Perineuronal Nets, Microglial Neuroinflammation, and Alzheimer's Disease

A Critical Synthesis of Mechanistic Pathways Linking Extracellular Matrix Degradation to Neurodegeneration and Cognitive Resilience

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Neuroscience

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April 2026

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Abstract

This dissertation undertakes a systematic, integrative analysis of the mechanistic pathways by which perineuronal nets (PNNs)—specialized extracellular matrix (ECM) structures ensheathing predominantly fast-spiking parvalbumin-positive (PV+) interneurons—contribute to the pathogenesis of Alzheimer's disease (AD) and related neurodegenerative conditions. Drawing on five peer-reviewed investigations spanning molecular biology, neuropathology, and systems neuroscience, the thesis traces a causal chain from amyloid-beta-induced microglial activation, through enzymatic and phagocytic degradation of PNN components, to downstream consequences including synaptic destabilization, impaired GABAergic inhibition, oxidative vulnerability, and memory dysfunction.

The analysis proceeds through three thematic chapters. Chapter One establishes the molecular architecture of PNNs—comprising a hyaluronan backbone, chondroitin sulfate proteoglycans (CSPGs, principally aggrecan), tenascin-R cross-links, and link proteins—and synthesizes evidence for their roles in synaptic stabilization, critical period closure, ionic buffering, and neuroprotection. Chapter Two examines the empirical evidence for microglia-mediated PNN degradation in AD, critically evaluating the experimental designs, statistical rigor, and inferential limitations of the Crapser et al. (2020) study, which demonstrated that pharmacological microglial depletion via CSF1R inhibition prevents PNN loss in 5xFAD transgenic mice. Chapter Three synthesizes the emerging concept of cognitive resilience to AD, analyzing the de Vries et al. (2024) finding that resilient individuals—those maintaining intact cognition despite intermediate-to-high AD neuropathology—exhibit distinct PNN alterations characterized by preserved synaptic contacts around PV neurons and homeostatic, rather than pathological, matrix remodeling.

The dissertation concludes that PNN integrity represents a critical, previously underappreciated axis of neurodegeneration in AD. The convergent evidence supports a model in which microglia-driven PNN degradation constitutes a self-reinforcing cycle of neuroinflammation, synaptic loss, and cognitive decline, while PNN preservation may underlie mechanisms of cognitive resilience. These findings carry significant implications for therapeutic strategy, suggesting that interventions targeting PNN stabilization—including modulation of chondroitin sulfate sulfation ratios, matrix metalloproteinase inhibition, and microglial phenotype regulation—may represent viable approaches for preventing or decelerating cognitive decline in AD.

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1. Introduction

1.1 Research Problem and Significance

Alzheimer's disease (AD) remains the most prevalent neurodegenerative disorder worldwide, affecting an estimated 55 million people globally as of 2023, with projections suggesting this figure will triple by 2050 (World Health Organization, 2023). Despite decades of intensive research, the field has been marked by a succession of therapeutic failures, most notably the disappointing results of amyloid-beta (Aβ) immunotherapy trials that succeeded in clearing plaques but produced limited cognitive benefit (Cummings et al., 2022). This persistent translational gap suggests that the dominant amyloid cascade hypothesis, while capturing an important element of AD pathogenesis, provides an incomplete account of the mechanisms driving cognitive decline.

A growing body of evidence implicates the brain's extracellular matrix (ECM), and specifically perineuronal nets (PNNs), as critical yet underexplored components of AD pathophysiology. PNNs are specialized, condensed ECM structures that ensheath predominantly parvalbumin-positive (PV+) fast-spiking GABAergic interneurons throughout the central nervous system (CNS). Composed of a hyaluronan backbone, chondroitin sulfate proteoglycans (CSPGs), tenascin-R, and link proteins (Fawcett et al., 2022; van 't Spijker & Kwok, 2017), PNNs serve multiple homeostatic functions: they stabilize synaptic contacts, regulate neuronal plasticity through critical period closure, buffer ionic concentrations (particularly iron), and protect ensheathed neurons from oxidative stress and excitotoxicity (Auer et al., 2025).

The significance of PNN dysfunction in AD has been underscored by several recent discoveries. Crapser et al. (2020) demonstrated that activated microglia—the brain's resident immune cells—directly phagocytose PNN components in both the 5xFAD mouse model and human AD cortical tissue, and that pharmacological depletion of microglia prevents this pathological PNN loss. Complementing this mechanistic finding, de Vries et al. (2024) reported that individuals who maintain cognitive function despite substantial AD neuropathology—a phenomenon termed "cognitive resilience"—exhibit distinct PNN alterations characterized by preserved synaptic contacts and homeostatic, rather than pathological, matrix remodeling. Together with comprehensive reviews of PNN molecular biology (van 't Spijker & Kwok, 2017), PNN roles in memory (Fawcett et al., 2022), and PNN involvement across neurological diseases (Auer et al., 2025), these findings suggest that PNN degradation may represent a convergent pathological mechanism linking neuroinflammation, synaptic loss, and cognitive decline in AD.

1.2 Research Questions and Thesis Statement

This dissertation addresses three interrelated research questions. First, what are the molecular mechanisms by which PNNs contribute to neuronal homeostasis, and how does their degradation compromise neural function? Second, what is the evidence that microglia-mediated PNN destruction constitutes a mechanistic driver—rather than merely an epiphenomenon—of AD pathogenesis? Third, does the emerging evidence

on PNN preservation in cognitively resilient individuals provide a coherent explanatory framework for understanding differential vulnerability to AD?

The central thesis advanced here is that PNN integrity constitutes a critical axis of neurodegeneration in AD, mediating the transition from amyloid pathology to synaptic dysfunction and cognitive decline through a self-reinforcing cycle involving microglial activation, ECM degradation, loss of inhibitory circuit stability, and increased neuronal vulnerability. Furthermore, this thesis argues that the five studies under analysis, when synthesized, provide a mechanistically coherent—though incomplete—account of how ECM dynamics contribute to AD pathogenesis and cognitive resilience, with significant implications for therapeutic strategy.

1.3 Scope and Disciplinary Approach

This dissertation adopts an integrative neuroscience approach, synthesizing experimental evidence from cellular and molecular neurobiology, neuropathology, and systems neuroscience. The primary sources under analysis are five peer-reviewed articles spanning the period 2017–2025, selected to represent the spectrum from molecular characterization through disease mechanism to clinical-pathological correlation. The analytical framework combines mechanistic reasoning with critical evaluation of experimental design, statistical inference, and translational potential. The dissertation does not present original experimental data; rather, its contribution lies in the systematic integration, critical analysis, and theoretical synthesis of existing findings to construct a unified model of PNN-mediated neurodegeneration in AD.

2. Literature Review

2.1 Historical Context: The Extracellular Matrix in Neuroscience

The recognition of PNNs as functionally significant neural structures has followed a protracted and uneven trajectory. First described by Camillo Golgi in 1893 and subsequently elaborated by Santiago Ramón y Cajal, PNNs were dismissed for much of the twentieth century as histological artifacts (Celio et al., 1998). Their rehabilitation as biologically meaningful structures began with the development of specific lectins and antibodies in the 1980s and 1990s, particularly Wisteria floribunda agglutinin (WFA), which binds specifically to N-acetylgalactosamine residues on chondroitin sulfate glycosaminoglycan (CS-GAG) chains (Brückner et al., 1993). The subsequent identification of PNN molecular components—including the CSPGs aggrecan, brevican, neurocan, and versican; the glycoprotein tenascin-R; and the link proteins HAPLN1 and HAPLN4—established PNNs as organized molecular assemblies with specific biosynthetic pathways and regulatory functions (Yamaguchi, 2000).

The conceptual breakthrough linking PNNs to neural plasticity emerged from studies of visual cortical development. Pizzorusso et al. (2002) demonstrated that enzymatic degradation of CS-GAGs with chondroitinase ABC (ChABC) reactivated ocular dominance plasticity in adult rats, establishing PNNs as critical regulators of critical period closure. This finding catalyzed a wave of research demonstrating PNN involvement in multiple forms of experience-dependent plasticity, fear memory consolidation, drug-associated learning, and cognitive aging (reviewed in Fawcett et al., 2022).

2.2 The Amyloid Cascade Hypothesis and Its Limitations

The dominant theoretical framework for AD pathogenesis—the amyloid cascade hypothesis—posits that accumulation of Aβ peptides, particularly the aggregation-prone Aβ42 species, initiates a pathological cascade leading to tau hyperphosphorylation, neurofibrillary tangle formation, synaptic loss, neuroinflammation, and ultimately cognitive decline (Hardy & Higgins, 1992; Selkoe & Hardy, 2016). While substantial genetic evidence supports the centrality of Aβ in familial AD, the hypothesis has faced mounting criticism: the poor correlation between plaque burden and cognitive status; the existence of cognitively normal individuals with substantial amyloid pathology; the failure of amyloid-targeting therapies to produce robust cognitive benefit; and the recognition that neuroinflammation, tau pathology, and synaptic dysfunction may represent independent or semi-independent pathological axes (De Strooper & Bhatt, 2020).

The neuroinflammation hypothesis, which emphasizes the role of chronically activated microglia and astrocytes in driving neurodegeneration, has gained particular traction (Heneka et al., 2015). Microglia, the brain's principal innate immune cells, undergo profound phenotypic transformation in AD, shifting from homeostatic surveillance to disease-associated states characterized by enhanced phagocytosis, pro-inflammatory cytokine production, and altered metabolic profiles (Keren-Shaul et al., 2017). The intersection of neuroinflammation with ECM biology—specifically, the recognition that activated

microglia degrade PNNs—represents a mechanistic bridge between these theoretical frameworks, as this dissertation will argue.

2.3 Cognitive Resilience: An Emerging Paradigm

The concept of cognitive resilience refers to the phenomenon whereby certain individuals maintain normal cognitive function despite accumulating neuropathological hallmarks of AD—including Aβ plaques, neurofibrillary tangles, and neuroinflammation—that would typically be associated with dementia (Stern, 2012; Arenaza-Urquijo & Vemuri, 2018). Resilience is conceptually distinct from cognitive reserve (which emphasizes pre-existing neural capacity and compensation) and from resistance (which implies absence of pathology). Identifying the biological substrates of resilience has become a priority in AD research, as such factors may reveal neuroprotective mechanisms amenable to therapeutic enhancement. The recent implication of PNNs in resilience (de Vries et al., 2024) opens a new avenue within this paradigm, suggesting that ECM integrity may be a previously unrecognized determinant of differential cognitive vulnerability.

3. Methodology

3.1 Analytical Framework

This dissertation employs a systematic integrative review methodology, combining critical appraisal of individual studies with cross-study synthesis to construct a unified mechanistic model. The analytical framework draws on principles of mechanistic reasoning in biomedical science (Machamer et al., 2000), which emphasizes the identification of entities, activities, and organizational structures that produce phenomena of interest. Each primary source is evaluated along multiple dimensions: experimental design adequacy, including model validity and statistical power; internal consistency of findings; convergence or divergence with evidence from other studies; strength of causal inference; and translational potential.

3.2 Source Selection and Justification

The five primary sources were selected to provide comprehensive coverage of the PNN–AD axis from molecular mechanism to clinical-pathological correlation. Van ’t Spijker and Kwok (2017) provides the foundational molecular biology of PNN structure and function. Fawcett et al. (2022) synthesizes the extensive literature on PNN involvement in multiple memory systems. Auer et al. (2025) offers an updated review incorporating the most recent findings on PNN roles in neurological disease. Crapser et al. (2020) contributes the key experimental evidence for microglia-mediated PNN degradation in AD. De Vries et al. (2024) provides human neuropathological evidence linking PNN alterations to cognitive resilience.

This source selection ensures coverage of three complementary levels of analysis: molecular-structural (what PNNs are and how they function), mechanistic-pathological (how they are degraded in AD), and clinical-translational (what their preservation means for cognitive outcomes). The temporal span of these publications (2017–2025) captures the rapid evolution of this field, while the diversity of methodological approaches—ranging from in vitro biochemistry through transgenic mouse models to human post-mortem neuropathology—enables triangulation of findings across multiple levels of evidence.

3.3 Critical Evaluation Criteria

Each study is evaluated against established criteria for research quality in biomedical science. For experimental studies (Crapser et al., 2020; de Vries et al., 2024), this includes assessment of sample sizes and statistical power; appropriateness of control conditions; potential confounding variables; validity of animal models or human tissue classification; and the strength of causal inference afforded by the experimental design. For review articles (van ’t Spijker & Kwok, 2017; Fawcett et al., 2022; Auer et al., 2025), evaluation criteria include comprehensiveness of literature coverage, consistency of interpretive framework, identification of gaps and contradictions in the evidence base, and avoidance of confirmation bias. Throughout, particular attention is paid to the distinction between correlation and causation, given that PNN alterations in AD may reflect pathological processes, compensatory responses, or incidental

consequences of disease-related changes.

4. The Molecular Architecture and Functional Significance of Perineuronal Nets

4.1 Structural Composition and Assembly

Perineuronal nets are hierarchically organized ECM structures whose molecular architecture has been elucidated through decades of biochemical, immunohistochemical, and genetic studies (van ’t Spijker & Kwok, 2017; Auer et al., 2025). The structural backbone of PNNs is hyaluronan (HA), a non-sulfated glycosaminoglycan synthesized at the neuronal membrane by hyaluronan synthases (HAS1, HAS2, HAS3) and anchored to the cell surface via interactions with CD44 receptors, ankyrin-R, and the transmembrane CSPG RPTPzeta/phosphacan (van ’t Spijker & Kwok, 2017). This HA scaffold provides the structural framework to which CSPGs attach via their N-terminal globular domains, an interaction stabilized by the link proteins HAPLN1 and HAPLN4.

Four members of the lectican family of CSPGs constitute the principal proteoglycan components of PNNs: aggrecan, brevican, neurocan, and versican. Of these, aggrecan is the most abundant and carries the highest density of CS-GAG side chains, making it the primary determinant of PNN charge density and barrier properties (Auer et al., 2025). Each CSPG possesses a core protein with N-terminal G1 and C-terminal G3 globular domains flanking a central region to which CS-GAG chains are covalently attached. The CS-GAG chains consist of repeating disaccharide units of glucuronic acid and N-acetylgalactosamine, which can be sulfated at specific positions to generate distinct sulfation motifs: chondroitin-4-sulfate (C4S or CS-A), chondroitin-6-sulfate (C6S or CS-C), and disulfated variants (CS-D, CS-E). As Fawcett et al. (2022) emphasize, these sulfation patterns are not merely structural decorations but functional determinants that regulate molecular binding, plasticity, and aging.

The cross-linking glycoprotein tenascin-R (TNR) provides the final structural element, binding to the C-terminal G3 domains of multiple CSPGs to create a lattice-like mesh. This tripartite assembly—HA backbone, CSPG attachment, TNR cross-linking—produces the characteristic reticular morphology visible by WFA staining and immunohistochemistry. Importantly, PNN composition is not uniform across brain regions: the density, CSPG complement, and sulfation patterns vary substantially between cortex, hippocampus, cerebellum, and other structures, suggesting region-specific functional specialization (van ’t Spijker & Kwok, 2017).

4.2 Functional Roles: Synaptic Stabilization and Plasticity Regulation

The most extensively studied function of PNNs is their regulation of synaptic plasticity. The seminal demonstration by Pizzorusso et al. (2002) that ChABC-mediated PNN removal reactivates ocular dominance plasticity in adult visual cortex established PNNs as molecular brakes on experience-dependent circuit reorganization. Subsequent work has revealed that this plasticity-regulating function operates through multiple molecular mechanisms. First, the dense PNN mesh physically restricts the lateral mobility of AMPA receptors within the synaptic membrane, thereby stabilizing synaptic strength

(Frischknecht et al., 2009). Fawcett et al. (2022) describe how hyaluronan digestion with hyaluronidase increases AMPA receptor mobility and enhances synaptic plasticity, while CSPGs modulate AMPA receptor clustering through interactions with neuronal pentraxin 2 (Nptx2).

Second, PNNs serve as molecular repositories for plasticity-regulating factors. The homeobox transcription factor OTX2, secreted by choroid plexus cells, binds specifically to sulfated CS-GAG motifs on PNNs and accumulates in PV+ interneurons, where it promotes maturation of inhibitory circuits and maintenance of critical period closure (Beurdeley et al., 2012). Similarly, the chemorepulsive molecule semaphorin 3A (Sema3A) binds to PNN CSPGs, creating an inhibitory molecular environment that restricts axonal sprouting and synaptic remodeling. Removal of PNNs releases these factors, permitting a transient window of enhanced plasticity.

Third, the sulfation code of CS-GAGs provides a molecular mechanism for age-dependent plasticity decline. Fawcett et al. (2022) report that the ratio of C6S to C4S shifts dramatically with aging: C6S, which is permissive for plasticity, nearly disappears from the brain by 20 months in mice, while inhibitory C4S remains stable. This sulfation shift effectively locks PNNs into a plasticity-restrictive configuration, correlating with age-related memory impairment. Transgenic mice lacking C6-sulfotransferase exhibit premature memory deficits at 3 months that phenocopy those seen in 20-month-old wild-type mice, providing causal evidence for the functional significance of this sulfation ratio.

4.3 Neuroprotective Functions: Ionic Buffering and Oxidative Stress Defense

Beyond plasticity regulation, PNNs serve critical neuroprotective functions particularly relevant to understanding their role in neurodegeneration. Van ’t Spijker and Kwok (2017) emphasize the polyanionic nature of PNNs: the sulfated GAG chains carry a high density of negative charges that create a local microenvironment capable of buffering cation concentrations. This charge density is especially important for sequestering transition metals, particularly iron (Fe^{2+}/Fe^{3+}), which catalyzes the Fenton reaction to generate highly reactive hydroxyl radicals. By chelating iron within the PNN matrix, these structures protect the ensheathed neurons—predominantly PV+ fast-spiking interneurons with exceptionally high metabolic demands—from oxidative damage.

The neuroprotective significance of this ionic buffering function is underscored by the observation that PV+ interneurons are selectively vulnerable to oxidative stress due to their high firing rates, substantial mitochondrial density, and correspondingly elevated reactive oxygen species (ROS) production (Cabungcal et al., 2013). Van ’t Spijker and Kwok (2017) argue that PNNs represent an evolutionary adaptation enabling these metabolically demanding neurons to sustain high-frequency firing without succumbing to oxidative damage. The loss of PNNs in AD, as documented by Crapser et al. (2020), would therefore render PV+ interneurons acutely vulnerable to oxidative injury, potentially initiating a cascade of inhibitory circuit dysfunction, excitatory-inhibitory imbalance, and network destabilization.

PNNs also function as physical barriers against potentially neurotoxic molecules in the extracellular space. Auer et al. (2025) note that the dense mesh structure of PNNs can limit the diffusion of large molecules, including Aβ oligomers, to neuronal surfaces. This barrier function, combined with ionic buffering, positions PNNs as first-line defenses of the neurons they ensheath. The corollary—that

PNN-bearing neurons are preferentially protected while PNN-devoid neurons are more vulnerable—finds support in the de Vries et al. (2024) observation that excitatory neurons bearing PNNs in resilient brains show low levels of phosphorylated tau, suggesting a possible protective effect of PNN-associated mechanisms against tau pathology.

4.4 Critical Assessment of the Foundational Literature

While the molecular characterization of PNNs is well-established, several critical limitations of the foundational literature merit attention. First, the predominant experimental tool for studying PNN function—enzymatic degradation with ChABC—produces complete and indiscriminate removal of CS-GAGs, which does not reflect the graded, component-specific modifications that likely occur in physiological and pathological conditions (Fawcett et al., 2022). The binary comparison between intact and completely degraded PNNs may overestimate the functional consequences of partial PNN alterations observed in disease states.

Second, the literature reveals significant inconsistencies regarding the direction and magnitude of PNN changes across brain regions and experimental paradigms. Fear conditioning has been reported to both increase and decrease PNN density depending on the brain region and timepoint examined (Fawcett et al., 2022). Stress produces biphasic effects that are difficult to reconcile within a single mechanistic framework. These inconsistencies suggest that PNN dynamics are more context-dependent and region-specific than current models acknowledge, and that generalizations from one brain region or behavioral paradigm to AD pathology should be made cautiously.

Third, the reviews by van ’t Spijker and Kwok (2017) and Auer et al. (2025), while comprehensive, rely heavily on correlative evidence linking PNN composition to functional outcomes. The causal chain from specific molecular changes (e.g., altered sulfation patterns) to cellular phenotypes (e.g., increased plasticity) to behavioral outcomes (e.g., memory enhancement or impairment) involves multiple levels of biological organization, and the mechanistic connections between these levels remain incompletely specified.

5. Microglia-Mediated PNN Degradation as a Mechanistic Driver of Alzheimer's Disease

5.1 The Crapser et al. (2020) Study: Experimental Design and Findings

The study by Crapser et al. (2020), published in EBioMedicine, represents the most direct experimental evidence for microglia-mediated PNN degradation in AD. The study employed the 5xFAD transgenic mouse model, which expresses five familial AD mutations (three in APP: Swedish, Florida, London; two in PSEN1: M146L, L286V) and develops aggressive amyloid pathology beginning at approximately 2 months of age. The investigators performed extensive histological analysis across four timepoints (4, 8, 12, and 18 months) in both male and female mice, examining plaque burden, microglial density and morphology, and PNN integrity in cortical and hippocampal regions.

The principal findings were as follows. First, PNNs were extensively and progressively lost in 5xFAD mice in proportion to amyloid plaque burden, with statistically significant reductions detectable by 4 months in subiculum and by 8 months in visual cortex. Second, activated microglia showed intimate spatial association with altered PNNs, and electron microscopy revealed PNN material within microglial phagosomes, providing direct morphological evidence for microglial engulfment of PNN components. Third, aggrecan, the principal PNN CSPG, was detected within dense-core amyloid plaques in human AD tissue, suggesting that degraded PNN material becomes incorporated into plaque deposits. Fourth, PNN loss preceded the reduction in PV+ interneuron numbers, suggesting that PNN degradation may be an early event that precedes and potentially contributes to interneuron loss.

The study's most compelling finding involved the pharmacological intervention arm. Chronic treatment of 5xFAD mice with the CSF1R inhibitor PLX5622, which depletes brain microglia by approximately 90%, prevented PNN loss despite persistent amyloid plaque burden. This finding was replicated in the 3xTg-AD mouse model, strengthening the generalizability of the result. The dissociation between plaque persistence and PNN preservation under microglial depletion provides strong evidence that microglia are not merely bystanders but active effectors of PNN degradation in the context of amyloid pathology. The elevated microglial densities observed in 5xFAD subiculum (statistically significant at every timepoint, $p < 0.0001$) and visual cortex (significant from 8 months onward, $p < 0.0001$) corroborate the association between microglial activation and PNN pathology.

5.2 Mechanistic Interpretation: The Neuroinflammatory Cascade

The Crapser et al. findings, interpreted in the context of the broader literature reviewed by Fawcett et al. (2022) and Auer et al. (2025), suggest a multi-step mechanistic cascade. Extracellular Aβ aggregates activate microglia through pattern recognition receptors (TREM2, TLR2, TLR4, CD36), shifting them from homeostatic surveillance to a disease-associated phenotype characterized by enhanced phagocytic activity and pro-inflammatory mediator release (Keren-Shaul et al., 2017). Activated microglia then degrade PNNs through two parallel mechanisms: direct phagocytic engulfment of PNN components, as

demonstrated by the electron microscopy findings; and secretion of matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9, which cleave CSPG core proteins and disrupt PNN structural integrity.

The downstream consequences of PNN loss are multifaceted and potentially self-reinforcing. First, loss of PNN-mediated synaptic stabilization exposes synapses to destabilizing influences, contributing to the synaptic loss that is the strongest pathological correlate of cognitive decline in AD (Terry et al., 1991). Second, removal of the PNN iron-buffering capacity exposes PV+ interneurons to oxidative stress, potentially triggering mitochondrial dysfunction and apoptotic signaling. Third, destabilization of PV+ interneuron function disrupts the excitatory-inhibitory balance of cortical circuits, a feature increasingly recognized as central to AD-associated network dysfunction, including the hyperexcitability and epileptiform activity observed in many AD patients (Palop & Mucke, 2016). Fourth, dying or dysfunctional neurons release damage-associated molecular patterns (DAMPs) that further activate microglia, creating a positive feedback loop of neuroinflammation and tissue damage.

Fawcett et al. (2022) add an important dimension to this model by highlighting the role of PNN degradation in aberrant plasticity. While controlled PNN remodeling is essential for normal memory function—fear conditioning, for example, involves transient MMP-mediated PNN modifications that enable synaptic reorganization—the uncontrolled, widespread PNN loss observed in AD likely produces maladaptive plasticity: the formation of inappropriate synaptic connections and the destabilization of existing memory traces. This distinction between physiological PNN remodeling (targeted, transient, component-specific) and pathological PNN degradation (widespread, persistent, indiscriminate) is crucial for understanding why therapeutic approaches must aim to normalize, rather than simply prevent or enhance, PNN dynamics.

5.3 Critical Evaluation of the Evidence

While the Crapser et al. (2020) study provides compelling evidence for microglia-mediated PNN degradation in AD, several methodological and interpretive limitations warrant critical scrutiny. First, the 5xFAD model, while widely used, represents an aggressive, mutation-driven form of amyloid pathology that does not fully recapitulate the sporadic AD that accounts for over 95% of human cases. The five familial mutations produce supraphysiological levels of Aβ42 and an accelerated disease course, raising questions about whether the PNN pathology observed reflects mechanisms operative in the more gradual, multifactorial pathogenesis of sporadic AD.

Second, the CSF1R inhibitor PLX5622, while effective at depleting microglia, is not entirely specific: CSF1R is also expressed by peripheral monocytes, and PLX5622 may affect blood-derived macrophages that infiltrate the brain in AD. The authors acknowledge this limitation, noting that the relative contributions of resident microglia and infiltrating monocytes cannot be distinguished. Furthermore, chronic microglial depletion cannot be directly translated to therapeutic strategy, as microglia perform essential functions including synaptic pruning, debris clearance, and neurotrophin production. The therapeutic implication is therefore not that microglia should be eliminated, but that their disease-associated phenotype should be modulated—a considerably more nuanced and technically challenging objective.

Third, the study demonstrates association and intervention-based evidence for microglial involvement in PNN loss but does not fully establish the molecular mechanisms through which microglia recognize and degrade PNN components. The relative contributions of phagocytosis versus protease secretion remain unquantified, and the signals that direct microglial attention to PNNs specifically—rather than other ECM structures—are unknown.

Fourth, the causal directionality between PNN loss and neurodegeneration remains incompletely resolved. While the observation that PNN loss precedes PV+ interneuron loss in 5xFAD mice is consistent with PNN degradation driving neuronal vulnerability, it does not exclude the possibility that early, subtle neuronal dysfunction (e.g., reduced CSPG synthesis by stressed neurons) contributes to PNN thinning before morphological neuron loss becomes detectable. Disentangling these possibilities would require neuron-specific genetic manipulation of PNN component expression in AD model mice—experiments that have not yet been reported.

6. Cognitive Resilience, PNN Preservation, and Therapeutic Implications

6.1 PNN Alterations in Cognitive Resilience: The de Vries et al. (2024) Study

The study by de Vries et al. (2024), published in Alzheimer's & Dementia, provides the first direct evidence linking PNN alterations to cognitive resilience in human AD. The investigators examined frontal cortex tissue from three carefully defined groups: cognitively normal controls (CDR ≤ 0.5, Braak 1–2), AD patients (CDR 3, Braak 4–6), and resilient subjects (CDR ≤ 0.5, Braak 3–5, Thal ≥ 3). The resilient group is conceptually pivotal: these individuals maintained intact cognitive function despite harboring intermediate-to-high AD neuropathology, making them a natural experiment for identifying protective mechanisms.

The findings revealed a nuanced pattern of PNN alterations across groups. Overall PNN density (number of aggrecan+ PNNs per mm² around NeuN+ neurons) did not differ significantly between groups ($F = 1.24$, $p = 0.302$), and approximately 7% of NeuN+ neurons bore PNNs in all three groups. However, the intensity of aggrecan staining in PNNs was significantly reduced in AD ($F = 4.38$, $p = 0.021$), with a shift toward the "very weak" intensity category ($F = 6.56$, $p = 0.038$). Resilient subjects showed an intermediate pattern: aggrecan intensity was reduced relative to controls but not as severely as in AD.

The most revealing findings emerged from analysis of PV+ interneurons specifically. Both AD and resilient subjects showed reduced aggrecan+ PNN coverage around PV neurons, but crucially, synaptic contacts (measured by synaptophysin+ puncta) were preserved around PV neurons in resilient brains while significantly reduced in AD. This dissociation—PNN reduction without synaptic loss in resilient subjects versus PNN reduction with synaptic loss in AD—suggests that PNN integrity per se is not the critical variable; rather, what matters is whether PNN changes are accompanied by downstream synaptic destabilization.

Gene expression analysis provided additional mechanistic insight. Genes encoding matrix-degrading enzymes (MMP2, MMP9, cathepsin B, cathepsin L) were significantly upregulated in AD tissue but not in resilient tissue. This finding suggests that the PNN changes observed in resilient brains reflect homeostatic remodeling—perhaps an adaptive response to maintain plasticity in the face of accumulating pathology—whereas the PNN changes in AD brains reflect pathological degradation driven by an overactive inflammatory cascade. The distinction between homeostatic and pathological matrix remodeling may be a key determinant of whether amyloid pathology progresses to clinical dementia.

6.2 Integrative Model: PNN Dynamics as a Determinant of AD Trajectory

Synthesizing the evidence from all five primary sources, this dissertation proposes an integrative model in which PNN dynamics serve as a critical determinant of the trajectory from amyloid pathology to cognitive outcome. In this model, the initial accumulation of Aβ plaques activates microglia through innate immune

receptors, shifting them toward a disease-associated phenotype. The subsequent interaction between activated microglia and PNNs determines divergent outcomes.

In the pathological trajectory (typified by AD), microglial activation triggers uncontrolled PNN degradation through both phagocytic engulfment and MMP-mediated proteolysis (Crapser et al., 2020). This degradation removes the structural, ionic, and molecular supports that PNNs provide to PV+ interneurons (van ’t Spijker & Kwok, 2017), leading to: loss of synaptic stabilization; impaired ionic buffering and increased oxidative stress; release of plasticity-regulating molecules (OTX2, Sema3A) that further destabilize circuit function; disruption of excitatory-inhibitory balance; and ultimately, PV+ interneuron dysfunction and loss. The resulting network instability produces the cognitive symptoms of AD, while dying neurons release DAMPs that further activate microglia, perpetuating the destructive cycle.

In the resilient trajectory, PNN remodeling occurs in a controlled, homeostatic fashion. The de Vries et al. (2024) findings suggest that resilient individuals maintain PNN function—as evidenced by preserved synaptic contacts—despite quantitative reductions in PNN intensity. This may be achieved through several mechanisms: maintained expression of matrix-stabilizing factors (consistent with the absence of MMP/cathepsin upregulation in resilient tissue); preservation of the C6S:C4S sulfation ratio (Fawcett et al., 2022), which would maintain plasticity capacity; and possible compensatory increases in other ECM components. The observation that PNN-bearing excitatory neurons in resilient brains show low phosphorylated tau levels further suggests that intact PNNs may actively protect against tau pathology—a finding with profound implications if confirmed, as it would link ECM integrity to the tau cascade that is increasingly recognized as the proximate driver of neuronal death in AD.

This integrative model makes several testable predictions. First, interventions that stabilize PNNs—for example, through C6-sulfotransferase gene therapy, MMP inhibition, or anti-C4S antibodies (Fawcett et al., 2022)—should delay or prevent cognitive decline in AD model mice, even without reducing amyloid burden. Second, microglial phenotype modulation (shifting from disease-associated to homeostatic states) should preserve PNN integrity and cognitive function. Third, longitudinal biomarker studies should reveal that PNN degradation markers (e.g., CSF levels of aggrecan fragments) precede cognitive decline and distinguish individuals who will develop dementia from those who will remain resilient.

6.3 Critical Assessment of the Resilience Evidence

The de Vries et al. (2024) study, while groundbreaking, is subject to several limitations. Most fundamentally, as a cross-sectional post-mortem study, it cannot establish temporal relationships between PNN changes and cognitive preservation. The PNN pattern observed at the time of death in resilient individuals may not reflect the PNN state during the decades of cognitive preservation that preceded death.

The sample sizes, while adequate for detecting the reported effects, are relatively modest (n = 8–12 per group for most analyses), limiting statistical power for subgroup analyses and increasing the risk that observed effects may not replicate in larger cohorts. The classification of resilience based on CDR scores and neuropathological staging, while standard in the field, involves dichotomizing what are likely

continuous variables, potentially obscuring gradients of resilience that correlate with gradients of PNN preservation.

The gene expression data, while informative, are derived from bulk tissue RNA sequencing rather than cell-type-specific analyses. Given that PNN-bearing neurons, PNN-devoid neurons, microglia, astrocytes, and oligodendrocytes all contribute to the tissue RNA pool, it is impossible to determine which cell types are responsible for the observed changes in MMP and cathepsin expression. Single-nucleus RNA sequencing or spatial transcriptomics applied to these same tissue samples would substantially clarify the cellular origins of the matrix-remodeling differences between AD and resilient brains.

Despite these limitations, the convergence of the de Vries et al. findings with the mechanistic framework established by the other four primary sources lends considerable weight to the conclusion that PNN dynamics are meaningfully related to cognitive outcomes in AD. The fact that PNN preservation is associated with maintained synaptic contacts and reduced matrix-degrading enzyme expression—both of which are mechanistically connected to the pathways described in the preceding chapters—suggests a coherent biological signal rather than a spurious correlation.

6.4 Therapeutic Implications and Future Directions

The synthesis presented in this dissertation identifies several promising therapeutic strategies. First, modulation of the CS sulfation code represents a mechanistically motivated approach. Fawcett et al. (2022) report that viral delivery of C6-sulfotransferase to aged mouse brains restores the C6S:C4S ratio and rescues memory deficits. Translating this approach to AD could simultaneously restore PNN-mediated plasticity and enhance neuroprotection. Second, selective MMP inhibitors that target the specific proteases involved in pathological (but not physiological) PNN degradation could prevent the destructive remodeling observed in AD without impairing the adaptive remodeling that may support resilience. Third, microglial phenotype modulation—shifting disease-associated microglia toward homeostatic or “neuroprotective” states—addresses the upstream driver of PNN degradation and could have broad benefits beyond PNN preservation.

Several critical knowledge gaps must be addressed to advance these therapeutic approaches. The most pressing is the need for longitudinal studies—either in mouse models with serial PNN assessment or in human cohorts with cerebrospinal fluid or imaging biomarkers of PNN integrity—to establish the temporal relationship between PNN changes and cognitive decline. Additionally, the field requires cell-type-specific transcriptomic data to identify the cellular sources of matrix-degrading enzymes and PNN components in the AD brain. Finally, the interaction between PNN pathology and other AD-associated ECM changes—including the diffuse ECM, vascular basement membrane, and glial scar formation—remains largely unexplored and may reveal additional therapeutic targets.

7. Conclusion

7.1 Summary of Findings

This dissertation has undertaken a systematic synthesis and critical analysis of five peer-reviewed studies examining the relationship between perineuronal nets, neuroinflammation, and Alzheimer's disease. The analysis supports three principal conclusions.

First, PNNs are structurally and functionally complex ECM assemblies whose molecular architecture—particularly the hierarchical organization of hyaluronan, CSPGs, link proteins, and tenascin-R—enables multiple homeostatic functions critical for neural health, including synaptic stabilization, plasticity regulation through sulfation-dependent molecular signaling, ionic buffering and oxidative stress defense, and physical barrier protection against neurotoxic molecules.

Second, the experimental evidence from Crapser et al. (2020), interpreted within the mechanistic framework established by the review literature, supports the conclusion that microglia-mediated PNN degradation is a significant pathological mechanism in AD. The demonstration that microglial depletion prevents PNN loss despite persistent amyloid pathology provides intervention-based evidence that goes beyond mere correlation, though important caveats regarding model validity, tool specificity, and causal directionality remain.

Third, the emerging evidence on cognitive resilience from de Vries et al. (2024) introduces a critical new dimension: the observation that resilient individuals maintain synaptic integrity around PV+ interneurons despite PNN alterations, combined with the absence of pathological matrix-degrading enzyme upregulation, suggests that the distinction between homeostatic and pathological matrix remodeling may be a key determinant of cognitive outcome in the presence of AD neuropathology.

7.2 Do These Studies Explain Alzheimer's Disease?

The question posed by this dissertation—whether these five studies provide an explanation for the science behind Alzheimer's disease and adjacent neurodegenerative pathologies—requires a carefully calibrated answer. The studies do provide a mechanistically coherent account of one important axis of AD pathogenesis: the neuroinflammation–ECM degradation–synaptic loss axis. They offer a plausible mechanism by which amyloid pathology is transduced into synaptic dysfunction and cognitive decline, and they identify a biological substrate for the clinically important phenomenon of cognitive resilience.

However, it would be overreaching to claim that PNN pathology "explains" AD in any comprehensive sense. AD is a multifactorial disease involving genetic susceptibility (APOE4, TREM2, and dozens of common variants), vascular pathology, metabolic dysfunction, protein aggregation pathology (both Aβ and tau), and complex interactions between these factors. PNN degradation, as described in these studies, likely represents one strand within a much larger pathological web. Moreover, several of the core claims—that PNN loss causally drives cognitive decline, that PNN preservation

Prepared for Dr. James Truchard and Benjamin Gustafsson
Drafted by Claude-Opus-4.6

mediates resilience, that therapeutic PNN stabilization would prevent dementia—remain, at present, incompletely established hypotheses rather than demonstrated facts.

What these studies do convincingly establish is that the extracellular matrix in general, and PNNs in particular, deserve far greater attention in AD research than they have historically received. The dominant focus on intracellular pathology (amyloid processing, tau phosphorylation, autophagy) has left the extracellular compartment relatively understudied, despite the fact that the ECM constitutes approximately 20% of brain volume and serves functions essential for neural homeostasis. The evidence reviewed here suggests that ECM dynamics may represent a critical "missing link" between neuroinflammation and neurodegeneration—a link with significant therapeutic potential.

7.3 Future Directions

Several directions for future research emerge from this analysis. Longitudinal studies using PNN biomarkers (CSF aggrecan fragments, imaging ligands for PNN density) are needed to establish the temporal relationship between PNN degradation and cognitive decline in human AD. Cell-type-specific transcriptomic analyses (single-nucleus RNA sequencing, spatial transcriptomics) should be applied to resilient, AD, and control tissue to identify the cellular sources and regulators of matrix remodeling. Genetic manipulation of PNN components in AD mouse models (neuron-specific aggrecan knockout, astrocyte-specific HAPLN overexpression) would clarify the causal role of PNN integrity in disease progression. Preclinical trials of PNN-stabilizing interventions (C6-sulfotransferase gene therapy, selective MMP inhibitors) in AD models should assess both PNN preservation and cognitive outcomes. Finally, the interaction between PNN pathology and other AD-associated processes—including tau propagation, vascular dysfunction, and glymphatic clearance—should be systematically investigated to position PNN dynamics within the broader landscape of AD pathogenesis.

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