

The Poisoned Service

*One Compartment, Two Jobs, and a Cargo That Disables
One of Them*

The synapse terminates its own structural instructions by destroying them,
and the peptide it manufactures disables the machinery that does the
destroying

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Abstract

A dendritic spine issues structural instructions and must withdraw them. For one class of instruction the withdrawal is not chemical but physical: the signal is terminated by destroying the molecule that carries it. Activity drives a proteasome-dependent fall in dendritic Ephexin5, a Rho-family exchange factor whose substrate preference is set by phosphorylation at tyrosine 361, and that same phosphorylation recruits the ubiquitin ligase that consigns the protein to the proteasome. The instruction and its expiry are written in one mark.

This paper places that architecture beside a second body of work that has never been joined to it. Amyloid- β 42 accumulates intraneuronally in multivesicular bodies, predominantly in postsynaptic compartments, before plaque pathology appears — and that accumulation inhibits proteasome and deubiquitinating-enzyme activity and impairs multivesicular-body sorting.

Read together, the two literatures describe one compartment with two jobs and a cargo that disables one of them. The peptide the synapse produces and retains degrades the proteolytic service on which the synapse depends to withdraw its own structural signals. The disease does not merely remove the licence for that destruction — amyloid drives the licensing receptor EphB2 into the proteasome — it also poisons the executioner. Two independent hits fall on one disposal step, and the substrate of that step is elevated in human Alzheimer hippocampus.

The claim yields a third explanation for that elevation, standing beside the two already in the literature. It is not only transcriptional induction, and not only a failure of licensing; it is predicted to be, in substantial part, the generic consequence of a poisoned proteolytic service acting on a substrate whose only off switch is destruction. The three have never been separated, because every human measurement reported to date is of total protein, and total protein is the one quantity that cannot distinguish them.

The argument is assembled entirely from published results, each taken as its authors reported it, and it is graded throughout for the preparation it came from. Sections 9 and 10 state what would refute it and what it does not claim, including one published finding that sits in direct tension with its central metaphor.

1. The problem: an instruction that cannot be withdrawn

A synapse is not a structure so much as a maintained relationship, and maintenance means the capacity to change and then to stop changing. A spine that grows must stop growing. A spine instructed to contract must be released from that instruction. The interesting question is not how the instruction is issued but how it ends.

For most signalling the answer is a phosphatase. A kinase adds a mark, a phosphatase removes it, and the system returns to baseline with every component still present. This is cheap, fast and reversible, and it is how the majority of synaptic signalling is terminated.

There is a second mechanism, and it is the subject of this paper. Some signals are terminated by removing the molecule that carries them. Where that is the design, the cell's capacity to stop signalling is a function of its proteolytic and sorting machinery rather than of its phosphatases — and anything that degrades that machinery degrades the cell's ability to issue a brief instruction at all, without touching a single kinase, receptor or ligand.

The question this paper asks is whether Alzheimer's disease interferes with that machinery in the specific compartment where this specific instruction is terminated, and whether it does so using the peptide that is the disease's own signature.

2. What is established about the instruction

Three results from two laboratories establish the architecture. Each is taken as published.

The licensing step. Ephexin5 is a Rho-family guanine nucleotide exchange factor that restrains excitatory synapse development until EphB receptor signalling triggers its phosphorylation, ubiquitination and degradation. The ligase is Ube3A, the gene mutated in Angelman syndrome. The removal of Ephexin5 is what permits EphB-dependent synapse development to proceed (Margolis et al., 2010).

The structure of this arrangement deserves to be stated plainly, because it is unusual. The phosphorylation that acts on the enzyme is also the phosphorylation that condemns it. Activation and expiry are not two events with two regulators; they

are one covalent mark read twice. A consequence follows immediately: for this protein, abundance and activity are not independent variables, and a measurement of one is partly a measurement of the other.

The service that executes it. The termination is proteolytic and it is activity-driven. Increased neural activity produces a proteasome-dependent reduction in the levels of Ephexin5 in neuronal dendrites. The same work found that reducing Ephexin5 increased spine outgrowth and increasing it decreased outgrowth — consistent with a brake — but also that lowering Ephexin5 *inhibited* new spine outgrowth in response both to global activity increases and to local glutamatergic stimulation, and that Ephexin5 was elevated on the dendritic shaft at the sites of future spines before those spines appeared. The authors concluded that the protein serves a dual role: a brake on overall outgrowth, and a necessary component of site-specific new spine formation (Hamilton et al., 2017).

The observation to hold is the middle one. The controlled variable is dendritic Ephexin5; the control is proteolytic; the trigger is activity. That is a disposal service operating on a schedule set by use.

What the mark actually decides. The mechanism of the duality was established in 2025. Ephexin5 activates both RhoA and Cdc42. At single spines during plasticity induced by high-frequency glutamate uncaging, it regulates the activation of Cdc42 but not of RhoA. Selectivity for Cdc42 is governed by tyrosine phosphorylation, itself regulated by neuronal activity; a phosphorylation-dead mutant at the key residue, Y361F, enhances Cdc42 activation while leaving RhoA activation unchanged. Functionally, spines in Ephexin5-knockout neurons failed to sustain activity-driven growth, and the Y361F phosphomutant rescued that growth (Petshow et al., 2025).

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 adult relevance of the protein is not its absence but its setting.

3. What is established about the cargo

A second programme, pursued independently and over a longer period, establishes where amyloid- β is and what it does to the compartment holding it.

Location. Immunoelectron microscopy localised amyloid- β 42 predominantly to the multivesicular bodies of neurons in normal mouse, rat and human brain. In amyloid precursor protein transgenic mice and in human Alzheimer brain, intraneuronal A β 42 increased with age and accumulated in multivesicular bodies within presynaptic and **especially postsynaptic** compartments. The accumulation was associated with abnormal synaptic morphology, and it occurred before plaque pathology (Takahashi et al., 200264463-x).

Two features of that result matter here. The peptide is inside the neuron, and its preferred address is the postsynaptic compartment — which is where the spine is, and therefore where the instruction of Section 2 is issued and withdrawn.

Effect on the compartment. The same laboratory then asked what the accumulation does to the sorting pathway holding it. Using epidermal growth factor receptor and TrkB trafficking as reporters in cultured neurons, they found that APP-mutant neurons showed impaired inactivation, degradation and ubiquitination of EGFR. Because EGFR degradation depends on translocation from the outer to the inner membrane of the multivesicular body, and that translocation is regulated by the ubiquitin–proteasome system, they tested the system directly: amyloid- β accumulation in APP-mutant neurons inhibited the activities of the proteasome and of deubiquitinating enzymes (Almeida et al., 2006).

This is the hinge of the present paper, and its provenance must be stated with it. The finding is in cultured APP-mutant neurons, and the readout is the trafficking of two receptor tyrosine kinases, neither of them Ephexin5. What it establishes is that intraneuronal amyloid degrades the capacity of this compartment to execute ubiquitin-dependent disposal. What it does not establish is that Ephexin5 in particular is among the substrates affected. Nobody has measured that.

4. One compartment, two jobs

Place the two sections side by side and a coincidence of address emerges that neither literature has drawn out.

The spine's structural instruction is terminated by ubiquitin-dependent proteolysis in the dendrite. The peptide that characterises Alzheimer's disease accumulates, before plaques, in multivesicular bodies of the postsynaptic compartment, and inhibits ubiquitin-dependent proteolysis there.

The claim is therefore not that amyloid is near the machinery. It is that the machinery which disposes of the peptide and the machinery which withdraws the structural instruction are, in the relevant respects, the same machinery — and the peptide is a cargo that degrades the service handling it.

This is the point at which the two programmes become one argument rather than two adjacent observations. The synapse manufactures a product on the trafficking apparatus it uses to run itself; the product impairs that apparatus; and one of the apparatus's duties is to end the synapse's own structural signalling. The failure is autocatalytic in form: the more the service is needed, the less of it there is.

And the disease strikes the same step twice. Amyloid- β oligomers bind the fibronectin-repeat domain of EphB2 and trigger the receptor's degradation in the proteasome; restoring EphB2 expression reverses deficits in long-term potentiation and memory in an Alzheimer model (Cissé et al., 2011). EphB signalling is what licenses the destruction of Ephexin5. So amyloid removes the licence at the same time as it poisons the executioner. One arm of the disposal step loses its instruction; the other loses its capacity.

The predicted consequence of two hits on one disposal step is that its substrate should accumulate. It does.

5. The human observation, and three explanations for it

Ephexin5 protein is highly elevated in the hippocampi of human Alzheimer patients. In mice expressing human amyloid precursor protein, removing Ephexin5 eliminated hippocampal dendritic spine loss and rescued behavioural deficits, and a selective reduction in the dentate gyrus of presymptomatic adolescent animals was sufficient to prevent cognitive impairment. The authors reported amyloid as sufficient to acutely promote the production of Ephexin5, framed the mechanism as reactivation of a developmental repressor, and concluded that strategies aimed at reducing Ephexin5 levels may be an effective approach to treating Alzheimer's disease (Sell et al., 2017).

The mouse results are not in dispute here and are taken exactly as published. What is at issue is the interpretation of the human elevation, for which three explanations are now available and none has been excluded.

Explanation one — transcriptional induction. This is the authors' own reading: amyloid reactivates expression of a developmental gene, and more protein is made. It is supported by their demonstration that amyloid acutely promotes Ephexin5 production in mature hippocampal neurons.

Explanation two — licensing failure. Amyloid depletes EphB2 (Cissé et al., 2011); EphB signalling is what marks Ephexin5 for destruction (Margolis et al., 2010); so the mark is not applied and the protein is not removed. On this reading the elevation is a consequence of a missing instruction rather than of increased synthesis.

Explanation three — a poisoned service. Intraneuronal amyloid inhibits proteasome and deubiquitinating-enzyme activity in the postsynaptic compartment (Almeida et al., 2006), and dendritic Ephexin5 is cleared by a proteasome-dependent route (Hamilton et al., 2017). On this reading the elevation requires no change in transcription and no loss of licensing: it is what happens to any substrate whose only off switch is destruction when the destroying service is impaired.

These are not rival theories of the disease. They are three routes to one observation, they are not mutually exclusive, and they have different therapeutic consequences. Induction argues for reducing synthesis. Licensing failure argues for restoring the receptor. A poisoned service argues for repairing the compartment — and warns specifically that lowering the substrate addresses neither the licence nor

the capacity.

6. Why the three have never been separated

The separation has not been attempted, and the reason is methodological rather than biological.

Every published human measurement of this protein is of total protein. Section 2 established that for Ephexin5 abundance and activity are two readings of one covalent mark. A total-protein elevation is therefore consistent with more synthesis, with less destruction, and with an unchanged enzyme that is simply failing to be cleared — and it cannot discriminate among them.

The quantity that discriminates is the ratio: the phosphorylated fraction of Ephexin5 at tyrosine 361, relative to total Ephexin5, in the same tissue.

- Under **induction**, both total and phosphorylated protein rise, and the fraction is roughly preserved, because the licensing machinery is intact and processes the extra substrate.
- Under **licensing failure**, total rises while the phosphorylated fraction falls, because the mark is not being applied.
- Under a **poisoned service**, the phosphorylated fraction rises, because the mark is applied normally and the marked protein is not removed — phosphorylated, ubiquitinated species should accumulate specifically.

The three predictions are distinct and the measurement is a ratio of two antibodies on one sample. That it has not been made is the most consequential gap in this area, and it is worth being explicit about why: no validated antibody against phospho-tyrosine-361 Ephexin5 appears in the published literature. The rate-limiting step in this entire argument is a reagent, not an idea.

7. What follows for the shape of the lesion

If the account in Section 4 is even partly right, the lesion at this node is one of specificity rather than of quantity.

A capacity model says the neuron's degradative throughput falls, and everything is cleared more slowly. That model predicts a correlated rise across proteasome substrates, and it prescribes increasing degradative flux.

The present account predicts something different and more awkward. The disease removes EphB2 by proteasomal degradation — the machinery working, on that substrate, with evident efficiency — while the substrate that EphB2 licenses for destruction accumulates. In the same neuron, in the same compartment, one arm of the ubiquitin–proteasome system over-runs and the other under-runs. No single scalar capacity variable predicts that pattern, and a bulk measurement of proteasome activity, autophagic flux or lysosomal enzyme content cannot detect it, because what has changed is which substrates are being handled rather than how many.

This matters because the instruction the two models give are opposite in order. A capacity model says induce flux. A specificity model says find out which arm is failing first, and note that increasing induction in a compartment that cannot complete disposal fills the neuron faster with vesicles it cannot empty.

8. What this forbids

It forbids lowering Ephexin5 systemically. Heterozygous loss-of-function mutations in *ARHGEF15*, the gene encoding Ephexin5, cause autosomal dominant hereditary cerebral small-vessel disease with osteoporotic fracture. The mechanism is RhoA/ROCK2 inactivation producing F-actin disorganisation in vascular smooth muscle and endothelial cells, with osteoblast dysfunction through Wnt/ β -catenin inhibition; transgenic mice carrying a patient variant developed small-vessel-disease pathology and behavioural phenotypes with severe osteoporosis (Ding et al., 2023). Cerebral small-vessel disease is itself a leading cause of vascular dementia.

A therapeutic strategy of chronic systemic Ephexin5 reduction is therefore a phenocopy of a Mendelian dementing vasculopathy with skeletal fragility, and one functional copy is demonstrably not enough in the adult human. Independently, the 2025 revision shows the protein is required for activity-driven spine growth, so the neuronal cost of lowering it is ablation of a growth arm rather than release of a brake.

The literature on this gene is small enough to characterise completely. It comprises thirty indexed papers, and it has split into two arms that do not cite one

another: a synaptic arm concerned with spine development and Alzheimer's disease, and a vascular, endothelial and oncological arm in which the Mendelian dementia finding sits. No paper in either arm discusses the other's conclusion. The therapeutic proposal and its contraindication have been in print simultaneously for three years without meeting.

It forbids total-protein readouts of this transducer. Where one mark sets both what an enzyme does and how long it survives, abundance and activity cannot be interpreted or targeted independently.

It forbids inducing degradative flux before the compartment can complete it. If the failing step is terminal — sorting, acidification, hydrolysis — then induction precedes repair at the cost of accumulating what cannot be emptied.

And it forbids inferring from a shared service to a shared fate. The same proteasome removes EphB2 and fails to remove Ephexin5. A drug acting on the machinery acts on both arms, and the sign of its net effect is not predictable from a capacity argument.

9. What would refute this

Each of the following is a published-result-shaped outcome that would count against the argument rather than merely complicate it.

- **The phosphorylated fraction of Ephexin5 is unchanged or reduced in Alzheimer cortex relative to age-matched control, with total protein elevated.** The poisoned-service explanation predicts accumulation of the marked species. A fall in the fraction would favour licensing failure and would remove the proteolytic-poisoning arm of the argument.
- **Ephexin5 elevation is shown to be transcriptional in human tissue,** by messenger RNA measurement in the same cases in which protein is elevated, with the phosphorylated fraction preserved. The elevation is then induction, and Sections 4 through 7 lose their subject.
- **Ephexin5 elevation precedes any detectable sorting or degradative abnormality** in a model carrying humanised amyloid precursor protein at endogenous levels. The ordering claim then fails and the elevation must be explained upstream of the compartment.

- **Proteasome substrates rise together in Alzheimer neurons.** The specificity claim of Section 7 is then unnecessary, a capacity deficit is sufficient, and the "misdirection" framing should be abandoned.
 - **Restoring EphB2 expression normalises Ephexin5 without any repair of compartment function.** Licensing is then rate-limiting and the service is not.
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10. Limits, and one finding in tension with the central metaphor

The argument is assembled from results obtained in different preparations, species and decades, and that assembly is its principal weakness. The instruction architecture is established in cultured rodent neurons and slice. The cargo results are established in cultured APP-mutant neurons, transgenic mice, and post-mortem human tissue by immunoelectron microscopy. The human Alzheimer elevation is a total-protein measurement in hippocampus. No single preparation contains the whole chain, and no result reported here was obtained in order to test it.

Three specific limits should be carried with the argument.

The ordering is not established. Nothing in the cited record demonstrates that sorting fails first and proteolytic execution second in the same human neurons. Early endosomal pathology, intraneuronal peptide observations and the Ephexin5 elevation come from different cohorts and endpoints. That each can be placed somewhere relative to plaque pathology does not order them relative to one another.

Mouse null and human haploinsufficiency are not the same experiment. *Arhgef15*-deleted mice are fertile with complete spermatogenesis (Wang et al., 2023), while human heterozygous loss of function produces a dementing vasculopathy. Tolerance of the null in one tissue and species is not evidence of safety in another.

And one published result sits in direct tension with the paper's framing. The 2025 work found that chemical long-term potentiation reduces phospho-tyrosine Ephexin5 sharply within minutes by *dephosphorylation* rather than degradation — the fall surviving proteasome inhibition (Petshow et al., 2025). The mark, then, does have a phosphatase-mediated off switch, and the claim that

termination at this surface is exclusively proteolytic is too strong as stated. The defensible version is narrower and is the one this paper adopts: the *mark* can be reset chemically, but the *protein* can only be removed by destruction, and it is the removal of dendritic protein that Hamilton and colleagues showed to be proteasome-dependent and activity-driven. A phosphatase returns the enzyme to its Cdc42-directed setting; it does not clear an accumulated exchange factor from a dendrite. Both statements are needed, and only the second supports the argument made here.

Finally, the scope. This is a claim about the execution of synaptic loss, not about what initiates the disease. It does not explain who develops Alzheimer's disease, why particular neurons fail first, or the substantial fraction of dementia not attributable to the pathologies conventionally measured.

11. The experiment this argues for

One measurement settles more than any other, and it is specified here in full so that it can be costed.

Array tomography of Alzheimer and age-matched control cortex, staining phospho-tyrosine-361 Ephexin5, total Ephexin5, ubiquitin, a postsynaptic marker and amyloid- β , read as a **joint distribution across individual synapses** rather than as a set of means. The three explanations of Section 5 predict different joint distributions, and a lysate averages the discriminating information away by construction.

Two conditions must be met before it can run. A validated antibody against phospho-tyrosine-361 must exist, which at present it does not; generating and qualifying it is the first and currently the only rate-limiting task. And the measurement should be performed first in perfusion-fixed model tissue rather than in post-mortem human cortex, because phospho-epitope lability after death biases a correlation-based readout toward the null — which, for a hypothesis whose central prediction is a *loss* of correlation, is the direction that would manufacture a false confirmation.

That last point is worth stating as a standing caution rather than a footnote. A hypothesis predicting reduced covariance is confirmed by anything that adds noise.

The experiment must therefore be designed to exclude its own most flattering artefact.

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