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The Clock and the Census

*Residence Time as the Common Variable in the Endosomal Traffic Jam,
Intraneuronal Amyloid, and the Licensing of Synaptic Proteolysis*

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October 2026

Three programmes describe one lesion — a failure of timed removal. The field measures a census. A census cannot report a clock.

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Abstract

Three bodies of work on Alzheimer's disease have been built in near-isolation from one another, and each has arrived at a lesion of the same unusual kind.

The first holds that the disease's upstream fault is a failure of endosomal retrieval: when the recovery arm of the sorting station stalls, everything held in the compartment dwells there longer than it should, and the consequences — amyloidogenic cleavage, loss of surface glutamate receptors, vesicular release of tau, blunted glial clearance — follow in parallel from that single mechanical fact. The second holds that the synapse manufactures amyloid- β on the same organelles it uses to transmit, that the variable which matters is not how much peptide is made but how much fails to leave, and that the lesion is the loss of the control loop coupling use to clearance rather than the presence of the peptide. The third holds that the control surface above the spine's actin cytoskeleton is terminated not by dephosphorylation but by destruction — that the tyrosine phosphorylation which activates each of the two opposed transducers above cofilin serine 3 is the same phosphorylation that condemns it — so that the capacity to deliver a brief, placed structural instruction is vested in a proteolytic service rather than in a signalling reaction.

Set side by side, these are three statements about one quantity: **how long a molecule stays where it is**. This paper takes that as its organising claim and develops its consequences.

The consequences are sharper than a thematic convergence. For any compartment at stationarity, the number of molecules present is the product of the rate at which they arrive and the mean time each one stays. All three programmes claim a lesion in the second factor. Almost all of the human evidence any of them possesses measures the first product — an abundance at one instant. An abundance cannot identify a residence time without an independent measure of flux, which means the three programmes are under-determined by their own human data, and under-determined in the same way. Several of the field's live contradictions — including two direct ones examined here — dissolve when that distinction is enforced; several of its favoured biomarkers are revealed as censuses offered as reports on a clock.

Four specific junctions are then proposed, each with the evidence for it graded and the experiment that would break it stated. **One:** amyloid-driven dephosphorylation of a routing tyrosine decides whether a glutamate receptor leaves the surface, and retromer-dependent retrieval decides whether it comes back; these are consecutive steps on one receptor that have never been placed in series, and the region that fails first in the disease is the region shown to run the retrieval step closest to capacity. **Two,** and the paper's keystone: Rho-associated kinase 2 has recently been reported to phosphorylate the cytoplasmic tail of the sorting receptor SORLA and thereby reduce its affinity for retromer — which places the kinase that writes the spine's collapse mark on the actin network at a second address, where it un-licenses the receptor that would resupply the spine. If that holds, contractile tone and the traffic jam are not two abnormalities but one self-sustaining lesion, and a clinically available kinase inhibitor already reported to rescue the actin phenotype should be re-examined for a recycling effect it was never tested for. **Three:** the retained intraneuronal peptide has been shown to inhibit both the

proteasome and the deubiquitinating enzymes, which is the mechanism by which one scalar insult can produce opposite changes in two substrates in the same compartment — the asymmetry a single clearance variable cannot predict. **Four:** loss of the sorting receptor and loading of the amyloid machinery produce, in two independent laboratories, the same measured phenotype of hyperexcitability with failed plasticity, and in both cases the authors conclude the peptide is not the sufficient cause.

What is established, what is inferred, and what is this paper's own is marked throughout and tabulated in a ledger. The synthesis is a claim about the execution of synaptic loss, not about what initiates the disease; it is neuron-centred at a moment when the sorting lesion's prevalence in ordinary late-onset disease may be glial; and its central therapeutic proposal — repair the compartment before supplying the signal — contains a conflict between two of its own sources over the direction in which endosomal acidity should be moved. That conflict is named rather than smoothed, and the experiment that resolves it is specified.

I. Three Programmes, One Variable

1.1 What this paper is about

Synapse loss remains the strongest structural correlate of cognitive impairment in Alzheimer's disease. In the study that established the point, neocortical synapse density carried a multivariate correlation of 0.96 with the Dementia Rating Scale, while plaque density contributed a minority of that model's strength (Terry et al., 1991). That observation obliges any account of the disease to say what happens, mechanically, at a synapse. It does not say which account is right, and for thirty years the field's answers have been organised around a different question — what starts the disease — for which the decisive evidence would have to come from human tissue at stages that are largely inaccessible.

The three bodies of work examined here are unusual in that none of them needs to win that argument. Each is, in its strongest form, a claim about a step that an upstream cause must pass through rather than a claim about which upstream cause came first. And each, on inspection, is a claim about the same physical quantity.

The endosomal traffic jam. Developed over two decades by Scott Small, Gregory Petsko and collaborators, this holds that the retrieval arm of endosomal sorting — retromer, with its cargo-selective receptors, of which SORLA is the most important here — is the disease's pathogenic hub. Jam the export step and residency time in the compartment rises for everything held there. The amyloid precursor protein and the enzyme that makes its first amyloidogenic cut spend longer together in an acidic lumen; glutamate receptors that should be returned to the postsynaptic membrane are not; a congested compartment matures into a multivesicular body and releases its contents, tau among them, as exosomes; and microglia, which use the same machinery to return their phagocytic receptors to the surface, clear less (Small et al., 2017; Small and Petsko, 2020).

The same machine. Developed over twenty-eight years by Gunnar Gouras and collaborators, this holds that the synapse manufactures amyloid- β on the organelles it uses to transmit — the two enzymes competing for the first cut of the precursor protein are enriched on synaptic vesicles (Lundgren et al., 2015) — and that the compartment in which the precursor is cut determines which peptide is made and whether it leaves (Greenfield et al., 1999). The programme's central turn is that the pathological variable is **retention rather than overproduction**: in diseased neurons, secretion of the peptide falls rather than rises, and the surface enzyme that normally degrades it is lost (Tampellini et al., 2011). The lesion, on this account, is the failure of the

loops that regulate the peptide, not the presence of the peptide (Roos et al., 2021; Martinsson et al., 2022).

The licensing tyrosine. This is the account advanced in the present line of work and re-established here from the primary record. A dendritic spine is an actin structure whose volume is the running balance of severing against stabilisation, and that balance is set at one residue, cofilin serine 3. The direction of change at that residue in human Alzheimer tissue is disputed and the dispute has not been resolved (Kim et al., 2013; Rush et al., 2018). The argument is that the sign is not recoverable at that residue, because two antagonistic Rho-family arms write the same phosphate there with opposite structural outcomes; that the information lives one layer up, at a tyrosine on each of the two transducers that set the sign; and that both of those transducers are built to the same unusual design — the tyrosine phosphorylation that activates is the tyrosine phosphorylation that licenses destruction (Margolis et al., 2010; Bock et al., 2004; Petshow et al., 2025). The consequence is that **transience at this control surface is achieved by destruction rather than by dephosphorylation**: a phosphatase resets a mark, but it cannot remove a transducer from a compartment.

Three literatures, three compartments, one quantity. The traffic jam is a theory of how long cargo stays in an endosome. The same machine is a theory of how long a peptide stays inside a neuron. The licensing architecture is a theory of how long a transducer stays in a dendrite. In every case the pathology is attributed not to the identity of a molecule and not, in the end, to its quantity, but to the duration of its stay.

1.2 What is claimed here, and what is not

This paper makes three claims of ascending strength and ascending exposure.

The first is a measurement claim, and it follows from elementary accounting. A residence-time lesion cannot be identified from an abundance measurement without an independent measure of flux. All three programmes assert residence-time lesions; almost all of their human evidence is abundance. This is not a criticism of any of them individually — it is a structural property of the instruments the field possesses — but it explains why each has the same unresolved gap at the same place, and it identifies which published results are exceptions.

The second is a set of mechanistic junctions, four of them, each named and graded. These are the paper's substantive contribution. They are proposals about where the three programmes meet in one cell, and each is stated so that a specific experiment would break it.

The third is a disease-level claim, and it is an inference. It is that the persistence of Alzheimer synaptic injury — the fact that a synapse exposed to a transient insult remains less able to respond after the insult has ended — is produced by the interaction of these residence-time failures

rather than by any one of them. That claim is graded as inference in Section 16 and its refutation conditions are stated in Section 15.

Four restrictions are honoured throughout and stated here rather than at the end.

It does not explain what initiates the disease. The account is compatible with amyloid-first, sorting-first, tau-first, inflammatory-first and vascular-first origins. That compatibility is a weakness as much as a strength: an argument consistent with every upstream story constrains none of them. The one ordering commitment made is in Section 11.4, and it can be broken.

It is neuron-centred, at a moment when that may be the wrong emphasis for sporadic disease. Section 12 states the problem in full: the strongest protective common variant at the *SORL1* locus appears to act through microglial expression, and the originating laboratory's own human imaging now places microglia between tau pathology and neurodegeneration.

It concerns the excitatory, spine-bearing pyramidal and stellate neurons of hippocampus and neocortex. The mechanics described are the mechanics of a spine. The cortex's most conspicuously vulnerable inhibitory element, the parvalbumin-positive fast-spiking interneuron, is characteristically aspiny, and no claim is made that it fails this way.

Nothing here is new experimental data. Primary studies support the individual mechanisms. The couplings between them are the proposals, and they are labelled as such.

1.3 Evidence grades, and how they are used

Claims are graded on a five-point maturity scale used consistently in the ledger of Section 16 and flagged in the text where it matters: **M1** human, population-scale or replicated across independent cohorts; **M2** human, single cohort or tissue series; **M3** animal, replicated or bidirectionally manipulated; **M4** animal or primary culture, single laboratory; **M5** in vitro, cell line, or inference from adjacent findings. Preprints are identified as preprints at every mention.

Three distinctions are enforced rather than assumed, because the argument turns on them.

A **level** is not an **object**. Much of the molecular literature on this disease reports how much of a protein or how much of a phosphorylation is present. Almost every mechanism described here is about whether a particular molecule is still in a particular compartment. These are different measurements and they can move in opposite directions.

Association is not **rate**, and rate is not **mediation**. Human tissue establishes anatomical and molecular associations at sampled stages and rarely measures flux. A pulse-chase shows that a molecule persists. Only a selective perturbation with rescue shows that the persistence causes a downstream defect.

Necessity and sufficiency are not **priority**. A convergence claim — whatever happens first must pass through here to produce this outcome — is adjudicated by intervention in the laboratory. An initiation claim is adjudicated by temporal priority in human tissue. Almost all the interventional evidence assembled below is of the first kind, and the paper does not spend it on the second.

2. Residence Time, and Why a Census Cannot Report a Clock

2.1 An accounting identity

Consider any compartment with a defined boundary — an early endosome, the lumen of a multivesicular body, the cytosol of a single dendritic branch — and one molecular species within it. Let λ be the mean rate at which molecules of that species enter, counted per unit time, and W the mean time a molecule spends inside before leaving by any route. At stationarity, the mean number present is

$$N = \lambda \cdot W$$

This is an accounting identity rather than a model. It requires only that the system be stationary over the interval of observation and that every molecule which enters eventually leaves; it makes no assumption about reaction order, compartment geometry, or the number of exit routes. Four consequences follow directly, and all four bear on the three programmes.

First, N is a product, so a change in dwell time and a change in arrival rate are indistinguishable within it. A doubling of W and a doubling of λ produce the same census. The observation that something has accumulated therefore carries no information about which factor changed. This is the formal content of the distinction between retention and overproduction that the intraneuronal-amyloid programme has pressed for two decades (Tampellini et al., 2011), and it is the reason that distinction cannot be settled by any measurement of how much peptide is present.

Second, W is set by all exits together, so a failure of one exit can be invisible in N . If recycling to the surface falls while delivery to the lysosome rises, dwell time may be unchanged, the census unchanged, and the cell nonetheless deprived of a receptor it needed returned. Conversely, a fall in two exits can show as accumulation without any change in entry. A single scalar called “clearance” is therefore the wrong variable: the relevant quantity is route-resolved.

Third — and this is why the identity is not merely pedantic — the chemistry that these programmes care about is a function of W , not of N . The probability that the amyloid precursor protein is cut by the enzyme that works best at acidic pH depends on how long the two occupy the same acidic lumen together, which is a dwell time. The probability that a condemned transducer is still present to be re-activated by the next input depends on how long it survives, which is a dwell time. The probability that a receptor can transduce a second signal depends on whether it was retrieved in time, which is a dwell time. In each case the pathological event is a functional of residence, and N is a quantity in which residence has been multiplied by something else.

Fourth, therefore, a residence-time hypothesis tested with an abundance assay is under-identified rather than imprecisely identified. The remedy is not a better abundance assay. It is a measurement of flux: a pulse and a chase, a labelled cohort followed in time, a stimulation and a recovery interval, an intervention whose effect is read as a change in rate.

An honest limit on the identity should be recorded at once. It applies at stationarity, to a compartment that can be treated as one well-mixed pool, over an interval short compared with the processes being compared. A neuron is not well mixed; a dendrite is not one pool; and a disease that unfolds over two decades is not stationary. The identity is accordingly a reasoning tool about what a snapshot can and cannot contain, not a quantitative model of a neuron. Used that way it is exact and it is useful, and the remainder of this section applies it to specific published results.

2.2 Published results that are dwell-time measurements

The three programmes between them contain a small number of measurements that do report W , and they are consistently the most informative results each programme possesses. They are also, consistently, the least-cited.

The intraneuronal pool turns over in hours. In cells producing amyloid- β , 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 rise in the precursor fragment produced by the first cut; in cells that had already accumulated internal aggregates, the correction did not occur (Roos et al., 2021). Read as a kinetic experiment rather than as a homeostasis experiment, this brackets the residence time of the intraneuronal pool at hours. The consequence is considerable and is rarely drawn: a pool whose dwell time is measured in hours but which rises over decades is not a vessel slowly filling. It is a steady state slowly shifting, and a steady state is set by rates. The disease's twenty-year accumulation is therefore a twenty-year change in λ or W , not a twenty-year integral of an unchanged imbalance — and the distinction is testable, because the two predict different responses to a step change in either factor. *Maturity M4: single laboratory, primary cells and cell lines.*

Secretion falls while the internal pool rises. Cultured neurons from Alzheimer-model mice secreted progressively *less* peptide with time in culture while normal neurons did not; the ability of synaptic activity to raise secretion and simultaneously lower the internal pool became impaired; and surface levels of neprilysin, the enzyme that degrades the peptide at the cell surface and whose colocalisation with A β 42 rises with activity, fell with time (Tampellini et al., 2011). In the identity, this is a fall in an exit rate with a rise in the census — which is to say, a measurement of W obtained by watching an exit. A pure overproduction account predicts that secretion rises. It fell. *Maturity M4, and the single most under-cited result in this corpus.*

The controlled variable in the sorting programme is a rate, and it has been moved in both directions. VPS35 was depleted in mouse hippocampal neurons and then restored with an optimised viral vector; accelerated cleavage of the precursor protein and loss of synaptic glutamate receptors followed the manipulation in both directions, and a neuronal retromer deficit was sufficient to induce the dystrophic microglial morphology characteristic of the disease, independently of tau (Qureshi et al., 2022). Bidirectional manipulation is the strongest design available for a rate hypothesis, because it shows that the phenotype tracks the manipulated step rather than accompanying it. *Maturity M3.*

A receptor's dwell time on the limiting membrane has been measured directly. Using the epidermal growth factor receptor and TrkB as reporters, neurons from precursor-mutant mice showed impaired inactivation, impaired degradation and impaired ubiquitination of the receptor after stimulation, and the accumulating peptide was shown to inhibit the activities of the proteasome and of deubiquitinating enzymes (Almeida et al., 2006). This is a stimulation-and-chase design whose read-out is how long a receptor persists in a state it should have left. It is, so far as this review can determine, the only published measurement of receptor residence under an amyloid load in a neuron, and Section 8 argues it is the mechanistic keystone of the whole synthesis. *Maturity M4.*

A cytosolic transducer's dwell time is activity-regulated. Increased neuronal activity produced a proteasome-dependent reduction in the levels of Ephexin5 in neuronal dendrites (Hamilton et al., 2017). The controlled variable is dendritic, the control is proteolytic, and the trigger is use. In the identity, activity acts on W . *Maturity M4.*

Five results, three programmes, one kind of measurement. Each one was obtained by watching a rate, and each is load-bearing for the programme that produced it.

2.3 Published results that are censuses offered as clocks

The complementary list is longer, and it includes most of what the field measures.

Fluid and imaging measures of amyloid. Cerebrospinal-fluid and plasma peptide concentrations and amyloid positron-emission tomography report, respectively, a stock in the extracellular space and a stock in the deposited phase. The pool that the intraneuronal account

identifies as the one that damages synapses — retained, inside the neuron, in the sorting compartments of the synapse — is measured by none of them. The same programme's own 2005 result showed, further, that the standard immunoassay systematically *underestimates* the peptide once it has assembled, because assembly occludes the sites the assay binds (Stenh et al., 2005), so the extracellular census is itself biased in a direction that depends on aggregation state. Trials select patients, stratify them and judge target engagement on a quantity the mechanism says is not the relevant one. *The point is not that these assays are useless; it is that they are stocks, in the wrong compartment, standing in for a rate.*

Bulk phospho-cofilin serine 3. This is a stock and, worse, a degenerate one. The contractile arm — RhoA through Rho-associated kinase to LIM-domain kinase — and the protrusive arm — Rac1 and Cdc42 through p21-activated kinase to the same LIM-domain kinase — both *add* the same phosphate to the same residue while producing opposite structural outcomes (Kang and Woo, 2019; Bamburg et al., 2021). The reelin pathway, which is protective, writes in the same direction as the destructive contractile arm (Chai et al., 2009). A mean phospho-serine-3 value is therefore uninformative about direction even if measured perfectly at a single synapse with no averaging at all, which is why two careful laboratories sampling different material obtained opposite signs in human Alzheimer tissue and could not reconcile them (Kim et al., 2013; Rush et al., 2018).

Total protein for a transducer whose abundance and activity are one mark. Phosphorylation of Ephexin5 at tyrosine 361 both biases the exchange factor's substrate selection and recruits the ligase that consigns it to the proteasome (Margolis et al., 2010; Petshow et al., 2025); reelin-induced tyrosine phosphorylation of Disabled-1 both transduces the protective signal and targets the adaptor for proteasomal degradation (Bock et al., 2004). Where one covalent modification sets both what an enzyme does and how long it survives, abundance and activity are two readings of one variable, and an elevation is as consistent with a failed signal as with an amplified one. Every published measurement of Ephexin5 in this disease is a measurement of total protein.

Soluble SORL1 in cerebrospinal fluid. This is the most instructive case, because it is a deliberate and intelligent attempt to measure pathway function in a living person, and because its result is exactly what the identity predicts. SORLA reaches the cell surface only if retromer-dependent recycling delivers it there, and the shed ectodomain reaches cerebrospinal fluid; so the soluble species was proposed as a read-out of recycling. In 218 participants, concentrations were significantly lower in carriers of protein-truncating and damaging missense *SORL1* variants, as the model predicts. In patients with sporadic Alzheimer's disease carrying no such variant, the measure did not differ from controls (de Waal et al., 2026). *Maturity M2.*

That pattern is the signature of a product-stock assay. The assay integrates expression, trafficking, shedding, release and clearance into one number. It has the sensitivity to detect a large,

constitutive, whole-cell reduction in the amount of receptor available to be shed — which is what a truncating variant produces. It does not have the sensitivity to detect a modest change in the dwell time of a cargo in a compartment, confined to a small region of cortex, in a cell type that may contribute little to the fluid pool. The negative result in sporadic disease is therefore a real and important constraint on the claim that the lesion is *common*, and it should not be explained away; but it is a constraint delivered by a census, and the claim it constrains is about a clock. Reading it as a defeat of the mechanism overstates what the measurement can carry, in exactly the direction the identity warns about.

Bulk degradative-capacity assays. Total proteasome activity, total autophagic flux and total lysosomal enzyme content are stocks of machine, not fluxes of substrate, and Section 8 argues that the lesion they are used to exclude is a change in *specificity* that none of them can detect. Human Alzheimer synaptic fractions illustrate the interpretive trap directly: they contain increased ubiquitinated substrates *and* increased proteasome components together (Tai et al., 2012). More machine with more tagged substrate is not more throughput. It is the signature of tags placed and not executed.

2.4 What follows for the three programmes jointly

Three conclusions, which organise the rest of the paper.

The three programmes have the same evidential gap in the same place, and it is not a coincidence. Each proposes a dwell-time lesion; each has strong interventional evidence for that lesion in animals or in cultured human neurons, where rates can be manipulated; and each has human evidence that is anatomical and abundance-based. This is why the sorting programme's word *common* remains unsupported, why the intraneuronal programme's human evidence is descriptive rather than mechanistic, and why the licensing architecture's central prediction has never been measured. The gap is instrumental, and it is shared.

The exceptions are the results worth building on. The five measurements in Section 2.2 are the ones that constrain anything, and the junctions proposed in Sections 6 to 9 are built on them rather than on the censuses.

And the identity supplies a positive research instruction, not only a caution. It says what to measure: a recovery interval. The functional phenotype that a residence-time lesion predicts is not a lower mean of anything. It is **impaired restoration of responsiveness after a controlled perturbation** — the synapse that answers the first stimulus and cannot answer the second. That endpoint is a rate by construction, it is accessible in slices and in cultured human neurons, and it is the endpoint against which every junction below is stated.

3. The Compartment: The Traffic Jam as a Theory of Dwell Time

3.1 Anatomy used as a filter

The sorting programme's method deserves separate statement from its hypothesis, because the method is the more transferable asset and because it explains why the molecule at the centre of the account was one nobody was looking for.

Expression profiling of diseased brain in the early 2000s returned candidate lists of unusable length, most of whose entries report the end state of a collapsing tissue rather than anything upstream. Rather than address this statistically, Small's group addressed it structurally: before generating data they specified how a molecule driving the disease ought to behave — abnormal in the entorhinal cortex, which is affected early; relatively spared in the dentate gyrus, which is not; and abnormal across a broad age span rather than only late — and used that specification as a filter on profiles generated from both regions, from cases and controls, across ages. The transcript that best conformed was *VPS35*, the core cargo-recognition component of the retromer complex; western blotting confirmed reduced VPS35 protein in the vulnerable regions and reduced VPS26 as well, and reducing VPS35 in culture altered amyloid- β levels (Small et al., 2005). *Maturity M2 for the human protein reduction.*

The cost of the filter should be stated as plainly as the benefit. A filter built from a model of the disease can return only molecules that fit the model; the prior built into the instrument is the very thing the hypothesis then asserts. That circularity is broken by the subsequent interventional work rather than by the 2005 paper, which is best read as a well-motivated nomination, and which its authors described that way. Two external checks are worth recording: turned on ordinary cognitive ageing the same instrument pointed to a different subregion and a molecular driver with nothing to do with trafficking (Pavlopoulos et al., 2013), and turned on psychosis it implicated different fields in the opposite metabolic direction (Small et al., 2011). An instrument that returns different answers when pointed at different disorders is measuring something about the disorders.

A note on a detail that has propagated in secondary accounts of this work, including in an earlier and less careful treatment of it in the present line of inquiry: the 2005 paper is sometimes said to have narrowed tens of thousands of transcripts to five surviving molecules. That specific count is not a finding of the paper, and it should not be repeated.

3.2 The mechanism is residency, not identity

The traffic-jam statement (Small et al., 2017) makes one move. Jam the export step, and residency time in the endosome rises — for everything held there, not only for the precursor protein. From that single mechanical fact the disease's separate pathologies follow in parallel rather than in series:

- **Amyloid.** The precursor protein and the enzyme that makes the first amyloidogenic cut, which works best at acidic pH, spend longer together in an acidic compartment, favouring that cut and the generation of the peptide and its carboxy-terminal fragments.
- **Synaptic failure.** Glutamate receptors that should be recycled back to the postsynaptic membrane are retained and diverted toward degradation. The synapse weakens without any cell dying.
- **Tau release.** A congested endosome matures into a multivesicular body; cytosolic contents, tau among them, are packaged into intraluminal vesicles and released when that body fuses with the membrane.
- **Glial change.** Microglia depend on the same recovery machinery to return phagocytic receptors to their surface, so the same lesion blunts clearance.

Read in the terms of Section 2, this is a theory whose independent variable is W and whose dependent variables are four dwell-dependent reactions. That is its principal structural virtue: it is the only one of the three programmes that states its lesion as a dwell time in so many words.

It is also the source of the architecture that makes the theory hard to refute. If endosomal recycling is the hub and the precursor protein, the secretases, apolipoprotein E and the immune receptors are spokes, then a spoke can be damaged directly — which is what the early-onset mutations do — and the products of a damaged spoke feed back on the hub, so both routes converge on the same downstream state (Small and Petsko, 2020). This reconciles the genetics of the two forms of the disease without demoting either. It also means that evidence that amyloid damages endosomes is not contrary evidence, that evidence of tau preceding amyloid is not contrary evidence, and that evidence of a glial lesion is not contrary evidence. A model with that property requires of itself an explicit statement of what would count against it, and Section 15 carries the version this paper can defend.

3.3 What is established

Three things, and they are strong.

The human genetics carry the direction of causation without a model organism.

Rare truncating variants in *SORL1*, the gene for the cargo receptor that delivers material to retromer, occur almost exclusively in people with Alzheimer's disease, and missense variants have

now been mapped onto the receptor's structural domains across a very large aggregated cohort, converting an undifferentiated list of rare variants into a graded interpretation (Andersen, de Waal et al., 2025). *Maturity M1*. A gene whose loss-of-function variants appear almost exclusively in people who develop the disease is not a bystander. It accounts for a small fraction of cases and its penetrance is incomplete, and both qualifications matter for scope rather than for direction.

The lesion is sufficient, and the demonstration is bidirectional. Depletion and repletion of VPS35 in mouse hippocampal neurons moved precursor-protein cleavage and synaptic glutamate-receptor loss in both directions (Qureshi et al., 2022); retromer-deficient mice and flies develop hippocampal dysfunction, neurodegeneration and amyloid accumulation (Muhammad et al., 2008); and depletion of the lipid kinase that organises endosomal traffic enlarges neuronal endosomes and enhances amyloidogenic processing, with the relevant lipid depleted in Alzheimer brain tissue (Morel et al., 2013). *Maturity M3*.

The pathway sits upstream of the tau arm, and this was shown only recently and from outside. Working in aged mice that develop tau pathology without any amyloid manipulation, an independent laboratory showed that transgenic up-regulation of SORLA reversed tau phosphorylation, tau seeding activity, synapse loss, impaired long-term potentiation and glial hyperactivation, with reversion of the disease signature on proteomic and single-nucleus transcriptomic analysis; deleting SORLA in the same background made seeding, aggregation and neuroinflammation worse (Huang et al., 2026). *Maturity M3*. This is the most consequential single result for the theory in the period under review: it tests the arm the dual-pathway proposal had claimed since 2008 and never reached in an animal (Small and Duff, 2008), it does so by intervention in both directions against established rather than incipient pathology, and it comes from outside the network of laboratories that generated the hypothesis. One qualification belongs with it: the transgene was crossed into the model rather than introduced after pathology had developed, so the result supports attenuation over the course of the model rather than therapeutic reversal from a treatment start in established disease.

3.4 What is not established

The word *common*. That a recycling failure is *causal* — sufficient to produce the disease's lesions — is now among the better-supported causal claims in the field. That the same failure is the operative lesion in most sporadic disease has never rested on more than inference from correlates, and the first direct assay of pathway function in living people did not support it (de Waal et al., 2026). Section 2.3 argues that this negative is a census result bearing on a clock claim, and that it therefore constrains without settling; Section 12 argues that the most productive available reading relocates the lesion rather than discarding it. Neither reading licenses treating the negative as absorbed. A pathway-function measurement that consistently fails to separate sporadic patients from age-matched controls in adequately powered cohorts would reduce the theory to an account of a rare monogenic subtype plus a druggable modifier, which would still be a substantial achievement and would not be the theory as stated.

The receptor's function is no longer a single function. The hypothesis rests its human-genetic weight on *SORL1* and interprets the gene through cargo delivery to retromer. A familial mutation in the receptor's ligand-binding domain has now been shown to reorganise its interactome around exosome biogenesis and to impair the release and neurotrophic quality of exosomes (Juul-Madsen et al., 2025), and an integrated study of human brain tissue, primary microglia and engineered stem-cell 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). This does not refute the endosomal account. It means that when human genetics attributes risk to *SORL1*, the pathway through which that risk operates has become an assumption rather than an inference.

The therapeutic record has not moved. Twenty-one years after the founding paper, the retromer-stabilising chaperones remain research compounds; their most recent and first independent animal test was by stereotaxic injection, where one of them reduced amyloid-related pathology, normalised synaptic gene expression and restored pathways associated with potentiation (Ramonet et al., 2025). The gene-therapy programme has been presented at a conference and not, at the time of writing, published as a peer-reviewed study (Andersen et al., 2024) — and should be cited as a conference abstract, not as a paper. A search of the public trials registry returns no interventional study of a retromer- or *SORL1*-directed agent in any indication. The reason is structural rather than a failure of science: an agent that repairs an upstream lesion should be given before the downstream lesions are established, which means long trials in minimally symptomatic people, which means a validated biomarker for enrolment, which is the thing that does not yet exist.

And the interests are disclosed and relevant to weighting. The strongest translational claims come from the group that generated the hypothesis, whose senior authors hold equity, patents and advisory relationships in the relevant commercial vehicles, as the papers themselves

state. Nothing about this is irregular and none of it bears on the validity of any individual result. It is a reason to weight the independent contributions of the last two years — the tauopathy rescue, the chaperone replication, the microglial genetics, the cerebrospinal-fluid assay — more heavily, and it is a point in the theory's favour that most of them were positive.

3.5 The regulated coupling, and why it matters here

One recent result from this programme is the hinge on which Section 7 turns, and it belongs in the description of the programme rather than in the synthesis.

The coupling between the cargo receptor and the retrieval machinery had been treated as constitutive. It is not. In a 2026 preprint, the group reports that phosphorylation of SORLA's cytoplasmic tail by Rho-associated kinase 2 reduces the receptor's affinity for retromer; that RhoGEF12, an upstream activator of that kinase, is upregulated in Alzheimer's disease; that a pharmacological inhibitor of RhoGEF12 increases endosomal SORLA–retromer association in mouse neurons; and that in human stem-cell-derived neurons the inhibitor reduces amyloid- β 40 and amyloid- β 42 in a SORL1-dependent manner, including in neurons carrying disease-associated mutations in either *APP* or *SORL1* (Qureshi et al., 2026, preprint). *Maturity M5: cultured neurons, preprint, no whole-animal test.*

Two features make this important beyond its immediate therapeutic proposal. It converts the drug problem from stabilising a multiprotein complex — hard, and the reason the chaperones stalled — into inhibiting a kinase upstream of a phosphorylation site, which is among the best-trodden paths in drug development. And the SORL1-dependence of the effect is an internal control the chaperone work lacked: the compound does nothing when the receptor is absent, which is what a pathway-specific agent should do.

But its largest consequence is not therapeutic, and the paper that reports it does not draw it. The kinase named is the kinase of the contractile arm above cofilin serine 3. Section 7 is about what follows.

4. The Cargo: The Same Machine as a Theory of Retention

4.1 Location determines product, and the first cut is on the synaptic vesicle

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

Using imaging in intact neurons, density-gradient separation and reconstitution outside the cell, the group established that the two principal peptide species are not made in the same place: the shorter is generated in the trans-Golgi network and packaged for export, whereas a population of the longer, aggregation-prone species is generated and *retained* in the endoplasmic reticulum in an insoluble state, with a further pool made in the trans-Golgi and exported (Greenfield et al., 1999). The conclusion is easy to state and took 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.

Where in the cell that geography lies was answered much later and more specifically than most summaries convey. The two enzymes competing for the first cut of the precursor — the one that begins the amyloid-producing route and the one that pre-empts it — are both strongly enriched on *synaptic vesicles* isolated from rat brain; the activity of the pre-empting enzyme was detectable in that fraction and the intermediate fragments produced by the first cut were enriched there too, while of the complex that performs the second cut only one component was enriched and active enzyme colocalised with the vesicle marker only sparsely (Lundgren et al., 2015). *Maturity M4*. 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 the peptide. It manufactures the precursor fragments locally, on the organelles it uses to transmit.

The human anatomy followed. The longer peptide accumulates within neurons in the regions vulnerable to the disease, in a distribution that appears to precede both plaques and tangles (Gouras et al., 2000); electron microscopy localised that intraneuronal peptide to multivesicular bodies inside synaptic terminals, within presynaptic and especially postsynaptic compartments, associated with abnormal synaptic structure **before** plaque pathology (Takahashi et al., 2002); and the peptide was then shown assembling into small soluble clusters inside those same processes and synapses (Takahashi et al., 2004). *Maturity M2 for the human localisation*. Independently, abnormalities of the internalisation pathway — swollen early sorting compartments — had been shown to precede amyloid deposition in sporadic disease and in Down syndrome (Cataldo et al., 2000). Two lines

converged on the same compartment from different directions, and the convergence has gone largely unexploited.

4.2 Retention, not overproduction — and the clearance coupling that fails

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

This programme points somewhere else, and the turn is the most consequential and least-cited thing in it. The variable that matters is not how much peptide is made but how much of it fails to leave. The distinction has a direct therapeutic consequence: a production problem is addressed by reducing synthesis, which is the strategy behind the secretase inhibitors that failed in trials; a retention problem is addressed by restoring export and degradation, which is a different target list and has scarcely been attempted.

The direct evidence is the result already discussed as a dwell-time measurement in Section 2.2, and it bears restating in its own terms. In cultured neurons from Alzheimer-model mice, secretion of the peptide fell with time in culture while normal neurons showed no such decline; the ability of synaptic activity to raise secretion and simultaneously lower the internal pool became impaired in diseased neurons and not in normal ones; and the mechanism was identified — synaptic activity normally increases the amount of surface neprilysin and its colocalisation with the longer peptide, and in diseased neurons neprilysin levels fell with time (Tampellini et al., 2011). The diseased synapse loses the activity-coupled clearance system that normally holds its local pool down. It is not making more; it is disposing of less, and the specific step that fails has a name.

It is worth recording in passing that the enzyme in question is under neuropeptide control — somatostatin regulates brain A β 42 through modulation of proteolytic degradation by this same enzyme (Saito et al., 2005) — and that somatostatin-expressing inhibitory neurons are among the populations lost in Alzheimer cortex. The programme has not pursued the thread, and it is one of several places where an obvious extension has been left undone.

What raises the retained pool has, by contrast, been documented extensively, and the breadth is structurally important rather than merely impressive. Normal ageing up-regulates internalisation of the precursor protein in aged neurons and aged brain, potentiating processing, and blocking production reverses the associated synapse loss (Burrinha et al., 2021). Apolipoprotein E internalised from astrocytes goes preferentially, in neurons, to the sorting and autophagy compartments of the neurites, where it intersects with the precursor and the peptide, and the E4 variant raises the neuronal pool (Konings et al., 2023). A precursor-protein 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). Interfering with the machinery that carries cargo into

multivesicular bodies traps the peptide inside the neuron and enlarges the compartment (Edgar et al., 2015; Willén et al., 2017a). Brain extract induces intracellular inclusions that persist across cell generations and seed further cells (Olsson et al., 2018; Roos et al., 2021). Loss of the surface degrading enzyme breaks the activity–clearance coupling (Tampellini et al., 2011). Chronic synaptic inhibition raises the internal pool (Tampellini et al., 2009, 2010). And cells already carrying aggregates lose the correction that would restore balance (Roos et al., 2021).

Eight entry conditions, causally independent of one another, one downstream compartment. Ageing is not apolipoprotein E4; a seed is not a mutation. What they share is where they arrive — which is the structure of a convergence claim, and which is why the account does not need, and its evidence does not supply, any particular reason for the rise.

4.3 Four routes from a loaded compartment to a failing synapse

The programme has identified four routes by which a loaded compartment produces a synapse that no longer works. They were established at different times by different methods, they are separable, and no one of them accounts for the whole phenotype.

Receptor withdrawal. In cultured neurons from precursor-mutant mice the earliest measurable change in synaptic composition was a reduction in the scaffolding protein that anchors glutamate receptors at the postsynaptic side, followed by reduced surface expression of an AMPA-receptor subunit; inhibiting the enzyme that produces the peptide blocked the changes, and adding synthetic peptide to normal neurons reproduced them (Almeida et al., 2005). In parallel, the peptide was shown to promote internalisation of NMDA receptors, with neurons from an Alzheimer model carrying fewer at the surface and inhibition of production restoring them; the pathway was traced through a nicotinic receptor, a phosphatase, and dephosphorylation of a specific tyrosine residue on the receptor subunit (Snyder et al., 2005). *Maturity M4, with intervention in both directions.* That last clause is the hinge of Section 6 and is developed there.

Autocatalysis. The most elegant result in the corpus, and the one Section 8 argues is the mechanistic keystone of this synthesis. Using two receptor systems as reporters, the group showed that peptide accumulation in precursor-mutant neurons impairs the multivesicular-body sorting pathway — receptor inactivation, degradation and ubiquitination were all impaired — and provided evidence that the accumulating peptide inhibits the proteasome *and the enzymes that remove ubiquitin tags* (Almeida et al., 2006). The structure of the 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, and which requires no external driver — whatever raised the pool initially need not still be present.

Structural failure. Compartments loaded with undegradable cargo distend. Interfering with the packaging machinery reproduces both the accumulation and the enlargement, establishing the swelling as a consequence of the sorting failure rather than an independent lesion (Willén et al., 2017a); three-dimensional reconstruction showed fibrillar material inside individual synaptic compartments, associated with abnormal morphology (Capetillo-Zarate et al., 2011); and accumulating intraneuronal peptide was associated with early changes in a microtubule-associated protein in neurites and synapses (Takahashi et al., 2013). This route explains something the others do not: the swollen, clubbed nerve endings that surround plaques in human tissue, described since the first decade of the twentieth century, which no purely biochemical account of synaptic dysfunction addresses.

Translational block. Lasting synaptic change requires new protein made locally on demand. The mTOR pathway is suppressed in hippocampal tissue from an Alzheimer model and in normal tissue exposed to the peptide, the intraneuronal peptide colocalises with mTOR, and potentiation can be rescued by restoring mTOR signalling (Ma et al., 2010); dysregulation of an elongation factor required for translation correlates with the plasticity impairments (Beckelman et al., 2016); and acute exposure to the E4 variant of apolipoprotein E causes a sustained calcium rise, sustained phosphorylation of that elongation factor and a sustained translational block, abolishing the translational response to NMDA-receptor stimulation (Ramakrishna et al., 2021). A synapse that cannot make new protein on demand cannot consolidate a change, whatever the state of its receptors.

That the routes are separable matters twice over. 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 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.

One further result qualifies all of them and is important for selective vulnerability: the association of the peptide and its precursor with synapses is *heterogeneous*, with preferential binding to excitatory rather than inhibitory neurons, and with the precursor's cleavage fragments accumulating earlier on the transmitting than on the receiving side (Willén et al., 2017b). Synapses are not uniformly exposed, and what determines which ones are is unresolved.

4.4 Activity is the regulator, and the designed dissociation

The relationship between activity and amyloid appears at first to be simple and bad: activity increases release into the extracellular space, and extracellular peptide 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 *inside* the neuron, promotes transport of the precursor to synapses and protects against peptide-related synaptic alterations, with the reduction mediated by neprilysin; and the synaptotoxicity of extracellular peptide itself requires ongoing processing of the precursor, so the external insult acts partly by way of the internal pool (Tampellini et al., 2009). Activity therefore has opposite signs in the two compartments: it raises what is outside and lowers what is inside.

The decisive test is the single experiment in this corpus that most clearly separates the programme's claims from plaque-centred accounts, and it is a designed dissociation rather than an observation. Chronic synaptic activity had 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 it directly with two independent means of chronic inhibition — surgical deafferentation of the barrel cortex and a benzodiazepine. Both reduced plaques. Both made the animals worse: prolonged inhibition exacerbated loss of a presynaptic marker relative to more active regions in Alzheimer-model but not normal mice, benzodiazepine treatment followed by washout exacerbated memory impairment in the model animals, and the deterioration occurred with *reduced* plaques and *elevated* intraneuronal peptide (Tampellini et al., 2010). *Maturity M4*. Plaque burden and synaptic-behavioural outcome were driven in opposite directions in the same animals, and outcome followed the internal pool. Whatever else is true of the programme, this experiment is a direct demonstration that the extracellular deposit is not the variable that tracks function.

Section 8.4 returns to this experiment and proposes that it measured something broader than amyloid clearance.

4.5 The lesion is the loss of the control loop, and the residue that complicates it

Sections 4.1 to 4.4 describe a system with a regulator. The programme's most recent decade has been about what happens when the regulation fails, and this is where it locates the actual lesion.

Neurons hold their firing rate near a set point despite changes in input, by multiplicatively adjusting the strength of their remaining connections and tuning their intrinsic excitability (Turrigiano, 2008). In 2022 the group showed this machinery is broken in the disease: neurons carrying elevated peptide became hyperactive, with calcium transients of increased frequency and amplitude, 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 (Martinsson et al., 2022). The cell has not simply become noisy. It has lost the ability to find its own set point. The parallel result on the amyloid homeostat itself — the eighty-per-cent fall and six-hour recovery that does not occur in cells already carrying aggregates (Roos et al., 2021) — converges on the same statement: the lesion is not the presence of the peptide

but the loss of the loops that regulate it. The peptide is present in every normal brain and is under control there.

Honesty requires recording a finding 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 the peptide at all became **just as hyperactive** as those flooded with peptide; and deleting the precursor altogether alters synaptic proteins and receptors, including raising a surface AMPA-receptor subunit, 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, and the account does not yet specify how much. This is an unresolved hole in an otherwise coherent structure; it is the group's own finding rather than a critic's; and Sections 9 and 10.2 argue that it is also the key to a five-year-old contradiction in a neighbouring literature.

4.6 Where the programme is weak

Four weaknesses bear on how much weight the synthesis below can put on it.

Over-expressing animals. The great majority of the cellular mechanism was established in mice engineered to over-produce the precursor protein, which 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 of an origin prediction in a knock-in went the other way (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 this synthesis leans on most heavily — impaired secretion with loss of surface neprilysin, and loss of homeostatic correction in cells already carrying aggregates — do not appear to have been independently reproduced outside the group. Both are single-laboratory results carrying a great deal of weight, and both are cited here as such.

Human evidence is descriptive rather than mechanistic. The human contributions establish that the phenomenon exists in human brain. None of the four routes of Section 4.3 has been demonstrated in human tissue.

The measurement problem is answered but not closed. The standing objection to the programme has always been that the antibodies used to detect a small peptide inside a cell full of its own precursor cannot distinguish the two. The response was to publish the objection — the group showed that the standard assay underestimates the peptide once assembled (Stenh et al., 2005), stated that detergent used in tissue processing can remove the intraneuronal pool altogether (Gouras et al., 2012), and recently reported that the standard antibody binds not only plaques but several

brain cell types and vessels (Wen et al., 2026) — and then to build methods that do not need an antibody. Synchrotron-based infrared micro-spectroscopy, which reports molecular structure directly, showed that the structural states of both peptide and precursor are altered in model brain *before* any plaque forms and that focal pre-plaque aggregates localise to synaptic terminals (Klementieva et al., 2017); dye-based three-dimensional reconstruction showed fibrillar material inside individual synaptic compartments (Capetillo-Zarate et al., 2011). Three methods with different failure modes agree qualitatively. None quantifies the intraneuronal pool absolutely, and the most decisive of them is technically demanding and narrowly replicated. A body of work that publishes the evidence against its own tools and then survives the argument has earned a different kind of confidence from one that has only ever been defended; what it has not yet earned is a number.

5. The Control Surface: Why Termination at the Spine Is Proteolytic

This section states the third programme compactly. It is re-established here from the primary record rather than asserted, because the junctions in Sections 6 to 9 depend on specific features of it, and because its first claim is a claim about what a widely used measurement cannot do.

5.1 The degenerate residue

Cofilin is the major actin-depolymerising factor of mammalian neurons and is switched by phosphorylation on serine 3: phosphorylated cofilin cannot bind filamentous actin and the network is stable, dephosphorylated cofilin binds and severs. LIM-domain kinases add the phosphate; the slingshot phosphatases and chronophin remove it (Bamburg et al., 2021; Kang and Woo, 2019).

The structural fact on which the first claim rests is standard cell biology, which is precisely why its consequence for the Alzheimer literature has gone unstated. The Rho-family GTPases that control spine actin do not form one pathway but two, with opposite structural outputs, and both write the same mark. RhoA activates Rho-associated kinase, which phosphorylates LIM-domain kinase on its activation-loop threonine, which phosphorylates cofilin at serine 3; the structural output is shrinkage. Rac1 and Cdc42 activate p21-activated kinase, which phosphorylates the *same* LIM-domain kinase on the *same* threonine, with the same consequence at serine 3; the structural output is growth and stabilisation.

Both arms raise phospho-serine-3, and they mean opposite things. This is not a subtlety about kinetics or subcellular pools: it is a many-to-one collapse in the information the mark carries. Even a

perfectly compartment-resolved measurement made at a single synapse with no averaging would not say which arm produced it. The degeneracy is in the variable, not in the sampling.

That architecture predicts the published contradiction. One laboratory reports amyloid *lowering* phospho-cofilin through an immune receptor, with enhanced cofilin signalling also seen in human Alzheimer brains, and the direction was independently reproduced with a natural receptor antagonist (Kim et al., 2013; Kawaguchi et al., 2022). Another, with no shared authorship, reports amyloid *raising* phospho-cofilin in the postsynaptic-enriched fraction of human Alzheimer cortex, where the phosphorylation is reported necessary and sufficient for synaptic impairment via actin stabilisation, with the clinically available Rho-kinase inhibitor fasudil preventing the stabilisation, the impairment and the loss (Rush et al., 2018). The 2018 paper opens by stating that the effect of amyloid on the actin cytoskeleton “remains unknown and contentious.” It still is. If phospho-serine-3 is the shared output of a collapsing arm, a growing arm and a receptor-driven phosphatase arm, which sign a given preparation returns is a function of which arm dominates the material sampled, and both results can be correct measurements of the same disease.

A caution against over-reading even the two-arm picture belongs here, because the paper that established the arms' spatial behaviour complicates them. Imaging RhoA and Cdc42 activity in single spines during induced potentiation, Murakoshi and colleagues found that both are activated by stimulation, that inhibiting the Rho-kinase pathway preferentially blocked the *initial* phase of spine growth while inhibiting the Cdc42 pathway blocked the *maintenance* of sustained plasticity, and — the detail that matters most below — that active RhoA diffused out of the stimulated spine and spread some five micrometres along the dendrite, whereas Cdc42 activation remained restricted to the stimulated spine with a steep gradient at the spine neck (Murakoshi et al., 2011). *Maturity M4*. So the contractile arm is not simply the collapse arm; it participates in growth, and its spatial extent is dendritic rather than synaptic. Both facts are used in Section 7, and the second is used in a way the original paper did not intend.

5.2 Two transducers, one design

The information the field wants is not lost. It has been sought at the wrong residue, because the arms differ upstream, at the level of which exchange factor is active on which GTPase. In the two transducers that set that difference, the governing mark is a tyrosine, and the two are built to the same unusual design.

Ephexin5. The product of *ARHGEF15* was identified as a Rho-family exchange factor, highly expressed in immature neurons, that negatively regulates excitatory synapse development until EphB receptor signalling triggers its phosphorylation, ubiquitination by the ligase Ube3A, and proteasomal degradation, which relieves the restraint and permits synaptogenesis (Margolis et al., 2010). *Maturity M4*. Note the kind of mechanism this is: the synapse is permitted to form not because

a brake has been switched off but because a brake has been **removed from the cell**. The signal is terminated by proteolysis, and the phosphorylation is the licence.

The simple brake reading was then substantially revised. Reducing Ephexin5 increases spine outgrowth and increasing it decreases outgrowth, consistent with a brake; but the protein is also elevated on the dendritic shaft at the sites of future new spines *before* those spines appear, and lowering it *inhibits* new spine outgrowth in response to global and local stimulation — and increased activity produces a proteasome-dependent reduction in dendritic Ephexin5 (Hamilton et al., 2017). The mechanism of the duality was established in 2025: Ephexin5 activates both RhoA and Cdc42; live imaging of GTPase biosensors at single spines showed that during plasticity it regulates the activation of Cdc42 but not of RhoA; the selectivity is set by tyrosine phosphorylation, which is itself activity-regulated and falls sharply within minutes of chemical potentiation by dephosphorylation rather than degradation; a phosphorylation-dead mutant at the key tyrosine enhances Cdc42 activation while leaving RhoA unchanged; and spines in knockout neurons fail to sustain activity-driven growth, rescued by full-length but not catalytically dead protein (Petshow et al., 2025). *Maturity M4*. Ephexin5 is therefore not a brake the adult brain has switched off. It is a switch the adult brain keeps and throws, and substrate selection is a property not of the enzyme but of its phosphorylation state.

The coincidence that has not been drawn out is that **the residue at which the switch is thrown is the residue whose phosphorylation recruits the ligase and consigns the enzyme to the proteasome**. Phosphorylation biases the exchange factor toward RhoA *and* marks it for destruction; dephosphorylation releases the Cdc42-directed activity *and* protects the protein from turnover. Abundance and activity are not independent variables in this system; they are two readings of one covalent modification.

Disabled-1. The opposing input to spine actin is reelin, and its architecture is the mirror image. Reelin binds the ectodomains of the very-low-density lipoprotein receptor and ApoER2 and induces tyrosine phosphorylation of the adaptor Disabled-1, through which the whole signal passes; loss of reelin or of both receptors hyperphosphorylates tau (Hiesberger et al., 1999). The actin limb was characterised a decade later: reelin signalling leads to serine-3 phosphorylation of cofilin and stabilises the cytoskeleton, through ApoER2, Disabled-1, Src-family kinases and the lipid kinase downstream (Chai et al., 2009).

And the same design. Reelin treatment targets Disabled-1 for degradation by the ubiquitin–proteasome pathway; tyrosine phosphorylation of the adaptor is required for that targeting; genetic deficiency in the adaptor's kinase Fyn prevents it; the degradation depends on both receptors in a gene-dose-dependent manner; and pharmacological blockade of the proteasome prevents formation of a proper cortical plate in slice culture, so the degradation is a required part of the signalling rather than an epiphenomenon (Bock et al., 2004). *Maturity M4/M5: primary embryonic*

neurons. Independently, reelin was shown to activate Src-family kinases in neurons in a manner dependent on both receptors and on the adaptor, with Disabled-1 protein levels *rising* in Fyn-deficient mice — the signature of impaired signalling (Bock and Herz, 2003; Arnaud et al., 2003). Later work established that the adaptor is multi-site and that the phosphotyrosine requirements for signalling and for degradation are not one residue: a particular tyrosine-containing recognition site is the important one for recruitment of the ubiquitin-ligase machinery that controls turnover, while others are dispensable for that response (Feng et al., 2007). The architecture is therefore a feedback system in which receptor activation both initiates downstream responses and promotes removal of the adaptor that supports them — and an elevated total Disabled-1 can mean diminished receptor-driven turnover, a downstream degradation defect, altered synthesis, or redistribution, which predict different phosphorylation patterns and different responses to ligand.

Table 1 — Two opposed transducers, one design

	Ephexin5	Disabled-1
role above cofilin Ser3	selects RhoA or Cdc42 as substrate	transduces reelin: stabilises actin, restrains the tau kinase
governing mark	phospho-tyrosine 361	phospho-tyrosine, site-specific for turnover
kinase writing it	EphB2; other tyrosine kinases uncharacterised	Fyn, with Src partially redundant
what the mark does, first	biases substrate toward RhoA	recruits the downstream lipid kinase; transduces
what the mark does, second	recruits Ube3A, then the proteasome	targets the adaptor to the ubiquitin–proteasome system
how the signal ends	destruction of the transducer	destruction of the transducer
the protein accumulates when	the licensing kinase is lost	signalling is impaired, or Fyn is absent

The parallel is verifiable directly from the sources tabulated, and the observation that the two opposed arms above one residue are built to the same self-terminating design is this line of work's structural contribution. Two features of it are load-bearing below. In each, the accumulation of the transducer is the biochemical signature of a *failed* signal rather than an amplified one. And in each, transience is achieved by proteolysis and not by dephosphorylation.

5.3 A third tyrosine, with the opposite polarity

A third instance belongs in the same frame, and it comes from inside the intraneuronal-amyloid programme rather than from this one — which is the first indication that the two literatures are describing one architecture.

The pathway by which amyloid withdraws NMDA receptors from the neuronal surface runs through a nicotinic receptor, calcineurin, and the striatal-enriched tyrosine phosphatase STEP, and the proximate event at the receptor is **dephosphorylation of tyrosine 1472 on the GluN2B subunit** (Snyder et al., 2005). That residue lies within an endocytic regulatory motif, so its phosphorylation governs adaptor access and therefore receptor residence at the surface.

The polarity is the reverse of the two transducers. At GluN2B, phosphorylation favours *retention*; dephosphorylation licenses *departure*. This is not an inconvenience to the argument; it is the reason the argument has to be made about architecture rather than about a generic meaning for phosphotyrosine. The consequence of a modified tyrosine depends on the protein and on the partner that reads the modified site. What the three instances share is the thing that matters: in each case a tyrosine phosphorylation is a routing instruction whose consequence is a change in how long the molecule stays where it is.

The phosphatase that writes the third instruction has itself been measured in human disease, and the measurement is the subject of Section 8.3.

5.4 The consequence: the off switch is a service

A cell that must deliver a *placed* and *brief* structural instruction to its cytoskeleton has a problem that a kinase–phosphatase pair does not solve well. A phosphatase resets the level of a mark, but the transducer remains in the compartment, available to be re-activated by any subsequent input including noise. If the instruction must be local — this spine, not its neighbour — and must not persist, removing the transducer is the more reliable termination. It is also self-limiting in a useful way: the more strongly the signal is transduced, the more completely the transducer is consumed, so the response is intrinsically pulse-like and cannot run away.

The price is a specific fragility, and it is the whole of this paper's interest in the architecture. A system that terminates by proteolysis has its temporal control vested in the proteolytic machinery rather than in the signalling machinery. Its off switch is not a chemical reaction that proceeds at the same rate regardless of cellular state. It is a **service** — provided by the ubiquitin–proteasome system at the cytosolic transducer, by endosomal sorting and the multivesicular body and the lysosome at the receptor. Anything that degrades that service degrades the cell's capacity to make brief signals, without touching a single kinase or receptor.

Three distinguishable proteolytic services act on the molecules in this account and the disease affects them differently. The **canonical ubiquitin–proteasome system** is the characterised route for both transducers and is also the route by which amyloid disposes of EphB2 (Cissé et al., 2011) — which makes it a participant in the disease rather than merely a casualty of it. A **dendritic, activity-regulated membrane proteasome** degrades intracellular proteins into peptides released directly into the extracellular space, where they act as selective activators of

NMDA receptors; its activity is promoted by stimulation (Türker et al., 2024). Its properties coincide closely with those of the reported activity-dependent, proteasome-dependent fall in dendritic Ephexin5, though whether the second degrades the first has not been tested and is stated here as a hypothesis rather than a finding. And the **endosomal-lysosomal system** handles the receptor limb. Separately, proteasomes themselves are redistributed into dendritic spines in an activity-dependent manner and their activity locally regulated (Bingol and Schuman, 2006), which links a physiological signal to the placement of the machinery as well as to its rate.

The unifying observation is that the spine's control surface has no off switch that is not a protease. Its kinases are shared, its phosphatases are non-specific and disease-engaged, and the only mechanism that reliably ends a local structural instruction is removal of the molecule that carried it.

That is the sentence on which this paper turns. If termination at the spine is a service provided by the sorting and degradative systems, then the two programmes of Sections 3 and 4 are not neighbouring accounts of a different compartment. They are measurements of the clock on which this control surface depends.

5.5 Misdirection rather than loss, and what it still owed

The disease's signature at this surface, on the account advanced in this line of work, is not a loss of proteolysis but a misdirection of it, and the argument reduces to two published findings about the same pathway in the same cell type.

The licence-writing receptor is destroyed too much. Amyloid- β oligomers bind the fibronectin-repeat domain of EphB2 and trigger the receptor's degradation *in the proteasome*; knockdown of EphB2 reduced NMDA-receptor currents and impaired potentiation in the dentate gyrus of non-transgenic mice, while increasing EphB2 in amyloid-bearing mice reversed deficits in potentiation and memory (Cissé et al., 2011). *Maturity M3, bidirectional in vivo.*

The effector is destroyed too little. Ephexin5 protein is elevated in the hippocampi of human Alzheimer patients and of model mice, amyloid acutely promotes its production, genetic removal eliminates hippocampal spine loss and rescues behaviour in the model, and reduction in the dentate gyrus of presymptomatic animals protects against later cognitive impairment (Sell et al., 2017). *Maturity M2 for the human elevation: a single report, no independent cohort, no cell-type resolution.*

EphB2 is the kinase whose signalling phosphorylates the licensing tyrosine and thereby recruits the ligase that destroys Ephexin5. Amyloid feeds that kinase to the proteasome. The proteasome then does not receive the substrate whose destruction the kinase was supposed to authorise. In the same neuron, in the same compartment, one arm of ubiquitin-dependent degradation over-runs while the arm it was supposed to license under-runs. The proteolytic system is not failing; it is executing the wrong instruction.

A second, independently evidenced route to the same accumulation should be recorded, because it changes what the observation licenses. In model mice, reduced Ube3A was found together with increased Ephexin5 and increased active RhoA and *unchanged* total EphB2; a chase experiment showed faster loss of the ligase after amyloid exposure, prevented by inhibition of a tyrosine kinase; depleting Ephexin5 prevented the measured RhoA activation and protected spines; and restoring the ligase was beneficial (Olabarria et al., 2019). *Maturity M4*. So Ephexin5 can rise through loss of ligase availability without any fall in total receptor. The causal question is accordingly more discriminating than “receptor down, effector up”: is the limiting step receptor signalling, ligase availability, catalytic execution, synthesis, or local transport? Several may coexist, and they predict different measurements.

Three things the account owed an explanation for, and did not have:

A mechanism for the sign asymmetry. A single scalar called clearance predicts that a receptor targeted for proteasomal degradation and a transducer targeted for proteasomal degradation move in the same direction. They do not. Section 8 supplies the mechanism, from the intraneuronal-amyloid programme.

A reason why the collapse arm, specifically, should be self-sustaining. The account requires that something restores RhoA bias after amyloid has stripped the licensing receptor, and left the candidate open. Section 7 proposes an answer that does not require a new kinase at the tyrosine at all.

An account of why this neuron. The architecture is general; the disease is not. Section 6.3 argues that the sorting programme has the answer, and that it is an answer about recycling demand.

6. First Weld: The Tyrosine Decides Departure, Retromer Decides Return

6.1 Two consecutive steps on one receptor, never placed in series

Take the single best-characterised receptor event in the intraneuronal-amyloid programme and follow it one step further than that programme follows it.

Amyloid- β promotes internalisation of NMDA receptors. The proximate molecular event is dephosphorylation of GluN2B tyrosine 1472 by STEP, acting downstream of a nicotinic receptor and calcineurin; neurons from an Alzheimer model carry fewer surface receptors, and inhibiting

peptide production restores them (Snyder et al., 2005). The receptor has now left the surface. The programme's account of what happens next is that the peptide accumulates in the multivesicular body (Takahashi et al., 2002) and that sorting through that compartment is impaired (Almeida et al., 2006).

Now take the sorting programme's central claim about the same compartment. After internalisation, whether a glutamate receptor is returned to the postsynaptic membrane or diverted toward degradation is decided by retromer-dependent retrieval, with cargo-selective receptors bringing specific proteins to the complex; jam that step and the receptor is retained and diverted (Small et al., 2017). The claim is not generic: the retrieval core that neurons are enriched in has been shown to mediate the recycling of glutamate receptors specifically, and of the cargo receptor SORLA itself (Simoes et al., 2021).

These are consecutive steps on the same molecule, and they have never been placed in series. The tyrosine decides whether the receptor departs. Retromer decides whether it returns. The surface receptor complement of a spine — which is to say its physiological strength — is the running balance of those two decisions, and the two literatures that measured them do not cite each other.

Stated in the terms of Section 2, the two steps are the two halves of a residence time. Internalisation sets the rate at which a receptor leaves the surface pool; retrieval sets the rate at which it comes back. Surface abundance is the product, and a fall in surface receptor is therefore consistent with more internalisation, less retrieval, or less delivery from elsewhere — the three being distinguishable only by a recovery measurement.

6.2 What each programme supplies the other

The weld is not decorative. Each side resolves something the other leaves open.

What sorting supplies to the amyloid account. The receptor-withdrawal route of Section 4.3 explains a loss of surface receptors. It does not explain *persistence* — why a synapse exposed to a transient peptide load should remain weak after the load has fallen. Internalisation is reversible by construction; a receptor in an endosome is a receptor that can come back. For the withdrawal to be lasting, the return must fail, and the sorting programme is the account of why the return fails. Without it the amyloid account describes an insult; with it, a lesion.

What the amyloid account supplies to sorting. The traffic-jam model requires a jam and explains its consequences. It is less specific about what fills the compartment in the first place in sporadic disease, where no truncating variant is present. The intraneuronal programme supplies a measured answer: a retained peptide pool that physically occupies the same compartment (Takahashi et al., 2002), that distends it (Willén et al., 2017a), and that inhibits the machinery which sorts it (Almeida et al., 2006). The sorting programme's own architecture already has a name for this

— a spoke feeding back on the hub — but the spoke has been treated as the precursor protein's processing rather than as a measured occupancy of the compartment with a measured effect on its machinery.

What both supply to the licensing architecture. Section 5.4 argued that the off switch of the spine's control surface is a service rather than a reaction. These two programmes are the specification of that service: one describes the retrieval decision that determines whether a receptor can transduce a second signal, the other describes what a loaded compartment does to the executor. The licensing account's weakest point is that it asserts a dependency on a clock without measuring the clock. These programmes have measured it.

6.3 The corollary: a region that recycles for a living

The licensing account explicitly disclaims an explanation of selective vulnerability — cofilin is ubiquitous and the disease is not. The sorting programme has an answer, and it is an answer of exactly the right shape.

Neurons are enriched in a second retrieval core organised around VPS26b rather than the ubiquitous VPS26a, and the two are not interchangeable: the VPS26b core is differentially dedicated to the *recycling* arm of endosomal traffic rather than to retrograde transport. Imaging mouse models, the trans-entorhinal cortex — the region that staging schemes identify as the earliest cortical site of tangle pathology — was the region most susceptible to VPS26b depletion, corroborated by electrophysiology, immunocytochemistry and behaviour; VPS26b was then found enriched in the trans-entorhinal cortex of human brains, where both it and SORLA are deficient in disease; and VPS26b was shown to mediate the recycling of glutamate receptors and of SORLA (Simoes et al., 2021). *Maturity M3 for the animal susceptibility, M2 for the human expression and deficiency.* The paper's own interpretation invokes the region's network properties, hypothesised to impose a heavy demand on endosomal recycling; the neurons of the entorhinal region carry unusually extensive dendritic arbours and occupy hub positions, so maintaining synaptic function across that surface loads the recycling machinery heavily. That demand argument is inference rather than demonstration and should be labelled so; what is demonstrated is the differential expression and the differential susceptibility.

Place that beside the cell the licensing architecture converges on. The Rho-kinase measurement is made in a postsynaptic-density-enriched fraction with glutamate-receptor insertion as its functional read-out (Rush et al., 2018). The Ephexin5 arm is characterised in hippocampal pyramidal neurons and dentate gyrus (Margolis et al., 2010; Sell et al., 2017; Hamilton et al., 2017). The reelin arm terminates on pyramidal neurons, where the adaptor is predominantly expressed (Pesold et al., 1999), and the receptor carrying the protective ligand is concentrated in entorhinal layer II, the prosubiculum–CA1 border and scattered neocortical pyramids (Ramsden et al., 2022).

It is the same map. And the coincidence has a mechanistic reading that neither programme has stated: *the region that fails first is the region shown to run the service on which the spine's off switch depends closest to its capacity.* A cell operating its recycling machinery near saturation is the cell in which a modest loss of capacity first produces a deficit — which is the sorting programme's own demand argument — and if termination of the spine's structural instructions is one of the things that machinery is doing, then that cell is also the first cell in which instructions stop being ended.

This is the paper's answer to the vulnerability question the licensing account could not answer, and it is an inference built on two established observations plus one hypothesis the sorting programme itself labels as such. It is graded accordingly.

6.4 Predictions, and what would break the weld

Three measurements follow, in ascending cost.

The licensing architecture should fail in a recycling-deficient neuron before any amyloid manipulation. In neurons depleted of VPS26b — the animal exists (Simoes et al., 2021) — the prediction is that dendritic Ephexin5 rises, the phosphorylated fraction of its licensing tyrosine falls, and the dispersion of phospho-cofilin serine 3 across individual synapses rises, all without any change in amyloid. **The weld fails if** the transducer pool is unchanged in a cell whose recycling core has been removed, because that would show the control surface does not in fact depend on this service.

Recovery, not level, should be the affected variable. In the same preparation, deliver a defined stimulation pulse and measure surface GluA1 and GluN2B return as a function of time, and the excursion and recovery of phospho-serine-3. The prediction is that the first response is near-normal and the second is not — impaired restoration of responsiveness after a controlled perturbation, which Section 2.4 identified as the phenotype a residence-time lesion predicts. **The weld fails if** the deficit is a lower initial response with normal recovery, which would indicate a steady-state loss of receptors rather than a failure of return.

The two decisions should be separable pharmacologically. Inhibiting STEP should reduce departure; enhancing retrieval should increase return. The prediction is that the two interventions are not redundant: at matched surface receptor abundance, they should differ in recovery kinetics, because they act on different terms of the product. **The weld fails if** they are interchangeable, which would indicate that one of them is not in fact rate-limiting under the conditions tested.

One caution on reagents, which applies to every proposal in this paper and is stated once. The site-specific phosphorylation measurements these experiments require are not all available in validated form for fixed adult tissue, and a project should not assume otherwise. A phospho-specific reagent needs qualification against the phosphopeptide and the unmodified peptide, a

non-phosphorylatable control, phosphatase treatment, and a preparation in which target identity is independently established; immunoprecipitation of total protein followed by a pan-phosphotyrosine read-out does not uniquely identify a site. Where the site-specific assay cannot be qualified, the claim should be narrowed rather than concealed behind an assay that measures something adjacent.

7. Second Weld: One Kinase Writes the Collapse Mark and Un-licenses the Retrieval Receptor

This is the paper's keystone and its most exposed claim. It is stated first as the three established facts it rests on, then as the inference, then as the ways the inference could be wrong, then as the experiment that decides it.

7.1 Rho-associated kinase at two addresses

Address one: the actin network. RhoA activates Rho-associated kinase, which phosphorylates LIM-domain kinase, which phosphorylates cofilin at serine 3 and prevents it binding filamentous actin. In Alzheimer-relevant preparations this arm is engaged: amyloid- β oligomers induced rapid actin stabilisation and increased phospho-cofilin within thirty minutes in primary cortical neurons; phospho-cofilin was elevated in the postsynaptic-enriched fraction of cortical samples from model mice and from human Alzheimer cases; the phosphorylation was reported necessary and sufficient for amyloid-induced synaptic impairment; and the clinically available Rho-kinase inhibitor fasudil prevented the actin stabilisation, the synaptic impairment and the synaptic loss, and restored the potentiation-induced insertion of the AMPA-receptor subunit GluA1 (Rush et al., 2018). *Maturity M2 for the human measurement, M4 for the mechanism.* The kinase family is elevated in Alzheimer brain and the inhibitor class is under investigation for the indication (Zheng et al., 2025, review).

Address two: the retrieval receptor. Phosphorylation of the cytoplasmic tail of SORLA by Rho-associated kinase 2 reduces the receptor's affinity for retromer; the upstream activator RhoGEF12 is upregulated in Alzheimer's disease; inhibiting RhoGEF12 increases endosomal SORLA-retromer association in mouse neurons; and in human stem-cell-derived neurons the inhibitor lowers both principal peptide species in a SORL1-dependent manner (Qureshi et al., 2026, preprint). *Maturity M5: cultured neurons, preprint, no whole-animal test.*

The spatial fact that connects them. Active RhoA does not stay in the spine. Imaged during induced single-spine plasticity, RhoA activity diffused out of the stimulated spine and spread

some five micrometres along the dendrite, persisting for more than half an hour, while Cdc42 activity remained restricted to the stimulated spine with a steep gradient at its neck (Murakoshi et al., 2011). *Maturity M4*.

7.2 The inference

Put the three together.

Of the two antagonistic arms above cofilin serine 3, only one has been shown to have a dendritic spatial extent, and it is the contractile arm. The dendritic shaft is where the early endosomes are. And the contractile arm's kinase has now been reported to phosphorylate, at that second address, the cytoplasmic tail of the receptor that delivers cargo to the retrieval machinery — reducing its affinity for that machinery.

The claim: Rho–ROCK tone does not merely write the collapse mark on the spine's actin network. It simultaneously un-licenses the retrieval step that would return to that spine the receptors it has lost and restore the service on which its off switch depends. Contractile tone and the endosomal traffic jam are, on this reading, not two abnormalities of one cell but two outputs of one signal.

Three consequences, each of which is the point of making the claim.

It makes the lesion self-sustaining without requiring a new kinase. Section 5.5 recorded that the licensing account needed something to restore RhoA bias after amyloid strips the receptor that writes the licence, and left the candidate open. This inference does not need one. If tone is already raised — by amyloid through the pathway Rush and colleagues characterised, by the upregulated upstream exchange factor the sorting programme reports in Alzheimer brain, or by both — then that same tone jams the compartment; the jammed compartment cannot restore the receptors or terminate the transducers; the failure of termination leaves the transducer pool in place; and a transducer pool left in place is a pool available to maintain tone. The loop closes, and it closes through two compartments rather than one. That is the shape of a lesion that persists after its initiating exposure has gone, which is the phenomenon this synthesis set out to explain.

It gives a measured mechanism to a feedback the sorting programme asserts architecturally. The hub-and-spoke model says that spokes feed back on the hub. Here is a named phosphorylation site at which a specific spoke — the Rho-family signalling that amyloid engages at the spine — acts directly on the hub's cargo receptor. That is a stronger statement than the architecture requires and a more testable one.

And it re-reads a published rescue. Fasudil, in the 2018 experiments, did two things that have been reported as one. It prevented the rise in phospho-cofilin, and it restored the potentiation-induced insertion of GluA1. Receptor insertion is a *delivery* event. On the inference

above, a Rho-kinase inhibitor should increase SORLA-retromer coupling and therefore receptor retrieval; so part of the restored insertion may have been a recycling rescue rather than an actin rescue. Nobody has asked. The figure exists, the compound is clinically available, and the question — was the rescue at the actin step or at the delivery step, or both — is answerable in a week by anyone with the preparation.

7.3 Five ways this could be wrong

The inference is this paper's own and it is graded as inference. Its exposures should be stated at full strength rather than in a limitations paragraph.

Isoform. The sorting result names Rho-associated kinase 2 specifically. The chain to cofilin is written by both isoforms of the kinase, and the published Alzheimer rescue used fasudil, which is not isoform-selective. If the effect on the cargo receptor is ROCK2-exclusive while the effect on cofilin is predominantly ROCK1, then the two arms share an upstream GTPase but not a kinase, and “one kinase, two addresses” must be weakened to “one pathway, two addresses.” That is a materially smaller claim, and the experiment in Section 7.5 distinguishes them.

Evidential status. The second address is a preprint, in cultured neurons, with no whole-animal test, from the group with the strongest disclosed commercial interest in the target. It may not survive review in its present form. Nothing in Section 7.2 should be carried forward if it does not.

Direction at the spine. The contractile arm is not simply the collapse arm. In the imaging study that supplies the spatial fact, inhibiting the Rho-kinase pathway preferentially blocked the *initial* phase of spine growth (Murakoshi et al., 2011). A tonic reduction of this arm is therefore not unambiguously beneficial at the spine, and the account advanced in this line of work is on record that the sign of a change in phospho-serine-3 is not recoverable from its level. Both cautions apply to the therapeutic reading in Section 13 and neither bears on whether the two addresses are connected.

Geometry. The five-micrometre spread of active RhoA was measured in rat hippocampal slice during induced potentiation, not in a chronically loaded dendrite in diseased tissue. Carrying that geometry across to disease is inference. It is, however, inference in the conservative direction: chronic tone would be expected to be less spatially confined than a pulse, not more.

Sufficiency. Even if tone un-licenses the receptor, the magnitude may be small compared with the loss of receptor expression, with ligase-route changes, or with the retained peptide's effect on the executor. A connection can be real and not rate-limiting. The experiment should therefore measure effect sizes, not only signs.

7.4 What the inference predicts about the human tissue

One prediction is worth separating, because it is cheap and because existing material may answer it.

If the collapse arm's tone acts at both addresses, then in human Alzheimer cortex the two markers should be related *across* regions and across cases: tissue with higher Rho-kinase pathway activity should show lower endosomal SORLA–retromer association and lower VPS26b-dependent recycling, and the relationship should be present in material from the trans-entorhinal region where both have been measured separately. The sorting programme has human tissue with SORLA and VPS26b quantified (Simoes et al., 2021); the Rho-kinase arm has human cortical fractions with phospho-cofilin quantified (Rush et al., 2018). **The prediction fails** if the two are unrelated at the level of donor-to-donor variation in material where both can be measured — which would indicate that whatever drives one does not drive the other in the human disease, however the biochemistry behaves in culture.

The usual cautions on post-mortem material apply with force here, and more to one marker than the other. Phosphorylation measures change rapidly after death and differ among proteins and sites (Wang et al., 2015), so a phospho-cofilin or phospho-tail measurement from autopsy tissue carries a preservation term that a total-protein measurement does not. A null result in autopsy material would therefore be weak evidence; a positive one, given that handling artefacts are unlikely to generate a specific inverse relationship between two unrelated assays, would be informative.

7.5 The discriminating experiment

One preparation, four read-outs, three compounds. In human stem-cell-derived excitatory neurons, with and without an amyloid- β oligomer challenge, apply fasudil (non-selective), a ROCK2-selective inhibitor, and the RhoGEF12 inhibitor at matched target engagement, and measure in parallel:

1. endosomal SORLA–retromer association;
2. surface GluA1 and GluN2B recovery after a defined stimulation pulse, as a time course rather than an endpoint;
3. phospho-cofilin serine 3, reported as a distribution across synapses and not as a mean;
4. the two peptide species, secreted and retained.

If the weld holds, all four move together under ROCK2 inhibition; the RhoGEF12 inhibitor reproduces the recycling and peptide effects with a smaller effect on cofilin; and fasudil moves everything, which would identify part of the 2018 rescue as a recycling rescue.

If the weld fails, fasudil normalises the cofilin distribution without improving retrieval or recovery, and the ROCK2-selective compound improves retrieval without changing the cofilin distribution. The two arms are then separable, the self-sustaining loop of Section 7.2 does not run

through a shared kinase, and the claim should be withdrawn to the weaker form: two Rho-family-dependent lesions in one cell, requiring separate interventions.

Either result is worth having, and neither requires a reagent that does not exist.

8. Third Weld: The Executor, and How One Insult Produces Two Signs

8.1 The result that supplies the missing mechanism

Section 5.5 left the licensing account owing an explanation. The disease's signature at the control surface is an asymmetry: a receptor targeted for proteasomal degradation is lost, while a transducer targeted for proteasomal degradation accumulates, in the same neuron and the same compartment. No account with a single scalar variable called clearance predicts that. If proteolysis were simply failing, the receptor would be *spared*, not lost.

The mechanism is in the intraneuronal-amyloid programme, in a paper the licensing account does not cite, and it is the keystone of this section.

Using two receptor systems as reporters, Almeida and colleagues showed that peptide accumulation in precursor-mutant neurons impairs the multivesicular-body sorting pathway, with receptor inactivation, degradation and ubiquitination all impaired after stimulation — and provided evidence that the accumulating peptide inhibits the activities of **the proteasome and of the deubiquitinating enzymes** (Almeida et al., 2006). *Maturity M4*.

That second clause is the one to hold. Inhibiting the proteasome lowers the rate at which tagged substrate is executed. Inhibiting the deubiquitinating enzymes lowers the rate at which tags are *removed*. A single insult that does both is not a reduction in a scalar called clearance. It is a change in the relation between two opposing rates — and the net effect on any given substrate depends on which of the two that substrate's turnover was limited by.

8.2 Why that inverts the sign for some substrates and not others

The point can be made with the minimum of formalism, and the formalism is worth writing down because it is what makes the claim falsifiable rather than merely suggestive.

Consider one substrate with an untagged pool U and a degradation-eligible tagged pool T . Let s be synthesis, k_{tag} effective tagging, k_{DUB} removal of the tag, k_{exec} productive degradation of

tagged substrate, and k_0 other loss from the untagged pool. The pools are substrate molecules, not free ubiquitin, and the rate constants are effective first-order quantities:

$$dU/dt = s - k_{\text{tag}} \cdot U + k_{\text{DUB}} \cdot T - k_0 \cdot U$$

$$dT/dt = k_{\text{tag}} \cdot U - (k_{\text{DUB}} + k_{\text{exec}}) \cdot T$$

At a steady state permitted by these assumptions, $T/U = k_{\text{tag}} / (k_{\text{DUB}} + k_{\text{exec}})$, and productive degradation is $\mathcal{J} = k_{\text{exec}} \cdot T$. Two things follow that bear directly on the asymmetry.

A snapshot of the tagged pool is under-determined. A larger T can result from more tagging, less deubiquitination, or less execution, and the ratio does not identify which. This is the Section 2 identity again, applied to a tag rather than to a compartment, and it is why the human cortical observation of increased ubiquitinated substrates together with increased proteasome components (Tai et al., 2012) does not distinguish inadequate capacity from defective targeting, sequestration, compensatory expression, or increased delivery.

An insult that lowers both k_{DUB} and k_{exec} does not have one sign. Two separate effects follow, and they act on different substrates.

Lowering k_{exec} slows execution. At the new steady state the pools must rise until the flux is restored to whatever synthesis supplies, so the substrate **accumulates** — which is the direction observed for the exchange factor of Section 5.5. Note that the accumulation is the robust prediction and the reduced flux is not: at a genuine steady state with unchanged synthesis, total degradative flux must return to its former value, and it is the *pool*, not the flux, that carries the lesion. This is the same reason a census is the wrong instrument, now applied one level down.

Lowering k_{DUB} raises the tagged fraction, since $T/U = k_{\text{tag}} / (k_{\text{DUB}} + k_{\text{exec}})$, and it does so by closing the escape route back to the untagged pool. That matters for a different class of substrate: one whose fate is decided not by proteasomal throughput but by whether a tag persists long enough to **commit** it to a one-way route — a receptor on a limiting membrane, waiting to be recognised and sorted into an intraluminal vesicle from which there is no return. For that substrate, a longer-lived tag is a greater probability of commitment, and the same insult that spares the first condemns it.

The direction therefore depends on which rate constant each substrate's fate was limited by, not on how much machine is present. That is what makes this a lesion of specificity rather than of capacity alone.

This is, in the terms of Section 2, a residence-time statement about a post-translational mark: the insult changes the dwell time of the tag, and two substrates with different dependence on that dwell time move in opposite directions.

The claim this licenses is modest and specific. It is not that the proteasome's capacity is unaffected — the same paper shows it is inhibited. It is that a lesion which simultaneously slows execution and slows tag removal is a lesion of *specificity* as well as of capacity, that such a lesion can produce opposite changes in two substrates in one compartment, and that the asymmetry the licensing account observed is therefore explained without needing a second, independent insult. The relevant alternatives are accordingly not “capacity loss” versus “misdirection” as mutually exclusive diagnoses. Both contribute, and their relative weight has to be measured. *Graded as inference (M5): the two directions have not been measured for these two substrates in one preparation.*

8.3 What the human cortex shows, on an enzyme whose job is to erase a routing tyrosine

One human measurement brings the executor failure and the tyrosine erasure of Section 5.3 into a single result, and it deserves to be stated carefully.

STEP61 levels rise progressively in the cortex of model mice over the first year and are elevated in the prefrontal cortex of **human Alzheimer brains**; the increase is accompanied by greater phosphatase activity, dephosphorylation of GluN2B tyrosine 1472 and reduced receptor subunits on neuronal membranes; peptide-enriched medium reproduces the changes in cortical cultures; in STEP-knockout cultures the peptide fails to internalise the receptor at all; blocking the proteasome raises STEP61 levels; and **STEP61-ubiquitin conjugates are increased** in wild-type cortical slices on peptide treatment and in aged model cortex (Kurup et al., 2010). *Maturity M2 for the human abundance, M3/M4 for the turnover mechanism.*

Read with Section 8.2, that is the predicted signature measured directly. Tags are being placed — conjugates up. The substrate is not being removed — abundance up, with proteasome blockade reproducing the accumulation. And the substrate in question is an enzyme whose function is to erase a routing tyrosine, so its persistence is not a generic proteostatic burden: it is a sustained instruction to withdraw a receptor from the surface. **The executor's failure and the erasure of a routing tyrosine are one event rather than two.**

Three cautions. The human finding is abundance and the turnover mechanism is from models, and the two should stay separately identified. The phosphatase's accumulation does not imply that it also acts on the licensing tyrosines of Section 5.2 — those would be new substrate claims requiring site-specific evidence, and a cellular response to manipulating the phosphatase could be indirect. And a rise in conjugated ubiquitin is compatible with reduced throughput but does not by itself establish it; the measured abundance of a machine is not a measurement of its productive flux. What the result establishes is a class of coupling — a change in the persistence of an intracellular enzyme altering the location of a receptor — demonstrated on an Alzheimer-relevant substrate in human cortex. That is enough for the architecture and not enough to assign all the neighbouring phosphotyrosines to the same phosphatase.

One further human observation, from a different group, corroborates the signature at the synapse itself. In human Alzheimer cortex, hyperphosphorylated tau oligomers accumulate at both presynaptic and postsynaptic terminals, are enriched in synaptoneurosomal fractions, appear to be ubiquitinated, and form stable oligomers resistant to denaturation — and their accumulation is associated with **both** increased ubiquitinated substrates and increased proteasome components (Tai et al., 2012). *Maturity M2*. More machine with more tagged substrate is the signature of tags placed and not executed, measured in human synaptic material rather than in a model. It is not, on its own, a measurement of throughput, and the authors do not claim it is.

8.4 Activity issues the licence, and the dissociation experiment measured more than amyloid

A striking coincidence links the intraneuronal programme's regulator to the licensing architecture's clock, and it generates the cheapest experiment in this paper.

Three proteolytic services on these molecules are activity-gated, and all three were characterised independently:

- surface neprilysin, which rises with activity and whose colocalisation with the peptide rises with it, and which is lost in diseased neurons (Tampellini et al., 2009, 2011);
- the dendritic membrane proteasome, whose activity is promoted by stimulation (Türker et al., 2024), alongside the activity-dependent redistribution of proteasomes into spines and local regulation of their activity (Bingol and Schuman, 2006);
- the proteasome-dependent fall in dendritic Ephexin5 that follows increased activity (Hamilton et al., 2017).

Activity, in other words, does not merely load the synapse. It issues the licence to terminate. And that re-reads the programme's designed dissociation experiment (Tampellini et al., 2010). Two independent means of chronic synaptic inhibition lowered plaques and worsened synapses and memory, with the internal peptide pool raised. The programme's interpretation is that the silenced synapse lost its activity-coupled amyloid clearance, which is well evidenced. The proposal here is that it lost something broader: **a chronically silenced synapse cannot terminate.** If the activity-gated services include the proteolytic removal of the spine's structural transducers, then chronic inhibition should leave those transducers in place, the licence unwritten, and the instruction unended — which would be a second, non-amyloid reason why the silenced synapse deteriorated.

The experiment is unusually cheap, and the tissue may already exist. In deafferented barrel cortex and in benzodiazepine-treated model and wild-type animals, measure dendritic Ephexin5 as total protein and as the phosphorylated fraction of its licensing tyrosine, with active RhoA and

active Cdc42 from the same tissue. **The prediction** is that chronic inhibition raises total Ephexin5 and lowers the phosphorylated fraction in both model and wild-type animals, because the service is activity-gated regardless of amyloid. **It fails** if the transducer pool is unchanged by chronic inhibition, which would confine the activity-gating to the amyloid-clearance arm and remove this reading of the dissociation.

Two qualifications. Deafferentation and a benzodiazepine alter many physiological demands at once, so a positive result identifies a correlate of chronic inhibition rather than a specific pathway, and a selective manipulation would be needed to close it. And the Ephexin5 measurement is a total-protein measurement unless the site-specific reagent can be qualified, with the consequences Section 6.4 described.

8.5 What this weld does and does not establish

It establishes a mechanism, from one programme, for an asymmetry observed in another, and it does so on a result that was published in 2006 and has not been used this way. It identifies a human measurement in which the two failures appear as one event. And it supplies a reading of the most-cited experiment in the intraneuronal corpus that extends it beyond amyloid.

It does not establish that the retained peptide is the cause of the asymmetry in human disease, which would require the two substrate directions to be measured together in human tissue, which has not been done. It does not establish that the executor is the rate-limiting failure rather than one of several. And it does not touch the question of what loaded the compartment in the first place — which is the subject of the next section, and which the two programmes answer differently.

9. Fourth Weld: One Phenotype, Two Lesions — Hyperexcitability Without Peptide

9.1 The same measured phenotype from opposite ends

The three preceding welds are proposals about mechanism. This one is an observation about two measurements, and it needs no inference at all to state.

From the amyloid side. Neurons carrying elevated amyloid- β become hyperactive, with calcium transients of increased frequency and amplitude; they fail to adapt those responses to imposed global changes in activity; they fail to adjust the axon initial segment, the structural

adaptation by which a neuron retunes its own excitability; and — the clause that matters here — neurons expressing a precursor protein engineered so that it **cannot yield the peptide at all** became just as hyperactive as those flooded with peptide (Martinsson et al., 2022). *Maturity M4*. The authors' conclusion is that the phenotype is one of dysregulated homeostatic plasticity, and that part of it belongs to the precursor protein rather than to the peptide.

From the sorting side. In human stem-cell-derived excitatory cortical neurons, loss of *SORL1* expression significantly changed many synaptic protein–protein interactions and patterns of expression; the deficient neurons were **hyperactive**, with the increased activity driven by glutamatergic neurotransmission; the hyperexcitability was attributed primarily to **mis-trafficking of synaptic proteins rather than to an overall increase in the peptide**; and the deficient neurons showed impaired synaptic plasticity (Williams et al., 2025, preprint). *Maturity M5: human cultured neurons, preprint*.

Two laboratories with no shared authorship, two unrelated lesions — an amyloid load in one case, loss of a sorting receptor in the other — one measured phenotype: hyperexcitability with failed plasticity. And in both cases the authors state, independently and for different reasons, that the peptide is not the sufficient cause.

9.2 What the convergence means, read through residence time

This is the cleanest available empirical connection between the two programmes, and it is a connection at the level of a phenotype rather than of a molecule, which makes it both more robust and less specific.

On the account of Section 2 it is what should be expected. Both lesions change where synaptic proteins are rather than how much peptide there is. One does so by jamming the retrieval step directly; the other by loading the compartment through which retrieval runs, and — on the measurement of Section 8.1 — by inhibiting the machinery that sorts it. If the controlled variable is residence, then two insults that act on residence by different routes should produce the same functional failure, and the failure should be a failure of *regulation* rather than of level. That is precisely what both papers report: not merely more firing, but the loss of the capacity to correct it, and the loss of plasticity.

Three further points sharpen the reading.

It supplies the amyloid programme's unresolved residue with an interpretation.

The finding that an amyloid-incompetent precursor construct produces equal hyperactivity has sat in the intraneuronal corpus as an awkward hole: some of the phenotype is the precursor's, and the account does not say how much. The sorting result gives the hole a shape. If hyperexcitability follows from mis-trafficking of synaptic proteins, then a construct that cannot make the peptide but

can still occupy and perturb the sorting compartments is expected to produce it. The residue is not an embarrassment to the convergence; it is evidence for it.

It argues against the sufficiency of the peptide, from both directions at once. This matters because it is the point on which both programmes are routinely misread. Neither is a claim that the peptide is unimportant; both are claims that the peptide is not the variable that tracks function. The designed dissociation of Section 4.4 made that case by driving plaque burden and outcome in opposite directions in the same animals. The sorting result makes it by producing the outcome with no increase in peptide at all.

And it identifies the functional endpoint the synthesis should be tested against. Both papers measure a regulatory failure — the inability to adapt, the inability to potentiate — rather than a level. That is the endpoint Section 2.4 derived from the accounting identity, arrived at independently by two laboratories measuring two different lesions.

9.3 Limits, stated at full strength

The sorting result is a preprint and should not be load-bearing until it is reviewed. Both results are in cultured neurons, where maturation, expression and stress responses differ from aged human cortex. Hyperexcitability is a common endpoint in Alzheimer models and has been reported downstream of many manipulations, including near plaques in vivo (Busche et al., 2008), so a shared endpoint is weaker evidence of a shared mechanism than a shared intermediate would be. Neither experiment measured the intermediate this paper proposes: neither reported receptor recovery kinetics after a pulse, and neither measured the transducers of Section 5.

The decisive version of the experiment is therefore obvious and has not been done. **In one preparation, compare a sorting lesion and an amyloid load against the same panel: receptor recovery after a defined pulse; dendritic transducer abundance and the phosphorylated fraction of its licensing tyrosine; the distribution of phospho-cofilin serine 3; and the excitability and plasticity read-outs both papers already use.** If the two lesions produce the same profile across the panel, the convergence is mechanistic. If they produce the same excitability with different profiles upstream, it is a shared endpoint reached by different routes — which would be a real and useful negative, because it would mean that hyperexcitability cannot be used as a read-out of either mechanism.

10. Two Directions in One Compartment

10.1 Sorting-first and cargo-first, both with interventional evidence

The two programmes disagree about the direction of causation inside the compartment they share, and the disagreement should be stated rather than dissolved by the word convergence.

The sorting account has the machinery fail first: retrieval stalls, cargo dwells, the precursor protein and the acid-preferring enzyme are held together, and the peptide is a consequence (Small et al., 2017). The intraneuronal account has the cargo fail the machinery: the peptide is retained, it inhibits the proteasome and the deubiquitinating enzymes, sorting through the multivesicular body is impaired, and the compartment distends (Almeida et al., 2006; Willén et al., 2017a).

Both have interventional evidence, in both directions, in the preparations each uses. Depleting and restoring the retrieval core moves the amyloid and receptor phenotypes both ways (Qureshi et al., 2022). Disrupting the packaging machinery that carries cargo into the multivesicular body traps the peptide and enlarges the compartment (Edgar et al., 2015; Willén et al., 2017a), and the peptide's accumulation reproduces the enlargement, which a dominant-negative manipulation of that machinery models.

The honest reading is that this is a feedback loop with interventional support on both limbs, and that an experiment which rescues at either limb will improve both endpoints without identifying the initiating event. That is not a failure of either programme; it is a property of coupled systems, and it has a specific methodological consequence. **An experiment seeking initiation requires temporal sampling before the coupled state forms. An experiment seeking reversibility requires intervention after it is established. These are different questions and they should be designed separately.** Much of the apparent conflict in this literature comes from results of the second kind being offered as answers to the first.

The useful question is therefore not which limb is first but which limb is rate-limiting in a given cell at a given time — and that is answerable, by the same route Section 2 prescribed: measure the flux, not the pool.

10.2 A five-year contradiction, dissolved by the cargo programme's own residue

The disagreement descends to a specific empirical contradiction in human stem-cell neurons, which is the sharpest unresolved item in the sorting literature.

In 2020, depleting *SORL1* in human neurons was reported to impair endosomal traffic **independent of amyloidogenic processing of the precursor protein** — the result the sorting-first model requires, obtained with inhibition of the enzyme that makes the amyloidogenic cut (Knupp et al., 2020). In 2021, a different group studying a truncating mutation in the same gene in the same kind of system reported that the endolysosomal dysfunction caused by loss of the receptor was **relieved by antisense-oligonucleotide reduction of the precursor protein**, concluding that presenilin-1, the precursor and the receptor act in a common pathway regulating the endolysosomal system (Hung et al., 2021). *Both M2*. Five years on, no study designed to adjudicate them has been published.

The resolution does not require a new experiment, and it comes from the intraneuronal programme's own unresolved residue.

“Precursor-dependent” and “amyloidogenic-processing-dependent” are not the same claim, and the two experiments tested different ones. Inhibiting the enzyme that makes the first amyloidogenic cut removes the β -cleaved products and the downstream peptide. It does not remove the precursor protein, and it does not remove the precursor protein's own synaptic functions. Reducing the precursor protein with an antisense oligonucleotide removes the precursor, all of its cleavage fragments including the carboxy-terminal fragment, and those functions, together.

Two facts from the cargo programme make that distinction substantive rather than semantic. A precursor construct engineered so that it cannot yield the peptide still produces the excitability phenotype (Martinsson et al., 2022). And deleting the precursor protein altogether alters synaptic proteins and receptors in a direction broadly opposite to the reductions seen in precursor-mutant neurons (Martinsson et al., 2019) — so the precursor has a synaptic function whose removal is not neutral. If the endosomal phenotype downstream of receptor loss depends on the precursor protein or on a fragment other than the peptide, then both published results are correct: the enzyme inhibitor should fail to rescue, and precursor depletion should succeed.

The adjudicating experiment follows directly and is straightforward. In one isogenic human-neuron series with the receptor depleted, compare four manipulations at verified target engagement and matched observation times: inhibition of the amyloidogenic enzyme; inhibition of the second cut, which allows particular carboxy-terminal fragments to accumulate rather than removing them; antisense reduction of the precursor; and reconstitution of a precursor construct that cannot yield the peptide. Measure endosome morphology, cargo flux, the fragments and the peptide pools together. **The prediction** is that precursor reduction rescues, enzyme inhibition does not, the second-cut inhibitor worsens the phenotype, and the amyloid-incompetent construct restores it — which would locate the relevant species in the precursor or in its carboxy-terminal fragment rather than in the peptide. **It fails**, and the sorting-first reading is strengthened, if the amyloid-incompetent construct leaves the rescue intact.

This is this paper's proposal and it is graded as inference. What is established is only that the two published results are not formally contradictory, because they manipulated different things.

10.3 The rival jam, and the experiment nobody has run

Within the endosomal camp there is a third account of what jams the compartment, and it is at least as well evidenced as either of the two above.

Nixon's group has argued for two decades that the endosomal abnormality is driven by the β -cleaved carboxy-terminal fragment of the precursor protein. The mechanism was specified: the fragment recruits the adaptor APPL1 to rab5-positive endosomes, where it stabilises the active form of rab5, producing accelerated endocytosis, endosome swelling and impaired axonal transport (Kim S et al., 2016). The account was then made independent of the precursor protein: in a mouse in which rab5 is over-activated directly, with no manipulation of the precursor, the animals reproduce endosome enlargement, accelerated AMPA-receptor endocytosis, spine loss, tau hyperphosphorylation, cholinergic neurodegeneration and memory impairment (Pensalfini et al., 2020); and a transgenic APPL1 mouse reproduced the same set (Jiang et al., 2025). The same group's account of the terminal stage — profound failure of autolysosomal acidification, with the peptide accumulating inside de-acidified compartments in neurons that go on to become the source of senile plaques — is a further, independent description of the same compartment failing (Lee et al., 2022). *Maturity M3 throughout.*

Note that this account's naming of the carboxy-terminal fragment as the pathogenic species is exactly the distinction Section 10.2 required, arrived at from a third direction.

The most valuable unperformed experiment in this area is a reciprocal rescue.

Does retromer enhancement rescue the rab5-overactivation animal, in which the compartment is jammed with no primary retrieval lesion? Does suppressing rab5 or its adaptor rescue the retromer-deficient animal? Both animals exist, in two laboratories, with reagents in hand. If retromer enhancement rescues an animal with no retromer lesion, these are two routes into one compartment failure and the traffic jam is the general description of it. If it does not, they are two different failures that happen to share an organelle, and the therapeutic implication is that an agent effective in one will be inert in the other.

10.4 What a feedback loop does to a claim of priority

One general point closes this section, because it applies to all three accounts and to this paper's own synthesis.

In a system with a feedback loop, “which came first” is not a question that intervention can answer and is not usually the question that matters therapeutically. What matters is whether the loop can be opened, and where. A rescue at either limb demonstrates that the manipulated process

contributes to maintaining the abnormal state; it says nothing about initiation. Conversely, a demonstration of temporal priority in human tissue — were it available — would say nothing about which limb is the better target, because the limb that started a loop need not be the limb that holds it closed.

The three programmes have spent a good deal of argument on priority. This paper's position is that the priority question is, for the synaptic-execution arm, largely unanswerable with present instruments and largely unnecessary; and that the productive questions are the ones in Sections 7.5, 8.4, 9.3, 10.2 and 10.3, every one of which is about a rate and none of which requires knowing what happened first.

II. The Composite: One Synapse, Three Clocks

11.1 The chain, stated in order

The three programmes can now be assembled into one account of a single excitatory synapse. What follows is a composite, not a finding: each link is supported by the citation given, and the chain as a chain has not been demonstrated in any one preparation. That is its status, and Section 11.3 says where it is measured and where it is assumed.

Ligand arrives and tone rises. Soluble amyloid- β oligomers engage their targets at the postsynaptic membrane; Rho-associated kinase activity rises, with phospho-cofilin elevated in the postsynaptic fraction of human Alzheimer cortex (Rush et al., 2018), and the upstream exchange factor that activates that kinase is reported upregulated in Alzheimer brain (Qureshi et al., 2026, preprint).

The receptors leave. The routing tyrosine on GluN2B is dephosphorylated by a phosphatase whose own abundance is rising because it is no longer being removed (Snyder et al., 2005; Kurup et al., 2010); the scaffolding protein falls and the AMPA-receptor subunit leaves the surface (Almeida et al., 2005); and the receptor that writes the licence at the first transducer is fed to an intact proteasome (Cissé et al., 2011). The protective arm's receptor is, in parallel, sequestered in intracellular compartments by the commonest genetic risk factor rather than lost or blocked (Chen et al., 2010).

They do not come back. Retrieval is the decision that determines return (Small et al., 2017; Simoes et al., 2021), and it has been un-licensed at the cargo receptor by the same tone that wrote

the collapse mark (Section 7). The compartment in which that decision is made is, in human tissue and before plaques, occupied by the peptide (Takahashi et al., 2002), distended (Cataldo et al., 2000; Willén et al., 2017a), and running a sorting pathway the peptide inhibits (Almeida et al., 2006).

The transducers are not removed. The executor is slowed and the tag's dwell time is extended (Almeida et al., 2006), so the arm of ubiquitin-dependent degradation that should have condemned the exchange factor under-runs while the arm acting on the receptor over-runs (Sell et al., 2017; Cissé et al., 2011; Section 8.2); ligase availability may fall independently (Olabarria et al., 2019); and the activity that would otherwise issue the licence is itself dysregulated, with the neuron having lost the capacity to find its own set point (Hamilton et al., 2017; Martinsson et al., 2022).

The output is read at a residue that cannot report it. Cofilin serine 3 is driven by chronic ligand occupancy and by tone from an unlicensed exchange factor rather than gated by the tyrosine above it, and the mark is the shared output of a collapsing arm, a growing arm and a protective arm (Kim et al., 2013; Rush et al., 2018; Chai et al., 2009).

And the clock that governs every step runs slower as a function of what it has failed to clear. The cargo inhibits the machinery that clears the cargo (Almeida et al., 2006); the feedback that couples the internal pool to the external one is lost in cells already loaded (Roos et al., 2021); and at the terminal step the transcriptional regulator of lysosomal biogenesis is itself inhibited by a pathological fragment of tau (Pollack et al., 2024), while its loss reduces lysosomal exocytosis of tau and worsens intraneuronal pathology and spreading (Xu et al., 2021).

11.2 Which programme measured which step

Table 2 — The composite chain and its evidential state

Step in the chain	Measured by	Strongest evidence	Grade
Tone rises at the postsynaptic membrane	licensing arm	phospho-cofilin elevated in human AD postsynaptic fraction; fasudil rescue in culture	M2 / M4
The routing tyrosine is erased and the receptor leaves	cargo programme	pathway traced to STEP and GluN2B Y1472; bidirectional intervention	M4
The phosphatase that erases it accumulates: tagged, not executed	external	STEP61 up in human AD prefrontal cortex; ubiquitin conjugates up	M2 / M3
Whether the receptor returns	sorting programme	depletion–repletion of the retrieval core moves receptor loss both ways	M3

Step in the chain	Measured by	Strongest evidence	Grade
The cargo receptor's coupling to retrieval is kinase-regulated	sorting programme	ROCK2 phosphorylation lowers affinity; inhibitor raises association	M5, preprint
The compartment is occupied, distended and before plaques	cargo programme	immunoelectron microscopy in human, mouse and rat	M2
The executor and the tag-removing enzymes are inhibited	cargo programme	receptor inactivation, degradation and ubiquitination impaired	M4
The transducer is not removed	licensing arm	Ephexin5 elevated in human AD hippocampus; removal rescues in model	M2 / M4
Activity gates the removal	licensing arm	activity-dependent, proteasome-dependent fall in dendritic Ephexin5	M4
The output residue cannot report its cause	licensing arm	two arms write the same mark; opposite human results	M5 (structural)
The clock degrades as a function of its own failure	cargo programme	correction lost in cells already carrying aggregates	M4

Two things are visible in that table and are the point of drawing it.

No single programme measured more than a third of the chain, and no programme measured two adjacent steps on the same molecule. The chain is a composite of three literatures, which is why it has not been assembled before and why its connections are inferences rather than findings.

The human grades cluster at the ends and thin in the middle. The occupancy of the compartment, the elevation of the transducer, the elevation of the phosphatase and the elevation of the output mark have all been measured in human tissue. Every step that is a *rate* — whether the receptor returns, whether the executor is slowed, whether activity gates removal — has been measured only in animals or cultures. That is the Section 2 gap, seen in one table.

11.3 Where the chain is assumed

Four joints carry inference rather than measurement, and they are the places to attack.

The kinase joint (Section 7): that the tone writing the collapse mark is the tone un-licensing the cargo receptor. Rests on a preprint and on an isoform assumption.

The executor joint (Section 8.2): that simultaneous inhibition of execution and of tag removal is what generates the sign asymmetry. The two substrate directions have not been measured in one preparation.

The service joint (Section 5.4): that the proteolytic systems the sorting and cargo programmes measure are in fact the systems on which the spine's control surface depends for termination. The characterised route for the first transducer is ligase-dependent and canonical; whether the dendritic membrane proteasome or the endosomal route contributes has not been tested.

The vulnerability joint (Section 6.3): that the region which runs recycling closest to capacity is first to fail because termination is one of the things that recycling does. Built on two established observations plus a demand argument its own authors label as inference.

11.4 A timetable, offered as a prediction

An ordering claim can be extracted, and it should be stated as a prediction with a stated failure condition rather than as a conclusion.

The sorting abnormality is independent of deposition: enlarged early endosomes are present in neocortical pyramidal neurons at preclinical stages, present in Down syndrome from twenty-eight weeks of gestation, accentuated by the $\epsilon 4$ allele, and — the control that makes the timing argument work — *normal* at advanced stages of familial disease caused by presenilin mutation, which is the form with the highest lifetime amyloid burden (Cataldo et al., 2000). *Maturity M2*. The transducer arm, by contrast, is gated on amyloid, since its first step requires the peptide to deplete the licensing receptor.

The prediction: in a model carrying humanised precursor sequence at endogenous levels, a sorting abnormality and impaired receptor recovery after a stimulation pulse precede the elevation of the dendritic transducer, which in turn precedes measurable dispersion of phospho-cofilin serine 3 across synapses.

It fails if the transducer elevation is found to precede any detectable sorting abnormality, or to be transcriptional rather than proteolytic in origin, in which case the misdirection reading has the order wrong and the elevation needs a different explanation.

Three cautions about ordering claims in general, which apply to this one. The available human studies do not establish any order among these lesions, because the measurements come from different cohorts, species, preparations and endpoints, and the fact that each can be placed relative to plaque pathology does not place them relative to one another. A cross-sectional human sample reflects both ongoing injury and survival selection: severely affected synapses may already be absent, so a weaker association among survivors is consistent with a mechanism that removed their

neighbours. And disease stage is not one anatomical coordinate — clinical severity, plaque burden, tau distribution and repair capacity need not advance at the same rate within a single brain.

12. The Cell That Receives the Cargo

12.1 The relocation of *common*

The synthesis assembled above is neuron-intrinsic, and the most important development in the sorting literature over the last two years points elsewhere. It is stated here at full strength because it bounds the whole paper.

Three results converge. 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**, with the protective allele associated 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). *Maturity M5*. The receptor's microglial biology is substantial and not endosomal in the narrow sense: deficiency induces endoplasmic-reticulum stress and interferon signalling, promotes lipid-droplet accumulation and impairs phagocytosis (Haq et al., 2026). And in a human positron-emission-tomography study using a microglial-density ligand alongside amyloid and tau tracers in 46 participants, microglial density colocalised with tau more often than with amyloid and **statistically mediated the relationship between tau and neurodegeneration** in limbic, temporal and parietal regions (Lao et al., 2026). *Maturity M2, with real limits: small, cross-sectional, a tracer whose interpretation as a pure density measure is contested, and mediation analysis on 46 participants establishing a hypothesis rather than a mechanism*. It is nonetheless notable that a laboratory whose signature model is neuron-centric has published a human study placing microglia in the causal position between the tau arm and neuronal loss.

Taken together these support a specific reframing: **the recycling lesion may be common in a cell the model was not written about**. The neuronal traffic jam is well evidenced in rare-variant carriers and in animals. The pathway's contribution to ordinary late-onset disease may run substantially through microglia — where the receptor governs lipid handling and inflammatory tone, where the common protective variant acts, and where the group's own imaging now places the mediating step. That reading also explains the negative biomarker of Section 2.3 without special pleading: a measure drawn from a neuronal read-out would not find a glial lesion. The two readings are not exclusive, since a neuronal retrieval deficit was already shown sufficient to induce a

dystrophic microglial morphology (Qureshi et al., 2022); the open question is which direction carries the weight in sporadic disease.

12.2 One machine, two cells

There is a symmetry here that belongs to this synthesis specifically, and it is the cargo programme's "same machine" claim reappearing across a cell boundary.

In human stem-cell-derived microglia, loss of the cargo receptor decreases lysosomal degradation and lysosomal enzyme activity through altered trafficking of the enzymes; phagocytic uptake of fibrillar amyloid- β and **of synaptosomes is increased while degradation is reduced**, so both substrates accumulate aberrantly in lysosomes; and lysosomal exocytosis is impaired (Mishra et al., 2025). *Maturity M2 in human cells*. A related study reported *reduced* uptake of the peptide and of synaptosomes in receptor-deficient microglia with impaired clearance (Haq et al., 2026) — so the direction of uptake is context-dependent and the two results should not be collapsed. What both show is disrupted processing.

The cell tasked with clearing synaptic material acquires the same disposal lesion as the cell losing synapses. That is not a metaphor: it is the same receptor, the same retrieval machinery, the same terminal compartment. The disposal failure is a property of a machine rather than of a cell type, which is the microglial form of the claim that the synapse makes amyloid on the organelles it runs on.

It also sets a measurement discipline that this literature frequently violates. Uptake, intracellular retention, digestion and exocytosis must be measured separately. Fluorescent material inside a phagocyte can increase because more entered or because less was processed; a brighter intracellular signal does not distinguish effective clearance from accumulation in an impaired cell. And uptake of isolated synaptosomes in a dish does not establish inappropriate removal of functioning synapses in vivo. This is the Section 2 identity again, in a phagocyte: the census is not the clock.

12.3 What this does to the synthesis's scope

Three consequences, and they are concessions rather than extensions.

The synthesis describes a neuron-autonomous execution step whose prevalence in sporadic disease is unestablished. If the common lesion is glial, the chain of Section 11 may be the correct account of what happens in the rare-variant carrier and in the animal, and a minority account of what happens in the ordinary patient. Nothing in the evidence assembled here settles that.

A complete synaptic account must distinguish three roles. Which cell initiates injury, which executes removal, and which retains the resulting material are separable, can be distributed,

and can be affected in different directions by the same intervention. A neuron-only culture establishes that an effect does not require microglia under those conditions; it does not determine the relative contribution in intact human brain. Persistence of injury after microglial depletion excludes a necessary contribution from the depleted population in that experiment; it does not establish neuronal autonomy.

And the experiment that would settle it has not been done. Conditional manipulation of the retrieval machinery or of the cargo receptor in microglia versus neurons, in the same animal background, with the same read-outs — including the receptor-recovery endpoint of Section 2.4 — would establish which cell carries the lesion and which contributes more to behaviour. A neuronal lesion has been shown to produce a microglial phenotype. Nobody has asked whether the microglial lesion produces the neuronal phenotype. Given that the human population's protection appears to act in microglia, that is not a peripheral question about a supporting cell type.

13. Therapeutic Consequences, Including a Conflict

13.1 Two programmes, one therapeutic category

The three accounts converge on a therapeutic category from opposite ends, and the convergence is the most immediately useful thing in this paper.

The licensing account's conclusion is that at a control surface terminated by destruction, every level-directed intervention is the wrong kind of intervention. Blocking the effector fails because cofilin is non-monotonic — it severs filaments at low occupancy and stabilises them at high occupancy — so a drug that pushes every synapse one way along a curve that turns over must convert some fraction of synapses from one failure mode into the other. Blocking the exchange factor fails because heterozygous loss-of-function mutations in *ARHGEF15* cause autosomal-dominant hereditary cerebral small-vessel disease with osteoporotic fracture, acting through inactivation of the same Rho pathway, with complete co-segregation in two families, two further sporadic cases, and a transgenic mouse recapitulating the pathology (Ding et al., 2023) — *maturity MI* — and because the protein is required for the spine growth that accompanies potentiation (Petshow et al., 2025). Blocking the shared kinase fails because Fyn transduces amyloid toxicity at the postsynaptic density and is simultaneously required for the protective arm to be applied *and* terminated (Arnaud et al., 2003; Bock et al., 2004). Every one of those failures is an attempt to fix a timing problem with a level intervention.

What the account points to instead is the restoration of the cell's capacity to terminate — and one published result occupies that category. Inhibition of the early endosome's principal proton-leak channel NHE6 reverses the ApoE4-induced recycling block of the reelin receptor and of the glutamate receptors co-endocytosed with it, and restores reelin's modulation of excitatory synapses (Xian et al., 2018). *Maturity M3*. Nothing was added to the signalling pathway and nothing blocked in it. The compartment was repaired and the signalling returned. An ApoE4 neuron does not have a ligand deficiency; it has a receptor in the wrong place (Chen et al., 2010), and no quantity of ligand fixes a receptor in the wrong place.

The sorting programme arrived at the same category from the other direction, and with a more developed pharmacology: stabilise the retrieval complex (Mecozzi et al., 2014; Mishra et al., 2023; Ramonet et al., 2025), raise the cargo receptor (Huang et al., 2026), or inhibit the kinase pathway that un-licenses their coupling (Qureshi et al., 2026, preprint). Its own therapeutic corollary is that if the hub is common to the spokes, repairing the hub should benefit patients whose primary lesion is in *any* spoke, including spokes the model does not name — so the indication does not depend on winning the argument about origins (Small and Petsko, 2020). That corollary is testable in a way the causal claim is not, and it is the argument for a trial.

Two independent lines, with no shared authorship and no shared vocabulary, have concluded that the intervention class to pursue is the one that restores capacity rather than adding or subtracting signal. That is the strongest available support for the general claim of this paper, and it is support of the right kind: a convergence of therapeutic logic arrived at from a receptor-recycling experiment on one side and from a cargo-sorting programme on the other.

13.2 The conflict, named

The convergence contains a contradiction that neither side has stated, and it concerns the direction in which endosomal acidity should be moved.

NHE6 leaks protons out of the early endosome. Inhibiting it therefore makes the compartment **more** acidic, and that is how the recycling block is relieved (Xian et al., 2018). The sorting account, meanwhile, rests part of its mechanism on the fact that the enzyme making the first amyloidogenic cut works best at **acidic** pH, which is why increased residency in an acidic lumen favours that cut (Small et al., 2017). The two therefore make opposite-signed predictions about what acidification does: on one account it restores receptor recycling and the capacity to signal; on the other it should increase amyloidogenic processing of whatever precursor is held in the compartment.

A third result constrains both. Chronic loss of NHE6 in human neurons produces lysosomal and autophagic abnormalities with elevated phosphorylated and sarkosyl-insoluble tau, reduced lysosomal number and protease activity, diminished autophagic flux and accumulation of an

autophagy substrate — partially rescued by two independent enhancers of autophagy–lysosomal function (Fernandez et al., 2022). *Maturity M2 in human neurons*. Chronic genetic loss of an exchanger is not the same manipulation as a particular pharmacological inhibition regimen in a recycling assay, and the two should not be equated. But together the three results establish that acidity is a regulated variable with an operating range rather than a quantity to be maximised.

The therapeutic aim is therefore not maximal acidification but restoration of the conditions under which the relevant cargos and enzymes both work — and whether such a window exists is an empirical question that has not been asked. The experiment is specified: in one human-neuron preparation, apply graded NHE6 inhibition and measure, in parallel, receptor recycling and surface recovery after a pulse; the two peptide species, secreted and retained; lysosomal enzyme maturation and protease activity; and cargo degradation. If there is a dose at which recycling is restored without raising the retained peptide pool or impairing hydrolysis, the licensing account's favoured intervention survives and acquires a therapeutic index. If recycling and peptide production move together across the whole range, then the two programmes' logics are in genuine conflict at this target and the repair must be attempted at a different step — of which the sorting programme now offers at least two.

This is the most useful thing a synthesis can produce: not a reconciliation, but a specific place where two accounts that agree in their conclusions disagree in their mechanism, with a measurement that decides it.

13.3 Order of operations, and what the order forbids

The ordering claim follows directly from the whole argument and is the most immediately actionable statement in this paper. **Repair capacity first; supply signal second.**

An agent that drives the protective arm — a ligand-directed agonist, a pathway-activating antibody — should be expected to underperform in exactly the population most likely to be enrolled, ApoE4 carriers, unless the recycling defect is addressed first or concurrently, because the receptor it needs is in the wrong compartment. The same ordering applies to the degradative arm, and here it becomes a prohibition: **an autophagy inducer given to a neuron that cannot complete the pathway fills it faster with vesicles it cannot empty.** If the failing step is terminal — sorting, acidification, hydrolysis — then increasing induction increases burden. Repair, then induce.

Four further prohibitions follow from the arguments above, and three of them bear on live programmes.

Lowering the exchange factor is forbidden as a chronic systemic strategy. The human loss-of-function phenotype is a dominant dementing vasculopathy with skeletal fragility (Ding et al., 2023). This does not establish that adult, partial, neuron-selective modulation

reproduces lifelong systemic genetic disease — that difference should be tested rather than assumed in either direction — but it removes the argument that made the target attractive, which was that a protein nearly absent from healthy adult tissue could be inhibited with impunity.

Total-protein read-outs of these transducers are uninterpretable. Where one covalent mark sets both what an enzyme does and how long it survives, abundance and activity are two readings of one modification, and an elevation is as consistent with a failed signal as with an amplified one.

Bulk phospho-cofilin is not an endpoint. It is the shared output of two antagonistic arms and a protective one, and its mean is uninformative about direction even when measured perfectly.

And bulk degradative-capacity assays cannot detect the lesion Section 8 describes. Total proteasome activity, total autophagic flux and total lysosomal enzyme content are measures of machine, and a change in the specificity of proteolysis does not appear in any of them.

13.4 Endpoints, and what should replace the ones in use

The accounting identity of Section 2 translates directly into an endpoint prescription, and it is more demanding than what is done now.

A mechanism-oriented intervention should be defined by the operation it changes, and its target-engagement assay should measure *that operation* rather than a distant marker chosen because it is convenient. Retrieval stabilisation aims to change a transport rate; its engagement assay should be a recycling rate, not a protein level. A fragment- or cargo-directed intervention aims to change production, location or persistence of a species; its engagement assay should follow that species.

The downstream benefit then needs independent demonstration, and three substitutions are routinely made that should not be. Corrected trafficking without restored physiological responsiveness is incomplete evidence of synaptic rescue. Improved spine density without preserved plasticity could reflect reduced elimination, altered development, or impaired remodelling. Increased intracellular uptake without productive digestion may worsen burden.

What should replace the current endpoints at this node is a joint, per-synapse measurement of the governing tyrosine and the governed serine with the GTPase state determined in the same tissue, and — as the functional endpoint — the recovery of a defined receptor response after a defined perturbation. Both are more difficult than what is done now. On the evidence assembled here neither is optional, because until residence is measured the field is choosing the direction of a therapy from variables that cannot carry the direction.

14. The Discriminating Experiments

Nine experiments, ranked by what they settle per unit of effort. Each is stated so that a specific result would count against the argument. None requires a reagent that does not exist, except where noted.

1. **Did fasudil rescue the actin step or the delivery step?** Re-examine the Rho-kinase rescue with endosomal cargo-receptor-retromer association and post-pulse surface receptor recovery as parallel read-outs alongside phospho-cofilin (Section 7.5). **Settles** whether contractile tone and the traffic jam are one lesion. Cheapest high-value experiment in the paper; the compound is clinically available.
2. **Does the licensing architecture fail in a recycling-deficient neuron with no amyloid?** In VPS26b-depleted neurons, measure dendritic transducer abundance, the phosphorylated fraction of its licensing tyrosine, and the per-synapse distribution of phospho-cofilin (Section 6.4). **Refutes** the service claim if the transducer pool is unchanged.
3. **Does chronic silencing leave the transducers in place?** Measure dendritic transducer abundance and phosphorylation in deafferented and benzodiazepine-treated animals, model and wild-type (Section 8.4). **Refutes** the licence-by-activity reading if unchanged. The tissue may already be banked.
4. **One panel, two lesions.** Compare a sorting lesion and an amyloid load in one preparation against receptor recovery, transducer state, cofilin distribution and the excitability and plasticity read-outs (Section 9.3). **Settles** whether the shared hyperexcitability phenotype reflects a shared mechanism or a shared endpoint.
5. **Adjudicate the precursor-dependence contradiction.** In one isogenic series with the cargo receptor depleted, compare amyloidogenic-enzyme inhibition, second-cut inhibition, antisense reduction of the precursor, and reconstitution with an amyloid-incompetent precursor construct (Section 10.2). **Settles** a five-year-old contradiction at the crux of the sorting hypothesis.
6. **Reciprocal rescue between the two jams.** Retromer enhancement in the rab5-overactivation animal; rab5 or adaptor suppression in the retromer-deficient animal (Section 10.3). **Settles** whether these are two routes into one compartment failure or two failures sharing an organelle. Both animals and all reagents exist.
7. **Is there an acidity window?** Graded NHE6 inhibition in one human-neuron preparation, with receptor recycling, both peptide species, lysosomal protease activity and cargo

degradation measured together (Section 13.2). **Settles** whether the two programmes' therapeutic logics conflict at this target.

8. **Which cell carries the lesion?** Conditional manipulation of the retrieval machinery in microglia versus neurons in one animal background, with the same read-outs including receptor recovery and behaviour (Section 12.3). **Settles** the relocation question that the negative human biomarker opened.
9. **The paired-residue measurement in human cortex.** Array tomography of Alzheimer and age-matched control cortex, reporting the *joint distribution* across single excitatory synapses of the phosphorylated fraction of the licensing tyrosine, total transducer, phospho-cofilin serine 3 and total cofilin, with active-RhoA and active-Cdc42 pulldowns from the same blocks. **Refutes** the degeneracy argument's consequence if the two marks covary in disease as in control. This is the primary falsifier of the licensing account, it requires one antibody the field does not have in validated form, and the design constraints are severe: antibodies must be qualified under the actual fixation and embedding conditions; segmentation rules must be fixed before diagnosis is revealed; the donor, not the synapse, is the unit of biological inference; normalising two channels by a shared denominator can manufacture correlation; and post-mortem phosphorylation changes are rapid and protein-specific (Wang et al., 2015), so a preservation pilot with biological replication at each delay is a precondition rather than a refinement.

15. What Would Refute This

Written as results that would end or narrow the argument, each achievable with existing methods.

The welds, each separately.

1. **Fasudil normalises the cofilin distribution without improving retrieval or post-pulse receptor recovery, and a ROCK2-selective inhibitor improves retrieval without changing the cofilin distribution.** The keystone of Section 7 fails; the two arms are separable; the self-sustaining loop does not run through a shared kinase, and the claim reduces to two Rho-family-dependent lesions requiring separate interventions.
2. **The transducer pool is unchanged in a neuron whose recycling core has been removed.** Section 5.4's claim that the spine's off switch is a service provided by these systems fails, and with it the structural basis for welding the three programmes at all.

3. **A capacity-deficit model is shown to predict the receptor and transducer directions jointly.** The specificity claim of Section 8 becomes unnecessary and the simpler framing should be preferred.
4. **The two lesions of Section 9 produce the same excitability phenotype with different upstream profiles.** The fourth weld reduces to a shared endpoint, and hyperexcitability cannot be used as a read-out of either mechanism.

The licensing architecture it rests on.

5. **The two phospho-marks covary in Alzheimer cortex as they do in control.** The degeneracy of the serine has no consequence in real tissue, the sign is recoverable where the field has been looking, and the architecture's central proposal is unnecessary.
6. **Total transducer protein predicts active RhoA as well as the phosphorylated fraction does.** The licensing architecture is not load-bearing in disease tissue.
7. **The reelin-induced destruction of Disabled-1 is shown not to occur in mature neurons.** The parallel between the two transducers is developmental only, and the general claim that this surface terminates by proteolysis loses one of its two pillars. The published demonstration is in cultured primary embryonic neurons (Bock et al., 2004) and has not, so far as can be determined, been repeated in adult tissue. This is the argument's most exposed empirical assumption.

The sorting programme it depends on.

8. **A panel of pathway-function measures consistently fails to separate sporadic patients from age-matched controls in adequately powered cohorts.** The claim that the recycling lesion is *common* fails, and the theory reduces to an account of a rare monogenic subtype plus a druggable modifier — which would make the synthesis here an account of the execution step in that subtype rather than in the disease.
9. **The precursor-dependence contradiction resolves against the sorting-first reading.** If the endosomal phenotype downstream of receptor loss requires the precursor protein and specifically its amyloidogenic products, the ordering inverts: the endosome becomes the compartment in which a precursor-driven lesion is expressed rather than the origin of it.
10. **Target engagement without clinical effect.** If an agent demonstrably enhances retrieval in the human brain, measurable by pathway biomarkers, and produces no benefit in appropriately early disease, the hub-and-spoke therapeutic corollary fails. This is the cleanest available test and it requires a trial that does not exist.

And the synthesis as a whole.

11. Well-controlled experiments find independent defects with no predicted interaction. If correcting one operation does not alter the effect of manipulating another where the model predicts dependence, the coupled explanation should be narrowed. Each component mechanism may remain valid while the claim that their interaction accounts for the persistent state is rejected.

That last condition deserves emphasis, because it is where syntheses of this kind characteristically fail. A modular hypothesis is useful only if its proposed connections can fail individually. It must not be allowed to become immune to evidence by treating every outcome as another form of convergence, and a hypothesis that survives only by repeatedly changing the relevant cell, time point or unmeasured intermediate has lost its explanatory value.

16. Ledger of Claims



Every load-bearing claim with its source and grade. **M1** human population-scale or multi-cohort; **M2** human single cohort or tissue series; **M3** animal replicated or bidirectional; **M4** animal or primary culture, single laboratory; **M5** in vitro, cell line, preprint, or inference.

Table 3 — Claims, sources and grades

Claim	Source	Grade
Synapse loss is the strongest structural correlate of cognitive impairment	Terry et al., 1991	M2
Enlarged early endosomes precede deposition; absent in advanced presenilin familial disease	Cataldo et al., 2000	M2
SORL1 truncating variants occur almost exclusively in cases; domain-level classification	Andersen, de Waal et al., 2025	M1
Retrieval-core depletion and repletion move amyloid processing and receptor loss in both directions	Qureshi et al., 2022	M3
Raising the cargo receptor reverses established tau pathology in aged animals; deletion worsens it	Huang et al., 2026	M3
A neuron-enriched retrieval core is dedicated to recycling, enriched in the first-failing region, deficient there in disease	Simoes et al., 2021	M3 / M2

Claim	Source	Grade
Soluble cargo-receptor ectodomain is low in variant carriers and does not differ in sporadic disease	de Waal et al., 2026	M2
Rho-associated kinase 2 phosphorylates the cargo receptor's tail and lowers its retromer affinity	Qureshi et al., 2026	M5, preprint
The first amyloidogenic cut occurs on synaptic vesicles	Lundgren et al., 2015	M4
Compartment determines which peptide is made and whether it leaves	Greenfield et al., 1999	M5
Intraneuronal Aβ42 accumulates in multivesicular bodies of postsynaptic compartments before plaques	Takahashi et al., 2002	M2
Secretion falls and surface neprilysin is lost in diseased neurons	Tampellini et al., 2011	M4, not independently replicated
The internal pool falls >80% in 3 h and recovers in 6 h; correction lost in loaded cells	Roos et al., 2021	M4, not independently replicated
The retained pool inhibits the proteasome and the deubiquitinating enzymes; receptor degradation and ubiquitination impaired	Almeida et al., 2006	M4
Chronic synaptic inhibition lowers plaques and worsens synapses and memory	Tampellini et al., 2010	M4
An amyloid-incompetent precursor construct produces equal hyperactivity; homeostatic plasticity lost	Martinsson et al., 2022	M4
Precursor deletion alters synaptic proteins opposite in direction to precursor-mutant neurons	Martinsson et al., 2019	M4
Cargo-receptor-null human neurons are hyperactive and plasticity-impaired, from mis-trafficking rather than peptide	Williams et al., 2025	M5, preprint
Both Rho-family arms raise phospho-cofilin Ser3 with opposite structural outcomes	Kang and Woo, 2019; Bamburg et al., 2021	M5 (canonical)
Active RhoA spreads ~5 μm along the dendrite and persists; Cdc42 stays in the spine	Murakoshi et al., 2011	M4
Aβ lowers phospho-cofilin via an immune receptor, in human AD brain; reproduced independently	Kim et al., 2013; Kawaguchi et al., 2022	M2 / M4
Aβ raises phospho-cofilin in human AD cortex; fasudil rescues, including GluA1 insertion	Rush et al., 2018	M2

Claim	Source	Grade
EphB signalling phosphorylates Ephexin5, recruiting Ube3A for proteasomal destruction	Margolis et al., 2010	M4
Substrate choice is set by the same tyrosine; the phospho-dead mutant biases to Cdc42; the protein is required for activity-driven growth	Petshow et al., 2025	M4
Dendritic Ephexin5 falls after activity, proteasome-dependently	Hamilton et al., 2017	M4
Ephexin5 is elevated in human Alzheimer hippocampus; removal rescues in model	Sell et al., 2017	M2 / M4
Reduced Ube3A with raised Ephexin5 and active RhoA at unchanged total EphB2	Olabarria et al., 2019	M4
Aβ binds EphB2 and triggers its proteasomal degradation; restoring EphB2 rescues	Cissé et al., 2011	M3
Reelin targets Dab1 for proteasomal degradation; Tyr phosphorylation required; Fyn deficiency prevents it	Bock et al., 2004	M4 / M5
Site-specific recruitment of the ligase machinery governs Dab1 turnover	Feng et al., 2007	M5
Aβ internalises NMDA receptors via STEP and dephosphorylation of GluN2B Y1472	Snyder et al., 2005	M4
STEP61 is elevated in human AD prefrontal cortex; its ubiquitin conjugates rise; proteasome blockade raises it	Kurup et al., 2010	M2 / M3
Human AD synapses show increased ubiquitinated substrates and proteasome components	Tai et al., 2012	M2
ApoE4 sequesters ApoER2 intracellularly, reducing surface receptor and reelin's effect	Chen et al., 2010	M3
NHE6 inhibition reverses the recycling block and restores reelin's modulation of synapses	Xian et al., 2018	M3
Chronic NHE6 loss in human neurons raises insoluble tau and impairs lysosomal function	Fernandez et al., 2022	M2
ARHGEF15 heterozygous loss of function causes dominant small-vessel disease with osteoporosis	Ding et al., 2023	M1
Cargo-receptor loss in human microglia increases uptake of synaptosomes while impairing degradation	Mishra et al., 2025	M2

Claim	Source	Grade
The protective common variant at the locus acts through microglial expression	Gorniak-Walas et al., 2026	M5
Microglial density mediates the tau–neurodegeneration relationship in human imaging	Lao et al., 2026	M2, small and cross-sectional
β CTF–APPL1–rab5 is a rival jam with bidirectional animal evidence	Kim S et al., 2016; Pensalfini et al., 2020; Jiang et al., 2025	M3
Residence time is the common variable, and an abundance assay cannot identify it	this paper	Derivation
The routing tyrosine decides departure and retromer decides return: consecutive steps, never placed in series	this paper	Observation
The region that fails first is the region running the termination service closest to capacity	this paper	Inference
One kinase writes the collapse mark and un-licenses the retrieval receptor; tone and jam are one lesion	this paper	Inference, on a preprint
Simultaneous inhibition of execution and of tag removal produces the sign asymmetry	this paper	Inference
Precursor-dependent is not amyloidogenic-processing-dependent; the 2020 and 2021 results are not contradictory	this paper	Inference
Part of the published Rho-kinase rescue may have been a recycling rescue	this paper	Prediction
A chronically silenced synapse cannot terminate, not only cannot clear	this paper	Prediction
An acidity window exists at which recycling is restored without raising the retained pool	this paper	Prediction, untested

Nine entries in that table are this paper's own, and not one of them has been tested. They are stated here rather than in a discussion section, and they are the first things a reader should attack.

Table 4 — The validity ledger: where the argument stands on the ground and where on inference

Tier	Basis	Claim	Why it sits there
Strong	imported, established	Synapse loss is the strongest structural correlate of cognitive impairment, and the endosomal abnormality is among the earliest disease-specific changes, independent of deposition.	Human material, replicated, with a presenilin control that makes the timing argument work. Both are imported rather than established here.
Strong	imported, established	A residence-time lesion cannot be identified from an abundance measurement without an independent measure of flux.	An accounting identity. It constrains what any snapshot can contain and it is the paper's formal spine.
Moderate	inference, this paper's own	The three programmes describe one lesion — a failure of timed removal — in three compartments of one cell, and the junctions between them are at named molecules.	The four welds. Each rests on established results; the couplings are proposals, and each has a stated falsifier.
Weak	predicted, untested	Contractile tone and the endosomal traffic jam are one self-sustaining lesion through a shared kinase.	The keystone. It rests on a preprint, on an isoform assumption, and on carrying a spatial measurement from induced plasticity into diseased tissue.
Weak	predicted, untested	Repairing the compartment restores the capacity to terminate, and an operating range exists in which that can be done without worsening the cargo.	The therapeutic claim, and the site of an unresolved conflict between two of the paper's own sources.

17. Scope: What Portion of the Disease This Claims

The largest failure mode of theories in this field is claiming everything and forbidding nothing. This section states the boundary.

What is claimed. An account of the *execution* of synaptic loss: the mechanical route by which an upstream cause becomes a synapse that no longer works, and specifically an account of why that failure persists after the initiating exposure has gone. To the extent that dementia in Alzheimer's disease is caused by synapse loss, this is a claim on the final common step, together with a claim about why the field's central measurements at that step have been unreadable.

What is not claimed, and these are large.

Initiation. Nothing here says what starts the disease. The account is compatible with amyloid-first, sorting-first, tau-first, inflammatory-first and vascular-first origins, which is a weakness as much as a strength. The one ordering commitment is in Section 11.4 and it can be broken.

Prevalence. Section 12 is the honest statement of the problem. If the recycling lesion is common in microglia rather than in neurons, the chain assembled here may be the correct account of the rare-variant carrier and the animal and a minority account of the ordinary patient.

Selective vulnerability, beyond one inference. Section 6.3 offers the sorting programme's regional answer as the best available, and that answer's own authors label its central step — the demand argument — as hypothesis. High expression of the relevant receptors is in any case necessary and plainly not sufficient, since neurons expressing them are spared in the same layer of the same section.

The non-synaptic contribution. Neuronal death, white-matter injury, vascular disease and the loss of whole compartments contribute to dementia by routes this account does not describe.

The inhibitory neuron. The mechanics described are the mechanics of a spine, and the cortex's most conspicuously vulnerable inhibitory cell is characteristically aspiny. The objection is accepted rather than argued around. No claim is made that the parvalbumin-positive interneuron fails this way; it probably does not.

Dementia not attributable to the pathologies conventionally measured. A substantial minority of dementia in community-based series is not explained by them, and a substantial minority of people meeting neuropathological criteria are not demented at death. This account speaks to neither group, and the second is a particular caution for every human measurement proposed here: resilient cases are not automatically a state of intact molecular coupling, and their value lies in testing which of several possibilities is actually present.

Tau propagation and regional spread. The sorting programme's account of release through the multivesicular body supplies a route; none of the three programmes supplies a mechanism for the stereotyped order of spread, and the regional specialisation that makes the onset argument strong contributes nothing to the spread argument.

The honest summary of scope: a claim about the last step of one route, that route being the one most of the field's structural evidence points at, with no claim over who enters the route or why, and with an unresolved question about which cell the route runs through in most patients. A reader looking for a theory of the disease's cause will not find one here and should not be told they have.

18. Conclusion: The Protein That Has Not Left

Three bodies of work, built in near-isolation, have converged on a lesion of the same kind without any of them naming the kind.

One says the disease's upstream fault is that cargo stays too long in a sorting compartment. One says the pathological variable is not how much peptide a neuron makes but how much of it fails to leave. One says that the spine's control surface has no off switch that is not a protease, so that the capacity to make a brief, placed structural instruction is vested in a disposal service rather than in a signalling reaction. These are three statements about residence time, in three compartments, in one cell.

Reading them that way does four things. It explains why all three have the same gap in the same place: each proposes a rate and each measures, in human tissue, a stock, and a stock cannot identify a rate. It identifies the small number of published measurements in all three corpora that do report a rate, and those turn out to be the results each programme leans on hardest and cites least. It dissolves two live contradictions — one about the direction of a phosphorylation at a residue that cannot report its own cause, one about whether an endosomal phenotype requires a precursor protein — by enforcing distinctions the measurements had blurred. And it locates four junctions at which the programmes meet on named molecules: the receptor whose departure is licensed by a tyrosine and whose return is decided by retromer; the kinase that writes the collapse mark on the actin network and, at a second address, un-licenses the receptor that would resupply it; the retained cargo that inhibits both the execution of a tag and its removal, and thereby condemns one substrate while sparing another; and the two unrelated lesions that produce, in two laboratories, the same hyperexcitable and unplastic neuron with the peptide excluded as the sufficient cause in both.

The therapeutic consequence is a category rather than a compound, and two of the three programmes reached it independently: restore the cell's capacity to terminate, and do it before supplying any signal. The conflict inside that category — one account wants the compartment more acidic to recover a receptor, the other notes that acidity is what makes the pathological cut — is named here rather than smoothed, because it is the kind of disagreement that a measurement settles and an argument does not.

What all of this asks of the field is a change of instrument rather than a change of belief. The measurements in use are censuses: how much peptide, how much phosphorylation, how much protein, how much machine. The lesion, on all three accounts, is a clock. A census taken at one

instant contains the clock multiplied by something else, and no refinement of the census recovers the factor. What recovers it is a pulse and a chase, a stimulation and a recovery interval, a rate moved in both directions — and the functional endpoint that follows, which is not a lower mean of anything but the synapse that answers the first stimulus and cannot answer the second.

Identify the protein that has not left. Determine why it has not left, and where it is acting while it stays. Then ask whether changing that fate restores the synapse's capacity to respond. That sequence turns a convergence of three literatures into a programme of causal investigation, and it is the only thing offered here that does not depend on which of the three accounts of the beginning turns out to be right.

References

- Almeida CG, Tampellini D, Takahashi RH, Greengard P, Lin MT, Snyder EM, Gouras GK. Beta-amyloid accumulation in APP mutant neurons reduces PSD-95 and GluR1 in synapses. *Neurobiology of Disease*. 2005;20(2):187–198. PMID 16242627.
- Almeida CG, Takahashi RH, Gouras GK. Beta-amyloid accumulation impairs multivesicular body sorting by inhibiting the ubiquitin-proteasome system. *Journal of Neuroscience*. 2006;26(16):4277–4288. PMID 16624948. doi:10.1523/JNEUROSCI.5078-05.2006
- 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.]
- 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.
- Andersson E, Schultz N, Saito T, Saido TC, Blennow K, Gouras GK, Zetterberg H, Hansson O. Cerebral A β deposition precedes reduced cerebrospinal fluid and serum A β 42/A β 40 ratios in the App^{NL-F/NL-F} knock-in mouse model of Alzheimer's disease. *Alzheimer's Research and Therapy*. 2023;15(1):64. PMID 36964585.
- Arnaud L, Ballif BA, Förster E, Cooper JA. Fyn tyrosine kinase is a critical regulator of disabled-1 during brain development. *Current Biology*. 2003;13(1):9–17. PMID 12526739.
- Bamburg JR, Minamide LS, Wiggan O, Tahtamouni LH, Kuhn TB. Cofilin and actin dynamics: multiple modes of regulation and their impacts in neuronal development and degeneration. *Cells*. 2021;10(10):2726. PMID 34685706.
- Beckelman BC, Day S, Zhou X, Donohue M, Gouras GK, Klann E, Keene CD, Ma T. Dysregulation of elongation factor 1A expression is correlated with synaptic plasticity impairments in Alzheimer's disease. *Journal of Alzheimer's Disease*. 2016;54(2):669–678. PMID 27567813.
- Bingol B, Schuman EM. Activity-dependent dynamics and sequestration of proteasomes in dendritic spines. *Nature*. 2006;441(7097):1144–1148. PMID 16810255.
- Bock HH, Herz J. Reelin activates SRC family tyrosine kinases in neurons. *Current Biology*. 2003;13(1):18–26. PMID 12526740.
- Bock HH, Jossin Y, May P, Bergner O, Herz J. Apolipoprotein E receptors are required for reelin-induced proteasomal degradation of the neuronal adaptor protein Disabled-1. *Journal of Biological Chemistry*. 2004;279(32):33471–33479. PMID 15175346.
- 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.
- Busche MA, Eichhoff G, Adelsberger H, Abramowski D, Wiederhold KH, Haass C, Staufenbiel M, Konnerth A, Garaschuk O. Clusters of hyperactive neurons near amyloid plaques in a mouse model of Alzheimer's disease. *Science*. 2008;321(5896):1686–1689. PMID 18802001.

- Capetillo-Zarate E, Gracia L, Yu F, Banfelder JR, Lin MT, Tampellini D, Gouras GK. High-resolution 3D reconstruction reveals intra-synaptic amyloid fibrils. *American Journal of Pathology*. 2011;179(5):2551–2558. PMID 21925470.
- Cataldo AM, Peterhoff CM, Troncoso JC, Gomez-Isla T, Hyman BT, Nixon RA. Endocytic pathway abnormalities precede amyloid β 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.
- Chai X, Förster E, Zhao S, Bock HH, Frotscher M. Reelin stabilizes the actin cytoskeleton of neuronal processes by inducing n-cofilin phosphorylation at serine3. *Journal of Neuroscience*. 2009;29(1):288–299. PMID 19129405.
- 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):12011–12016. PMID 20547867.
- Cissé M, Halabisky B, Harris J, Devidze N, Dubal DB, Sun B, Orr A, Lotz G, Kim DH, Hamto P, Ho K, Yu GQ, Mucke L. Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. *Nature*. 2011;469(7328):47–52. PMID 21113149.
- de Waal MWJ, van der Lee SJ, Lunding M, Boonkamp L, Barrett N, Monti G, Jensen AMG, Vaegter CB, Raska J, Cesnarikova S, Sedmik J, Trieu C, Weiss MM, van Spaendonk R, Vermunt L, Ozhegov G, Tesi N, Hulsman M, Janulien D, Moller A, Bohaciakova D, van der Flier WM, 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.
- Ding X, Chen Y, Guo C, Fu Y, Qin C, Zhu Q, Wang J, Zhang R, Tian H, Feng R, Liu H, Liang D, Wang G, Teng J, Li J, Tang B, Wang X. Mutations in ARHGEF15 cause autosomal dominant hereditary cerebral small vessel disease and osteoporotic fracture. *Acta Neuropathologica*. 2023;145(5):681–705. PMID 36929019.
- Edgar JR, Willén K, Gouras GK, Futter CE. ESCRTs regulate amyloid precursor protein sorting in multivesicular bodies and intracellular amyloid- β accumulation. *Journal of Cell Science*. 2015;128(14):2520–2528. PMID 26002056.
- Feng L, Allen NS, Simo S, Cooper JA. Cullin 5 regulates Dab1 protein levels and neuron positioning during cortical development. *Genes and Development*. 2007;21(21):2717–2730. doi:10.1101/gad.1604207
- Fernandez MA, Bah F, Ma L, Lee Y, Schmidt M, Welch E, Morrow EM, Young-Pearse TL. Loss of endosomal exchanger NHE6 leads to pathological changes in tau in human neurons. *Stem Cell Reports*. 2022;17(9):2111–2126. PMID 36055242.
- 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.
- Gouras GK, Tsai J, Naslund J, Vincent B, Edgar M, Checler F, Greenfield JP, Haroutunian V, Buxbaum JD, Xu H, Greengard P, Relkin NR. Intraneuronal A β 42 accumulation in human brain. *American Journal of Pathology*. 2000;156(1):15–20. PMID 10623648.
- Gouras GK, Willén K, Tampellini D. Critical role of intraneuronal A β in Alzheimer's disease: technical challenges in studying intracellular A β . *Life Sciences*. 2012;91(23–24):1153–1158. PMID 22727791.
- Greenfield JP, Tsai J, Gouras GK, Hai B, Thinakaran G, Checler F, Sisodia SS, Greengard P, Xu H. Endoplasmic reticulum and trans-Golgi network generate distinct populations of Alzheimer beta-amyloid peptides. *Proceedings of the National Academy of Sciences USA*. 1999;96(2):742–747. PMID 9892704.

- Hamilton AM, Lambert JT, Parajuli LK, Vivas O, Park DK, Stein IS, Jahncke JN, Greenberg ME, Margolis SS, Zito K. A dual role for the RhoGEF Ephexin5 in regulation of dendritic spine outgrowth. *Molecular and Cellular Neuroscience*. 2017;80:66–74. PMID 28185854.
- Haq I, Ngo JC, Roy N, Lee E, Choudhury MA, Soni RK, Teich AF, Mayeux RP, De Jager PL, He Y, Wu X, Bennett DA, 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.
- Hiesberger T, Trommsdorff M, Howell BW, Goffinet A, Mumby MC, Cooper JA, Herz J. 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):481–489. PMID 10571241.
- Huang H, Shi CH, Yang W, Piña-Crespo J, Bhatnagar J, Curatolo J, Murad R, Shah P, Campos A, Houser A, Porritt RA, Zhang T, Xiao Q, Feng S, Yip KY, Huang TY. SORLA up-regulation suppresses pathological effects in aged tauopathy mouse brain. *Science Advances*. 2026;12(29):eaed6825. doi:10.1126/sciadv.aed6825
- Hung C, Tuck E, Stubbs V, van der Lee SJ, Aalfs C, van Spaendonk R, Scheltens P, Hardy J, Holstege H, Livesey FJ. SORL1 deficiency in human excitatory neurons causes APP-dependent defects in the endolysosome-autophagy network. *Cell Reports*. 2021;35(11):109259. PMID 34133918.
- Jiang Y, Sachdeva K, Goulbourne CN, Berg MJ, Peddy J, Stavrides PH, Pensalfini A, Pawlik M, Malampati S, Whyte L, Basavarajappa BS, Subbanna S, Bleiwas C, Smiley JF, 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.
- Juul-Madsen K, Rudolph IM, Gomes JP, Meyer K, Ovesen PL, Gorniak-Walas M, Kokoli M, Telugu NS, von Tangen Sivertsen M, Febbraro F, Sutherland DS, Palmfeldt J, Diecke S, Andersen OM, Selbach M, Willnow TE. Familial Alzheimer's disease mutation identifies novel role of SORLA in release of neurotrophic exosomes. *Alzheimer's and Dementia*. 2025;21(9):e70591. PMID 40928027.
- Kang DE, Woo JA. Cofilin, a master node regulating cytoskeletal pathogenesis in Alzheimer's disease. *Journal of Alzheimer's Disease*. 2019;72(s1):S131–S144. PMID 31594228.
- Kawaguchi Y, Matsubayashi J, Kawakami Y, Nishida R, Kurihara Y, Takei K. LOTUS suppresses amyloid β -induced dendritic spine elimination through the blockade of amyloid β binding to PirB. *Molecular Medicine*. 2022;28(1):154. PMID 36510132.
- Kim S, Sato Y, Mohan PS, Peterhoff C, Pensalfini A, Rigoglioso A, Jiang Y, Nixon RA. Evidence that the rab5 effector APPL1 mediates APP- β CTF-induced dysfunction of endosomes in Down syndrome and Alzheimer's disease. *Molecular Psychiatry*. 2016;21(5):707–716. PMID 26194181.
- Kim T, Vidal GS, Djuricic M, William CM, Birnbaum ME, Garcia KC, Hyman BT, Shatz CJ. Human LirB2 is a β -amyloid receptor and its murine homolog PirB regulates synaptic plasticity in an Alzheimer's model. *Science*. 2013;341(6152):1399–1404. PMID 24052308.
- Klementieva O, Willén K, Martinsson I, Israelsson B, Engdahl A, Cladera J, Uvdal P, Gouras GK. Pre-plaque conformational changes in Alzheimer's disease-linked A β and APP. *Nature Communications*. 2017;8:14726. PMID 28287086.
- Knupp A, Mishra S, Martinez R, Braggins JE, Szabo M, Kinoshita C, Hailey DW, Small SA, Jayadev S, Young JE. Depletion of the AD risk gene SORL1 selectively impairs neuronal endosomal traffic independent of amyloidogenic APP processing. *Cell Reports*. 2020;31(9):107719. PMID 32492427.

- Konings SC, Nyberg E, Martinsson I, Torres-Garcia L, Klementieva O, Guimas Almeida C, Gouras GK. Apolipoprotein E intersects with amyloid- β within neurons. *Life Science Alliance*. 2023;6(8):e202201887. PMID 37290814.
- Kurup P, Zhang Y, Xu J, Venkitaramani DV, Haroutunian V, Greengard P, Nairn AC, Lombroso PJ. A β -mediated NMDA receptor endocytosis in Alzheimer's disease involves ubiquitination of the tyrosine phosphatase STEP61. *Journal of Neuroscience*. 2010;30(17):5948–5957. PMID 20427654. doi:10.1523/JNEUROSCI.0157-10.2010
- Lao PJ, Lee S, Talmasov D, Dass D, Chikwem N, Johnson A, Smith A, Guzman D, Okafor A, Houlihan H, Heuer L, Sanchez T, Maldonado A, Palacios C, Rossano S, Andrews H, Reitz C, Kreisl WC, Noble JM, Qureshi YH, Small SA. Inflammatory alterations mediate tau-associated neurodegeneration. *Brain Communications*. 2026;8(4):fcag242. PMID 42404434.
- Lee JH, Yang DS, Goulbourne CN, Im E, Stavrides P, Pensalfini A, Chan H, Bouchet-Marquis C, Bleiwas C, Berg MJ, Huo C, Peddy J, Pawlik M, Levy E, Rao M, Staufenbiel M, Nixon RA. Faulty autolysosome acidification in Alzheimer's disease mouse models induces autophagic build-up of A β in neurons, yielding senile plaques. *Nature Neuroscience*. 2022;25(6):688–701. PMID 35654956.
- Lundgren JL, Ahmed S, Schedin-Weiss S, Gouras GK, Winblad B, Tjernberg LO, Frykman S. ADAM10 and BACE1 are localized to synaptic vesicles. *Journal of Neurochemistry*. 2015;135(3):606–615. PMID 26296617.
- Ma T, Hoeffler CA, Capetillo-Zarate E, Yu F, Wong H, Lin MT, Tampellini D, Klann E, Blitzer RD, Gouras GK. Dysregulation of the mTOR pathway mediates impairment of synaptic plasticity in a mouse model of Alzheimer's disease. *PLoS One*. 2010;5(9):e12845. PMID 20862226.
- Margolis SS, Salogiannis J, Lipton DM, Mandel-Brehm C, Wills ZP, Mardinly AR, Hu L, Greer PL, Bikoff JB, Ho HH, Soskis MJ, Sahin M, Greenberg ME. EphB-mediated degradation of the RhoA GEF Ephexin5 relieves a developmental brake on excitatory synapse formation. *Cell*. 2010;143(3):442–455. PMID 21029865.
- Martinsson I, Capetillo-Zarate E, Faideau M, Willén K, Esteras N, Frykman S, Tjernberg LO, Gouras GK. APP depletion alters selective pre- and post-synaptic proteins. *Molecular and Cellular Neuroscience*. 2019;95:86–95. PMID 30763689.
- Martinsson I, Quintino L, Garcia MG, Konings SC, Torres-Garcia L, Svanbergsson A, Stange O, England R, Deierborg T, Li JY, Lundberg C, Gouras GK. A β /amyloid precursor protein-induced hyperexcitability and dysregulation of homeostatic synaptic plasticity in neuron models of Alzheimer's disease. *Frontiers in Aging Neuroscience*. 2022;14:946297. PMID 35928998.
- 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, 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, Yu R, Staniszewski A, Planel E, Herman M, Ho L, Kreber R, Honig LS, Ganetzky B, Duff K, Arancio O, Small SA. Retromer deficiency observed in Alzheimer's disease causes hippocampal dysfunction, neurodegeneration, and A β accumulation. *Proceedings of the National Academy of Sciences USA*. 2008;105(20):7327–7332. PMID 18480253.
- Murakoshi H, Wang H, Yasuda R. Local, persistent activation of Rho GTPases during plasticity of single dendritic spines. *Nature*. 2011;472(7341):100–104. PMID 21423166. doi:10.1038/nature09823
- Olabarria M, Pasini S, Corona C, Robador P, Song C, Patel H, Lefort R. Dysfunction of the ubiquitin ligase E3A Ube3A/E6-AP contributes to synaptic pathology in Alzheimer's disease. *Communications Biology*. 2019;2:111. PMID 30937395.
- Olsson TT, Klementieva O, Gouras GK. Prion-like seeding and nucleation of intracellular amyloid- β . *Neurobiology of Disease*. 2018;113:1–10. PMID 29414379.
- 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, Bleiwas C, Goulbourne CN, Stavrides PH, Jiang Y, Lee JH, Darji S, Pawlik M, Huo C, Peddy J, Berg MJ, Smiley JF, 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.
- Pesold C, Liu WS, Guidotti A, Costa E, Caruncho HJ. Cortical bitufted, horizontal, and Martinotti cells preferentially express and secrete reelin into perineuronal nets, nonsynaptically modulating gene expression. *Proceedings of the National Academy of Sciences USA*. 1999;96(6):3217–3222. PMID 10077664.
- Petshow S, Coblentz A, Hamilton AM, Sarkar D, Anisimova M, Flores JC, Zito K. Activity-dependent regulation of Cdc42 by Ephexin5 drives synapse growth and stabilization. *Science Advances*. 2025;11(13):eadp5782. PMID 40138406.
- Pollack SJ, Dakkak D, Guo T, Chennell G, Gomez-Suaga P, Noble W, Jimenez-Sanchez M, Hanger DP. Truncated tau interferes with the autophagy and endolysosomal pathway and results in lipid accumulation. *Cellular and Molecular Life Sciences*. 2024;81(1):304. PMID 39009859.
- 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, Végh B, Petsko GA, Young JE, Závodszy 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.
- Ramakrishna S, Jhaveri V, Konings SC, Nawalpur B, Chakraborty S, Holst B, Schmid B, Gouras GK, Freude KK, Muddashetty RS. APOE4 affects basal and NMDAR-mediated protein synthesis in neurons by perturbing calcium homeostasis. *Journal of Neuroscience*. 2021;41(42):8686–8709. PMID 34475200.
- 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.
- Ramsden CE, Keyes GS, Calzada E, Horowitz MS, Zamora D, Jahanipour J, Sedlock A, Indig FE, Moaddel R, Kapogiannis D, Maric D. Lipid peroxidation induced ApoE receptor-ligand disruption as a unifying hypothesis

- underlying sporadic Alzheimer's disease in humans. *Journal of Alzheimer's Disease*. 2022;87(3):1251–1290. PMID 35466940.
- 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 A β and its disruption of A β homeostasis. *Acta Neuropathologica*. 2021;142(4):669–687. PMID 34272583.
- Rush T, Martinez-Hernandez J, Dollmeyer M, Frandemich ML, Borel E, Boisseau S, Jacquier-Sarlin M, Buisson A. Synaptotoxicity in Alzheimer's disease involved a dysregulation of actin cytoskeleton dynamics through cofilin 1 phosphorylation. *Journal of Neuroscience*. 2018;38(48):10349–10361. PMID 30341179.
- Sahlin C, Lord A, Magnusson K, Englund H, Almeida CG, Greengard P, Nyberg F, Gouras GK, Lannfelt L, Nilsson LN. The Arctic Alzheimer mutation favors intracellular amyloid-beta production by making amyloid precursor protein less available to alpha-secretase. *Journal of Neurochemistry*. 2007;101(3):854–862. PMID 17448150.
- Saito T, Iwata N, Tsubuki S, Takaki Y, Takano J, Huang SM, Suemoto T, Higuchi M, Saido TC. Somatostatin regulates brain amyloid β peptide A β 42 through modulation of proteolytic degradation. *Nature Medicine*. 2005;11(4):434–439. PMID 15778722.
- Saito T, Matsuba Y, Mihira N, Takano J, Nilsson P, Itohara S, Iwata N, Saido TC. Single App knock-in mouse models of Alzheimer's disease. *Nature Neuroscience*. 2014;17(5):661–663. PMID 24728269.
- Sell GL, Schaffer TB, Margolis SS. Reducing expression of synapse-restricting protein Ephexin5 ameliorates Alzheimer's-like impairment in mice. *Journal of Clinical Investigation*. 2017;127(5):1646–1650. PMID 28346227.
- 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, Qureshi YH, Feng X, Kothiya M, Cortes E, Patel V, Kannan S, Kim YH, Chang KT, Hussaini SA, Moreno H, Di Paolo G, 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. doi:10.1016/j.celrep.2021.110182
- 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, Duff K. Linking Abeta and tau in late-onset Alzheimer's disease: a dual pathway hypothesis. *Neuron*. 2008;60(4):534–542. PMID 19038212.
- Small SA, Schobel SA, Buxton RB, Witter MP, Barnes CA. A pathophysiological framework of hippocampal dysfunction in ageing and disease. *Nature Reviews Neuroscience*. 2011;12(10):585–601. PMID 21897434.
- 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.
- Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, Nairn AC, Salter MW, Lombroso PJ, Gouras GK, Greengard P. Regulation of NMDA receptor trafficking by amyloid-beta. *Nature Neuroscience*. 2005;8(8):1051–1058. PMID 16025111.

- Stenh C, Englund H, Lord A, Johansson AS, Almeida CG, Gellerfors P, Greengard P, Gouras GK, Lannfelt L, Nilsson LN. Amyloid-beta oligomers are inefficiently measured by enzyme-linked immunosorbent assay. *Annals of Neurology*. 2005;58(1):147–150. PMID 15984012.
- Tai HC, Serrano-Pozo A, Hashimoto T, Frosch MP, Spires-Jones TL, Hyman BT. The synaptic accumulation of hyperphosphorylated tau oligomers in Alzheimer disease is associated with dysfunction of the ubiquitin-proteasome system. *American Journal of Pathology*. 2012;181(4):1426–1435. PMID 22867711. doi:10.1016/j.ajpath.2012.06.033
- Takahashi RH, Milner TA, Li F, Nam EE, Edgar MA, Yamaguchi H, Beal MF, Xu H, Greengard P, Gouras GK. Intraneuronal Alzheimer A β 42 accumulates in multivesicular bodies and is associated with synaptic pathology. *American Journal of Pathology*. 2002;161(5):1869–1879. PMID 12414533.
- Takahashi RH, Almeida CG, Kearney PF, Yu F, Lin MT, Milner TA, Gouras GK. Oligomerization of Alzheimer's β -amyloid within processes and synapses of cultured neurons and brain. *Journal of Neuroscience*. 2004;24(14):3592–3599. PMID 15071107.
- Takahashi RH, Capetillo-Zarate E, Lin MT, Milner TA, Gouras GK. Accumulation of intraneuronal β -amyloid 42 peptides is associated with early changes in microtubule-associated protein 2 in neurites and synapses. *PLoS One*. 2013;8(1):e51965. PMID 23372648.
- Tampellini D, Rahman N, Gallo EF, Huang Z, Dumont M, Capetillo-Zarate E, Ma T, Zheng R, Lu B, Nanus DM, Lin MT, Gouras GK. Synaptic activity reduces intraneuronal A β , promotes APP transport to synapses, and protects against A β -related synaptic alterations. *Journal of Neuroscience*. 2009;29(31):9704–9713. PMID 19657023.
- Tampellini D, Capetillo-Zarate E, Dumont M, Huang Z, Yu F, Lin MT, Gouras GK. Effects of synaptic modulation on β -amyloid, synaptophysin, and memory performance in Alzheimer's disease transgenic mice. *Journal of Neuroscience*. 2010;30(43):14299–14304. PMID 20980585.
- Tampellini D, Rahman N, Lin MT, Capetillo-Zarate E, Gouras GK. Impaired β -amyloid secretion in Alzheimer's disease pathogenesis. *Journal of Neuroscience*. 2011;31(43):15384–15390. PMID 22031884. doi:10.1523/JNEUROSCI.2986-11.2011
- 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.
- Türker F, Brennan A, Margolis SS. Neuronal membrane proteasome-derived peptides modulate NMDAR-dependent neuronal signaling to promote changes in gene expression. *Molecular Biology of the Cell*. 2024;35(1):ar6. PMID 37910253.
- Turrigiano GG. The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell*. 2008;135(3):422–435. PMID 18984155.
- Wang Y, Zhang Y, Hu W, Xie S, Gong CX, Iqbal K, Liu F. Rapid alteration of protein phosphorylation during postmortem: implication in the study of protein phosphorylation. *Scientific Reports*. 2015;5:15709. PMID 26511732.
- Wen G, Lindblom N, Zhan X, Garcia-Ryde M, Deierborg T, Kobre-Flatmoen A, Gouras GK. A β antibodies target not only amyloid plaques but also distinct brain cells and vessels. *Alzheimer's and Dementia*. 2026;22(1):e71121. PMID 41566565.
- Willén K, Edgar JR, Hasegawa T, Tanaka N, Futter CE, Gouras GK. A β accumulation causes MVB enlargement and is modelled by dominant negative VPS4A. *Molecular Neurodegeneration*. 2017a;12(1):61. PMID 28835279.

- Willén K, Sroka A, Takahashi RH, Gouras GK. Heterogeneous association of Alzheimer's disease-linked amyloid- β and amyloid- β protein precursor with synapses. *Journal of Alzheimer's Disease*. 2017b;60(2):511–524. PMID 28869466.
- Williams CA, Rose SE, Stamenkovic V, Smith SEP, Young JE. The Alzheimer's disease risk gene SORL1 is a regulator of excitatory neuronal function. *bioRxiv*. 2025;2025.07.28.667194. [Preprint.] PMID 40766397. doi:10.1101/2025.07.28.667194
- Xian X, Pohlkamp T, Durakoglulil MS, Wong CH, Beck JK, Lane-Donovan C, Plattner F, Herz J. Reversal of ApoE4-induced recycling block as a novel prevention approach for Alzheimer's disease. *eLife*. 2018;7:e40048. PMID 30375977.
- Xu Y, Du S, Marsh JA, Horie K, Sato C, Ballabio A, Karch CM, Holtzman DM, Zheng H. TFEB regulates lysosomal exocytosis of tau and its loss of function exacerbates tau pathology and spreading. *Molecular Psychiatry*. 2021;26(10):5925–5939. PMID 32366951.
- Young JE, Holstege H, Andersen OM, Petsko GA, Small SA. On the causal role of retromer-dependent endosomal recycling in Alzheimer's disease. *Nature Cell Biology*. 2023;25(10):1394–1397. PMID 37803174.
- Zheng C, Xia W, Zhang J. Rock inhibitors in Alzheimer's disease. *Frontiers in Aging*. 2025;6:1547883. PMID 40182055.