The Crowded Cleft

The extracellular half of the plasticity switch — how a sulfated matrix gates the delivery of astrocytic cholesterol to the neuronal membrane, and what a raft theory of Alzheimer's disease requires from the space its ingredient must cross

Cholesterol · Apolipoprotein E · Heparan sulfate · The perineuronal net · Reelin · ApoER2

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Brain lipoprotein particles are about twenty nanometres across. The extracellular space they must cross is about forty, and it is not empty. A theory that makes the delivery of astrocytic cholesterol the rate-limiting step in the consolidation of a synapse has described the cell that releases the parcel and the cell that receives it, and has left out the road.

Contents

Abstract

There is a theory of Alzheimer's disease which holds that memory formation runs in two stages — a generative stage that proposes candidate synapses and a resolving stage that selects among them — and that the transition between the two is thrown by a physical event: the assembly of cholesterol-ordered domains in the neuronal plasma membrane. Because neurons cannot manufacture usable quantities of their own cholesterol, the transition depends on delivery from astrocytes, and the disease, on this account, is a brain arrested between the two stages, generating candidates it can never resolve, with hyperphosphorylated tau and secreted amyloid-β as the debris of a compensation that cannot succeed.

The theory is unusual in identifying a *stage* of a normal process at which a disease could sit, and in insisting that pathological molecules be given jobs in health before they are given jobs in disease. It has one prediction that held in human material: that the lesion in the cholesterol supply chain lies in neuronal uptake and not in astrocytic synthesis. It also has four structural defects. It absorbs contradictory measurements through a two-population device that renders its central variables unfalsifiable. After a late revision it possesses exactly one route to the stalled switch, having discarded its second. It describes the failing populations rather than observing them. And it treats the delivery of cholesterol as a two-body problem between an astrocyte and a neuron, when it is a three-body problem: between an astrocyte, a neuron, and the space in between.

This paper supplies the third body. Brain lipoprotein particles are on the order of twenty nanometres across; the extracellular space they must cross is on the order of forty, and it is not empty. It is a hydrated lattice of hyaluronan, lecticans, link proteins and heparan sulfate proteoglycans whose composition changes with age and with disease. Sulfated glycosaminoglycan content rises in the Alzheimer hippocampus, heparan sulfate rises with it, and the transcripts of the enzymes that write the sulfation pattern rise too. Apolipoprotein E binds heparan sulfate through a defined basic interface, and it does so isoform-specifically, in an order that inverts the clinical one: E4 binds most tightly, E3 less, E2 less again, and the Christchurch variant least of all — the variant carried homozygously by the one person known to have resisted an autosomal-dominant Alzheimer mutation for three decades.

The proposal made here is that the sulfated matrix and the neuronal surface constitute **two competing beds of heparan sulfate for one ligand**, that the rate-limiting step in the supply of cholesterol to the neuronal membrane is the outcome of that competition, and that the disease is a drift in it. On this reading, the highest-affinity apolipoprotein is not the best deliverer but the most easily detained, and resilience is conferred not by binding the sugar better but by binding it worse. That is a directional claim about a measurable quantity, and it is the reason for making it: the theory being extended cannot presently be falsified by any measurement of cholesterol or of raft abundance in Alzheimer tissue, and an addition that does not restore a sign is not worth making.

Four further arguments follow. The matrix is already load-bearing inside the theory it is being added to, and unelaborated: detachment from matrix is what internalises the membrane's own organising proteins, and the protease that performs the detachment during normal plasticity is the one whose chronic activation would hold the switch open. That protease has a named driver, and the driver is a neuromodulatory system whose earliest tau pathology in the human brain long precedes the cortical disease. The matrix exists in two states — a condensed perineuronal net that shields the neurons it surrounds from tau pathology, and a diffuse interstitial matrix that impedes traffic — and the disease is better read as a shift between them than as an increase or a decrease in either; this supplies an *observable* replacement for the two neuronal populations the original theory had to posit. And the receptor at which all of this converges, ApoER2, is simultaneously an apolipoprotein E receptor on the delivery chain, a heparan-sulfate-dependent reelin receptor that suppresses tau phosphorylation, a raft-resident signalling protein, and the target of the aldehyde crosslinking proposed by an unrelated programme. Four literatures meet at one receptor, and no two of them cite each other.

The paper states each addition with the direction it commits to, grades every claim, and closes with a cell-type map naming which neuron each part of the argument concerns, ten experiments, the results that would refute the proposal, and an account of what remains unresolved — including a genuine tension between the sulfation changes reported in disease and the sulfation chemistry the reelin interface is thought to require.

Note on sources and citation

Three kinds of source are used here and they are not of equal standing, so they are marked.

Published primary literature is cited in the ordinary way and carries the argument wherever a claim is graded above *plausible*.

Two unpublished hypothesis papers are cited because their central proposals are load-bearing and no published version states them in the same form: the entrapment proposal of Boche (2020), and the glycosaminoglycan proposal of Quintero (2020), both submitted to the Oskar Fischer Prize competition and held in that competition's public record. Where either is cited for a *mechanism*, the underlying experimental work is cited alongside it and the hypothesis paper is credited only with the assembly. Where either is cited for the assembly alone, that is stated.

One theory is cited in two versions — a 2020 statement and the final published account (Rappoport, 2025) — because the argument of this paper turns in one place on a claim the author held in the first and withdrew from the second. Both are identified at the point of use.

Grades used throughout are those stated in Chapter 4. Preprint status, where it applies, is given at every point of use and not only in the reference list.

Part One — The Incomplete Switch

1. Introduction: A Switch With Only One Half Described

1.1 The shape of the problem

Every theory of a supply failure has to say three things: what is supplied, by whom, and to whom. Very few say anything about the journey.

The theory this paper extends says that the brain's ability to convert experience into structure depends on the arrival of cholesterol at the neuronal plasma membrane; that neurons cannot make usable quantities of it themselves; that astrocytes make it, package it onto apolipoprotein E particles, and release it; and that the neuron takes it up through receptors of the low-density-lipoprotein receptor family. It then locates the disease at the last of those steps. Uptake fails; the membrane cannot assemble the ordered domains that consolidation requires; the neuron proposes candidate synapses it can never select among; and the accumulated debris of that failure is what a pathologist eventually sees.

The argument is a good one, and its best move is an argument from physical chemistry rather than from statistics. Cholesterol is not glutamine and it is not lactate. It has a large hydrophobic domain, so it cannot diffuse freely through an aqueous compartment and it cannot ride a small transporter. Its delivery requires a particle, a receptor, an endosome and a route to the plasma membrane — heavy machinery with no redundancy of the relevant kind, since the cholesterol a stressed neuron makes for itself cannot substitute for delivered cholesterol in building plasma-membrane domains. If one is looking for a step in the maintenance of a synapse that has no backup, this is a defensible place to look.

But the chain as stated has three links and the physical problem has four. Between release by the astrocyte and capture by the neuron there is a journey, and the journey is the part nobody wrote down.

1.2 Twenty nanometres in forty

The brain's lipoprotein particles are discoidal to spherical assemblies in the region of twenty nanometres across (Stukas et al., 2015). The extracellular space through which they must travel is, in the living brain, on the order of forty nanometres wide (Nicholson and Hrabetova, 2017) — below the resolution of light microscopy, and barely twice the diameter of the object that has to get through it.

That geometry alone would be worth a chapter. It is made considerably more interesting by the fact that the space is not water. It is a hydrated lattice: hyaluronan chains, the chondroitin-sulfate-bearing lecticans that decorate them, link proteins and tenascins that stabilise the assembly, and heparan sulfate proteoglycans distributed through the interstitium and concentrated at basement membranes and cell surfaces. Diffusion of small molecules through this compartment is close to free. Diffusion of macromolecules considerably smaller than a lipoprotein particle is already hindered, and hindrance increases under exactly the conditions that accompany neurodegeneration (Nicholson and Syková, 1998).

Put the two facts together and a question follows that the supply-chain theory never asks. If the rate-limiting resource for synaptic consolidation is carried on a twenty-nanometre particle through a forty-nanometre gel whose composition changes with age, then the state of the gel is a term in the rate equation. It may not be the largest term. But a theory that omits it entirely is not a theory of the supply chain; it is a theory of two of its four steps.

1.3 The claim of this paper

The proposal here has one sentence at its centre.

> *The neuronal surface and the extracellular matrix are two beds of sulfated glycosaminoglycan competing for the same apolipoprotein-bearing particle, the delivery of cholesterol to the plasma membrane is the outcome of that competition, and Alzheimer's disease involves a drift in it toward the matrix.*

Everything else in this paper is either the evidence for that sentence, a consequence of it, or an honest account of what it does not cover.

Three properties of the claim are worth stating at the outset, because they are the reasons for making it rather than some other addition.

It has a sign. The theory being extended has been criticised, correctly, for absorbing measurements in either direction. The competing-beds claim does not have that freedom. It says that matrix-bed capacity rises in disease, that the neuronal bed does not rise with it, that particles are detained in proportion to their affinity for the sugar, and that the apolipoprotein isoforms should therefore rank for detention in an order opposite to their rank for delivery. Each of those is a directional statement about a quantity that can be measured, and the ranking has already been measured in vitro.

It restores a second route. In its 2020 form the theory had two independent ways of reaching a chronically stalled switch: failure of cholesterol supply, and failure of reelin signalling through its lipoprotein receptors. The second was withdrawn in the final version. Chapter 13 argues the withdrawal was a mistake, and that the two routes turn out not to be independent at all — they run through the same sugar.

It is already inside the theory, unelaborated. This is the point on which the whole paper rests, and it is not an interpretive liberty. In the theory's own description of candidate generation, the extracellular protease matrix metalloproteinase-9 digests matrix and adhesion molecules, and — verbatim — *detachment from matrix triggers endocytosis of raft components including caveolin-1*. Matrix state is therefore already upstream of membrane-domain state in the author's own account of the normal process. He states it once and never returns to it. The disease is then built entirely on the failure of a supply that arrives *through* the matrix, without asking what the matrix is doing to the supply or to the membrane.

1.4 What is being added, and to what

The additions of this paper fall into four groups, and the order of the parts follows them.

The crossing (Part Two) supplies the missing station: the geometry of the space, the chemistry of the lattice, the isoform-specific affinity that governs detention, and an account of entrapment as a lesion with a direction. It also records a convergence that ought to be uncomfortable for both parties: two independent hypothesis papers derive the same regional prediction — that vulnerability tracks plasticity demand — from the same supply chain, and place the lesion at different points along it. That makes them jointly testable rather than rival.

What the matrix does to the membrane (Part Three) follows the protease. If matrix detachment internalises the membrane's organising proteins, then a chronically active matrix protease is a mechanism for holding a plasticity switch open, and the theory acquires something it conspicuously lacks: a driver for chronicity, rather than an assertion of it. Part Three also proposes replacing the theory's two posited neuronal populations with an observable variable — whether a neuron wears a condensed perineuronal net — and confronts the fact that this same matrix, in that state, is protective.

The second route (Part Four) restores reelin and its receptor, shows that the receptor's engagement is heparan-sulfate-dependent and therefore subject to the same competition, and argues that ApoER2 is the node at which four unconnected literatures meet.

The rest of the chain (Part Five) deals with what the theory leaves out downstream and alongside: what happens to the cholesterol after uptake, whether the delivered lipid is chemically intact, whether a theory of supply can decline to have a theory of disposal, and who actually removes a synapse once it has been marked.

Part Six states the sign commitments in a single table, grades every claim, gives ten experiments, and sets out what would refute the proposal.

1.5 What this paper does not claim

It does not claim that matrix entrapment is the cause of Alzheimer's disease. It claims that it is a step in a chain the disease runs through, that the step has been left out, and that putting it back changes what the chain predicts.

It does not claim that the raft-gated switch is correct. That mechanism remains untested as a mechanism, and Chapter 22 grades it accordingly. The additions made here would be worth making even if the switch turns out to be wrong, because the delivery problem they describe is a problem for any theory in which the neuronal membrane must be rebuilt in order for a synapse to be consolidated.

And it does not claim novelty for the components. Every molecule named in this paper has a literature. What is claimed is that these literatures have been kept apart by the accident that one of them is written by membrane biophysicists, another by glycobiologists, a third by neuropathologists and a fourth by developmental neuroscientists, and that when they are put together they generate experiments none of them would run alone.

2. The Theory Being Extended, Stated at Its Strongest

2.1 Two stages, and the reason there must be two

The disease theory is downstream of a theory of normal plasticity, and cannot be assessed without it.

The plasticity theory begins from an engineering problem. When an animal executes a response with a novel component and survives, the pathways involved should be made more available in future. But the brain does not know which of the many synapses, boutons and branch points active during that response were the ones that mattered. It cannot simply strengthen the correct elements, because it has not identified them.

The proposed solution is the one adaptive immunity uses and the one evolution uses: generate a surplus of candidates and then select among them. Plasticity therefore runs in two stages.

Candidate generation destabilises the existing arrangement and produces an excess of potential modification sites — new spines, new boutons, new branch points, existing synapses marked for potentiation. Destabilisation is required rather than incidental, because a stable structure cannot generate new geometry. Extracellularly, matrix metalloproteinase-9 digests matrix and adhesion molecules; intracellularly, tau is phosphorylated and thereby inactivated, releasing the cross-links that hold the existing cytoskeleton in place; calcium-permeable receptors are inserted so that activity produces large calcium influx.

Competition resolution then enhances the winners, eliminates the losers, and restabilises. Calcium influx is brought back down; calcium-permeable AMPA receptors are exchanged for calcium-impermeable ones; GluN2B-containing NMDA receptors switch to GluN2A; winners acquire a stable actin cytoskeleton cross-linked to microtubules by dephosphorylated tau; losers are retracted.

None of the components is novel and the theory does not claim otherwise. What is claimed is that these constitute one canonical two-stage process with a defined transition between them, and an identification of what throws the switch.

2.2 The gate as a physical object

The distinctive claim is that the transition is not triggered by a timer, a counter, or a threshold on activity. It is triggered by the assembly of plasma-membrane domains ordered by cholesterol, sphingomyelin and glycolipids — the structures through which the membrane connects to the cytoskeleton, to the extracellular matrix, and to the synaptic partner across the cleft.

The reasoning is that resolution requires capabilities only such domains provide. A winning spine cannot be stabilised without anchoring receptors and scaffolds in position, and stable membrane anchoring of the relevant proteins depends on palmitoylation, which targets them to ordered domains. Neurotrophin receptors and the insulin receptor are anchored and trafficked through caveolin-1-containing domains. The neuron cannot begin to consolidate anything until it has built the platform on which consolidation is physically performed.

Then the load-bearing step: the rate-limiting ingredient in that platform is cholesterol, and the neuron must import it. Brain cholesterol does not cross the blood–brain barrier in significant amounts; it is synthesised locally, principally by astrocytes, packaged onto apolipoprotein E particles by ABCA1, released, and taken up through the low-density-lipoprotein receptor and LRP1 (Dietschy and Turley, 2004; Wahrle et al., 2004).

The switch, therefore, is a supply check. Cholesterol arrival signals that an astrocyte process is close enough to service the new synapse, and therefore that consolidating a synapse at this location is worth doing.

2.3 Disease as chronic non-resolution

If domain assembly is impaired but not abolished, the neuron neither completes plasticity nor abandons it. Both stages run simultaneously, at lower agent concentrations than either would use in health, and for far longer.

Three consequences follow, and the second is the theory's best move.

The staging of agent changes is predicted: early in disease both generation and resolution agents should be elevated; later, as resources deplete, resolution should rise while generation falls.

Chronicity inverts the sign of the neuron's own compensation. Many plasticity agents are double-edged — one agent produces opposite effects at different concentrations, because it engages a high-affinity receptor at low concentration and recruits a low-affinity receptor in addition at high concentration. Calcium is the canonical instance: high or fast calcium activates calcium/calmodulin-dependent kinase II and marks winners; low calcium activates calcineurin and eliminates losers. Chronicity means lower concentrations, so chronic signalling is tilted toward the elimination edge. The neuron's attempt to compensate — continued candidate generation, which would normally drive cholesterol synthesis and rescue the situation — instead produces

degeneration. **The compensation is the pathology, and a partial failure is worse than a complete one.**

Chronic signalling then desensitises receptors and pathways through ordinary negative feedback, so signalling degrades further and the lesion deepens itself.

2.4 Jobs in health for the molecules of disease

The theory's most durable contribution is methodological, and it is worth separating from the mechanism because it survives independently of it. The operating principle is that one cannot understand a disease molecule without an account of its job in health, and that the absence of such an account is why the field's dominant theory has not converged.

The account given is specific. The amyloid precursor protein manages cholesterol during plasticity: its α-route is a candidate-generation agent, occurring outside ordered domains, stimulated by reduced membrane cholesterol and promoting cholesterol synthesis — the neuron requesting cholesterol while it builds candidates and waits. Amyloid-β terminates candidate generation and removes losers: β-cleavage requires cholesterol and begins once domains have formed, and the peptide then exerts negative feedback on cholesterol synthesis, cancelling the request once it has been filled. Tau cross-links the cytoskeletons of winners: phosphorylation inactivates it, so tau is phosphorylated everywhere during generation to release the existing structure, and dephosphorylated specifically in winners so that it can cross-link microtubules to actin.

This triad is uncomfortable in the way good proposals are uncomfortable. It says amyloid-β is supposed to remove synapses. It says the field's central pathological marker, phospho-tau, is the default state of tau in any neuron currently learning something.

2.5 The regional argument

If the lesion is in plasticity, the most vulnerable regions should be those with the highest plasticity demand. The theory identifies the earliest tau-bearing regions — entorhinal cortex, hippocampus, locus coeruleus — as exactly the regions where continuous plasticity is required, and then does something more discriminating with the hippocampal subfields: CA1 and subiculum, much more vulnerable than dentate gyrus and CA3, support familiar scenes, while dentate gyrus and CA3 encode new ones, and most life experience consists of familiar scenes with an element of novelty — the precise condition that triggers candidate generation without triggering wholesale new encoding.

Chapter 9 returns to this argument, because a second and entirely independent hypothesis derives the same regional prediction from the same supply chain while locating the lesion elsewhere on it. When two theories agree on a prediction and disagree on a mechanism, the prediction stops being evidence for either and the disagreement becomes the experiment.

2.6 The prediction that held

The theory made one prediction that is specific, mechanistically committed, contrary to the simpler reading of its own supporting literature, and testable in human material. It concerns which step of the supply chain fails.

A theory that merely said cholesterol is low in Alzheimer's disease would be agnostic about the step. This one was not: the lesion is at **uptake**, not synthesis. Five years later, human cerebrospinal fluid showed preserved astrocytic efflux with significantly reduced neuronal uptake, and apolipoprotein E4-bearing particles delivering less cholesterol to neurons than E3 particles (Borràs et al., 2025).

That is the best single result in the theory's favour, it arrived from a group with no stake in it, and it is the reason this paper extends the theory rather than replacing it. It is also — and this is the argument of Part Two — a result with two available explanations, only one of which the theory considered. Reduced uptake with preserved efflux is what a broken receptor looks like. It is also what a detained particle looks like.

3. Four Structural Defects

3.1 Symmetry

The central charge against the theory is that it cannot be wrong.

Double-edged plasticity, chronicity, and a two-population model of neurons together make almost every direction of almost every measurement a confirmation. The two-population move is the clearest instance: neurons in which chronic cholesterol production eventually succeeds form domains normally, show no tau pathology, over-produce amyloid-β and generate plaques; neurons in which it does not show plasticity failure, tau pathology and amyloid pathology. That elegantly explains why plaques are common in cognitively normal older people and correlate weakly with symptoms — plaque as the signature of a solved problem — and it simultaneously renders the theory unfalsifiable by any measurement of cholesterol or of membrane-domain abundance in Alzheimer tissue.

The device is not illegitimate in itself. Cellular heterogeneity is real, and a theory that ignored it would be worse. What makes it a defect is that the two populations are *posited from the theory* rather than identified by an independent criterion. If one could say in advance which neurons belong to which population — by a marker, an anatomy, a cell class — the device would become a prediction. Chapter 12 proposes exactly that substitution.

3.2 A single supply chain

In the 2020 statement of the theory there were two independent routes to a chronically stalled switch: failure of cholesterol delivery, and failure of reelin signalling through its lipoprotein receptors. Reelin carried real load. Its role was given as axonal elongation, synapse formation and the termination of consolidation-phase plasticity; its receptor ApoER2 was described as domain-dependent and crucial to final synaptic adhesion; and defective reelin signalling was offered explicitly as a route to a chronic stalled state independent of cholesterol.

By the final version reelin appears in a single paragraph as a candidate-generation agent, the consolidation-termination function is gone, the receptor's role is gone, the genetics is gone, and reelin is listed among topics left to future work.

The revision made the theory more parsimonious and simultaneously more fragile. Everything now runs through one supply chain, and the theory's account of *why entorhinal layer two* fails first became a general argument about novelty rather than a molecular one.

The timing was also unfortunate, in a way that matters for Part Four. The demotion happened across exactly the interval in which reelin produced the strongest human resilience result in the field.

3.3 Posited populations, unobserved

Related to the first defect but distinct from it: the theory describes the failing cells rather than pointing at them. It requires that some neurons succeed in scaling up cholesterol production and some fail, and it assigns the pathology accordingly, but it does not say which neurons, and it offers no way to tell them apart before the fact.

A theory of selective vulnerability that cannot name its vulnerable population in advance is doing the work of description, not prediction. This is the defect with the clearest remedy, and it is the one this paper is most confident about.

3.4 A two-body model of a three-body problem

The fourth defect is the one this paper is built around, and it is the least discussed because it does not look like a defect. It looks like a simplification.

The chain is stated as: astrocytes synthesise, astrocytes package and release, neurons take up. Three steps, three verbs, two cells. The space between the two cells appears nowhere in the chain, and the theory's one commitment about the location of the lesion — uptake rather than synthesis — is a choice between the first and third steps of a chain whose second step is the one that involves a journey.

This matters for three reasons.

It matters *empirically*, because the result that most supports the theory — reduced neuronal uptake with preserved astrocytic efflux — is exactly the signature that particle detention would produce, and the study that produced it was not designed to separate the two.

It matters *for falsifiability*, because the matrix supplies directional, measurable variables where the intracellular account supplies bidirectional ones.

And it matters *internally*, because the theory already assigns the matrix a role and never follows it. Matrix metalloproteinase-9 is named as a candidate-generation agent, and matrix detachment is named as the trigger for internalisation of caveolin-1 and other domain components. The theory therefore already holds that **the state of the matrix controls the state of the membrane domain**. It simply never asks what happens when the matrix state is chronically wrong.

4. Method, Grades, and the Rule That Governs Additions

4.1 The failure mode of extension

The characteristic way a paper like this one goes wrong is by making its target theory better at explaining and worse at predicting. Adding mechanisms increases coverage. Coverage is not the objective; a framework that accommodates every result is exactly what the original theory has been criticised for, and importing nine new mechanisms without discipline reproduces the defect at a larger scale.

The rule adopted here is therefore restrictive, and it is applied to every addition in this paper:

> *An addition is admissible only if it does one of two things: supplies a* **second independent route** *to the phenomenon the theory explains, or supplies a* **variable with a declared direction** *that the theory's existing devices cannot absorb. Additions that only increase explanatory reach are rejected.*

Chapter 20 collects every directional commitment made in this paper into a single table, so that the paper can be checked against its own rule.

A second discipline is inherited from the theory itself and extended. The theory's most transferable idea — that a spatially propagating agent can implement a binary local decision through nothing more than an affinity difference between two receptors — is also the source of its symmetry problem, because used carelessly it licenses accommodating any observation. The discipline that makes it a tool rather than an excuse is to specify, in advance and for each agent, the concentration at which the edge flips and the receptor pair that implements it. That discipline is here extended to structures: for every structural variable added, the direction it must move is stated before the evidence is reviewed.

4.2 Grades

Every substantive claim in this paper carries one of five grades, and the ledger in Chapter 21 assigns one to each.

Established — supported by direct evidence in human material, or by convergent experimental evidence, and not seriously contested.

Well-supported — good evidence, some of it indirect or from model systems.

Plausible — coherent and consistent with the evidence, not directly tested.

Inference — a join between two literatures, generating a prediction, resting on no direct evidence of its own. Original proposals of this paper are, with two exceptions, of this kind, and they are marked.

Contradicted — evidence runs the other way.

The distinction between *plausible* and *inference* is deliberate and is the one most often elided in synthesis papers. A plausible claim is one somebody might have tested and has not. An inference is a claim that exists only because two literatures were put side by side, and its status is that of a hypothesis until somebody runs the experiment. Chapters 13, 14 and 12 contain the paper's principal inferences.

4.3 What counts as evidence about a human being

Where a claim is made about the human disease, human evidence is required and its type is named: post-mortem tissue, in vivo imaging, cerebrospinal fluid, genetics, or a clinical trial. Where only model-system evidence exists, the claim is graded no higher than *plausible* regardless of how good the model work is.

This is applied with particular strictness to the sulfation chemistry of Part Two, because that literature is largely biochemical and its bridge to human tissue rests on a small number of studies.

4.4 On citing a competition submission

Two of the sources used here were submitted to a prize competition and not published in a journal. They are used because their central assemblies are not stated in that form anywhere else, and because the paper would be dishonest if it presented their arguments as its own. The convention adopted is that such a source may be credited with an *assembly* or a *proposal*, never with a *result*; every result attributed to them is separately cited to its primary source; and no claim is graded above *plausible* on their authority alone.

4.5 What would make this paper wrong

Stated at the outset rather than at the end, because a paper that only says this in its final chapter has usually decided not to mean it.

If sulfated glycosaminoglycan content and heparan sulfate abundance in the interstitial matrix of the Alzheimer hippocampus turn out not to rise; if apolipoprotein isoform affinity for heparan sulfate turns out not to predict retention in tissue; if lipoprotein particle mobility in aged or diseased brain tissue turns out to be unimpaired; or if restoring matrix permeability turns out to leave neuronal cholesterol delivery unchanged — then the central proposal of this paper is false, in a way

that no adjustment of the surrounding argument can rescue.

Chapter 23 states the full set.

Part Two — The Crossing

5. Twenty Nanometres in Forty

5.1 The dimensions

Two numbers organise this chapter.

Brain lipoprotein particles, assembled on apolipoprotein E and lipidated by ABCA1, are on the order of **twenty nanometres** in diameter, comparable to the high-density lipoproteins of plasma (Stukas et al., 2015; Wahrle et al., 2004). Apolipoprotein E genotype affects the distribution: E4 carriers' cerebrospinal fluid contains smaller complexes than E3 carriers' (Heinsinger et al., 2016), and induced pluripotent stem-cell-derived astrocytes from E4 homozygotes produce particles that are smaller, carry less cholesterol, and support neurons less well than E3 particles by measures of viability and synaptic protein expression (Zhao et al., 2017).

The extracellular space of the living brain is on the order of **forty nanometres** wide, occupying roughly a fifth of tissue volume, with a tortuosity that already slows diffusion of even freely diffusing small molecules relative to free solution (Nicholson and Hrabetova, 2017; Nicholson and Syková, 1998).

A twenty-nanometre object in a forty-nanometre channel is not diffusing through a solvent. It is negotiating a passage.

5.2 What hindrance means for a rate-limiting step

Diffusion in the brain interstitium is characterised by a tortuosity factor and by a volume fraction, and both worsen with the conditions that accompany neurodegeneration. Hindrance rises steeply with the ratio of particle size to channel width, and it rises further where the channel is not merely narrow but filled — where a hydrated polyanionic gel occupies the space and the traveller carries a complementary charge.

The theory being extended requires cholesterol delivery to be rate-limiting for the consolidation of a synapse. If that is so, then any term that lowers the delivery rate is a term in the disease equation, and the terms available are: how much is made, how well it is packaged, how far it must travel, how obstructed the path is, how strongly the path binds the traveller, and how efficiently the destination captures it. The theory addresses the first, the second and the last. This paper addresses the middle three.

The distinction is not academic. A theory of impaired uptake predicts that the fault is in the neuron and that repairing the neuron will restore delivery. A theory of impaired transit predicts that

the neuron is competent, that delivery is restored by clearing the path, and — crucially — that a measurement of neuronal receptor function will look normal while delivery is failing. The two predictions differ, and no published study separates them.

5.3 The result that is compatible with both

The single strongest result in favour of the theory extended here is that human cerebrospinal fluid shows preserved astrocytic efflux with significantly reduced neuronal uptake, and that E4-bearing particles deliver less cholesterol to neurons than E3 particles (Borràs et al., 2025).

That result was reached with reconstituted particles and a cellular uptake assay. It establishes that the neuron receives less. It does not establish where the loss occurs, because the assay by construction removes the tissue: the particle and the neuron meet in a dish, without forty nanometres of charged gel between them.

The honest statement is therefore that the result confirms a *delivery* deficit and is silent on its *location*. It is compatible with a receptor lesion. It is equally compatible with a particle whose surface chemistry causes it to be detained by matrix — since the same surface chemistry that governs detention also governs receptor engagement, and the dish measures only the second. Chapter 7 argues that the two are the same molecular interface read at two addresses, which is why one assay cannot separate them, and Chapter 22 gives the assay that could.

5.4 Why the journey has been invisible

There is a structural reason this step has gone unexamined, and it is worth naming because it explains why an obvious idea is not already in the literature.

The extracellular space of the brain cannot be seen in a fixed section. Fixation collapses it; conventional electron microscopy shrinks it to near nothing; light microscopy cannot resolve it. Its dimensions are known largely from real-time iontophoresis with ion-selective microelectrodes and from diffusion analysis in living tissue — techniques belonging to a small biophysical community with almost no overlap with the community that studies apolipoprotein E (Nicholson and Syková, 1998; Nicholson and Hrabetova, 2017).

The result is that the compartment through which every extracellular molecule in the brain must travel is, in the working picture of most cell biologists, a gap rather than an object. A theory can pass an apolipoprotein particle from one cell to another across that gap without ever asking what the gap is made of, and nobody notices the omission, because everyone else is drawing the same diagram.

6. The Lattice

6.1 What the space is made of

The brain's extracellular matrix is not the collagen-dominated matrix of other tissues. It is a hydrated, highly charged assembly whose backbone is hyaluronan — an unsulfated, extremely long glycosaminoglycan synthesised at the plasma membrane and extruded directly into the space — decorated by the lectican family of chondroitin sulfate proteoglycans (aggrecan, brevican, neurocan, versican), stabilised by link proteins, and cross-linked by tenascins. Distributed through it, and concentrated at cell surfaces and basement membranes, are the heparan sulfate proteoglycans: the membrane-anchored syndecans and glypicans, and the secreted basement-membrane proteoglycans of which perlecan is the principal (Fawcett et al., 2019).

The assembly of this compartment's chemistry into an account of Alzheimer's disease is not original to this paper. It was set out, in a form that no published review states, in a hypothesis paper submitted to the same competition as the entrapment proposal (Quintero, 2020), and several of the specific human measurements used in §6.2 and §6.3 were brought together there. The convention of Chapter 4 applies: the assembly is credited, and every result is cited to its primary source.

Three properties of this assembly matter here.

It is **polyanionic**. Sulfate and carboxylate groups give the lattice a high negative charge density, and the mobility of any positively charged species through it is a function of electrostatic interaction rather than of size alone.

It exists in **two states**. The diffuse interstitial matrix fills the space between cells; the condensed perineuronal net is a lattice of the same molecular families organised into a dense, aggrecan-rich sheath around the somata and proximal dendrites of a defined subset of neurons. These are not two names for one thing. They differ in composition, in density, in the enzymes that build and remove them, and — Chapter 12 argues — in what they do to the neuron they surround.

It is **rebuilt**. The matrix is not inert scaffolding but a turned-over structure, digested by matrix metalloproteinases and by the hyaluronidases and heparanases, and re-synthesised. That turnover is under activity-dependent control, and it is the reason a theory of plasticity has a protease in it at all.

6.2 What changes in disease

The relevant human measurements are few and they point in a consistent direction.

In the Alzheimer hippocampus, total sulfated glycosaminoglycan content is significantly **increased** relative to control tissue — 2.31 ± 0.06 against 1.84 ± 0.14 micrograms per milligram — with chondroitin sulfate and heparan sulfate as the major species and heparan sulfate specifically increased in the disease tissue (Huynh et al., 2019). The same study found that glycosaminoglycans isolated from Alzheimer hippocampus bound tau with a 5.2-fold greater capacity than control glycosaminoglycans, and that the transcripts of several of the enzymes that write the sulfation pattern were elevated: N-deacetylase/N-sulfotransferase-2, the 3-O-sulfotransferases HS3ST2 and HS3ST4, heparan sulfate C5-epimerase, and heparanase.

Growth-factor handling shifts in parallel and not uniformly: disease-derived glycosaminoglycans bound heparin-binding EGF-like growth factor and pleiotrophin considerably more strongly, and fibroblast growth factors 1 and 2 and VEGF-165 considerably less, with a reduced capacity to potentiate the mitogenic activity of the latter (Huynh et al., 2019). This matters because it demonstrates that the change is not a simple increase in a uniform polymer. The bed does not merely grow; it is rewritten, and its ligand preferences change.

Sulfation increases are reported outside the brain in the same disease. Proteoglycans from Alzheimer skin fibroblasts show a higher chondroitin-to-heparan sulfate ratio and elevated sulfation of both — 56 per cent for chondroitin sulfate, 27 per cent for heparan sulfate — with altered disaccharide composition in secreted heparan sulfate (Zebrower et al., 1992). That the signature appears in a peripheral cell from the same patients is evidence that at least part of it is constitutional rather than a local reaction to plaques.

Heparan sulfate accumulates early and in the right places. It is present in neurons and in amyloid-bearing lesions of Alzheimer's disease and of Down syndrome (Snow et al., 1990), and it appears in the diffuse plaques of hippocampus but not of cerebellum in the same brains (Snow et al., 1994) — an anatomical dissociation this paper returns to in Chapter 9, because it matches the regional prediction of the theory being extended.

The non-glycan components change too. Collagen IV, laminin and fibronectin are reported upregulated in the cerebral cortex in early disease, consistent with a stiffening or fibrosis of the interstitium (Boche, 2020, and references therein).

6.3 The affinities that make the lattice a trap

The lattice does not merely obstruct. It binds, and the binding constants are not small.

Vascular-cell-derived proteoglycans were compared directly for their binding to amyloid-β: the heparan sulfate proteoglycan perlecan bound with by far the highest affinity, at two sites with dissociation constants of 8.3 × 10-11 and 4.2 × 10-8 molar, while the dermatan sulfate proteoglycans decorin and biglycan bound more weakly and the chondroitin sulfate proteoglycan versican did not bind at all (Snow et al., 1995). A picomolar site on a matrix component for a peptide that is carried

in the core of the very particle that must cross the matrix is not a detail.

Sulfated glycosaminoglycans also assemble tau into filaments with the morphology of those found in disease (Goedert et al., 1996), and the *pattern* of sulfation — not merely its extent — controls the kinetics and the filament structure that result, with 2-O-sulfation slowing aggregation of a tau fragment (Townsend et al., 2020). The lattice is therefore not a passive obstruction in either direction: it templates one of the disease's two defining aggregates and it detains the carrier of the other.

6.4 What this chapter licenses and what it does not

It licenses the statement that the compartment through which brain cholesterol travels changes in Alzheimer's disease, in composition, in charge and in ligand preference, and that the direction of the change in the hippocampus is toward more sulfated glycosaminoglycan and more heparan sulfate. That is graded **well-supported** — human tissue, but a small number of studies, one of them the principal source for several of the specifics.

It does not license the statement that particles are detained. Nobody has measured the mobility of an apolipoprotein E particle in aged or diseased human brain tissue. That measurement is the first of the experiments in Chapter 22, and until it exists the central mechanism of this paper is an **inference**.

7. The Sulfation Code and the Competing Beds

7.1 One interface, two addresses

Apolipoprotein E binds heparin and heparan sulfate through a defined basic surface on its amino-terminal domain, mapped to the region around residues 130 to 143 and identified as one of two heparin-binding domains in the intact protein (Cardin et al., 1986; Weisgraber et al., 1986; Dong et al., 2001). The same interface serves two functions that are almost never discussed together.

At the **neuronal surface**, cell-surface heparan sulfate proteoglycans act as co-receptors that concentrate lipoprotein particles and hand them to LRP1 and the low-density-lipoprotein receptor for internalisation. Binding the sugar here is part of how the particle gets in.

In the **interstitial matrix**, the same chemistry encounters a bed of the same polymer that has no receptor behind it. Binding the sugar here is how the particle stops moving.

This is the observation on which the paper turns. The molecular feature that makes an apolipoprotein good at being captured by a neuron is the same feature that makes it good at being detained by the space in front of the neuron. Whether a given affinity helps or harms is therefore not a property of the affinity. It is a property of the *ratio of the two beds*.

7.2 The ranking, and why it should be surprising

Apolipoprotein isoforms differ in their affinity for heparin, and the order is now well established in vitro: **E4 binds most tightly, E3 less, E2 less again**, and the Christchurch variant — an arginine-to-serine substitution at residue 136, squarely within the heparin-binding interface (Wardell et al., 1987) — binds least of all, at roughly forty per cent of the relative affinity of E3.

Set that beside the clinical ranking. E4 is the major risk allele; E3 is neutral; E2 is protective; and homozygosity for Christchurch is associated with the most striking resistance to autosomal-dominant Alzheimer's disease yet documented in a human being — a carrier of the *PSEN1* E280A mutation in the Antioquia kindred who remained free of mild cognitive impairment until her early seventies, nearly three decades beyond the kindred's median onset, with a very high amyloid burden and limited entorhinal tau (Arboleda-Velasquez et al., 2019).

Affinity for the sugar tracks risk, monotonically, across four variants.

That correlation is a problem for the naive reading of the interface. If heparan sulfate binding is how the particle is captured and delivered, then tighter binding should mean better delivery, and better delivery should mean lower risk. The observed order is the opposite of that in every position.

7.3 The competing-beds proposal

The proposal of this paper is that the correlation is explained by a competition, and that the competition has drifted.

Consider the particle's fate as a partition between two sinks. One sink is the neuronal surface: heparan sulfate proteoglycans backed by an endocytic receptor, so that binding is productive and terminates in delivery. The other is the interstitial matrix: heparan sulfate with no receptor behind it, so that binding is unproductive and terminates in detention. Both sinks recognise the same basic interface on the same apolipoprotein.

Three consequences follow, and each is directional.

First, raising the particle's affinity for the polymer raises occupancy at *both* sinks, but the effect on net delivery depends on which sink has more sites. Where the matrix bed is small, higher affinity is close to neutral or mildly favourable, because the surface sink is receptor-backed and therefore continually emptied. Where the matrix bed is large, higher affinity is unfavourable, because the unproductive sink competes for a particle that has a finite residence time before it is degraded.

Second, the disease should be a change in bed ratio rather than in affinity, since affinity is set at conception by genotype and the disease is not. The measured direction of change in the human hippocampus — more sulfated glycosaminoglycan, more heparan sulfate, altered sulfotransferase transcripts (Huynh et al., 2019) — is an increase in the unproductive bed. Whether the productive bed rises with it has not been measured, and the proposal requires that it does not.

Third, the genotype effect should be *conditional on age*, because it is conditional on bed ratio. In a young brain with a small interstitial bed, higher-affinity apolipoprotein should deliver as well or better; in an aged brain with a large one, it should deliver worse. This is not a rescue invented for convenience. It is a specific and awkward commitment, and it happens to be the one place where the theory being extended has been contradicted in the direction opposite to its prediction: in young human cellular systems, E4 astrocytes over-supply cholesterol and expand neuronal ordered domains rather than starving them, a result obtained by direct imaging of the domains themselves (Lee et al., 2021, 2025). A model in which the sign of the E4 effect on delivery inverts with the growth of the matrix bed predicts exactly that discrepancy, and predicts that it resolves in aged tissue.

That is the paper's principal **inference**. It is stated with its refutation attached in Chapter 23, and it is the subject of experiments one, two and three.

7.4 The rival reading, stated fairly

There is an established alternative explanation of the same correlation, and it was the reading offered by the group that characterised the Christchurch variant.

Heparan sulfate proteoglycans mediate the cellular uptake of tau, and apolipoprotein E binding to heparan sulfate participates in the step by which amyloid pathology is converted into tau pathology. On that reading, reduced apolipoprotein–heparan sulfate binding is protective because it limits tau propagation, not because it frees a lipoprotein particle to move. The Christchurch carrier's phenotype — very high amyloid with limited entorhinal tau and preserved cognition — fits that account directly, and a monoclonal antibody raised against the apolipoprotein E 130–143 interface reduces heparin binding, which the authors proposed as a druggable target.

Three things should be said about this.

It is well-evidenced and this paper does not dispute it. The tau-uptake route is graded here as **well-supported**, higher than the detention route.

It is not exclusive of the proposal made here. Both readings say that the apolipoprotein–heparan sulfate interface is the pivot; they differ on which cargo matters. A single interface can govern two traffics.

And the two readings are **separable by experiment**, which is what makes stating both worthwhile rather than diplomatic. The tau reading predicts that the protective effect of reduced binding appears only downstream of amyloid, requires tau, and does nothing for cholesterol delivery. The detention reading predicts that reduced binding improves particle mobility and neuronal cholesterol delivery in aged tissue, in the complete absence of tau. Experiment four is that cross.

7.5 The complication this paper cannot resolve

Honesty requires recording a tension that runs against the proposal, and it is chemical rather than conceptual.

The reelin interface discussed in Part Four depends specifically on **N-sulfated** heparan sulfate domains, and the tau-serving interactions are associated with **3-O-** and **6-O-**sulfated domains. The human hippocampal data show transcripts for both kinds of enzyme rising in disease: N-deacetylase/N-sulfotransferase-2 alongside the 3-O-sulfotransferases (Huynh et al., 2019). If N-sulfation is rising, the reelin-staging chemistry should be improving, which is not what the disease looks like.

Three candidate resolutions exist and this paper does not choose between them.

Transcript abundance is not domain availability: the enzymes compete for the same substrate chains, and an increase in several sulfotransferase transcripts can produce a redistribution rather than a uniform increase.

The measurement is of bulk hippocampal tissue and cannot distinguish the interstitial bed from the perineuronal and cell-surface beds, which is precisely the distinction the proposal requires. A bulk increase in N-sulfation located in the interstitium is compatible with a decrease at the neuronal surface.

Or the reelin limb of Part Four is wrong about which domains it needs.

The tension is real, it is recorded in the ledger as an unresolved contradiction, and experiment six is designed to settle it. A synthesis that hid it would be worth less than one that names it.

8. Entrapment as a Lesion With a Direction

8.1 The proposal

The assembly this chapter draws on was stated in a hypothesis paper submitted to the Oskar Fischer Prize competition and not, in this form, published: that impaired movement of lipoprotein particles in the ageing extracellular space is the primary abnormality in Alzheimer's disease, and that the key features of the disease follow from it (Boche, 2020).

Its argument runs as follows. Cholesterol delivery from glia to neurons is uniquely non-redundant in the brain, because apolipoprotein E is effectively the only extracellular carrier and the blood–brain barrier excludes a peripheral supply. The particles are about twenty nanometres and the space is about forty. With age, and under the influence of hypertension, diabetes, obesity, inflammation and physical inactivity, the matrix changes and the narrow space becomes compromised — "fibrosed" — impeding the movement of, and trapping, lipoprotein particles between cells. Neuronal cholesterol deficiency follows, and with it impaired synaptic plasticity.

The account then does something a supply-failure theory usually cannot: it explains the deposits. Amyloid-β is poorly soluble in water and is carried in the hydrophobic core of lipoprotein particles (Biere et al., 1996; Beffert and Poirier, 1996; Cole and Ard, 2000). Trapping the particles immobilises the peptide. As detained particles degrade and rupture, the peptide is released into an aqueous environment where it aggregates — which is why apolipoprotein E co-localises with plaques, and why amyloid accumulates in vessel walls where basement-membrane matrix detains the same particles. Cholesterol deficiency then thins the neuronal membrane and shifts secretase cleavage toward the longer and more aggregation-prone species, so the initial seeds grow — a step for which the proposal assembles evidence from the membrane-thickness literature rather than supplying its own.

8.2 What is being taken from it, and what is not

This paper does not adopt the claim that entrapment is the primary cause of Alzheimer's disease. That claim is graded here as **plausible** and no higher, for the reason given in Chapter 6: nobody has measured particle mobility in aged human brain tissue, and a hypothesis about a rate cannot be established without a measurement of the rate.

What is adopted is narrower and, for the argument of this paper, sufficient: that **transit is a step**, that the step has a state, and that the state changes with age and disease. That is enough to make the matrix a term in the delivery equation, which is all the extension requires.

The stronger claim is worth stating precisely because it is falsifiable in a way that the theory it is being joined to is not, and because it makes a prediction that no intracellular account makes: that clearing the path restores delivery in a neuron whose receptors were never broken.

8.3 Why entrapment has a sign and receptor failure does not

The theory being extended locates the lesion in uptake and explains contrary measurements through two populations of neuron. Entrapment cannot do that, and the constraint is worth spelling out because it is the whole reason for preferring an extracellular addition to an intracellular one.

Matrix state is a property of a *place*, not of a cell. It can be measured in a section, in a volume, by diffusion analysis in living tissue, by the mobility of a tracer of defined size. It does not have two populations. A tracer either moves or it does not, and if the proposal is right the change is regional and should follow the regional gradient of the disease.

Three directional commitments follow, and they are entered in the sign table of Chapter 20.

Interstitial matrix density and sulfated glycosaminoglycan content in vulnerable regions **rise** with age and with disease stage.

The effective diffusion coefficient of a twenty-nanometre tracer in the interstitium of vulnerable regions **falls** with age and with disease stage, and falls further than that of a small-molecule tracer measured in the same tissue.

Neuronal cholesterol delivery measured *in tissue* **falls** further than neuronal receptor competence measured *in dissociated culture* from the same donor — the mismatch being the size of the transit lesion.

The third of these is the discriminating measurement of this paper, and no version of the receptor-failure account predicts it.

8.4 The awkwardness that must be conceded

An entrapment account inherits an obligation, and it is the same one the theory it extends inherits: to say why removing plaques produces clinical benefit at all.

It does produce benefit. Anti-amyloid antibodies slow decline modestly and reproducibly, with larger effects at lower baseline tau. On an account in which the deposit is the residue of a detained and ruptured carrier, that benefit has to come from somewhere other than removing a cause.

The available answer is that the deposit is a *sink* that worsens the transit problem it was produced by — it occupies interstitial volume, it nucleates further matrix change around it, and it sustains a glial state that stiffens the compartment. Removing it partially relieves each. That answer predicts the observed shape of the trial results, but it is post hoc, and it is labelled here as an interpretation rather than a prediction. The long-term follow-up of active immunisation, in which plaque clearance did not halt clinical decline (Holmes et al., 2008), is consistent with it and does not establish it.

9. Two Theories, One Regional Prediction

9.1 The convergence

The theory being extended derives regional vulnerability from plasticity demand: the regions that fail first are the ones that must continuously modify themselves, which is why entorhinal cortex, hippocampus and locus coeruleus lead, and why the hippocampal subfields that handle familiar scenes with novel elements are more vulnerable than those that encode entirely new ones.

The entrapment proposal derives regional vulnerability from plasticity demand: synaptic remodelling requires membrane to be built, membrane requires delivered cholesterol, and so the deficit is most pronounced where plasticity is greatest — hippocampus and association cortex — and least or absent where plasticity is least, in primary motor and sensory cortex, cerebellum and spinal cord, which is the hierarchical distribution of functional loss the clinical syndrome actually follows (Mesulam, 2000; Boche, 2020).

These are the same derivation. Two hypothesis papers, written without knowledge of each other, take the same supply chain, apply the same premise about where demand is highest, and reach the same anatomical prediction. They differ only in where on the chain they locate the failure — one at the neuron's receptor, the other in the space in front of it.

9.2 Why the agreement is not evidence

It is tempting to read convergence as corroboration. It is not, and the reason is instructive.

The regional prediction follows from the *premise* the two share — that cholesterol delivery is rate-limiting for plasticity — and not from either lesion. Any theory built on that premise makes the same prediction, so the prediction cannot discriminate between them. Worse, the premise is close to unfalsifiable by regional data alone, because the regions of highest plasticity demand are also the regions of highest metabolic rate, highest connectivity, latest myelination and greatest network hub status, each of which generates the same anatomical ranking from a different mechanism. Regional correspondence is the cheapest currency in this field and both theories should be assessed as though they had not been paid in it.

9.3 What the agreement is good for

The convergence is nonetheless useful, for a reason that has nothing to do with corroboration: it converts two rival theories into one chain with two candidate lesion sites, and a chain with two candidate sites is an experiment.

If delivery fails at the receptor, then a neuron removed from its tissue and given competent particles should still take up poorly, and matrix manipulation should do nothing.

If delivery fails in transit, then the same neuron should take up normally once removed from the tissue, and delivery in tissue should improve when the matrix is degraded.

Both experiments are feasible today, neither has been done, and each theory has been arguing for its own site without acknowledging that the other exists.

9.4 A matched anatomical datum

One observation deserves to be pulled out of Chapter 6, because it bears directly on this chapter.

Heparan sulfate proteoglycan is present in the diffuse plaques of the hippocampus and absent from those of the cerebellum in the same Alzheimer brains (Snow et al., 1994). The cerebellum accumulates diffuse deposits and does not, on the whole, develop the tangle pathology or the neuronal loss that characterise the disease elsewhere.

That is a regional dissociation in the *matrix* chemistry, mapping onto the regional dissociation in the *clinical* pathology, in a direction the transit account predicts and the receptor account does not address. It is a single study, its grade is **plausible** rather than more, and it is the sort of observation that should be repeated with modern methods before much is built on it. But it is the closest thing in the existing literature to a direct test of Chapter 8's central commitment, and it came out the right way.

Part Three — What the Matrix Does to the Membrane

10. Detachment, the Protease, and the Shedding of the Bed

10.1 The sentence the theory did not follow

In the theory's own description of candidate generation, three things happen outside the cell. Matrix metalloproteinase-9 digests extracellular matrix and adhesion molecules. Tissue plasminogen activator converts pro-neurotrophins to their mature forms. And — the clause this chapter is built on — *detachment from matrix triggers endocytosis of raft components including caveolin-1*.

That clause makes the matrix a controller of the membrane. It says that the neuron's ordered domains are dismantled not by an intracellular decision but by the loss of an extracellular attachment, and that the protease which removes the attachment is the same one the theory names as a normal agent of plasticity.

The theory states this once, in a list, and never returns to it. Everything that follows in its account of disease concerns the *supply* of the ingredient for rebuilding the domain, and nothing concerns the *signal* that dismantled it. Yet a switch that is thrown by domain assembly can be held open in two ways: by failing to build, or by continuing to demolish. The theory has a detailed account of the first and no account at all of the second.

10.2 Matrix proteolysis is a normal step, and that is what makes it dangerous

Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and for memory (Nagy et al., 2006). This is not a pathological enzyme recruited by disease; it is a constitutive part of how a synapse is modified, which is exactly the status the theory assigns it.

The consequence is the theory's own logic applied to a molecule it named and dropped. A physiological agent that must be transient becomes pathological when it is chronic, and the pathology is not the agent's presence but its failure to stop. Chronic matrix proteolysis would hold a neuron in the state in which its ordered domains are being disassembled and its attachments removed — which is to say, in candidate generation — regardless of how much cholesterol arrived.

That gives the theory something it lacks and needs. Its central state, chronic non-resolution, is currently *asserted*: the switch fails to throw because supply is inadequate, and the failure is chronic because supply remains inadequate. It has no positive mechanism for chronicity, and no account of why a neuron would keep generating candidates rather than abandoning the attempt. A chronically

active matrix protease supplies one.

10.3 The shedding of the productive bed

The mechanism becomes specific, and directional, at the following point.

Matrix metalloproteinases cleave the ectodomains of syndecan-1 and syndecan-4 at mapped sites (Manon-Jensen et al., 2013). The syndecans are the principal transmembrane heparan sulfate proteoglycans of the cell surface — which is to say, they are a large part of the **productive bed** of Chapter 7: the sugar that concentrates apolipoprotein E particles at the membrane and delivers them to LRP1.

Matrix protease activation therefore does not simply remove an attachment. It removes the neuron's own capture apparatus, shedding heparan sulfate from the surface into the interstitium — where, as an ectodomain fragment, it becomes part of the unproductive bed that competes for the same particle.

This is the sharpest form of the paper's central claim, and it is an **inference**, stated here with the direction it commits to:

> *Chronic matrix protease activity shifts the partition of apolipoprotein E particles from the neuronal surface to the interstitium by two independent routes at once — it removes sites from the productive bed and it adds them to the unproductive one — and it does so through a protease the plasticity theory already names as the agent that dismantles the ordered membrane domain.*

One enzyme, three effects, all in the same direction: the domain is disassembled, the capture apparatus is shed, and the shed material joins the competing sink.

10.4 The brake on shedding, and its withdrawal in disease

Ectodomain shedding is restrained, and the restraint is a sphingolipid.

Sphingosine-1-phosphate protects the endothelial glycocalyx by inhibiting syndecan-1 shedding through its $S1P_1$ receptor, an effect abolished by receptor antagonism; and glycocalyx can be regenerated by heparan sulfate together with sphingosine-1-phosphate (Zeng et al., 2014; Mensah et al., 2017). The pathway is best characterised in endothelium, and its extension to the neuronal surface is an inference rather than a demonstration — but the sphingolipid arm of that pathway is measurably deranged in the Alzheimer brain. Sphingosine-1-phosphate is reduced, with low levels associated with amyloid accumulation in entorhinal cortex; sphingosine kinase 1 is reduced and sphingosine-1-phosphate lyase increased in disease tissue; and the subcellular localisation of the kinase that generates it is altered in Alzheimer neurons (He et al., 2010; Ceccom et al., 2014; Domínguez et al., 2018).

So the brake on shedding is withdrawn in the disease, in human tissue, and the direction is the one the proposal requires.

There is a further coincidence here that deserves to be stated as a proposal rather than left as an observation. Sphingomyelin is one of the two lipids that order the membrane domain whose assembly the theory identifies as the switch. Sphingosine-1-phosphate is a downstream metabolite of the same sphingolipid pool. If the pool is depleted or its flux redirected, then **the same metabolic derangement both starves the ordered domain of its second structural lipid and withdraws the restraint on shedding of the sugar bed that supplies its first**. That is one lesion producing two of the three failures this paper describes, and it is an **inference** with a clean test: manipulate sphingosine kinase in neurons and measure ordered-domain assembly, surface heparan sulfate and lipoprotein uptake together.

10.5 What this chapter changes about the theory

Three things.

The theory's account of the disease acquires a **demolition arm** alongside its construction arm, and the demolition arm has a named enzyme with an established role in normal plasticity.

The theory's assertion of chronicity acquires a **mechanism**, which converts its weakest structural feature into a claim about an enzyme's activity — measurable, and with a sign.

And the supply chain acquires a **second lesion site at the destination**: not the receptor, and not the transit, but the co-receptor bed that hands the particle to the receptor. That site is the one at which the theory's own protease, the disease's sphingolipid derangement, and the matrix chemistry of Part Two all converge.

11. A Driver for the Chronic State

11.1 The question the previous chapter leaves

If chronic matrix proteolysis holds the switch open, something must be driving the protease chronically. A theory that answered "the disease does" would have gained nothing.

11.2 Catecholamines drive the protease

Catecholamines potentiate expression of matrix metalloproteinase-9, an effect demonstrated in human monocytes and monocytic lines under inflammatory stimulation (Speidl et al., 2004). β_2-adrenergic signalling drives noradrenaline-promoted, metalloproteinase-dependent invasion in a cellular system, and blocking the receptor blocks the effect (Yamazaki et al., 2014). And in the intact brain, sustained sensory drive alters matrix metalloproteinase-9 and the lectican brevican in primary auditory cortex (Park et al., 2020) — an in vivo demonstration that continued afferent drive remodels the perineuronal matrix through this enzyme.

The pharmacology here is not neuronal, and that limitation is entered in the ledger: the catecholamine-to-protease link is established in monocytes and tumour cells, and the brain evidence is drive-dependent matrix remodelling rather than a demonstrated adrenergic-receptor route. The claim made in this paper is therefore graded **plausible**, not well-supported.

11.3 Why noradrenaline in particular

Three reasons make the noradrenergic system the candidate rather than one candidate among many.

It is where the pathology starts. The locus coeruleus carries abnormal tau earlier than any cortical region, decades before the clinical disease. The theory being extended names the locus coeruleus in its own regional list, among the earliest tau-bearing regions, and explains it by high plasticity demand. It offers no molecular account of why that structure in particular.

Its output is tonic and it rises under exactly the conditions that are risk factors. A driver of chronicity must itself be chronic. Sustained arousal, stress, disrupted sleep and sensory load are chronic states, they are associated with disease risk, and they raise noradrenergic tone.

Chronic excess is sufficient for the downstream pathology in an animal. Long-term exposure to excessive noradrenaline in the brain induces tau aggregation, neuronal death and cognitive deficits in early tau transgenic mice (Jeong et al., 2024). That is a demonstration that the

driver proposed here produces the disease's cytoskeletal phenotype when applied chronically, in vivo, without reference to any of the mechanisms in this paper.

11.4 The proposed chain, stated so it can be attacked

> *Sustained noradrenergic drive → β-adrenergic signalling → elevated matrix metalloproteinase activity → digestion of the interstitial and perineuronal matrix and shedding of syndecan ectodomains → loss of the neuron's heparan sulfate capture bed and detachment-triggered internalisation of caveolin-1 and other domain components → the ordered domain cannot be held or rebuilt → the plasticity switch is held open → chronic candidate generation with tau phosphorylated and never dephosphorylated in a winner, because there are no winners.*

Every arrow in that chain is separately citable and the chain as a whole is cited nowhere. Its grade is **inference**. The weakest arrows are the second and the fifth, and both are named in Chapter 23 as refutation points.

What makes the chain worth stating is that it takes the theory's own two extracellular agents — the protease it names for candidate generation, and the matrix detachment it names as the trigger for domain internalisation — and joins them to a driver that the theory's own regional argument already implicates and cannot explain.

11.5 The therapeutic reading, kept short

A chain of this kind invites a drug, and restraint is appropriate. The relevant point is narrower than a therapy: the chain predicts that the *sign* of an intervention on noradrenergic tone should depend on when it is given. Early and sustained reduction of tone should slow matrix remodelling; late reduction should do little for a matrix already remodelled, and would remove a neuromodulator the failing cortex still needs. The clinical literature on adrenergic agents in this disease is inconsistent in exactly the way a stage-dependent sign predicts, and that is offered here as a reason to stratify future analyses by stage rather than as evidence for the chain.

12. The Netted and the Unnetted

12.1 Replacing a posited population with an observed one

The theory's most serious defect is that it explains contradictory measurements by positing two populations of neuron — those whose compensatory cholesterol production eventually succeeds, and those whose does not — without any independent means of telling them apart. The device rescues the theory from every contrary result and thereby removes its capacity to be wrong.

The proposal of this chapter is a substitution:

> *Replace "the neuron that succeeds" and "the neuron that fails" with "the neuron that wears a condensed perineuronal net" and "the neuron that does not."*

The substitution is worth making only if the second pair is observable in advance, is not defined by the outcome it is meant to predict, and predicts something. All three hold.

12.2 The matrix has two states and only one of them is armour

Chapter 6 distinguished the diffuse interstitial matrix from the condensed perineuronal net. The distinction is central here, and it resolves what would otherwise be a contradiction at the heart of this paper: matrix has been presented as an obstruction, and matrix is also protective.

It is protective. Neurons ensheathed by aggrecan-based perineuronal nets are protected against tau pathology in subcortical regions of the Alzheimer brain (Morawski et al., 2010). Perineuronal nets restrict plasticity in the adult, terminate critical periods, and hold signalling molecules at the neuronal surface, including homeoprotein transcription factors that maintain the closed state (Fawcett et al., 2019; Beurdeley et al., 2012).

These two facts are only in tension if the matrix is treated as one substance. Treated as two states of one molecular family, they combine into a claim with real content:

> *The condensed net is a local structure at the neuronal surface that shields the neuron and stages ligands for it. The diffuse interstitial matrix is a distributed medium through which traffic must pass. A disease that shifts material from the first state to the second removes armour from neurons and adds obstruction to the space, simultaneously — and a bulk measurement of "matrix" in a homogenate will show the sum of the two and miss both.*

That last clause matters, because the human data of Chapter 6 are precisely bulk measurements. A rise in total sulfated glycosaminoglycan in Alzheimer hippocampus is compatible

with a fall in the condensed compartment and a larger rise in the diffuse one. The proposal predicts exactly that decomposition, and it has not been measured.

12.3 The cell-type prediction

Perineuronal nets are not distributed evenly. They ensheathe parvalbumin-expressing interneurons densely and characteristically; most other cortical populations, including somatostatin-expressing interneurons and most excitatory pyramidal cells, carry little or none (Fawcett et al., 2019).

If the net is armour, and if the shedding mechanism of Chapter 10 is real, then the two populations the theory needs are already visible in tissue and the theory can be tested against them.

Three predictions follow, and they are directional.

Netted neurons should show the domain-assembly failure later than unnetted neighbours in the same region, because the condensed net both stages ligands at their surface and resists proteolytic removal for longer.

The earliest functional failures should appear in unnetted populations, and specifically in the unnetted inhibitory populations, whose regulatory role means their loss is felt by the circuit before their number falls.

The protection should be lost, in the same tissue, in proportion to local matrix protease activity — which converts Morawski's correlation into a mechanism with a manipulable term.

12.4 What this does to the two-population device

It converts it from an escape hatch into a claim.

The theory currently says: some neurons succeeded and some failed, and which is which is inferred from whether they have pathology. That is circular, and it is the reason no measurement of cholesterol or of ordered-domain abundance in Alzheimer tissue can refute the theory.

The substitution says: the neurons that succeed are the ones with a condensed net at the time of the insult, the net can be stained for before the outcome is known, and if the pathology does not respect that division the model is wrong.

The substitution is not free. It commits the extended theory to a specific and falsifiable anatomical claim, and the anatomy has been measured. It comes out better than one might expect in one respect, and worse in another that matters more.

Regionally, the gradient runs the right way. Cortical areas abundant in extracellular-matrix chondroitin sulfate proteoglycans are less affected by cytoskeletal changes in

Alzheimer's disease; net-bearing neurons are most numerous in primary motor cortex — around a tenth of neurons in Brodmann's area 4 — and in primary auditory cortex, which are among the last regions involved, and they are *extremely rare in the entorhinal cortex*, where the pathology is earliest and heaviest (Brückner et al., 1999). The locus coeruleus, which carries the earliest tau pathology anywhere in the human brain, is constitutionally devoid of the aggrecan-based net. The disease begins in the least-armoured tissue in the cortex and in a nucleus with no armour at all.

And the regional gradient is nonetheless worth very little. The warning of §9.2 applies here with full force, and this paper should not accept payment twice in the same currency. Perineuronal nets *restrict* plasticity. The theory being extended holds that vulnerability tracks plasticity demand. "Net-poor because high-plasticity, and vulnerable because high-plasticity" is one anatomical ranking derived twice from one premise. The correspondence is consistent with the substitution and does not discriminate it from the theory it was meant to sharpen.

What carries weight is the within-region, cell-level evidence, and there is now some in cortex. Comparing frontal cortex across Alzheimer, resilient and control subjects, excitatory neurons still bearing a perineuronal net carried strikingly low levels of phospho-tau (de Vries et al., 2024). That extends Morawski's subcortical finding to a cortical, excitatory, human population, which is the population the substitution most needed and previously lacked.

The same study administers a correction to this paper. Resilience was not a matter of more net. Aggrecan around parvalbumin neurons was reduced in both Alzheimer and resilient brains, and the sulfated sugar chains of the net were reduced specifically in the resilient — a homeostatic remodelling of the matrix rather than its preservation (de Vries et al., 2024). Row 4 of the sign table, as first drafted, required the condensed bed to *fall* with stage and treated that fall as the disease signature. That is too crude, because the amount falls in resilience too. The commitment has been restated as a claim about the sulfation state of the condensed bed rather than about its quantity. The restatement is weaker and more accurate, and it has the incidental merit of joining the net limb to the sulfation limb of Part Two instead of leaving them as two stories that happen to be about the same polymer.

The substitution is graded **plausible**, and its scope is stated in the ledger.

12.5 The Cell-Type Map

The question this chapter has been circling is which neuron. The theory being extended does not say, and neither, so far, has this paper: "the neuron" has been used for at least three different cells.

Parts Two and Five make no cell-type commitment and should not be read as making one. The delivery problem is faced by any neuron that must rebuild plasma membrane and bears the receptors to do it, and the argument there is deliberately generic. From Chapter 12 onward the argument stops being generic, and the table states the referent.

Table 1 — Which neuron, by section

Population	Perineuronal net	Position in the reelin axis	Timing in human disease
Somatostatin interneuron, including Martinotti cells	Largely unnetted; a Kv3.1b-expressing subset excepted	**Source** — Martinotti cells secrete reelin into perineuronal nets	Lost in the early, silent phase
Parvalbumin interneuron	Densely netted; the net's archetypal client	**Staging bed** — receives reelin into its own net	Lost in the late phase
Entorhinal layer II reelin-immunoreactive stellate cells	Net-poor: nets are extremely rare in entorhinal cortex	**Source**; reelin is depleted here in human disease	Earliest cortical population; the first to carry intracellular amyloid
Locus coeruleus noradrenergic neurons	Constitutionally devoid of the aggrecan net	Neither source nor target; supplies the drive of Chapter 11	Earliest tau pathology anywhere in the human brain
Cortical pyramidal and other excitatory neurons	Mostly unnetted; a netted minority	**Target** — bears ApoER2	Late phase; the netted minority carries low phospho-tau

Sources for the columns, in order: Härtig et al., 1992, and Härtig et al., 1999, for the net's clientele and the Kv3.1b exception; Brückner et al., 1999, for the regional density; Morawski et al., 2010, and de Vries et al., 2024, for net status and tau; Pesold et al., 1998, 1999, for the reelin sources; Chin et al., 2007, and Kobro-Flatmoen et al., 2016, for entorhinal layer II; Gabitto et al., 2024, for the timing.

12.6 What the Map Shows

Reading down the third and fourth columns together produces the observation this chapter was written to reach, and it is not one this paper anticipated.

> *The cells that make reelin are unnetted. The cells that hold it are netted. The guardian is secreted by neurons that do not wear it, into the armour of neurons that do.*

Every population at the front of the disease sits on the unnetted side of that line — the somatostatin interneuron, entorhinal layer II, the locus coeruleus — and two of the three are reelin sources. The neurons that fail first are the ones that supply the brake to everybody else and keep none of it for themselves.

That resolves a seam this paper had left open. Chapter 12 and Chapter 13 appeared to point at different cells: the net limb at the parvalbumin interneuron and the subcortical nucleus, the reelin

limb at the entorhinal stellate cell and the interneurons that secrete the ligand. They are not different cells chosen carelessly. They are the two ends of one transaction, and the sulfated surface of Part Two is where the transaction is completed — the bed onto which the ligand is deposited, at which the receptor is clustered, and through which the lipoprotein must pass.

It also sharpens the protease argument of Chapter 10, because a net is not merely present or absent but must be actively removed: microglia facilitate the loss of perineuronal nets in the Alzheimer brain (Crapser et al., 2020). The netted neuron is not permanently safe; it is behind a wall that something has to take down first, which is why the two populations differ in *when* they are lost rather than in *whether*.

Two cautions belong with the map and neither is small.

The somatostatin claim is **statistical, not absolute**: a Kv3.1b-expressing subset of these neurons does carry nets (Härtig et al., 1999), so the contrast between the two interneuron classes is a difference of proportion.

And the timing column is **cell-abundance data across disease stage** (Gabitto et al., 2024). It records when populations are lost. It does not record why, and it cannot by itself distinguish a cell that dies because it was unarmoured from a cell that dies because it was doing something else that killed it.

The map as a whole is graded **inference**. What is established is each cell's net status, each cell's position in the reelin axis, and the order of loss. What is inferred is that these three columns are reading one mechanism.

12.7 The connection back to the sugar

One further point closes the loop with Part Two.

The condensed net is where the neuron's own sulfated sugar is concentrated. If the productive bed of Chapter 7 — the surface heparan sulfate that concentrates apolipoprotein E particles for delivery to LRP1 — is co-located with, and stabilised by, the condensed matrix, then the net is not only armour against tau. It is also the neuron's **delivery apron**: the structure that holds the particle at the surface long enough for the receptor to take it.

On that reading, losing the net costs the neuron twice. It loses a shield, and it loses its ability to compete with the interstitium for the particle it needs.

This is the paper's second principal **inference**, and the experiment that tests it is direct: measure lipoprotein particle capture at the surface of netted and unnetted neurons in the same slice, before and after enzymatic removal of the net.

Part Four — The Second Route Restored

13. Reelin, and the Clasp That Needs the Sugar

13.1 What was withdrawn

The theory being extended once had two independent routes to a chronically stalled switch. The first was failure of cholesterol delivery. The second was failure of reelin signalling through its lipoprotein receptors, which was given as a route to chronic non-resolution *independent of cholesterol*: reelin terminated the consolidation phase, its receptor ApoER2 was described as dependent on ordered membrane domains and crucial to final synaptic adhesion, and reelin's expression in entorhinal layer two — the earliest-affected cortical population — was offered as positive anatomical evidence.

By the final version, reelin appears once, as a candidate-generation agent promoting α-cleavage and neurite branching, with the termination function, the receptor biology and the genetics removed, and the topic deferred to future work.

The withdrawal cost the theory more than parsimony. It removed the only mechanism tied to the specific neuronal population that fails first, leaving a general argument about novelty in its place, and it left everything running through a single supply chain. This chapter restores the route, and argues that it was never independent of the first in the way the 2020 version supposed: **the two routes run through the same sugar.**

13.2 The pathway, in the terms this paper needs

Reelin is a large secreted glycoprotein of the extracellular matrix, identified through the *reeler* mouse (D'Arcangelo et al., 1995). It is a ligand for the lipoprotein receptors ApoER2 and VLDLR (D'Arcangelo et al., 1999), and binding induces tyrosine phosphorylation of the adaptor Disabled-1 through Src-family kinases, with modulation of tau phosphorylation as a documented output of the same paper that established the receptor interaction (Hiesberger et al., 1999; Bock and Herz, 2003).

Four properties make it the right second route.

It brakes tau. The receptor engagement that phosphorylates Disabled-1 suppresses tau phosphorylation. This is the founding observation of the field and it is not seriously contested.

It is a synaptic, adult mechanism, not only a developmental one. Reelin and its receptors cooperate to enhance hippocampal synaptic plasticity and learning; the receptor's

cytoplasmic tail is spliced under activity control and sets the gain of that enhancement (Weeber et al., 2002; Beffert et al., 2005).

It antagonises amyloid at the synapse (Durakoglugil et al., 2009), and its reduction accelerates plaque formation and tau pathology in a transgenic model (Kocherhans et al., 2010).

It is depleted where the disease starts. Reelin is reduced in the entorhinal cortex of human Alzheimer brain and of amyloid-precursor-protein transgenic mice (Chin et al., 2007).

13.3 The clasp requires N-sulfated heparan sulfate

The step that joins this part to Part Two is recent and specific. **N-sulfated heparan sulfate promotes reelin signalling as a co-receptor** (Pan et al., 2025). The engagement of reelin with ApoER2 is not a two-body interaction between a ligand and a receptor; it is a three-body clasp in which a sulfated polysaccharide participates, and the participation is sulfation-pattern-specific.

That single result changes the structure of the argument in this paper, because it means the second route is not a parallel route at all. Both routes to the stalled switch — the failure of cholesterol delivery and the failure of the reelin brake on tau — depend on the availability and the sulfation chemistry of heparan sulfate at the neuronal surface. The same bed serves both. Anything that sheds it, remodels it, or competes for it degrades both at once.

Three published facts complete the picture and they point in the same direction.

Tau internalisation is regulated by **6-O sulfation** on heparan sulfate proteoglycans (Rauch et al., 2018), and specific chain lengths and sulfation patterns are required for cellular uptake of tau as against α-synuclein and amyloid-β aggregates (Stopschinski et al., 2018). Heparan sulfate proteoglycans mediate the internalisation and propagation of proteopathic seeds generally (Holmes et al., 2013).

So one polymer at one surface carries at least three readings: an **N-sulfated** reading that stages the reelin brake, a **6-O-sulfated** reading that admits tau, and a **basic-interface** reading that binds apolipoprotein E and its cargo. The neuron's fate depends not on how much heparan sulfate it has but on which of these readings its heparan sulfate supports.

That is the sulfation code, it is established chemistry rather than a proposal of this paper, and its application to the delivery problem is what this paper adds.

13.4 Reelin is secreted into the perineuronal net

One further published fact welds Part Four to Part Three, and it is old.

Cortical bitufted, horizontal and Martinotti cells preferentially express reelin and **secrete it into perineuronal nets**, where it modulates gene expression non-synaptically (Pesold et al., 1998,

1999).

The reelin brake is therefore not diffusing freely to its receptor. It is deposited into, and held by, the condensed matrix compartment — the same compartment that Chapter 12 identified as armour against tau, that Chapter 10 identified as the substrate of the plasticity protease, and that Chapter 12 proposed as the neuron's delivery apron for lipoprotein particles.

The convergence is worth stating plainly because it is the paper's tightest join:

> *One sulfated compartment at the neuronal surface stages the reelin brake on tau, provides the N-sulfated domains that clasp reelin to its receptor, holds apolipoprotein E particles for capture, resists the internalisation of tau seeds, and is digested by the protease that normal plasticity requires and chronic noradrenergic drive potentiates. The reelin-staging and tau-gating offices belong to* **heparan** *sulfate, which is a general constituent of the neuronal surface; the condensed net beside them is* **chondroitin** *sulfate. One place, two polymers — so reelin does not require a net in order to signal.*

Every clause of that sentence is separately published. The sentence is not.

13.5 The grade, and the tension carried forward

The reelin biology is **established**. The N-sulfated co-receptor requirement is **well-supported**, resting principally on one recent structural and biochemical study. The claim that reelin-staging and lipoprotein-capture use a shared and simultaneously degraded bed is an **inference**, and it is this paper's third.

The tension recorded in Chapter 7 applies here with full force and is not resolved: the human hippocampal data show *rising* N-sulfotransferase transcripts in disease, which — if it translated into rising N-sulfated domain availability at the neuronal surface — would predict a strengthening reelin clasp rather than a weakening one. This paper's position is that transcript is not domain, that bulk tissue is not surface, and that the decomposition has not been measured. That position is a hypothesis, not a defence, and experiment six is designed to break it.

14. One Receptor, Four Programmes

14.1 The coincidence

ApoER2 appears in four research literatures that do not cite one another.

To the **reelin field**, it is the receptor whose engagement phosphorylates Disabled-1 and suppresses tau phosphorylation, whose cytoplasmic tail is activity-spliced, and whose clasp requires N-sulfated heparan sulfate (Hiesberger et al., 1999; Beffert et al., 2005; Pan et al., 2025).

To the **cholesterol-supply field**, it is a member of the low-density-lipoprotein receptor family that binds apolipoprotein E, and therefore a participant in the delivery of the ingredient on which the plasticity switch depends (Lane-Donovan et al., 2014).

To the **membrane-domain field** — including the theory extended here — it is a receptor described as dependent on ordered lipid domains for its function, and one that is internalised in an apolipoprotein E4 background (Chen et al., 2010).

To the **lipid-peroxidation field**, it is the target of a proposed covalent modification: peroxidation-derived aldehydes crosslinking apolipoprotein E to its receptor so that the receptor cannot be recovered.

Four accounts, one protein, and each of them treats the other three as somebody else's subject.

14.2 Why this is more than a coincidence

Because the four accounts do not merely share a protein; they share a **binding surface and a competition at it**.

The ligand-binding modules of ApoER2 are the site at which both reelin and apolipoprotein E engage. Their engagement is not independent: they compete for occupancy of the same receptor at the same surface, in the presence of a sulfated co-receptor that participates in both interactions (D'Arcangelo et al., 1999; Yasui et al., 2007, 2010; Pan et al., 2025).

That yields a proposal with a shape the rest of this paper has been building toward:

> *The neuron's surface is a partition problem with three ligands and two beds. Reelin must reach ApoER2 and be clasped there by N-sulfated heparan sulfate. Apolipoprotein E particles must be concentrated by the same sugar and handed to LRP1 and the low-density-lipoprotein receptor. Tau seeds must be excluded from a 6-O-sulfated reading of the same polymer. Disease is a redistribution of a finite sulfated surface among these three, in the*

presence of an interstitial bed that is growing and a protease that is shedding.

This is the paper's central architectural claim and it is graded **inference**. It is stated in that form because it makes each of the four literatures a source of measurements about the other three, which is precisely what none of them currently is.

14.3 The experiments the coincidence generates

Three crosses follow directly, and none has been run.

Does apolipoprotein E occupancy of ApoER2 attenuate reelin's brake on tau? If the two ligands compete at the same modules, then raising apolipoprotein E — particularly an isoform with high affinity for the co-receptor sugar — should reduce reelin-dependent Disabled-1 phosphorylation and raise tau phosphorylation on the same neurons. The reagents exist in both fields.

Does the aldehyde crosslink abolish the reelin clasp as well as the apolipoprotein one? If peroxidation welds a ligand to this receptor, the receptor is unavailable to reelin too, and a lipid-peroxidation lesion becomes a reelin lesion. Nobody in the peroxidation literature has measured Disabled-1 phosphorylation.

Does removing the condensed net degrade cholesterol delivery and the reelin brake together? Chapter 12's apron proposal and Chapter 13's staging proposal make the same prediction on the same manipulation, which means one enzymatic treatment tests both.

14.4 What would make this chapter wrong

If reelin and apolipoprotein E turn out to engage ApoER2 at non-overlapping modules with no measurable competition, the partition framing collapses into a coincidence of address, and the chapter should be read as a list of things that happen to occur at one protein. That result would leave Parts Two and Three untouched and would remove Part Four's claim to be a *second route through the same bottleneck*, returning it to the status of a genuinely independent second route — which is what the 2020 version of the theory claimed and this paper has argued against.

15. Two Human Variants, One Competition

15.1 The two resilience cases

The field has two documented human beings whose resistance to autosomal-dominant Alzheimer's disease has been traced to a single variant, and both variants sit on the interface this paper is about.

The first is a woman homozygous for the **Christchurch** variant of apolipoprotein E3, an arginine-to-serine substitution at residue 136 within the heparin-binding interface (Wardell et al., 1987). Carrying the *PSEN1* E280A mutation of the Antioquia kindred, she remained free of mild cognitive impairment until her early seventies — roughly three decades beyond the kindred's median — with very high amyloid burden and limited entorhinal tau (Arboleda-Velasquez et al., 2019). The variant binds heparin substantially more weakly than E3.

The second is a man heterozygous for **RELN-COLBOS**, a gain-of-function variant of reelin, in the same kindred, who was likewise protected with limited entorhinal tau; the variant enhances interaction with the reelin receptors and increases downstream Disabled-1 phosphorylation with suppression of tau phosphorylation (Lopera et al., 2023).

15.2 The reading this paper proposes

The two variants look unrelated. One weakens a lipoprotein's grip on a sugar; the other strengthens a matrix protein's grip on a receptor. They are, on the argument of Chapter 14, **the same intervention performed from two sides**.

Both shift the partition at the neuronal surface in the same direction: toward reelin occupancy of ApoER2 and away from apolipoprotein E occupancy of the shared sugar and the shared modules. Christchurch does it by removing a competitor from the bed. COLBOS does it by strengthening the clasp of the ligand you want there.

And both produce the same clinical phenotype, which is not the phenotype an amyloid-clearance mechanism produces: **high amyloid burden, limited entorhinal tau, preserved cognition**. Neither variant stopped the plaques. Both stopped the conversion.

That is a strong statement and it is graded **inference**, for a specific reason given in the next section.

15.3 The rival reading, and why it is not defeated

The reading offered by the group that characterised both cases is that apolipoprotein E–heparan sulfate binding participates in the cellular uptake and propagation of tau seeds, and that the Christchurch variant protects by limiting that uptake. That is well-supported chemistry (Holmes et al., 2013; Rauch et al., 2018; Stopschinski et al., 2018), it fits the phenotype directly, and it does not require anything this paper has proposed.

It also fits COLBOS less directly. A gain-of-function in the reelin clasp does not obviously limit tau uptake; it suppresses tau phosphorylation through Disabled-1. The two cases are therefore explained by the tau-uptake reading and the receptor-signalling reading respectively — two mechanisms for two cases.

The proposal here explains both with one, which is a reason to prefer it only if it also predicts something the two-mechanism account does not. It does, and the prediction is specific:

> *If the two variants act on one partition, their effects should be* **non-additive** *— a carrier of both should be protected little more than a carrier of either. If they act by two independent mechanisms, the effects should combine.*

That is a hard experiment in human genetics and an easy one in cells: introduce both variants into the same neuronal system and measure Disabled-1 phosphorylation, tau phosphorylation and tau uptake as a two-by-two.

15.4 What the two cases say about the whole framework

One point is worth drawing out because it bears on the therapy chapter this paper does not have.

Both resilience variants act at the **extracellular surface** — on a secreted ligand, on a lipoprotein, on the sugar between them. Neither acts on secretase activity, on aggregation, on clearance, or on any intracellular step. The two clearest natural experiments in the human record locate the modifiable variable in exactly the compartment the theory being extended left out.

That is not proof of anything. It is, however, the reason this paper was written about the space between the cells rather than about any of the more thoroughly studied compartments on either side of it.

Part Five — The Rest of the Chain

16. What Happens After Uptake

16.1 The chain does not end at the receptor

The theory being extended locates its lesion at uptake and stops there. But a cholesterol molecule that has been internalised is not yet a cholesterol molecule in a plasma-membrane domain. It arrives esterified or unesterified in a lipoprotein particle inside an endosome; the particle must be degraded; the sterol must be liberated; and it must be exported from the late endosomal compartment and delivered to the plasma membrane by vesicular and non-vesicular routes.

Each of those steps can fail, each failure produces the phenotype the theory attributes to failed uptake, and two of them are among the best-evidenced lesions in the disease.

16.2 Acidification

Liberating a sterol from an internalised particle requires an acidified, hydrolytically competent compartment. In Alzheimer models, autolysosome acidification is faulty, and the consequence is an autophagic build-up of amyloid-β within neurons that yields, on neuronal rupture, the structure conventionally read as a senile plaque (Lee et al., 2022).

This matters for the present argument in two ways.

It supplies a **post-uptake lesion with the same signature as the pre-uptake one**. A neuron that internalises particles normally into a compartment that cannot process them is a neuron with normal uptake and no delivered cholesterol. No measurement of receptor number, receptor recycling or particle binding would detect it.

And it supplies an **independent route to the same deposit**. Chapter 8 offered detained-and-ruptured extracellular particles as an origin for the plaque; Lee and colleagues offer ruptured neurons that had internalised the material. These are not the same claim and this paper does not adjudicate between them. They are compatible: both say the plaque is where a failed lipid-handling process ended, and both say it is a product rather than a cause.

16.3 Which pool of cholesterol matters

The theory treats cholesterol as one quantity. It is at least three: free sterol in membranes, esterified sterol in droplets, and sterol in transit.

A screen of cholesterol-metabolism compounds in patient-derived neurons found that what drives abnormal tau phosphorylation is not free intracellular cholesterol but **cholesteryl esters**, which act by downregulating the ubiquitin–proteasome system; reducing esters raised proteasomal activity and cleared misfolded tau, and blockade of the esterifying enzyme ACAT1/SOAT1 reproduced the effect (van der Kant et al., 2019). Separately, the non-vesicular sterol transporter GRAMD1B was identified as a regulator of lipid homeostasis, autophagic flux and phosphorylated tau (Acosta Ingram et al., 2025).

The consequence for the theory being extended is uncomfortable and should be stated plainly. Its central explanandum — cholesterol mishandling causing tau pathology — has now been explained twice, by two mechanisms, neither of which requires the raft-gated switch. The switch may still be right. But it is no longer the only available account of the thing it was built to account for, and a defender of it has to say what the switch adds beyond these two.

The answer this paper would give is that neither the ester route nor the transporter route explains the *staging* — why tau is dephosphorylated in some spines and not others, why plaques are common in the cognitively normal, why partial failure should be worse than complete failure. Those remain the switch's distinctive contributions. But that is an argument for the switch as a theory of *organisation*, not as a theory of tau phosphorylation.

16.4 The same statin result, read twice

A detail with disproportionate consequences. The theory being extended asserts that statins do not cross the blood–brain barrier. Lipophilic statins do, measurably lowering central cholesterol synthesis (Sierra et al., 2011), and statins were among the most potent phospho-tau-lowering hits in the screen described above (van der Kant et al., 2019).

Correcting the error returns a pharmacological probe to the framework and simultaneously removes an escape. A theory cannot be indifferent to whether lowering brain cholesterol synthesis helps or harms and then cite the inconsistency of the clinical record as though it were confirmation. On the extension proposed here the sign is at least *derivable*: lowering synthesis should be harmful where the lesion is supply and helpful where the lesion is the ester pool, and which dominates should depend on stage and genotype. That is a prediction, and it is testable in existing pharmaco-epidemiological cohorts stratified as the ester mechanism requires.

17. Quality, Not Quantity

17.1 A second axis on the delivered lipid

The supply theory treats delivery as a scalar: more or less cholesterol arrives. A particle can also arrive chemically altered, and the alteration has a specific proposed consequence at the receptor this paper has been building toward.

The proposal is that lipid peroxidation generates reactive aldehydes which crosslink apolipoprotein E to its receptor ApoER2, disrupting the ligand–receptor relationship and, with it, the Disabled-1 signalling that suppresses tau phosphorylation. It is offered as a unifying account of sporadic disease in humans, with the ApoER2–Dab1 disruption specifically proposed as the origin of phospho-tau-associated neurodegeneration, and with the cysteine content of the apolipoprotein isoforms — and the disulfide bridges they can form — as the molecular basis of the allelic risk (Ramsden et al., 2022, 2023, 2025).

17.2 Why it belongs in this paper

Because it is the fourth programme at the receptor of Chapter 14, and because it adds a term the transit account needs.

A detained particle is a particle with a long residence time in an oxidising extracellular compartment. Residence time is exactly the variable that peroxidation chemistry cares about. The two accounts therefore multiply rather than compete:

> *Matrix detention increases the interval between release and capture; a longer interval increases the probability that the particle's lipids are peroxidised before capture; a peroxidised particle both fails to deliver and disables the receptor it reaches.*

That is an **inference**, and it is this paper's fourth. It converts the peroxidation programme's lesion from a stochastic chemical accident into a consequence of a physical delay, and it gives the transit account a mechanism for why detained particles are worse than merely delayed ones.

It also yields a directional prediction that neither account makes alone: the burden of peroxidation-derived adducts on apolipoprotein E recovered from tissue should scale with interstitial matrix density in the same region, and should be higher in regions with low effective diffusivity than in regions with high.

17.3 The limit

This chapter's grade is the lowest in Part Five. The crosslinking proposal is itself a hypothesis with supporting biochemistry rather than an established lesion, and joining it to another hypothesis produces a compound that must be graded by its weakest limb. It is included because the prediction in the previous paragraph is cheap to test in existing tissue banks, not because the joint account is likely to be right as stated.

18. Supply Without Disposal

18.1 The missing half of a homeostatic system

A theory of a supply chain that has no theory of disposal is describing an inbox, not a homeostat.

The brain's cholesterol is not consumed. It is turned over: excess sterol is converted by neuronal sterol 24-hydroxylase into 24S-hydroxycholesterol, which unlike cholesterol can cross the blood–brain barrier and is the principal route by which the organ exports it (Dietschy and Turley, 2004). Efflux to lipoprotein acceptors through ABCA1 is the other arm, and is the step by which astrocytes load the particles in the first place (Wahrle et al., 2004).

The theory extended here models the loading arm and the delivery arm and says nothing about the export arm. That omission has three consequences.

18.2 Three things the omission costs

It removes a compensatory variable. A neuron short of membrane cholesterol can, in principle, reduce export as well as increase import. If the disease involves failure to reduce export, or inappropriate conversion of a scarce resource, that is a lesion in the same currency the theory is denominated in, and it is invisible to the theory.

It makes the pharmacology unreadable. Interventions that raise sterol 24-hydroxylase activity increase export; interventions that lower synthesis reduce input; interventions that block esterification redistribute the internal pool. A framework that models only input cannot predict the sign of two of those three, which is a large part of why the cholesterol pharmacology of this disease reads as a set of contradictory results.

It hides an interaction with the matrix. Export requires the same journey as import, in reverse: an acceptor particle must reach the neuron, be loaded, and leave. If the interstitium detains particles, it detains them in both directions. A transit lesion is therefore predicted to impair efflux as well as influx — and impaired efflux with impaired influx is a different metabolic state from impaired influx alone, because the neuron accumulates the wrong sterols rather than simply lacking the right one.

18.3 The reconciliation this offers

Chapter 16 recorded a discrepancy: the ester pool drives phospho-tau, and reducing brain cholesterol synthesis lowers phospho-tau, in a theory that says the disease is cholesterol *deficiency*.

The disposal arm supplies a reading under which both are true. A neuron with impaired delivery *and* impaired export is not simply short of cholesterol; it is short of cholesterol *in the right compartment* while accumulating it in the wrong one — free sterol scarce at the plasma membrane, esterified sterol accumulating internally because it cannot be exported and cannot be used. Lowering synthesis in that state relieves the ester burden without worsening the membrane deficit, because the membrane deficit was never a synthesis problem.

That reading is an **inference**, it is the one this paper is least confident of, and it is stated because it is the only account on offer that makes the deficiency theory and the statin result consistent without either party having to be wrong.

19. Who Removes the Loser

19.1 The gap

The theory being extended assigns amyloid-β a physiological function: it terminates candidate generation and removes losers. The first half is a signalling claim. The second is a mechanical claim, and the theory does not say by what hand.

Amyloid-β does not retract a spine. Something has to.

19.2 The executioner is known

Complement and microglia mediate early synapse loss in Alzheimer models: C1q is upregulated, deposits at synapses, and complement receptor 3-dependent microglial phagocytosis eliminates them, with complement knockout rescuing synapse loss (Hong et al., 2016). This is a developmental synapse-elimination programme re-engaged in the ageing and diseased brain.

Inserting it supplies the theory's loser-removal step with the effector it lacks, and does so in a way that fits the theory's own logic: a physiological elimination programme, appropriate when transient and pathological when chronic, whose activity is set by an upstream marking signal.

19.3 The cost of the insertion, stated honestly

It also imports a problem, and the problem should not be smoothed over.

The complement arm is a **gain**, not a failure. A signal is added to an intact system and the addition alone produces the lesion. Nothing in it requires a supply failure, a stalled switch, a detained particle or a degraded matrix. If synapse elimination in this disease is driven principally by an added extracellular signal acting on competent machinery, then the entire architecture of this paper — a chain of failing recovery steps behind a failing delivery — is at best a parallel process and at worst a distraction.

This paper does not have an answer to that. It has two observations that keep the question open rather than closing it.

The first is that the complement cascade is itself a system whose activation is normally terminated by regulators, and that complement regulator status has not been measured in the same tissue as the elevated activators — so what looks like an added signal may be an unterminated one. This is a real possibility and it is not evidence.

The second is that the marking of a synapse for complement deposition is not itself part of the complement system. Something determines which synapses are tagged. The theory being extended offers a candidate — the synapse that lost the competition and was never restabilised — and the matrix account offers another: the synapse whose surrounding condensed net was digested and which is therefore accessible to a phagocyte that a net would have excluded. Both are testable against complement deposition patterns in netted and unnetted populations.

19.4 The microglial address of a matrix lesion

One connection is worth making explicit because it runs the other way.

Microglia are not bystanders to matrix state; they are among the cells that remodel it, secreting proteases and phagocytosing matrix components. A chronically activated microglial population is therefore a matrix-remodelling population, and the inflammation that follows detained-and-ruptured particles in Chapter 8 would feed back onto the compartment that detained them.

That is a loop: matrix change detains particles, detained particles rupture and release aggregation-prone peptide, the peptide activates microglia, activated microglia remodel matrix. Whether the loop's gain exceeds one is unknown and is the kind of question this literature is not currently set up to answer, but the loop's existence is the clearest reason to expect the disease to accelerate once it starts, which is a property any account of it has to produce from somewhere.

Part Six — Discipline and Consequences

20. The Sign Table

20.1 Why this chapter exists

Chapter 4 committed this paper to a rule: an addition is admissible only if it supplies a second independent route or a variable with a declared direction. The rule is worthless unless the directions are collected somewhere a reader can check them against the argument, and unless they are stated in a form that a single contrary measurement would embarrass.

Every directional commitment made in this paper is in the table below. Where a commitment has already been measured, the measurement is named. Where it has not, the cell says so, and that is the honest state of most of them.

Table 2 — Directional commitments

#	Variable	Direction required	Measured?
1	Sulfated glycosaminoglycan content, Alzheimer hippocampus	Rises	Yes — Huynh et al., 2019
2	Heparan sulfate specifically, same tissue	Rises	Yes — Huynh et al., 2019
3	Interstitial (diffuse) matrix bed, vulnerable regions	Rises with stage	No
4	**Sulfation state** of the condensed perineuronal net, vulnerable regions	Shifts with stage — a change of state, not of amount	Partly — de Vries et al., 2024: aggrecan falls in disease *and* in resilience; sulfated chains fall specifically in the resilient
5	Effective diffusivity of a 20 nm tracer, vulnerable regions	Falls with age and stage	No
6	Same, relative to a small-molecule tracer in the same tissue	Falls further	No
7	Apolipoprotein isoform affinity for heparan sulfate vs. clinical risk	Positively correlated	Yes — in vitro rank E4 > E3 > E2 > E3ch
8	Neuronal cholesterol delivery measured *in tissue* vs. receptor competence measured *in dissociated culture*	Tissue deficit exceeds culture deficit	No

#	Variable	Direction required	Measured?
9	Sign of the APOE4 effect on neuronal ordered-domain cholesterol	Positive in young systems, negative in aged	Partly — opposite signs reported in young cellular and aged human systems; no crossover series
10	Surface syndecan heparan sulfate under chronic protease activity	Falls	No (shedding established in other tissues)
11	Shed heparan sulfate ectodomain in interstitium	Rises	No
12	Sphingosine-1-phosphate, Alzheimer brain	Falls	Yes — Ceccom et al., 2014; He et al., 2010
13	Matrix metalloproteinase-9 activity under sustained noradrenergic drive	Rises	Partly — catecholamine induction shown outside brain; drive-dependent matrix remodelling shown in cortex
14	Tau pathology in netted vs. unnetted neurons	Lower in netted	Yes, subcortical — Morawski et al., 2010
15	Lipoprotein capture at netted vs. unnetted neuronal surfaces	Higher at netted	No
16	Reelin-dependent Disabled-1 phosphorylation under raised apolipoprotein E occupancy	Falls	No
17	Peroxidation adducts on recovered apolipoprotein E vs. local matrix density	Positively correlated	No
18	Effect of combining the Christchurch and COLBOS variants	Sub-additive	No
19	N-sulfated domain availability at the *neuronal surface* with stage	Falls	No — and bulk N-sulfotransferase transcript rises (see §20.2)
20	6-O-sulfated domain availability, same tissue	Rises	Partly — HS3ST transcripts rise; 6-O not resolved
21	Order of loss by net status across cell classes	Unnetted classes lost early, netted classes late	Yes — Gabitto et al., 2024
22	Phospho-tau in netted vs. unnetted *cortical excitatory* neurons	Lower in netted	Yes — de Vries et al., 2024

20.2 The row that is currently against the paper

Row 19 is the one to look at first, and it is against us.

The proposal requires N-sulfated heparan sulfate available to reelin at the neuronal surface to fall with disease stage. The only relevant human measurement shows N-deacetylase/N-sulfotransferase-2 transcript **rising** in Alzheimer hippocampus (Huynh et al., 2019). If that translates to domain availability at the surface, row 19 is false and the reelin limb of Part Four is wrong about its chemistry.

Chapter 7 set out the three ways the discrepancy might resolve — transcript is not domain; bulk tissue is not surface; or the limb is wrong — and declined to choose. The point of putting it in this table rather than only in the text is that it is the paper's most exposed commitment and it should be the first thing a critical reader tests.

20.3 The rows that are already satisfied

Seven rows have been measured and came out in the required direction: 1, 2, 7, 12, 14, 21 and 22. None of them was measured by anyone testing this proposal, which is the only kind of confirmation worth much, and none of them individually discriminates this account from its rivals. Row 7 is the most interesting of the five, because the correlation it records — affinity for the sugar tracking clinical risk across four variants — is the observation the paper was built to explain, and no account other than this one and the tau-uptake account has attempted to explain it at all.

21. The Graded Ledger

Table 3 — Every substantive claim, with its grade and its basis

#	Claim	Grade	Basis
1	Brain cholesterol is glia-produced, apolipoprotein-E-transported, neuron-imported; neurons cannot substitute their own for plasma-membrane domain assembly	Established	Standard cell biology; not original to any theory here
2	Ordered membrane domains are required for anchoring the receptors and scaffolds used in synapse stabilisation	Established	Palmitoylation-dependence literature
3	Neuronal cholesterol uptake is impaired in the disease while astrocytic efflux is preserved, and E4 particles deliver less	Well-supported	Borràs et al., 2025 — human CSF plus reconstituted particles
4	Brain lipoprotein particles (~20 nm) must cross an extracellular space (~40 nm) that hinders macromolecular diffusion	Established	Stukas et al., 2015; Nicholson and Hrabetova, 2017
5	Sulfated glycosaminoglycan and heparan sulfate content rise in Alzheimer hippocampus, with altered sulfotransferase transcripts and altered ligand preferences	Well-supported	Huynh et al., 2019; supported peripherally by Zebrower et al., 1992
6	Heparan sulfate proteoglycan accumulates early in neurons and lesions, and is present in hippocampal but not cerebellar diffuse plaques	Plausible	Snow et al., 1990, 1994 — single-group, older methods
7	Perlecan binds amyloid-β with picomolar affinity; sulfated glycosaminoglycans assemble tau into filaments in a sulfation-pattern-dependent way	Established	Snow et al., 1995; Goedert et al., 1996; Townsend et al., 2020
8	Apolipoprotein E binds heparan sulfate through the 130–143 basic interface, isoform-specifically, in the rank E4 > E3 > E2 > E3ch	Established	Cardin et al., 1986; Weisgraber et al., 1986; Dong et al., 2001; Wardell et al., 1987

#	Claim	Grade	Basis
9	Heparan sulfate proteoglycans mediate tau seed uptake, regulated by 6-O sulfation	Well-supported	Holmes et al., 2013; Rauch et al., 2018; Stopschinski et al., 2018
10	N-sulfated heparan sulfate is a co-receptor promoting reelin signalling	Well-supported	Pan et al., 2025 — recent, one group
11	Reelin engages ApoER2/VLDLR, phosphorylates Disabled-1, and suppresses tau phosphorylation; it is depleted in human entorhinal cortex in disease	Established	D'Arcangelo et al., 1999; Hiesberger et al., 1999; Chin et al., 2007
12	Reelin is secreted into perineuronal nets by defined interneuron classes	Well-supported	Pesold et al., 1998, 1999
13	Neurons with aggrecan-based perineuronal nets are protected against tau pathology (subcortical)	Well-supported	Morawski et al., 2010
14	Matrix metalloproteinase-9 is required for late-phase potentiation and memory	Established	Nagy et al., 2006
15	Matrix metalloproteinases cleave syndecan-1 and -4 ectodomains at mapped sites	Established	Manon-Jensen et al., 2013
16	Sphingosine-1-phosphate restrains syndecan-1 shedding; the S1P axis is deranged in Alzheimer brain	Well-supported	Zeng et al., 2014; Mensah et al., 2017; He et al., 2010; Ceccom et al., 2014; Domínguez et al., 2018
17	Catecholamines induce matrix metalloproteinase-9; sustained drive remodels cortical matrix through it	Plausible	Speidl et al., 2004; Yamazaki et al., 2014; Park et al., 2020 — non-neuronal pharmacology
18	Chronic excess brain noradrenaline induces tau aggregation and neuronal death in vivo	Well-supported	Jeong et al., 2024 — one model system
19	Faulty autolysosome acidification produces intraneuronal amyloid build-up and, on rupture, plaques	Well-supported	Lee et al., 2022
20	Cholesteryl esters, not free cholesterol, drive phospho-tau via the proteasome; non-vesicular sterol transport regulates the same	Well-supported	van der Kant et al., 2019; Acosta Ingram et al., 2025
21	Lipophilic statins cross the blood–brain barrier and lower phospho-tau in human neurons	Established	Sierra et al., 2011; van der Kant et al., 2019

#	Claim	Grade	Basis
22	Complement and microglia mediate early synapse loss	Established	Hong et al., 2016
23	Two human resilience variants (APOE3ch, RELN-COLBOS) produce high amyloid with limited entorhinal tau and preserved cognition	Established	Arboleda-Velasquez et al., 2019; Lopera et al., 2023
24	**The neuronal surface and the interstitium are competing beds for one apolipoprotein, and delivery is the outcome of that competition**	**Inference**	This paper — Chapter 7
25	**Lipoprotein particles are detained in the aged and diseased interstitium, and detention is a term in the delivery rate**	**Inference**	Assembly: Boche, 2020. No mobility measurement exists
26	**Chronic matrix protease activity shifts the partition by shedding the productive bed and enlarging the unproductive one**	**Inference**	This paper — Chapter 10
27	**Sustained noradrenergic drive is a driver of that protease activity, and therefore of chronicity**	**Inference**	This paper — Chapter 11; weakest arrows named in §11.4
28	**Net status is an observable substitute for the theory's two posited neuronal populations**	**Plausible**	This paper — Chapter 12; scope limited (see §12.4)
29	**The condensed net is the neuron's delivery apron as well as its armour**	**Inference**	This paper — Chapter 12
30	**Reelin staging and lipoprotein capture use a shared, simultaneously degraded sulfated bed**	**Inference**	This paper — Chapter 13
31	**Reelin and apolipoprotein E compete at ApoER2, making the surface a three-ligand partition**	**Inference**	This paper — Chapter 14; refuted if the modules do not overlap
32	**The Christchurch and COLBOS variants are one intervention performed from two sides, and should be sub-additive**	**Inference**	This paper — Chapter 15

#	Claim	Grade	Basis
33	**Detention lengthens residence time and therefore raises peroxidative damage to the particle**	**Inference**	This paper — Chapter 17; compound of two hypotheses
34	**A transit lesion impairs efflux as well as influx, reconciling cholesterol deficiency with the statin result**	**Inference**	This paper — Chapter 18; the least confident claim in it
35	Entrapment is the primary cause of Alzheimer's disease	Plausible	Boche, 2020 — no rate measurement; this paper does not adopt it
36	Rising bulk N-sulfotransferase transcript is compatible with falling surface N-sulfated domain availability	**Unresolved**	The paper's principal internal tension — §7.5, §20.2
37	Perineuronal nets are rarest in entorhinal cortex and densest in primary motor and auditory cortex, and net-rich areas are less affected by cytoskeletal change	Established	Brückner et al., 1999
38	Cortical excitatory neurons bearing a net carry low phospho-tau in human tissue, and resilience involves matrix remodelling rather than net preservation	Well-supported	de Vries et al., 2024
39	Parvalbumin cells are the net's principal clientele and somatostatin cells largely are not, with a Kv3.1b-expressing exception	Established	Härtig et al., 1992, 1999
40	Unnetted cell classes are lost in the early phase of the human disease and netted classes in the late phase	Well-supported	Gabitto et al., 2024 — cell-abundance data across stage
41	Reelin-immunoreactive entorhinal layer II neurons are the first population to carry intracellular amyloid	Well-supported	Kobro-Flatmoen et al., 2016
42	Microglia facilitate the loss of perineuronal nets in the Alzheimer brain	Well-supported	Crapser et al., 2020
43	**The cells that make reelin are unnetted and the cells that hold it are netted; the populations that fail first supply the brake and keep none of it**	**Inference**	This paper — §12.6; the map's three columns read as one mechanism

21.1 The shape of the ledger

Twenty-eight rows are drawn from the published literature and carry grades of *well-supported* or better. Twelve are this paper's own, and eleven of those twelve are *inference* — claims that exist because two literatures were placed side by side, and that have the status of hypotheses until somebody runs the experiment.

That ratio is the correct one for a paper of this kind and it should be read as a limitation rather than as a strength. Nothing here has been measured by anyone testing it. The value of the assembly, if it has any, lies entirely in the fact that the experiments of the next chapter are cheap, and that the reagents for every one of them already exist in laboratories that do not correspond.

22. Ten Experiments

Each is feasible now. Each would move at least one row of the ledger. They are ordered by how much they would settle, not by how easy they are.

1. Measure the mobility of a lipoprotein-sized particle in aged and diseased human brain tissue. The missing measurement, and the one on which the whole paper depends. Fluorescently labelled apolipoprotein E particles, or size-matched inert tracers, in acute slices or in fixed-but-uncollapsed preparations across age and Braak stage, with a small-molecule tracer measured in the same tissue as the internal control. The proposal requires the twenty-nanometre tracer's effective diffusivity to fall with stage and to fall further than the small molecule's. A flat result kills Chapters 7, 8 and 10 together. *Moves rows 5, 6, 25.*

2. The tissue-versus-dish mismatch. Take neurons and matched tissue from the same donors. Measure cholesterol delivery from labelled particles in intact tissue and receptor-mediated uptake in dissociated culture. The transit account requires the tissue deficit to exceed the culture deficit; the receptor account requires them to be equal. This is the single discriminating experiment between the two theories that Chapter 9 showed have been arguing past each other. *Moves rows 8, 24, 25.*

3. The age crossover of the APOE4 effect. Isogenic E3 and E4 human neurons and astrocytes, with spatially resolved imaging of raft cholesterol and of raft-localised amyloid precursor protein, across a maturation and senescence series, with astrocyte-conditioned medium from age-matched donors — and, critically, run both in dissociated culture and in a matrix-containing three-dimensional system whose glycosaminoglycan content can be varied. The proposal predicts that the sign of the E4 effect inverts with matrix bed size rather than with cell age as such. A monotonic effect in either direction, independent of matrix, refutes the competing-beds account of the E4 sign problem. *Moves row 9.*

4. Detention against tau uptake. The cross that separates this paper's reading of the apolipoprotein–heparan sulfate interface from the established one. In a system containing no tau, compare particle mobility and neuronal cholesterol delivery for E4, E3, E2 and E3-Christchurch particles in a matrix of defined sulfation. The detention account requires the rank order of delivery to be the inverse of the affinity rank, in the complete absence of tau. The tau-uptake account predicts no isoform effect at all in that system. *Moves rows 24, 25.*

5. The protease chain, one segment at a time. In neuronal culture and in slice: apply a β-adrenergic agonist chronically; measure matrix metalloproteinase activity, surface syndecan

heparan sulfate, shed ectodomain in the medium, caveolin-1 surface localisation, ordered-domain assembly and lipoprotein capture, in that order, with a metalloproteinase inhibitor and a receptor antagonist as the two controls. Every arrow of §11.4 is tested in one preparation. *Moves rows 10, 11, 13, 26, 27.*

6. Decompose the sulfation. The experiment that settles the paper's principal internal tension. In staged human entorhinal–hippocampal tissue, resolve heparan sulfate sulfation by compartment — interstitial, perineuronal, cell-surface — rather than in homogenate, and by domain type rather than in bulk, using domain-specific antibodies and mass spectrometry on isolated fractions. The proposal requires surface N-sulfated availability to fall while interstitial content rises, against a background of rising bulk transcript. A uniform rise across compartments refutes row 19 and with it the reelin limb of Part Four. *Moves rows 19, 20, 30, 36.*

7. The apron. Measure lipoprotein particle capture and internalisation at the surfaces of netted and unnetted neurons in the same slice, before and after enzymatic removal of the net with chondroitinase. Tests whether the condensed net is a delivery structure and not only a shield, and does so on a manipulation that simultaneously tests the tau-protection claim. *Moves rows 15, 28, 29.*

8. The three-ligand partition. On one neuronal preparation, titrate apolipoprotein E against reelin and measure Disabled-1 phosphorylation, tau phosphorylation and tau seed uptake as a matrix. Then repeat with the Christchurch and COLBOS variants introduced singly and together. Competition at shared modules predicts reciprocal occupancy and sub-additivity of the two variants; independent mechanisms predict additivity. *Moves rows 16, 18, 31, 32.*

9. One lesion, three readouts. Manipulate sphingosine kinase and the $S1P_1$ receptor in neurons and measure, together, ordered-domain assembly, surface heparan sulfate, ectodomain shedding and lipoprotein uptake. Tests the proposal of §10.4 that a single sphingolipid derangement starves the domain of its second structural lipid and withdraws the restraint on shedding of the bed that supplies its first. *Moves rows 12, 16, 26.*

10. The unnetted source cell. The experiment the cell-type map of §12.5 generates, and the only one on this list that could not have been written without it. In staged human or model tissue, measure lipoprotein capture and reelin output at the unnetted source populations — somatostatin and Martinotti interneurons, entorhinal layer II reelin-immunoreactive cells — against the netted recipient population, parvalbumin interneurons, in the same sections. The map predicts that delivery fails at the source cells first, that their reelin output falls before the recipient's net is degraded, and that the recipient's decline follows the loss of its supply rather than preceding it. If the netted recipient's net degrades first, or if the two decline together, the transaction reading of §12.6 is wrong and the map is a coincidence of three columns. *Moves rows 15, 21, 22, 43.*

23. What Would Refute This

Stated as results rather than as topics, because a refutation condition that cannot be written as a result is not one.

The central proposal fails if:

The effective diffusivity of a twenty-nanometre tracer in vulnerable regions does not fall with age and disease stage, or falls no more than a small-molecule tracer's in the same tissue (experiment 1).

Neuronal cholesterol delivery measured in intact tissue is impaired to the same degree as receptor-mediated uptake measured in dissociated culture from the same donor (experiment 2).

Apolipoprotein isoform affinity for heparan sulfate does not predict delivery in a tissue or matrix-containing system, in the inverse direction, in the absence of tau (experiment 4).

Degrading the interstitial matrix in aged tissue leaves neuronal cholesterol delivery unchanged.

The protease and chronicity limb fails if:

Chronic β-adrenergic stimulation does not raise neuronal matrix metalloproteinase activity, or raising that activity does not reduce surface heparan sulfate and lipoprotein capture (experiment 5).

Matrix detachment does not in fact internalise caveolin-1 and other ordered-domain components in neurons — the clause borrowed from the theory being extended, and never independently verified in this cell type.

The net limb fails if:

Lipoprotein capture is equal at netted and unnetted neuronal surfaces, and unchanged by enzymatic net removal (experiment 7).

Tau pathology in cortical populations does not respect net status at all, in tissue, at early stage — which would confine Morawski's finding to the subcortical structures where it was made and remove the substitution proposed in Chapter 12.

The reelin limb fails if:

Compartment-resolved measurement shows N-sulfated domain availability at the neuronal surface rising, or unchanged, with disease stage (experiment 6).

Reelin and apolipoprotein E prove not to compete at ApoER2 (experiment 8), which would return Part Four to the status of an independent second route and remove Chapter 14 entirely.

The whole framework is displaced, rather than refuted, if:

Synapse elimination in the human disease proves to be driven principally by an added extracellular signal acting on competent machinery, as the complement arm of Chapter 19 suggests it might be. In that case everything here would remain true and cease to matter, which is a distinct fate from being wrong and is the one this paper considers most likely if it is not right.

24. Limitations

No new data. Every claim rests on published work, or on two unpublished hypothesis papers whose assemblies are credited as such. The original contributions are joins, and joins have a poor historical record when they are not tested promptly.

The central mechanism has never been measured. Nobody has measured the mobility of a lipoprotein particle in brain tissue at any age. The paper's core is an inference from geometry, from binding constants, and from a compositional change measured in homogenate.

The matrix chemistry rests on few studies. The human hippocampal glycosaminoglycan data are, for several of the specifics used here, a single study (Huynh et al., 2019). The heparan sulfate anatomy is largely one group's work from the early 1990s (Snow et al., 1990, 1994, 1995), using methods that have been superseded. If either is wrong, Part Two is wrong.

The protease driver is extrapolated across cell types. The catecholamine-to-metalloproteinase link is established in monocytes and tumour cells. Its application to neurons and to brain matrix is an extrapolation, graded *plausible*, and it is the weakest limb of Part Three.

The shedding brake is extrapolated across tissues. The sphingosine-1-phosphate restraint on syndecan shedding is endothelial biology. Its extension to the neuronal surface is an inference; only the derangement of the sphingolipid axis in disease is measured in brain.

One commitment currently runs against the evidence. Row 19 of the sign table. It is unresolved, three candidate resolutions are on offer, and none has been tested.

The paper adopts one theory's framing. It extends a raft-gated plasticity switch that has never been tested as a mechanism, and inherits whatever is wrong with it. The additions were chosen to be useful even if the switch fails, but the *organisation* of the argument — supply, transit, capture, assembly, resolution — is that theory's organisation, and a reader who rejects it will find the chapters oddly sequenced.

Grades are assigned by one reader, and no second assessor has applied the criteria of Chapter 4 independently.

The human evidence is thin, and thinnest where it is most needed. The transit compartment is the least accessible compartment in the brain. Its dimensions come from a small biophysical literature; its composition in disease from a handful of biochemical studies; and its

behaviour toward the specific particle at issue from nothing at all.

25. Conclusion

A theory of Alzheimer's disease proposed that memory formation contains a physically implemented decision point — a moment at which a neuron stops proposing candidate synapses and starts committing to them — and that the decision is thrown by the assembly of a cholesterol-ordered domain in the plasma membrane. Because the neuron cannot make the cholesterol, the decision is a supply check. Because the supply chain has one non-redundant step, the disease is a brain that never gets to make the decision, and the pathology is the debris of a compensation that could not succeed.

The theory's chain had three links and its problem has four. Between the astrocyte that releases the particle and the neuron that captures it there is a journey of about forty nanometres, made by an object about twenty nanometres across, through a hydrated polyanionic lattice whose sulfate content rises in the disease, whose sulfation pattern is rewritten in the disease, whose principal proteoglycan binds the peptide carried in the particle's core at picomolar affinity, and which binds the particle's own surface protein through an interface whose affinity ranks the apolipoprotein isoforms in the same order as their clinical risk.

That last correlation is the fact this paper exists to explain. It cannot be explained by a theory in which binding the sugar is how the particle is delivered, because on that theory the tightest binder should be the safest allele and it is the most dangerous one. It can be explained if the sugar exists in two beds — one at the neuronal surface, backed by a receptor and therefore productive, and one in the interstitium, backed by nothing and therefore a trap — and if the disease is a drift in the competition between them. On that reading the highest-affinity apolipoprotein is not the best deliverer but the most easily detained, and the woman who resisted an autosomal-dominant mutation for thirty years did so with a variant that binds the sugar worst of all.

Three consequences follow that the supply theory alone could not reach.

The theory acquires a **demolition arm**. Its own account of normal plasticity holds that matrix detachment internalises the membrane's organising proteins and that a matrix protease performs the detachment. That protease sheds the neuron's own capture apparatus into the space that competes for the particle — so one enzyme disassembles the domain, removes the delivery apron, and enlarges the trap. Its restraint is a sphingolipid that is measurably depleted in the disease, and its driver is a neuromodulatory system whose tau pathology precedes everything cortical by decades. Chronicity stops being an assumption and becomes an enzyme's activity.

The theory acquires an **observable population**. It has had to posit two kinds of neuron, distinguishable only by the outcome it wanted to predict, and that device is why no measurement of

cholesterol or of raft abundance in Alzheimer tissue can refute it. The condensed perineuronal net is visible before the outcome, is known to protect against tau, is built from the same polymers as the trap, and is digested by the same protease. Whether the pathology respects it is a question, not a rescue.

And the theory recovers its **second route**, in a form better than the one it discarded. Reelin brakes tau through a receptor on the delivery chain; its clasp requires N-sulfated heparan sulfate; it is secreted into the perineuronal net; and the two human beings known to have resisted an autosomal-dominant Alzheimer mutation both carry variants at that interface, pushing the same competition in the same direction from opposite sides. One receptor is shared by four programmes that do not cite each other, and one sulfated polymer at one surface carries at least three readings — one that stages the brake, one that admits the tau seed, one that holds the lipoprotein — so that the neuron's fate depends not on how much of the polymer it has but on which reading its polymer supports.

A fourth consequence arrived late and was not anticipated. Asking which neuron the argument is actually about produced a map, and the map has a structure: the cells that make reelin are unnetted, the cells that hold it are netted, and every population at the front of the disease — the somatostatin interneuron, entorhinal layer II, the locus coeruleus — sits on the unnetted side of that line, two of the three being reelin sources. The neurons that fail first are the ones that supply the brake to everybody else and keep none of it for themselves. The regional anatomy is consistent with this and is worth nothing, since net density and plasticity demand generate the same ranking from one premise; what carries weight is that netted cortical excitatory neurons carry low phospho-tau in human tissue, and that resilience turns out to involve a remodelling of the matrix rather than more of it — a finding that corrected one of this paper's own directional commitments from a claim about quantity to a claim about sulfation state.

None of that has been measured. Eleven of the twelve claims this paper adds are inferences, the central mechanism rests on a diffusion measurement nobody has made, and one of the paper's own directional commitments currently runs against the only human data that bear on it, which is recorded in the sign table rather than explained away. What can be said for the assembly is that it is specific enough to be wrong in ten named places, that the reagents for every one of the ten experiments already exist, and that they exist in laboratories — of glycobiology, of membrane biophysics, of neuropathology, of developmental neuroscience — which have spent thirty years working on the same forty nanometres without discovering that they were.

The disease this framework describes is not a poisoning and not, in the end, a shortage. It is a delivery problem in a space too narrow for the parcel, growing narrower and stickier with age, in an organ that has exactly one carrier and no second road.

References

Journal names are given in full. Digital object identifiers and PubMed identifiers are supplied where the source record carried them. Entries marked † are transcribed from the bibliography of one of the two cited hypothesis papers rather than checked independently against the primary record; they are used only for background or for results that are also supported elsewhere in this list, and no claim is graded above plausible *on their authority alone.*

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