REVIEW

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Alcohol-induced structural and cellular brain alterations: molecular and histopathological mechanisms

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Abstract

Chronic alcohol consumption is a leading cause of acquired neurodegeneration with well-documented structural and ultrastructural brain alterations. This review analyzes the cellular and molecular mechanisms underlying alcohol neurotoxicity, integrating findings from animal models, human *post-mortem* studies, and neuroimaging investigations. Ethanol crosses the blood–brain barrier and generates toxic metabolites including acetaldehyde and reactive oxygen species, triggering oxidative stress, lipid peroxidation, and mitochondrial dysfunction. Chronic exposure induces glutamatergic and gamma-aminobutyric acid (GABA)ergic adaptations leading to excitotoxicity during withdrawal. Cell death occurs through apoptotic, necrotic, and necroptotic pathways, while microglial and astrocytic activation perpetuates neuroinflammation. Histopathological (HP) changes include selective neuronal loss in the prefrontal cortex, hippocampus, and cerebellum, dendritic simplification, and synaptic alterations. White matter pathology manifests as demyelination and axonal degeneration. Associated thiamine deficiency produces characteristic lesions in the mammillary bodies, thalamus, and cerebellar vermis. Neuroimaging techniques provide valuable HP correlates and biomarkers for disease monitoring. While some changes demonstrate partial reversibility with abstinence through remyelination and synaptic plasticity, extensive neuronal loss remains irreversible. Understanding these mechanisms is essential for developing neuroprotective therapeutic strategies.

Keywords: alcohol, neurotoxicity, neurodegeneration, oxidative stress, excitotoxicity, neuroinflammation.

Introduction

Chronic alcohol consumption is a leading cause of acquired neurodegeneration worldwide, with well-documented structural and ultrastructural brain changes observed through histological and neuroimaging studies [1]. Ethanol readily crosses the blood–brain barrier (BBB) owing to its amphiphilic properties (octanol/water partition coefficient ~0.5), achieving tissue concentrations equivalent to plasma levels within 6–10 minutes of ingestion [2].

At the cellular level, alcohol neurotoxicity involves disruption of membrane ionic homeostasis, mitochondrial (MT) dysfunction with energy collapse, activation of intrinsic and extrinsic apoptotic pathways, and neuronal cytoskeletal disorganization [3]. These processes manifest histologically as selective neuronal loss, Wallerian and non-Wallerian axonal degeneration, segmental demyelination, reactive astrogliosis, and persistent microglial activation [4].

Beyond direct neurotoxic effects, chronic alcohol exposure interacts with multiple pathophysiological processes that amplify neurological vulnerability. Clinical observations demonstrate significant biochemical alterations in patients with comorbid mental illness and alcohol use disorder, including disrupted neurotransmitter systems and oxidative stress markers [5]. Systemically, alcohol-induced cardiovascular dysfunction, ranging from hypertension and cardiomyopathy to arrhythmias, compromises cerebral perfusion and contributes to vascular cognitive impairment [6]. Metabolic disturbances associated with chronic alcohol use, including dysregulated glucose homeostasis and lipid metabolism, further exacerbate neuronal injury through mechanisms involving insulin resistance and inflammatory signaling [7]. Importantly, alcohol-related immune dysregulation may accelerate neurodegenerative processes through systemic inflammation and BBB compromise [8]. Moreover, experimental evidence suggests that acute

inflammatory insults, such as sepsis, can synergistically promote pathological protein aggregation and neuronal loss in vulnerable brain regions, highlighting the complex interplay between alcohol neurotoxicity, systemic inflammation, and neurodegenerative cascades [9].

This review provides a comprehensive analysis of the cellular and molecular mechanisms underlying alcohol neurotoxicity, with emphasis on histopathological (HP) and ultrastructural changes in the central nervous system (CNS). We integrate findings from animal models, human *post-mortem* studies, and advanced neuroimaging investigations.

Molecular mechanisms of alcohol neurotoxicity

Ethanol metabolism and generation of toxic metabolites

The brain metabolizes ethanol through pathways that partially overlap with hepatic metabolism yet exhibit distinct features with particular relevance to neurotoxicity. Understanding these pathways has been a focus of extensive research over the past decades [10].

Alcohol dehydrogenase pathway

Early studies demonstrated limited cerebral alcohol dehydrogenase (ADH) expression compared to the liver; subsequent immunohistochemical (IHC) investigations revealed that ADH1B and ADH1C isoforms are expressed in both neurons and astrocytes [11, 12]. This pathway converts ethanol to acetaldehyde, a highly reactive compound recognized for its direct cytotoxic properties [13]. Research established that acetaldehyde forms protein adducts through reactions with free amino groups, particularly lysine and cysteine residues [14, 15]. Furthermore, studies have identified deoxyribonucleic acid (DNA) adducts, notably N^2-ethyl-2'-deoxyguanosine (N^2-Et-dG), with demonstrated mutagenic potential [16]. Experimental evidence also indicates that acetaldehyde inhibits MT complex I [nicotinamide dinucleotide reduced form (NADH):ubiquinone oxidoreductase] through covalent binding, thereby compromising cellular energy production [17].

Microsomal ethanol oxidizing system (MEOS)

A substantial body of literature has documented that cytochrome P450 2E1 (CYP2E1) is significantly induced during chronic alcohol consumption [18]. IHC studies in both animal models and human *post-mortem* tissue have demonstrated increased CYP2E1 expression in cortical and hippocampal neurons [19, 20]. Notably, CYP2E1-mediated ethanol oxidation generates reactive oxygen species (ROS), including superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH) through the Fenton reaction in the presence of ferrous ions (Fe^{2+}) [21]. These findings have been consistently replicated across multiple experimental paradigms.

Catalase pathway

Although historically considered a minor contributor, evidence suggests that catalase (CAT) in peroxisomes and mitochondria oxidizes ethanol in the presence of H_2O_2, accounting for approximately 60–70% of cerebral ethanol metabolism [13]. This pathway may assume greater significance under conditions of elevated oxidative stress.

Oxidative stress and lipid peroxidation

The brain's particular vulnerability to oxidative damage has been recognized for decades and remains a central theme in alcohol neurotoxicity research [22]. Several intrinsic characteristics account for this susceptibility: the brain consumes approximately 20% of total body oxygen despite representing only 2% of body mass, neuronal membranes contain high concentrations of polyunsaturated fatty acids (PUFAs) including docosahexaenoic acid and arachidonic acid, certain regions such as the *substantia nigra* and *globus pallidus* harbor elevated free iron concentrations, and antioxidant capacity is relatively limited compared to other tissues [23, 24].

Molecular mechanisms of lipid peroxidation

The process of lipid peroxidation has been extensively characterized in the context of alcohol-induced brain injury. Initiation occurs when •OH abstract hydrogen atoms from *bis*-allylic carbons of PUFAs, generating carbon-centered lipid radicals [25, 26]. During the propagation phase, these radicals react with molecular oxygen to form lipid peroxyl radicals, which subsequently abstract hydrogen from adjacent PUFAs, yielding lipid hydroperoxides and perpetuating the chain reaction [27]. This self-amplifying cascade has been demonstrated in numerous *in vitro* and *in vivo* studies [27–29].

Toxic end products

Among the most extensively studied lipid peroxidation products is 4-hydroxynonenal (4-HNE), a highly reactive α,β-unsaturated aldehyde [30]. Research has demonstrated that 4-HNE forms protein adducts through Michael addition reactions with cysteine, histidine, and lysine residues [31]. Of particular relevance to excitotoxicity, studies have shown that 4-HNE inhibits excitatory amino acid transporter (EAAT)1 and EAAT2 glutamate transporters, thereby impairing synaptic glutamate clearance [32]. Malondialdehyde (MDA), another well-characterized end product, forms Schiff bases with protein amino groups and DNA bases, generating adducts such as M_1dG (pyrimido-purinone) [33]. Isoprostanes, particularly 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), have emerged as reliable biomarkers of oxidative stress in clinical studies and have been shown to exert cerebral vasoconstrictive effects [34].

Disruption of antioxidant systems

Multiple investigations have documented that chronic alcohol consumption reduces the activity of key antioxidant enzymes, including superoxide dismutase (SOD) (both cytosolic Cu/Zn-SOD1 and MT Mn-SOD2), glutathione peroxidase (GPx), and CAT [35–38]. Reduced glutathione (GSH) depletion occurs through several mechanisms: conjugation with acetaldehyde, increased consumption for H_2O_2 and lipid hydroperoxide reduction, and inhibition of gamma-glutamylcysteine synthetase (γ-GCS), the rate-limiting enzyme in *de novo* GSH synthesis [39]. These findings have been consistently observed across animal models and human studies.

Mitochondrial dysfunction

Mitochondria have emerged as central targets of alcohol

neurotoxicity, with damage occurring at multiple levels. This understanding has evolved through decades of research employing biochemical, ultrastructural, and molecular approaches [3].

Studies examining electron transport chain function have revealed deficits at multiple complexes. Complex I (NADH:ubiquinone oxidoreductase) activity is reduced by 30–40% in the prefrontal cortex of chronic alcohol consumers, attributed to acetaldehyde–protein adduct formation and oxidation of Fe–S centers [3]. Research has also demonstrated increased superoxide generation at the quinol-oxidation (Q_o) site of Complex III (ubiquinol:cytochrome *c* oxidoreductase) through reverse electron flow [40]. Complex IV (cytochrome *c* oxidase) shows reduced oxygen affinity due to oxidative modifications of catalytic subunits, further compromising cellular respiration [41].

Other experimental studies have shown that ethanol and its metabolites reduce $\Delta\Psi$m from normal values of approximately -180 mV to -100 to -120 mV. This depolarization compromises adenosine triphosphate (ATP) synthesis *via* ATP synthase (Complex V), impairs import of nuclear-encoded MT proteins, and disrupts MT calcium ions (Ca^{2+}) homeostasis. These changes have been documented using fluorescent probes and electrophysiological techniques in both isolated mitochondria and intact cells [42].

There is also considerable evidence that indicates that calcium overload, oxidative stress, and ATP depletion trigger mitochondrial permeability transition pore (mPTP) opening. This protein complex, comprising cyclophilin D in the matrix, adenine nucleotide translocator in the inner membrane, and voltage-dependent anion channel in the outer membrane, has been extensively characterized. Studies have demonstrated that mPTP opening leads to osmotic matrix swelling, outer membrane rupture, and release of proapoptotic factors including cytochrome *c*, apoptosis-inducing factor (AIF), second mitochondria-derived activator of caspases (Smac)/direct inhibitor of apoptosis protein (IAP)-binding protein with low isoelectric point (pI) (DIABLO), and endonuclease G (EndoG) [43].

Cell death mechanisms

Research over the past two decades has revealed that alcohol-induced neuronal death occurs through multiple, often overlapping pathways. The relative contribution of each pathway depends on the severity and duration of exposure, regional vulnerability, and cellular context.

Apoptosis, which is the intrinsic (MT) pathway, has been extensively characterized by alcohol neurotoxicity models. The molecular sequence involves activation of B-cell lymphoma-2 (Bcl-2) homology 3 (BH3)-only proteins [Bcl-2 interacting mediator of cell death (Bim), BH3 interacting-domain death agonist (Bid), p53 up-regulated modulator of apoptosis (PUMA), Noxa] by stress signals, followed by Bax/Bak oligomerization in the outer MT membrane. This leads to MT outer membrane permeabilization (MOMP) and cytochrome *c* release [44]. Studies have demonstrated subsequent apoptosome formation through assembly of cytochrome *c*, apoptotic protease activating factor 1 (Apaf-1), and procaspase-9, leading to activation of effector caspases (caspase-3, -6, -7). These caspases cleave numerous substrates including inhibitor of caspase-activated DNase (ICAD; activating caspase-activated DNase and causing DNA fragmentation), nuclear lamins, poly[adenosine diphosphate (ADP)-ribose] polymerase-1 (PARP-1), and cytoskeletal proteins [45].

Histological studies have characterized the morphological features of neuronal apoptosis, including peripheral chromatin condensation, karyorrhexis, cytoplasmic retraction with preserved membrane integrity, and apoptotic body formation. Importantly, this process occurs without local inflammatory response, a feature termed "silent" phagocytosis [46].

Evidence from multiple laboratories indicates that ethanol increases expression of death ligands [FasL, tumor necrosis factor-alpha (TNF-α), TNF-related apoptosis-inducing ligand (TRAIL)] and their corresponding receptors [Fas/cluster of differentiation (CD)95, TNF receptor 1 (TNFR1), death receptor (DR)4/DR5)] in brain tissue. The signaling cascade involves adaptor protein recruitment [Fas-associated protein with death domain (FADD), TNFR1-associated death domain (TRADD)], death-inducing signaling complex (DISC) formation, and caspase-8 activation. Studies have shown that caspase-8 either directly cleaves effector caspases or amplifies the signal through MT pathway engagement *via* Bid cleavage [47].

Under conditions of severe ATP depletion or caspase inhibition, research has demonstrated that cell death proceeds through necrotic or necroptotic pathways. Unlike apoptosis, these processes are characterized by cellular swelling, plasma membrane rupture, and release of damage-associated molecular patterns (DAMPs), triggering local inflammation. Recent studies have elucidated the molecular machinery of necroptosis, involving receptor-interacting serine/threonine-protein kinase (RIPK)1 and RIPK3 kinases, mixed lineage kinase domain like pseudokinase (MLKL) phosphorylation and oligomerization, and pore formation leading to osmotic lysis [48].

Emerging evidence also indicates that ethanol disrupts autophagy, a process essential for neuronal homeostasis. Acute exposure inhibits mechanistic target of rapamycin complex 1 (mTORC1), inducing autophagy, whereas chronic exposure impairs autophagosome–lysosome fusion. This results in accumulation of autophagosomes and undegradable substrates, contributing to neurodegeneration through failed protein clearance. These findings have generated considerable interest in autophagy modulation as a potential therapeutic target [49].

Glutamatergic excitotoxicity

The glutamatergic system undergoes profound adaptations during chronic alcohol consumption, with particularly severe consequences during withdrawal. This phenomenon, termed "kindling", has been extensively studied and has significant clinical implications.

Electrophysiological and pharmacological studies have established that ethanol acutely inhibits *N*-methyl-D-aspartate (NMDA) receptors, with particular sensitivity conferred by the GluN2B subunit, through mechanisms involving transmembrane domain residues. Concurrently, ethanol potentiates gamma-aminobutyric acid (GABA)-A receptors, particularly extrasynaptic δ-containing subtypes, through allosteric modulation. These combined effects produce the characteristic sedative and anxiolytic properties of acute alcohol intoxication [50].

Prolonged alcohol exposure triggers homeostatic adaptations that maintain neuronal function in the presence of the drug. Studies have demonstrated 50–100% upregulation of GluN2B-containing NMDA receptors in cortical and hippocampal regions. Research has also shown increased expression of calcium-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors lacking the GluA2 subunit, alongside downregulation of GABA-A receptor α_1 and γ_2 subunits and reduced GABA synthesis and release [51].

The clinical and experimental significance of these adaptations becomes apparent during alcohol withdrawal. In the absence of ethanol, the resulting excitatory/inhibitory imbalance triggers glutamatergic hyperactivation with massive Ca^{2+} influx through upregulated NMDA receptors. Studies using calcium imaging techniques have documented cytosolic Ca^{2+} elevation from approximately 100 nM to 1–10 μM. This calcium overload activates deleterious enzymes including calpain (degrading cytoskeletal and synaptic proteins), phospholipase A2 (releasing proinflammatory eicosanoids), and neuronal nitric oxide (NO) synthase (producing peroxynitrite). These processes culminate in MT energy failure and neuronal death, providing the rationale for pharmacological interventions targeting glutamatergic transmission during detoxification [52].

Neuroinflammation

Neuroinflammation has emerged as a critical component of alcohol-related brain damage (ARBD), with research over the past decade substantially advancing our understanding of the underlying mechanisms.

Microglia, the resident immune cells of the CNS, respond robustly to ethanol through pattern recognition receptors. Pioneering work demonstrated that ethanol activates Toll-like receptor (TLR)4 signaling by promoting its recruitment into lipid rafts and triggering the release of endogenous danger signals such as high mobility group box 1 (HMGB1). Subsequent studies showed that ethanol induces TLR4/TLR2 heterodimerization, amplifying the neuroinflammatory response, while receptor for advanced glycation endproducts (RAGE) detection of oxidatively modified proteins provides an additional activation mechanism [53].

The TLR4 signaling cascade has been extensively mapped, involving myeloid differentiation primary response 88 (MyD88) and TIR-domain-containing adapter-inducing interferon-beta (TRIF) adaptor recruitment, interleukin (IL)-1 receptor-associated kinase (IRAK)1/4 and TNFR-associated factor 6 (TRAF6) activation, and nuclear factor-kappa B (NF-κB) nuclear translocation driving pro-inflammatory gene transcription. Key cytokines produced include TNF-α (inducing apoptosis and disrupting synaptic transmission), IL-1β (potentiating excitotoxicity through NMDA receptor modulation), and IL-6 (with context-dependent effects) [54].

Recent research has highlighted the role of the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome in alcohol-induced neuroinflammation. This complex is activated by ROS, potassium ions (K^+) efflux, and metabolic byproducts of alcohol metabolism. Inflammasome assembly recruits apoptosis-associated speck-like protein containing a CARD (ASC) and activates caspase-1, enabling IL-1β and IL-18 maturation. Studies have also implicated this pathway in pyroptosis, a form of inflammatory cell death that may contribute to neuronal loss [55].

Astrocytes respond to alcohol-induced injury through well-characterized morphological and functional changes. Histological studies have distinguished isomorphic astrogliosis (hypertrophy without scarring) from anisomorphic astrogliosis (with glial scar formation). Research has documented increased glial fibrillary acidic protein (GFAP) and vimentin expression, cellular hypertrophy, and process elaboration [56].

The functional consequences of astrogliosis have received considerable attention. Studies have demonstrated impaired glutamate reuptake through EAAT2 downregulation, altered extracellular K^+ buffering, neurovascular coupling dysfunction, BBB disruption, and aberrant gliotransmitter release. These findings underscore the dual nature of astrocytic responses, with both protective and detrimental aspects depending on context and severity [57].

Histopathological brain changes

Figure 1 (A–D) provides a comparative analysis of control and alcohol-exposed cerebral cortex, emphasizing alcohol-associated alterations in both gray and white matter. This overview is complemented by Figure 2 (A–F), which offers an in-depth IHC characterization of the underlying structural lesions.

Neuronal changes

The neuropathological consequences of chronic alcohol consumption have been documented through extensive *post-mortem* studies spanning several decades. These investigations have revealed a characteristic pattern of selective neuronal vulnerability that correlates with clinical manifestations.

Quantitative neuropathological studies have established that neuronal loss follows a distinct topographic distribution reflecting differential regional vulnerability.

In the prefrontal cortex, stereological analyses have demonstrated reduced neuronal density in layers III and V, predominantly affecting pyramidal neurons. *Post-mortem* studies estimate 15–23% neuronal loss in severe alcoholism, accompanied by perikaryal atrophy with reduced somatic volume. These findings correlate with the executive dysfunction and personality changes commonly observed in affected individuals [58].

Hippocampal involvement in ARBD presents a more nuanced picture. While rodent models have consistently demonstrated preferential vulnerability of *Cornu Ammonis* (CA)1 and CA3 pyramidal neurons and reduced neurogenesis in the dentate gyrus subgranular zone, a key stereological study found that chronic alcohol consumption does not cause hippocampal neuron loss in humans without concurrent Wernicke–Korsakoff pathology. Rather, hippocampal damage in human alcoholics appears mediated primarily by glial cell loss, volumetric reduction, and functional impairment rather than frank neuronal death. In contrast, alcoholics with Wernicke–Korsakoff syndrome show more pronounced hippocampal pathology. These findings, combined with neuroimaging evidence of hippocampal atrophy, provide a structural basis for the memory impairments characteristic of ARBD [59].

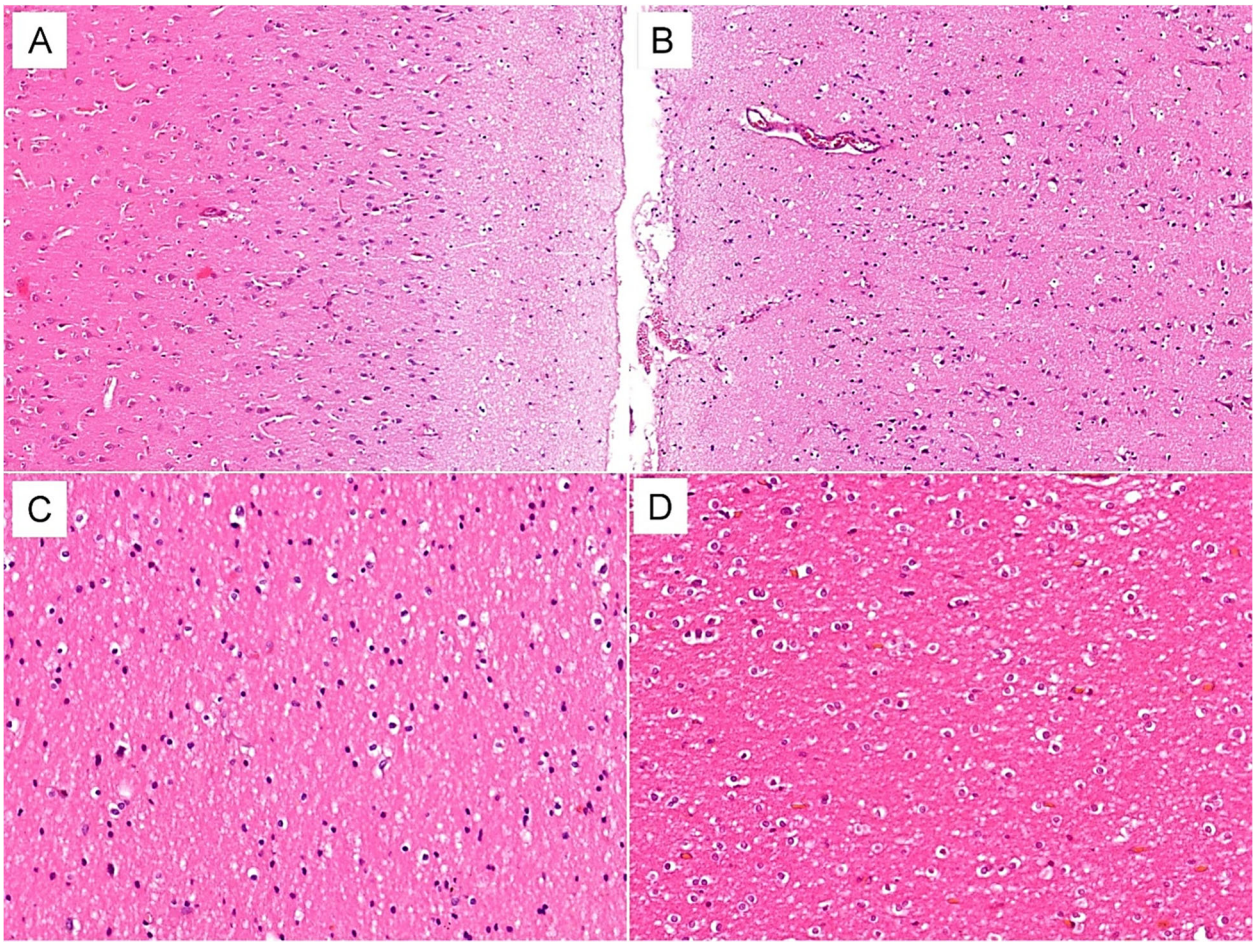

Figure 1 – ***Histopathological HE chronic alcohol-associated cerebral cortex changes: (A) Control cortex showing gray matter with preserved cortical architecture, normal neuronal density and morphology; (B) Alcohol-exposed cortex demonstrating gray matter with reduced neuronal density below molecular layer, degenerative neuronal changes, and neuropil rarefaction; there is an apparent increase in small glial nuclei consistent with reactive gliosis, with focal perivascular/pericellular clear spaces suggestive of edema/tissue loosening; (C) Control cortex white matter showing a compact fibrillary background with scattered oligodendroglia nuclei and no significant vacuolation; (D) Alcohol-exposed cortex white matter showing rarefaction with prominent vacuolation/spongy change, compatible with myelin/axonal injury and reactive glial response. These features support chronic alcohol-associated neurodegeneration, with neuronal loss and gliosis in gray matter and vacuolar white matter injury. HE staining: (A and B) 200×; (C and D) 400×. HE: Hematoxylin–Eosin.***

Cerebellar pathology has been particularly well characterized. Neuropathological studies have documented marked Purkinje cell loss in the anterior superior vermis, with density reductions of 21–40% reported in severe cases. Notably, a dose-dependent relationship has been demonstrated, with moderate consumption (41–80 g/day) associated with approximately 15% Purkinje cell loss, increasing to over 33% at higher intake levels. Accompanying changes include variable granular layer thinning, molecular layer narrowing, and Bergmann gliosis. Stereological studies have added nuance to these findings, demonstrating that in alcoholics without Wernicke's encephalopathy, perikaryal volume reduction (~20%) may be more prominent than absolute cell loss, suggesting that cellular atrophy precedes frank neurodegeneration. The topographic progression of lesions follows a characteristic pattern, beginning in the anterior superior vermis and extending to the anterior superior hemisphere, anterior inferior hemisphere, and eventually the posterior vermis in the most severe cases. These findings correlate with the ataxia and gait disturbances frequently observed clinically, as confirmed by positron emission tomography (PET) studies demonstrating vermian hypometabolism specifically in alcoholic patients with ataxia [60].

The basal forebrain has received attention due to its relevance to cognitive function. While chronic alcoholism without concurrent Wernicke–Korsakoff pathology does not appear to cause significant cholinergic neuron loss in the *nucleus basalis* of Meynert, studies have demonstrated substantial neuronal depletion (~47%) in patients with Korsakoff's disease, resulting in reduced cortical cholinergic innervation. This pathology contributes to the severe cognitive and attentional deficits characteristic of Korsakoff's syndrome and shows notable overlap with Alzheimer's disease (AD) neuropathology, where even greater *nucleus basalis* degeneration (up to 70%) has been documented [61].

Beyond neuronal loss, substantial research has focused on alterations in surviving neurons. Golgi impregnation studies and morphometric analyses have revealed significant reduction in total dendritic arbor length, decreased dendritic branching complexity, and simplified dendritic geometry [62].

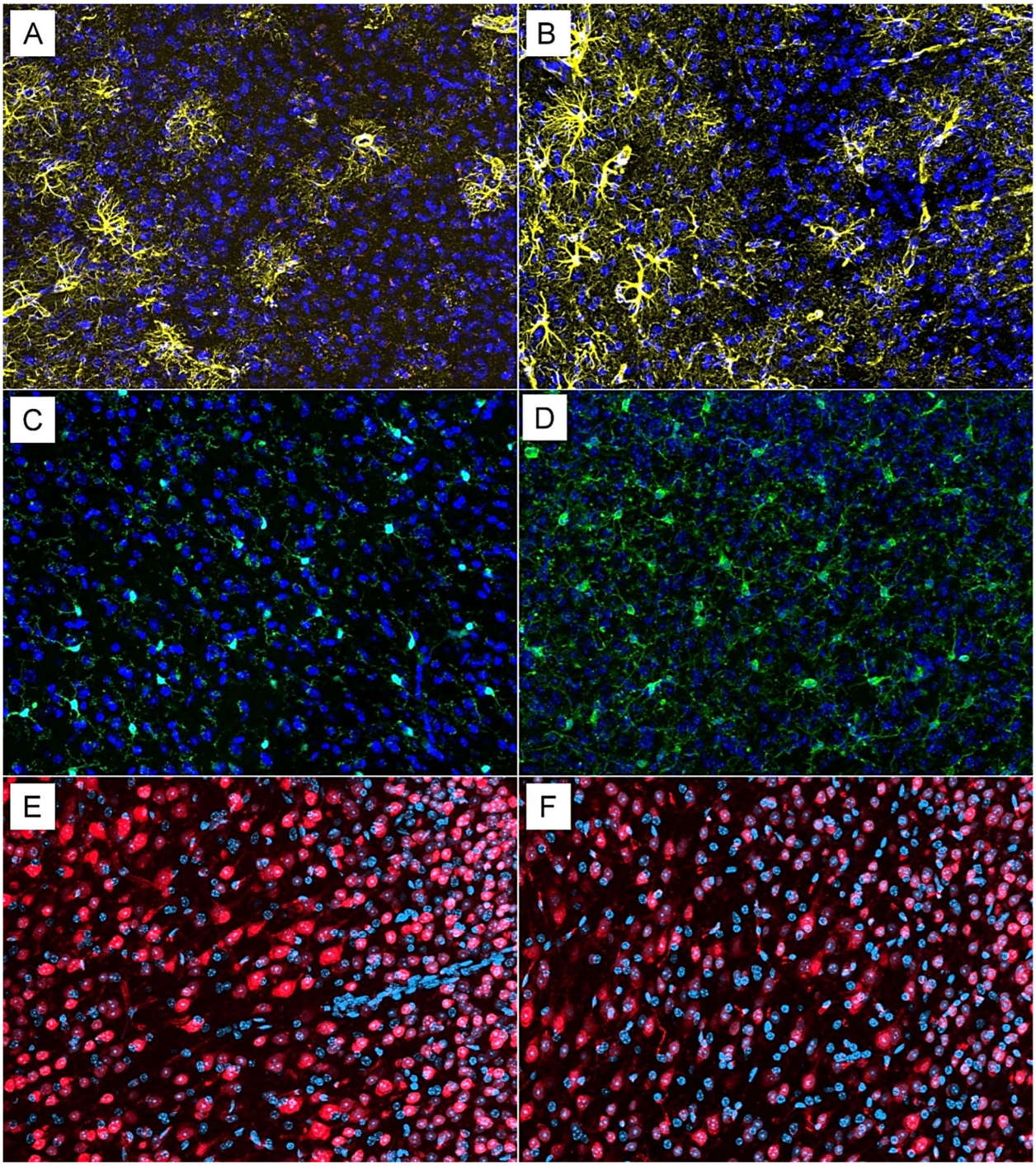

Figure 2 – ***Histopathological, immunofluorescence, chronic alcohol-associated cerebral cortex changes (400×): (A) Control cortex showing minimal astroglia activation (GFAP, yellow); (B) Alcohol-exposed cortex demonstrating marked reactive astrogliosis (increased GFAP immunoreactivity, yellow, underlying enlarged and overlapping cell processes); (C) Control cortex showing minimal microglial activation (IBA1 immunoreactivity, green); (D) Alcohol-exposed cortex demonstrating marked reactive microgliosis (increased IBA1 immunoreactivity, green, underlying enlarged bodies, thick and blunt processes); (E) Control cortex showing preserved neuronal density (NeuN, red); (F) Alcohol-exposed cortex demonstrating heterogeneous neuronal preservation with an overall reduction in NeuN-positive neurons (red). These alcohol-associated alterations are consistent with chronic neuroinflammatory signaling and cumulative excitotoxic and oxidative injury, resulting in neuronal loss and sustained glial activation. GFAP: Glial fibrillary acidic protein; IBA1: Ionized calcium-binding adaptor molecule 1; NeuN: Neuronal nuclei.***

Dendritic spine pathology has been documented in chronic alcoholism. The only human *post-mortem* Golgi study demonstrated significantly reduced spine density on cortical pyramidal neurons, though the magnitude and direction of changes are region-specific and context dependent. Animal models reveal complex, bidirectional alterations: some paradigms show spine loss while others demonstrate increased spine density, particularly during withdrawal. Spine morphological changes also vary by brain region and exposure paradigm, precluding simple generalizations. At the molecular level, chronic ethanol exposure and withdrawal produce widespread neuroadaptations in postsynaptic density

proteins that regulate dendritic spine morphology, though the specific patterns differ across brain regions and between active exposures *versus* withdrawal states [63, 64].

Ultrastructural studies using electron microscopy (EM), primarily in cerebellar Purkinje neurons of chronically ethanol-fed rats, have provided insights into subcellular pathology. The most consistently reported finding is progressive dilation of the smooth endoplasmic reticulum (SER) within dendritic shafts and spines, linked to decreased sarcoplasmic/endoplasmic reticulum calcium adenosine triphosphatase (ATPase) (SERCA) pump levels and disturbed calcium homeostasis. This SER dilation precedes and accompanies dendritic regression and is followed by formation of degenerating bodies composed of membrane whorls and vesicular structures. Notably, the total number of synapses on Purkinje neurons is reduced following chronic ethanol treatment, though this loss appears reversible with abstinence. These ultrastructural findings suggest that intracellular organelle dysfunction, particularly disrupted calcium signaling within the endoplasmic reticulum (ER), may represent a key mechanism preceding dendritic degeneration [65].

The neuronal cytoskeleton undergoes significant alterations in chronic alcoholism, with implications for axonal transport and cellular integrity. Microtubule pathology has attracted particular interest due to parallels with neurodegenerative diseases. Studies have documented *tau* protein hyperphosphorylation at multiple sites including Ser202, Thr205, Ser396, and Ser404. This leads to microtubule destabilization and impaired axonal transport. While *tau* aggregation into fibrillar structures occurs, research suggests this pathology is less extensive than in AD [66].

Neurofilament (NF) abnormalities have been documented through IHC studies, including aberrant phosphorylation of heavy NF (NF-H) subunits, perikaryal NF accumulation, and disrupted axonal caliber. Actin dynamics are also affected, with studies demonstrating dysregulated actin turnover in dendritic spines, altered Rho guanosine triphosphatase (GTPase) signaling [Ras homolog family member A (RhoA), Ras-related C3 botulinum toxin substrate 1 (Rac1), cell division cycle 42 (Cdc42)], and consequent changes in spine morphology and synaptic plasticity [67].

The morphological characteristics of neuronal death have been extensively characterized using multiple techniques.

Light microscopy with Hematoxylin–Eosin (HE) staining reveals pyknotic hyperchromatic nuclei, cytoplasmic condensation with increased eosinophilia, cellular retraction with pericellular halo, and the classical "red neuron" appearance indicating acute neuronal death [68].

IHC studies have provided molecular confirmation of apoptotic mechanisms, demonstrating positivity for cleaved caspase-3, nuclear DNA fragmentation detected by terminal deoxynucleotidyl transferase deoxyuridine triphosphate (dUTP) nick end labeling (TUNEL) method, cytochrome *c* translocation from mitochondria to cytosol, increased Bax expression, and reduced Bcl-2 [69].

EM has revealed the ultrastructural hallmarks of apoptosis: marginal chromatin condensation, nuclear fragmentation (karyorrhexis), formation of membrane-bound apoptotic bodies, and preserved organelle integrity in early stages [70].

White matter changes

White matter pathology in chronic alcoholism has gained increasing recognition, particularly with advances in neuroimaging that reveal the extent of involvement *in vivo*.

Multiple mechanisms contribute to myelin damage, as established through experimental and human studies. Direct oligodendrocyte toxicity occurs through oxidative stress, while TNF-α and glutamate mediate oligodendrocyte apoptosis. Research has also demonstrated impaired myelin lipid synthesis related to essential fatty acid deficiency and interference with myelinating gene expressions including myelin basic protein (*MBP*), proteolipid protein (*PLP*), and myelin-associated glycoprotein (*MAG*).

Special histological stainings have characterized the features of alcohol-related demyelination: myelin pallor on Luxol fast blue staining, intramyelinic vacuolization (lamellar splitting), myelin "bubble" formation, and axonal spheroids representing organelle accumulation in demyelinated zones. Neuropathological studies have identified predominantly affected regions, including the *corpus callosum* (particularly the genu and body), frontal white matter, cerebellar peduncles, and internal capsule. These findings have been corroborated by diffusion tensor imaging (DTI) studies showing reduced fractional anisotropy (FA), increased mean diffusivity, and altered radial diffusivity in corresponding regions [60].

Several patterns of axonal degeneration in ARBD were observed: Wallerian degeneration secondary to neuronal soma or proximal axon injury, "dying-back" degeneration with initial distal involvement and retrograde progression, and segmental degeneration with multifocal involvement.

Ultrastructural studies have documented the features of axonal pathology, including axonal swelling with organelle accumulation (axonal spheroids), NF disorganization, swollen mitochondria with disrupted cristae, ER vacuolization, and terminal axonal fragmentation [71].

IHC markers have proven valuable for detecting axonal degeneration. Amyloid precursor protein (APP) accumulation serves as an early marker, while phosphorylated NF positivity in abnormal locations and ubiquitin positivity in axonal spheroids indicate more advanced pathology.

Beyond demyelination, primary oligodendrocyte pathology has been documented. Quantitative studies have demonstrated 20–30% reduction in oligodendrocyte numbers in frontal white matter, decreased oligodendrocyte precursor cell (OPC) density, and inhibited OPC differentiation into mature oligodendrocytes. Functional alterations include reduced expression of myelinating transcription factors [oligodendrocyte transcription factor 2 (Olig2), sex determining region Y (SRY)-box transcription factor 10 (Sox10), myelin regulatory factor (Myrf)], decreased synthesis of myelin basic protein and proteolipid protein, and impaired trophic support for axons.

EM has revealed ultrastructural changes including degeneration of oligodendrocyte processes, myelin lamellae separation, abnormal cytoplasmic inclusions, and ER dilation [71].

Glial changes

Glial responses to alcohol-induced injury have received increasing attention as their contribution to both pathology and potential recovery has become apparent. Astrocytic

responses have been extensively characterized through neuropathological studies. HP classification distinguishes isomorphic astrogliosis, characterized by hypertrophy without proliferation or scar formation, from anisomorphic astrogliosis with reorganization and glial scar formation. The pattern observed depends on injury severity and chronicity.

IHC markers have proven valuable for characterizing astrogliosis. GFAP expression increases 2–5 fold in affected areas, vimentin is re-expressed in reactive astrocytes, S100B serves as an activation marker with elevated cerebrospinal fluid (CSF) levels, and aquaporin-4 shows redistribution from vascular end-feet. Morphological changes documented through immunohistochemistry and Golgi staining include cell body hypertrophy, process thickening and elongation, territorial domain expansion, and domain overlap between neighboring astrocytes indicating loss of normal domain organization [72].

Research has elucidated the functional consequences of astrogliosis, including impaired glutamate reuptake through EAAT2 downregulation, altered extracellular K^+ buffering *via* modified Kir4.1 expression, neurovascular unit dysfunction, and BBB disruption. These changes have significant implications for neuronal function and survival [73].

Microglial responses to chronic alcohol exposure have been characterized using morphological and IHC approaches. Studies have documented the morphological transformation from the resting state, with ramified morphology and thin processes, to the activated state with process retraction, enlarged cell body, and amoeboid morphology. Research has attempted to characterize activation phenotypes, with M1 (proinflammatory) microglia producing TNF-α, IL-1β, IL-6, NO, and ROS, and M2 (anti-inflammatory/reparative) microglia producing IL-10, transforming growth factor-beta (TGF-β), and neurotrophic factors. However, studies increasingly recognize that the *in vivo* spectrum is more complex than this dichotomy suggests. IHC markers employed in research include ionized calcium-binding adaptor molecule 1 (IBA1) as a pan-microglial marker, CD68 for active phagocytosis, major histocompatibility complex class II [MHC-II; human leukocyte antigen-DR isotype (HLA-DR)] with increased expression upon activation, and phenotype-specific markers including CD86 and inducible NO synthase (iNOS) (M1) and CD206 and arginase 1 (Arg1) (M2). Neuropathological studies have characterized the distribution of microgliosis, showing maximum intensity in the frontal cortex, hippocampus, and periventricular white matter, with colocalization in areas of neuronal loss and perivascular aggregation [74].

Thiamine deficiency-associated lesions

The interaction between alcohol toxicity and nutritional deficiency produces distinctive neuropathological features that have been recognized since the classical descriptions by Wernicke and Korsakoff.

In Wernicke encephalopathy, the molecular basis of thiamine deficiency has been well established through biochemical studies. Thiamine serves as an essential cofactor for several key enzymes: pyruvate dehydrogenase converting pyruvate to acetyl-CoA, α-ketoglutarate dehydrogenase in the Krebs cycle, transketolase in the pentose phosphate pathway, and branched-chain α-ketoacid dehydrogenase. Research has documented the biochemical consequences of deficiency, including 50–60% reduction in ATP production in vulnerable regions, lactate accumulation with tissue acidosis, decreased neurotransmitter synthesis, impaired myelin and membrane lipid synthesis, and increased vulnerability to oxidative stress due to reduced nicotinamide adenine dinucleotide phosphate (NADPH) generation [75].

The mammillary bodies exhibit the most consistent and severe pathology. Neuropathological studies have documented severe atrophy with 50–80% volume reduction, massive neuronal loss with replacement gliosis, perivascular petechial microhemorrhages, hemosiderin-laden macrophages, and capillary proliferation. These changes are virtually pathognomonic of Wernicke encephalopathy. Thalamic involvement, particularly of dorsomedial nuclei, has been well characterized. Studies report laminar necrosis, acute-phase edema and spongiosis, and chronic-phase neuronal loss and gliosis, with predominant involvement of magnocellular and parvocellular components. This pathology correlates with the memory impairment characteristic of Korsakoff syndrome. The periaqueductal gray matter demonstrates periependymal edema and vacuolization, neuronal degeneration, myelin loss in adjacent tracts, and reactive gliosis. These changes account for the oculomotor abnormalities frequently observed clinically. Cerebellar pathology in the anterior vermis includes marked Purkinje cell loss, molecular layer atrophy, Bergmann gliosis, and efferent tract degeneration. While overlapping with direct alcohol toxicity, thiamine deficiency contributes substantially to cerebellar damage. EM studies of Wernicke lesions have revealed neuronal cytoplasmic edema, MT swelling with cristolysis, ER dilation, Golgi apparatus fragmentation, severe astrocytic edema (Alzheimer's type II astrocytes), and vascular wall alterations including endothelial swelling and thickened basement membranes [76].

Regional neuroanatomical correlates of chronic alcohol-related brain injury and clinical manifestations correlations are presented in Table 1.

Imaging findings and histopathological correlations

Neuroimaging has transformed our understanding of ARBD by enabling *in vivo* visualization of macro pathological changes. Correlating imaging findings with neuropathological substrates enhances interpretation and provides potential biomarkers for clinical monitoring. Figure 3 (A–D) illustrates key neuroimaging features of chronic alcohol-related brain injury, contrasting computed tomography (CT) and magnetic resonance (MR) findings.

CT imaging

CT imaging of the brain in chronic ethanol consumers commonly reveals structural changes associated with long-term alcohol exposure. The most frequently observed findings include generalized cerebral atrophy, particularly in the frontal lobes, widened cortical sulci, and an enlarged ventricular system, all resulting from the loss of brain parenchyma. CT scans can sometimes also find problems like subdural hematomas, which are more likely to happen in people with alcohol use disorder because they are more likely to fall and their brains are shrinking. Advanced cases

can show signs consistent with conditions like Wernicke encephalopathy, although magnetic resonance imaging (MRI) is generally more sensitive for detecting such metabolic or microstructural changes. Overall, CT is still a useful and quick way to look at brain damage and sudden problems in people who drink a lot of alcohol [77].

Table 1 – ***Regional neuroanatomical correlates of chronic alcohol-related brain injury: HP features, quantitative deficits, and clinical manifestations***

Brain region	HP changes	Quantitative findings	Clinical correlates
Prefrontal cortex	Neuronal loss in layers III and V (pyramidal neurons); perikaryal atrophy; dendritic simplification	15–23% neuronal loss; 20–40% reduction in dendritic arbor length	Executive dysfunction; personality changes; impaired decision-making
Hippocampus	*Cornu Ammonis* 1 and 3 pyramidal cell loss; GABAergic interneuron depletion; reduced subgranular zone neurogenesis	30–50% reduction in dendritic spine density	Memory impairment; learning deficits
Cerebellum (*anterior vermis*)	Purkinje cell loss (lobules I–V); granular layer degeneration; Bergmann gliosis	Up to 40% Purkinje cell reduction	Ataxia; gait disturbances; dysmetria
Corpus callosum	Demyelination; axonal degeneration; intramyelinic vacuolization	Reduced fractional anisotropy on DTI	Impaired interhemispheric integration
Mammillary bodies	Severe atrophy; neuronal loss with replacement gliosis; perivascular microhemorrhages; hemosiderophages	50–80% volume reduction	Anterograde amnesia (Korsakoff syndrome)
Thalamus (dorsomedial nuclei)	Laminar necrosis; edema and spongiosis (acute); neuronal loss and gliosis (chronic)	Magnocellular and parvocellular involvement	Memory consolidation deficits
Periaqueductal gray matter	Periependymal edema; neuronal degeneration; myelin loss	Variable involvement	Oculomotor abnormalities
Basal forebrain (*nucleus basalis* of Meynert)	Cholinergic neuron loss; reduced cortical cholinergic innervation	Variable neuronal loss	Cognitive and attentional deficits

DTI: Diffusion tensor imaging; GABA: Gamma-aminobutyric acid; HP: Histopathological.

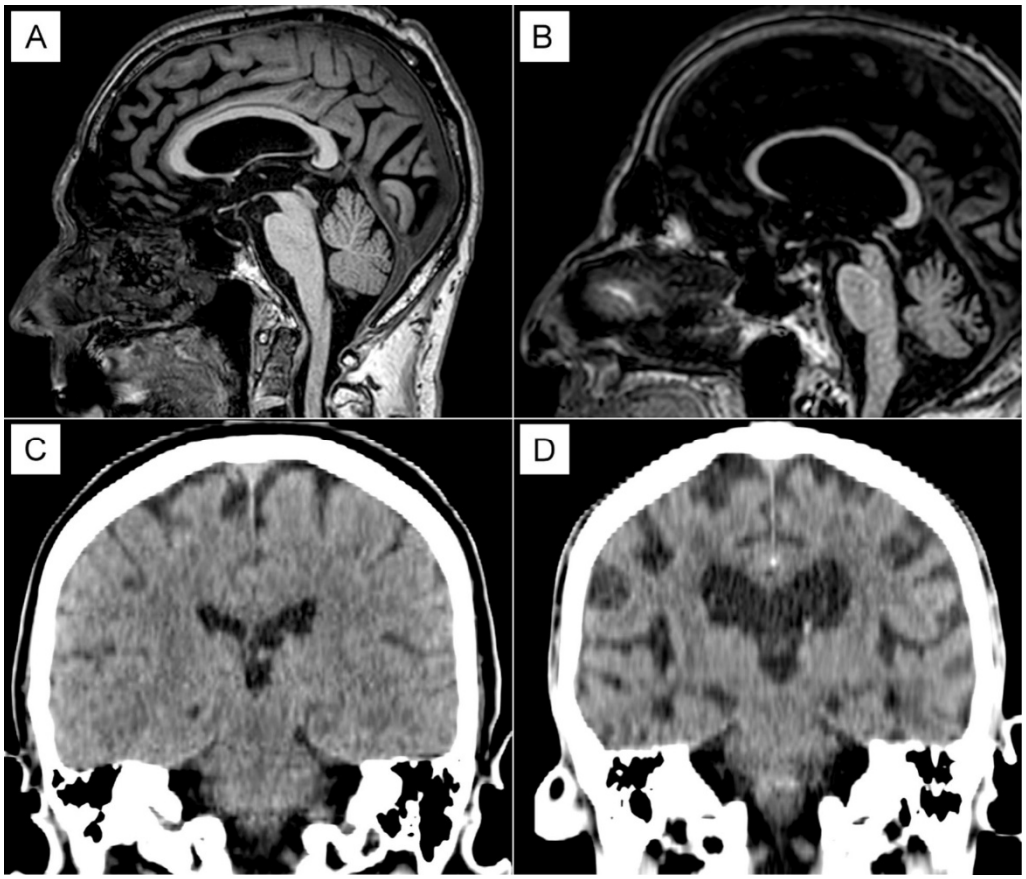

Figure 3 – ***Neuroimaging chronic alcohol-associated cerebral cortex changes: (A and B) Midline sagittal T1-weighted MR images of age-matched individuals showing the cerebellar vermis: (A) Normal vermian morphology and volume; (B) Severe vermian atrophy with vermian thinning and widening of cerebellar folial fissures; (C and D) Coronal CT images at the level of the ventricular system: (C) Preserved cerebral parenchymal volume with normal pericerebral CSF spaces and ventricular size; (D) Diffuse cerebral atrophy with enlargement of pericerebral CSF spaces and ex vacuo ventricular dilatation. These findings are compatible with chronic alcohol-associated neurodegeneration, characterized by disproportionate cerebellar (vermian) atrophy and diffuse supratentorial volume loss, with ex vacuo ventricular enlargement secondary to parenchymal atrophy. MR is more sensitive than CT for detecting and characterizing these changes, particularly highlighting posterior fossa involvement and subtle cortical/subcortical volume loss, whereas CT primarily demonstrates established atrophy and ventricular dilatation. CSF: Cerebrospinal fluid; CT: Computed tomography; MR: Magnetic resonance.***

Structural MRI

Regarding the atrophy of the brain, combined imaging–neuropathological studies have provided insight into the histological basis of cortical atrophy seen on MRI. Subcortical white matter volume reduction, dendritic arbor simplification, neuronal loss and neuropil reduction, and changes in extracellular space followed by compensatory CSF space dilatation are all contributing factors. Research comparing volumetric measurements to histology indicates that roughly 10–15% of neurons are lost for every 1% decrease in prefrontal cortex volume. Crucially, studies show that edema and possibly reversible inflammatory changes may cause MRI-measured atrophy to overestimate actual neuronal loss. Hippocampal volume loss, diencephalic structure atrophy, and involvement of the periventricular white matter and basal ganglia are all reflected in ventricular enlargement (dilation), which is a sign of central atrophy. Ventricular size may partially return to normal with prolonged abstinence, according to longitudinal studies [78].

Diffusion tensor imaging

DTI has emerged as a sensitive tool for detecting white matter microstructural changes, with specific parameters reflecting distinct pathological processes.

The main contributing factors, demyelination, axonal loss, tract disarray, and inflammation with edema are all correlated with a decrease in FA. Primary axonal degeneration is suggested by decreased axial diffusivity, whereas demyelination is more precisely indicated by increased radial diffusivity. Imaging and HP studies have been combined to establish these relationships.

DTI tractography has identified predominantly affected white matter tracts and their functional correlates: superior longitudinal fasciculus involvement correlates with executive dysfunction, *corpus callosum* changes impair interhemispheric integration, uncinate fasciculus damage relates to emotional dysregulation, and cerebellar peduncle pathology underlies ataxia and dysmetria [79].

Magnetic resonance spectroscopy

Magnetic resonance spectroscopy (MRS) provides metabolic information that complements structural imaging, with specific metabolites reflecting distinct cellular populations and processes.

N-acetyl-aspartate (NAA), synthesized in neuronal mitochondria, serves as a marker of neuronal integrity and viability. Studies have consistently demonstrated reduced NAA in alcohol-affected regions, reflecting MT dysfunction and neuronal loss. Myo-inositol (mI), localized predominantly in astrocytes, increases with reactive astrogliosis. A reduced NAA/mI ratio has emerged as a useful marker indicating gliosis accompanying neuronal loss. Choline (Cho) reflects membrane and myelin turnover. Research has shown that increased Cho may indicate active demyelination or inflammation, while reduced Cho in later stages suggests tissue loss. Creatine serves as an energy metabolism marker and remains relatively stable, providing an internal reference for ratio calculations. Glutamate/glutamine (Glx) measurements have proven clinically relevant, with Glx elevation during withdrawal indicating glutamatergic hyperexcitability and chronic glutamate reduction reflecting synaptic loss [80, 81].

Reversibility of changes and neuroplasticity

The potential for recovery following alcohol cessation has significant clinical implications and has been the subject of extensive research.

Recovery mechanisms

When it comes to abstinence-related cellular processes, one method of structural recovery is remyelination. Research has shown that OPCs can be recruited and differentiated with myelin sheath reformation; however, the regenerated sheaths are notably thinner than the original myelin. The degree of oligodendrocyte and axonal loss limits the amount of remyelination [82].

Increased dendritic spine density, reactive synaptogenesis, strengthening of preexisting synapses through long-term potentiation, and synaptic receptor re-expression are just a few of the processes that make up synaptic plasticity and contribute to functional recovery. Improvements in metabolite profiles and white matter integrity have been reported by neuroimaging studies to coincide with these cellular alterations.

Adult neurogenesis, while limited to specific niches (subgranular and subventricular zones), resumes following abstinence. However, research suggests its contribution to functional recovery is modest compared to synaptic plasticity.

Even though the brain can regenerate, some alterations cannot be reversed. Complete axonal degeneration is irreversible, replacement gliosis (glial scar) endures, severe mammillary body atrophy shows little recovery, and extensive neuronal loss cannot be replaced. These results highlight how crucial early intervention and long-term abstinence are to optimizing recovery potential [83].

Emerging therapeutic strategies: biomaterial-based interventions

The limitations of endogenous recovery mechanisms have prompted investigation of novel therapeutic approaches to enhance neuroregeneration following ARBD. Among these, biocompatible hydrogel scaffolds have emerged as promising platforms for targeted neural tissue repair. Recent advances in biomaterial science have enabled development of injectable hydrogels capable of providing a three-dimensional microenvironment that mimics the brain's extracellular matrix. These systems can be engineered to deliver neurotrophic factors, antioxidants, or anti-inflammatory agents directly to damaged brain regions [84]. Hydrogel-based delivery systems have shown particular promise in ischemic and hemorrhagic stroke models, where they facilitate localized and sustained release of bioactive molecules, promoting neuroprotection and functional recovery [84, 85]. The versatility of hydrogel formulation extends to peripheral nerve repair, where emerging materials and therapeutic applications have shown the capacity to support axonal regrowth and Schwann cell proliferation [86]. Gelatin-based hydrogels, in particular, represent a multifunctional vehicle for cellular, molecular, and pharmacological therapy in peripheral nerve regeneration, offering excellent biocompatibility and tunable degradation properties [87, 88]. In the domain of spinal cord injury,

bioactive hydrogels with emphasis on gelatin and its derivatives have shown capacity to support neuronal survival and axonal regeneration within the hostile post-injury microenvironment [89]. Functionalized hydrogels incorporating growth factors or stem cell populations represent a potential strategy to address the irreversible neuronal loss characteristic of severe ARBD. While still in preclinical stages, such approaches have shown promise in traumatic brain injury (TBI) models, where experimental devices have been developed to reproduce various types of TBIs in laboratory settings, enabling systematic study of lesion mechanisms and evaluation of therapeutic interventions [90]. Translation of hydrogel-based therapies to clinical practice faces significant challenges, including precise delivery methods, optimal timing of intervention, and long-term biocompatibility in the brain microenvironment. Nevertheless, continued development of these biomaterial approaches, supported by growing evidence from stroke, peripheral nerve injury, spinal cord injury, and TBI models, may eventually complement traditional abstinence-based recovery strategies, particularly for patients with extensive irreversible ARBD.

Conclusions

Alcohol neurotoxicity represents a complex, multifactorial process involving interdependent molecular and cellular mechanisms. Research has established that oxidative stress with lipid peroxidation, MT dysfunction with energy collapse, glutamatergic excitotoxicity, activation of both intrinsic and extrinsic apoptotic pathways, and chronic neuroinflammation mediated by microglia and reactive astrocytes all contribute to brain damage.

HP changes documented through decades of neuropathological research reflect these molecular processes and include selective neuronal loss with characteristic topographic distribution, dendritic and synaptic alterations, white matter demyelination and axonal degeneration, and reactive gliosis. Associated thiamine deficiency adds an additional layer of pathology, producing characteristic lesions in metabolically vulnerable structures.

The correlation of neuroimaging findings with histological substrates has enabled an integrated understanding of ARBD and provided biomarkers for monitoring disease progression and therapeutic response. While some changes demonstrate partial reversibility with abstinence through remyelination, synaptic plasticity, and neurogenesis, extensive neuronal loss and glial scarring remain permanent.

A thorough understanding of these mechanisms, derived from animal models, human *post-mortem* studies, and neuroimaging investigations, is essential for developing effective neuroprotective and neuroregenerative therapeutic strategies in the context of chronic alcohol consumption.

Conflict of interests

The authors declare that they have no conflict of interests.

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