The Stacked Phases

Four short papers on the course of adult cognitive disease

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Preface

These four papers describe one disease, one step at a time.

They are short on purpose. The corpus they are drawn from runs to two hundred volumes, several of them book-length, and that is the right form for an argument that has to be checkable line by line. It is the wrong form for someone who wants to know what the model says. Each paper here summarises a stretch of that larger work and names, at every point, the primary study and the longer volume a reader can go and check.

The model measures the disease by what is lost, where and when, rather than by what accumulates. Read that way, the published record settles into an order: connections go first in a few small nuclei of the brainstem and basal forebrain, then in the hippocampus, then in the cortex. The three stretches are called Phase I, Phase II and Phase III, and they begin, roughly, in the third decade of life, the sixth and the eighth.

One term is fixed throughout. The model measures function in **connections**, not in cells. A neuron that has lost its connections but is still alive is silenced rather than dead, and the difference matters, because a silenced neuron may in principle be recovered. Synapse loss is also the structural change that tracks cognitive decline most closely — better than plaque burden, better than tangle burden — which is why it is the unit used across the corpus.

The phases were first drawn as a relay, each handing over to the next. They are now drawn as stacked steps, and the change is not cosmetic. No step ends when the next begins. Phase I continues underneath Phase II; both continue underneath Phase III. A person of seventy-five is carrying all three at once, and the brainstem is still failing while the cortex fails. That is a more faithful picture of a disease that runs for fifty years, and the papers are written to it.

Parts One to Three take one step each. Part Four is different: it describes a single piece of cellular machinery that has a station in all three, and is therefore not a fourth step but the axis the other three stand on. It was written last and is best read last, though each part stands alone.

Claims are graded where they are made, using four grades. **Established** means replicated in human material by more than one group. **Probable** means good evidence with a gap named. **Inference** means a conclusion

drawn by us, from evidence gathered for another purpose. **Speculative** means coherent and untested. Every part ends with a ledger of its own claims and the results that would overturn them, and the claims that are ours rather than the literature's are marked as ours in every one.

That discipline is not decoration. Almost nothing in this account can be measured directly in a living person, and in a field whose primary objects cannot be seen, declaring exactly what you are standing on is what substitutes for observation. A companion paper, *The Dial and the Decade*, asks what could in principle be checked in a living person and what could not; readers who want to know how much weight these four papers can bear should read it alongside them.

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Part One

What the Brainstem Stops Sending

Brainstem and basal forebrain neurons in early Alzheimer's disease: which functions fade with age, and what each one withdraws from the forebrain

The base of the stack. Five small nuclei that each make one chemical messenger and send it almost everywhere, one forebrain neighbour that depends on them, and the cell that survives because it needs nothing they stop supplying.

Abstract

The first phase of the disease, in the model this site has built from the published record, begins in a handful of small nuclei at the base of the brain. Each of them makes one chemical messenger and sends it almost everywhere: noradrenaline from the locus coeruleus, serotonin from the raphe, acetylcholine from the nucleus basalis, dopamine from the ventral tegmental area. None of them carries information in the ordinary sense. They set the conditions under which the rest of the brain works: how awake it is, how well it sleeps, what it attends to, which memories it keeps, how much effort a goal is worth, and how fast it disposes of its own waste.

This paper is the first of a short series that explains the model one phase at a time. It asks two plain questions of each population. What does it supply? And what about that supply fades as a person ages towards the disease? The answers are drawn from the corpus's longer papers on each nucleus, and the primary studies those papers rest on.

Three findings organise the account. First, these neurons fail early and fail in function long before they die. Abnormal tau is already present in the locus coeruleus in the second and third decades of life, yet its neurons are not lost in number until the disease is half over; the axons go first, and the cell bodies decades later. Second, the supplies they withdraw are not only cognitive. Two of them are brakes on amyloid itself: serotonin tonically suppresses its production, and somatostatin, made by a forebrain interneuron that depends on cholinergic drive, licenses its disposal through the enzyme neprilysin. Two more, noradrenaline and acetylcholine, hold the brain's immune cells at rest. Third, the losses are selective in a way that teaches something. A cholinergic nucleus in the brainstem, the pedunculopontine, accumulates tangles and keeps its neurons, because it does not depend on a growth factor that the disease cuts off; what dies is not the cholinergic cell but the dependent one.

The model now draws its three phases as stacked steps rather than a sequence of hand-overs. This paper describes the base of that stack. It is a step that does not end when the next begins: the brainstem keeps failing underneath everything that follows. The paper closes with a summary table, a graded ledger of its claims, and the results that would undo them.

How to read this paper

This is a short paper by design. Each section below summarises a longer volume in the corpus, and each of those volumes carries its own full argument, evidence and ledger. Where this paper states a number it gives the primary source; where it states an interpretation it names the corpus paper that makes it, so the reader can go and check.

Claims are graded where they are made, using the four grades the corpus uses throughout. **Established** means replicated in human material by more than one group. **Probable** means good evidence with a gap, such as a single cohort or an animal result not yet confirmed in people. **Inference** means a conclusion drawn here, or in the corpus, from evidence gathered for another purpose. **Speculative** means coherent and untested.

One term needs fixing at the start. The model measures function by **connections**, not by cells. A neuron that has lost its connections but is still alive is silenced rather than dead, and the difference matters, because a silenced neuron may in principle be recovered. Synapse loss is the structural change that tracks cognitive decline most closely, which is why it is the unit used across the corpus.

1. The Base of the Stack

For most of its history the disease has been described by what accumulates in the cortex: plaques of amyloid between cells, tangles of tau inside them. The model this site has assembled describes it instead by what is lost, where and when. Read that way, the published record settles into an order. Connections are lost first in a few small nuclei of the brainstem and basal forebrain, then in the hippocampus, then in the cortex. The corpus calls these three stretches Phase I, Phase II and Phase III.

The phases were first drawn as a relay, each handing over to the next. They are now drawn as stacked steps. Phase I begins in the third decade of life. Phase II begins in the sixth, on top of it. Phase III begins in the eighth, on top of both. No step ends when the next begins. A person of seventy-five is

carrying all three, and the brainstem is still failing underneath the hippocampus and the cortex. The steps are joined by two specified transitions, which the corpus calls bridges: the first is the withdrawal of noradrenaline from the brain's immune cells, the second the digestion of the protective net around inhibitory neurons.

This paper is about the bottom step. It covers five populations of neurons and one forebrain neighbour:

- the **locus coeruleus**, which makes noradrenaline;
- the **raphe nuclei**, which make serotonin;
- the **nucleus basalis of Meynert**, which makes acetylcholine for the cortex;
- the **ventral tegmental area**, which makes dopamine for memory and motivation;
- the **pedunculopontine nucleus**, a brainstem cholinergic group that turns out to be the control case;
- and the **somatostatin interneuron**, a forebrain cell that is not part of the brainstem but is among the first casualties of what the brainstem stops sending.

The nucleus basalis sits in the basal forebrain rather than the brainstem proper. It is included because the model groups it with the brainstem nuclei: it shares their architecture, their early failure and their kind of job.

2. What the Population Shares

The neurons of the first step look alike in four ways, and the likeness is the beginning of an explanation for why they go first.

An enormous axon. Each of these neurons sends a thin, branching, mostly unmyelinated axon across very large territories. A single coerulean neuron accompanies some twenty metres of cerebral capillary (Matchett and colleagues, 2021); a dorsal raphe neuron's reconstructed axon in the rat runs to nearly nineteen centimetres (*The Unpicked Seam*). Maintaining a structure of that size costs energy continuously, and supplying its far end depends on

transport along the whole of its length.

A pacemaker that does not rest. These neurons fire on their own, tonically, through the waking day. The firing imposes a continuous load of calcium and a continuous demand for energy. The locus coeruleus fires at about two spikes per second in waking, below one in slow-wave sleep, and almost not at all in REM sleep (Aston-Jones and Bloom, 1981), so sleep is the only time the load is lifted.

No protective net. Many neurons elsewhere in the brain are wrapped in a lattice of extracellular matrix, the perineuronal net. In subcortical regions of the Alzheimer brain, neurons that carry such a net are protected against tau pathology (Morawski and colleagues, 2010). The coerulean and raphe neurons are constitutionally without one.

A single, broadcast job. Each population supplies one modulator to the whole forebrain. Its loss produces no paralysis, no focal sign and no single symptom that sends a patient to a neurologist. It produces changes that are easily mistaken for ordinary ageing: worse sleep, less drive, poorer attention, a little more forgetting.

Two of these four properties are about energy and one is about protection. That is why the corpus calls Phase I *Bioenergetic Ignition*: the first failure is a failure to sustain an expensive structure, and it is metabolic before it is anything else (*The Shape of the Disease*). The grade on that interpretation is **inference**. The neuropathology it explains is **established**.

3. The Locus Coeruleus: Noradrenaline

What it supplies

The locus coeruleus is a nucleus of a few tens of thousands of neurons on each side of the pons, no larger than a grain of rice. It is the brain's main source of noradrenaline, and it supplies it to the entire cortex, the hippocampus, the thalamus and the cerebellum. Four of its supplies matter here.

- **Waking and attention.** Noradrenaline sets the depth of wakefulness and the gain of attention. The nucleus's firing anticipates shifts in the

sleep–wake cycle (Aston-Jones and Bloom, 1981).

- **The architecture of sleep.** The nucleus has to oscillate in one sleep state and fall silent in another. During non-REM sleep, slow infra-slow oscillations of noradrenaline drive the vasomotion that pumps the brain's glymphatic clearance; abolishing them abolishes the clearance (Hauglund and colleagues, 2025). During REM sleep it falls silent.
- **A brake on the brain's immune cells.** Noradrenaline holds microglia in their resting state. When the locus coeruleus is lesioned in amyloid-bearing mice, microglia lose their ability to clear amyloid and inflammation rises (Heneka and colleagues, 2010).
- **The consolidation of memory.** Coerulean axons release dopamine as well as noradrenaline in the hippocampus, and that release helps decide which everyday memories persist (Takeuchi and colleagues, 2016).

What fades, and when

The locus coeruleus is the first place in the human brain to accumulate abnormal tau. Pretangle material is demonstrable there from the second and third decades of life (Braak and Del Tredici, 2011), across a survey of 2,332 brains spanning ages one to one hundred (Braak and colleagues, 2011). In brains with no cortical tangles at all, 7.9 per cent of coerulean neurons already carry hyperphosphorylated tau inclusions (Ehrenberg and colleagues, 2017). **Established.**

The neurons themselves are not lost for a long time. Stereological counts across the whole staging series find neuronal number preserved through Braak stages 0 to II and falling only from stage III (Theofilas and colleagues, 2017). The interval between the appearance of tau and the death of the neuron has been put at about a quarter of a century (Matchett and colleagues, 2021). By amnestic mild cognitive impairment about thirty per cent of the neurons are gone, with a further quarter lost by mild-to-moderate dementia (Kelly and colleagues, 2017), and the end-stage loss is the largest of any subcortical nucleus (Zarow and colleagues, 2003). **Established.**

What fails in the interval is the axon. In an amyloid knock-in mouse, noradrenergic fibres in a coerulean target field are 14 per cent lost at two months, 27 per cent at three and 33 per cent at six, while the number of coerulean cell bodies is indistinguishable from normal at twelve months. In human tissue the matching fibre degeneration is already present at Braak

stages 1 to 2 (Meyer and colleagues, 2025). The axon is not lost because the cell died. It is removed while the cell is alive: microglia in the target field recognise the coerulean axons and engulf them, and reducing that engulfment genetically preserves both the axons and the function they serve (Meyer and colleagues, 2025). *The Coerulean Clock* argues that what marks the axon for removal is the neuron's own overactive firing, calls this the axon clock, and holds that it, not the death of the cell body, is the clinically relevant one. **Probable**: the mouse result is strong and the human fibre data agree; the firing mechanism is the corpus's **inference**.

The corpus offers a chemical reason why this nucleus tangles first. The locus coeruleus expresses monoamine oxidase A, and the oxidation of its own transmitter by that enzyme produces a reactive aldehyde, DOPEGAL. DOPEGAL activates an enzyme, asparaginyl endopeptidase, that cuts tau into a fragment prone to aggregate (Kang and colleagues, 2020; Zhang and colleagues, 2014). APOE4, the strongest genetic risk factor for the disease, inhibits the transporter that packages noradrenaline into vesicles and so leaves more of it in the cytoplasm to be oxidised (Kang and colleagues, 2021). *The First Ember* sets out the chain. **Probable** in its parts, measured in model systems; the joined chain is **inference**.

What the loss costs

The first clinical sign of a failing locus coeruleus is not forgetting. It is broken sleep. In cognitively unimpaired older adults, worse structural integrity of the nucleus already tracks more frequent awakenings at night (Van Egroo and colleagues, 2021). The model is careful about direction: it does not argue that poor sleep causes the disease. The coerulean tau comes first, and disturbed sleep is among the earliest things it produces. But once present, disturbed sleep becomes a lever on what follows, because the nucleus is also the pump for night-time clearance.

The loss of the brake on microglia is the first bridge of the model, the point at which Phase I sets off Phase II. The same ascending axons that withdraw noradrenaline from the hippocampus are the route by which, on the model's reading, tau is carried there. That joint is graded **inference** throughout the corpus and is the part it most wants other laboratories to test (*The Coerulean Interface*; *The Shape of the Disease*).

4. The Raphe Nuclei: Serotonin

What it supplies

The raphe nuclei lie along the midline seam of the brainstem and contain nearly all of the brain's serotonin. The rostral group, and the dorsal raphe nucleus above all, supplies the forebrain. *The Unpicked Seam* lists seven things that supply does; three matter most here.

- **A brake on amyloid production.** This is the least expected and the most consequential. Serotonin signalling lowers the level of amyloid-β between cells in mice, and people exposed to selective serotonin reuptake inhibitors carry less amyloid on imaging (Cirrito and colleagues, 2011). In healthy volunteers, a single course of citalopram slowed the production of amyloid-β in cerebrospinal fluid by 37 per cent (Sheline and colleagues, 2014). The route runs through the 5-HT4 receptor, which binds and traffics ADAM10, the enzyme that cuts the amyloid precursor protein in the harmless direction (Cochet and colleagues, 2013).
- **Sleep and the night.** Serotonin consolidates sleep, and it is the chemical substrate for melatonin.
- **Mood, but less than assumed.** The serotonergic loss in Alzheimer's disease is not the cause of its depression. Comparing four groups at post-mortem, Alzheimer brains were markedly depleted of dorsal raphe serotonergic neurons, brains from primary late-life depression were not, and Alzheimer patients with and without depression did not differ (Hendricksen and colleagues, 2004).

What fades, and when

The raphe fails early, after the locus coeruleus and before the cortex. Braak's survey places abnormal tau in the serotonergic raphe at pretangle stage c, before any cortical involvement (Braak and colleagues, 2011), and the dorsal raphe shows phospho-tau change before the transentorhinal region does (Grinberg and colleagues, 2009). At Braak stage 0, 2.6 per cent of dorsal raphe neurons already carry tau inclusions (Ehrenberg and colleagues, 2017). **Established.**

It fails severely. A meta-analysis of sixty-seven studies ranks raphe neuron loss at an effect size of 1.79 standard deviations, behind the nucleus basalis and the locus coeruleus but well ahead of the substantia nigra (Lyness and colleagues, 2003); direct counts put the loss near forty per cent of the nucleus (Aletrino and colleagues, 1992). **Established.**

The raphe teaches something by what it lacks. It does not carry the coeruleus's chemical liability: its neurons express monoamine oxidase B rather than A, so they do not make DOPEGAL in the same way. Yet they tangle nearly as early. *The Unpicked Seam* draws the conclusion that the shared cause of early failure is architectural rather than chemical: the long arbor, the pacemaker and the missing net. **Inference**, and a useful one, because it says where to look.

What the loss costs

A nucleus that begins to fail in the third decade is withdrawing a tonic brake on amyloid production across the whole cortex, decades before the first plaque is counted (*The Unpicked Seam*; *The Fifty-Year Prescription*). That is a Phase I event with a Phase III consequence, and it is the clearest example in this paper of how the bottom step feeds the top one.

The trial record is close to uniformly negative, and the corpus reports it in full. The 5-HT6 antagonist idalopirdine failed three trials enrolling 2,525 patients (Atri and colleagues, 2018). Citalopram reduced agitation in Alzheimer's disease while worsening cognition and prolonging the cardiac QT interval (Porsteinsson and colleagues, 2014). The corpus's reading is that the target was right and the timing was wrong: the evidence describes a lesion that matters in midlife, and every trial enrolled people decades later. That reading is **inference**, and it is testable.

5. The Nucleus Basalis: Acetylcholine

What it supplies

The nucleus basalis of Meynert supplies acetylcholine to the cortex. Its loss gave the field its first mechanism and its first drug class (Whitehouse and colleagues, 1981, 1982). *The Gate and the Ransom* argues that the field misread what the nucleus does, and what it loses first.

- **Attention, not storage.** Acetylcholine in the cortex rises in sub-second bursts when a cue is detected, and the timing of those bursts predicts whether the cue is noticed (Parikh and colleagues, 2007). The nucleus is a gate on attention and plasticity rather than a memory store.
- **The difference between sleep states.** Cholinergic neurons fire with the theta rhythm in waking and REM sleep and almost stop in slow-wave sleep. That silence is needed: raising acetylcholine during slow-wave sleep in healthy people abolishes the consolidation of declarative memory (Gais and Born, 2004). The signal is a contrast, not a level.
- **A brake on inflammation.** Loss of basal forebrain volume in cognitively normal adults with abnormal amyloid and tau tracks rising markers of microglial activation, TREM2 and complement C3 (Schmitz and colleagues, 2020). Like the locus coeruleus, the nucleus may hold the brain's immune cells at rest. **Probable.**

What fades, and when

The nucleus does not die first. It lets go of its growth factor first. Counting neurons that display TrkA, the receptor for nerve growth factor, Mufson and colleagues found about 197,000 in the cognitively normal, 106,000 in mild cognitive impairment and 87,000 in Alzheimer's disease: a loss of nearly half, complete before dementia (Mufson and colleagues, 2000). Counting the same nucleus for the enzyme that makes acetylcholine found about 210,000 cholinergic neurons per hemisphere in the normal elderly and no significant loss in mild cognitive impairment or early disease (Gilmor and colleagues, 1999). Cortical choline acetyltransferase activity is unchanged or even raised in mild cognitive impairment (DeKosky and colleagues, 2002). Read together, with the caution that they are different series, the numbers describe a cell that is still there and still cholinergic, and has stopped displaying the receptor it cannot live without. **Probable.**

The reason is that the nucleus holds its survival on loan. It depends on nerve growth factor supplied by the cortex it projects to, and that factor is

released as a precursor that must be matured outside the cell by a protease cascade and is degraded by MMP-9 (Bruno and Cuello, 2006). In mild cognitive impairment the precursor rises (Peng and colleagues, 2004), MMP-9 activity rises (Bruno and colleagues, 2009), and TrkA falls while the co-receptor p75 is unchanged (Counts and colleagues, 2004). Nerve growth factor protein is increased throughout the Alzheimer brain while falling in the nucleus basalis itself (Scott and colleagues, 1995). *The Gate and the Ransom* reads this as a failure of delivery: the factor is made, but not matured and not received. **Probable** for each measurement; the reading is **inference**.

In living people, basal forebrain volume falls early and predicts later degeneration of the entorhinal cortex, not the other way round (Schmitz and Spreng, 2016; Fernández-Cabello and colleagues, 2020). **Probable.**

What the loss costs

The cost is attention, the depth of the difference between sleep states, and a brake on inflammation. The therapeutic lesson is sharp. The disease flattens a rhythm; a cholinesterase inhibitor raises an average. The drugs work, and the effect is small (Birks, 2006), which the corpus argues is the predictable result of treating a loss of contrast with a steady supply. **Inference.**

There is a second cost, carried by a different cell, and it is taken up in Section 8.

One further point about this cell belongs to the fourth paper in this series rather than to this one. Because its axon is so long, the growth factor it depends on is picked up at the far end and carried home on a sorting compartment called an endosome, so a fault in that compartment cuts the line between the terminal and the nucleus; *The Long Way Back* sets out the evidence that this is where the lesion sits.

6. The Ventral Tegmental Area: Dopamine

What it supplies

The ventral tegmental area, in the floor of the midbrain, is the source of the brain's mesolimbic and mesocortical dopamine. *The Price and the Permission* names its two jobs in its title.

- **The price.** Dopamine sets how much effort a goal is worth. Its withdrawal is the best-characterised chemistry of lost motivation.
- **The permission.** Dopamine does not carry the content of a memory, but it decides whether the memory is kept. A dopaminergic signal in the hippocampus, arriving in the hours around an event, converts a decaying trace into a lasting one (Takeuchi and colleagues, 2016).

What fades, and when

In amyloid-bearing mice, the dopaminergic neurons of this nucleus begin dying at three months of age, before any plaque has formed, and the loss is confined to the mesolimbic neurons; the neighbouring substantia nigra, which fails first in Parkinson's disease, is untouched (Nobili and colleagues, 2017). Every classical theory of dopaminergic vulnerability predicts the opposite ranking, which is itself informative. **Probable**, in models.

The human evidence is thinner, and the corpus says so precisely. The nucleus was tied to dementia in a 1988 case series of six brains (Torack and Morris, 1988). Midbrain volume and connectivity are reduced in people with mild cognitive impairment and track the cognitive signature of the disease (De Marco and Venneri, 2018). But there is no published unbiased count of dopaminergic neurons in the human ventral tegmental area in Alzheimer's disease. The nucleus has an experimental literature, an imaging literature, and no autopsy census. **Inference** for the human claim.

What the loss costs

The clinical signature is apathy. In cognitively normal people apathy carries roughly three times the odds of progressing to mild cognitive impairment and twice the odds of progressing to dementia, and in mild cognitive impairment it shortens the time to conversion (*The Price and the Permission*, which reviews the meta-analyses). Apathy is the second of the three early complaints most often written off as ageing. Methylphenidate improves it in patients (Mintzer and colleagues, 2021), which fits a dopaminergic lesion without proving one. **Established** for the prognostic association; **inference** for the attribution to this nucleus.

7. The Pedunculopontine Nucleus: The Cell That Survives

Not every nucleus at the base of the stack dies, and the one that does not is as informative as the ones that do.

The pedunculopontine nucleus is a cholinergic group in the upper brainstem, a few millimetres from the locus coeruleus. With its neighbour, the laterodorsal tegmental nucleus, it supplies most of the acetylcholine that reaches the thalamus, and it is part of the circuitry of REM sleep. It has every risk factor the field attributes to the cells this disease destroys: it is cholinergic, large, diffusely projecting and metabolically expensive, and it accumulates Alzheimer tau. And it keeps its neurons. Woolf and colleagues found tangles but no loss of cell bodies (Woolf and colleagues, 1989); design-based stereology found neuron number indistinguishable from control (Eser and colleagues, 2018). The one dissenting study measured a reduction of large neurons only (Jellinger, 1988), which *The Standing Relay* reads as shrinkage rather than death. **Established.**

The reason is a molecular difference noticed in 1989 and not read against this disease until the corpus did so. Nucleus basalis neurons carry high levels of the nerve growth factor receptor; pedunculopontine neurons do not (Mesulam and colleagues, 1989). The pedunculopontine neuron is not dependent on the supply the disease cuts off. What the disease kills, on this

reading, is not the cholinergic cell but the dependent cell. **Inference**, from two established findings.

The nucleus does not die, but its function may still fade. Tau in the pedunculopontine nucleus tracks a lengthened delay to REM sleep and a smaller share of it, and in the Framingham cohort a smaller share of REM sleep predicted dementia (Pase and colleagues, 2017). Whether the channel from this nucleus to the thalamus still carries its traffic has never been measured (*The Standing Relay*).

8. The First Neighbour: Somatostatin and Neprilysin

The somatostatin interneuron is not a brainstem cell. It lives in the hippocampus and cortex, and the corpus files it with Phase II. It is included here because it is among the first cells to suffer from what the brainstem stops sending, and because its loss removes the second of the two amyloid brakes this paper has to describe.

What it supplies

The oldest finding in this disease that is not about acetylcholine is that somatostatin is depleted from the Alzheimer cortex (Davies, Katzman and Terry, 1980). The peptide does two things that matter here.

- **It licenses the disposal of amyloid.** Somatostatin raises the activity of neprilysin, the brain's main amyloid-β-degrading enzyme. Mice lacking somatostatin have altered hippocampal neprilysin and more of the aggregation-prone form of amyloid-β (Saito and colleagues, 2005). The pathway runs from the somatostatin receptor through α-endosulfine to ATP-sensitive potassium channels, and acting on those channels activates neprilysin, reduces amyloid deposition and rescues memory in mice (Watamura and colleagues, 2022). The SST1 and SST4 receptors carry the effect between them; deleting both reduces neprilysin, worsens amyloid pathology, and an agonist at both reverses it (Nilsson and colleagues, 2026). **Established** in animals, by loss and by gain of function.

- **It gates the input to memory.** The somatostatin interneurons of the hippocampus control how the entorhinal cortex's input reaches CA1 (Leão and colleagues, 2012). *The Homeward Vector* argues that this gate is what lets an internal map be reset by landmarks, and that losing it is why people with early disease get lost. That is **inference**.

What fades, and when

Somatostatin interneurons are lost early, in cognitively still-normal stages, in the largest single-cell atlas of the disease (Gabitto and colleagues, 2024). In an amyloid model, the interneurons that gate the CA1 input fail at both ends, and the mechanism identified was reduced cholinergic drive (Schmid and colleagues, 2016). That is the link to the bottom step: the acetylcholine the nucleus basalis stops sending is part of what keeps this cell working. **Probable.**

What the loss costs

The Unnetted Governor states the consequence as a loop. Somatostatin licenses the disposal of amyloid; amyloid injures the somatostatin cell; fewer somatostatin cells mean less disposal, and therefore more amyloid. The loop amplifies itself, and it starts early. The corpus adds a claim of address: the neprilysin that depends on somatostatin receptors is concentrated in the very layer of the hippocampus where the entorhinal input arrives and where the somatostatin cell's axon ends (Nilsson and colleagues, 2026). The two facts are measured; the joining is **inference**, and the volume names it as its most important untested claim.

Getting lost is the third of the early complaints. With broken sleep from the locus coeruleus and apathy from the ventral tegmental area, it completes the set the model predicts should come before forgetting.

9. What the Step Adds Up To

Table 1 gathers the populations in one place.

Table 1. The bottom step, population by population. Timing for each population is given in its section; dates are nominal and individuals differ.

Population	Messenger	What it supplies	What fades first	Corpus paper
Locus coeruleus	Noradrenaline	Waking, attention, night-time clearance, a brake on microglia	The axon, decades before the cell body	*The Coerulean Clock*
Raphe nuclei	Serotonin	A brake on amyloid production, sleep, melatonin substrate	Tau after the coeruleus, before cortex; ~40% loss	*The Unpicked Seam*
Nucleus basalis	Acetylcholine	Attention, the contrast between sleep states, a brake on inflammation	The growth-factor receptor, before the cell	*The Gate and the Ransom*
Ventral tegmental area	Dopamine	Effort and motivation; the permission to keep a memory	Mesolimbic neurons, in mice before plaques	*The Price and the Permission*
Pedunculopontine	Acetylcholine	Thalamic acetylcholine, REM sleep	Function, not number: tangles without loss	*The Standing Relay*
Somatostatin interneuron	Somatostatin	Licence for neprilysin to clear amyloid; the input gate to memory	Early loss; driven partly by lost cholinergic input	*The Unnetted Governor*

Read across, four things stand out.

Function goes before the cell. The coerulean axon is removed while its cell body lives. The nucleus basalis gives up its receptor before it gives up its transmitter, and its transmitter before it dies. The pedunculopontine nucleus tangles and never dies at all. In each case there is a long interval in which the neuron is silenced rather than lost. That interval is where the model locates its hope, because a silenced neuron may be recoverable and a dead one is not.

The step does not end. None of these populations recovers when Phase II begins. Their loss of connections keeps rising through the sixth, seventh and eighth decades. A therapy aimed at this step is therefore not only a therapy for the young: the step is still active in the old, even if the window for some treatments has closed.

The step loosens two brakes on amyloid and two on inflammation. Serotonin brakes amyloid production; somatostatin licenses its disposal. Noradrenaline and acetylcholine hold microglia at rest. All four supplies fall in the first step. That is how the bottom of the stack sets up the top: the amyloid that defines the disease's later decades, and the inflammation that defines its middle, are both made easier by what the brainstem has already stopped sending.

The first symptoms are not memory symptoms. Broken sleep, apathy and getting lost are the three complaints the model predicts first, and each maps to a population in this paper. They are the complaints most often written off as ordinary ageing.

10. Graded Ledger

Table 2. Each claim in this paper, with its grade and the sources it rests on.

#	Claim	Grade	Basis
1	Abnormal tau appears first in the locus coeruleus, from the 2nd–3rd decade	Established	Braak and Del Tredici 2011; Braak et al. 2011; Ehrenberg et al. 2017
2	Coerulean neuron number is preserved to Braak II and falls from III	Established	Theofilas et al. 2017; Kelly et al. 2017
3	Coerulean axons are lost while the cell body lives	Probable	Meyer et al. 2025 (mouse; human fibre data concordant)
4	The shared cause of early brainstem failure is architectural (arbor, pacemaker, no net)	Inference	*The Unpicked Seam*; Morawski et al. 2010; Matchett et al. 2021

#	Claim	Grade	Basis
5	DOPEGAL–AEP chemistry contributes to coerulean tau	Probable (parts); inference (chain)	Kang et al. 2020, 2021; Zhang et al. 2014
6	Noradrenaline holds microglia at rest; its loss is the first bridge	Probable (mouse); inference (bridge)	Heneka et al. 2010; *The Coerulean Interface*
7	Raphe loss is early, severe and not the cause of depression	Established	Lyness et al. 2003; Grinberg et al. 2009; Hendricksen et al. 2004
8	Serotonin tonically suppresses amyloid production	Probable	Cirrito et al. 2011; Sheline et al. 2014; Cochet et al. 2013
9	Nucleus basalis neurons lose TrkA before they lose their transmitter or die	Probable	Mufson et al. 2000; Gilmor et al. 1999; DeKosky et al. 2002
10	The nucleus basalis lesion is a failure of growth-factor delivery	Inference	Bruno and Cuello 2006; Peng et al. 2004; Scott et al. 1995
11	Ventral tegmental dopamine neurons are lost before plaques, sparing the nigra	Probable (mouse only)	Nobili et al. 2017
12	The pedunculopontine nucleus tangles without losing neurons	Established	Woolf et al. 1989; Eser et al. 2018
13	Growth-factor dependence, not cholinergic phenotype, marks the cells that die	Inference	Mesulam et al. 1989; *The Standing Relay*
14	Somatostatin licenses neprilysin-mediated amyloid disposal	Established (animal)	Saito et al. 2005; Watamura et al. 2022; Nilsson et al. 2026
15	Somatostatin interneuron failure is driven in part by lost cholinergic input	Probable	Schmid et al. 2016
16	The first step continues to worsen after the second begins	Inference	Site connection-loss curves; *The Shape of the Disease*

11. What Would Change This Account

The account above would need rewriting if any of the following were found.

- A human stereological count showing ventral tegmental dopamine neurons intact in Alzheimer's disease. The nucleus would then leave the first step.
- Evidence that coerulean axon loss in people follows, rather than precedes, the loss of the cell body. The axon clock would fail, and with it the case for treating the axon rather than the cell.
- A brainstem nucleus that is growth-factor dependent and survives, or one that is independent and dies. Either would break the reading offered for the pedunculopontine contrast.
- A properly timed trial, in midlife, of serotonergic or somatostatin-receptor treatment that changes neither amyloid production nor disposal. The amyloid brakes would then be smaller than claimed.
- Longitudinal evidence that brainstem connection loss stops or reverses once hippocampal involvement begins. The step model would revert to a relay.

The next paper in this series takes up the second step: the hippocampus, and the brain's immune cells as they lose their resting state.

Corpus papers drawn on

The Shape of the Disease (Phase overview). *The Coerulean Clock*: The Timing of Locus Coeruleus Loss in Alzheimer's Disease: Axon Before Soma. *The First Ember*: Tau Kindling in the Locus Coeruleus: Asparaginyl Endopeptidase, DOPEGAL and PP2A. *The Coerulean Interface*: The Brainstem Origin of Alzheimer's. *The Unpicked Seam*: The Raphe Nuclei in Alzheimer's Disease as a Subject in Their Own Right. *The Fifty-Year Prescription*. *The Gate and the Ransom*: The Cholinergic Nucleus Basalis in Alzheimer's Disease: Why the Receptor Is Surrendered. *The Price and the Permission*: The Ventral Tegmental Area in Alzheimer's Disease: Vulnerability, Trajectory, Disinhibition. *The*

Standing Relay: The Pedunculopontine Nucleus in Alzheimer's Disease: Tangle Burden Without Neuron Loss. *The Unnetted Governor*: The Somatostatin Interneuron in Alzheimer's Disease. *The Homeward Vector*: Wandering in Alzheimer's Disease. All are available in full at AdultCognitiveDisease.com.

References

Aletrino MA, Vogels OJ, Van Domburg PH, Ten Donkelaar HJ. Cell loss in the nucleus raphes dorsalis in Alzheimer's disease. *Neurobiology of Aging*. 1992;13(4):461-8. PMID 1508296. doi:10.1016/0197-4580(92)90073-7

Aston-Jones G, Bloom FE. Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *The Journal of Neuroscience*. 1981;1(8):876-86. PMID 7346592. doi:10.1523/JNEUROSCI.01-08-00876.1981

Atri A, Frölich L, Ballard C, Tariot PN, Molinuevo JL, Boneva N, et al. Effect of Idalopirdine as Adjunct to Cholinesterase Inhibitors on Change in Cognition in Patients With Alzheimer Disease: Three Randomized Clinical Trials. *JAMA*. 2018;319(2):130-142. PMID 29318278. doi:10.1001/jama.2017.20373

Birks J. Cholinesterase inhibitors for Alzheimer's disease. *Cochrane Database of Systematic Reviews*. 2006;2006(1):CD005593. PMID 16437532. doi:10.1002/14651858.CD005593

Braak H, Del Tredici K. The pathological process underlying Alzheimer's disease in individuals under thirty. *Acta Neuropathologica*. 2011;121(2):171-81. PMID 21170538. doi:10.1007/s00401-010-0789-4

Braak H, Thal DR, Ghebremedhin E, Del Tredici K. Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *Journal of Neuropathology and Experimental Neurology*. 2011;70(11):960-9. PMID 22002422. doi:10.1097/NEN.0b013e318232a379

Bruno MA, Cuello AC. Activity-dependent release of precursor nerve growth factor, conversion to mature nerve growth factor, and its degradation by a protease cascade. *Proceedings of the National Academy of Sciences USA*. 2006;103(17):6735-40. PMID 16618925. doi:10.1073/pnas.0510645103

Bruno MA, Mufson EJ, Wuu J, Cuello AC. Increased matrix metalloproteinase 9 activity in mild cognitive impairment. *Journal of Neuropathology and Experimental Neurology*. 2009;68(12):1309-18. PMID 19915485. doi:10.1097/NEN.0b013e3181c22569

Cirrito JR, Disabato BM, Restivo JL, Verges DK, Goebel WD, Sathyan A, et al. Serotonin signaling is associated with lower amyloid-β levels and plaques in transgenic mice and humans. *Proceedings of the National Academy of Sciences USA*. 2011;108(36):14968-73. PMID 21873225. doi:10.1073/pnas.1107411108

Cochet M, Donneger R, Cassier E, Gaven F, Lichtenthaler SF, Marin P, et al. 5-HT4 receptors constitutively promote the non-amyloidogenic pathway of APP cleavage and interact with ADAM10. *ACS Chemical Neuroscience*. 2013;4(1):130-40. PMID 23336052. doi:10.1021/cn300095t

Counts SE, Nadeem M, Wuu J, Ginsberg SD, Saragovi HU, Mufson EJ. Reduction of cortical TrkA but not p75(NTR) protein in early-stage Alzheimer's disease. *Annals of Neurology*.

2004;56(4):520-31. PMID 15455399. doi:10.1002/ana.20233

Davies P, Katzman R, Terry RD. Reduced somatostatin-like immunoreactivity in cerebral cortex from cases of Alzheimer disease and Alzheimer senile dementa. *Nature*. 1980;288(5788):279-80. PMID 6107862. doi:10.1038/288279a0

De Marco M, Venneri A. Volume and Connectivity of the Ventral Tegmental Area are Linked to Neurocognitive Signatures of Alzheimer's Disease in Humans. *Journal of Alzheimer's Disease : JAD*. 2018;63(1):167-180. PMID 29578486. doi:10.3233/JAD-171018

DeKosky ST, Ikonomovic MD, Styren SD, Beckett L, Wisniewski S, Bennett DA, et al. Upregulation of choline acetyltransferase activity in hippocampus and frontal cortex of elderly subjects with mild cognitive impairment. *Annals of Neurology*. 2002;51(2):145-55. PMID 11835370. doi:10.1002/ana.10069

Ehrenberg AJ, Nguy AK, Theofilas P, Dunlop S, Suemoto CK, Di Lorenzo Alho AT, et al. Quantifying the accretion of hyperphosphorylated tau in the locus coeruleus and dorsal raphe nucleus: the pathological building blocks of early Alzheimer's disease. *Neuropathology and Applied Neurobiology*. 2017;43(5):393-408. PMID 28117917. doi:10.1111/nan.12387

Eser RA, Ehrenberg AJ, Petersen C, Dunlop S, Mejia MB, Suemoto CK, et al. Selective Vulnerability of Brainstem Nuclei in Distinct Tauopathies: A Postmortem Study. *Journal of Neuropathology and Experimental Neurology*. 2018;77(2):149-161. PMID 29304218. doi:10.1093/jnen/nlx113

Fernández-Cabello S, Kronbichler M, Van Dijk KRA, Goodman JA, Spreng RN, Schmitz TW. Basal forebrain volume reliably predicts the cortical spread of Alzheimer's degeneration. *Brain : a Journal of Neurology*. 2020;143(3):993-1009. PMID 32203580. doi:10.1093/brain/awaa012

Gabitto MI, Travaglini KJ, Rachleff VM, Kaplan ES, Long B, Ariza J, et al. Integrated multimodal cell atlas of Alzheimer's disease. *Nature Neuroscience*. 2024;27(12):2366-2383. PMID 39402379. doi:10.1038/s41593-024-01774-5

Gais S, Born J. Low acetylcholine during slow-wave sleep is critical for declarative memory consolidation. *Proceedings of the National Academy of Sciences USA*. 2004;101(7):2140-4. PMID 14766981. doi:10.1073/pnas.0305404101

Gilmor ML, Erickson JD, Varoqui H, Hersh LB, Bennett DA, Cochran EJ, et al. Preservation of nucleus basalis neurons containing choline acetyltransferase and the vesicular acetylcholine transporter in the elderly with mild cognitive impairment and early Alzheimer's disease. *The Journal of Comparative Neurology*. 1999;411(4):693-704. PMID 10421878.

Grinberg LT, Rüb U, Ferretti RE, Nitrini R, Farfel JM, Polichiso L, et al. The dorsal raphe nucleus shows phospho-tau neurofibrillary changes before the transentorhinal region in Alzheimer's disease. A precocious onset?. *Neuropathology and Applied Neurobiology*. 2009;35(4):406-16. PMID 19508444. doi:10.1111/j.1365-2990.2009.00997.x

Hauglund NL, Andersen M, Tokarska K, Radovanovic T, Kjaerby C, Sørensen FL, et al. Norepinephrine-mediated slow vasomotion drives glymphatic clearance during sleep. *Cell*. 2025;188(3):606-622.e17. PMID 39788123. doi:10.1016/j.cell.2024.11.027

Hendricksen M, Thomas AJ, Ferrier IN, Ince P, O'Brien JT. Neuropathological study of the dorsal raphe nuclei in late-life depression and Alzheimer's disease with and without depression. *The American Journal of Psychiatry*. 2004;161(6):1096-102. PMID 15169699. doi:10.1176/appi.ajp.161.6.1096

Heneka MT, Nadrigny F, Regen T, Martinez-Hernandez A, Dumitrescu-Ozimek L, Terwel D, et al. Locus ceruleus controls Alzheimer's disease pathology by modulating microglial functions through norepinephrine. *Proceedings of the National Academy of Sciences USA*. 2010;107(13):6058-63. PMID 20231476. doi:10.1073/pnas.0909586107

Jellinger K. The pedunculopontine nucleus in Parkinson's disease, progressive supranuclear palsy and Alzheimer's disease. *Journal of Neurology, Neurosurgery, and Psychiatry*. 1988;51(4):540-3. PMID 3379428. doi:10.1136/jnnp.51.4.540

Kang SS, Liu X, Ahn EH, Xiang J, Manfredsson FP, Yang X, et al. Norepinephrine metabolite DOPEGAL activates AEP and pathological Tau aggregation in locus coeruleus. *The Journal of Clinical Investigation*. 2020;130(1):422-437. PMID 31793911. doi:10.1172/JCI130513

Kang SS, Ahn EH, Liu X, Bryson M, Miller GW, Weinshenker D, et al. ApoE4 inhibition of VMAT2 in the locus coeruleus exacerbates Tau pathology in Alzheimer's disease. *Acta Neuropathologica*. 2021;142(1):139-158. PMID 33895869. doi:10.1007/s00401-021-02315-1

Kelly SC, He B, Perez SE, Ginsberg SD, Mufson EJ, Counts SE. Locus coeruleus cellular and molecular pathology during the progression of Alzheimer's disease. *Acta Neuropathologica Communications*. 2017;5(1):8. PMID 28109312. doi:10.1186/s40478-017-0411-2

Leão RN, Mikulovic S, Leão KE, Munguba H, Gezelius H, Enjin A, et al. OLM interneurons differentially modulate CA3 and entorhinal inputs to hippocampal CA1 neurons. *Nature Neuroscience*. 2012;15(11):1524-30. PMID 23042082. doi:10.1038/nn.3235

Lyness SA, Zarow C, Chui HC. Neuron loss in key cholinergic and aminergic nuclei in Alzheimer disease: a meta-analysis. *Neurobiology of Aging*. 2003;24(1):1-23. PMID 12493547. doi:10.1016/s0197-4580(02)00057-x

Matchett BJ, Grinberg LT, Theofilas P, Murray ME. The mechanistic link between selective vulnerability of the locus coeruleus and neurodegeneration in Alzheimer's disease. *Acta Neuropathologica*. 2021;141(5):631-650. PMID 33427939. doi:10.1007/s00401-020-02248-1

Mesulam MM, Geula C, Bothwell MA, Hersh LB. Human reticular formation: cholinergic neurons of the pedunculopontine and laterodorsal tegmental nuclei and some cytochemical comparisons to forebrain cholinergic neurons. *The Journal of Comparative Neurology*. 1989;283(4):611-33. PMID 2545747. doi:10.1002/cne.902830414

Meyer C, Niedermeier T, Feyen PLC, Strübing FL, Rauchmann BS, Karali K, et al. Early Locus Coeruleus noradrenergic axon loss drives olfactory dysfunction in Alzheimer's disease. *Nature Communications*. 2025;16(1):7338. PMID 40781079. doi:10.1038/s41467-025-62500-8

Mintzer J, Lanctôt KL, Scherer RW, Rosenberg PB, Herrmann N, van Dyck CH, et al. Effect of Methylphenidate on Apathy in Patients With Alzheimer Disease: The ADMET 2 Randomized Clinical Trial. *JAMA Neurology*. 2021;78(11):1324-1332. PMID 34570180. doi:10.1001/jamaneurol.2021.3356

Morawski M, Brückner G, Jäger C, Seeger G, Arendt T. Neurons associated with aggrecan-based perineuronal nets are protected against tau pathology in subcortical regions in Alzheimer's disease. *Neuroscience*. 2010;169(3):1347-63. PMID 20497908. doi:10.1016/j.neuroscience.2010.05.022

Mufson EJ, Ma SY, Cochran EJ, Bennett DA, Beckett LA, Jaffar S, et al. Loss of nucleus basalis neurons containing trkA immunoreactivity in individuals with mild cognitive impairment and early Alzheimer's disease. *The Journal of Comparative Neurology*. 2000;427(1):19-30. PMID 11042589. doi:10.1002/1096-9861(20001106)427:1<19::aid-cne2>3.0.co;2-a

Nilsson P, Sörgjerd K, Kakiya N, Sasaguri H, Watamura N, Johansson L, et al. Somatostatin receptor subtypes 1 and 4 regulate neprilysin, the major amyloid-β degrading enzyme in brain. *Journal of Alzheimer's Disease : JAD*. 2026;109(2):651-666. PMID 41255128. doi:10.1177/13872877251392782

Nobili A, Latagliata EC, Viscomi MT, Cavallucci V, Cutuli D, Giacovazzo G, et al. Dopamine neuronal loss contributes to memory and reward dysfunction in a model of Alzheimer's disease. *Nature Communications*. 2017;8:14727. PMID 28367951. doi:10.1038/ncomms14727

Parikh V, Kozak R, Martinez V, Sarter M. Prefrontal acetylcholine release controls cue detection on multiple timescales. *Neuron*. 2007;56(1):141-54. PMID 17920021. doi:10.1016/j.neuron.2007.08.025

Pase MP, Himali JJ, Grima NA, Beiser AS, Satizabal CL, Aparicio HJ, et al. Sleep architecture and the risk of incident dementia in the community. *Neurology*. 2017;89(12):1244-1250. PMID 28835407. doi:10.1212/WNL.0000000000004373

Peng S, Wuu J, Mufson EJ, Fahnestock M. Increased proNGF levels in subjects with mild cognitive impairment and mild Alzheimer disease. *Journal of Neuropathology and Experimental Neurology*. 2004;63(6):641-9. PMID 15217092. doi:10.1093/jnen/63.6.641

Porsteinsson AP, Drye LT, Pollock BG, Devanand DP, Frangakis C, Ismail Z, et al. Effect of citalopram on agitation in Alzheimer disease: the CitAD randomized clinical trial. *JAMA*. 2014;311(7):682-91. PMID 24549548. doi:10.1001/jama.2014.93

Saito T, Iwata N, Tsubuki S, Takaki Y, Takano J, Huang SM, et al. Somatostatin regulates brain amyloid beta peptide Abeta42 through modulation of proteolytic degradation. *Nature Medicine*. 2005;11(4):434-9. PMID 15778722. doi:10.1038/nm1206

Schmid LC, Mittag M, Poll S, Steffen J, Wagner J, Geis HR, et al. Dysfunction of Somatostatin-Positive Interneurons Associated with Memory Deficits in an Alzheimer's Disease Model. *Neuron*. 2016;92(1):114-125. PMID 27641495. doi:10.1016/j.neuron.2016.08.034

Schmitz TW, Nathan Spreng R. Basal forebrain degeneration precedes and predicts the cortical spread of Alzheimer's pathology. *Nature Communications*. 2016;7:13249. PMID 27811848. doi:10.1038/ncomms13249

Schmitz TW, Soreq H, Poirier J, Spreng RN. Longitudinal Basal Forebrain Degeneration Interacts with TREM2/C3 Biomarkers of Inflammation in Presymptomatic Alzheimer's Disease. *The Journal of Neuroscience*. 2020;40(9):1931-1942. PMID 31915256. doi:10.1523/JNEUROSCI.1184-19.2019

Scott SA, Mufson EJ, Weingartner JA, Skau KA, Crutcher KA. Nerve growth factor in Alzheimer's disease: increased levels throughout the brain coupled with declines in nucleus basalis. *The Journal of Neuroscience*. 1995;15(9):6213-21. PMID 7666203. doi:10.1523/JNEUROSCI.15-09-06213.1995

Sheline YI, West T, Yarasheski K, Swarm R, Jasielec MS, Fisher JR, et al. An antidepressant decreases CSF Aβ production in healthy individuals and in transgenic AD mice. *Science Translational Medicine*. 2014;6(236):236re4. PMID 24828079. doi:10.1126/scitranslmed.3008169

Takeuchi T, Duszkiewicz AJ, Sonneborn A, Spooner PA, Yamasaki M, Watanabe M, et al. Locus coeruleus and dopaminergic consolidation of everyday memory. *Nature*. 2016;537(7620):357-362. PMID 27602521. doi:10.1038/nature19325

Theofilas P, Ehrenberg AJ, Dunlop S, Di Lorenzo Alho AT, Nguy A, Leite REP, et al. Locus coeruleus volume and cell population changes during Alzheimer's disease progression: A stereological study in human postmortem brains with potential implication for early-stage biomarker discovery. *Alzheimer's & Dementia*. 2017;13(3):236-246. PMID 27513978. doi:10.1016/j.jalz.2016.06.2362

Torack RM, Morris JC. The association of ventral tegmental area histopathology with adult dementia. *Archives of Neurology*. 1988;45(5):497-501. PMID 3358699. doi:10.1001/archneur.1988.00520290025008

Van Egroo M, van Hooren RWE, Jacobs HIL. Associations between locus coeruleus integrity and nocturnal awakenings in the context of Alzheimer's disease plasma biomarkers: a 7T MRI study. *Alzheimer's Research & Therapy*. 2021;13(1):159. PMID 34560904. doi:10.1186/s13195-021-00902-8

Watamura N, Kakiya N, Nilsson P, Tsubuki S, Kamano N, Takahashi M, et al. Somatostatin-evoked Aβ catabolism in the brain: Mechanistic involvement of α-endosulfine-Kchannel pathway. *Molecular Psychiatry*. 2022;27(3):1816-1828. PMID 34737456. doi:10.1038/s41380-021-01368-8

Whitehouse PJ, Price DL, Clark AW, Coyle JT, DeLong MR. Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. *Annals of Neurology*. 1981;10(2):122-6. PMID 7283399. doi:10.1002/ana.410100203

Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR. Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science (New York, N.Y.)*. 1982;215(4537):1237-9. PMID 7058341. doi:10.1126/science.7058341

Woolf NJ, Jacobs RW, Butcher LL. The pontomesencephalotegmental cholinergic system does not degenerate in Alzheimer's disease. *Neuroscience Letters*. 1989;96(3):277-82. PMID 2717053. doi:10.1016/0304-3940(89)90391-1

Zarow C, Lyness SA, Mortimer JA, Chui HC. Neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases. *Archives of Neurology*. 2003;60(3):337-41. PMID 12633144. doi:10.1001/archneur.60.3.337

Zhang Z, Song M, Liu X, Kang SS, Kwon IS, Duong DM, et al. Cleavage of tau by asparagine endopeptidase mediates the neurofibrillary pathology in Alzheimer's disease. *Nature Medicine*. 2014;20(11):1254-62. PMID 25326800. doi:10.1038/nm.3700

Part Two

The Hands That Let Go

The brakes that hold microglia in check: what makes a microglion different from a macrophage, and which restraints fail in Alzheimer's disease

The second step. Nine restraints hold the brain's immune cell at rest; seven of them weaken. What happens to amyloid when the cells that clear it stop clearing, and the inflammasome arm that leads out of this step and into the next.

Abstract

The first paper in this series described the base of the stack: the brainstem and basal forebrain neurons whose supplies begin to fade in the third decade of life. Two of those supplies, noradrenaline and acetylcholine, do not act on other neurons alone. They act on the brain's immune cell, the microglion, and they hold it in check. This paper is about what happens when that holding fails.

The field's usual verb is wrong. Microglia are not *activated*, as though a switch were thrown on a cell at rest. They are *released*. The microglion possesses the full destructive equipment of a tissue macrophage — phagocytosis, protease release, complement tagging, cytokine production — at all times, and it is prevented from using that equipment by a braking system that must be renewed continuously and at metabolic cost. What makes a microglion different from an ordinary macrophage is not its machinery. It is the restraint.

This paper inventories that braking system. Nine brakes are described, grouped by what retires each one:

- **The standing order.** A continuous instruction from the surrounding tissue, carried by TGF-β, that manufactures microglial identity itself. Remove it and the cell reverts to a macrophage within hours.
- **The two diffuse brakes.** Noradrenaline from the locus coeruleus and somatostatin from cortical interneurons, both withdrawn in the first step of the stack.
- **The four contact brakes.** Signals held on the neuronal surface and read on touch: CD200, **fractalkine (CX3CL1, read by the microglial receptor CX3CR1)**, CD47 and the sugar coat read by the inhibitory Siglecs. The fractalkine brake is the one this paper gives its own treatment, because it fails twice over — the neuron that displays the ligand is sick, and the receptor is among the first badges the microglion sheds.
- **The matrix brake.** The protective lattice around certain neurons, which keeps the microglion physically away from the cell surface.
- **The ceiling.** The microglion's own gain control, lowered by ageing.

Three conclusions follow. First, the brakes fail in a particular order, and the order explains the shape of the second step: the diffuse brakes go early, with their operating neurons; the contact brakes follow the lesion rather than causing it; and the ceiling falls in everyone with age, which is why this is a disease of the old. Second, one brake grows *stronger* with age rather than weaker, which means "restore the brakes" cannot be applied uniformly. Third, the two neurons that operate the diffuse brakes are the two that lack the matrix brake themselves — the system's long-range governors are among its least protected cells.

A section on amyloid runs beside the inventory. The first step has already been retarding amyloid for thirty years by three separate routes — serotonin brakes its production, somatostatin licenses its disposal, noradrenaline enables both its cellular clearance and its nightly drainage — so the deposit that names the disease is what accumulates when those brakes have gone and the microglion then stops clearing. The paper closes with the step's exit: a released microglion assembles its inflammasome, which suppresses clearance, seeds more amyloid, and licenses the enzymes that digest the protective lattice. That is the transition into the third step and the subject of the next paper.

How to read this paper

This is the second of the short papers on the model's three stacked steps. It follows *What the Brainstem Stops Sending* and assumes its conclusions, which are summarised in Section 1 for a reader starting here. Each section below condenses a longer volume in the corpus; those volumes carry the full argument and their own ledgers.

The grades are the corpus's four. **Established** means replicated in human material by more than one group. **Probable** means good evidence with a gap — a single cohort, or an animal result not yet confirmed in people. **Inference** means a conclusion drawn here, or in the corpus, from evidence gathered for another purpose. **Speculative** means coherent and untested.

One caution governs the whole paper. Most of what is known about microglial brakes comes from mice, and mouse microglia are not human microglia. Where a brake rests only on rodent work, the text says so, and the ledger in Section 11 grades it accordingly.

1. Where the First Paper Left Off

The first paper described five populations of neurons at the base of the stack and asked what each supplies. Four of its findings carry into this one.

Function goes before the cell. The locus coeruleus accumulates abnormal tau from the second and third decades of life, loses its axons across the following decades, and does not lose neurons in number until the disease is half over (Braak and Del Tredici, 2011; Theofilas and colleagues, 2017; Meyer and colleagues, 2025). The nucleus basalis stops displaying its growth-factor receptor long before it stops making acetylcholine, and stops making acetylcholine long before it dies (Mufson and colleagues, 2000; Gilmor and colleagues, 1999). A brake operated by such a neuron therefore fails when the neuron's *output* fails, which is decades before the neuron's death.

The step does not end. Phase I keeps worsening underneath Phase II and Phase III. The brainstem is still failing in the eighth decade.

Two of the withdrawn supplies are brakes on this cell. Noradrenaline holds microglia at rest (Heneka and colleagues, 2010). So, on the evidence assembled in the corpus, does somatostatin. Both fall in the first step.

The first step loosens, it does not trigger. Nothing in the first paper claims that the brainstem *causes* the microglial turn. It removes restraints. What follows is the subject of this paper.

The model's name for the second step is the **Microglial Bridgehead**, placed in the sixth and seventh decades and centred on the hippocampus. The name is apt in one way and misleading in another. It is apt because this is where the disease establishes itself in the structures that make memory. It is misleading if it suggests an invasion. Nothing invades. A cell that was always present, and always capable of this, stops being held back.

2. The Cell, and Why It Is Not a Macrophage

Every tissue in the body has resident macrophages: cells of the myeloid lineage that eat debris, digest it, call for help and clear up afterwards. The microglion is the brain's. It arrives early — microglia derive from primitive macrophages of the yolk sac, not from blood monocytes (Ginhoux and colleagues, 2010) — and thereafter it is largely self-renewing and very long-lived.

What distinguishes it is not what it can do. It is what it is forbidden to do, and the forbidding is extrinsic.

The microglion is not resting. The phrase "resting microglia" survives in textbooks and should not. In the healthy brain the cell is the most motile object there is, its fine processes extending and retracting continuously, surveying the whole of the tissue on a timescale of hours. A cell that never stops moving is not at rest. It is restrained: doing a great deal, and prohibited from certain other things.

Its identity is a subscription, not a settlement. The decisive experiment is Butovsky's. The molecular badge set by which a microglion is recognised — P2RY12, TMEM119, SALL1, HEXB, OLFML3, CX3CR1 and the rest — is not an intrinsic property of the lineage. It is a transcriptional programme installed and maintained from outside by TGF-β signalling, and microglia removed from the brain and put in culture lose it within hours (Butovsky and colleagues, 2014). Silencing the pathway in the living animal does the same (Zöller and colleagues, 2018). Conversely, supply TGF-β, cholesterol and IL-34 in a defined medium and the identity can be held outside the brain (Bohlen and colleagues, 2017). **Established.**

That is an unusual arrangement, and it has a consequence the rest of this paper depends on. The microglion's character is only as durable as the health of the tissue around it. A cortex whose neurons are sick issues its microglia a weaker order, and a less-braked microglion damages more neurons. The loop closes on itself.

What a disease mechanism now has to explain. If activation were a switch, the question would be what throws it, and the candidates would be amyloid, tau or an infection. The corpus has taken that question seriously and found the provocations real but insufficient: proteinopathy provokes an ungoverned cell far more effectively than a governed one (*The Corruption of the Gardener*). If restraint is instead the default and its lapse the event, the questions become which restraints exist, what retires each, and in what order they go. That is an inventory, and Sections 3 to 9 are it.

The practical difference is not semantic. If microglia are activated, the rational drug is an antagonist: block the receptor, inhibit the kinase, neutralise the cytokine. The field has produced many such agents and none has altered the course of the disease. If microglia are released, the rational drug is an agonist: restore the instruction, re-engage the brake. That is a different pharmacology, and it has barely been tried. **Inference**, and the corpus's own argument (*The Double Release*).

3. The Standing Order

The first brake is not a brake on any particular behaviour. It is the instruction that makes the cell the kind of thing that has brakes at all.

What it is. Transforming growth factor β is present continuously in healthy brain tissue. It engages a receptor on the microglion whose inner part phosphorylates the proteins SMAD2 and SMAD3; these enter the nucleus and switch on the homeostatic badge set (Butovsky and colleagues, 2014; reviewed by Spittau and colleagues, 2020). The signal comes from the surrounding neurons and astrocytes. It must be re-issued continuously.

How it fails in Alzheimer's disease. Three lines converge and none is decisive alone. The type-II TGF-β receptor is reduced in the Alzheimer brain, and impairing neuronal TGF-β signalling promotes both neurodegeneration and amyloid pathology (Tesseur and colleagues, 2006). Neurofibrillary tangles appear to interfere with SMAD2/3 signalling inside neurons, sequestering the transducer (Chalmers and Love, 2007). And a cortex losing synapses and neurons simply has fewer sources issuing the order. **Probable.** No study has yet measured SMAD-driven transcription in microglia across the human

Braak series, which is the measurement this brake most needs.

Why it cannot be the whole answer. The standing order fails slowly and everywhere, in proportion to how sick the tissue already is. It cannot explain why a microglion turns in the entorhinal cortex while the occipital cortex is intact. For that you need a brake with an address, and the next six have one.

4. The Two Diffuse Brakes

These are the brakes the first paper's neurons operate. They reach the microglion by diffusion from a distant cell, so they govern tone across a whole territory rather than deciding individual cases.

Noradrenaline, from the locus coeruleus

The anatomy makes this the most extensive braking signal in the brain. One nucleus of a few tens of thousands of neurons, tonically active through the waking day, addresses the entire microglial population at once. Microglia carry the β2-adrenergic receptor, and it is among the seventeen rhodopsin- and adhesion-family receptors found to be reliably transcribed by human microglia (Hsiao and colleagues, 2021). **Established.**

What it does. Three functions, each established separately. It suppresses the inflammatory programme: lesioning the locus coeruleus in an amyloid-bearing mouse raises microglial inflammatory gene expression, impairs the migration and phagocytosis of amyloid, and worsens the pathology, while supplying the missing transmitter reverses it (Heneka and colleagues, 2010). It constrains surveillance: in the awake mouse, noradrenaline acting at the β2 receptor dials down microglial process motility, which is released again in sleep (Stowell and colleagues, 2019; Liu and colleagues, 2019). And it constrains the disease-associated transition itself: β2 receptor message is very low in microglia near plaques at every age tested and falls with age in those further away; deleting the receptor from microglia before plaques form worsens amyloid burden and neuritic damage (Le and colleagues, 2025). **Probable**, in models.

The complication, and it matters for therapy. The brake can be withdrawn at the microglial end while the brainstem end is still producing

transmitter — indeed while it is producing more than normal, since surviving coerulean neurons compensate before they fail. A receptor that is no longer there cannot be agonised. This is the strongest reason to doubt that simply raising noradrenaline would restore the brake (*The Double Release*). **Inference.**

Somatostatin, from cortical and hippocampal interneurons

The first paper introduced the somatostatin interneuron as the neighbour that fails early, and described its licence over neprilysin, the enzyme that clears amyloid. The corpus makes a further claim: the same peptide is a microglial brake.

The evidence. Cultured microglia transcribe somatostatin receptors, and somatostatin suppresses their proliferation (Feindt and colleagues, 1998). Somatostatin given before an inflammatory challenge in the intact rat brain suppresses microglial activation, lowers inflammatory mediators, and prevents the neuronal loss the challenge would otherwise cause (Bai and colleagues, 2015). A selective agonist at the fourth somatostatin receptor increases microglial uptake of amyloid-β while suppressing inflammatory output (Schober and colleagues, 2021), and in a mouse model raises neprilysin 9.3-fold and insulin-degrading enzyme 14.8-fold (Sandoval and colleagues, 2019). This brake is unusual in damping the inflammatory arm while *releasing* the clearing arm. **Probable**, in rodents.

Where it is weak, stated plainly. The same survey lists seventeen such receptors in human microglia and no somatostatin receptor among them (Hsiao and colleagues, 2021). That is not a demonstration of absence — the survey did not set out to test for them, and scarce receptors are easily missed — but it is a real asymmetry with the noradrenergic brake, whose receptor *is* on the list. An indirect route, in which somatostatin acts on the neuron and the microglion quiets because its neighbour is healthier, has not been excluded by any experiment yet performed. **The corpus grades its own claim here as the weakest in the inventory, and so does this paper.**

How close in time do the two fail?

The Double Release puts the question carefully and answers it with error bars. Dated by first lesion, the locus coeruleus leads by twenty-five to thirty years. Dated by the death of the operating neuron, the order may reverse, because somatostatin interneurons are lost in the early preclinical phase while coerulean neurons are not lost until Braak III (Gabitto and colleagues, 2024; Theofilas and colleagues, 2017). Dated by the event that actually matters — the moment the microglion stops being restrained — the two fall within the same fifteen-year preclinical band, and the best estimate of the offset is zero to ten years with a confidence interval spanning zero. The correct picture is a near-simultaneous double release rather than a relay. **Inference**, and the volume's central claim.

One consequence deserves stating. Because the two fail within the resolution of any instrument used to measure either, no human tissue has ever been examined during a period of single-brake failure. Every released microglion yet described in an Alzheimer brain has lost both hands.

5. The Contact Brakes, and the One That Was Missed

The diffuse brakes set the tone of a whole territory. The contact brakes decide individual cases: this process, this synapse, now. Each is a molecule held on the neuronal surface and read by a receptor on the microglial one. The logic is the immune system's logic of a "self" signal: *do not eat me, I am one of yours*.

Fractalkine and CX3CR1

This is the brake Jim Ray raised, and the corpus mentions it in a dozen places without ever giving it a section of its own. It deserves one, for three reasons.

It is unusual in its physics. Fractalkine (CX3CL1) is a chemokine, but unlike other chemokines it is not released to diffuse. It is tethered to the neuron's membrane, so its signal is delivered only on contact. The neuron is therefore not broadcasting a general request for calm; it is saying, to one microglial process at one point of touch, *not here*. A brake with that geometry

can protect one synapse while leaving its neighbour unprotected.

It is both a brake and a badge. CX3CR1, the receptor that reads fractalkine, is one of the core markers of the homeostatic microglion — part of the same badge set that the standing order of Section 3 maintains. This is what makes it structurally different from the other contact brakes, and it is the point the corpus has not emphasised. The fractalkine brake fails twice over, by two independent routes. The *ligand* is lost as the neuron that displays it sickens and dies. The *receptor* is shed as the microglion leaves its homeostatic state, and that shedding is among the earliest and most reliable events in the transition — in single-cell studies of the disease-associated microglion, the loss of P2RY12, TMEM119 and CX3CR1 is the most consistent feature of the change, more consistent than anything the cell switches on (Keren-Shaul and colleagues, 2017; Krasemann and colleagues, 2017). So the brake can be released from the microglial side before the neuron it protects has lost anything at all.

What its loss does. Mice lacking the receptor show dysregulated, neurotoxic microglia in three separate experiments in living animals: an inflammatory challenge, a toxic model of Parkinson's disease, and a model of motor neuron disease (Cardona and colleagues, 2006). **Probable.**

The complication, stated rather than smoothed. The brake is not uniformly protective. Losing CX3CR1 *reduces* amyloid burden in some models while worsening tau pathology in others. The corpus reads this as another instance of the context-dependence that afflicts TREM2, where the same receptor helps at one stage and harms at another. Any therapy aimed here would have to specify which pathology, in which decade. **Established** that the direction is context-dependent; **inference** as to why.

Why it belongs with the sick neuron rather than the healthy one. This brake, like the other three below, requires a neuronal surface that is present and correctly decorated. It therefore fails *downstream* of the lesion. It is best read as the mechanism by which a lesion spreads locally, not as a cause of the lesion — which makes it indispensable to the pathology and a poor early target.

CD200 and CD200R

The founding demonstration: mice lacking CD200 carry a myeloid compartment in a permanently more activated state, their microglia showing an activated shape and a faster response to injury (Hoek and colleagues, 2000). The Alzheimer evidence is unusually direct for a contact brake. Both CD200 and its receptor are significantly reduced in the Alzheimer hippocampus and inferior temporal gyrus, and not in the cerebellum (Walker and colleagues, 2009). Affected regions yes, spared region no — exactly the pattern a lesion-following brake should show. **Established.**

CD47 and SIRPα

The cleanest proof that microglial pruning is held back by a stop signal comes from development rather than disease. CD47 sits on neurons, SIRPα on microglia; their levels track the peak of synaptic refinement in the visual system; and mice lacking either show increased microglial engulfment of inputs and fewer synapses (Lehrman and colleagues, 2018). What this establishes is the architecture — that synapse elimination in the healthy brain is limited by a stop signal, and not only driven by an eat-me signal. The complement literature has largely omitted that half. The direct Alzheimer evidence is thinner, and this paper does not overstate it. **Established** in development; **speculative** in the disease.

The sugar coat, and the brake that should be released

The neuronal surface carries a coat of sialic acid, read as "self" by inhibitory receptors called Siglecs. Two matter. CD22 rises on aged microglia and blocks their phagocytosis; blocking CD22 in turn restores the clearance of debris, amyloid oligomers and α-synuclein in living mice, shifts microglia back toward a homeostatic state, and improves cognition in aged animals (Pluvinage and colleagues, 2019). CD33 is an Alzheimer risk gene whose product inhibits microglial uptake of amyloid (Griciuc and colleagues, 2013). **Established** in models; the human genetics for CD33 is **established**.

These are brakes that grow *stronger* with age, not weaker. They are the reason "restore the brakes" cannot be a slogan applied uniformly, and Section 11 returns to the contradiction.

6. The Matrix Brake

The sixth brake is not chemical. It is a structure.

Certain neurons wear a dense lattice of extracellular matrix around the cell body and the nearest dendrites — the perineuronal net, built of hyaluronan, aggrecan, brevican, link proteins and tenascin-R, with holes left at synaptic contacts. Among other jobs, it keeps the microglion physically away from the neuronal surface.

The evidence that it is a brake. Neurons wearing aggrecan-based nets are protected against tau pathology in subcortical regions of the Alzheimer brain (Morawski and colleagues, 2010). Resilience to Alzheimer's disease is associated with alterations in these nets in human tissue (de Vries and colleagues, 2025). And the microglial half of the relation is direct: microglia facilitate the loss of perineuronal nets in the Alzheimer brain, and depleting microglia preserves them (Crapser and colleagues, 2020). **Established.**

The relationship is therefore adversarial and reciprocal. The net excludes the microglion; the microglion digests the net. It is the only brake in the inventory that its own target attacks.

The observation that organises this paper. Parvalbumin interneurons wear dense nets. Somatostatin interneurons largely do not. The locus coeruleus does not. So the two neurons that operate the diffuse brakes of Section 4 are themselves the two that lack the matrix brake. The system's two long-range governors are among its least protected cells, and that is why the first step of the stack falls where it does. **Inference**, and the structural heart of *The Double Release*.

7. The Ceiling

The last brake is not imposed from outside at all. It is the microglion's own limit on how far it will travel when something provokes it — a gain control rather than an instruction.

MEF2C. This transcription factor acts in microglia as a restraint on the inflammatory response. Deczkowska and colleagues established the chain: the aged brain carries chronic type-I interferon; interferon-β in the adult brain induces an ageing-like microglial signature and impairs cognition; the interferon milieu lowers microglial *Mef2C*; and mice lacking it in microglia mount an exaggerated response to challenge, with behavioural consequences (Deczkowska and colleagues, 2017). *MEF2C* is additionally an Alzheimer risk gene, which places the same molecule in the genetics and the ageing biology at once. **Probable.**

Why this is the ceiling and not another brake. MEF2C does not tell the microglion what to do. It determines how far the cell goes when something else does. That is what makes it the system's gain control, and it explains the epidemiology: age is the dominant risk factor for this disease, and what ageing does to this system is lower the ceiling, so that provocations a young brain absorbs produce in an old brain a response the tissue cannot tolerate.

A consequence for timing. Because the ceiling falls continuously from midlife in everyone, the *same* brake failure has different consequences at different ages. Withdrawing the somatostatin brake at fifty in a brain with a high ceiling may produce nothing observable; the identical withdrawal at seventy may produce a great deal. The brakes cannot be dated in isolation — they must be read against a ceiling that is itself falling.

8. The Order of Failure

Table 1 gathers the nine brakes.

Table 1. The braking system, grouped by what retires each brake. "When" is relative to the lesion in the tissue, not to a calendar age.

Brake	How it reaches the microglion	What retires it	When
The standing order (TGF-β/SMAD)	Continuously, from surrounding tissue	Sickness of the source tissue; tangles sequester the transducer	Gradual, late-loading

Brake	How it reaches the microglion	What retires it	When
Noradrenaline → β2 receptor	Diffusion from the brainstem	Loss of coerulean output; and loss of the receptor itself	Early
Somatostatin → its receptors	Diffusion from local interneurons	Loss of the peptide, before the cell that makes it	Early
Fractalkine → CX3CR1	Contact with the neuronal membrane	Ligand lost with the neuron; receptor shed with the badge set	Both early and late
CD200 → CD200R	Contact	Withdrawal of the ligand from the sick neuron	Follows the lesion
CD47 → SIRPα	Contact	Loss of the stop signal at the synapse	Follows the lesion
Sialic acid → CD22, CD33	Contact	Nothing: this brake *strengthens* with age	Inverted
The perineuronal net	Physically, by excluding the cell	Digestion by the microglion it restrains	Late; the exit into the third step
The ceiling (MEF2C)	Internal to the cell	Ageing, through chronic interferon	Continuous from midlife

Read down the table, three patterns emerge.

The diffuse brakes go first, and they go together. They are the only two whose failure precedes the local lesion, because they depend on the output of a distant neuron rather than on the health of the neuron next door. They are therefore the only two that could in principle be targeted before the damage they permit.

The contact brakes follow the lesion. All four require a neuronal surface that is present and properly decorated. They matter enormously to how the damage spreads, and they are poor candidates for early intervention.

The system has no single point of failure, which is why it fails slowly. When the noradrenergic brake is withdrawn, the others continue to operate and are sufficient to hold a homeostatic-like state for a time. That is why the second step does not follow immediately on the first. But the released brake

raises the demand on those remaining, and the cumulative demand eventually exceeds what an ageing supply can meet (*The Locus Coeruleus Bridge*). **Inference.**

9. What the Released Microglion Does

A microglion that has lost its restraints does not do one new thing. It does several, and the corpus's reading is that the various named states in the literature — disease-associated, lipid-droplet-accumulating, dystrophic — are variants of one event rather than distinct programmes. The most reliable feature of all of them is not what the cell switches on, which varies between datasets, but what it switches off: the homeostatic badge set, including the fractalkine receptor (Keren-Shaul and colleagues, 2017; Krasemann and colleagues, 2017; Marschallinger and colleagues, 2020). **Probable**, and the corpus's own synthesis.

Four outputs matter for what follows.

It tags and eats synapses. The classical complement cascade — C1q marks a synapse, C3 is cleaved, and the microglial receptor CR3 engulfs what has been marked — is the mechanism of normal developmental pruning, and it is reactivated in the Alzheimer brain to eliminate synapses that should not be eliminated, before plaques form (Hong and colleagues, 2016). This is the clearest single mechanism linking a released microglion to the loss of connections the model measures throughout. **Established** in models.

It digests the matrix. Released microglia secrete matrix-degrading enzymes, MMP-9 among them, which break down the perineuronal net; depleting microglia preserves the nets (Crapser and colleagues, 2020). Section 10 follows that arm to the exit from this step.

It stops finishing its work. A cell under metabolic strain does not go quiet; it stops clearing and starts broadcasting. Andreasson's programme traces that inversion to a lipid switch acting through the EP2 receptor, and removing the receptor from myeloid cells restores a youthful cytokine profile and rescues memory in old mice (*The Starving Housekeeper*). **Probable**, in rodents; every causal result in that programme is murine.

And it compacts plaques, which is protective. The disciplined microglion wraps a deposit and compacts it into an inert core, insulating the surrounding neurites. TREM2 is central to that barrier (Wang and colleagues, 2016; Ulland and colleagues, 2017), which is why TREM2 variants raise risk (Guerreiro and colleagues, 2013; Jonsson and colleagues, 2013). This is the strongest reason to resist reading the released microglion as simply harmful: the same cell, restrained, is doing work the brain cannot do without (*The Gardener's Restraint*).

10. What Happens to Amyloid

The disease is named for a deposit, and so far this paper has mentioned it only in passing. That is deliberate: the brakes come first, and amyloid is best understood as something the braking system handles. But the handling is where the second step does its most consequential work, and it is also where the first step turns out to have been acting all along.

Amyloid is made continuously, and disposal is the variable. A healthy brain produces amyloid-β throughout life. What changes with age and disease is not chiefly how much is made but how much is taken away, and there are three routes of removal.

- **Enzymatic.** Neprilysin is the rate-limiting enzyme for the aggregation-prone form of the peptide: tracing labelled amyloid-β injected into the rat brain showed it degraded through this enzyme, and inhibiting the enzyme produced deposition of the animal's own peptide (Iwata and colleagues, 2000). **Established.**
- **Cellular.** Microglia take the peptide up and digest it in their lysosomes, and a restrained microglion also compacts a deposit into an inert core (Section 9).
- **Drainage.** The peptide is washed out of the tissue during sleep, by a flow that noradrenaline oscillations drive (Hauglund and colleagues, 2025).

The first step already retards amyloid, by three different routes. This is the point Dr. Truchard's note makes, and it deserves stating plainly, because it changes what the second step inherits. The populations of paper one are not

merely upstream of the amyloid problem; each of them is holding part of it back.

- **Serotonin brakes production.** The raphe's tonic signal drives the harmless cleavage of the amyloid precursor, and a single course of a serotonergic drug slowed amyloid production in healthy people by 37 per cent (Sheline and colleagues, 2014; Cirrito and colleagues, 2011).
- **Somatostatin licenses enzymatic disposal.** The peptide from the cortical interneuron raises neprilysin activity, through a defined receptor pathway (Saito and colleagues, 2005; Nilsson and colleagues, 2026).
- **Noradrenaline enables cellular clearance and nightly drainage.** Lesioning the locus coeruleus impairs microglial migration and phagocytosis of amyloid, and supplying the missing transmitter restores both (Heneka and colleagues, 2010); the same nucleus's slow oscillations drive the sleeping brain's clearance (Hauglund and colleagues, 2025).

Read together, these say something the three-phase picture can obscure. **By the time the microglion is released, the brain has already lost one brake on amyloid production and two on its removal.** The deposit that defines the disease is not simply the product of the second step. It is what accumulates when the first step has been failing for thirty years and the second step then stops clearing.

When does the microglion lose its ability to clear? Not at a single moment, and not by one mechanism. Four measurable changes remove the capacity, and they are separable in principle.

- **The inhibitory receptors rise.** CD33 inhibits microglial uptake of amyloid and is raised in microglia in the Alzheimer brain (Griciuc and colleagues, 2013); CD22 rises on aged microglia, and blocking it restores clearance (Pluvinage and colleagues, 2019). These are the Siglec brakes of Section 5, strengthening when they should not.
- **The cell fills with fat.** Lipid-droplet-accumulating microglia, which build up with age in mouse and human brain, are defective in phagocytosis, produce high levels of reactive oxygen species, and secrete inflammatory cytokines (Marschallinger and colleagues, 2020).
- **The barrier apparatus fails.** The TREM2-built barrier that compacts a plaque is what stands between a diffuse, neurotoxic deposit and an inert

one (Wang and colleagues, 2016; Ulland and colleagues, 2017).

- **The inflammasome shuts clearance down.** This is the most direct answer to the question, and it is taken up next.

The inflammasome, and the toxins it licenses

The NLRP3 inflammasome is a sensor assembled inside the microglion. Its activation is the event that converts a cell that has stopped clearing into a cell that is actively doing harm, and the sequence is unusually well characterised.

Amyloid activates it, by being eaten. The microglion takes up fibrillar amyloid, the peptide damages the lysosome, cathepsin B is released into the cytoplasm, and the sensor assembles (Halle and colleagues, 2008). **Established.** The cell is set off by the very material it was trying to dispose of.

It is activated in the human disease, and removing it protects animals. Active caspase-1 is strongly raised in human mild cognitive impairment and Alzheimer brain. Mice carrying familial Alzheimer mutations and lacking either NLRP3 or caspase-1 were largely protected from the loss of spatial memory, showed reduced inflammasome activity, **enhanced clearance of amyloid**, and less deposition (Heneka and colleagues, 2013). **Established** in models; the human finding is a measurement of activation, not of causation.

Activation suppresses clearance, which closes a loop. A systemic inflammatory challenge reduced microglial clearance of amyloid in amyloid-bearing mice, and deleting NLRP3 blocked that reduction (Tejera and colleagues, 2019). Inhibiting the sensor pharmacologically does the converse: it stimulates uptake of the peptide, reduces its accumulation, and improves cognition in the same model (Dempsey and colleagues, 2017). So the amyloid that activates the inflammasome causes the cell to clear less amyloid. **Probable**, and the clearest self-amplifying loop in the second step.

It seeds more amyloid directly. The activated sensor builds a particle called an ASC speck, which the microglion releases. Released specks bind amyloid-β rapidly and increase its aggregation, and injecting them into amyloid-bearing mice spread the pathology, while the spread failed in mice lacking the speck protein and was blocked by an antibody against it (Venegas and colleagues, 2017). **Probable**: a striking result from one programme, not yet independently replicated in human tissue.

And it licenses the enzymes that destroy the protective lattice. The cytokine the inflammasome exists to mature, interleukin-1β, is the principal inducer of the aggrecan-degrading proteases — the ADAMTS aggrecanases and matrix metalloproteinases — that cleave the core of the perineuronal net. This is the best-characterised route from a cytokine to a protease in all of matrix biology. But it was characterised in cartilage, and the corpus grades it honestly: **established** in the joint, **conjecture** in the brain, where the intermediate steps have not been measured (*One Sensor, Two Collapses*). The net itself is also directly vulnerable, because it protects the cell it wraps against oxidative stress and is itself degraded by that stress (Cabungcal and colleagues, 2013).

That last arm is the exit from this step. A microglion that has lost its restraints, stopped clearing, and assembled its inflammasome is a cell that secretes the enzymes which digest the matrix brake — the last brake in the inventory, and the one whose loss defines the third step.

A step underneath all of this, treated in the fourth paper. Every route described in this section ends in digestion, and digestion happens in the lysosome. A microglion with all nine brakes intact but a failed lysosome takes material in and does not break it down; *The Long Way Back* gives the evidence that this disposal step fails in the same cell, in the direction that makes matters worse.

One concession, which the corpus states and this paper repeats. The inflammasome is an amplifier, not the initiator. It is activated by a pathology that began upstream of it, in a brain whose brakes were already going. Nothing in this section claims that the microglion starts the disease.

11. Two Contradictions the Inventory Must Carry

An honest inventory has to state where it argues against itself.

Not every brake should be restored. Seven of the nine brakes weaken. The Siglec brakes strengthen, and strengthening is the problem: an aged microglion that cannot phagocytose is not a safe microglion but a useless one,

and blocking CD22 improves clearance and cognition in aged mice (Pluvinage and colleagues, 2019). So the therapeutic instruction is not "restore restraint" but "restore the *right* restraints" — damp the inflammatory arm, release the clearing arm. The somatostatin brake is the only one in the inventory that does both at once, which is the strongest argument for pursuing it and the reason its weak human evidence matters so much.

Context-dependence is not a detail. CX3CR1 and TREM2 both change sign depending on model, stage and which pathology is measured. A brake that protects against tau while permitting amyloid, or that helps early and harms late, cannot be targeted without specifying the decade. The corpus's position is that this is a genuine property of the system rather than noise in the literature, and that it is the main reason single-target immune therapies have failed. **Inference.**

12. Graded Ledger

Table 2. Each claim in this paper, with its grade and the evidence it rests on.

#	Claim	Grade	Basis
1	Microglia derive from yolk-sac macrophages, not blood monocytes	Established	Ginhoux et al. 2010
2	Microglial identity is installed and maintained extrinsically by TGF-β	Established	Butovsky et al. 2014; Zöller et al. 2018; Bohlen et al. 2017
3	The standing order is impaired in Alzheimer's disease	Probable	Tesseur et al. 2006; Chalmers and Love 2007
4	Noradrenaline restrains microglial inflammation and surveillance	Established (models)	Heneka et al. 2010; Stowell et al. 2019; Liu et al. 2019
5	The β2 receptor is lost from plaque-associated microglia	Probable	Le et al. 2025 (mouse)

#	Claim	Grade	Basis
6	Somatostatin is a microglial brake	Probable (rodent); weakest in the inventory	Feindt et al. 1998; Bai et al. 2015; Schober et al. 2021; Sandoval et al. 2019
7	No somatostatin receptor appears in the human microglial GPCR survey	Established	Hsiao et al. 2021
8	The two diffuse brakes fail within the same preclinical band	Inference	*The Double Release*; Gabitto et al. 2024; Theofilas et al. 2017
9	Fractalkine is a contact brake whose loss causes microglial neurotoxicity	Probable	Cardona et al. 2006
10	CX3CR1 is shed early as part of the homeostatic badge set	Established	Keren-Shaul et al. 2017; Krasemann et al. 2017
11	The fractalkine brake therefore fails by two independent routes	Inference	This paper, from rows 9 and 10
12	CD200 and its receptor are reduced in affected regions and not in spared ones	Established	Walker et al. 2009; Hoek et al. 2000
13	CD47–SIRPα limits synaptic pruning	Established (development); speculative (AD)	Lehrman et al. 2018
14	The Siglec brakes strengthen with age and should be released	Established (models)	Pluvinage et al. 2019; Griciuc et al. 2013
15	The perineuronal net is a physical brake, and microglia digest it	Established	Morawski et al. 2010; Crapser et al. 2020; de Vries et al. 2025
16	The operators of the diffuse brakes are themselves unnetted	Inference	*The Double Release*
17	MEF2C sets a ceiling that ageing lowers through interferon	Probable	Deczkowska et al. 2017

#	Claim	Grade	Basis
18	Loss of the homeostatic signature is the common event behind every named microglial state	Probable	Keren-Shaul et al. 2017; Krasemann et al. 2017; Marschallinger et al. 2020
19	Complement-tagged synapses are eaten before plaques form	Established (models)	Hong et al. 2016
20	Restrained microglia compact plaques, and TREM2 builds that barrier	Established	Wang et al. 2016; Ulland et al. 2017; Guerreiro et al. 2013; Jonsson et al. 2013
21	Neprilysin is the rate-limiting enzyme for amyloid-β disposal	Established	Iwata et al. 2000
22	The first step retards amyloid by three routes: production, enzymatic disposal, clearance and drainage	Inference, from established parts	Sheline et al. 2014; Saito et al. 2005; Heneka et al. 2010; Hauglund et al. 2025
23	Amyloid activates the NLRP3 inflammasome through lysosomal damage	Established	Halle et al. 2008
24	The inflammasome is activated in human AD; its deletion protects animals and enhances clearance	Established (models); activation measured in humans	Heneka et al. 2013
25	Inflammasome activation suppresses microglial amyloid clearance	Probable	Tejera et al. 2019; Dempsey et al. 2017
26	Released ASC specks cross-seed amyloid	Probable; one programme	Venegas et al. 2017
27	Interleukin-1β induces the proteases that strip the perineuronal net	Established in cartilage; conjecture in brain	*One Sensor, Two Collapses*

13. What Would Change This Account

- A measurement of SMAD-driven transcription in human microglia across the Braak series showing the standing order intact. The first brake would leave the inventory.
- Human microglia shown to carry no functional somatostatin receptor, with the rodent effect traced to an action on neurons instead. The second diffuse brake would become an indirect one, and the near-simultaneous double release would lose half its substance.
- A human series showing CX3CR1 lost only after the neurons that supply its ligand have died. The two-route failure of Section 5 would collapse into one.
- Evidence that the homeostatic badge set is shed *after*, not before, the destructive programme begins. The whole framing of release rather than activation would need rewriting.
- A single-target immune therapy that works across stages. The context-dependence of Section 11 would be noise after all.
- A measurement in brain showing that interleukin-1β does not induce the aggrecan-degrading proteases there. The exit into the third step would need another mechanism.

The next paper takes up the third step: the digestion of the protective lattice, the failure of inhibition, and the loss of connections that makes the illness visible.

Corpus papers drawn on

The Double Release: The Brakes on Microglial Activation in Alzheimer's Disease — A Graded Inventory. *The Corruption of the Gardener*: The Drivers of Microglial Dysfunction, Ranked by Causal Weight. *The Gardener's Restraint*: Microglial Restraint as Resilience. *The Second Brake*: Amyloid-β Restrains Microglia Twice. *Homeostatic Microglial Collapse*. *The Locus Coeruleus Bridge*: The Locus Coeruleus as Phase Bridge. *The DAM Trajectory*: Deczkowska's Programme and the Matrix-Degrading Arm. *The Complement-Pruning Substrate*: Beth Stevens's Programme. *The Starving Housekeeper*: Andreasson's EP2, IDO1 and TREM1 Programme. *The Silenced Eraser*. *What the Brainstem Stops Sending* (paper one of this series). All are available in full at AdultCognitiveDisease.com.

References

Bai L, Zhang X, Li X, Liu N, Lou F, Ma H, et al. Somatostatin prevents lipopolysaccharide-induced neurodegeneration in the rat substantia nigra by inhibiting the activation of microglia. *Molecular Medicine Reports*. 2015;12(1):1002-8. PMID 25777539. doi:10.3892/mmr.2015.3494

Bohlen CJ, Bennett FC, Tucker AF, Collins HY, Mulinyawe SB, Barres BA. Diverse requirements for microglial survival, specification, and function revealed by defined-medium cultures. *Neuron*. 2017;94(4):759-773.e8. PMID 28521131. doi:10.1016/j.neuron.2017.04.043

Braak H, Del Tredici K. The pathological process underlying Alzheimer's disease in individuals under thirty. *Acta Neuropathologica*. 2011;121(2):171-81. PMID 21170538. doi:10.1007/s00401-010-0789-4

Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, et al. Identification of a unique TGF-β-dependent molecular and functional signature in microglia. *Nature Neuroscience*. 2014;17(1):131-43. PMID 24316888. doi:10.1038/nn.3599

Cabungcal JH, Steullet P, Morishita H, Kraftsik R, Cuenod M, Hensch TK, et al. Perineuronal nets protect fast-spiking interneurons against oxidative stress. *Proceedings of the National Academy of Sciences USA*. 2013;110(22):9130-5. PMID 23671099. doi:10.1073/pnas.1300454110

Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, et al. Control of microglial neurotoxicity by the fractalkine receptor. *Nature Neuroscience*. 2006;9(7):917-24. PMID 16732273. doi:10.1038/nn1715

Chalmers KA, Love S. Neurofibrillary tangles may interfere with Smad 2/3 signaling in neurons. *Journal of Neuropathology and Experimental Neurology*. 2007;66(2):158-67. PMID 17279001. doi:10.1097/nen.0b013e3180303b93

Cirrito JR, Disabato BM, Restivo JL, Verges DK, Goebel WD, Sathyan A, et al. Serotonin signaling is associated with lower amyloid-β levels and plaques in transgenic mice and humans. *Proceedings of the National Academy of Sciences USA*. 2011;108(36):14968-73. PMID 21873225. doi:10.1073/pnas.1107411108

Crapser JD, Spangenberg EE, Barahona RA, Arreola MA, Hohsfield LA, Green KN. Microglia facilitate loss of perineuronal nets in the Alzheimer's disease brain. *EBioMedicine*. 2020;58:102919. PMID 32745992. doi:10.1016/j.ebiom.2020.102919

de Vries LE, Bahnerth A, Swaab DF, Verhaagen J, Carulli D. Resilience to Alzheimer's disease associates with alterations in perineuronal nets. *Alzheimer's & Dementia*. 2025;21(2):e14504. PMID 39737731. doi:10.1002/alz.14504

Deczkowska A, Matcovitch-Natan O, Tsitsou-Kampeli A, Ben-Hamo S, Dvir-Szternfeld R, Spinrad A, et al. Mef2C restrains microglial inflammatory response and is lost in brain ageing in an IFN-I-dependent manner. *Nature Communications*. 2017;8(1):717. PMID

28959042. doi:10.1038/s41467-017-00769-0

Dempsey C, Rubio Araiz A, Bryson KJ, Finucane O, Larkin C, Mills EL, et al. Inhibiting the NLRP3 inflammasome with MCC950 promotes non-phlogistic clearance of amyloid-β and cognitive function in APP/PS1 mice. *Brain, Behavior, and Immunity*. 2017;61:306-316. PMID 28003153. doi:10.1016/j.bbi.2016.12.014

Feindt J, Schmidt A, Mentlein R. Receptors and effects of the inhibitory neuropeptide somatostatin in microglial cells. *Molecular Brain Research*. 1998;60(2):228-33. PMID 9757047. doi:10.1016/s0169-328x(98)00184-3

Gabitto MI, Travaglini KJ, Rachleff VM, Kaplan ES, Long B, Ariza J, et al. Integrated multimodal cell atlas of Alzheimer's disease. *Nature Neuroscience*. 2024;27(12):2366-2383. PMID 39402379. doi:10.1038/s41593-024-01774-5

Gilmor ML, Erickson JD, Varoqui H, Hersh LB, Bennett DA, Cochran EJ, et al. Preservation of nucleus basalis neurons containing choline acetyltransferase and the vesicular acetylcholine transporter in the elderly with mild cognitive impairment and early Alzheimer's disease. *The Journal of Comparative Neurology*. 1999;411(4):693-704. PMID 10421878.

Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science (New York, N.Y.)*. 2010;330(6005):841-5. PMID 20966214. doi:10.1126/science.1194637

Griciuc A, Serrano-Pozo A, Parrado AR, Lesinski AN, Asselin CN, Mullin K, et al. Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. *Neuron*. 2013;78(4):631-43. PMID 23623698. doi:10.1016/j.neuron.2013.04.014

Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, et al. TREM2 variants in Alzheimer's disease. *The New England Journal of Medicine*. 2013;368(2):117-27. PMID 23150934. doi:10.1056/NEJMoa1211851

Halle A, Hornung V, Petzold GC, Stewart CR, Monks BG, Reinheckel T, et al. The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nature Immunology*. 2008;9(8):857-65. PMID 18604209. doi:10.1038/ni.1636

Hauglund NL, Andersen M, Tokarska K, Radovanovic T, Kjaerby C, Sørensen FL, et al. Norepinephrine-mediated slow vasomotion drives glymphatic clearance during sleep. *Cell*. 2025;188(3):606-622.e17. PMID 39788123. doi:10.1016/j.cell.2024.11.027

Heneka MT, Nadrigny F, Regen T, Martinez-Hernandez A, Dumitrescu-Ozimek L, Terwel D, et al. Locus ceruleus controls Alzheimer's disease pathology by modulating microglial functions through norepinephrine. *Proceedings of the National Academy of Sciences USA*. 2010;107(13):6058-63. PMID 20231476. doi:10.1073/pnas.0909586107

Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, et al. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature*. 2013;493(7434):674-8. PMID 23254930. doi:10.1038/nature11729

Hoek RM, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, et al. Down-regulation of the macrophage lineage through interaction with OX2 (CD200). *Science (New York, N.Y.)*. 2000;290(5497):1768-71. PMID 11099416. doi:10.1126/science.290.5497.1768

Hong S, Beja-Glasser VF, Nfonoyim BM, Frouin A, Li S, Ramakrishnan S, et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science (New York, N.Y.)*. 2016;352(6286):712-716. PMID 27033548. doi:10.1126/science.aad8373

Hsiao CC, Sankowski R, Prinz M, Smolders J, Huitinga I, Hamann J. GPCRomics of homeostatic and Disease-Associated human microglia. *Frontiers in Immunology*. 2021;12:674189. PMID 34054860. doi:10.3389/fimmu.2021.674189

Iwata N, Tsubuki S, Takaki Y, Watanabe K, Sekiguchi M, Hosoki E, et al. Identification of the major Aβ1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. *Nature Medicine*. 2000;6(2):143-50. PMID 10655101. doi:10.1038/72237

Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson PV, Snaedal J, et al. Variant of TREM2 associated with the risk of Alzheimer's disease. *The New England Journal of Medicine*. 2013;368(2):107-16. PMID 23150908. doi:10.1056/NEJMoa1211103

Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, et al. A unique microglia type associated with restricting development of Alzheimer's disease. *Cell*. 2017;169(7):1276-1290.e17. PMID 28602351. doi:10.1016/j.cell.2017.05.018

Krasemann S, Madore C, Cialic R, Baufeld C, Calcagno N, El Fatimy R, et al. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity*. 2017;47(3):566-581.e9. PMID 28930663. doi:10.1016/j.immuni.2017.08.008

Le LHD, Feidler AM, Rodriguez LC, Cealie M, Plunk E, Li H, et al. Noradrenergic signaling controls Alzheimer's disease pathology via activation of microglial β2 adrenergic receptors. *Brain, Behavior, and Immunity*. 2025;128:307-322. PMID 40245958. doi:10.1016/j.bbi.2025.04.022

Lehrman EK, Wilton DK, Litvina EY, Welsh CA, Chang ST, Frouin A, et al. CD47 protects synapses from excess Microglia-Mediated pruning during development. *Neuron*. 2018;100(1):120-134.e6. PMID 30308165. doi:10.1016/j.neuron.2018.09.017

Liu YU, Ying Y, Li Y, Eyo UB, Chen T, Zheng J, et al. Neuronal network activity controls microglial process surveillance in awake mice via norepinephrine signaling. *Nature Neuroscience*. 2019;22(11):1771-1781. PMID 31636449. doi:10.1038/s41593-019-0511-3

Marschallinger J, Iram T, Zardeneta M, Lee SE, Lehallier B, Haney MS, et al. Lipid-droplet-accumulating microglia represent a dysfunctional and proinflammatory state in the aging brain. *Nature Neuroscience*. 2020;23(2):194-208. PMID 31959936. doi:10.1038/s41593-019-0566-1

Meyer C, Niedermeier T, Feyen PLC, Strübing FL, Rauchmann BS, Karali K, et al. Early locus coeruleus noradrenergic axon loss drives olfactory dysfunction in Alzheimer's disease. *Nature Communications*. 2025;16(1):7338. PMID 40781079. doi:10.1038/s41467-025-62500-8

Morawski M, Brückner G, Jäger C, Seeger G, Arendt T. Neurons associated with aggrecan-based perineuronal nets are protected against tau pathology in subcortical regions in Alzheimer's disease. *Neuroscience*. 2010;169(3):1347-63. PMID 20497908. doi:10.1016/j.neuroscience.2010.05.022

Mufson EJ, Ma SY, Cochran EJ, Bennett DA, Beckett LA, Jaffar S, et al. Loss of nucleus basalis neurons containing trkA immunoreactivity in individuals with mild cognitive impairment and early Alzheimer's disease. *The Journal of Comparative Neurology*. 2000;427(1):19-30. PMID 11042589. doi:10.1002/1096-9861(20001106)427:1<19::aid-cne2>3.0.co;2-a

Nilsson P, Sörgjerd K, Kakiya N, Sasaguri H, Watamura N, Johansson L, et al. Somatostatin receptor subtypes 1 and 4 regulate neprilysin, the major amyloid-β degrading enzyme in brain. *Journal of Alzheimer's Disease : JAD*. 2026;109(2):651-666. PMID 41255128. doi:10.1177/13872877251392782

Pluvinage JV, Haney MS, Smith BAH, Sun J, Iram T, Bonanno L, et al. CD22 blockade restores homeostatic microglial phagocytosis in ageing brains. *Nature*. 2019;568(7751):187-192. PMID 30944478. doi:10.1038/s41586-019-1088-4

Saito T, Iwata N, Tsubuki S, Takaki Y, Takano J, Huang SM, et al. Somatostatin regulates brain amyloid beta peptide Abeta42 through modulation of proteolytic degradation. *Nature Medicine*. 2005;11(4):434-9. PMID 15778722. doi:10.1038/nm1206

Sandoval K, Umbaugh D, House A, Crider A, Witt K. Somatostatin receptor Subtype-4 regulates mRNA expression of Amyloid-Beta degrading enzymes and microglia mediators of phagocytosis in brains of 3xTg-AD mice. *Neurochemical Research*. 2019;44(11):2670-2680. PMID 31630317. doi:10.1007/s11064-019-02890-6

Schober J, Polina J, Walters F, Scott N, Lodholz E, Crider A, et al. NNC 26-9100 increases Aβ1-42 phagocytosis, inhibits nitric oxide production and decreases calcium in BV2 microglia cells. *PloS One*. 2021;16(7):e0254242. PMID 34237100. doi:10.1371/journal.pone.0254242

Sheline YI, West T, Yarasheski K, Swarm R, Jasielec MS, Fisher JR, et al. An antidepressant decreases CSF Aβ production in healthy individuals and in transgenic AD mice. *Science Translational Medicine*. 2014;6(236):236re4. PMID 24828079. doi:10.1126/scitranslmed.3008169

Spittau B, Dokalis N, Prinz M. The role of TGFβ signaling in microglia maturation and activation. *Trends in Immunology*. 2020;41(9):836-848. PMID 32741652. doi:10.1016/j.it.2020.07.003

Stowell RD, Sipe GO, Dawes RP, Batchelor HN, Lordy KA, Whitelaw BS, et al. Noradrenergic signaling in the wakeful state inhibits microglial surveillance and synaptic plasticity in the mouse visual cortex. *Nature Neuroscience*. 2019;22(11):1782-1792. PMID 31636451. doi:10.1038/s41593-019-0514-0

Tejera D, Mercan D, Sanchez-Caro JM, Hanan M, Greenberg D, Soreq H, et al. Systemic inflammation impairs microglial Aβ clearance through NLRP3 inflammasome. *The EMBO Journal*. 2019;38(17):e101064. PMID 31359456. doi:10.15252/embj.2018101064

Tesseur I, Zou K, Esposito L, Bard F, Berber E, Can JV, et al. Deficiency in neuronal TGF-beta signaling promotes neurodegeneration and Alzheimer's pathology. *The Journal of Clinical Investigation*. 2006;116(11):3060-9. PMID 17080199. doi:10.1172/JCI27341

Theofilas P, Ehrenberg AJ, Dunlop S, Di Lorenzo Alho AT, Nguy A, Leite REP, et al. Locus coeruleus volume and cell population changes during Alzheimer's disease progression:

A stereological study in human postmortem brains with potential implication for early-stage biomarker discovery. *Alzheimer's & Dementia*. 2017;13(3):236-246. PMID 27513978. doi:10.1016/j.jalz.2016.06.2362

Ulland TK, Song WM, Huang SC, Ulrich JD, Sergushichev A, Beatty WL, et al. TREM2 maintains microglial metabolic fitness in Alzheimer's disease. *Cell*. 2017;170(4):649-663.e13. PMID 28802038. doi:10.1016/j.cell.2017.07.023

Venegas C, Kumar S, Franklin BS, Dierkes T, Brinkschulte R, Tejera D, et al. Microglia-derived ASC specks cross-seed amyloid-β in Alzheimer's disease. *Nature*. 2017;552(7685):355-361. PMID 29293211. doi:10.1038/nature25158

Walker DG, Dalsing-Hernandez JE, Campbell NA, Lue LF. Decreased expression of CD200 and CD200 receptor in Alzheimer's disease: a potential mechanism leading to chronic inflammation. *Experimental Neurology*. 2009;215(1):5-19. PMID 18938162. doi:10.1016/j.expneurol.2008.09.003

Wang Y, Ulland TK, Ulrich JD, Song W, Tzaferis JA, Hole JT, et al. TREM2-mediated early microglial response limits diffusion and toxicity of amyloid plaques. *The Journal of Experimental Medicine*. 2016;213(5):667-75. PMID 27091843. doi:10.1084/jem.20151948

Zöller T, Schneider A, Kleimeyer C, Masuda T, Potru PS, Pfeifer D, et al. Silencing of TGFβ signalling in microglia results in impaired homeostasis. *Nature Communications*. 2018;9(1):4011. PMID 30275444. doi:10.1038/s41467-018-06224-y

Part Three

The Cage and the Clock

The perineuronal net, the parvalbumin interneuron and the forty-hertz rhythm in late Alzheimer's disease: what breaks when the last brake is digested

The third step. A protective lattice is digested by three converging arms, the cell it protected loses the ability to keep time, and the cortex becomes noisy rather than quiet. At the synapse, four receptor systems deliver contradictory instructions.

Abstract

This is the third and last of the short papers on the model's three stacked steps. The first described the base: brainstem and basal forebrain neurons whose supplies fade from the third decade, and which have been retarding amyloid all along. The second described the step above it: the brain's immune cell, released from nine restraints, which stops clearing amyloid and begins to secrete the enzymes that digest the only brake left. This paper is about what happens when they finish.

The last brake is physical. A condensed lattice of sugar and protein, the perineuronal net, wraps a particular kind of inhibitory neuron and keeps the microglion away from its surface. The cell inside is the parvalbumin interneuron, whose job is timing: it fires fast and precisely, inhibits its neighbours in rhythm, and generates the forty-hertz cortical oscillation on which coordinated cortical activity depends. For six decades it is the best-protected cell in the cortex. Then the net is digested, and it becomes the worst-affected one.

Four consequences follow, and the paper takes them in order. **The net goes**, by proteolysis, and with it the cell's protection against the oxidative cost of its own fast firing. **The parvalbumin cell fails**, which is a failure of inhibition rather than of excitation — and the counter-intuitive result is that removing a brake produces noise, not silence: the Alzheimer cortex is hyperexcitable, carries subclinical epileptiform activity in a substantial minority of patients, and declines faster when it does. **The clock stops**, and with it the mechanism that keeps assemblies of excitatory neurons in phase; the excitatory cells then fail for want of coordination rather than for want of drive. **And at the synapse itself**, amyloid acts through a set of named receptors — the restraint receptor LilrB2 with its complement ligand C4d, the exchange factor Ephexin5 below EphB2, and the reelin–ApoER2 clasp that normally antagonises amyloid at that very membrane — which together describe synapse loss as misdirection rather than damage: nothing is broken, and the instructions have changed.

The paper carries two honest difficulties on its face. The forty-hertz entrainment literature, which is the most visible therapeutic programme aimed at this step, has a failure to replicate at its centre. And the corpus's own analysis argues that preserving the net and driving the rhythm are not two halves of one therapy, because the intervention that preserves the net makes the rhythm worse. The series closes with what the three steps imply

together, and with the measurement that would settle the most important open question in all three.

How to read this paper

This paper follows *What the Brainstem Stops Sending* and *The Hands That Let Go*, and assumes their conclusions, which are summarised in Section 1. As in those papers, each section below condenses a longer corpus volume, and those volumes carry the full argument and their own ledgers.

The grades are the corpus's four. **Established** means replicated in human material by more than one group. **Probable** means good evidence with a gap. **Inference** means a conclusion drawn here, or in the corpus, from evidence gathered for another purpose. **Speculative** means coherent and untested.

Two cautions govern this paper in particular. First, the unit throughout the series is the **connection**, not the cell, because synapse loss is the structural change that tracks cognitive decline most closely — the finding that opened this literature and has never been overturned (Terry and colleagues, 1991; Selkoe, 2002). Second, this step contains the field's most active therapeutic programme and its sharpest replication dispute, and both are reported here in full.

1. Where the Series Has Got To

The first step begins in the third decade, in a handful of small nuclei that each supply one chemical messenger to the whole forebrain. Their axons fail long before their cell bodies, and their supplies fade with them. Three of those supplies hold amyloid back: serotonin brakes its production, somatostatin licenses the enzyme that disposes of it, noradrenaline enables both its cellular clearance and its drainage during sleep. Two of them — noradrenaline and acetylcholine — also hold the brain's immune cell at rest.

The second step begins around the sixth decade, on top of the first. The microglion is not switched on; it is let go, as nine restraints fail in a particular

order. Once released it stops clearing amyloid, assembles the NLRP3 inflammasome, which suppresses clearance further and seeds more amyloid, and secretes the proteases that digest the perineuronal net.

Neither step has ended. That is the point of drawing the model as stacked layers rather than a relay. A person in the eighth decade carries all three at once: the brainstem still failing underneath, the microglion still released, and now a third thing beginning on top of both.

The third step is the one in which the disease becomes visible to other people. It is also the one where almost every drug ever trialled has been given.

2. The Cage: What the Perineuronal Net Is and Does

The perineuronal net is a condensed lattice of extracellular matrix wrapped around the cell body and nearest dendrites of certain neurons. It is built of hyaluronan as a backbone, lecticans — aggrecan and brevican above all — as the load-bearing proteins, link proteins that fasten them to the backbone, and tenascin-R as a cross-link. It is not a shapeless coating. It is a structure, with holes left open at synaptic contacts, and it does several jobs at once (Fawcett and colleagues, 2019).

- **It excludes.** The net physically restricts access to the neuronal surface, which is the office that matters for this series: it is the brake that keeps the released microglion of the second step away from the cell it would otherwise strip.
- **It protects against the cell's own chemistry.** Fast-spiking neurons pay a high metabolic price, and that price is paid in oxidative stress. Nets protect these cells against it, and degrading the net makes them, and the fast rhythms they generate, more vulnerable (Cabungcal and colleagues, 2013). **Established.**
- **It protects against tau.** In subcortical regions of the Alzheimer brain, neurons wearing aggrecan-based nets are protected from tau pathology (Morawski and colleagues, 2010). **Established.**

- **It sets the cell's firing properties.** The net is not inert scaffolding: its brevican component gates the parvalbumin cell's function in an activity-dependent way, organising the ion channels and the inputs the cell needs to fire fast (Favuzzi and colleagues, 2017). **Established.**
- **It closes the critical period.** Nets assemble as juvenile plasticity ends, and removing them in an adult reopens it — in part by *decreasing* inhibition and increasing gamma activity (Lensjø and colleagues, 2017). That result is worth holding onto, because it is the clearest demonstration that what the net does to the network is not simply protective: it is regulatory.

And it is a brake its own target attacks. Microglia facilitate the loss of perineuronal nets in the Alzheimer brain, and chronic depletion of microglia prevents the loss, despite plaques persisting (Crapser and colleagues, 2020). **Established** in models. In human tissue, resilience to Alzheimer's disease — intact cognition despite the pathology — is associated with distinct alterations in these nets, and in people with the disease the genes for net-degrading enzymes are raised (de Vries and colleagues, 2025). **Probable**, from one well-designed human series.

3. How the Cage Is Opened

The corpus calls this transition the **proteolytic turn**, and its argument is that three chemically distinct arms converge on the same structure at the same time.

- **The protease arm.** The inflammasome of the second step matures interleukin-1β, and interleukin-1β is the principal inducer of the aggrecan-degrading enzymes — the ADAMTS aggrecanases and matrix metalloproteinases, MMP-9 chief among them — that cleave the net's core protein. This is the best-characterised route from a cytokine to a protease in matrix biology, but it was characterised in cartilage. The corpus grades it **established** in the joint and **conjecture** in the brain, where the intermediate steps have not been measured (*One Sensor, Two Collapses*).
- **The iron arm.** Iron liberated from failing oligodendrocytes catalyses oxidative damage to the same structure, which is already the cell's

defence against oxidative stress (*The Proteolytic Turn*).

- **The complement arm.** Complement proteins tag what is to be removed, and the tagging reaches the same neighbourhood.

Why three arms matter therapeutically. The corpus's conclusion from this is unusually practical and unusually firm: because three mechanistically distinct arms converge, interdicting any one of them produces partial protection while the other two continue. This window, on the corpus's reading, admits **no single-target intervention** (*The Proteolytic Turn*). **Inference**, and the strongest prediction the third step makes.

4. The Cell: Why the Parvalbumin Interneuron Is the One That Matters

Inside the cage is a particular cell, and its job explains the shape of the whole step.

What it does. The parvalbumin interneuron is a fast-spiking inhibitory cell that synapses on the cell bodies and initial segments of excitatory neurons — the places from which it can veto an output rather than merely shade it. It does not carry content. It supplies timing: it inhibits its neighbours in fast, precise rhythm, keeping cortical activity from running away with itself and holding assemblies of excitatory cells in phase.

Why it is protected for so long, and then is not. It wears the densest net in the cortex. On the model's accounting of connection loss, it is the best-protected of the five cell types counted for six decades — and then, when the lattice is digested, it becomes the worst-affected, losing some 84 per cent of its connections against 54 per cent for the excitatory cells around it. That crossing point is the model's explanation for why the last phase arrives so much faster than the first (*The Shape of the Disease*). **The curves are the model's own and are not a measurement**; the ordering, not the percentages, is the claim.

One correction the corpus has already made, and keeps. A blind re-reading of the evidence found parvalbumin involvement to be late and

functional, with **somatostatin** interneurons failing earlier. The ordering claim survives; the identity of the terminal cell is held more loosely than it once was. The somatostatin cell, as the first paper described, largely has no net at all — which is consistent with it failing earlier and for a different reason.

The decisive causal experiment is from the other direction. Restoring Nav1.1 — the sodium channel the cell needs in order to fire fast — specifically in parvalbumin interneurons corrects network dysfunction and cognitive deficits in an amyloid model (Verret and colleagues, 2012). **Established** in models. That is the cleanest demonstration in the literature that the inhibitory cell's loss of *capacity*, rather than its death, is doing the damage.

5. The Clock: Forty Hertz, and What Stops

What the rhythm is. When parvalbumin cells fire in synchrony they impose a cortical oscillation at roughly forty cycles a second. Gamma rhythm is not an epiphenomenon of busy cortex; it is the mechanism by which populations of excitatory neurons are held in a common timeframe, so that signals arriving together are read together.

It falls early, before plaques. Driving fast-spiking parvalbumin interneurons at gamma frequency reduced amyloid in a mouse model, and the behaviourally driven rhythm was already reduced before plaques formed or cognition declined (Iaccarino and colleagues, 2016). **Probable**, in models.

What driving it appears to do. The programme that followed is the most ambitious therapeutic effort aimed at this step. Multisensory forty-hertz stimulation reduced pathology and improved cognition across models (Martorell and colleagues, 2019); it entrained the rhythm in deeper structures and preserved neurons and synapses in two models of neurodegeneration (Adaikkan and colleagues, 2019); and it promoted the glymphatic clearance of amyloid, the same drainage route the first paper's noradrenaline drives at night (Murdock and colleagues, 2024).

And the replication that the corpus insists on reporting. A separate laboratory, recording with multisite probes in the visual cortex, entorhinal

cortex and hippocampus of two amyloid models, found that forty-hertz flickering light **did not engage native gamma oscillations** in those regions and did not suppress amyloid (Soula and colleagues, 2023). The corpus's position is that this does not refute the clinical programme, which is being tested on its own endpoints, but that it bears directly on the mechanism the programme claims. **The dispute is live and should be presented as live.**

The corpus's own warning, which is sharper than either. It is tempting to pair the two interventions of this step: preserve the lattice, then exercise the cell it protects. *The Price of the Net* argues that this pairing does not survive the evidence. The manipulation that preserves the net — suppressing microglia — **worsens** gamma disruption, increases network hyperactivity, and increases epileptiform discharge in the same animals. Matrix integrity, the intrinsic excitability of the fast-spiking cell, and the network's excitatory–inhibitory set-point are three variables, and only the first is improved by removing microglia. The two interventions act on different variables with different signs, and were joined by an inference about shared substrate rather than by any experiment that ran them together. **Inference**, and the most useful negative result in this part of the corpus.

6. Noise, Not Silence: Why Losing a Brake Produces Hyperactivity

The intuitive picture of a failing brain is one that goes quiet. This step does the opposite, and the evidence is unusually direct.

What is observed. Clusters of hyperactive neurons appear near amyloid plaques in a mouse model, and the measured cause is a relative *decrease* in synaptic inhibition (Busche and colleagues, 2008). Aberrant excitatory activity, non-convulsive seizure activity and compensatory remodelling of inhibitory circuits are documented across amyloid models (Palop and colleagues, 2007), and interneuron dysfunction is the organising theme of the field's principal review of the subject (Palop and Mucke, 2016). **Established** in models.

In patients. Extended monitoring found subclinical epileptiform activity in 42.4 per cent of Alzheimer patients with no history of seizures, against 10.5 per cent of age-matched controls — and the patients with it declined roughly twice as fast on a global cognitive measure, 3.9 points a year against 1.6 (Vossel and colleagues, 2016). **Established**, in one well-conducted prospective series.

The direction of the effective drug tells you the direction of the lesion. Of a panel of antiepileptic drugs, only levetiracetam reduced abnormal spike activity in an amyloid model, and chronic treatment reversed the circuit remodelling, the synaptic deficits and the learning failure (Sanchez and colleagues, 2012). A low dose reduced hippocampal hyperactivation and improved memory in people with amnestic mild cognitive impairment (Bakker and colleagues, 2012). In a randomised crossover trial in Alzheimer's disease the drug did **not** improve the primary endpoint in the group as a whole, but improved performance on spatial memory and executive tasks in the prespecified subgroup with epileptiform activity (Vossel and colleagues, 2021). **Established** that the effect is confined to the hyperexcitable subgroup; **inference** that this is the correct dosing rather than a failed drug.

The rival account, taken seriously. The corpus's adjudication volume gives the opposite reading a fair hearing: that the disease is at bottom a failure of excitatory transmission, and the seizures are what an over-inhibited network does. That is not a fringe position — seizures genuinely can arise from too much inhibition, as in absence epilepsy. But in absence epilepsy the rational and demonstrated therapy is to *reduce* inhibition, and in Alzheimer's disease every intervention that works runs the other way. The adjudication goes to disinhibition (*Low Signal, High Noise*). **Inference**, with the rival's strongest leg conceded.

And the consequence for the excitatory neuron. On this reading the excitatory cell fails not for want of drive but for want of coordination. It is left firing without restraint, in a network whose timing has gone, which is both excitotoxic and uninformative. That is why the model counts connections rather than cells, and why the loss of an inhibitory cell shows up as a loss of excitatory function.

7. At the Synapse: Four Receptors and One Instruction

The preceding sections are about a circuit. This one is about a single synapse, and about what amyloid does there. The corpus's reading, assembled across two volumes, is that **nothing at the synapse is broken**. The molecules are present, often in normal amounts. What has changed is where they are, what covalent marks they carry, and therefore which instruction they are executing. The corpus's phrase is *misdirection, not damage* (*The Hand and the Address*).

Amyloid at the glutamate synapse

Soluble amyloid oligomers, rather than the deposit, are the species that act here. They alter the trafficking of NMDA-type glutamate receptors, promoting their removal from the surface (Snyder and colleagues, 2005). Oligomers extracted **directly from human Alzheimer cortex** inhibit long-term potentiation, enhance its reversal, and reduce dendritic spine density in normal rodent hippocampus — the demonstration that the agent in the human brain confers the deficit (Shankar and colleagues, 2008). **Established.** A receptor complex for this action has been identified: oligomers bound to cellular prion protein couple to Fyn kinase through metabotropic glutamate receptor 5 (Um and colleagues, 2013). **Probable.**

The restraint receptor: PirB, LilrB2, and C4d

This is the receptor set Dr. Truchard asked for, and it is the most developed account of how a synapse is instructed to disassemble.

What the receptor normally does. PirB in the mouse, LilrB2 in the human, is a neuronal receptor that *restrains* plasticity throughout life. Deleting it leaves ocular-dominance plasticity in the visual cortex unrestricted (Syken and colleagues, 2006), and it regulates dendritic spine density cell-autonomously (Vidal and colleagues, 2016). Animals lacking it form more spines and stabilise new ones. **Established.**

What amyloid does to it. PirB and LilrB2 are receptors for soluble amyloid oligomers, binding at nanomolar affinity through the same two domains that bind their normal ligands; engagement enhances cofilin signalling — the same

signalling seen in human Alzheimer brain — and deleting the receptor rescues both long-term potentiation and memory in an amyloid model (Kim and colleagues, 2013). **Established** in models; the human arm is a tissue measurement.

And the ligand that was found later. C4d, a durable cleavage fragment of complement component 4, binds LilrB2 and PirB with nanomolar affinity, colocalises with the receptor at excitatory synapses in human cortex, is elevated in Alzheimer's disease, and mediates synapse pruning (Brott and colleagues, 2025). **Probable**: a recent result from one laboratory. Its importance for this series is that it joins the complement arm of Section 3 to the receptor arm of this one — the same protein family that tags a synapse for a microglion also speaks directly to the neuron's own restraint receptor. The neuron, on this reading, disassembles its own spine.

The exchange factor: EphB2 and Ephexin5

A second account describes the machinery inside the spine rather than the receptor on its surface.

The normal arrangement. Ephexin5 is a RhoA exchange factor that acts as a brake on excitatory synapse formation; EphB receptor signalling degrades it, and that degradation is what permits a synapse to be built (Margolis and colleagues, 2010). **Established.**

What fails. Amyloid oligomers bind EphB2 and trigger its degradation; reducing EphB2 impairs NMDA currents and potentiation, and restoring it in an amyloid model reverses both the plasticity deficit and the memory impairment (Cissé and colleagues, 2011). **Established** in models. With EphB2 gone, the brake is no longer removed: Ephexin5 accumulates, and reducing its expression ameliorates the Alzheimer-like impairment in mice (Sell and colleagues, 2017). **Probable.**

So the spine is not demolished. It is forbidden to rebuild — which is why the corpus describes this limb as a ratchet rather than a weapon.

The clasp: reelin and ApoER2

The fourth receptor system is the one that normally opposes all of this at the same membrane.

What it is. Reelin, secreted in the adult cortex by inhibitory interneurons, binds the lipoprotein receptors ApoER2 and VLDLR, which phosphorylate the adaptor Disabled-1 — and mice lacking either the ligand or both receptors show hyperphosphorylation of tau (Hiesberger and colleagues, 1999). **Established.** The same signal enhances synaptic strength: ApoER2 sits in the postsynaptic density in a complex with the NMDA receptor, reelin markedly enhances long-term potentiation through it, and the enhancement is abolished when the receptors are deleted (Weeber and colleagues, 2002; Beffert and colleagues, 2005). **Established.**

Why it belongs in this paper. Reelin signalling **antagonises amyloid at the synapse**. It prevents the suppression of potentiation and of NMDA-receptor function caused by amyloid at concentrations comparable to the diseased brain — but at high amyloid concentrations the protection is overwhelmed and the reelin-dependent phosphorylation is completely blocked (Durakoglugil and colleagues, 2009). **Established** in slices. The clasp is a brake on this step in the same sense that serotonin is a brake on amyloid production in the first: real, measurable, and finite.

And the genotype term. ApoE4 selectively impairs the recycling of ApoER2, sequestering it along with glutamate receptors inside the cell, so that reelin can no longer enhance synaptic function — and the ability of reelin to protect slices against human Alzheimer brain extract is severely impaired in mice carrying the human ApoE4 isoform (Chen and colleagues, 2010). **Probable.** This is one of the few places in the series where the strongest common genetic risk factor acts on a named mechanism at a named membrane.

Why this is called misdirection rather than damage

Two features of the receptor account deserve stating separately, because they change what kind of lesion this is.

The receptor's own working limb has never been pointed at its assigned targets. The cytoplasmic tail of LilrB2 carries inhibitory motifs that recruit the phosphatases SHP-1 and SHP-2 — the machinery by which the receptor would actually deliver its instruction inside the cell. That was reported in a single clause of the founding paper, and it has one demonstrated neuronal consequence, on a third-party receptor kinase in the same membrane. Searching the indexed literature for the receptor or its phosphatases paired with EphB2, with Ephexin5 or with Disabled-1 returns nothing on point (*The*

Hand and the Address). The hand is described; what it grips is not. **Inference**, from an absence, and stated as such.

And the field's standard measurement cannot see the lesion. If the disease-relevant change is where a molecule sits and what covalent mark it carries, rather than how much of it there is, then homogenising a piece of cortex and reporting a mean is systematically blind to it. The corpus's address claim survives in three of four regulators examined and fails in the fourth — and the failure is informative, because in the two cases where abundance was never measured, the method used could not have seen what moved. **Inference**, and the reason the corpus asks for single-synapse measurements rather than better blots.

What the four accounts say together, and where they collide

Read side by side, the four describe one synapse receiving several instructions at once: a restraint receptor engaged by amyloid and by complement, an exchange-factor brake that can no longer be lifted, and a clasp that would oppose both but is overwhelmed and, in ApoE4 carriers, mislocated.

The corpus does not pretend they agree. *The Hand and the Address* shows that where the receptor account and the exchange-factor account are joined, they **predict opposite signs at the same two residues**, and that the single measurement which would separate them — the phosphorylated fraction of Ephexin5 and of Tiam1, reported jointly across single synapses — has not been attempted in disease tissue although the reagent has existed for sixteen years. That is the most specific unperformed experiment in this series. **Inference**, and the corpus's own.

A supply question runs underneath all four of these receptors and is taken up in the fourth paper. Each is held at the membrane only as long as it is returned there faster than it is removed, which makes a synapse a balance rather than a stock; *The Long Way Back* argues that the return side of that balance is where this disease does its damage, and marks the extension to these four receptors as untested.

Amyloid at two concentrations, and the receptor we have left out

Everything above describes what amyloid does to a synapse. It says nothing about what the molecule is *for*, and the omission distorts the picture — it makes the account read as a list of poisonings rather than as a regulator pushed past its range.

The peptide is required, not merely tolerated. Healthy brains make amyloid-β continuously, in proportion to how hard their neurons are working. At low picomolar concentrations a preparation of amyloid-β42 markedly *increases* long-term potentiation and enhances both reference and contextual fear memory, while high nanomolar concentrations produce the familiar reduction (Puzzo and colleagues, 2008). The dose-response is biphasic.

The stronger version of the result is a subtraction rather than an addition. Removing the animal's *own* peptide — with an antibody against rodent amyloid-β, or by silencing murine precursor protein — **impairs** plasticity and memory in a healthy brain, and adding human amyloid-β42 back rescues the deficit (Puzzo and Arancio, 2013). A molecule whose removal breaks memory in a normal animal is not waste. **Established** in rodent preparations; no human equivalent exists and none is likely.

Where the useful effect happens, and through what. It is not a gentler version of the damage described above. At 200 picomolar, oligomeric amyloid-β acts **presynaptically**: it raises the frequency of miniature excitatory currents, reduces paired-pulse facilitation and increases the number of docked vesicles, which is to say it augments neurotransmitter release. Postsynaptically it lengthens the postsynaptic density and raises the plasticity proteins — phosphorylated CREB and CaMKII, and brain-derived neurotrophic factor — converting early potentiation into late potentiation and short-term memory into long-term. The effects require the peptide to be outside the cell, and they require **α7 nicotinic acetylcholine receptors** (Gulisano and colleagues, 2019). **Established** in models.

That receptor is not one of the four this section is about. It is a fifth, and it is cholinergic.

The four receptors, asked the same question. Setting the positive literature against this section's four produces an asymmetry worth stating plainly rather than smoothing.

Receptor system	What amyloid does at disease concentrations	Is a physiological role known here?
NMDA-type glutamate receptors	Removed from the surface; potentiation suppressed	No. The beneficial effect is presynaptic and α7-dependent, and reaches this receptor only downstream
PirB / LilrB2, with C4d	Bound at nanomolar affinity; cofilin signalling enhanced	No physiological-concentration role reported
EphB2 with Ephexin5	EphB2 degraded; the brake on rebuilding is never lifted	No physiological-concentration role reported
Reelin with ApoER2	Protection against amyloid, overwhelmed at high concentration	**The concentration axis is already here** — reelin preserves potentiation at disease-comparable amyloid and is completely blocked above it (Durakoglugil and colleagues, 2009)
α7 nicotinic receptors	Not characterised as a damage route in this section	**Yes** — this is where the useful effect acts

So the positive role is documented at one receptor, absent from the literature at two, and already implicit at a third without having been named as such. That is a statement about where the field has looked, not a claim that the other three have no physiological arm.

What this implies for the stack, and it is ours. The useful arm of amyloid runs through an acetylcholine receptor. Acetylcholine is what the nucleus basalis supplies, and its withdrawal is a Phase I event — the first paper in this series. The harmful arms run through four postsynaptic receptors that have nothing to do with it, and they are engaged at Phase III concentrations.

Read across the stack, that is a two-stroke failure of a single molecule. **Phase I removes the channel through which amyloid does its useful work; Phase III raises amyloid to the concentrations at which it does harm.** The beneficial arm is lost first and the harmful arms are gained second, in the order the steps occur.

Inference, and ours. No study has measured the α7 arm and the four-receptor arm in the same preparation, and the cholinergic loss and the amyloid rise have never been staged against each other in one animal. It is stated here because it is the kind of claim the series was built to make visible,

and because it names an experiment: raise amyloid to picomolar levels in a preparation with cholinergic input intact and again with it removed, and see whether the benefit survives.

8. What the Third Step Adds Up To

Table 1. The third step, element by element.

Element	What it normally does	What fails	How firm
Perineuronal net	Excludes microglia; protects against oxidative stress and tau; sets fast-firing properties	Digested by proteases, iron and complement acting together	Net loss in AD: established. The three-arm convergence: inference
Parvalbumin interneuron	Perisomatic inhibition; generates the forty-hertz rhythm	Loses the capacity to fire fast, before it dies	Restoring Nav1.1 rescues the network: established in models
Forty-hertz rhythm	Holds excitatory assemblies in a common timeframe	Falls before plaques; driving it is therapeutic in some hands and inert in others	Disputed; the replication failure is live
Excitatory neurons	Carry the content	Fire without restraint, in a network whose timing has gone	Hyperexcitability established in models and in patients
The synapse	Builds, strengthens and prunes under instruction	Receives contradictory instructions through LilrB2, EphB2–Ephexin5 and reelin–ApoER2	Each limb probable or established; their joint reading is inference

Three things follow.

Function goes before the cell, again. This has been true at every step of the series. The brainstem axon is removed while its cell body lives; the nucleus basalis surrenders its receptor before its transmitter; the microglion loses its badge set before it does harm; and here the parvalbumin cell loses its capacity to fire fast before it is lost, while the synapse receives wrong

instructions rather than suffering breakage. **That is the series' single most consistent finding, and it is the ground of whatever hope the model carries.**

The step is a convergence, not a chain. Three proteolytic arms meet on one structure, and four receptor systems meet at one membrane. Single-target intervention is predicted to fail here specifically, and the record of single-target immune and anti-amyloid therapy in late disease is consistent with that.

And almost every drug ever trialled has been given here. If the model is even approximately right, the third step is the last few years of a forty-year process, and the target lies further left.

9. What This Step Implies for Treatment

Four things follow for therapy, and they are unusually specific.

No single-target drug in the turn window. Three arms converge on the net. Interdicting one leaves two running. The corpus states this as a flat prediction (*The Proteolytic Turn*).

Anti-excitability treatment belongs to the subgroup that has the excitability. Levetiracetam helped where epileptiform activity was documented and not otherwise, across an animal model, a mild-cognitive-impairment study and a randomised trial (Sanchez and colleagues, 2012; Bakker and colleagues, 2012; Vossel and colleagues, 2021). That is a dosing instruction, not a failure.

Do not assume the net and the rhythm are one therapy. They act on different variables with opposite signs on excitability, and the experiment that would settle it — microglial suppression against forty-hertz entrainment in one cohort, with net integrity, fast-spiking excitability, gamma power and epileptiform burden as four separate endpoints — has not been run (*The Price of the Net*).

And the target is capacity, not survival. The Nav1.1 result says that restoring the inhibitory cell's ability to fire corrects the network while the cell is still there (Verret and colleagues, 2012). Every step in this series has the

same shape, and this is where it is most directly testable.

10. Graded Ledger

Table 2. Each claim in this paper, with its grade and the evidence it rests on.

#	Claim	Grade	Basis
1	Synapse loss is the structural change that tracks cognition most closely	Established	Terry et al. 1991; Selkoe 2002
2	The perineuronal net protects fast-spiking cells against oxidative stress	Established	Cabungcal et al. 2013
3	Net-bearing neurons are protected against tau	Established	Morawski et al. 2010
4	The net gates parvalbumin-cell function; removing it reopens plasticity and raises gamma	Established	Favuzzi et al. 2017; Lensjø et al. 2017; Fawcett et al. 2019
5	Microglia digest the net; depleting them preserves it	Established (models)	Crapser et al. 2020
6	Human resilience is associated with altered nets	Probable	de Vries et al. 2025
7	Three arms converge on the net, so no single-target drug can work in this window	Inference	*The Proteolytic Turn*; *One Sensor, Two Collapses*
8	The parvalbumin cell is best protected for six decades, then worst affected	Inference; the curves are the model's own	*The Shape of the Disease*
9	Somatostatin interneurons fail earlier; the terminal cell's identity is held loosely	Probable	Blind re-reading recorded in the corpus; Gabitto et al. 2024
10	Restoring Nav1.1 in parvalbumin cells rescues network and cognition	Established (models)	Verret et al. 2012

#	Claim	Grade	Basis
11	Gamma falls before plaques, and driving it reduces pathology	Probable, and disputed	Iaccarino et al. 2016; Martorell et al. 2019; Adaikkan et al. 2019; Murdock et al. 2024
12	Forty-hertz light does not entrain native gamma in two models	Established, as a negative result	Soula et al. 2023
13	Preserving the net and driving the rhythm are not complementary	Inference	*The Price of the Net*
14	The Alzheimer cortex is hyperexcitable, from reduced inhibition	Established (models)	Busche et al. 2008; Palop et al. 2007; Palop and Mucke 2016
15	Subclinical epileptiform activity affects ~42% of patients and doubles the rate of decline	Established, one series	Vossel et al. 2016
16	Anti-excitability treatment helps where hyperexcitability is documented, and not otherwise	Established	Sanchez et al. 2012; Bakker et al. 2012; Vossel et al. 2021
17	Human-brain-derived amyloid oligomers impair plasticity and spine density	Established	Shankar et al. 2008; Snyder et al. 2005; Um et al. 2013
18	LilrB2/PirB restrains plasticity and is a nanomolar amyloid receptor	Established	Syken et al. 2006; Kim et al. 2013; Vidal et al. 2016
19	C4d is a high-affinity LilrB2 ligand, elevated in AD, and prunes synapses	Probable; one laboratory	Brott et al. 2025
20	Amyloid degrades EphB2; Ephexin5 then accumulates and forbids rebuilding	Established (EphB2); probable (Ephexin5)	Cissé et al. 2011; Margolis et al. 2010; Sell et al. 2017
21	Reelin–ApoER2 signalling antagonises amyloid at the synapse and is overwhelmed at high concentrations	Established (slices)	Durakoglugil et al. 2009; Weeber et al. 2002; Beffert et al. 2005; Hiesberger et al. 1999

#	Claim	Grade	Basis
22	ApoE4 impairs ApoER2 recycling and disables the reelin protection	Probable	Chen et al. 2010
23	The receptor and exchange-factor accounts predict opposite signs at the same residues	Inference	*The Hand and the Address*

11. What Would Change This Account

- A human series showing the perineuronal net intact in late Alzheimer cortex. The whole step would need rebuilding.
- A single-target intervention at one of the three proteolytic arms that fully preserves the net. The convergence argument of Section 3 would fail.
- Evidence that the parvalbumin cell dies before it loses its fast-firing capacity. The therapeutic case for acting on capacity rather than survival would go.
- A properly powered trial of forty-hertz entrainment that succeeds on a clinical endpoint while the replication failure stands. The mechanism would then need an account that does not depend on native entrainment.
- A joint measurement of the Ephexin5 and Tiam1 phosphorylation marks across single synapses showing both rising together. One of the two synaptic accounts would be eliminated.
- Levetiracetam helping a whole unselected Alzheimer population. Hyperexcitability would be general rather than a subgroup property.

12. The Three Steps Together

This series has described a disease that begins in the third decade in a handful of brainstem neurons, loosens a braking system on the brain's immune cell in the sixth, and digests a protective lattice in the eighth — with no step ending when the next begins.

Three conclusions hold across all three papers.

Function fails before cells die, everywhere. At every step the measurable loss is of capacity, connection or instruction, and not of the cell. A neuron that has lost its inputs is in a different condition from a dead one, and the model's claim is that much of what is lost in the late step is the former.

The brakes are the story. Amyloid production is braked by serotonin, its disposal licensed by somatostatin, its clearance enabled by noradrenaline; the microglion is restrained by nine separate mechanisms; the parvalbumin cell is caged; the synapse is held by a reelin clasp. The disease is better described as the sequential failure of restraints than as the accumulation of a toxin.

And the target lies further left than the trials have gone. That is the practical conclusion the three papers share, and the one most worth attacking.

The measurement that would settle the most. Whether a silenced neuron can be recovered is the open question on which the hopeful reading of this whole model rests. It has not been answered, and nothing in these three papers answers it.

Corpus papers drawn on

The Shape of the Disease. *The Proteolytic Turn*. *One Sensor, Two Collapses* and *The Net and the Speck*. *The Price of the Net*. *Low Signal, High Noise*: Excitation and Inhibition in Alzheimer's Disease. *The Hand and the Address*: LilrB2, C4d, Ephexin5 and Reelin in Alzheimer's Disease — Misdirection, Not Damage. *No Switch but the Endosome*. *The Architect's Handshake*: The Reelin–ApoER2–Heparan Sulfate Interface. *The Matrix of Collapse* and *The Perineuronal Turn*. *Convergent Synaptic Collapse*. *The Unnetted Governor*. Papers one and two of this series, *What the Brainstem Stops Sending* and *The Hands That Let Go*. All are available in full at AdultCognitiveDisease.com.

References

Adaikkan C, Middleton SJ, Marco A, Pao PC, Mathys H, Kim DN, et al.. Gamma entrainment binds Higher-Order brain regions and offers neuroprotection. *Neuron*. 2019;102(5):929-943.e8. PMID 31076275. doi:10.1016/j.neuron.2019.04.011

Bakker A, Krauss GL, Albert MS, Speck CL, Jones LR, Stark CE, et al.. Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. *Neuron*. 2012;74(3):467-74. PMID 22578498. doi:10.1016/j.neuron.2012.03.023

Beffert U, Weeber EJ, Durudas A, Qiu S, Masiulis I, Sweatt JD, et al. Modulation of synaptic plasticity and memory by Reelin involves differential splicing of the lipoprotein receptor Apoer2. *Neuron*. 2005;47(4):567-79. PMID 16102539. doi:10.1016/j.neuron.2005.07.007

Brott BK, Raissi AJ, Micheva KD, Vielmetter J, Mendes MS, Baccus CJ, et al.. C4d, a high-affinity LilrB2 ligand, is elevated in Alzheimer's disease and mediates synapse pruning. *Proceedings of the National Academy of Sciences USA*. 2025;122(38):e2519253122. PMID 40966293. doi:10.1073/pnas.2519253122

Busche MA, Eichhoff G, Adelsberger H, Abramowski D, Wiederhold KH, Haass C, et al.. Clusters of hyperactive neurons near amyloid plaques in a mouse model of Alzheimer's disease. *Science (New York, N.Y.)*. 2008;321(5896):1686-9. PMID 18802001. doi:10.1126/science.1162844

Cabungcal JH, Steullet P, Morishita H, Kraftsik R, Cuenod M, Hensch TK, et al. Perineuronal nets protect fast-spiking interneurons against oxidative stress. *Proceedings of the National Academy of Sciences USA*. 2013;110(22):9130-5. PMID 23671099. doi:10.1073/pnas.1300454110

Chen Y, Durakoglugil MS, Xian X, Herz J. ApoE4 reduces glutamate receptor function and synaptic plasticity by selectively impairing ApoE receptor recycling. *Proceedings of the National Academy of Sciences USA*. 2010;107(26):12011-6. PMID 20547867. doi:10.1073/pnas.0914984107

Cissé M, Halabisky B, Harris J, Devidze N, Dubal DB, Sun B, et al. Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. *Nature*. 2011;469(7328):47-52. PMID 21113149. doi:10.1038/nature09635

Crapser JD, Spangenberg EE, Barahona RA, Arreola MA, Hohsfield LA, Green KN. Microglia facilitate loss of perineuronal nets in the Alzheimer's disease brain. *EBioMedicine*. 2020;58:102919. PMID 32745992. doi:10.1016/j.ebiom.2020.102919

de Vries LE, Bahnerth A, Swaab DF, Verhaagen J, Carulli D. Resilience to Alzheimer's disease associates with alterations in perineuronal nets. *Alzheimer's & Dementia*. 2025;21(2):e14504. PMID 39737731. doi:10.1002/alz.14504

Durakoglugil MS, Chen Y, White CL, Kavalali ET, Herz J. Reelin signaling antagonizes β-amyloid at the synapse. *Proceedings of the National Academy of Sciences USA*. 2009;106(37):15938-43. PMID 19805234. doi:10.1073/pnas.0908176106

Favuzzi E, Marques-Smith A, Deogracias R, Winterflood CM, Sánchez-Aguilera A, Mantoan L, et al.. Activity-Dependent gating of parvalbumin Interneuron function by the perineuronal net protein brevican. *Neuron*. 2017;95(3):639-655.e10. PMID 28712654. doi:10.1016/j.neuron.2017.06.028

Fawcett JW, Oohashi T, Pizzorusso T. The roles of perineuronal nets and the perinodal extracellular matrix in neuronal function. *Nature Reviews Neuroscience*. 2019;20(8):451-465. PMID 31263252. doi:10.1038/s41583-019-0196-3

Gabitto MI, Travaglini KJ, Rachleff VM, Kaplan ES, Long B, Ariza J, et al. Integrated multimodal cell atlas of Alzheimer's disease. *Nature Neuroscience*. 2024;27(12):2366-2383. PMID 39402379. doi:10.1038/s41593-024-01774-5

Gulisano W, Melone M, Ripoli C, Tropea MR, Li Puma DD, Giunta S, Cocco S, Marcotulli D, Origlia N, Palmeri A, Arancio O, Conti F, Grassi C, Puzzo D. Neuromodulatory action of picomolar extracellular Aβ42 oligomers on presynaptic and postsynaptic mechanisms underlying synaptic function and memory. *Journal of Neuroscience*. 2019;39(30):5986-6000. PMID 31127002.

Hiesberger T, Trommsdorff M, Howell BW, Goffinet A, Mumby MC, Cooper JA, et al. Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron*. 1999;24(2):481-9. PMID 10571241. doi:10.1016/s0896-6273(00)80861-2

Iaccarino HF, Singer AC, Martorell AJ, Rudenko A, Gao F, Gillingham TZ, et al.. Gamma frequency entrainment attenuates amyloid load and modifies microglia. *Nature*. 2016;540(7632):230-235. PMID 27929004. doi:10.1038/nature20587

Kim T, Vidal GS, Djurisic M, William CM, Birnbaum ME, Garcia KC, et al.. Human LilrB2 is a β-amyloid receptor and its murine homolog PirB regulates synaptic plasticity in an Alzheimer's model. *Science (New York, N.Y.)*. 2013;341(6152):1399-404. PMID 24052308. doi:10.1126/science.1242077

Lensjø KK, Lepperød ME, Dick G, Hafting T, Fyhn M. Removal of perineuronal nets unlocks juvenile plasticity through network mechanisms of decreased inhibition and increased gamma activity. *The Journal of Neuroscience*. 2017;37(5):1269-1283. PMID 28039374. doi:10.1523/JNEUROSCI.2504-16.2016

Margolis SS, Salogiannis J, Lipton DM, Mandel-Brehm C, Wills ZP, Mardinly AR, et al.. EphB-mediated degradation of the RhoA GEF Ephexin5 relieves a developmental brake on excitatory synapse formation. *Cell*. 2010;143(3):442-55. PMID 21029865. doi:10.1016/j.cell.2010.09.038

Martorell AJ, Paulson AL, Suk HJ, Abdurrob F, Drummond GT, Guan W, et al.. Multi-sensory gamma stimulation ameliorates Alzheimer's-Associated pathology and Improves cognition. *Cell*. 2019;177(2):256-271.e22. PMID 30879788. doi:10.1016/j.cell.2019.02.014

Morawski M, Brückner G, Jäger C, Seeger G, Arendt T. Neurons associated with aggrecan-based perineuronal nets are protected against tau pathology in subcortical

regions in Alzheimer's disease. *Neuroscience*. 2010;169(3):1347-63. PMID 20497908. doi:10.1016/j.neuroscience.2010.05.022

Murdock MH, Yang CY, Sun N, Pao PC, Blanco-Duque C, Kahn MC, et al.. Multisensory gamma stimulation promotes glymphatic clearance of amyloid. *Nature*. 2024;627(8002):149-156. PMID 38418876. doi:10.1038/s41586-024-07132-6

Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N, et al.. Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron*. 2007;55(5):697-711. PMID 17785178. doi:10.1016/j.neuron.2007.07.025

Palop JJ, Mucke L. Network abnormalities and interneuron dysfunction in Alzheimer disease. *Nature Reviews Neuroscience*. 2016;17(12):777-792. PMID 27829687. doi:10.1038/nrn.2016.141

Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, et al. Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. *Proceedings of the National Academy of Sciences USA*. 2012;109(42):E2895-903. PMID 22869752. doi:10.1073/pnas.1121081109

Selkoe DJ. Alzheimer's disease is a synaptic failure. *Science (New York, N.Y.)*. 2002;298(5594):789-91. PMID 12399581. doi:10.1126/science.1074069

Sell GL, Schaffer TB, Margolis SS. Reducing expression of synapse-restricting protein Ephexin5 ameliorates Alzheimer's-like impairment in mice. *The Journal of Clinical Investigation*. 2017;127(5):1646-1650. PMID 28346227. doi:10.1172/JCI85504

Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, et al.. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nature Medicine*. 2008;14(8):837-42. PMID 18568035. doi:10.1038/nm1782

Puzzo D, Privitera L, Leznik E, Fà M, Staniszewski A, Palmeri A, Arancio O. Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. *Journal of Neuroscience*. 2008;28(53):14537-14545. PMID 19118188.

Puzzo D, Arancio O. Amyloid-β peptide: Dr. Jekyll or Mr. Hyde? *Journal of Alzheimer's Disease*. 2013;33(Suppl 1):S111-S120. PMID 22735675.

Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, et al.. Regulation of NMDA receptor trafficking by amyloid-beta. *Nature Neuroscience*. 2005;8(8):1051-8. PMID 16025111. doi:10.1038/nn1503

Soula M, Martín-Ávila A, Zhang Y, Dhingra A, Nitzan N, Sadowski MJ, et al.. Forty-hertz light stimulation does not entrain native gamma oscillations in Alzheimer's disease model mice. *Nature Neuroscience*. 2023;26(4):570-578. PMID 36879142. doi:10.1038/s41593-023-01270-2

Syken J, Grandpre T, Kanold PO, Shatz CJ. PirB restricts ocular-dominance plasticity in visual cortex. *Science (New York, N.Y.)*. 2006;313(5794):1795-800. PMID 16917027. doi:10.1126/science.1128232

Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, et al.. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of

cognitive impairment. *Annals of Neurology*. 1991;30(4):572-80. PMID 1789684. doi:10.1002/ana.410300410

Um JW, Kaufman AC, Kostylev M, Heiss JK, Stagi M, Takahashi H, et al.. Metabotropic glutamate receptor 5 is a coreceptor for Alzheimer aβ oligomer bound to cellular prion protein. *Neuron*. 2013;79(5):887-902. PMID 24012003. doi:10.1016/j.neuron.2013.06.036

Verret L, Mann EO, Hang GB, Barth AM, Cobos I, Ho K, et al.. Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. *Cell*. 2012;149(3):708-21. PMID 22541439. doi:10.1016/j.cell.2012.02.046

Vidal GS, Djurisic M, Brown K, Sapp RW, Shatz CJ. Cell-Autonomous regulation of dendritic spine density by PirB. *eNeuro*. 2016;3(5). PMID 27752542. doi:10.1523/ENEURO.0089-16.2016

Vossel KA, Ranasinghe KG, Beagle AJ, Mizuiri D, Honma SM, Dowling AF, et al. Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Annals of Neurology*. 2016;80(6):858-870. PMID 27696483. doi:10.1002/ana.24794

Vossel K, Ranasinghe KG, Beagle AJ, La A, Ah Pook K, Castro M, et al. Effect of levetiracetam on cognition in patients with Alzheimer disease with and without epileptiform activity: a randomized clinical trial. *JAMA Neurology*. 2021;78(11):1345-1354. PMID 34570177. doi:10.1001/jamaneurol.2021.3310

Weeber EJ, Beffert U, Jones C, Christian JM, Forster E, Sweatt JD, et al. Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. *The Journal of Biological Chemistry*. 2002;277(42):39944-52. PMID 12167620. doi:10.1074/jbc.M205147200

Part Four

The Long Way Back

Endosomal recycling failure in Alzheimer's disease: retromer, SORL1 and why the lesion sits on the return leg

Not a fourth step but the axis the other three stand on. A cell sorts what it takes in and either destroys it or sends it back; the sending-back is separate machinery, and it is the part that fails — in three places, which are the three steps.

Abstract

The three papers before this one describe three steps of the disease, each with its own decade and its own cast of cells. This paper describes something that runs beside all three.

Every cell in the body takes material in from its own surface, sorts it in a compartment called the early endosome, and then either destroys it or sends it back out. The sending-back is a separate piece of machinery from the taking-in, and it is the piece that fails in this disease. A cargo receptor called SORLA, made by the gene *SORL1*, recognises what is to be saved. A three-protein complex called retromer collects it. A mechanical step then pulls the membrane into a tube and pinches the tube free. If that last step does not happen, the compartment does not empty, and everything waiting in it waits longer — not only the one protein that was being returned.

That single mechanical fact has consequences in three places, and the three places are the three phases. In the brainstem and basal forebrain neurons of Phase I, the endosome is not merely a sorting station but the vehicle that carries a survival signal up a very long axon. In the disposal machinery of Phase II, the same compartment matures into the lysosome, and both the neuron and the microglion depend on it finishing the job. At the synapse of Phase III, the receptors that make a connection work are held at the membrane by a balance between the rate at which they are removed and the rate at which they are returned.

So this is not a fourth step. It is the axis the other three stand on, and it is the only element of the model with a station in all three.

One further finding organises the paper, and it is the corpus's own. Nine long-running research programmes have each spent two or three decades asking what goes wrong at the synapse in this disease, and arrived at nine apparently different answers. Set along the itinerary of one neuron, each occupies a station on a single circuit, and at every station there is a forward leg and a return leg. In eight of the nine, the reported lesion is on the return leg, and the forward leg is frequently not merely intact but increased. That asymmetry is this paper's spine, and it is graded as an inference drawn from other people's published work, not as a finding of our own.

The paper closes with what has been given to people, with the measurement problem that stands in the way of giving them more, a graded ledger, and the results that would undo the account.

How to read this paper

This is the fourth of four short papers and the only one that is not about a phase. The first three can be read in any order; this one is best read after them, because it keeps pointing back at their cells.

Claims are graded where they are made, using the four grades the corpus uses throughout. **Established** means replicated in human material by more than one group. **Probable** means good evidence with a gap, such as a single cohort, or an animal result not yet confirmed in people. **Inference** means a conclusion drawn here, or elsewhere in the corpus, from evidence gathered for another purpose. **Speculative** means coherent and untested.

Two pieces of vocabulary are fixed at the start, because the whole paper turns on them.

A **cycle** is any step a cell performs in two directions: a forward leg that makes, delivers, cuts, engages or instructs, and a **return leg** that recovers, releases, clears, disposes, resolves or restabilises. The distinction is not decorative. A drug that slows a forward leg and a drug that restores a return leg are different kinds of object, they are tested differently, and almost everything tried in this disease so far has been the first kind.

Recycling, in this paper, always means the return leg of membrane traffic specifically: a protein taken into a cell being brought back out to where it was, rather than being destroyed. It is the ordinary word for an ordinary cellular operation, and it is not a metaphor.

1. Why This Is Not a Fourth Step

The three papers before this one are built on a clock. Phase I begins in the third decade of life, in a handful of small nuclei at the base of the brain. Phase II begins in the sixth, when the brain's immune cells lose the restraints that had held them at rest. Phase III begins in the eighth, when the protective matrix around inhibitory neurons is digested and the cortex loses its timing.

The steps are stacked rather than sequential: none ends when the next begins, and a person of seventy-five is carrying all three.

The obvious thing to do with a fourth mechanism is to look for a fourth decade and give it a step of its own. That does not work here, and the reason it does not work is the most useful thing about it.

The endosomal machinery has no decade of its own because it has a station in every one of them. In Phase I it is a transport problem: the signal that keeps a brainstem neuron alive travels to the cell body on an endosome, and if the endosome is deranged the signal does not arrive. In Phase II it is a disposal problem: the same compartment matures into the lysosome, and two different cell types depend on it to finish digesting what they have taken in. In Phase III it is a supply problem: the receptors that make a synapse work are held at the membrane only as long as they are returned there faster than they are removed.

Drawn on the stacked diagram, the three phases are steps and this is a vertical axis cutting through all three. That is not a rhetorical convenience. It is the testable claim of this paper, and Section 3 states what each phase's station is and how old the person is when it is reached.

An omission to declare. The three papers of this series between them run to some twenty-three thousand words and mention this machinery barely at all: no sustained treatment of retromer, of *SORL1*, or of the return leg appears in any of them. That is a real gap and this paper exists to close it. The gap was not deliberate, and it is worth naming because it shows how easily an axis that runs through everything can be left out of an account organised by steps. Each of the three papers now carries one forward-pointing sentence at the place where its own station on this axis belongs.

2. The Machine, in Plain Words

This section is self-contained. A reader who already knows what retromer is can skip to Section 3; nothing later in the paper assumes more than is set out here.

2.1 One counter, two trays

A neuron is continually pulling material in from its own surface — receptors, lipids, whatever has bound to them, and some of its own membrane along with them. All of it arrives at a single compartment, the **early endosome**. That compartment is the cell's sorting counter, and everything that arrives there has one of two fates. It goes onward, to the late endosome and then the lysosome, where it is broken up. Or it is recovered and sent back: to the cell surface, or backwards to the Golgi apparatus for re-use.

Think of a post room with one counter and two trays: one to the incinerator, one return-to-sender. The incinerator tray is the easy operation, because gravity is on its side — a compartment left alone matures towards the lysosome. The return tray needs work, and three separate things have to go right.

2.2 Three things that have to go right

Something has to recognise what is worth saving. That is the job of a cargo receptor. The one that matters most in this disease is **SORLA**, the protein made by the gene *SORL1*. It binds the amyloid precursor protein and directs it away from the route that produces amyloid, and it does so by gripping the recovery machinery with its own short tail inside the cell.

Something has to collect it. That is **retromer**: a complex of three proteins — VPS35, VPS26 and VPS29 — that assembles on the surface of the endosome. VPS35 is the scaffold and does the cargo recognition; VPS26 sits at one end and engages the cargo receptors; VPS29 sits at the other and recruits regulatory partners. Retromer does not have one cargo. In Scott Small's recurring phrase it is a *master conductor*: whatever the neuron needs returned passes through it. That is the structural reason one fault can produce several pathologies at once.

Something has to physically get the parcel off the counter. The membrane has to be deformed into a narrow tube, and the tube has to be pinched free so that it can travel. Proteins called sorting nexins do the deforming; the pinching — the **fission** step — is performed by scission machinery of the dynamin class, and it is regulated. This is the step that is easiest to overlook and the most recently identified as a drug target, which Section 9 returns to.

A parcel that is recognised, collected and tubulated but never cut loose has not been returned. The counter is still occupied.

2.3 Why a jam is worse than a blockage

Here is the move that makes this a disease mechanism rather than a piece of cell biology (Small et al., 2017). Stall the exit, and **residency time** in the endosome rises — for everything held there, not only for the protein whose return was being attempted.

Two properties of that compartment turn raised residency into pathology.

It is **acidic**. BACE1, the enzyme that makes the first, amyloid-producing cut in the amyloid precursor protein, works best at acidic pH. So substrate and enzyme held together for longer in an acidic space makes more amyloid, and more of the C-terminal fragments that precede it.

It is **where synaptic receptors wait**. The glutamate receptors that make a dendritic spine responsive are not permanent fixtures. They are removed and returned continuously, and between cycles they sit in this compartment. Receptors held there are receptors not at the synapse.

From that one mechanical fact, four pathologies follow in parallel rather than in series:

- **Amyloid.** Precursor protein and BACE1 spend longer together in an acidic compartment; the amyloidogenic cut is favoured.
- **Synaptic failure.** Glutamate receptors that should go back to the postsynaptic membrane are retained and diverted towards degradation. The synapse weakens without any cell dying.
- **Tau release.** A congested endosome matures into a multivesicular body; cytosolic contents, tau included, are packaged into internal vesicles and released when that body fuses with the surface — a route out of the cell.
- **Glial change.** Microglia use the same recovery machinery to return their own phagocytic receptors to their surface, so the same lesion blunts clearance.

That parallelism is the claim. It is why this is an axis and not a step: four effects, one cause, no ordering required among them.

2.4 What the evidence for it actually is

It is worth arranging the support by what each kind of study can establish, because the summaries in circulation do not.

Association in human tissue, which is where most readers meet this account, is the weakest of the four tiers. VPS35 and VPS26 are reduced in vulnerable regions (Small et al., 2005); VPS26b and SORLA are reduced in human trans-entorhinal cortex in disease (Simoes et al., 2021). Both are post-mortem, and therefore from the end of the process. **Established** as association; silent on direction.

Perturbation in models is stronger. Retromer-deficient mice develop memory and synaptic dysfunction with raised endogenous amyloid-β, and retromer-deficient flies carrying human precursor protein and BACE develop neuronal loss and amyloid aggregates (Muhammad et al., 2008). Phosphatidylinositol-3-phosphate, which organises endosomal traffic, is depleted in Alzheimer tissue, and reducing the kinase that makes it enlarges neuronal endosomes and enhances amyloidogenic processing (Morel et al., 2013).

Depletion and repletion is the strongest design available in an animal, and it is the one that matters. VPS35 was depleted in mouse hippocampal neurons and then restored with an optimised viral vector; accelerated precursor-protein cleavage and loss of synaptic glutamate receptors both followed the manipulation in **both** directions. The same study found that a deficit in the *neuron's* retromer was by itself sufficient to induce the dystrophic microglial morphology characteristic of the disease, independently of tau (Qureshi et al., 2022).

Human genetics is the only part of the case that carries the direction of causation without a model organism, and Section 8 takes it up.

So the account does not mainly rest on having seen jams in human brains. It rests on the fact that inducing the lesion produces the phenotype, that reversing it removes the phenotype, and on a gene.

3. The Two Clocks

Jim's question when this paper was commissioned was a question about time: when does each of these failures become important? The honest answer has two parts, because the pathway has two clocks and they are not the same clock.

3.1 Clock one: the machine gets slower

The capacity of this machinery declines with age in everybody, disease or no disease. The clearest demonstration is also the one that runs the right way round. In cultured mouse neurons aged *in vitro*, internalisation of the amyloid precursor protein **rises** with neuronal age, and that rise is what drives the age-related synapse loss: blocking amyloid production reversed the synaptic decline of the aged neurons, and driving internalisation upward in young neurons reproduced it (Burrinha et al., 2021). Taking-in goes up; the return leg does not keep pace.

That finding needs a discipline set beside it. The dentate gyrus — the one part of the hippocampal circuit this disease spares — has its own age-related decline, traceable to a different molecular lesion entirely (Pavlopoulos et al., 2013). Ordinary age-related memory loss and this disease are separable, they have different molecular signatures in different subregions, and an account of the disease that explains ordinary ageing equally well has explained neither.

Clock one is slow, general and present in everyone. By itself it produces forgetting, not dementia.

3.2 Clock two: the load arrives

What makes this a disease is that the load on a declining machine rises at particular ages, and each rise belongs to one of the three phases. Table 1 states what is known about when.

Table 1 — What is known about when, on this axis.

Approximate age	What is reported	Where	Grade
Before birth, and through childhood	Enlarged early endosomes in neurons in Down syndrome, in some neurons before birth	Human tissue (Cataldo et al., 2000)	Established
Third to fifth decade	No direct human measurement of this machinery exists in this window	—	Gap
Before amyloid deposition, sporadic disease	Markedly enlarged early endosomes with increased delivery of digestive enzymes to them, at early neuropathological stages	Human neocortex (Cataldo et al., 1997, 2000)	Established
Mild cognitive impairment, before dementia	Rab GTPases up-regulated in individually captured cholinergic basal forebrain neurons	Human tissue, single-cell expression (Ginsberg et al., 2011)	Established
Across the clinical transition	Endosomal regulatory genes up-regulated in individually captured CA1 pyramidal neurons, from normal ageing through mild cognitive impairment to disease	Human tissue, single-cell expression (Ginsberg et al., 2010)	Established
Throughout, independent of disease	Precursor-protein internalisation rises with neuronal age and drives synapse loss	Cultured mouse neurons (Burrinha et al., 2021)	Probable in people
Any age, modifier	*APOE* ε4 accentuates the endosomal enlargement	Human tissue (Cataldo et al., 2000); neuronal models	Probable

Four things in that table deserve emphasis.

The abnormality is **early**. Among the structural abnormalities of this disease, enlargement of the early endosome has the best claim to be the first thing that happens. In Down syndrome it is present decades before dementia and, in some neurons, before birth.

It is early **relative to amyloid**, which is the claim that costs something. The 1997 and 2000 reports found it at early neuropathological stages and before deposition. That is a statement about order in human tissue, and it is the reason this machinery is treated as upstream rather than consequential.

The **third to fifth decade is empty**. There is no human measurement of this machinery in the window during which Phase I is, on the model's own account, already under way. This is the largest single hole in the paper and it is stated as a hole, not smoothed over. The corpus's Phase I evidence for those decades is tau in the locus coeruleus, not endosomes.

And the up-regulations are **up**, not down. In both Ginsberg studies what rises is the machinery, in the cells that are about to fail. Section 7 argues that this is the signature of a return leg that is not keeping up, rather than of a forward leg that has gone wrong — and that the distinction is the single most useful thing the corpus has to offer about this literature.

4. The First Address: The Long Axon

4.1 A cell with a distance problem

The neurons of Phase I have a problem that no other cell in this story has, and it is purely geometric. A cholinergic neuron of the nucleus basalis sits at the base of the brain and sends a single thin axon to the cortex — a journey of several centimetres, which for a cell is an enormous distance. The survival signal it depends on, nerve growth factor, is not made at the cell body. It is picked up at the far end, by the axon terminal, and it has to be carried all the way home.

It is carried on an endosome. For this cell, the compartment described in Section 2 is not only a sorting counter; it is the vehicle. A sorting fault in any other cell makes a mess locally. In this cell it cuts the line between the terminal and the nucleus.

4.2 What the evidence shows

The evidence here is unusually direct, and it comes almost entirely from Ralph Nixon's laboratory.

Trophic signalling from Rab5 endosomes declines early in both Alzheimer's disease and Down syndrome. In human tissue, the Rab GTPases are transcriptionally up-regulated in individually captured cholinergic basal forebrain neurons in mild cognitive impairment — that is, before dementia is diagnosed (Ginsberg et al., 2011). **Established** in human material.

In animals, the lesion is sufficient on its own. Directly over-activating Rab5 — with no amyloid mutation, no tau, nothing else — recapitulates prodromal and neurodegenerative features of the disease, including cholinergic degeneration (Pensalfini et al., 2020). So does over-expressing APPL1, a Rab5 effector on the early endosome, which reproduces the endosomal and synaptic dysfunction together with cholinergic neurodegeneration (Jiang et al., 2025). An earlier study established the link in the other direction, showing that APPL1 mediates the endosome dysfunction induced by the precursor protein's β-cleaved fragment in Down syndrome and Alzheimer's disease (Kim et al., 2016). **Established** in the animals.

And it is reversible in the animals by three independent routes: raising nerve growth factor, lowering the β-cleaved fragment, or reversing Rab5 activation pharmacologically. Three routes to the same rescue is a strong argument that the pathway, and not some incidental consequence of the manipulation, is what is doing the damage.

4.3 What Nixon says the tributaries are

Asked directly, in correspondence in November 2026, what tips the endosome off balance in the sporadic disease, Nixon answered that the C-terminal fragment, cholesterol and *APOE* ε4 are all tributaries to the river of Rab5 activation, and that over-activation blocks the transport of both nerve growth factor and brain-derived neurotrophic factor. His group has extended the same account to frontotemporal dementia and is preparing a review of these early tributaries across Alzheimer's and Parkinson's disease.

This is recorded here as his framing, given to us directly, and it is **not yet a published claim in that form**. It is included because it is the clearest available statement of how several apparently separate risk factors could act on one step, and because it is falsifiable: it predicts that each tributary, applied alone, raises Rab5 activity in the same cells.

4.4 What this contributes to the first paper

What the Brainstem Stops Sending makes an argument from a control case. The pedunculopontine nucleus is a cholinergic group in the brainstem that accumulates tangles and nonetheless keeps its neurons, and the reason it offers is that this nucleus does not depend on the growth factor the disease cuts off. What dies is not the cholinergic cell but the dependent one.

Read alongside this section, that control case becomes a prediction about traffic. If the lesion is a failure to deliver a trophic signal up a long axon on an endosome, then the cell that does not need that delivery should survive, whatever else accumulates in it — which is what is observed. The two arguments are the same argument approached from opposite ends.

This is an **inference**, and the gap in it should be named: nobody has measured endosomal transport in the pedunculopontine nucleus. The nucleus is rarely studied at all, because it requires dedicated dissection.

5. The Second Address: The Disposer

5.1 Downhill is not automatic

The second address is the far end of the same compartment. An endosome that is not emptied matures: late endosome, then lysosome, where the contents are digested. That maturation needs acid. The lysosome runs at a pH around 4.5, maintained by a pump in its membrane, and its digestive enzymes do not work without it.

Two different cells in this story depend on that step, and both acquire the same lesion.

5.2 The neuron

If acidification fails, nothing downstream digests, and the consequence in the neuron is not a quiet accumulation. In mouse models, faulty autolysosome acidification induces an autophagic build-up of amyloid-β inside neurons, and those neurons die in place in a characteristic morphology, the cell body itself becoming a senile plaque (Lee et al., 2022). **Established** in the models; the corresponding human study is now accepted for publication.

What jams the pump. For years the acidification failure was a described fact without a named cause. Nixon's laboratory supplied one. The β-cleaved fragment of the amyloid precursor protein — the uncut stub usually written C99 — when phosphorylated on a single tyrosine, binds into a pocket on the V0a1 subunit of the pump and competitively blocks the rest of the pump from assembling, lowering its activity. Reducing that phosphorylation restores the pump and lysosomal function in Down syndrome fibroblasts and in the brains of Down syndrome model mice; reducing it *below* normal constitutive levels raises pump assembly above baseline, which suggests the fragment tunes the pump in health as well as disabling it in disease (Im et al., 2023). **Established** in human fibroblasts and mouse brain.

The detail worth holding on to is which part of the fragment does it. The sequence that binds the pump is the YENPTY motif — the tag whose ordinary job is to signal that the protein should be pulled in from the cell surface. The same few residues that drive a protein into the endosome, once phosphorylated, disable the pump at the far end of the same pathway. This paper's axis and this section's disposal step turn out to be two functions of one molecule, which is the subject of a companion paper, *The Same Defendant*.

Two consequences deserve drawing out.

On mitochondria. Damaged mitochondria can only pile up if the disposal system has already failed: the recycling machinery still wraps them but cannot digest them. The tagging is competent; the failure is in degradation. On this account "mitophagy" is not an independent event, and a bioenergetic story in which mitochondrial failure is the first domino has the order the wrong way round. This is Nixon's position, stated plainly, and it is a live disagreement rather than a settled matter.

On where the plaque comes from, a dispute this paper does not resolve. On the account just given, the plaque arises from the cell body of a neuron

that ruptures where it stands. On Gunnar Gouras's account, the accumulated peptide relocalises to the axon terminals and deposits far from the soma — and there is a measurement: somatic amyloid in CA1 fell by roughly forty per cent as plaques appeared at the corresponding terminals (Roos et al., 2021). As descriptions of how a raised internal pool damages a synapse the two compose. As accounts of the plaque's origin they do not, and both cannot be right.

5.3 The microglion

The second cell is the one charged with clearing up, and what happens to it is worse than a simple loss of capacity.

In human induced-pluripotent-stem-cell microglia, loss of SORLA decreased lysosomal degradation and lysosomal enzyme activity through altered trafficking of the enzymes themselves. Phagocytic uptake of fibrillar amyloid-β **and of synaptosomes** was *increased* while degradation was *reduced*, so both substrates accumulated aberrantly inside lysosomes, and lysosomal exocytosis — the route by which a cell gets rid of indigestible material — was impaired (Mishra et al., 2025). **Supported**: one group, one system, human cells.

The direction is the important part. The cell responsible for removing synaptic material takes in more of it and digests less. That is not a brake failing off; it is a swallow with no stomach behind it.

And there is a route from the neuron to the microglion that needs no amyloid at all. In the depletion-and-repletion study already cited, a deficit in the *neuron's* retromer was by itself sufficient to induce the dystrophic microglial morphology characteristic of the disease, independently of tau (Qureshi et al., 2022). **Established** in mouse.

5.4 What this contributes to the second paper

The Hands That Let Go is built around nine brakes — the restraints that make a microglion different from an ordinary macrophage, and that are released one by one across the sixth and seventh decades. This section does not add a tenth brake, and it would be a mistake to file it as one.

The brakes decide **whether** the microglion acts. This decides whether the action **finishes**. A microglion with every brake intact and a failed lysosome still accumulates what it has swallowed; a microglion with every brake

released and an intact lysosome does damage of an entirely different kind. The two failures are independent, they are measured differently, and the second paper's account of amyloid clearance — three removal routes, four mechanisms of loss — is incomplete without the digestion step underneath all of them.

This is an **inference** from the two bodies of work set side by side. No study has manipulated a brake and the lysosome in the same microglion.

6. The Third Address: The Synapse

6.1 A synapse is a balance, not a stock

The third address is the one that matters most for cognition, because synapse loss remains the strongest structural correlate of cognitive decline in this disease — better than plaque burden, better than tangle burden, established for three decades and now measurable in living people (DeKosky and Scheff, 1990; Terry et al., 1991; Mecca et al., 2020).

A dendritic spine is, functionally, a set of receptors held at a membrane: glutamate receptors that make it responsive, neurotrophin receptors that make it maintainable, and a precursor protein whose own function remains contested but whose residence in the endosome is the measured variable. Each of those is removed from the surface by endocytosis and returned, or not, by retrieval.

So the quantity that sets a synapse's strength is not a stock but a **balance** — the rate at which receptors leave against the rate at which they come back. That is the sentence this whole paper exists to establish, and it changes what a therapy would have to do.

6.2 Three cargoes, one receptor, one step

The result that establishes it is four years old and has not entered the summaries.

Working in isogenic human induced-pluripotent-stem-cell neurons engineered to have *SORL1* either depleted or over-expressed, Mishra and

colleagues tracked three transmembrane proteins through the endosomal network and tested the functional consequence with surface-recycling and lysosomal-degradation assays. The three were the amyloid precursor protein; TRKB, the receptor for brain-derived neurotrophic factor; and GLUA1, the subunit that carries most fast excitatory transmission in the forebrain (Mishra et al., 2022).

Trafficking of **all three** cargoes was altered by manipulating SORLA. Depletion significantly impaired the recycling pathway for the precursor protein **and** for the glutamate receptor, at the level of the recycling endosome and of delivery to the cell surface. Raising SORLA **enhanced** recycling for both. And the glutamate-receptor effect was not merely morphological: it registered as a change in network activity on a multi-electrode array. **Established** in human neurons, by bidirectional manipulation — the strongest design available short of a person.

The therapeutic reading changes accordingly. On the amyloid reading, restoring SORLA lowers a toxic product. On the supply reading, restoring SORLA returns a glutamate receptor to a membrane and makes a neuron answerable to brain-derived neurotrophic factor again. These are not the same benefit, they would be measured differently, and only the first has ever been used as the end-point of a preclinical study of this pathway.

6.3 Why the disease starts where it starts

The model's account of place belongs here, because it is an account about recycling capacity specifically.

Neurons carry a second retromer core, built around VPS26b rather than VPS26a, and dedicated to the recycling arm. The two forms are not redundant. The trans-entorhinal cortex — the small piece of cortex where this disease reliably begins — depends on that recycling-dedicated core most heavily, and in the diseased human brain both VPS26b and SORLA are depleted there (Simoes et al., 2021). **Probable**: one group, human tissue, end-stage.

The region the disease starts in is the region that was already running closest to its recycling capacity. That is an explanation of selective vulnerability that does not invoke amyloid at any point, and it is the single most attractive feature of this account.

6.4 What this contributes to the third paper

The Cage and the Clock devotes a section to four receptors and one instruction: amyloid acting at the NMDA receptor, PirB and LilrB2 with C4d as a ligand, EphB2 with Ephexin5, and reelin with ApoER2. Each of those is a surface receptor on a dendrite, and Section 6.1 says what a surface receptor is: a balance between removal and return.

So the endosomal axis is the supply side of every one of them. That is the natural reading, and it has to be marked clearly as an **inference**, because the supporting work does not exist. Nobody has measured PirB, LilrB2, EphB2 or ApoER2 recycling through SORLA or through retromer. The three cargoes that have been measured are the three named above. Extending the finding to the receptors of the third paper is a prediction this paper makes, not a result it reports, and Section 10 lists it among the things that would change the account if tested and found false.

7. The Finding: Eight of Nine on the Return Leg

7.1 Nine answers to one question

Nine long-running research programmes have each spent two to three decades asking what happens, mechanically, at the synapse in this disease. They arrived at nine apparently different answers: a sterol, an aldehyde, a proteolytic fragment, a sorting complex, a retained peptide, a scaffolding protein, a surface protease, an inhibitory receptor, a competition signal. Read one at a time they look like rival origin stories, and the field has largely read them that way.

Set them instead along the itinerary of a lipid and a membrane protein through the compartments of one neuron, and each occupies a distinct station on a single circuit. At every station there is a cycle. **In eight of the nine, the reported lesion is on the return leg.**

Table 2 — The nine programmes, by leg.

Programme	Forward leg — intact or increased	Return leg — the reported lesion
Astrocytic cholesterol supply (Rappoport)	Candidate generation runs on	Resolution never begins
Peroxidation at the receptor (Ramsden)	Ligand binds; is internalised	Acid release fails; ApoER2 not recovered
The uncleared lipid signal (Area-Gómez)	C99 made; the correction runs	Clearance of C99 fails; signal stays on
Retromer-dependent sorting (Small)	Endocytosis normal or accelerated	Retrieval jams; residency time rises
Intraneuronal retention (Gouras)	Production up in loaded cells	Secretion falls; surface neprilysin lost
The membrane platform (Head)	Ligand present; receptors made	Platform unmaintained; receptors delocalise
Surface proteolysis (Margolis)	Activity-driven synthesis runs on	Surface proteasome falls; trimming fails
The system of restraint (Shatz, Brott)	Ligand engagement rises	Restraint not lifted; cofilin stays active
Competitive plasticity (Huang)	Axons compete; signal secreted	Monomer lost to aggregate; no resolution

Five of the nine state this in their own summary vocabulary, without using the word: *retention rather than overproduction*, *the traffic jam*, *a homeostatic correction left switched on*, *chronic candidate generation*, *failure of local disposal*.

A tenth programme — the endosomal–lysosomal–autophagy account that supplies most of Sections 4 and 5 — is absent from the table only because its object is a network rather than the synapse. It makes the point more explicitly than any of the nine.

7.2 Why this is not a tautology

The obvious objection is that "return leg" is elastic enough to accommodate anything, and that any lesion can be described as a failure to undo something. The answer is that the account makes a prediction a tautology could not, and the prediction has been tested.

A disease of over-production predicts products elevated and machinery normal. A disease of **failed return** predicts something stranger: products

elevated, return machinery reduced, *and forward machinery up-regulated in compensation*, because a homeostat that detects a shortage at the surface responds by making more.

The third is documented. Amyloid production is up-regulated in cells carrying an internal load, because the homeostat has detected a low external pool (Roos et al., 2021). Precursor-protein internalisation rises in aged neurons (Burrinha et al., 2021). Across the thirteen stations of the full circuit, forward-leg up-regulation is reported at six.

It also explains the thing a step model explains badly: how a modest deficit becomes a progressive one over twenty years. Because the cycle **stalls** rather than stopping, the cell keeps paying the forward cost for a return that never arrives — small at any instant, compounding over decades. No accelerating insult is required, and none has been found.

7.3 The exception, recorded rather than absorbed

One programme does not fit, and it is recorded here rather than argued away.

C4d is a nanomolar ligand of LilrB2. It rises with age and further in Alzheimer cortex, and delivered to normal adult cortex it strips dendritic spines — a loss abolished in animals lacking PirB (Brott et al., 2025). That is a **forward-leg excess**: a signal is added, and the addition alone produces the lesion. Hence eight of nine, not nine.

That the exception is the newest mechanism in the set, and has the cleanest demonstration in normal tissue, is a caution against over-reading the pattern. A ninth programme breaking the rule in the most rigorous available design is exactly how a real pattern starts to come apart.

7.4 How this claim is graded

The return-leg finding is the corpus's own and is graded **Inference**, at the strength the word implies. It is a structural reading of other people's published work, arrived at by placing nine independent literatures on one itinerary. No experiment has been done to test it as such. It has not been peer-reviewed. Its value is that it is cheap to check — anyone who knows one of these nine literatures can look at one row and say whether it is fairly stated — and that it makes the therapeutic prediction in Section 9.

What would weaken it: a station where the lesion is demonstrably on the forward leg and the return leg is intact, as C4d may already be; or a demonstration that the forward-leg up-regulations are artefacts of post-mortem tissue or of the culture models in which some were measured.

8. The Genetics Agree, With One Complication

Everything to this point is mechanism, and mechanism does not establish direction. Genetics does, and here it does.

8.1 The strongest single link

Rare truncating variants in *SORL1* are found almost exclusively in people who have Alzheimer's disease. In December 2025 these were mapped across the receptor's structure in a very large cohort, assigning pathogenicity by domain rather than treating the gene as a single unit (Andersen, de Waal et al., 2025). **Established**.

A gene whose loss-of-function variants appear almost exclusively in people who develop the disease is not a bystander, and no amount of argument about what is downstream of what can make it one. This is the causal spine of the whole account.

The mechanism of one variant has been resolved to the step this paper is about. The p.Y1816C variant causes autosomal dominant Alzheimer's disease by impairing the receptor's **dimerisation on the endosome** — the molecular event that governs its association with retromer (Jensen et al., 2024; mechanism established in Jensen et al., 2023). A dominant Alzheimer mutation whose demonstrated lesion is a failure to engage the recovery machinery is as direct as this kind of evidence gets.

8.2 The four classes, stated honestly

The convergence argument usually offered alongside this gives endosomal recycling the status of a hub on which the disease's other genetic risk factors act as spokes (Small and Petsko, 2020), and is usually stated as four separate classes of Alzheimer risk gene converging on endosomal function. The classes are real. They do not contribute equally, and the corpus has said so before.

- **Endosomal trafficking genes —** *SORL1*, *BIN1*, *PICALM*, *CD2AP* — act on membrane traffic directly, immediately and mechanistically, and *SORL1* carries the rare-variant evidence above.
- **Precursor-processing genes —** *APP*, *PSEN1*, *PSEN2* — act conditionally. Amyloidogenic cleavage happens mostly in the acidic endosomal lumen, so a jam favours that cut — but this is a consequence of prior endosomal dysfunction, not independent evidence of it, and the temporal primacy is exactly what is in dispute.
- **Lipid handling —** *APOE* — acts indirectly. The ε4 variant interferes with endosomal recycling, but its effects are not confined to this compartment.
- **Innate immunity —** *TREM2* — acts indirectly and in a different cell. Microglial phagocytic receptors need retrieval machinery to get back to the surface, so a recycling failure blunts clearance — a parallel mechanism in a parallel cell type, not the neuronal lesion.

Labelling all four as equally converging obscures a difference in kind. A hub that four gene classes converge on is a strong claim about explanatory reach; a hub that one class acts on directly while three touch it obliquely is a weaker one, and the weaker reading is what the evidence supports.

The broader picture is consistent without being decisive. The largest association study of the disease identified seventy-five loci, and pathway analysis places endocytosis and lysosomal function among the most strongly enriched pathways — alongside immunity and lipid metabolism, not instead of them (Bellenguez et al., 2022).

8.3 The complication: which cell carries the risk

One recent result makes the genetics harder to use, and it should not be buried.

The strongest known protective common variant at the *SORL1* locus was examined in donor-derived and genome-engineered stem-cell lines, and its effect was found to be uniquely linked to functional expression of the receptor **in microglia**, the protective allele associating with higher expression and reduced pro-inflammatory responses. The effect appeared in donor lines but not in isogenic lines carrying the variant alone, which the authors read as a haplotype acting in context rather than a single sufficient nucleotide (Gorniak-Walas et al., 2026). A separate integrated study of human brain tissue, primary microglia and engineered lines found that SorLA deficiency induces endoplasmic-reticulum stress and interferon signalling, promotes lipid-droplet accumulation and impairs phagocytosis, with the lipid-droplet phenotype reproduced in neurons (Haq et al., 2026).

The consequence is uncomfortable and worth stating plainly. When human genetics attributes risk to *SORL1*, the cell type through which that risk operates has become an open question rather than a settled one. The neuron-centred reading developed in Sections 4 and 6 is a reading of what the receptor does **in a neuron**. It is not a demonstration that the neuronal arm is where the population-level risk is carried.

9. Therapy, and the Measurement Problem

9.1 Why this pathway is attractive to a drug developer

The anti-amyloid antibodies now licensed slow decline modestly (van Dyck et al., 2023). This account offers a specific reason the effect might be smaller than the completeness of plaque removal would predict: the internal and external pools are actively coupled, and lowering the external pool in a cell that already carries an internal load may *increase* production rather than reduce it (Roos et al., 2021). That is a forward-leg intervention meeting a return-leg lesion. The alternative is to restore a recovery step, and three classes of agent exist, at very different stages.

9.2 Stabilise the complex

Retromer is a three-protein complex, which means it has a weakest interface, which means it is a candidate for stabilisation by a small molecule rather than by a gene. Pharmacological chaperones were designed to do exactly that and were shown to limit precursor-protein processing (Mecozzi et al., 2014). In a human stem-cell model they reduced tau phosphorylation **independently of the precursor protein —** which is the result that makes the hub claim more than rhetoric (Young et al., 2018). They rescue the endosomal pathology produced by *SORL1* defects in human neurons (Mishra et al., 2023). And in September 2025 an independent laboratory gave a retromer chaperone to amyloid-model mice and reported pathology falling and synaptic gene expression recovering (Ramonet et al., 2025).

Probable. The honest caveat is that the early-generation chaperones have modest potency and short half-lives, and are better described as research tools than as candidate medicines. Independent replication in an animal is nonetheless the most encouraging development in this pathway.

9.3 Raise the receptor

If loss of SORLA function causes disease in people, restoring it should help. A gene-therapy programme exists, and its obstacle is mundane: the human *SORL1* gene is too large for the viral vector, so truncated versions retaining the critical domains have had to be engineered (Andersen et al., 2024, conference abstract only).

The most important recent result tested the arm this programme had claimed since 2008 and never reached in an animal: raising SORLA in **aged** tauopathy mice attenuated tau phosphorylation, seeding, synapse loss and glial activation, and deleting it made all of them worse (Huang et al., 2026).

A correction of ours belongs here. The corpus's August 2026 paper described that study as reversing established pathology. It did not. The receptor was raised from the beginning, not introduced after the damage had accumulated; the authors' own word is *attenuate*. The paper has been corrected. What the result shows is protection across the life of the animal. Reversal from a treatment start — which is what a patient would need — remains untested in this pathway by anyone.

9.4 Target the fission step

The newest line targets the mechanical step from Section 2.2 rather than the complex itself. RhoGEF12 regulates the endosomal SORL1–retromer step, and inhibiting it is reported to be therapeutic in human neuronal models of the disease (Qureshi et al., 2026, preprint). A kinase or an exchange factor is a far more tractable drug target than a three-protein complex, which is the strategic argument for it.

This is **Speculative** as a therapy and is given one paragraph deliberately. It is a 2026 preprint. It sits inside an unresolved dispute about the sign of the relevant signalling — whether the kinase arm should be inhibited or supported depends on measurements that two laboratories report with opposite signs in the same cell type, and the corpus treats that contradiction as unadjudicated. Readers who want that argument should go to *The Three Cargoes*, which sets it out at length. It is mentioned here only so that its absence from the rest of this paper is deliberate rather than an oversight.

9.5 The measurement problem

The obstacle in front of all three classes is not the drug. It is that **there is no accepted way to measure endosomal recycling function in a living person.**

That has four consequences. You cannot tell which patients have the lesion, so you cannot select for a trial. You cannot tell whether a dose engaged the target, so a negative result cannot distinguish a wrong hypothesis from a wrong dose. You cannot tell whether the pathway is still worth repairing at the stage at which patients present. And you cannot establish the thing this paper most needs — whether the lesion precedes the pathology in individual people rather than in autopsy series.

Three candidate read-outs exist, all early. Spinal-fluid proteins have been linked to retromer-mediated traffic in mice and in humans, offering a panel rather than a single marker (Simoes et al., 2020). Soluble SORL1 in cerebrospinal fluid has been proposed as a marker of the functional impact of rare *SORL1* variants (de Waal et al., 2026). And a luminescent reporter assay now quantifies SORL1 ectodomain shedding and retromer-dependent recycling activity, though in cells rather than in people (Fazeli et al., 2026).

The corpus's recommendation is unchanged and is stated here as the paper's main practical conclusion: **measure it in people before treating it.** A pathway this well supported mechanically and this poorly instrumented

clinically is a pathway that will produce an ambiguous trial, and an ambiguous trial in this field sets the question back a decade.

10. Graded Ledger

#	Claim	Grade	Resting on	What would overturn it
1	Early endosomes are enlarged in Alzheimer neurons before amyloid deposition, and in Down syndrome decades before dementia	Established	Cataldo 1997, 2000; Ginsberg 2010, 2011 (human)	A powered human series finding the change only after deposition
2	Stalling endosomal exit raises residency time for all cargo, not only the precursor protein	Established	Small 2017; Morel 2013	Export blocked with non-APP cargo residency unchanged
3	Inducing retromer deficiency produces the phenotype; restoring retromer removes it	Established (mouse)	Qureshi 2022; Muhammad 2008	Failure of the repletion arm elsewhere
4	SORLA traffics and recycles GLUA1 and TRKB as well as APP	Established (human neurons)	Mishra 2022, bidirectional	A bidirectional human-neuron study showing APP alone
5	Rab5 over-activation alone is sufficient to produce cholinergic degeneration	Established (mouse)	Pensalfini 2020; Jiang 2025; Kim 2016	Failure to replicate outside the originating laboratory
6	Rare truncating *SORL1* variants occur almost exclusively in people with the disease, and one dominant mutation acts by impairing SORLA dimerisation on the endosome	Established	Andersen, de Waal 2025; Jensen 2024, 2023	Comparable variant frequency in controls

#	Claim	Grade	Resting on	What would overturn it
7	The trans-entorhinal cortex depends on a recycling-dedicated retromer core and is depleted of it in disease	Probable	Simoes 2021 (one group, end-stage)	Independent series showing no regional VPS26b difference
8	Precursor-protein internalisation rises with neuronal age and drives synapse loss	Probable in people	Burrinha 2021 (cultured mouse)	Human data showing internalisation flat with age
9	Microglial lysosomal digestion fails while uptake rises, in the same cell	Supported	Mishra 2025 (one group, human iPSC)	Uptake and degradation shown falling together
10	Retromer stabilisation reduces tau phosphorylation independently of APP	Probable	Young 2018; Mishra 2023; Ramonet 2025	The independent animal replication failing to hold
11	In eight of nine synapse-directed programmes, the lesion is on the return leg	**Inference (ours, not peer-reviewed)**	Nine published literatures; forward-leg up-regulation at six of thirteen stations	A station with a forward-leg lesion and intact return; or the up-regulations proving artefactual
12	The endosomal axis is the supply side of the four synaptic receptors in Phase III	**Inference (ours, untested)**	Mishra 2022 for three cargoes; extension by analogy	PirB, EphB2 or ApoER2 recycling shown unaffected
13	The pedunculopontine nucleus survives because it does not require endosomal trophic delivery	**Inference (ours, untested)**	Paper 1's control case read against Section 4	Endosomal transport measured there and found deranged
14	C99, cholesterol and *APOE* ε4 act as tributaries to Rab5 activation	**Probable; Nixon's framing, given in correspondence, not yet published in this form**	Correspondence, Nov 2026; Kim 2016 for the C99 arm	Any one tributary alone failing to raise Rab5 activity

#	Claim	Grade	Resting on	What would overturn it
15	There is no validated human read-out of endosomal recycling function	Established (by absence)	Simoes 2020; de Waal 2026; Fazeli 2026, all early	A validated, replicated human assay

11. What Would Change This Account

Four results would change it, and two of them are cheap.

A human series finding endosomal enlargement only after amyloid deposition. This is the load-bearing claim of the whole axis. The existing evidence is from 1997 and 2000, from one group, in small series. It has never been attempted at modern scale. If the order reversed, the pathway would become a consequence of the disease rather than an upstream driver, and almost everything in Sections 4 to 6 would have to be re-read as sequelae.

A measurement of this machinery in the third to fifth decade. Table 1 has a hole where Phase I is. If the machinery is intact in those decades in people who later develop the disease, then the axis does not run beside Phase I and the paper's central structural claim is wrong for the earliest third of the lifespan.

A forward-leg lesion with an intact return leg. The C4d result may already be this. A second clean example would move the return-leg finding from an organising structure to a tendency with exceptions, which is materially weaker.

A trial. No return-leg agent in this pathway has been given to a person. Until one is, most of Section 10 describes cells and animals. The measurement problem in Section 9.5 is what stands between the pathway and that trial, and it is the thing most worth the field's money.

Where this sits in the series

What the Brainstem Stops Sending describes the base of the stack: the small nuclei that fail from the third decade, and what each withdraws from the forebrain. *The Hands That Let Go* describes the second step: the nine restraints that keep the brain's immune cells at rest, and what happens as they are released. *The Cage and the Clock* describes the third: the digestion of the perineuronal net, the failure of fast-spiking inhibition, and what amyloid does at the synapse.

This paper describes the axis that runs through all three, and it is the only one of the four that can be read as a claim about every phase at once. Its three addresses — the long axon, the disposer, the synapse — correspond to the three steps in order, and the correspondence is the argument.

Corpus papers drawn on

Causal and Common (the endosomal theory, and Small's programme assessed); *The Three Cargoes* (SORL1 as a supply line, and the ROCK2 sign problem); *The Other Half of the Cycle* (thirty-three recovery steps, graded, with the eight drugs already given to people); *The Acid Test* (Nixon's endosomal–lysosomal–autophagy account); *The Same Machine* (Gouras, intraneuronal retention); *No Switch but the Endosome* (restraint and termination); *The Common Shape* (the return-leg finding, stated for a general scientific reader); *The Gate and the Ransom* (the nucleus basalis).

References

Andersen OM, de Waal MWJ, Monti G, Tesi N, Jensen AMG, de Geus C, van Spaendonk R, Vogel M, van der Lee SJ, Olsen JG, Hulsman M, Holstege H. Domain mapping of disease mutations reveals pathogenic SORL1 variants in Alzheimer's disease. *Molecular Neurodegeneration*. 2025;20(1):122. PMID 41327266.

Andersen OM, Qureshi YH, Jensen AMG, Williams A, Young JE, Roy M, Klein J, Armstrong R, Merchant KM. AAV9-SORL1 gene therapy for Alzheimer's disease. *Alzheimer's and Dementia*. 2024;20(Suppl 1):e088971. [Conference abstract.] PMCID PMC11713639.

Bellenguez C, Kucukali F, Jansen IE, et al. New insights into the genetic etiology of Alzheimer's disease and related dementias. *Nature Genetics*. 2022;54(4):412-436. PMID 35379992.

Brott BK, Xu M, Djurdjevic I, Tsai HC, Shatz CJ, et al. C4d, a high-affinity LilrB2 ligand, is elevated in Alzheimer's disease and mediates synapse pruning. *Proceedings of the National Academy of Sciences USA*. 2025. PMID 40966293.

Burrinha T, Martinsson I, Gomes R, Terrasso AP, Gouras GK, Almeida CG. Upregulation of APP endocytosis by neuronal aging drives amyloid-dependent synapse loss. *Journal of Cell Science*. 2021;134(9):jcs255752. PMID 33910234.

Cataldo AM, Barnett JL, Pieroni C, Nixon RA. Increased neuronal endocytosis and protease delivery to early endosomes in sporadic Alzheimer's disease: neuropathologic evidence for a mechanism of increased beta-amyloidogenesis. *Journal of Neuroscience*. 1997;17(16):6142-6151. PMID 9236226.

Cataldo AM, Peterhoff CM, Troncoso JC, Gomez-Isla T, Hyman BT, Nixon RA. Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome: differential effects of APOE genotype and presenilin mutations. *American Journal of Pathology*. 2000;157(1):277-286. PMID 10880397.

DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Annals of Neurology*. 1990;27(5):457-464. PMID 2360787.

de Waal MWJ, van der Lee SJ, Lunding M, et al., Andersen OM, Teunissen CE, Holstege H. Soluble SORL1 in cerebrospinal fluid as a marker for functional impact of rare SORL1 variants. *Alzheimer's and Dementia*. 2026;22(2):e71042. PMID 41685556.

Fazeli E, Jan A, Huber AK, Jensen AMG, Fazeli E, Klein J, Merchant K, Andersen OM. A luminescent reporter assay to quantify SORL1 ectodomain shedding and retromer-dependent endosome recycling activity. *Journal of Biological Chemistry*. 2026;302(2):111136. PMID 41519200.

Ginsberg SD, Alldred MJ, Counts SE, Cataldo AM, Neve RL, Jiang Y, Wuu J, Chao MV, Mufson EJ, Nixon RA, Che S. Microarray analysis of hippocampal CA1 neurons implicates early endosomal dysfunction during Alzheimer's disease progression.

Biological Psychiatry. 2010;68(10):885-893. PMID 20655510.

Ginsberg SD, Mufson EJ, Alldred MJ, Counts SE, Wuu J, Nixon RA, Che S. Upregulation of select rab GTPases in cholinergic basal forebrain neurons in mild cognitive impairment and Alzheimer's disease. *Journal of Chemical Neuroanatomy*. 2011;42(2):102-110. PMID 21669283.

Gorniak-Walas M, Telugu NS, Rudolph IM, Diecke S, Willnow TE. Alzheimer's disease risk single nucleotide polymorphism rs11218343 is linked to functional expression of SORL1 in microglia. *Alzheimer's and Dementia*. 2026;22(4):e71390. PMID 42002722.

Haq I, Ngo JC, Roy N, et al., Olah M, Sher F. Alzheimer's disease risk protein SorLA regulates ER homeostasis and lipid metabolism in human microglia, with conserved effects in neurons. *Acta Neuropathologica*. 2026;151(1):37. PMID 41942750.

Huang H, Shi CH, Yang W, et al., Huang TY. SORLA up-regulation suppresses pathological effects in aged tauopathy mouse brain. *Science Advances*. 2026;12(29):eaed6825. PMID 42467771.

Im E, Jiang Y, Stavrides PH, Darji S, Erdjument-Bromage H, Neubert TA, Choi JY, Wegiel J, Lee JH, Nixon RA. Lysosomal dysfunction in Down syndrome and Alzheimer mouse models is caused by v-ATPase inhibition by Tyr-phosphorylated APP βCTF. *Science Advances*. 2023;9(30):eadg1925. PMID 37494443.

Jensen AMG, Kitago Y, Fazeli E, Vaegter CB, Small SA, Petsko GA, Andersen OM. Dimerization of the Alzheimer's disease pathogenic receptor SORLA regulates its association with retromer. *Proceedings of the National Academy of Sciences USA*. 2023;120(4):e2212180120. PMID 36652482.

Jensen AMG, Raska J, Fojtik P, et al., Sleegers K, Bohaciakova D, Holstege H, Andersen OM. The p.Y1816C variant causes impaired endosomal dimerization and autosomal dominant Alzheimer's disease. *Proceedings of the National Academy of Sciences USA*. 2024;121(37):e2408262121. PMID 39226352.

Jiang Y, Sachdeva K, Goulbourne CN, et al., Mathews PM, Nixon RA. Increased neuronal expression of the early endosomal adaptor APPL1 replicates Alzheimer's disease-related endosomal and synaptic dysfunction with cholinergic neurodegeneration. *Journal of Neuroscience*. 2025;45(29):e2331242025. PMID 40514243.

Kim S, Sato Y, Mohan PS, Peterhoff C, Pensalfini A, Rigoglioso A, Jiang Y, Nixon RA. Evidence that the rab5 effector APPL1 mediates APP-betaCTF-induced dysfunction of endosomes in Down syndrome and Alzheimer's disease. *Molecular Psychiatry*. 2016;21(5):707-716. PMID 26194181.

Lee JH, Yang DS, Goulbourne CN, et al., Staufenbiel M, Nixon RA. Faulty autolysosome acidification in Alzheimer's disease mouse models induces autophagic build-up of Abeta in neurons, yielding senile plaques. *Nature Neuroscience*. 2022;25(6):688-701. PMID 35654956.

Mecca AP, Chen MK, O'Dell RS, et al., Carson RE, van Dyck CH. In vivo measurement of widespread synaptic loss in Alzheimer's disease with SV2A PET. *Alzheimer's and Dementia*. 2020;16(7):974-982. PMID 32400950.

Mecozzi VJ, Berman DE, Simoes S, Vetanovetz C, Awal MR, Patel VM, Schneider RT, Petsko GA, Ringe D, Small SA. Pharmacological chaperones stabilize retromer to limit APP processing. *Nature Chemical Biology*. 2014;10(6):443-449. PMID 24747528.

Mishra S, Knupp A, Szabo MP, Williams CA, Kinoshita C, Hailey DW, Wang Y, Andersen OM, Young JE. The Alzheimer's gene SORL1 is a regulator of endosomal traffic and recycling in human neurons. *Cellular and Molecular Life Sciences*. 2022;79(3):162. PMID 35226190.

Mishra S, Knupp A, Kinoshita C, Williams CA, Rose SE, Martinez R, Theofilas P, Young JE. Pharmacologic enhancement of retromer rescues endosomal pathology induced by defects in the Alzheimer's gene SORL1. *Stem Cell Reports*. 2023;18(12):2434-2450. PMID 37949073.

Mishra S, Morshed N, Sidhu SB, Kinoshita C, Stevens B, Jayadev S, Young JE. The Alzheimer's disease gene SORL1 regulates lysosome function in human microglia. *Glia*. 2025;73(7):1329-1348. PMID 40183375.

Morel E, Chamoun Z, Lasiecka ZM, Chan RB, Williamson RL, Vetanovetz C, Dall'Armi C, Simoes S, Point Du Jour KS, McCabe BD, Small SA, Di Paolo G. Phosphatidylinositol-3-phosphate regulates sorting and processing of amyloid precursor protein through the endosomal system. *Nature Communications*. 2013;4:2250. PMID 23907271.

Muhammad A, Flores I, Zhang H, et al., Arancio O, Small SA. Retromer deficiency observed in Alzheimer's disease causes hippocampal dysfunction, neurodegeneration, and Abeta accumulation. *Proceedings of the National Academy of Sciences USA*. 2008;105(20):7327-7332. PMID 18480253.

Pavlopoulos E, Jones S, Kosmidis S, Close M, Kim C, Kovalerchik O, Small SA, Kandel ER. Molecular mechanism for age-related memory loss: the histone-binding protein RbAp48. *Science Translational Medicine*. 2013;5(200):200ra115. PMID 23986399.

Pensalfini A, Kim S, Subbanna S, et al., Basavarajappa BS, Nixon RA. Endosomal dysfunction induced by directly overactivating Rab5 recapitulates prodromal and neurodegenerative features of Alzheimer's disease. *Cell Reports*. 2020;33(8):108420. PMID 33238112.

Qureshi YH, Berman DE, Marsh SE, Klein RL, Patel VM, Simoes S, Kannan S, Petsko GA, Stevens B, Small SA. The neuronal retromer can regulate both neuronal and microglial phenotypes of Alzheimer's disease. *Cell Reports*. 2022;38(3):110262. PMID 35045281.

Qureshi YH, Williams CA, Hajdu I, Kannan S, Govindarajan A, Vegh B, Petsko GA, Young JE, Zavodszky P, Small SA. RhoGEF12 regulates endosomal SORL1-retromer and its inhibition is therapeutic in human neuronal models of Alzheimer's disease. *bioRxiv*. 2026;2026.03.06.709427. [Preprint.] PMID 41889823.

Ramonet D, Daerr A, Hallbeck M. Stabilizing the retromer complex rescues synaptic dysfunction and endosomal trafficking deficits in an Alzheimer's disease mouse model. *Acta Neuropathologica Communications*. 2025;13(1):190. PMID 40931359.

Roos TT, Garcia MG, Martinsson I, Mabrouk R, Israelsson B, Deierborg T, Kobro-Flatmoen A, Tanila H, Gouras GK. Neuronal spreading and plaque induction of intracellular Abeta and its disruption of Abeta homeostasis. *Acta Neuropathologica*.

2021;142(4):669-687. PMID 34272583.

Simoes S, Neufeld JL, Triana-Baltzer G, Moughadam S, Chen EI, Kothiya M, Qureshi YH, Patel V, Honig LS, Kolb H, Small SA. Tau and other proteins found in Alzheimer's disease spinal fluid are linked to retromer-mediated endosomal traffic in mice and humans. *Science Translational Medicine*. 2020;12(571):eaba6334. PMID 33239387.

Simoes S, Guo J, Buitrago L, et al., Andersen OM, Small SA. Alzheimer's vulnerable brain region relies on a distinct retromer core dedicated to endosomal recycling. *Cell Reports*. 2021;37(13):110182. PMID 34965419.

Small SA, Kent K, Pierce A, Leung C, Kang MS, Okada H, Honig L, Vonsattel JP, Kim TW. Model-guided microarray implicates the retromer complex in Alzheimer's disease. *Annals of Neurology*. 2005;58(6):909-919. PMID 16315276.

Small SA, Simoes-Spassov S, Mayeux R, Petsko GA. Endosomal traffic jams represent a pathogenic hub and therapeutic target in Alzheimer's disease. *Trends in Neurosciences*. 2017;40(10):592-602. PMID 28962801.

Small SA, Petsko GA. Endosomal recycling reconciles the Alzheimer's disease paradox. *Science Translational Medicine*. 2020;12(572):eabb1717. PMID 33268506.

Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Annals of Neurology*. 1991;30(4):572-580. PMID 1789684.

van Dyck CH, Swanson CJ, Aisen P, et al., Iwatsubo T. Lecanemab in early Alzheimer's disease. *New England Journal of Medicine*. 2023;388(1):9-21. PMID 36449413.

Young JE, Fong LK, Frankowski H, Petsko GA, Small SA, Goldstein LSB. Stabilizing the retromer complex in a human stem cell model of Alzheimer's disease reduces TAU phosphorylation independently of amyloid precursor protein. *Stem Cell Reports*. 2018;10(3):1046-1058. PMID 29503090.