The Same Machine

How the synapse fails in Alzheimer's disease — an evaluation of Gunnar Gouras's cell biology, twenty-eight years of work, and why it explains how the disease damages the brain without having to explain how it begins

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A nerve cell manufactures amyloid on the same organelles it uses to run its synapses. One machine, two jobs — so a fault in it is necessarily both an amyloid event and a plasticity event, whatever caused the fault. This is an account of how synapses fail that needs no theory of how the disease starts, and is stronger for it.

Contents

Abstract

A nerve cell manufactures amyloid-β on the same organelles it uses to run its synapses. The enzymes that make the first cut in the amyloid precursor protein are enriched on synaptic vesicles. The compartments that hold the peptide are the compartments that recycle glutamate receptors, deliver lipid, and return worn cargo to the cell body for disposal. One machine does both jobs. That single structural fact is what Gunnar Gouras's laboratory has established, piece by piece, since 1998, and it has a consequence his own framing has tended to obscure: a fault in that machine is necessarily both an amyloid event and a plasticity event, and it is so whatever caused the fault.

This paper evaluates the whole programme rather than any one claim of it, and it asks a specific question of the work: what does it establish about how synapses fail, considered separately from the proposition that this is where the disease starts?

The answer assembled here has five parts. **Location determines product.** Different compartments make different peptides — the longer, aggregation-prone amyloid-β42 is made and retained in ways the shorter species is not — so where a molecule is cut determines what is made and what happens to it. **The pathology is one of retention, not overproduction.** The most under-cited finding in this corpus is that amyloid secretion becomes *impaired* in diseased neurons, and that the enzyme which normally degrades the peptide at the cell surface is lost from it. **A loaded compartment damages the synapse by at least four partly independent routes:** withdrawal of glutamate receptors from the surface; inhibition of the protein-disposal system, so that the accumulating cargo disables the machinery that would clear it; physical distension and structural failure of the compartment itself; and suppression of the local protein synthesis on which lasting plasticity depends. **Synaptic activity is the regulator** — use lowers the retained pool and protects the synapse, and in the programme's most striking experiment, chronically reducing activity in a mouse model *lowered plaque burden while worsening synapse loss and memory*. **The lesion is the loss of the control loop**, not the presence of the peptide: in diseased neurons the coupling between activity and clearance fails, the homeostatic machinery that holds firing near a set point is disrupted, and cells already carrying an internal load stop correcting at all.

Read this way the account is *cause-agnostic by construction*. At least eight distinct upstream conditions raise the retained intraneuronal pool — ageing, apolipoprotein E4, precursor-protein mutations, gene dosage, trafficking-gene variants, seeded aggregates, loss of the degrading enzyme, and reduced activity itself — and the downstream mechanism is the same in each case. A theory of that shape does not compete with theories of onset; it is the layer they must pass through to produce a symptom. This is why the work survives the difficulties of the amyloid cascade as an origin story: it never needed one.

Gouras has nonetheless argued for one, under the name of the inside-out amyloid hypothesis, and this evaluation concludes that the claim costs him more than it earns. It is the part of the programme with no quantitative human support, it is what most summaries foreground, and it makes a body of convergence evidence look like a rival origin story it cannot be adjudicated as. The weaknesses are stated plainly: heavy reliance on precursor-over-expressing animals, thin independent replication of the two most consequential results, an unresolved residue of synaptic effects that belong to the precursor protein rather than to amyloid at all, and no clinical candidate in twenty-eight years. What stands, and stands well, is the best available cell-biological account of how a synapse in this disease actually stops working.

1. Introduction: What Kind of Theory Is This?

1.1 A programme, not a paper

Gunnar Gouras began publishing on amyloid in 1998, in work with Paul Greengard's group in New York, and has continued for twenty-eight years, latterly at Lund University in Sweden. The output runs to roughly a hundred papers. It is not a single hypothesis defended over time; it is a programme with a consistent object of study — where the amyloid-β peptide is made inside a nerve cell, what it does there, and what that has to do with the failure of synapses.

The programme has stated itself several times: at length in a 2010 review of intraneuronal amyloid and synapse pathology (Gouras et al., 2010) and a 2015 review of the peptides and the plaques (Gouras et al., 2015), and compactly in a 2020 submission to the Oskar Fischer Prize, *The synaptic endosome: at the intersection of synapse pathology and apolipoprotein E4*, which distils the position into six pages. None of these is a substitute for evaluating the laboratory work, and each states the position more strongly than the data behind it. The prize submission is used here as the author's own summary of what he takes the work to mean, not as its evidence.

Programmes of this kind are hard to evaluate. Individual papers are reviewed at publication; hypotheses are debated in the abstract; but the thing itself — a sustained line of laboratory work with a cumulative shape — is rarely assessed as a whole. That is what this paper attempts.

1.2 The framing problem

Alzheimer's disease research has a structural bias toward theories of onset. The field's organising question for thirty years has been *what starts it* — the amyloid cascade hypothesis is precisely an answer to that question, and was restated as such at its twenty-fifth anniversary (Selkoe and Hardy, 2016) — and every mechanistic account is pulled toward answering that question whether or not its evidence supports one. Gouras's work has been pulled in the same direction. It is usually presented — including by him — as a rival origin story: the disease begins inside the neuron, at the synapse, and plaques are the residue.

This framing does the work a disservice, for a reason that is worth stating at the outset. Theories of onset are adjudicated by temporal priority in human tissue, and human tissue gives one frozen frame of a process that runs for two decades. Almost no theory of onset in this field can be settled, which is why so many coexist. Theories of *convergence* are different. They claim that whatever the first

event, it must pass through a particular mechanism to produce a particular symptom, and they are adjudicated by necessity and sufficiency — questions that can be answered in the laboratory.

Nearly all of the evidence in this corpus is of the second kind.

1.3 What this evaluation asks

Two questions organise what follows.

What does this body of work establish about how a synapse fails? Not what causes the disease — what happens, mechanically, at the point where the damage that matters clinically actually occurs. Synapse loss remains the best structural correlate of cognitive decline, better than plaques or tangles (Terry et al., 1991; Selkoe, 2002), so this is not a peripheral question.

Can that account stand without a claim about origins? If it can, then its standing is independent of how the argument about initiation is eventually settled, and it should be read and cited differently from the way it usually is.

Sections 2 to 9 assemble the mechanism from the primary work. Section 10 answers the second question. Sections 11 to 13 state the weaknesses, the refutation conditions, and the practical consequences.

2. Location Determines Product

2.1 Two compartments, two peptides

The founding result of the programme is not about disease at all. It is about geography.

The amyloid-β peptide is cut from a larger membrane protein, the amyloid precursor protein, by two successive enzymatic cuts. The second cut is imprecise, and produces peptides of slightly different length — principally forty amino acids (Aβ40) and forty-two (Aβ42). The two-residue difference matters enormously: Aβ42 is stickier, aggregates far more readily, and is the species enriched in plaques.

In 1999, working with Jeffrey Greenfield and Huaxi Xu in Greengard's laboratory, Gouras helped establish that these peptides are not made in the same place (Greenfield et al., 1999). Using three independent approaches — imaging in intact neurons, density-gradient separation of cell compartments, and reconstitution of the reaction outside the cell — they showed that Aβ40 is generated exclusively in the trans-Golgi network and packaged into secretory vesicles for export, whereas a population of Aβ42 is generated and *retained* in the endoplasmic reticulum in an insoluble state. A further pool of Aβ42 is made in the trans-Golgi network and exported.

The conclusion is easy to state and its consequences take twenty years to unfold: **the compartment in which the precursor is cut determines which peptide is made and whether it leaves the cell.** Amyloid is not one substance produced at one site. It is a family of products, sorted by geography, some destined for export and some not.

The year before, Gouras had shown that neurons themselves generate and regulate the different peptide variants (Gouras et al., 1998) — which is to say the cell is not a passive site of a chemical accident but an active regulator of what it makes.

2.2 The enzymes sit on the synaptic vesicle

If geography determines product, the next question is where in the cell the relevant geography lies. Part of the answer arrived much later, and it is more specific than most summaries convey.

Working with Susanne Frykman and colleagues at the Karolinska Institute, the group showed that the two enzymes competing for the first cut of the precursor protein — BACE1, which begins the amyloid-producing route, and ADAM10, which pre-empts it — are both strongly enriched on *synaptic vesicles* isolated from rat brain (Lundgren et al., 2015). ADAM10 enzymatic activity was detectable in the vesicle fraction; the intermediate fragments produced by the first cut were enriched

there too. Of the complex that performs the *second* cut, only one component was enriched, and active enzyme co-localised with the synaptic-vesicle marker only sparsely.

The precision of that result is worth pausing on. **The first step of amyloid production happens on the machinery of neurotransmitter release. The last step happens elsewhere.** The synapse is not merely exposed to amyloid; it manufactures the precursor fragments locally, on the organelles it uses to transmit.

A companion paper showed that nerve terminals release the peptide by a route that does not require electrical activity, and so operates continuously (Lundgren et al., 2014).

2.3 Amyloid inside the human neuron

The programme's best-known result came in 2000. Examining human brain tissue, Gouras and colleagues reported that Aβ42 accumulates *within* neurons in the regions vulnerable to Alzheimer's disease, in a distribution that appears to precede both plaques and tangles (Gouras et al., 2000). Two years later, using electron microscopy, the group localised that intraneuronal peptide to multivesicular bodies — a late compartment of the sorting system — inside synaptic terminals, and found it associated with abnormal synaptic structure before plaque pathology was present (Takahashi et al., 2002). In 2004 they showed the peptide assembling into small soluble clusters inside those same processes and synapses (Takahashi et al., 2004).

Independently, Anne Cataldo, Ralph Nixon and colleagues had shown that abnormalities of the internalisation pathway — swollen early sorting compartments — precede amyloid deposition in sporadic Alzheimer's disease and in Down syndrome (Cataldo et al., 2000). The two lines converged on the same compartment from different directions.

2.4 What the founding decade established

By the middle of the 2000s the programme had put three propositions in place, and they are the load-bearing ones:

- amyloid is made inside the cell, in specific compartments, and the compartment determines the product;
- the first step of its production occurs on synaptic machinery;
- a fraction is retained rather than exported, and the retained fraction is enriched in the aggregation-prone species, accumulating inside vulnerable human neurons.

Nothing in those three propositions says anything about what starts Alzheimer's disease. They describe a normal cell biology with an abnormal load.

3. The Retained Fraction

3.1 Retention, not overproduction

The dominant reading of amyloid in this disease is quantitative: there is too much of it. Genetics supports that reading for the early-onset familial forms, where mutations raise production or shift the ratio toward the stickier species.

Gouras's work points somewhere else, and this is the most under-cited turn in the whole corpus. The variable that matters is not how much peptide is made but **how much of it fails to leave.**

The distinction has consequences. A production problem is addressed by reducing synthesis — the strategy behind secretase inhibitors, which failed in trials. A retention problem is addressed by restoring export and degradation, which is a different target list entirely and has scarcely been attempted.

3.2 Impaired secretion, and the enzyme that fails

The direct evidence arrived in 2011, in a short paper that deserves to be better known (Tampellini et al., 2011).

Cultured neurons from Alzheimer-model mice were compared with normal neurons over time in culture. In the diseased neurons, the amount of amyloid *secreted* fell with time — it did not rise. Normal neurons showed no such decline. Moreover, the ability of synaptic activity to increase secretion and simultaneously reduce the pool inside the neuron became impaired in diseased neurons and not in normal ones.

The group then identified a mechanism. Synaptic activity normally increases the amount of neprilysin — an enzyme that degrades amyloid — at the cell surface, and increases its co-localisation with Aβ42. In the diseased neurons, neprilysin levels fell with time in culture.

Put together: **the diseased synapse loses the activity-coupled clearance system that normally keeps its local amyloid pool low.** It is not making more; it is disposing of less, and the specific disposal step that fails has a name.

This is a mechanistic statement about synaptic function that makes no claim about disease initiation whatsoever.

It is also worth noting, in passing, that the enzyme in question is under neuropeptide control: somatostatin regulates brain Aβ42 through modulation of proteolytic degradation by this same enzyme (Saito et al., 2005), and somatostatin-expressing inhibitory neurons are among the populations lost in Alzheimer cortex. The programme has not pursued this thread, and it is one of several places where an obvious extension has been left undone.

3.3 What raises the retained pool

If retention is the variable, then anything that increases it enters the same pathway. The programme and its collaborators have documented a striking number of distinct entry points.

Table 1 — Conditions shown to raise the retained intraneuronal pool

Entry condition	Mechanism demonstrated	Source
Normal ageing	Internalisation of the precursor protein is up-regulated in aged neurons and aged brain, potentiating processing and amyloid production; blocking production reverses the associated synapse loss	Burrinha et al., 2021
Apolipoprotein E4	Internalised apolipoprotein E meets the precursor and the peptide inside neurites; the E4 variant raises neuronal Aβ42; degradative capacity declines with time in culture	Konings et al., 2023; Nyberg et al., 2025
Precursor-protein mutation	The Arctic mutation reduces the precursor's presence at the cell surface, making it less available to the pre-empting enzyme and shifting production to intracellular sites	Sahlin et al., 2007
Disrupted sorting machinery	Interfering with the packaging system that carries cargo into multivesicular bodies traps amyloid inside the neuron and enlarges the compartment	Edgar et al., 2015; Willén et al., 2017a
Seeded aggregates	Brain extract induces intracellular amyloid inclusions that persist across cell generations and can seed further cells	Olsson et al., 2018; Roos et al., 2021
Loss of the degrading enzyme	Surface neprilysin falls in diseased neurons, breaking the activity–clearance coupling	Tampellini et al., 2011
Reduced synaptic activity	Chronic synaptic inhibition raises intraneuronal amyloid	Tampellini et al., 2009, 2010
Prior intracellular load	Cells already carrying aggregates lose the homeostatic correction that normally restores balance	Roos et al., 2021

Eight conditions; one downstream compartment. The importance of this table for the argument of Section 10 is that the entries are *causally independent of one another*. Ageing is not apolipoprotein E4; a seed is not a mutation. What they share is where they arrive.

4. Four Routes From a Loaded Compartment to a Failing Synapse

The question this paper is built around — how does the work explain synaptic dysfunction — is answered here. The programme has identified four routes from a loaded compartment to a synapse that no longer works. They are separable, they were established at different times by different methods, and no one of them is sufficient to account for the whole phenotype.

4.1 Receptor withdrawal

A synapse works because receptors sit in its membrane. Take them out and the synapse is structurally present but functionally silent.

Two papers from 2005 established that the retained peptide does exactly this, by two different receptor systems.

In cultured neurons from precursor-mutant mice, the earliest measurable change in synaptic composition was a reduction in PSD-95 — the scaffolding protein that anchors glutamate receptors at the receiving side of a synapse — followed by reduced surface expression of the glutamate receptor subunit GluA1 (Almeida et al., 2005). The causal attribution was tested in both directions: inhibiting the enzyme that produces amyloid blocked the changes, and adding synthetic amyloid to normal neurons reproduced them.

In parallel, working with Greengard, the group showed that amyloid promotes the internalisation of NMDA receptors — the coincidence detectors that gate lasting synaptic change — and that neurons from an Alzheimer model carry fewer of them at the surface (Snyder et al., 2005). Inhibiting amyloid production restored surface expression. The pathway was traced through a nicotinic receptor, a phosphatase, and dephosphorylation of a specific tyrosine residue on the receptor subunit.

These are not observations of correlation. They are interventions in both directions on a named molecular pathway, and they establish that the retained pool is sufficient to withdraw the receptors on which synaptic function depends.

4.2 The autocatalytic step

The second route is the one that turns a problem into a progressive one, and it is the most elegant result in the corpus.

Multivesicular bodies are the compartments that sort membrane proteins for disposal. Cargo destined for destruction is tagged with a small protein, ubiquitin, and the tagging system is regulated by the proteasome — the cell's protein-shredding machine.

In 2006, using two receptor systems as reporters, the group showed that amyloid accumulation in precursor-mutant neurons impairs this sorting pathway: receptor inactivation, degradation and tagging were all impaired, and the group provided evidence that the accumulating amyloid inhibits the proteasome and the enzymes that remove ubiquitin tags (Almeida et al., 2006).

The structure of that finding is a feedback loop with the wrong sign. **The cargo disables the machine that would clear the cargo.** Once the retained pool passes a threshold, its own clearance becomes progressively harder, which is a mechanism for why a slow accumulation should accelerate rather than plateau. It is also a mechanism that requires no external driver: whatever raised the pool initially need not still be present.

4.3 Structural failure of the compartment

The third route is physical, and it is where the programme's imaging work sits.

Compartments loaded with undegradable cargo distend. Interfering with the packaging machinery reproduces both the amyloid accumulation and the enlargement, establishing that the swelling is a consequence of the sorting failure rather than an independent lesion (Willén et al., 2017a). In brain tissue, three-dimensional reconstruction from confocal imaging showed fibrillar amyloid inside individual synaptic compartments, associated with abnormal morphology, and in places appearing to pierce the cell membrane; in dendrites, rising intraneuronal fibrillar signal tracked falling levels of a structural neurofilament marker (Capetillo-Zarate et al., 2011). Accumulating intraneuronal Aβ42 was associated with early changes in the microtubule-associated protein MAP2 in neurites and synapses (Takahashi et al., 2013).

This route explains something the other three do not: the swollen, clubbed nerve endings that surround plaques in human tissue, which have been described since 1907 and which no purely biochemical account of synaptic dysfunction addresses.

4.4 The translational arm

The fourth route is the one most often left out of summaries, and it is independent of trafficking altogether.

Lasting synaptic change requires new protein to be made locally, at the synapse, on demand. Two signalling systems govern this, and the programme has implicated both.

The mTOR pathway, which licenses protein synthesis, is suppressed in hippocampal tissue from an Alzheimer model and in normal tissue exposed to amyloid; intraneuronal Aβ42 co-localises

with mTOR; and long-term potentiation — the electrophysiological signature of synaptic strengthening — can be rescued in the model by inhibitors of glycogen synthase kinase 3, which act by restoring mTOR signalling, or by deleting a protein that restrains it (Ma et al., 2010). Separately, dysregulation of an elongation factor required for translation was found to correlate with the plasticity impairments (Beckelman et al., 2016).

The apolipoprotein E work reaches the same arm by another road. Acute exposure of neurons and isolated nerve terminals to the E4 variant causes a significant fall in overall protein synthesis and abolishes the translational response to NMDA-receptor stimulation. The mechanism runs through calcium: E3 produces a brief calcium transient and a transient dip in synthesis that recovers, while E4 produces a sustained calcium rise through both NMDA receptors and voltage-gated channels, sustained phosphorylation of the elongation factor eEF2, and sustained translational block (Ramakrishna et al., 2021).

A synapse that cannot make new protein on demand cannot consolidate a change, whatever the state of its receptors.

4.5 Why four routes rather than one

Table 2 — Four routes from a loaded compartment to a failing synapse

Route	What fails	Key evidence	Causal test performed
Receptor withdrawal	Glutamate and NMDA receptors leave the surface; PSD-95 falls	Almeida et al., 2005; Snyder et al., 2005	Blocked by inhibiting amyloid production; reproduced by adding amyloid
Autocatalysis	Amyloid inhibits the proteasome and de-ubiquitinating enzymes, impairing the sorting pathway that would clear it	Almeida et al., 2006	Reporter-receptor trafficking impaired in mutant neurons
Structural failure	Compartments distend; fibrils form inside synapses; cytoskeletal markers fall	Capetillo-Zarate et al., 2011; Takahashi et al., 2013; Willén et al., 2017a	Enlargement reproduced by disrupting the packaging machinery
Translational block	Local protein synthesis required for lasting plasticity is suppressed	Ma et al., 2010; Beckelman et al., 2016; Ramakrishna et al., 2021	Potentiation rescued by restoring mTOR signalling; E4 effect traced to calcium and eEF2

That the routes are separable matters for two reasons. It explains why the synaptic phenotype is robust — four partly redundant mechanisms will not be abolished by blocking one — and it predicts that single-target therapy at this level should underperform, which is what has happened.

It also means the account is not the tautology it can appear to be. "Amyloid damages synapses" is uninformative. "A retained intraneuronal pool withdraws surface receptors, disables its own clearance, distends the compartment, and blocks local translation, by four pathways with distinct effectors" is a mechanism, and each clause is separately falsifiable.

4.6 Not every synapse

One further result qualifies all of the above and is important for understanding selective vulnerability.

Using immunogold electron microscopy and confocal imaging, the group found the association of amyloid and its precursor with synapses to be *heterogeneous*: the peptide binds a subset of synapses, with preferential binding to excitatory rather than inhibitory neurons, and the precursor's cleavage fragments accumulate earlier on the transmitting side than the receiving side, consistent with a higher rate of processing in axons (Willén et al., 2017b).

Synapses are therefore not uniformly exposed. Whatever determines which ones are is unresolved, and it is one of the more interesting open questions the programme has generated.

5. Activity Is the Regulator

5.1 Use lowers the retained pool

The relationship between neural activity and amyloid appears at first to be simple and bad: activity increases amyloid release into the extracellular space, and extracellular amyloid harms synapses. On that reading, thinking is toxic.

The programme showed the picture is inverted at the compartment that matters. Synaptic activity reduces the pool of amyloid *inside* the neuron, promotes transport of the precursor protein to synapses, and protects against amyloid-related synaptic alterations; the reduction of the intraneuronal pool is mediated by neprilysin (Tampellini et al., 2009). The same paper established that the synaptotoxicity of extracellular amyloid itself requires ongoing processing of the precursor — that is, the external insult acts partly by way of the internal pool.

Gouras has since set out the argument that ageing, metabolic demand and synaptic activity converge on this balance as a single problem (Gouras, 2019).

So activity has opposite signs in the two compartments: it raises what is outside and lowers what is inside. If the inside pool is the one that damages synapses, activity is protective, and its protective mechanism is enzymatic clearance at the surface.

5.2 The experiment that separates plaques from function

The decisive test came in 2010, and it is the single experiment in this corpus that most clearly separates the programme's claims from plaque-centred accounts (Tampellini et al., 2010).

Chronic synaptic activity had previously been shown to raise plaque burden, and chronic inhibition to lower it — suggesting, on a plaque-centred view, that quieting the brain should help. The group tested this directly, using two independent means of chronic synaptic inhibition: surgical deafferentation of the barrel cortex, and administration of a benzodiazepine.

Both reduced plaques. Both made the animals worse. Prolonged synaptic inhibition exacerbated the loss of the presynaptic marker synaptophysin relative to more active brain regions, in Alzheimer-model but not normal mice. Benzodiazepine treatment followed by washout exacerbated memory impairment in the model animals. And the deterioration occurred in the setting of *reduced* plaques and *elevated* intraneuronal amyloid.

This is a designed dissociation. Plaque burden and synaptic-behavioural outcome were driven in opposite directions in the same animals, and outcome followed the intraneuronal pool rather than the deposit. Whatever else is true of the programme, this experiment is a direct empirical demonstration that the extracellular deposit is not the variable that tracks function.

5.3 What it predicts about sedation and reserve

Two implications follow, one clinical and one epidemiological, and both should be stated with the caution owed to a mouse experiment.

The clinical one concerns sedation. If chronic reduction of synaptic activity worsens synaptic and memory outcomes in a model of this disease despite lowering deposits, then long-term benzodiazepine use in older adults at risk is a plausible harm with a specific proposed mechanism. This is a testable epidemiological prediction, and it has not been tested against this mechanism.

The epidemiological one concerns cognitive reserve. The observation that education, occupational complexity and continued mental engagement associate with later onset (Stern, 2012) is usually explained in terms of network redundancy — more connections, more tolerance of loss. The mechanism here is different and additive: activity buys enzymatic clearance at the synapse, so a more-used synapse carries a lower internal load. The brain regions that are most continuously active across a lifetime are also those with the earliest deposition (Buckner et al., 2005), and the two facts are not in conflict on this account — those regions secrete the most and therefore deposit the most, while being intracellularly protected until the clearance coupling fails.

6. The Lesion Is the Loss of the Control Loop

Sections 3 to 5 describe a system with a regulator: production geographically constrained, export and enzymatic degradation coupled to use, and a homeostatic relationship between the pool inside the cell and the pool outside it. The programme's most recent decade has been about what happens when that regulation fails, and this is where the account locates the actual lesion.

6.1 Homeostatic plasticity fails

Neurons hold their own firing rate near a set point despite changes in the input they receive, by multiplicatively adjusting the strength of their remaining connections and by tuning their intrinsic excitability (Turrigiano, 2008). This machinery is what makes a neural circuit stable.

In 2022 the group showed it is broken in this disease (Martinsson et al., 2022). Neurons carrying elevated amyloid became hyperactive, showing calcium transients of increased frequency and amplitude — consistent with earlier reports of hyperactive neurons near plaques (Busche et al., 2008). But the more consequential findings concerned regulation rather than level. The diseased neurons failed to adapt their calcium responses to imposed global changes in activity, and failed to adjust the length of the axon initial segment — a structural adaptation neurons use to retune their own excitability. Precursor protein levels were themselves shown to be regulated by chronic changes in activity.

The cell has not simply become noisy. It has lost the ability to find its own set point.

6.2 Feedback is lost in cells already loaded

A parallel result concerns the amyloid homeostat itself (Roos et al., 2021).

In cells producing amyloid, removing the peptide from the surrounding medium caused the internal pool to fall by more than eighty per cent within three hours, and to recover fully within six — accompanied by a significant rise in the precursor fragment produced by the first cut. The cell had detected the external drop and increased production to compensate. That is a functioning homeostat, and its existence is itself a notable finding: the internal and external pools are actively coupled, not merely connected by diffusion.

In cells that had accumulated internal aggregates, the correction did not occur. The authors conclude that under conditions of high internal and low external amyloid, production may be

permanently up-regulated, and connect this to the very large increase in amyloid between a young brain and a diseased one.

Two independent lines therefore converge on the same statement: **the lesion is not the presence of amyloid but the loss of the loops that regulate it.** The peptide is present in every normal brain and is under control there. What the disease adds is not the substance but the failure of governance.

6.3 The residue: what the precursor protein itself does

Honesty requires recording a result from the same group that complicates its own account.

In the 2022 excitability paper, neurons expressing a precursor protein engineered so that it *cannot* yield amyloid at all became just as hyperactive as those flooded with the peptide. And in 2019 the group had shown that deleting the precursor protein altogether alters synaptic proteins and receptors — including raising surface GluA1 — in a direction broadly *opposite* to the reductions seen in precursor-mutant neurons (Martinsson et al., 2019).

The plain reading is that some part of the synaptic phenotype belongs to the precursor protein rather than to the peptide cut from it. That protein has a normal synaptic function, the programme has demonstrated as much, and the account does not yet specify how much of the disease phenotype is peptide and how much is precursor.

This is an unresolved hole in an otherwise coherent structure, and it is the group's own finding rather than a critic's.

7. The Measurement Problem, and How It Was Answered

7.1 The objection, stated first by Gouras

The entire claim about intraneuronal amyloid rests on being able to detect a small peptide inside a cell that is full of the larger protein it was cut from. The most widely used antibodies recognise a region present in both. An antibody that cannot distinguish the peptide from its own precursor will stain a neuron containing only precursor.

This objection has been the standing criticism of the programme for twenty-five years. Its most candid statements have come from Gouras himself.

In 2005 the group demonstrated that the standard immunoassay used across the field systematically *underestimates* amyloid when the peptide has assembled into small clusters — because the assembly hides the sites the assay binds (Stenh et al., 2005). The implication was uncomfortable for everyone, including them: much of the field's quantification of soluble amyloid has been measuring the wrong thing in the wrong direction.

In 2012 Gouras published a paper whose subtitle is *technical challenges in studying intracellular Aβ*, which states plainly that detection difficulties have made the topic "remarkably controversial," that standard biochemical methods underestimate the intraneuronal pool, and — most damaging to his own case — that detergent used in tissue processing can remove it altogether (Gouras et al., 2012). A researcher writing the strongest available critique of his own principal observation is unusual, and it should be weighed in evaluating the programme's reliability.

7.2 Structure without antibodies

The response was to develop methods that do not depend on antibodies at all, and this is the programme's most important methodological contribution.

Working with Oxana Klementieva at Lund, the group applied synchrotron-based infrared micro-spectroscopy to brain tissue. Infrared spectroscopy reports on molecular structure directly — it detects the β-sheet conformation characteristic of aggregation as a physical signature, without a reagent that must recognise a sequence. Combined with non-denaturing gel separation and conformation-dependent antibodies, this showed that the structural states of both the peptide and its precursor are altered in Alzheimer-model brain *before* any plaque forms, and that focal aggregates

preceding plaque formation localise to synaptic terminals (Klementieva et al., 2017).

The method was then pushed to super-resolution, imaging structurally distinct amyloid aggregates directly in neurons (Klementieva et al., 2020), and extended by a series of technical papers on infrared imaging of primary neurons and correlative chemical imaging.

A second antibody-independent line came from dye-based imaging: three-dimensional reconstruction using thioflavin S, a fluorescent dye that binds β-sheet structure rather than a sequence, showed fibrillar material inside individual synaptic compartments (Capetillo-Zarate et al., 2011).

7.3 What the same laboratory has since conceded

The programme has continued to publish against its own tools. In 2026 it reported that the standard antibody, injected into Alzheimer-model mice by three routes, bound not only plaques but hippocampal pyramidal neurons, microglia, astrocytes, oligodendrocytes, perivascular macrophages and blood vessels (Wen et al., 2026). A companion study found that when labelled peptide is applied to human Alzheimer brain sections, a significant fraction of what it binds is not other amyloid but anti-amyloid antibodies already sequestered inside the plaques (Takahashi et al., 2025).

7.4 What is now settled

The fair position is that the objection has been substantially, though not entirely, answered.

That intraneuronal material with β-sheet structure accumulates in synaptic compartments before plaques appear is now supported by three methodologically independent lines: immunostaining, structure-sensitive dye imaging with three-dimensional reconstruction, and label-free infrared spectroscopy. Agreement across methods with different failure modes is the strongest form of evidence available short of a definitive assay.

What is not settled is quantity. None of these methods gives an absolute measure of how much peptide is inside a human neuron at a given disease stage, and the infrared work — the most decisive of them — requires specialist instrumentation, has been performed largely in model systems, and has not been widely replicated outside the collaboration that developed it.

8. Where Genetic Risk Enters

The largest common genetic risk factor for late-onset Alzheimer's disease is the E4 variant of apolipoprotein E, the brain's principal lipid carrier. Any account of synaptic failure must say how that risk becomes a synaptic defect, and this programme's answer is that it enters at the same node, by both of the arms described in Section 4.

The trafficking arm. Apolipoprotein E internalised from astrocytes behaves differently depending on the receiving cell: in non-neuronal cells it goes mostly to lysosomes, but in neurons it goes preferentially to sorting and autophagy compartments *of the neurites* — the same distal compartments the rest of the programme concerns. In neurons from Alzheimer-model mice it intersects intracellularly with the precursor protein and the peptide, and the E4 variant raises the amount of Aβ42 in the neuron, both endogenous and internalised (Konings et al., 2023).

The translational arm. As described in Section 4.4, E4 suppresses basal and activity-driven protein synthesis in neurons and nerve terminals by perturbing calcium handling (Ramakrishna et al., 2021). The group has also shown that neuronal and astrocytic apolipoprotein E isoforms differentially affect neuronal excitability (Konings et al., 2021).

The timing. A recent result adds an important qualification. In mature primary neurons, apolipoprotein E knockout, E3 and E4 show *no* major differences in compartment appearance or function, and adapt similarly to increased synaptic activity. Differences emerge only with prolonged time in culture: aged E4 neurons show reduced degradative capacity, fewer active lysosomal compartments, and a tendency to accumulate cholesterol in the system when supplied with it (Nyberg et al., 2025).

That the largest genetic risk factor for a disease of ageing produces no measurable defect in young neurons and a clear one in old ones is the kind of result that makes a mechanism credible. It also means the genetic risk is not a constant burden but a slowly widening gap — which is the shape the epidemiology has always suggested and which mechanism has rarely supplied.

9. Where the Failure Goes Next

Synaptic failure in one neuron would be a local event were it not transmissible. The programme has pursued three ways in which it is not.

Seeding is intracellular, and heritable across cell generations. Treating cells with brain extract from Alzheimer-model mice induces amyloid inclusions in a subset of them; those cells continue to produce oligomeric amyloid across multiple rounds of division; and lysates from them induce aggregation in previously untreated cells (Olsson et al., 2018). This is a cellular model of templated seeding in which the template is an *intracellular* species. It matters because most seeding work has concerned extracellular material, and because it supplies a route by which a loaded compartment in one cell becomes a loaded compartment in another.

Loss travels along connections. Destroying the subiculum reduces the spread of amyloid pathology to the regions connected to it, implicating connectivity rather than proximity (George et al., 2014). Injecting brain extract into one hippocampus induces plaques and produces measurable changes in *connected* regions: intraneuronal amyloid in entorhinal cortex layer II rose at six weeks and fell by sixteen, amyloid in CA1 pyramidal cell bodies on the injected side fell by roughly forty per cent as plaques appeared at the corresponding terminals, and inhibitory neurons beside the induced plaques declined by thirty-two per cent (Roos et al., 2021).

The tau junction. Amyloid accumulation and abnormal tau phosphorylation were shown to develop concomitantly *within synaptic terminals* in two model systems (Takahashi et al., 2010) — placing the interaction of the disease's two proteins at the synapse rather than in the cell body. A candidate molecular link was later identified: a direct, high-affinity interaction between Aβ42 and the kinase GSK3α, which stimulates tau hyperphosphorylation, with the two proteins co-localising in neurites of mature neurons (Dunning et al., 2016). This finding has not been much taken up, and its status is a single-group result awaiting independent confirmation.

10. Why the Account Does Not Need an Origin Story

10.1 The structure of a convergence claim

Return to the distinction drawn in Section 1.2.

An *initiation* claim asserts temporal priority: this happens first, and the rest follows. Its evidence must be temporal and, for a human disease with a twenty-year preclinical phase, must come from human tissue sampled at stages that are largely inaccessible.

A *convergence* claim asserts necessity: whatever happens first, it must pass through here to produce this outcome. Its evidence is interventional — block the mechanism and the outcome disappears; drive the mechanism and the outcome appears — and it can be obtained in the laboratory.

Look at what the evidence in Sections 4 to 6 actually is. Inhibiting amyloid production restores PSD-95 and surface glutamate receptors (Almeida et al., 2005) and restores surface NMDA receptors (Snyder et al., 2005). Blocking amyloid production reverses the synaptic decline of aged neurons, and driving internalisation upward in young neurons reproduces it (Burrinha et al., 2021). Restoring mTOR signalling rescues long-term potentiation (Ma et al., 2010). Disrupting the packaging machinery reproduces the amyloid accumulation and compartment enlargement (Willén et al., 2017a). Reducing synaptic activity worsens synapses and memory while lowering plaques (Tampellini et al., 2010).

Every one of these is a statement about necessity or sufficiency for the *synaptic phenotype*. Not one is a statement about priority in the human disease. The corpus is convergence evidence throughout.

Combine that with Table 1. Eight causally independent conditions raise the retained intraneuronal pool, and the four routes of Section 4 run the same way regardless of which one did it. **The mechanism specifies what happens after the pool rises. It does not require, and its evidence does not supply, any particular reason for the rise.**

This is not a hedge or a retreat. It is a structural property of the account, and it has a consequence worth stating directly: **the account does not compete with theories of onset.** It does not compete with Nixon's account of autophagy–lysosomal failure, which describes a different compartment at a later stage and composes with this one rather than contradicting it (Lee et al., 2022). It does not compete with tau-first accounts, with inflammation-first accounts, or with vascular

accounts. Any of those, if true, must still produce its cognitive symptom by damaging synapses, and this is the best-specified available description of how synapses in this disease are damaged.

A convergence theory is *strengthened* by the plurality of upstream causes, because each one that funnels into the same mechanism is another demonstration that the mechanism is the common path.

10.2 What the inside-out hypothesis costs

Gouras has nonetheless made an initiation claim, repeatedly and by name. He has called his position "the inside-out amyloid hypothesis" (Gouras, 2014), and the position holds that plaques form principally from the rupture of amyloid-laden neurons and processes, so that the deposit is the residue of a cellular failure rather than its cause.

The 2020 prize submission states it at maximum strength. Its summary sentence declares that "the central cause of Alzheimer's disease is a vulnerability to age of synapses and their associated neurites at the level of synaptic endosomes," and that amyloid plaques and neurofibrillary tangles "are end-stage remains" of that process. Every element of the mechanism assembled in Sections 3 to 6 appears somewhere in those six pages; but the document leads with the origin claim, and the mechanism arrives as its support rather than as the finding in its own right. That is the inversion this evaluation is arguing against, in the author's own most compact statement of his position.

The proposition is coherent and it is supported in model systems: fibrils inside synaptic compartments appearing to pierce the membrane (Capetillo-Zarate et al., 2011); intracellular sources inducing plaques (Roos et al., 2021); structural change preceding deposition (Klementieva et al., 2017). It also has an intellectual pedigree running back to Oskar Fischer's 1907 reading of the plaque as a process rather than an object.

But it costs the programme more than it earns, for four reasons.

It is the one part with no quantitative human support. A finished plaque is the same object however it formed; the peptide carries no record of its origin; human tissue gives a single frozen frame; and the alternative route is independently demonstrated — dilute brain extract injected into the extracellular space nucleates deposition in a dose- and time-dependent manner (Meyer-Luehmann et al., 2006). Both routes are real, and no measured answer exists for their relative contribution in human brain.

It converts a convergence claim into a competition. Stated as an origin story, the work becomes a rival to the amyloid cascade rather than a specification of the step the cascade never supplied, and it invites dismissal from anyone committed to the cascade — for whom it is not a completion but a challenge.

It is what gets summarised. The synaptic mechanism, which is the strong part, travels under the banner of the plaque claim, which is the weak part. Reviews of this work routinely lead with plaque genesis and reach the receptor, autocatalytic, structural and translational routes late or not at all.

It has been tested once and was not supported. In a mouse engineered to carry humanised amyloid sequence at normal expression levels rather than over-expressing the precursor — the design point of the knock-in series being to avoid exactly the artefacts of over-expression (Saito et al., 2014) — the fall in cerebrospinal-fluid and serum amyloid ratios *coincided with* the appearance of plaques rather than preceding them, contrary to the proposal that the earliest fluid changes reflect intracellular accumulation before deposition (Andersson et al., 2023). Gouras is a co-author on that paper.

10.3 The version that should be cited

The account that the evidence actually supports can be stated in one paragraph, and it makes no claim about origins:

> In Alzheimer's disease a rising pool of amyloid-β retained within the sorting compartments of the synapse degrades synaptic function by four partly independent routes — withdrawal of glutamate and NMDA receptors from the surface, inhibition of the protein-disposal system that would clear the peptide, physical distension and structural failure of the compartment, and suppression of the local protein synthesis on which lasting plasticity depends. Synaptic activity normally holds that pool down by driving enzymatic degradation at the cell surface, and the lesion of the disease is the failure of that regulation rather than the presence of the peptide. The mechanism is engaged by any condition that raises the retained pool, of which at least eight are documented.

Every clause of that is supported by interventional evidence in the corpus. None of it depends on how the disease begins, and none of it is threatened if the origin turns out to be somewhere else entirely.

11. Where the Programme Is Weak

An evaluation that returned only strengths would not be one. Seven weaknesses, in descending order of seriousness.

The dependence on over-expressing animals. The great majority of the cellular mechanism was established in mice engineered to over-produce the precursor protein — Tg2576, Tg19959, APP/PS1, 5xFAD. These systems force large intracellular loads and are exactly the systems in which an intraneuronal-accumulation account would be expected to look best. Knock-in models expressing humanised sequence at normal levels were created to remove this artefact (Saito et al., 2014); the programme has not systematically rebuilt its case in them, and the one published test in a knock-in went against a prediction (Andersson et al., 2023). This is the most consequential gap and the most tractable.

Thin independent replication of the load-bearing results. The two findings on which this evaluation leans most heavily — impaired secretion with loss of surface neprilysin (Tampellini et al., 2011), and loss of the homeostatic correction in cells already carrying aggregates (Roos et al., 2021) — do not appear, so far as this review could determine, to have been independently reproduced outside the group. Both are single-laboratory results carrying a great deal of weight.

The unresolved precursor-protein residue. As Section 6.3 records, a construct incapable of yielding amyloid produces equal hyperactivity, and deleting the precursor alters synaptic proteins in the opposite direction to the mutant. Some of the phenotype is the precursor's, and the account does not say how much. Until it does, attributions of synaptic dysfunction to amyloid within this corpus carry an unquantified error term.

Human evidence is descriptive rather than mechanistic. The human contributions — intraneuronal Aβ42 (Gouras et al., 2000), its localisation to multivesicular bodies in synaptic terminals (Takahashi et al., 2002), co-occurrence with tau at synapses (Takahashi et al., 2010), and cross-model proteomics against post-mortem hippocampus (Pomeshchik et al., 2023) — establish that the phenomenon exists in human brain. None of the four mechanistic routes has been demonstrated in human tissue.

The measurement problem is answered but not closed. Three independent methods agree qualitatively; none quantifies the intraneuronal pool absolutely; the most decisive of them is technically demanding and narrowly replicated.

No therapeutic candidate in twenty-eight years. The programme has generated a specific and unusual target list, and none of it has reached the clinic. An early demonstration that antibodies internalised against the amyloid domain of the precursor reduce neuronal amyloid and protect against synaptic alterations (Tampellini et al., 2007) was never developed. This is not unique to this laboratory, but it is a fair criticism of a programme now approaching three decades.

Breadth at the cost of depth in the recent period. The output of the last five years spans α-synuclein, oligodendrocyte biology, early-life stress, sphingosine-1-phosphate signalling and immunotherapy pharmacology alongside the core programme. Some of this is productive collaboration; some of it reads as dispersion at the point where the core account most needed consolidation in knock-in models.

12. What Would Refute It

The account of Section 10.3 is falsifiable, and five results would damage or destroy it.

1. If raising the retained intraneuronal pool in a knock-in model, without raising the extracellular pool, produced no synaptic phenotype, the central claim fails. This is the experiment the programme most needs to do.

2. If restoring surface neprilysin in loaded neurons failed to restore the coupling between activity and clearance, the named mechanism of Section 3.2 fails and the impaired-secretion result would require a different explanation.

3. If synaptic protein loss in precursor-knockout neurons ran in the *same* direction as in precursor-mutant neurons, the attribution of the phenotype to amyloid rather than to the precursor protein would collapse. The existing result runs the other way, which supports the account; a failure to replicate it would not.

4. If blocking any single one of the four routes abolished the whole synaptic phenotype, the claim that they are partly independent is wrong, and the account should be simplified to one mechanism.

5. If the β-sheet signal detected by infrared spectroscopy inside pre-plaque neurons proved to arise from something other than amyloid — a real possibility, since the method reports structure rather than identity — the principal antibody-independent support for the founding observation would be lost.

13. Consequences for Measurement and Treatment

13.1 The pool that matters has no clinical assay

This is the sharpest practical consequence of the whole programme and it is rarely stated.

Every amyloid biomarker in clinical use reports either the extracellular soluble pool (cerebrospinal fluid, plasma) or the deposited pool (positron emission tomography). The pool this account identifies as the one that damages synapses — retained, intraneuronal, in synaptic compartments — is measured by none of them. Trials therefore select patients, stratify them, and judge target engagement on a quantity the mechanism says is not the relevant one.

Compounding this, the group's own 2005 result showed that the standard immunoassay underestimates soluble amyloid once it has assembled (Stenh et al., 2005), so even the extracellular measurement is biased in a direction that depends on aggregation state.

Developing a proxy for the intraneuronal pool — whether by imaging, by neuron-derived extracellular vesicles, or by a conformation-sensitive assay — is the most valuable thing that could be done for this account, and it would be valuable regardless of whether the account is right, because it would allow the question to be settled.

13.2 The target list

The mechanism suggests interventions that are not the ones the field has pursued:

- **Restore the activity–clearance coupling.** The specific lesion is loss of surface neprilysin in diseased neurons. Restoring it, or the signalling that recruits it, targets the retention problem at the step shown to fail.
- **Protect the protein-disposal step.** The autocatalytic route runs through inhibition of the proteasome and de-ubiquitinating enzymes; preserving that step should slow the feed-forward loop rather than merely reducing its input.
- **Address the translational arm.** The mTOR and eEF2 routes are pharmacologically approachable and are not addressed by any amyloid-directed therapy.
- **Reduce production rather than remove deposits.** The ageing work showed that blocking amyloid production reverses synaptic decline in aged neurons (Burrinha et al., 2021).

That is a different intervention from clearing plaques, and it has not been tested as a prevention strategy in the way the mechanism implies.

13.3 What it says about the antibodies

The current anti-amyloid antibodies slow decline modestly (van Dyck et al., 2023; Sims et al., 2023). This account offers a specific reason why the effect might be smaller than the completeness of plaque removal predicts: the 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). The group has since argued that immunotherapy should be understood as engaging a multicellular clearance system rather than removing a deposit (Zhan et al., 2026).

This is a testable prediction with a clear experiment: administer an antibody to a model animal and measure the intracellular pool and the precursor fragment, not the plaque burden.

13.4 A note on sedation

The 2010 inhibition experiment implies that chronic reduction of synaptic activity may be harmful in this disease despite lowering deposits. If that transfers to humans, it bears on the long-term prescribing of sedatives to older adults at risk. It is a mouse result and should not be over-read, but it is a specific, mechanistically grounded hypothesis that epidemiology could examine.

14. Conclusion

The synapse makes amyloid on the machinery it uses to work. That is the fact this programme has established, across twenty-eight years and by a dozen methods, and it is why the two pathologies of this disease — a peptide that aggregates and a brain that stops learning — are not two problems but one.

What follows from it is a mechanism, and it is more detailed than any competing account of synaptic failure in Alzheimer's disease. A retained intraneuronal pool withdraws the receptors a synapse needs, disables the disposal system that would clear it, distends the compartment that holds it, and shuts down the local protein synthesis that lasting plasticity requires. Activity normally holds the pool down by enzymatic clearance at the surface. In disease that coupling is lost, along with the homeostatic machinery that sets the neuron's firing rate and the feedback that couples the pool inside the cell to the pool outside. The lesion is the loss of control, not the presence of the substance.

The account is engaged by ageing, by apolipoprotein E4, by precursor mutation, by gene dosage, by trafficking-gene variants, by seeds, by enzyme loss, and by disuse. It therefore needs no origin story, and this evaluation's central conclusion is that it should stop being given one. The inside-out hypothesis is the weakest thing Gouras has argued and the thing he is most often cited for; the cell biology of synaptic failure is the strongest thing he has built and the thing he is least cited for. That inversion is worth correcting, and correcting it costs the programme nothing, because everything of value in it survives the subtraction.

The weaknesses are real and mostly fixable. The case needs rebuilding in animals that do not over-produce the protein under study. Two load-bearing results need independent replication. The share of the phenotype belonging to the precursor protein rather than to amyloid needs to be settled, and it is the group's own data that raise the question. None of these is a refutation; all are outstanding work.

And there is one thing the programme has done that is rarer than any result. It has published, repeatedly and at its own expense, the evidence that its principal tools are unreliable — that the standard assay underestimates what it measures, that detergent can wash away the very pool under study, and that the antibody underpinning its signature observation binds half the cell types in the brain. Then it built methods that do not need the antibody, and found the same thing. A body of work that argues against itself in public and survives the argument has earned a different kind of confidence from one that has only ever been defended.

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