in a stereotactic frame. Two holes are drilled on the skull to place the cannula into the lateral cerebral ventricles and the STZ is administered into cerebral ventricles [41].

Table 4 Streptozotocin-Induced Alzheimer's Model with Biomarkers and Morphological Changes

Route of administration and dose	Biomarkers & histopathological changes	Behavioral changes	References
0.3, 1 & 3 mg/kg ICV (3 different groups)	↑ NFTs and Aβ Enlarged lysosomes, Golgi hypertrophy, pyknotic nuclei, and nuclear envelope invaginations were observed in the hippocampus, parietal cortex, and corpus callosum.	Fear memory loss	[40]
C57BL/6 mice 3 mg/kg ICV	↓ synapsin, ChAT, ↑ Aβ, tau hyperphosphorylation in hippocampus region	Impaired object recognition memory	[39]
3 mg/kg, ICV	Uptake of [^{18}F] FDG was significantly lower - indicating glucose hypometabolismin whole brain	Impaired object recognition memory.	[42]
1.5 mg/kg on day 1 and day3	↓ SOD, CAT, GSH ↑ MDA – in hippocampus Neuronal loss and degeneration were observed in the cerebral cortex and hippocampus.	Learning and Spatial memory impairment	[37]
3 mg/kg ICV	↓ Ach, NEP ↑ AChE in hippocampus	Learning and Spatial memory impairment Decrease object Recognition memory Spatial confusion	[43]
3 mg/kg ICV	↑ APP mRNA levels, MAPT, NfκB, GSK3α, and GSK3β in hippocampus and cerebral cortex	Impaired cognitive flexibility Fear memory loss	[44]
3mg/kg ICV	↑ Phosphorylated AKT (AKT pT308), GFAP, Iba1, GSK-3β, GSK-3α Activation ERK1/2, JNK and CaMKII in hippocampus	Increased exploratory activities Indicating impaired spatial memory in these rats	[36]
Mice 3 mg/kg ICV	↓ GSH, SOD ↑ MDA, NO, TNF-α, IL-6, AChE ↓ CREB, BDNF, NGF in hippocampus	Learning & decision-making deficit. Decreased memory retention Loss of fear memory	[41]
Mice 3 mg/kg ICV	↓ GSH, CAT, SOD ↑ β amyloid, GSSG/GSH, AChE in hippocampus	Increase Anxiety	[45]

➢ *Colchicine Induced AD:*

Colchicine in rats imitates the human AD in numerous aspects including memory loss, oxidative stress and hyperphosphorylation of tau protein [46]. Administration of colchicine through ICV route causes it to bind with tubulin which is an essential structural protein that helps stabilize the

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microtubules which performs axonal transport of the essential molecules within neurons thereby causing tau hyperphosphorylation and dwell as generating ROS; ultimately leading to neurodegeneration and a condition analogous to [47]. Furthermore, colchicine decreased the levels of antioxidant enzymes such as glutathione S-transferase, glutathione peroxidase, catalase, glutathione reductase and superoxide dismutase leading to increased ROS. Colchicine induces inflammatory response in CNS which leads to release of certain proinflammatory cytokines and chemokines specifically in the cortex and hippocampus. The levels of TNF-α, IL-1β, IL-6, and IL-10 increases indicating microglial activation and lasting inflammation in CNS [47]. Colchicine induction is also known to elevate the levels of nitrite as3well as myeloperoxidase, further indicating neuroinflammation [48]. Colchicine triggers neuronal degeneration, impacting the cholinergic system alongside reducing acetylcholine transferase activity. Colchicine induced AD causes noticeable cholinergic dysfunctions [49]. It also alters dopamine, glutamate serotonin, and GABA and communication between the nerve cells in the brain. Colchicine causes morphological hippocampal changes like arrangement of neurons a reduction of cell number [49].

Table 5 Colchicine-Induced Alzheimer's Model with Biomarkers and Morphological Changes

Model and route of administration and dose	Biomarkers & histopathological changes	Behavioral changes	References
Rats 15 µg ICV	↑ BACE1, IL-6 mRNA, TNF-α mRNA MCP-1 mRNA, COX-2 and iNOS mRNA expression, Aβ ↓ GSH in cerebral cortex and hippocampus	Learning and Spatial memory impairment	[47]
Rats 15µg ICV	↑ NF-kB, P. carbonyl, AChE, Aβ, NF-kB, IL-1β ↓ BDNF, GPx, SOD, GR and CATin hippocampus	Learning and Spatial memory impairment	[50]
Rats 15µg ICV	↑ MDA, NO, AChE ↓ GSH, SOD Impaired mitochondrial enzyme complex (I–IV) in whole brain	Learning and Spatial memory impairment	[49]
Rats 15µg ICV	↑ TNF α, IL-1β, NO ↓ WBC, serum corticosterone levels Chromatolysis & neurodegenerationin hippocampus	The Working Memory Errors Reference Memory Errors	[51]
Rats 15µg ICV	↑ Astrocytes, sharp dendritic margins of astrocytes expressed, BAP positive cells, GFAP positive cells, Iba1 positive microglia in hippocampus and Prefrontal cortex	Demonstrated learning disability and memory impairment	[46]
Rats 15 µg ICV	↑ TNF-α, NF-κB, IL-1β, IL-6, IL-10, NO, MPO ↓ GSH, GPx, GR, GST, BDNF in hippocampus	Decreased the cognitive performance	[48]

➢ *Okadaic Acid Induced AD:*

In experimental mice, the toxin okadaic acid (OKA) can cause phenotypes similar to those of Alzheimer's dementia (AD) [52]. As a strong and specific inhibitor of the serine/threonine phosphatases PP1 and PP2A, okadaic acid (OKA) is frequently used to mimic Alzheimer's disease (AD)-like lesions in lab settings. In mammalian brains, PP2A is the most significant serine/threonine phosphatase. People with AD are said to have reduced PP2A activity in their brains [53]. Tau protein is hyperphosphorylated when OKA is administered intrahippocampally or intracerebroventricularly (ICV). Additionally, the brain produces amyloid beta (Aβ) and amyloid precursor protein (APP) as a result of this stimulation [53].Central cholinergic dysfunction may result from OKA-induced neurotoxicity and neurodegeneration [54]. Oxidative damage, cellular inflammation, and neurodegenerative alterations are brought on by ICV microinjection of OKA. Antioxidants like SOD and catalase also decline as a result of it [52]. Neurotoxicity results from OKA's induction of tau hyperphosphorylation and inhibition of α7, α4, and α3 nicotinic acetylcholine receptor (nAChR) subunit expression [55]. In the rat brain, OKA results in mitochondrial malfunction, which is a critical step in memory loss and apoptotic cell death [53].

Lower mitochondrial membrane potential (MMP), a gauge of mitochondrial integrity and health, is linked to OKA exposure [52]. In vivo Okadaic acid (ICV) can cause neurodegeneration and heat shock protein production in the rat hippocampal region [55].

- *Administration of Okadaic Acid:*

By intravenous injection, a dose of around 100 to 500 ng (nanograms) is given. [53]. Intranasal administration is another method (Subramanian et al., 2016). Any aesthetic substance, such as thiopentone sodium (45 mg/kg, i.p.), is employed [56]. To reveal the skull, a midline sagittal incision is made in the scalp while the head is positioned in a stereotaxic frame [53]. Using the stereotaxic coordinates of 0.8 mm posterior, 1.3 mm lateral to the midline, and 3.5 mm

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ventral with regard to the bregma, burr holes are bored in the skull bilaterally over the lateral ventricles [54]. In addition to suturing the skin, antibacterial powder may be used. Rats can be fed a regular pellet diet and oral glucose while being observed [56].

Table 6 Okadaic Acid-Induced Alzheimer's Model with Biomarkers and Morphological Changes

Model and route of administration and dose	Biomarkers & histopathological changes	Behavioral changes	References
(200 ng) 100 ng ICV	↑ AChE,TNF-α,TGF-β,IL-1β,NF-κB p65, Caspase 3, MDA, NO ↓ CAT, SOD, GSHin hippocampus and cortex	Learning and Spatial memory impairment	[56]
500 ng intra-nasal route	↑ p-tau levels, p-tau/t-tau ratio in hippocampus	Barnes Maze Task: significant impairment spatial learning and memory	[57]
200 ng ICV	↑ CDK5, GSK3β at Tyrosine 216, Tau protein levels at pSer214 (S214), pThr231 (T231), pThr205 (T205), pThr181 (T181) sites. ↓ GSK3β at Serine 9 (S9), PP2A Dark and reduced neurons with shrivelled nucleiin hippocampus	Learning and Spatial memory impairment	[57]
200 ngICV	↑ AChE activity, NO, Malondialdehyde, IL-1β, IL-6, TNF-α Pyramidal cells with shrunken shapes and deeper staining in hippocampus in whole brain	Impaired object recognition memory Spatial memory impairment Decreased retention	[54]
200ng/kg ICV	↑ TNF-α, IL-6, MDA, NO, Protein carbonyl, Caspase1, caspase3, p-tau ↓SOD, GSH, GPx, GR, CAT, AChE, BACE1, ↓ density of CA1 neurons in nissil's staining and ↑ GFAP in hippocampus	Working memory deficit, Decreased discrimination index deficits in associative learning and memory,	[52]
200ng/kg ICV twice with 3-day interval	↑ Aβ40, MDA, ↓GFAP, GPx, LDH Neuronal loss& swelling, nuclear pyknosis, neuronophagia and reduced density of Nissl bodies in hippocampus and cerebral cortex	Impaired Spatial and learning memory	[58]

➢ *Cadmium Induced AD:*

Cadmium (Cd), a toxic environmental pollutant, is a recognized risk factor for accelerating neurodegenerative disorders such as Alzheimer's disease (AD) [59]. Human exposure to Cd occurs through industrial emissions, contaminated food and water, and tobacco smoke [60]. Research indicates that Cd can traverse the blood-brain barrier (BBB) and accumulate in brain tissue, inducing neurotoxicity [59]. Notably, Cd exposure has been associated with elevated Aβ peptide levels and β-amyloid plaque formation, central pathological features of AD [61]. Studies demonstrate that Cd enhances Aβ immunoreactivity in the brain, particularly when combined with elements like arsenic and lead, which synergistically upregulated APP, BACE-1, and PSEN1 expression, promoting Aβ aggregation [59,62]. Additionally, Cd increases Aβ dodecamers in aged mouse brains, which correlate with aging and mild cognitive impairment [14,63]. Mechanistically, Cd induces oxidative stress by elevating reactive oxygen species (ROS) production and suppressing antioxidant defenses, including nuclear factor-erythroid 2-related factor 2 (Nrf2) and hemeoxygenase-1 (HO-1)[64]. It also promotes neuroinflammation via BBB leakage, microglial activation, and immune cell infiltration, triggering pro-inflammatory cytokine release [59]. Furthermore, Cd disrupts neuronal calcium homeostasis, impairing synaptic plasticity and exacerbating AD-related pathology [65].

Cd exposure compromises autophagy, a critical process for degrading misfolded proteins and damaged organelles. By inhibiting autophagosome-lysosome fusion and impairing lysosomal function, Cd reduces cathepsin B (CTSB) activity, leading to defective protein clearance, APP accumulation, and neuronal death [61]. Cd also alters acetylcholinesterase (AChE) activity in the brain, with inhibition causing acetylcholine buildup at synapses, disrupting neuronal signaling [66,67]. Paradoxically, cholinesterase inhibition may confer neuroprotection under certain conditions [68]. Cd activates ROS-dependent AKT/mTOR and mitochondrial apoptotic pathways in neurons, contributing to synaptic and memory deficits [67]. Chronic exposure reduces dopamine and serotonin levels, elevates lipid peroxidation, and induces learning impairments and hyperactive behaviors [66,69]. Collectively, these mechanisms underscore Cd's multifaceted role in neurodegeneration and cognitive decline.

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Table 7 Cadmium-Induced Alzheimer's Model with Biomarkers and Morphological Changes

Model route of administration and dose	Biomarkers & histopathological changes	Behavioral changes	References
5 mg/kg for 2 months orally	Pyramidal layers, shrunken nerve cells with, lost processes, swollen mitochondria and astrocytes, disorganized axons and capillaries in cerebral cortex		[60]
$CdCl_2$50mg/kg/week Orally for 4 weeks	↓ NA, 5-HT, DA ↑ DOPAC and HVA in hippocampus and cerebral cortex	Increased anhedonia, anxiogenic behavior, increased locomotion, poor recognition memory	[66]
$CdCl_2$ 4.5 mg/kg Intraperitoneally for 30 days	↑ LPO, NO ↓ DA, NE, and 5-HT, AChE, GSH, SOD, CAT, GPx, GR mRNA expression of Sod2, CAT, GPx, and Gsr downregulation, Nos2 gene upregulation-inflammatory cell infiltration, deeply stained nuclei indicating deathin cerebral cortex		[67]
$CdCl_2$ 5 mg/kg/day for 21 days orally	↑ MDA, DA, MPO, NO, IL-6 ↓ SOD, AChE, BChE, 5-HT, ↓ Na+/K+-ATPase, CAT, GSH Vacuolation andseparation of purkinje cell layerfrom granular layerin cerebrum		[64]
Cadmium 2.5 mg/kg intraperitoneal 14 days	↑ IL-6, TNF- α, AChE, Adenosine Deaminase Activity ↓ IL-10in Prefrontal cortex		[59]
5 mg/kg orally for 6 weeks daily	↑ MDA, Bax, Caspase-3 ↓ GSH, Bcl2, SNAP-25, synapsin, PSD-95, ↑ p-PTEN (Ser380), p-mTOR (Ser2448) and p-Akt (Thr308) ↓ p-AMPK (Thr172), Shrinkage in pyramidal cells, enlarged glial cellsin hippocampus	Learning and Spatial memory impairment	[70]
5mg/kg Cadmium chloride for 6 days	↑ levels of MDA,IFN-γ and NF-kB ↓SOD, GST, CAT, AChE, MAOin whole brain Hyper cellularity, increased apoptotic cells, vacuolation of neutrophil and few red neurons in cerebral cortex		[69]
6 mg/kg of Cd-solution orally for 21 days	↓ Na+/K+ activity, SOD, ↓AChE, GPx, GSHin whole brain		[68]

➢ *Amyloid β-Induced Toxicity Rat Model:*

Soluble amyloid-beta (Aβ) oligomers, key neurotoxic agents in Alzheimer's disease (AD), disrupt synaptic plasticity and memory by overwhelming Aβ clearance mechanisms, leading to accumulation in monomeric, oligomeric, and fibrillar states [71]. To model AD, researchers employ diverse Aβ administration strategies:

- **Intracerebroventricular (ICV) injections**: Aβ1–42 in rats induces hippocampal neurodegeneration and memory deficits, while repeated oligomeric Aβ injections replicate early AD stages [71,72].
- **Combined models**: Co-administration of Aβ25–35, aluminum trichloride ($AlCl_3$), and thalamic TGF-β1 mimics multifactorial AD pathology [73].
- **Peripheral Aβ effects**: Blood-derived Aβ migrates to the brain, exacerbating cerebral amyloid angiopathy (CAA) and plaque deposition, as shown in parabiosis studies [74].
- Aβ toxicity involves multiple pathways:
- **Oxidative stress**: Triggers ROS, lipid peroxidation, and antioxidant suppression [72].
- **Neuroinflammation**: Activates microglia/astrocytes, releasing neurotoxic nitric oxide [71].
- **Synaptic dysfunction**: Oligomeric Aβ impairs long-term potentiation (LTP), critical for memory [71].
- **Vascular damage**: Aβ-driven amyloid angiopathy disrupts cerebral blood flow, impairing nutrient delivery [74].
- Genetic risk factors like APOE4 accelerate amyloid deposition pre-symptomatically [72]. Methodologically, Aβ fragments are tailored to study specific pathologies:
- **Aβ25–35**: Rapid aggregation for acute toxicity studies.
- **Aβ1–42**: Forms soluble oligomers/protofibrils for chronic memory assessments (Sharma et al., 2016; Sellers et al., 2018).

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Commercial Aβ peptides (e.g., Sigma-Aldrich) are prepared via protocols such as hexafluoroisopropanol (HFIP) solubilization and oligomerization [75], enabling precise modeling of Aβ's role in AD pathogenesis.

Table 8 Amyloid β-Induced Alzheimer's Model with Biomarkers and Morphological Changes

Model and route of administration and dose	Biomarkers & histopathological changes	Behavioral changes	References
5 µl Oligomeric amyloid-β (1–42) peptide Intrahippocampal injection	↑ MDA, TNF-α, IL-1β, ↓ CAT, Bcl-2 ↑ in the mRNA expression of NMDA receptor 2A and 2B subunits, changes in hippocampal circuitry associated with neurodegeneration	Increased number of errors and longer times to complete the maze Increased locomotion	[65]
Amyloid beta (1-40) 5 µg ICV	↑ IL-1β, IL-6, TNF, MPO activity, caspase 3, MDA, Nitrite, NF-kB, AChE, GFAP, DNA fragmentation, Protein carbonyl ↓CAT, SOD, GSH, ↓CA1 pyramidal neurons in hippocampus	Navigation errors Reduction in Discrimination ratio % Fear memory loss Increased working and references memory errors	[76]
Amyloid beta (25-35) Intra hippocampal 6µl for 2 weeks	↓ PS amplitude in LTP induction test ↑ EPSPs in LTP induction test	Decreased memory retention Fear memory loss	[77]
5 µl ICV Human Aβ1–42 peptide induction	↑ Glutamate, DA, 5-HT, NE, cystatin, Kynurenine ↓Melatonin	Impaired social memory and induced anhedonia	[4]
Amyloid beta (1-42) 100ICV	↑ Monocular inflammatory cells in between hepatocytes and abnormal congested blood vessels are detected, necrotic lesions in the liver and kidney ↓Urea	Decreased memory retention Fear memory loss	[72]
Biotin-Ab1–42 (ANA24640) ICV 10 µmol		Object Recognition memory deficit	[78]
Amyloid-β $(Aβ)_{1–42}$ peptide 10 µg ICV		Fear memory loss Impaired Long-term memory and Associative memory	[79]
ICR mice injected 10 µM ICV		Fear memory loss Learning and Spatial memory impairment Object Recognition memory deficit Working memory impairment deficits in associative learning and memory	[80]

➢ *Other Heavy-Metal Induced AD*

Emerging evidence implicates environmental heavy metals, such as lead (Pb) and manganese (Mn), in exacerbating Alzheimer's disease (AD) pathology through mechanisms including oxidative stress, neuroinflammation, amyloid-beta (Aβ) aggregation, and tau hyperphosphorylation [81]. Lead, a pervasive neurotoxicant, demonstrates long-term AD risks: early-life exposure to low Pb levels elevates amyloid precursor protein (APP) and Aβ in aged rats, suggesting latent AD susceptibility [82]. Pb also disrupts the blood-brain barrier, induces neuronal damage, and correlates with cognitive deficits, mirroring AD-associated memory impairments [83]. Clinically, elevated blood Pb levels are frequently observed in AD patients, reinforcing its pathogenic role [83].

Similarly, chronic manganese exposure accelerates amyloid pathology. In 3×Tg-AD mice, prolonged Mn treatment increases cortical and hippocampal Aβ peptides and plaque deposition, driven by microglia-mediated pro-inflammatory cytokine release [84]. Mechanistically, Mn promotes amyloidogenic APP processing and Aβ production, highlighting neuroinflammation-Aβ crosstalk [84]. Both Pb and Mn synergize with intracellular signaling cascades to amplify Aβ aggregation and tau hyperphosphorylation, the latter contributing to neurofibrillary tangle formation [83]. These findings underscore heavy metal-exposed rodent models as pivotal tools for dissecting AD pathways and evaluating therapeutics.

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Table 9 Heavy Metals-Induced Alzheimer's Model with Biomarkers and Morphological Changes

Model and route of administration and dose	Biomarkers & histopathological changes	Behavioral changes	References
Gestational rats, Through drinking water 0.02% of lead acetate (109 ppm)	↓ Glucose metabolism in hippocampus, olfactory bulb and amygdala ↓GLUT 4 protein in the hippocampus of Pb induced offspring ↑ blood Pb levels ↓ fEPSP slope of LTP in the CA1 region, basal and apical dendritic spine density of CA1 region of in hippocampus	Learning and Spatial memory impairment	[85]
C57BL/6J mice & APP/PS1 - 100 ppm Lead acetate in drinking water from 3 weeks of their age until they were 4 months old	↑ Cu concentrations, CTR1(Copper Transporter 1), TNF-α and IL-6, COX17 ↓ Mitochondrial Membrane Potential, PRX3 in hippocampus & serum	learning and memory deficits Spatial memory retention Impaired object recognition memory	[82]
APP/PS1/Tau triple transgenic AD (3×Tg-AD) mice -108 mg $MnCl_2•4H_2O$ dissolved in 300 ml of distilled drinking water) for 5 months	↑ Mn, APP mRNA, APP, Aβ levels, BACE1, PS1, C99, C83, IL-1β, TNF-α ↓ ADAM10 in hippocampus and cerebral cortex		[84]
Mixture of aluminium, cadmium and fluoride 50 mg/kg, 5 mg/kg and 20 mg/ kg, respectively for 90 days orally	↑ TNF-α, IL-1β, IL-16, IL-12, Aβ 40 & 42, AChE, Monoamine oxidase, GSH, GPx, No, Tau protein mRNA, APP mRNA in hippocampus and serum Degenerating pyramidal neurons with pyknotic nuclei, neuron swelling in hippocampus		[81]

➢ *Sodium Azide Induced AD:*

Animal models employing sodium azide (NaN_3) have emerged as valuable tools for studying Alzheimer's disease (AD), particularly to replicate mitochondrial dysfunction and oxidative stress—hallmark features of AD pathology. NaN_3, a potent mitochondrial toxin, inhibits cytochrome c oxidase (COX-IV), a critical enzyme in the electron transport chain. This inhibition disrupts ATP production and elevates reactive oxygen species (ROS), mimicking the bioenergetic deficits and oxidative damage observed in AD neurons [62,86]. Notably, these effects extend to amyloidogenic pathways: NaN_3 increases β-site APP-cleaving enzyme 1 (BACE1) activity, accelerating amyloid-beta (Aβ) accumulation, which further destabilizes mitochondrial dynamics and perpetuates a destructive cycle of neurodegeneration [62,86].

Mitochondrial dysfunction induced by NaN_3 also intersects with tau pathology. By impairing glucose metabolism and activating glycogen synthase kinase-3β (GSK-3β), a kinase that phosphorylates tau and regulates mitochondrial permeability transition pores (mPTP); NaN_3 models replicate the dual Aβ/tau pathology characteristic of AD [87,88]. This disruption of neural bioenergetics leads to neuronal death, astrogliosis, and cytoskeletal breakdown, particularly in the prefrontal cortex (PFC) and hippocampus, regions critical for memory and cognition [89].Importantly, NaN_3's synergistic effects with other toxins, such as ($AlCl_3$), exacerbate AD-like features. Combined exposure amplifies mitochondrial failure, depletes antioxidant defences, and accelerates neurodegeneration, offering a robust model for testing neuroprotective therapies [89]. These models underscore mitochondrial dysfunction as both an early driver and a consequence of AD progression.

Table 10 Sodium Azide-Induced Alzheimer's Model with Biomarkers and Morphological Changes

Model and route of administration and dose	Biomarkers & histopathological changes	Behavioral changes	References
Rats 12.5mg/kg Sodium azide (NaN3) for first 5 days and 10mg/kg for next 9 days, intraperitoneally	↑ APP protein level, BACE1, PS1 positive cells, Aβ (1-42), Tau protein, MDA, NO, AChE, AMP, ADP, Ca ↓ COX-IV, SOD, CAT, GSH, ATP in cerebral cortex and hippocampus	Learning and Spatial memory impairment Spatial confusion	[62]
Rats 0.5 mg/kg/h Sodium azide (NaN3) for 28 days Implanted under skin	↓ number, density and area of BDNF positive cells in the, ACh, SOD, GSH, CAT, mitochondrial COX, NGF ↑ Aβ(1-42) ↓ BDNF positive cells in the hippocampal CA1 region		[86]
Rats 15 mg/kg NaN_3 for 14 days	↓ SOD, GPx, ↑ LDH		[89]

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	Cytoplasmic fragmentation, aggregated nuclear material, pyknotic changes, disorderly pyramidal neurons, clustered cell bodies, extruded cytoplasmic contents, in PFC and Hippocampus		
Rats 20 mg/kg for 5 days orally	↑ MAPT, iNOS, Bax, BCL-2 ↓ 2 (MAP2) ↓brain weight, Neuronal damage, clustered pyknotic pyramidal neurons, Perineural spaces, increased astroglia size		[90]

- *Transgenic Models: The Following Transgenic Models are Discussed in Detail.*

✓ TgF344-AD rats
✓ 5xFAD mice
✓ APP/PS1 mice
✓ 3×Tg-AD
✓ tau P301

Transgenic animal models, especially mice, play a pivotal role in studying Alzheimer's disease (AD) mechanisms and evaluating potential therapies in a living organism. To replicate features of human AD, researchers genetically engineer these models by introducing human genes linked to familial Alzheimer's disease (fAD), such as amyloid-β precursor protein (APP) and presenilin 1 (PS1) or presenilin 2 (PS2), often through overexpression or targeted mutations [10,40]. These models are evaluated for their "face validity" based on how closely their biological or behavioral traits mirror human AD symptoms, and for "construct validity" when they incorporate human APP or PSEN1 genes with specific AD-causing mutations, mimicking the genetic basis of the disease [91].

The genetic background of the mice themselves can influence behavioral outcomes and the progression of amyloid plaque formation, introducing variability in study results [92]. Among the most widely used models, the 5XFAD strain stands out for its rapid and severe pathology. These mice develop amyloid plaques as early as three months of age, reflecting an aggressive, early-onset AD phenotype. In contrast, APPswePS1dE9 mice express a humanized APP gene with the Swedish mutation alongside a PS1 gene lacking exon 9. This combination results in a slower progression of amyloid pathology, offering a model with delayed but robust plaque development [93]. Together, these models provide versatile tools for dissecting disease mechanisms and testing therapeutic interventions at different stages of AD progression.

Table 11 Transgenic Models of Alzheimer's Disease with Biomarkers and Morphological Changes

Model	Onset of AD/ Dose and route Route of administration	Biomarkers & histopathological changes	Behavioral changes	References
TgF344-AD rats	4 months	↑ Iba 1, GFAP, CCK+, Aβ in hippocampus & medial prefrontal cortex	Impaired spatial and learning memory	[94]
APPswePS1ΔE9 mice	6 to 8 months	↑ in Aβ, Iba1, microglia cell counts in cortex and hippocampus	Impaired object recognition memory	[95]
5× FAD Mice	5 months	↓ small vessel density in the cerebral cortical vessels ↓Vessel length density ↑ Aβ plaque density in cerebral cortex		[96]
Tau-P301S	6 months	↑tau hyperphosphorylation in the hippocampal CA1 and DA regions ↑ astrogliosis microgliosis ↑ IL-1β, TNF-α, IKK-β, p-P65/P65 ratio in whole brain	Impaired spatial and learning memory Impaired object recognition memory	[6]
3×Tg-AD mice + Amyloid β induction	8 months 50 mg/kg daily for 8 weeks Intraperitoneally	↓ Synaptophysin Levels, UCP2, MDA ↓ APP, BACE1 in hippocampus	Impaired object recognition memory learning memory impairments decreased discrimination index	[75]
5xFAD + Ethanol	2 months Oral administration of ethanol 5g/kg for 10 consecutive days	↑GFAP immunoreactivity, in all hippocampal subregions (CA1, CA2/3, DG), microglial activity, Ethanol	Impaired spatial and learning memory Lower object discrimination index	[97]

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		exposure- ↑ FJC+ cells in CA1 and DG regions		

- *Biomarkers and their Measurement Methods*

Alzheimer's disease begins its assault on the brain years before memory fades—a silent progression demanding tools to unmask its covert advance. Biomarkers rise to this challenge, serving as molecular sentinels that detect amyloid-β accumulation, tau misfolding, and neuroinflammatory cascades long before cognitive symptoms emerge [96]. In rodent models, these biomarkers take on dual roles: they validate the relevance of experimental paradigms (e.g., Aβ42/40 ratios in transgenic mice, oxidative stress markers in toxin-exposed rats) while compensating for the inherent limitations of each model. For instance, when $AlCl_3$ models fail to replicate neurofibrillary tangles, CSF p-tau assays step in to quantify tauopathy, bridging gaps between artificial systems and human pathology [37]. This synergy transforms biomarkers from passive indicators into active translators, decoding fragmented insights from disparate models into a unified roadmap for therapeutic discovery [8].

III. PATHOLOGICAL BIOMARKERS

A. *Amyloid-β (Aβ):*

Alzheimer's disease (AD) is driven by amyloid-β (Aβ) plaque accumulation and tau tangle formation, as outlined in the amyloid cascade hypothesis [94]. Rodent models, such as APP/PS1 transgenics and toxin-induced paradigms (e.g., $AlCl_3$, LPS), recapitulate Aβ overproduction and tauopathy [37], mirroring the APP processing pathways described in earlier sections [78]. These models reveal a critical insight: biomarkers like Aβ42/40 ratios and phosphorylated tau (p-tau) in biofluids emerge *years* before cognitive deficits, paralleling the silent progression of human AD. For example, PET imaging of amyloid plaques in transgenic mice correlates with CSF Aβ levels (see Table 3), while plasma p-tau217 elevations in toxin-exposed rodents align with synaptic loss. By linking these biomarker trajectories to behavioral outcomes—such as spatial memory deficits in scopolamine models—researchers decode AD's preclinical phase, prioritizing therapeutic strategies (e.g., Aβ immunotherapy, tau kinase inhibitors) for early intervention.

➢ *Methods Adapted to Measure Aβ Protein*

- *ELISA:*

Quantifies Aβ1-42 levels in cortical/hippocampal homogenates and Aβ40/42 ratios in interstitial fluid (ISF) using monoclonal antibodies [98].

- *Histological Staining:*

✓ Congo red: Detects amyloid plaques in hippocampal tissue (Ahmadi et al., n.d.).

✓ X-34: Permeabilized brain sections are stained (10 μM X-34, 40% ethanol, 0.02 M NaOH) and analysed via slide scanning/ImageJ to quantify plaque burden [98].

- *PET Imaging:*

Amyloid radiotracers (e.g., ^{18}F-labeled ligands) map Aβ deposition in vivo, though limited by spatial resolution [96].

B. *Tau Protein:*

Tau, encoded by *MAPT*, stabilizes microtubules in healthy neurons, regulating cytoskeletal integrity and axonal transport [90]. Its activity is modulated by mRNA splicing and post-translational modifications (e.g., phosphorylation), as detailed in earlier sections on tau regulation (see "Mechanisms of Tauopathy"). In Alzheimer's disease (AD), dysregulation of these processes drives tau hyperphosphorylation and aggregation into neurofibrillary tangles (NFTs), disrupting neuronal architecture and accelerating neurodegeneration [62,89]. These pathological changes, mirrored in rodent models like P301L tau mice (refer to Table 5), underscore tau's dual role as both a structural scaffold and a mediator of synaptic failure in AD.

➢ *Methods Adapted to Measure Tau Protein*

- **Tau-PET Imaging:** Utilizes radiotracers (e.g., ^{18}F-flortaucipir) to non-invasively map tau neurofibrillary tangles in living brains, correlating deposition patterns with regional atrophy for AD subtyping [62].
- **Thioflavin-T Staining:** Post-mortem histological technique where Thioflavin-T fluoresces upon binding β-sheet structures in amyloid-beta plaques; indirectly highlights coexisting tau pathology due to Aβ-tau code position [71].
- **TMS-EEG:** Combines transcranial magnetic stimulation (TMS) with electroencephalography (EEG) to assess tau-induced disruptions in cortical excitability and oscillatory activity, predicting therapeutic efficacy [99].
- **Western Blot:** Separates tau isoforms via gel electrophoresis, probing tissue lysates with phosphorylation-specific antibodies (e.g., AT8) to detect hyperphosphorylated tau in NFTs [62].
- **ELISA:** Employs monoclonal antibodies in sandwich assays to quantify total or phosphorylated tau (e.g., p-tau181) in CSF/plasma, critical for early AD diagnosis [15].
- **AT8 Immunohistochemistry**: Labels hyperphosphorylated tau (Ser202/Thr205 epitopes) in fixed brain sections, enabling spatial mapping of NFTs in hippocampal/cortical regions [40].

IV. NEUROINFLAMMATORY BIOMARKERS

A. *TNF-α:*

Tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine, drives neuroinflammation in Alzheimer's disease (AD) by activating TNFR-mediated apoptotic and inflammatory pathways [100]. TNF-α exacerbates Aβ plaque formation and tau hyperphosphorylation [3], while Aβ accumulation itself triggers microglial/astrocytic TNF-α release—a destructive

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feedback loop amplifying neurodegeneration [81]. These mechanisms, detailed in earlier sections on neuroinflammation (see "Neuroinflammatory Pathways in AD"), position TNF-α as a critical mediator linking Aβ toxicity, tau pathology, and synaptic loss.

➢ *Methods Adapted to Measure TNF-α*

- **ELISA**: Quantifies TNF-α concentrations in hippocampal homogenates, serum, or brain lysates using standardized kits. Results are expressed as µg TNF-α/mg total protein [101].

➢ *Immunohistochemistry (IHC):*

- **Tissue Preparation**: Paraffin-embedded rat brain sections (4 µm) are deparaffinized (xylene/ethanol) and undergo antigen retrieval in citrate buffer (100°C).
- **Blocking & Staining**: Sections are blocked with 3% goat serum/PBS-Triton X-100, then incubated with primary antibodies (72 hours, 4°C) and FITC/TRITC-labelled secondary antibodies (2 hours).
- **Signal Amplification**: Biotinylated secondary antibodies and avidin-biotin-HRP/DAB enhance signals.
- **Imaging**: Counterstained sections are visualized via confocal microscopy to localize TNF-α [97].

B. IL1β& IL6:

IL-1β and IL6are a pro-inflammatory cytokine [51]. In the aged brain, over-stimulated proinflammatory cytokines have been produced [93].The pathogenicity of early-stage AD is mostly attributable to the inflammatory response [3].

➢ *Methods Adapted to measureIL-1β & IL-6*

- ELISA kits are used to determine IL-1β and IL-6 concentrations in tissue homogenates [59].
- IHC can be used to evaluate the levels of IL-1β and IL-6[102].
- Western blot analysis is used to detect the expression of inflammatory markers [28].

C. GFAP (Glial Fibrillary Acidic Protein):

Glial fibrillary acidic protein (GFAP), a marker of astrocyte activation, reflects reactive gliosis in Alzheimer's disease (AD). While hyperactive, pro-inflammatory astrocytes exacerbate neurodegeneration (as outlined in neuroinflammatory pathways), hypoactive astrocytes compromise neuronal support, and worsening synaptic dysfunction. Elevated GFAP levels correlate with microglial activation and spatial learning deficits in AD models [94], reinforcing its role as a biomarker of glial-driven pathology [97].

➢ *Methods Adapted to Measure GFAP*

- *Immunohistochemistry (IHC):*

✓ **Tissue Preparation:** Coronally sliced brain tissue is sectioned (free-floating), deparaffinized, and subjected to antigen retrieval in citrate buffer (100°C).

✓ **Staining:** Blocking with 3% goat serum/PBS-Triton X-100 to reduce non-specific binding.

Incubation with rabbit anti-GFAP primary antibody, followed by biotinylated goat anti-rabbit secondary antibody. Signal amplification using Avidin-Biotin Complex (ABC) and visualization with 3,3'-Diaminobenzidine (DAB).

✓ **Imaging & Analysis:** Confocal microscopy quantifies GFAP+ pixels in brain tissue [97].

- *Western Blot:*

✓ **Protein Extraction & Separation**: Proteins extracted from brain tissue are separated via SDS-PAGE gel electrophoresis [13,24].

Transfer to PVDF/nitrocellulose membranes using semi-dry/wet methods.

✓ **Detection:** Membranes blocked (e.g., 5% BSA) to minimize non-specific binding.

Incubation with GFAP-specific primary antibodies (overnight, 4°C) and HRP-conjugated secondary antibodies.

✓ **Quantification:** Protein bands visualized via chemiluminescence and quantified using densitometry (e.g., ImageJ). Normalized to loading controls (e.g., β-actin) [30].

D. Iba1:

Iba1 (also known as allograft inflammatory factor 1 (AIF-1)) is used as a marker for microglial activation1. Increased Iba1 expression in brain tissue of AD patients indicates microglial activation [95].

➢ *Methods Adapted to Measure Iba1*

- **Western Blot:** Brain lysates are separated via SDS-PAGE, transferred to membranes, and probed with anti-Iba-1 primary antibodies. Immunoreactive bands are visualized using chemiluminescence and quantified via densitometry [6].
- **Stereological Cell Counting:** Iba-1+ microglia in brain sections are counted using Stereo Investigator software. Sections are imaged under a microscope, and unbiased stereological parameters (e.g., grid size, counting frame) are applied to estimate cell density [94].
- **Immunohistochemistry (IHC):** Brain sections are incubated with Iba-1 primary antibodies, followed by fluorophore-/HRP-conjugated secondary antibodies. Stained sections are imaged via microscopy, and Iba-1+ area is quantified using ImageJ [95].
- **Flow Cytometry:** Microglia are isolated, fixed, and stained with Annexin V-FITC (apoptosis marker) and propidium iodide (viability dye). Iba-1 expression is quantified using fluorescently labeled antibodies and flow cytometric analysis [103].

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V. OXIDATIVE STRESS MARKERS

A. Superoxide Dismutase (SOD):

Superoxide dismutase (SOD), a primary antioxidant enzyme, scavenges free radicals by catalyzing the dismutation of superoxide radicals into hydrogen peroxide (H_2O_2) and molecular oxygen. This activity mitigates oxidative stress, a key contributor to neurological disorders such as Alzheimer's disease [49,62].

➢ *Methods Adapted to Measure SOD*

- *Spectrophotometric Assays*

✓ **NBT Method:** Superoxide reduces nitro blue tetrazolium (NBT) to blue formazan. SOD activity is calculated as the protein concentration required to inhibit 50% of NBT reduction. Absorbance is measured at 560 nm, with results expressed as µmol/min/mg protein [12,15].
✓ **Pyrogallol Assay**: Auto-oxidation of pyrogallol in potassium phosphate buffer is monitored at 325 nm for 3 minutes. SOD activity is reported in U/mg protein [25].
✓ **Xanthine Oxidase Assay:** Supernatants are incubated with xanthine oxidase and xanthine in phosphate buffer (30 minutes), measuring superoxide-driven reactions [15].
✓ **Hydroxylamine Auto-Oxidation:** Hydroxylamine, EDTA, sodium carbonate, and NBT react in a system where absorbance at 560 nm is recorded every 30 seconds for 2 minutes [49].

- *Native Page:*

Brain extracts are separated on non-denaturing polyacrylamide gels. Gels are stained with NBT, riboflavin, and TEMED, then exposed to light to visualize achromatic SOD bands against a blue formazan background [71].

B. Catalase (CAT):

Catalase (CAT) is an enzyme that neutralizes hydrogen peroxide (H_2O_2) by converting it into water (H_2O) and oxygen (O_2), utilizing manganese or iron as a cofactor [3]. This process supports neuroprotection by reducing oxidative stress and maintaining molecular homeostasis in the central nervous system (CNS), as noted by [92].

➢ *Methods Adapted to Measure CAT*

- To measure CAT activity, researchers commonly employ a spectrophotometric method that tracks the decomposition of H_2O_2 at 240 nm. In this approach, a reaction mixture containing phosphate buffer and brain homogenate supernatant is prepared, and the decline in absorbance over time is recorded to determine enzymatic activity [25].

- Colorimetric test kits can assess total antioxidant capacity (TAC) in brain homogenate samples, offering a streamlined method for evaluating oxidative stress markers [104].

C. Glutathione (GSH):

GSH a tripeptide composed of glutamic acid, cysteine, and glycine, is a critical antioxidant that protects cells from oxidative damage [25]. It functions as a primary defense mechanism against oxidative stress by directly neutralizing free radicals or serving as a substrate for glutathione peroxidase, an enzyme that detoxifies hydrogen peroxide (H_2O_2). Present in both reduced (active) and oxidized forms, GSH participates in various cellular processes, ensuring the mitigation of oxidative damage and supporting cellular homeostasis [7].

➢ *Methods Adapted to Measure GSH*

- GSH levels are quantified by reacting the sample with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), using reduced glutathione as a reference standard. Absorbance is measured at 412 nm to determine GSH concentration [105].
- **Ellman's method:** This method employs a reaction mixture containing tissue homogenate supernatant, sodium phosphate buffer, and DTNB. Absorbance changes at 412 nm are monitored to assess GSH activity, reflecting its antioxidant capacity [25].
- **Colorimetric assay kits:** Commercial glutathione assay kits provide a standardized approach to measure endogenous antioxidant GSH levels, enabling rapid and reproducible quantification without extensive sample preparation [105].

D. Malondialdehyde (MDA):

Increased MDA levels indicate the extent of lipid peroxidation in the brain. In age-related neurodegenerative disease, lipids and proteins are primary target components that undergo lipid modification by free radicals[25].

➢ *Methods Adapted to Measure MDA*

- *Spectrophotometric Assays (TBA Reaction)*

✓ **Method A**: Tissue homogenate is reacted with thiobarbituric acid (TBA) in acidic conditions (95°C), and absorbance is measured at 534 nm [104].
✓ **Method B**: Homogenate mixed with SDS, acetic acid, and TBA is incubated (95°C), centrifuged with n-butanol/pyridine, and the organic layer's absorbance is read at 532 nm [25].
✓ **Method C**: Supernatant combined with TCA, glacial acetic acid, and TBA is boiled (15 min), cooled, and measured at 532 nm [12].
✓ **Method D**: TBARS solution is added to supernatant, boiled, cooled on ice, centrifuged, and analyzed at 532 nm [15].
✓ Calculation: MDA concentration is determined using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$, expressed as nmol/mg protein [12].

- *Colorimetric Kits:*

Pre-packaged kits (e.g., Biodiagnostic Company, Egypt) quantify MDA in brain homogenate via standardized protocols [104].

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- *HPLC:*

High-performance liquid chromatography (HPLC) separates and quantifies MDA with high specificity [15].

E. Nitric Oxide (NO):

NO is a free radical that can combine with an O_2• anion to produce peroxynitrite (ONOO-), which can damage and destroy neurons by attacking many biological molecules [62]. NaN_3-induced oxidative stress occurs through excessive synthesis of NO. It may be one reactive radical involved in degenerative cascades leading to neuronal cell death [90].

➢ *Methods Adapted to Measure NO*

- **Colorimetric Assay with Griess Reagent:** Determine nitrite accumulation, an indicator of nitric oxide production, by using a colorimetric assay with Griess reagent. In this method, supernatant from hippocampal homogenate and serum are mixed with Griess reagent, incubated, and then absorbance is read spectrophotometrically at 550 nm [106].

VI. SYNAPTIC AND NEURONAL BIOMARKERS

A. BDNF:

Brain-derived neurotrophic factor (BDNF), a critical protein involved in neurogenesis and synaptic plasticity, plays a pivotal role in AD pathogenesis. It supports neuronal survival, enhances learning and memory, and contributes to visual recognition memory via the BDNF/CREB signaling pathway. Notably, studies demonstrate that conditional BDNF delivery from astrocytes can rescue memory deficits, highlighting its therapeutic potential in mitigating AD-related cognitive decline [22].

➢ *Methods Adapted to Measure BDNF*

- **ELISA:** Cytokine levels are quantified using an ELISA plate reader. Tissue samples are first incubated with primary antibodies, followed by peroxidase-conjugated secondary antibodies, enabling colorimetric detection of target proteins [59].
- **Western blot:** The levels of the target protein BDNF can be determined using western blot, with β-actin serving as a loading control. Densitometric analysis can be performed using a gel image analysis program [107]. The data are corrected by background subtraction and normalized against beta-actin [13].
- **Quantitative RT-PCR (qPCR):** The total RNA of brain tissue is extracted, and cDNA synthesis is completed. Quantitative PCR is then carried out. The target mRNA expression is normalized to ACTB, and the results are calculated using an equation [104].

B. AChE:

Acetylcholinesterase (AChE) is an enzyme responsible for breaking down acetylcholine (ACh), a neurotransmitter critical for synaptic signaling in the nervous system [59]. In Alzheimer's disease, AChE activity is significantly elevated in the brain [14]. This hyperactivity depletes ACh levels, impairing cholinergic neurotransmission and contributing to central nervous system dysfunction. The resulting deficits in synaptic communication correlate with declines in learning, memory, and cognitive performance [12].

➢ *Methods Adapted to Measure AChE*

- **Ellman's method:** AChE activity is determined calorimetrically by measuring the absorbance of a yellow product formed when thiocholine (released from acetylthiocholine hydrolysis) reacts with dithiobisnitrobenzoate (DTNB). Absorbance is read at 412 nm [12].
- **Microplate Reader Assay:** Activity is quantified in 96-well plates by monitoring absorbance changes over time. Enzyme activity is calculated based on reaction kinetics [59].
- **ELISA Method:** Commercially available ELISA kits are used to assay AChE activity in tissue samples[22].
- High-performance liquid chromatography (HPLC) measures ACh levels in samples, providing indirect insights into cholinergic activity[15].

C. Choline Acetyltransferase (ChAT):

ChATa key enzyme in learning and memory processes, catalyzes the synthesis of acetylcholine from choline and acetyl-CoA [9]. Acetylcholine, the neurotransmitter produced through this reaction, plays a critical role in modulating memory formation and learning[16]. Studies demonstrate that spatial memory training enhances acetylcholine release in the brain, with elevated levels strongly correlating with improved performance in spatial memory tasks [108].

➢ *Methods Adapted to Measure ChAT*

- ChAT activity is assayed by quantifying acetylcholine (ACh) synthesis via the enzymatic reaction: Acetyl-CoA + choline → ACh + Coenzyme A. The reaction's progress is measured spectrophotometrically at an optical density of 324 nm, with enzyme activity expressed as units per gram of wet tissue [108].

- *Western Blot*

✓ **Sample Preparation**: Tissue/cell lysates are prepared in lysis buffer (protease/phosphatase inhibitors), centrifuged, and supernatants collected. Total protein is quantified using BCA or Bradford assays.
✓ **Electrophoresis & Transfer**: Proteins are separated via SDS-PAGE and transferred to PVDF/nitrocellulose membranes using semi-dry or wet transfer systems.
✓ **Antibody Incubation**: Membranes are incubated with primary antibodies (overnight, 4°C), washed, and treated with HRP-conjugated secondary antibodies.
✓ **Detection & Analysis**: Protein bands are visualized using enhanced chemiluminescence (ECL) and imaged with a CCD camera. Band intensity is quantified via densitometry (e.g., Image J) and normalized to β-actin/GAPDH [39].

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D. BuChE:

BuChE (pseudocholinesterase) and acetylcholinesterase (AChE) are both enzymes in the cholinesterase family. While AChE is well-characterized for its primary role in hydrolyzing acetylcholine, BuChE's physiological substrates—such as butyrylcholine and its precise biological functions remain less clearly understood compared to AChE [3]. Despite these differences, both enzymes contribute to acetylcholine catabolism, a process critical to regulating neurological functions, including learning, memory, and higher-order cognitive processes [49].

➢ *Methods Adapted to Measure BuChE*

- **Ellman's Colorimetric Assay:** Standard Protocol: Hydrolysis of butyrylthiocholine iodide is measured at 412 nm to quantify BuChE activity [109].
- Modified Protocol: Cholinergic biomarkers, including BuChE, are assessed at 450 nm using optimized conditions [3].
- **Enzyme Kinetics:** Lineweaver-Burk Plots: Analyze inhibition mechanisms (competitive/non-competitive) by plotting 1/[S] vs. 1/V to determine inhibition constants (Ki, αKi) [109].
- **Inhibition Studies:** IC_{50}: Concentration of inhibitor required to reduce BuChE activity by 50%.
- Percent Inhibition: Activity reduction at fixed inhibitor concentrations, evaluating efficacy and off-target effects [109].

E. Dopamine (DA):

Dopamine (DA), a key regulator of cognition and emotion, is implicated in Alzheimer's disease (AD) progression through its interaction with amyloid-beta (Aβ) aggregation pathways [37], as detailed in earlier sections on neurotransmitter crosstalk (see "Aβ and Synaptic Dysfunction"). Dysregulated dopaminergic signaling also underlies neuropsychiatric comorbidities like psychosis and major depressive disorder (MDD) [4,15], highlighting its dual role in neurodegeneration and mood regulation. These insights position dopaminergic modulators as potential therapeutics for AD-related cognitive and behavioral deficits.

➢ *Methods Adapted to Measure DA*

- **HPLC-ECD:** Brain tissue is homogenized in HPLC-grade methanol, centrifuged, and the supernatant stored at -80°C. DA levels are quantified using high-performance liquid chromatography with electrochemical detection [14,104].
- **UPLC-MS/MS:** Separation is achieved using a dedicated column with a mobile phase of formic acid–water/methanol–water. Parameters such as MRM transitions, cone voltages, and collision energies are optimized for sensitivity [14].
- **LC-MS/MS:** Hippocampal samples are homogenized in ice-cold formic acid-methanol, vortexed, centrifuged, and analyzed via LC-MS/MS for precise DA quantification [22].

F. Gamma-Aminobutyric Acid (GABA):

GABA is a crucial inhibitory neurotransmitter within the central nervous system. GABA is a key inhibitory neurotransmitter in the central nervous system [15].Severe Alzheimer's disease cases are associated with notable decreases in GABA concentration, potentially contributing to behavioral and psychiatric symptoms [15]. The endogenous amyloid precursor protein (APP) is highly expressed in a subset of GABAergic interneurons in the hippocampus, potentially contributing to AD plaque pathology [94].

➢ *Methods Adapted to Measure GABA*

- *Chromatography-Based Methods*

✓ UHPLC-Q-TOF-MS: Ultra high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry analyzes neurotransmitters, including GABA, with high resolution [22].
✓ LC-MS/MS: Quantifies GABA in rat hippocampal tissue using a single-run protocol. Samples are homogenized under conditions preventing degradation/oxidation, and results are normalized to total protein concentration [22].
✓ Spectrofluorometric Assay: GABA derivatized with o-phthalaldehyde/mercaptopropionic acid emits fluorescence, enabling hypothalamic GABA quantification [14].

- **ELISA:** Commercial kits measure GABA levels in biological samples, with results normalized to protein content [3].
- **qPCR:** Assesses mRNA expression of GABAergic system components (e.g., GAD67, GABA receptors) to infer synaptic GABA dynamics.

G. Serotonin:

Serotonin a monoamine, it is also called 5-hydroxytryptamine (5HT) [104]. Serotonin is associated with sleep-wake regulation [110]. Serotonin affects functions in the central nervous system, such as alertness, wakefulness, and aggression[45]. Loss of serotonin 5-HT2A receptors in the postmortem temporal cortex correlates with rate of cognitive decline in Alzheimer's disease [111].

➢ *Methods Adapted to Measure Serotonin*

- **HPLC**: Serotonin levels can be determined by HPLC with electrochemical detection (HPLC-ECD) [14].
- **UPLC-MS/MS (Ultra-Performance LC system with a Xevo TQ MS spectrometer)**: This method involves chromatographic separation using a Venusil ASB C18 HPLC column and a mobile phase consisting of formic acid–water and methyl alcohol–water [37].
- **ELISA (Enzyme-Linked Immunosorbent Assay):** Although not specified for serotonin, ELISA is used to measure other biochemical markers related to Alzheimer's disease [14].
- **Spectrofluorometric method:** The cortex and cerebellum are separated, weighed, and processed for 5-HT estimations. Rat brain cortex and cerebellum are homogenized in cold acidified m-butanol using glass

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homogenizer tubes and a motor-driven Teflon pestle. The homogenate is then centrifuged. An aliquot of the butanol is then used for analysis [112].

VII. GENETIC AND EPIGENETIC BIOMARKERS

A. Amyloid Precursor Protein mRNA (mAPP):

Alzheimer's disease (AD) is driven by dysregulated amyloid precursor protein (APP) processing, which favors amyloidogenic cleavage by β- and γ-secretases to generate neurotoxic Aβ peptides [113]. These peptides aggregate into plaques, disrupting synaptic function (see "Synaptic Dysfunction in AD") and triggering neuroinflammatory cascades. Notably, APP expression varies regionally, with CA1 pyramidal neurons exhibiting ~1.7-fold higher levels than dentate gyrus granule cells [114]. Pathogenic APP mutations (e.g., Swedish, Arctic) exacerbate Aβ production and plaque deposition [115], accelerating dendritic atrophy and neuronal loss, processes detailed in earlier sections on AD progression.

➢ *Methods Adopted to measureAPP mRNA*

- *Quantitative PCR (qPCR) for APP mRNA Analysis*

✓ **Tissue Preparation:** Total RNA is extracted from homogenized brain tissue (hippocampus/prefrontal cortex) using Trizol, with purity/concentration evaluated via Nanodrop (A260) [16,44].
✓ **cDNA Synthesis:** RNA (1 μg) is reverse-transcribed using kits containing $MgCl_2$, dNTPs, oligo-dT primers, RNase inhibitor, and reverse transcriptase. RNA is pre-incubated at 70°C to disrupt secondary structures, followed by synthesis at 42°C [21].
✓ **qPCR Assay:**Primers: APP-specific primers (e.g., F: 5′-GGATGCGGAGTTCGGACATG-3′; R: 5′-GTTCTGCATCTGCTCAAAG-3′) amplify target sequences [21,116].
✓ Reagents: SYBR Green/ROX master mix (e.g., Bio-Rad CFX Connect systems).
✓ **Cycling Conditions:** Initial denaturation: 95°C, 1–10 min.
✓ Amplification: 30–40 cycles of denaturation (94–95°C), annealing (54–60°C), and extension (72°C).
✓ Final elongation: 72°C, 5–10 min (Khalaf et al., 2019; Sarathlal et al., 2021).
✓ **Data Analysis**: Relative Quantification: $2^{-\Delta\Delta CT}$ method normalizes APP CT values to reference genes (e.g., GAPDH, IMPDH2) [21].

B. Tau protein mRNA:

As detailed in earlier sections on tauopathy, hyperphosphorylated tau destabilizes microtubules, forming neurofibrillary tangles (NFTs) that disrupt neuronal integrity and synaptic function [40]. These aggregates, hallmark features of AD, correlate with neurodegeneration and cognitive decline, reinforcing tau's role in disease progression [20,37].

➢ *Methods Adapted to Measure Tau mRNA*

Tau (MAPT) mRNA levels are quantified in brain regions like the hippocampus or prefrontal cortex using qPCR [44].

- **RNA Extraction**: Total RNA is isolated from homogenized tissue using TRIzol or RNeasy Mini kits, with genomic DNA removed via on-column DNase treatment. RNA concentration is determined spectrophotometrically (Nanodrop) at 260 nm [21,44].
- **cDNA Synthesis**: RNA (500 ng–1 μg) is reverse-transcribed using kits containing oligo-dT primers, dNTPs, MgCl2, and reverse transcriptase. Incubation steps include 70°C (10 min) to eliminate RNA secondary structures and 42°C (1 hr) for cDNA synthesis [21,44].
- **qPCR Assay**: Amplification is performed on systems like ABI PRISM or StepOne using SYBR Green kits and gene-specific primers [44].
- **PCR Conditions**: Protocols include initial denaturation (95°C, 10 min), 40 cycles of denaturation (95°C, 15 s) and annealing/extension (60°C, 60 s), followed by melting curve analysis (60–95°C)[113].
- **Primers**: MAPT-specific primers target mRNA sequences [41].
- **Data Analysis**: Relative expression is calculated using the $2^{-\Delta\Delta CT}$ method, normalized to reference genes (e.g., GAPDH) and MIQE guidelines [21,113].

VIII. CONCLUSION

Rodent models remain indispensable in Alzheimer's disease (AD) research, offering mechanistic insights into amyloidogenesis, tauopathy, neuroinflammation, and mitochondrial dysfunction, yet their true value lies in their integration with biomarker discovery, therapeutic innovation, and translational rigor. While sodium azide (NaN_3) or cadmium models elucidate mitochondrial ROS-driven pathology for testing antioxidants, and transgenic systems (e.g., 5xFAD) or Aβ-infused rodents replicate amyloid/tau cascades for evaluating anti-aggregation therapies, these models must be contextualized alongside biomarkers such as CSF p-tau, plasma Aβ42/40 ratios, and neuroimaging correlates (e.g., PET-detected amyloid) to bridge preclinical findings to human diagnostics. Environmental toxin models (e.g., aluminum, lead) directly link AD risk to pollutant exposure, enabling studies on detoxification strategies, while LPS or STZ paradigms highlight neuroinflammation-cytokine interplay for screening immunomodulators. However, species-specific limitations, such as rodents' abbreviated lifespan, divergent tau isoforms, and lack of spontaneous amyloidosis, underscore the need for complementary approaches, including humanized models (e.g., iPSC-derived neuron xenografts) and multi-omics profiling to capture AD's genetic-environmental complexity.

Emerging technologies like CRISPR-edited risk-gene models, in vivo two-photon imaging, and AI-driven behavioral analysis refine mechanistic precision, while standardized protocols for biomarker quantification (e.g., harmonized ELISA kits, MRI parameters) must address reproducibility gaps. Ethically, balancing rapid induction

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(e.g., scopolamine's acute deficits) with chronic, progressive models (aged transgenics) ensures alignment with AD's temporal dynamics. Looking ahead, hybrid paradigms—merging toxin exposure with genetic susceptibility—and patient-derived models will better mimic sporadic AD's multifactorial etiology, accelerating therapies tailored to individualized risk profiles. Ultimately, advancing AD research demands a synergistic triad: *mechanistically precise models, clinically validated biomarkers, and ethically grounded innovation*, ensuring preclinical breakthroughs translate into meaningful patient outcomes.

IX. SUMMARY

- **Model Limitations**: No single model fully captures AD; hybrid paradigms better reflect human disease complexity.
- **Biomarker Utility**: CSF p-tau and plasma Aβ42/40 ratios bridge preclinical and clinical research.
- **Environmental Links**: Heavy metals (e.g., cadmium) and mitochondrial toxins highlight environmental AD risks.
- **Translational Tools**: Emerging tools like CRISPR and patient-derived models enhance translational potential.

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ABBREVIATIONS

- **AD** – Alzheimer's Disease
- **Aβ** – Amyloid-beta
- **NFTs** – Neurofibrillary Tangles
- **APP** – Amyloid Precursor Protein
- **PS1/PS2** – Presenilin 1/Presenilin 2
- **BACE1** – Beta-site APP Cleaving Enzyme 1
- **CSF** – Cerebrospinal Fluid
- **PET** – Positron Emission Tomography
- **MRI** – Magnetic Resonance Imaging
- **LPS** – Lipopolysaccharide
- **STZ** – Streptozotocin
- **ICV** – Intracerebroventricular
- **ELISA** – Enzyme-Linked Immunosorbent Assay
- **qPCR** – Quantitative Polymerase Chain Reaction
- **HPLC** – High-Performance Liquid Chromatography
- **BDNF** – Brain-Derived Neurotrophic Factor
- **AChE** – Acetylcholinesterase
- **ChAT** – Choline Acetyltransferase
- **BuChE** – Butyrylcholinesterase
- **GABA** – Gamma-Aminobutyric Acid
- **5-HT** – 5-Hydroxytryptamine (Serotonin)
- **MDA** – Malondialdehyde
- **SOD** – Superoxide Dismutase
- **CAT** – Catalase
- **GSH** – Glutathione
- **GFAP** – Glial Fibrillary Acidic Protein
- **Iba1** – Ionized Calcium-Binding Adapter Molecule 1
- **TNF-α** – Tumor Necrosis Factor-alpha
- **IL-1β/IL-6** – Interleukin-1 beta/Interleukin-6
- **NF-κB** – Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
- **LTP** – Long-Term Potentiation
- **ROS** – Reactive Oxygen Species
- **RNS** – Reactive Nitrogen Species
- **DA** – Dopamine
- **NE** – Norepinephrine
- **GSSG** – Oxidized Glutathione
- **GPx** – Glutathione Peroxidase
- **GR** – Glutathione Reductase
- **GST** – Glutathione S-Transferase
- **Nrf2** – Nuclear Factor Erythroid 2–Related Factor 2
- **HO-1** – Heme Oxygenase-1
- **GSK-3β** – Glycogen Synthase Kinase-3 beta
- **CDK5** – Cyclin-Dependent Kinase 5
- **mTOR** – Mechanistic Target of Rapamycin
- **AMPK** – AMP-Activated Protein Kinase
- **MAPK** – Mitogen-Activated Protein Kinase
- **TLR4** – Toll-Like Receptor 4
- **RAGE** – Receptor for Advanced Glycation Endproducts
- **HMGB1** – High Mobility Group Box 1
- **iNOS** – Inducible Nitric Oxide Synthase
- **COX-2** – Cyclooxygenase-2
- **CAA** – Cerebral Amyloid Angiopathy
- **APOE4** – Apolipoprotein E epsilon 4 allele
- **iPSC** – Induced Pluripotent Stem Cells
- **CRISPR** – Clustered Regularly Interspaced Short Palindromic Repeats
- **EEG** – Electroencephalography
- **TMS** – Transcranial Magnetic Stimulation
- **BBB** – Blood-Brain Barrier
- **CNS** – Central Nervous System
- **PNS** – Peripheral Nervous System
- **GWAS** – Genome-Wide Association Study
- **LC-MS/MS** – Liquid Chromatography-Tandem Mass Spectrometry
- **UPLC-MS/MS** – Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry
- **FACS** – Fluorescence-Activated Cell Sorting
- **MACS** – Magnetic-Activated Cell Sorting
- **PP2A** – Protein Phosphatase 2A
- **Bcl-2** – B-cell lymphoma 2
- **Bax** – Bcl-2-associated X protein
- **NEP** – Neprilysin
- **CHOP** – C/EBP Homologous Protein

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- **LRP1** – Low-Density Lipoprotein Receptor-Related Protein 1
- **MAO** – Monoamine Oxidase
- **CTSB** – Cathepsin B
- **ADAM-17** – A Disintegrin and Metalloproteinase 17
- **Seladin-1** – Selective Alzheimer's Disease Indicator-1
- **MPO** – Myeloperoxidase
- **MHC-II** – Major Histocompatibility Complex Class II
- **MAPT** – Microtubule-Associated Protein Tau
- **NGF** – Nerve Growth Factor
- **VEGF** – Vascular Endothelial Growth Factor
- **CREB** – cAMP Response Element-Binding Protein
- **SNAP-25** – Synaptosomal-Associated Protein 25
- **PSD-95** – Postsynaptic Density Protein 95
- **Synapsin** – Synaptic vesicle protein
- **UCP2** – Uncoupling Protein 2
- **GSK3α** – Glycogen Synthase Kinase 3 alpha
- **FJC** – Fluoro-Jade C
- **ICR** – Institute of Cancer Research (mice strain)
- **mAChR** – Muscarinic Acetylcholine Receptor
- **MCP-1** – Monocyte Chemoattractant Protein-1
- **LPO** – Lipid Peroxidation
- **LDH** – Lactate Dehydrogenase
- **CYP2E1** – Cytochrome P450 2E1
- **IL-10/12/18** – Interleukin-10/12/18
- **COX-IV** – Cytochrome c Oxidase Subunit IV
- **PP1** – Protein Phosphatase 1

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