The Contested Serine

One residue, four laboratories, two opposite signs — and why the disease is a loss of control rather than a displacement

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Four independent laboratories write to serine 3 of cofilin in Alzheimer's disease, and two of them report opposite directions in human tissue without addressing each other. All four, unremarked, converge on the dendritic spines of excitatory pyramidal neurons. Because cofilin severs actin at low occupancy and bundles it at high occupancy, the effector is non-monotonic and the residue has no pathological direction — only a pathological loss of range. The paper's original claim is that the lesion is the loss of governed control at that residue rather than a displacement of its mean; it has never been measured, and Section VI states the experiment that would break it.

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Abstract

Synapse loss remains the strongest structural correlate of cognitive impairment in Alzheimer's disease. In the study that established this, neocortical synapse density carried a multivariate correlation of 0.96 with the Dementia Rating Scale, while plaque density contributed only 26 per cent of that strength (Terry et al., 1991). Any account of the disease must therefore eventually say what happens, mechanically, at a dendritic spine — and a dendritic spine is an actin structure whose volume is set by the balance between filament severing and filament stabilisation.

That balance is governed by one protein, cofilin-1, through phosphorylation at one residue: **serine 3**.

This paper reports that four independent laboratories have converged on that residue in this disease, and that **they disagree about the direction of the change, in human tissue, and the disagreement has not been stated in print.** Reelin, acting through ApoER2 and Disabled-1, *phosphorylates* serine 3 and stabilises the cytoskeleton (Chai et al., 2009). Amyloid-β, acting through the receptor LilrB2 and its murine orthologue PirB, produces "enhanced cofilin signalling" — that is, *dephosphorylation* — reported in mouse and detected in human Alzheimer brain (Kim et al., 2013), a direction independently reproduced with a natural receptor antagonist (Kawaguchi et al., 2022). And amyloid-β, acting through Rho-associated kinase, *increases* serine-3 phosphorylation in the post-synaptic fraction of **human Alzheimer cortex**, where that phosphorylation is reported to be necessary and sufficient for synaptic impairment (Rush et al., 2018).

Two of these describe amyloid removing the phosphate. One describes amyloid adding it. All three describe human Alzheimer tissue. The 2018 paper states that the effect of amyloid on the actin cytoskeleton "remains unknown and contentious" and does not resolve the conflict; no subsequent paper has.

This is not an academic difficulty. Rho-kinase inhibitors are in active development for Alzheimer's disease, and a 2025 review of the class notes — without apparent alarm — that "activation **or** inhibition of ROCKs changes dendritic/synaptic structures" (Zheng et al., 2025). **The sign of the intended therapy is unresolved at the residue through which it acts.**

The resolution proposed here is that both findings are correct and the question is malformed. Cofilin's action on actin is **non-monotonic**: it severs filaments at low cofilin-to-actin ratios and stabilises them at high ratios (Bamburg and Bernstein, 2016). A residue whose effector inverts across its own range does not have a pathological direction. It has a pathological *loss of range*.

The claim advanced is therefore a **convergence claim**, and it is precise: Alzheimer's synaptic failure is executed at cofilin serine 3 **on the dendritic spines of excitatory glutamatergic**

pyramidal neurons of neocortex and hippocampus — the one cell on which all four literatures land without any of them saying so — and the lesion is the loss of governed, transient, local control of that residue, not a displacement of its mean in either direction. Bulk-tissue measurement, which averages across synapses whose local receptor complement differs, cannot see this and will return whichever sign the sampling favours. That is the most economical explanation of why three careful laboratories disagree.

The claim generates a hard, cheap, falsifying prediction that nobody has tested: **in Alzheimer cortex the synapse-to-synapse variance of phospho-serine-3 cofilin will be elevated relative to control even where the mean is unchanged.** The method required — array tomography of individual excitatory synapses in human cortex — has already been applied to these exact synapses by one of the disputing groups. The experiment is a re-analysis, not a new programme.

Four consequences follow. Bulk phospho-cofilin is disqualified as a trial endpoint. Monotonic Rho-kinase therapy is predicted to help one stratum of patients and harm another, which is what a failed trial with a positive subgroup looks like. The therapeutic target is not the kinase or the phosphatase but the restoration of signal-driven control — for which the only characterised physiological system is reelin through ApoER2, where a human gain-of-function allele is already known to confer resistance to autosomal-dominant Alzheimer's disease (Lopera et al., 2023). And because reelin's receptor activation obligately requires **N-sulfated heparan sulfate** (Pan et al., 2025) — the same polymer through which pathological tau enters neurons — a heparin mimetic deployed to block tau propagation would silence the reelin brake by the identical chemistry. That conflict is verified at drug level and has not been stated.

A note on the perineuronal net, which is frequently invoked in this area. The net is a **chondroitin**-sulfate structure and reelin's requirement is for **heparan** sulfate, a component of the diffuse matrix that surrounds all central nervous tissue (Fawcett et al., 2022). Reelin therefore does not need a net in order to signal, and the two can be separated experimentally with a pair of enzymes.

I. What kind of claim this is

Claims about Alzheimer's disease fail most often not because the evidence is weak but because the evidence is of the wrong kind for the claim being made. A claim about what *initiates* the disease requires evidence about the earliest affected people. A claim about what the disease *converges on* requires evidence that multiple upstream causes arrive at one place. The two are routinely conflated, and a convergence result is presented as an origin story.

This paper makes a **convergence claim** and nothing more. It does not say what starts Alzheimer's disease. It says that however the disease starts — and it plainly starts in more than one way — the path to synaptic failure passes through the loss of control of a single named residue, and that this is why the therapeutic literature at that residue contradicts itself.

Three restrictions follow and are honoured throughout.

It does not explain who gets the disease. Nothing here addresses initiation, selective vulnerability, or why one person with amyloid becomes demented and another does not. Those are the important questions and this is not an answer to them.

It applies to the synaptic-execution arm. Alzheimer's dementia is not a single road. A substantial fraction of dementia is not attributable to the pathologies usually measured, and a substantial fraction of people meeting neuropathological criteria are not demented. Section IX states what portion of the disease this paper claims and what portion it does not.

Its central positive proposal is untested. The reconciliation offered in Section V is an inference from published facts, not a result. It is graded as such in Section XI, and Section X states what would refute it.

II. Why one residue

A dendritic spine is a bag of actin. Its volume, and therefore the strength of the synapse it carries, is the running balance of two opposed processes: nucleation and elongation of actin filaments, and their severing and turnover.

Severing is performed by cofilin, the major actin-depolymerising factor of mammalian neurons. Cofilin 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, downstream of Rho-associated kinase, add the phosphate; the slingshot phosphatases and chronophin remove it.

Three properties make this residue the natural place to look for the lesion.

It is the convergence point by construction. Every receptor system that alters spine structure must eventually alter the actin network, and the great majority of them do so through the Rho-family GTPases and thence through this residue. A signal that changes spine volume and does *not* pass through cofilin is the exception.

It is directly implicated in this disease in human tissue. Inclusions containing cofilin and actin are prominent in hippocampal and cortical neurites of post-mortem Alzheimer brain, and are most prominent in neurites contacting amyloid deposits (Minamide et al., 2000). These rods are present in Alzheimer brain and not in normal brain (Bamburg and Bernstein, 2016).

And it is druggable, right now. Rho-kinase inhibitors exist, one of them is clinically available, and the class is under active investigation for this disease (Zheng et al., 2025). Whatever is true at this residue has immediate consequences for what is put into patients.

2.1 Which neuron

Cofilin is ubiquitous and Alzheimer's disease is not, so a claim at this residue is worthless until it names a cell. The four literatures below have never been read together, and when they are, they are found to have converged on **one cell type without any of them saying so**: the **excitatory glutamatergic pyramidal neuron of neocortex and hippocampus, at its dendritic spines**.

The spine-density experiments are performed on layer 5 pyramidal neurons, and the receptor and its complement ligand colocalise at *excitatory* synapses of human cerebral cortex (Brott et al., 2025). The phospho-cofilin measurement is made in a post-synaptic-density-enriched synaptosome

fraction, and its functional readout is the insertion of the AMPA-receptor subunit GluA1 — a glutamatergic synapse by definition (Rush et al., 2018). The plasticity deficits are hippocampal long-term potentiation and visual-cortical ocular dominance (Kim et al., 2013). And the reelin arm terminates on the same cell: Disabled-1, the adaptor through which the entire reelin signal is transduced, is expressed **predominantly in pyramidal neurons** (Pesold et al., 1999).

This convergence on one cell is not a premise of the argument. It is a result of reading the four preparations side by side, and it is worth stating because it is what makes them commensurable at all.

It also fixes what this paper is *not* about. The parvalbumin-positive fast-spiking interneuron — the cell most often nominated as the cortex's vulnerable inhibitory element — is characteristically aspiny or sparsely spiny, and receives its excitatory input on dendritic shafts and soma rather than on spines. The mechanics described here do not transfer to it directly, and no claim is made that they do. Section IX.1 sets out how that cell nevertheless bears on the argument.

III. Four laboratories writing to serine 3

What follows sets out each finding in its own terms, with the direction of the change stated explicitly. The gradings in the right-hand column of Section XI's ledger use a five-point maturity scale: **M1** human, population-scale or replicated across independent cohorts; **M2** human, single cohort or tissue series; **M3** animal, replicated; **M4** animal, single laboratory; **M5** in vitro or inference from adjacent findings.

3.1 Reelin adds the phosphate, and this stabilises

Chai and colleagues showed that reelin signalling leads to serine-3 phosphorylation of n-cofilin, that phosphorylation at serine 3 renders n-cofilin unable to depolymerise filamentous actin and thereby stabilises the cytoskeleton, and that the chain runs through the lipoprotein receptor ApoER2, the adaptor Disabled-1, Src-family kinases and PI3-kinase. Phosphorylation was localised to the leading processes of migrating neurons as they approached the reelin-containing marginal zone, and immunostaining for phospho-cofilin in dissociated reeler neurons rose significantly after incubation in reelin-containing medium (Chai et al., 2009).

The physiological reading offered by the authors is important and is retained here: reelin-induced stabilisation *anchors* the leading process. It is a stop signal delivered at a place, and its value lies in being local and transient.

A structural fact about this arm must be recorded, because it is routinely lost when reelin is discussed as a protective system. **The cell that makes reelin is not the cell that responds to it.** In adult cortex reelin is expressed primarily in GABAergic neurons and secreted extrasynaptically into the perineuronal matrix, but only by a defined subset: interneurons expressing neuropeptide Y or somatostatin are reelin-positive, a small number of calbindin cells are, and **none of the parvalbumin-expressing cells are** — basket and chandelier cells are "often immunopositive to parvalbumin, but never to reelin." Disabled-1, the adaptor through which the signal is read, is expressed **predominantly in pyramidal neurons** (Pesold et al., 1999).

So the reelin signal is written by one interneuron class into a shared extracellular compartment and read by a different, excitatory cell. The residue in dispute is on the reader.

Direction: phosphorylation up. Consequence: stabilisation. Context: developmental, mouse and culture. Maturity M4.

3.2 Amyloid removes the phosphate, through an immune receptor

Kim and colleagues reported that murine PirB and its human orthologue LilrB2, present in human brain, are receptors for soluble amyloid-β oligomers with nanomolar affinity; that the first two extracellular immunoglobulin domains mediate the interaction; and that engagement leads to **enhanced cofilin signalling, also seen in human Alzheimer brains**. In mice the deleterious effect of amyloid oligomers on hippocampal long-term potentiation required PirB, and in a transgenic model PirB contributed to adult memory deficits and mediated loss of synaptic plasticity in juvenile visual cortex (Kim et al., 2013).

"Enhanced cofilin signalling" means more active cofilin, which means **less** phosphate at serine 3.

The same laboratory has since shown that C4d, a complement cleavage product of previously unknown function, binds LilrB2 and PirB with nanomolar affinity; that C4d and LilrB2 colocalise at excitatory synapses in human cerebral cortex and with amyloid-β in Alzheimer's disease; that both C4 and C4d increase with age and more so in Alzheimer's; and that infusing C4d into wild-type mouse cortex significantly reduces dendritic spine density, with the loss **completely prevented** by knockout of PirB (Brott et al., 2025).

Direction: phosphorylation down. Consequence: destabilisation. Context: mouse, with human tissue corroboration. Maturity M3 for the mechanism, M2 for the human colocalisation and elevation.

3.3 An independent laboratory reproduces the dephosphorylation

Kawaguchi and colleagues, working on an unrelated problem — an endogenous antagonist of the Nogo receptor and PirB called LOTUS — showed that LOTUS inhibits amyloid-β binding to PirB; that in cultured hippocampal neurons from LOTUS-overexpressing transgenic mice **amyloid-induced dephosphorylation of cofilin** and amyloid-induced loss of PSD-95 were both suppressed; that the amyloid-induced fall in dendritic spine density was improved; and that human LOTUS inhibits amyloid binding to human LilrB2 in the same way (Kawaguchi et al., 2022).

This matters as corroboration of the *direction*, not as therapy. A different laboratory, a different country, a different tool — a natural competitive antagonist rather than a knockout — reproduced the specific chain: block the ligand at the receptor and the **dephosphorylation** does not occur.

Direction: phosphorylation down. Consequence: destabilisation. Independent of the originating group. Maturity M4.

3.4 Amyloid adds the phosphate, in human Alzheimer cortex

Rush and colleagues, working in Grenoble with no shared authorship with any of the above, reported **elevated** phospho-cofilin-1 in the post-synaptic-enriched fraction of synaptosomes from cortical samples of APP/PS1 mice **and of human Alzheimer cases**. In primary cortical neurons, amyloid-β oligomers induced rapid actin **stabilisation** and **increased** phospho-cofilin within thirty minutes. Fluorescence recovery after photobleaching and calcium imaging in neurons expressing active or inactive cofilin mutants indicated that cofilin phosphorylation is **necessary and sufficient** for amyloid-induced synaptic impairment via actin stabilisation, occurring *before* the formation of cofilin-actin rods. The clinically available Rho-kinase inhibitor fasudil prevented the actin stabilisation, the synaptic impairment and the synaptic loss by blocking cofilin phosphorylation; amyloid also blocked the potentiation-induced insertion of the AMPA-receptor subunit GluA1 in a fasudil-sensitive manner (Rush et al., 2018).

The paper opens by stating that how amyloid-β affects the actin cytoskeleton "remains unknown and **contentious**," and notes that **others have described increased cofilin phosphorylation in Alzheimer patients**. It does not cite or address the opposite finding from the receptor literature.

Direction: phosphorylation up. Consequence: stabilisation. Context: human Alzheimer cortex plus mouse and culture. Maturity M2 for the human measurement.

3.5 And the rods require the phosphate to be absent

Minamide and colleagues showed that mediators of neurodegeneration induce rod-like inclusions of cofilin and actin in axons and dendrites, that rods form spontaneously in neurons overexpressing **active** cofilin — indicating that activation by dephosphorylation is *sufficient* to induce them — and that persistent rods disrupt microtubules and degenerate the distal neurite without killing the neuron (Minamide et al., 2000).

Bamburg and colleagues have since established the biochemistry: cofilin binds cooperatively along ADP-actin subunits, and — the fact on which this paper turns — **severs filaments at low cofilin-to-actin ratios and stabilises them at high ratios**. Rod formation requires oxidation of cofilin to disulfide-linked dimers, and rods sequester cofilin, block transport and exacerbate mitochondrial membrane-potential loss. In roughly 20 per cent of hippocampal neurons rods form by a slower receptor-mediated route requiring cellular prion protein, NADPH oxidase and G-protein-coupled receptors (Bamburg and Bernstein, 2016; Bamburg et al., 2021).

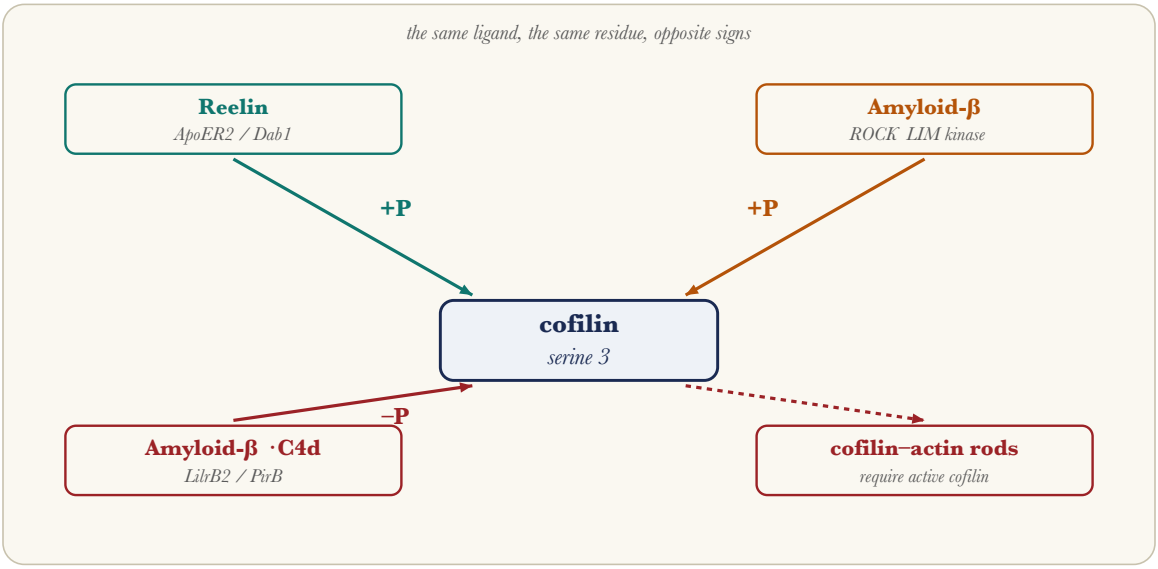
Direction: requires phosphorylation down. Consequence: initially severing, then — at high occupancy — bundling and blockade. Maturity M2 for rods in human Alzheimer brain, M4 for the induction mechanism.

IV. The contradiction, stated plainly

	writes to Ser3	direction	actin result	human data?	source
Reelin → ApoER2/Dab1	yes	**phosphorylate**	stabilise	no	Chai 2009
Aβ → LilrB2/PirB	yes	**dephosphorylate**	destabilise	yes, AD brain	Kim 2013
Aβ → PirB (antagonist test)	yes	**dephosphorylate**	destabilise	human LilrB2 binding	Kawaguchi 2022
Aβ → ROCK → LIMK	yes	**phosphorylate**	stabilise	yes, AD cortex	Rush 2018
Cofilin–actin rods	requires low pSer3	**dephosphorylate**	bundle, block transport	yes, AD brain	Minamide 2000

Rows two and four are the same ligand, the same residue, the same disease, and opposite signs, each supported by measurement in human Alzheimer tissue.

Figure 1 · One residue, four writers — and two of them disagree in sign



Reelin through ApoER2 phosphorylates serine 3 and stabilises the actin network. Amyloid-β through Rho-kinase and LIM kinase also phosphorylates it. Amyloid-β through LilrB2 — and the complement fragment C4d through the same receptor — dephosphorylates it. Cofilin–actin rods, present in Alzheimer brain and not in normal brain, require the phosphate to be absent. Amyloid-β appears twice, on the left and on the right, writing to the same residue in the same disease with opposite signs — and both directions are reported from human tissue.

There are only four ways this can be resolved, and it is worth being explicit about them because three are commonly assumed and none of the three is satisfactory.

One of the measurements is wrong. Possible, and not demonstrated. Both groups used appropriate methods; the Grenoble measurement is a fractionated synaptosome preparation from human cortex, the Stanford observation is of cofilin signalling in human Alzheimer brain. Neither has been retracted or failed replication, and the dephosphorylation direction has independent support from a third laboratory.

They are measuring different compartments. Almost certainly true in part — a post-synaptic-density-enriched synaptosome fraction is not the same object as whole neurites or bulk cortex. But this is a description of the problem rather than a resolution: it says the answer depends on where you look, which is the thing that needs explaining.

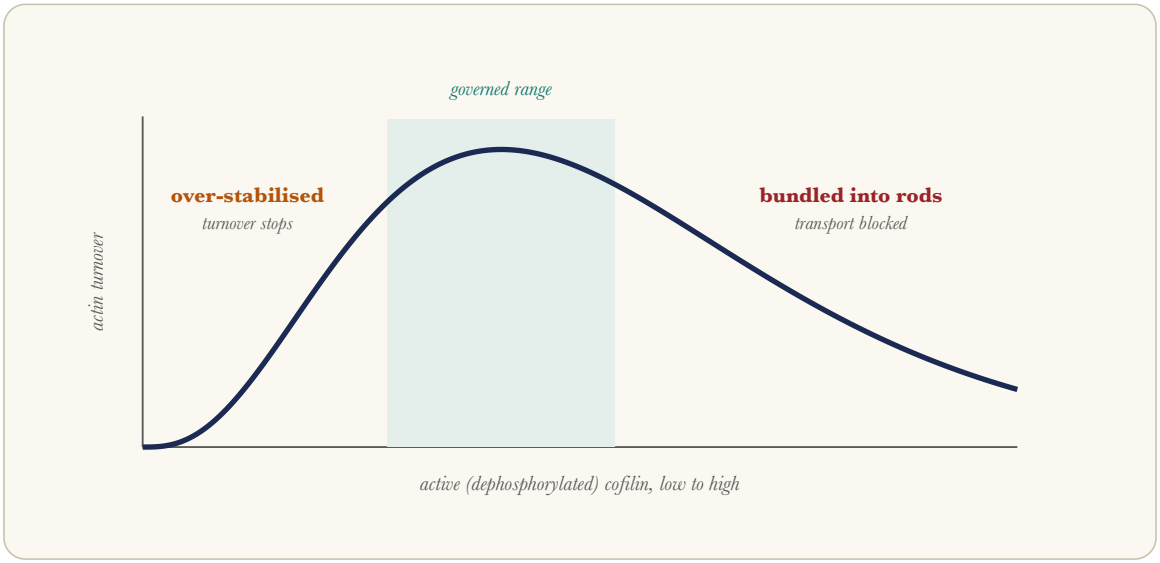
They are measuring different disease stages. Rush and colleagues explicitly place their phosphorylation event *before* rod formation, which requires dephosphorylation. A temporal sequence — phosphorylation early, dephosphorylation later — is the most attractive of the conventional resolutions and may well be part of the answer. It does not by itself explain why a bulk measurement in end-stage human cortex should come out on the phosphorylated side, when the rods are present by then.

Or the residue has no pathological direction. This is the possibility the field has not taken up, and it follows directly from a fact already established in the same literature.

V. The reconciliation: a non-monotonic effector under lost governance

The fact is Bamburg's: cofilin **severs filaments at low cofilin-to-actin ratios and stabilises them at high ratios** (Bamburg and Bernstein, 2016). The relationship between active cofilin and actin stability is not a line. It is a curve that turns over.

Figure 2 · The effector is non-monotonic: it severs, then it bundles



Cofilin severs filaments at low occupancy and stabilises them at high occupancy, so actin turnover rises to a peak and then collapses. A residue whose effector inverts across its own range has no pathological direction. Both departures from the governed band are pathological, by different routes: too much phosphate and turnover stops, too little and the protein bundles actin into rods that block transport. There is no safe direction of travel from the healthy state.

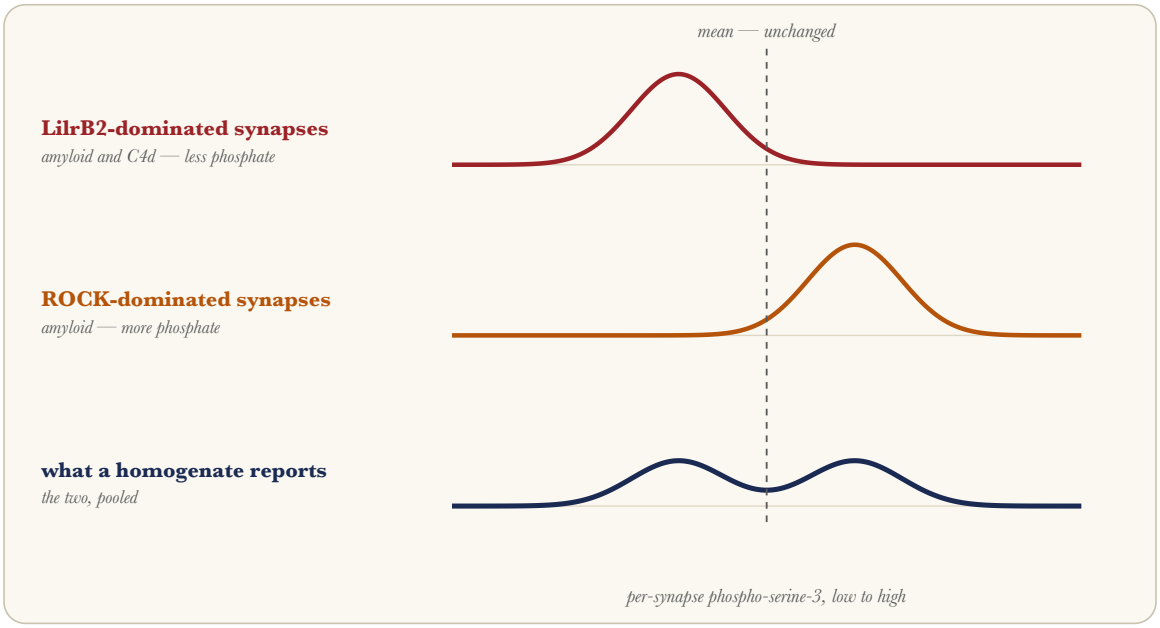
Three consequences follow, and together they dissolve the contradiction.

First, both directions of change are pathological, by different routes. Drive serine-3 phosphorylation up and cofilin is withdrawn from the filament: the network over-stabilises, turnover stops, and the structural plasticity a spine needs in order to potentiate is lost — which is precisely the phenotype Rush and colleagues measured, including the failure of GluA1 to insert after potentiation. Drive phosphorylation down and active cofilin rises: filaments sever, and then, as occupancy climbs past the turnover point and oxidation cross-links the protein, the same molecule bundles actin into rods that block transport and strangle the distal neurite — which is precisely the

phenotype Minamide, Bamburg and colleagues described. **There is no direction of travel from the healthy state that is safe.**

Second, a bulk measurement of a non-monotonic system is uninterpretable. Suppose two populations of synapses in the same cortex: one dominated by LilrB2 engagement, where amyloid and C4d drive dephosphorylation, and one dominated by Rho-kinase tone, where amyloid drives phosphorylation. A homogenate reports the mean. The mean will move in whichever direction the more abundant population dictates, which depends on the region sampled, the fraction prepared, the stage of disease and the case series. Two careful laboratories sampling differently will obtain opposite signs, publish both, and be unable to reconcile them — because the quantity they are comparing is not the quantity that matters.

Figure 3 · Why a bulk measurement cannot see the lesion



If synapses dominated by LilrB2 engagement are displaced one way and synapses dominated by Rho-kinase tone the other, a homogenate returns a mean that has barely moved. Two careful laboratories sampling different fractions will obtain opposite signs, publish both, and be unable to reconcile them — because the quantity they are comparing is not the quantity that matters.

Third, and this is the claim: the lesion is the loss of range, not the displacement of the mean. In the healthy spine, serine 3 is driven in both directions on demand, transiently and locally — that is what the reelin result actually describes, a stop signal delivered at a place and then released. Potentiation requires a brief local excursion into severing and a return. The pathological state is one in which the residue can no longer be driven both ways: it is pinned, in different directions at different synapses, by chronic ligand occupancy that no longer encodes anything. **The disease is not that cofilin is too active or too inactive. It is that cofilin has stopped**

being a signal and become a setting.

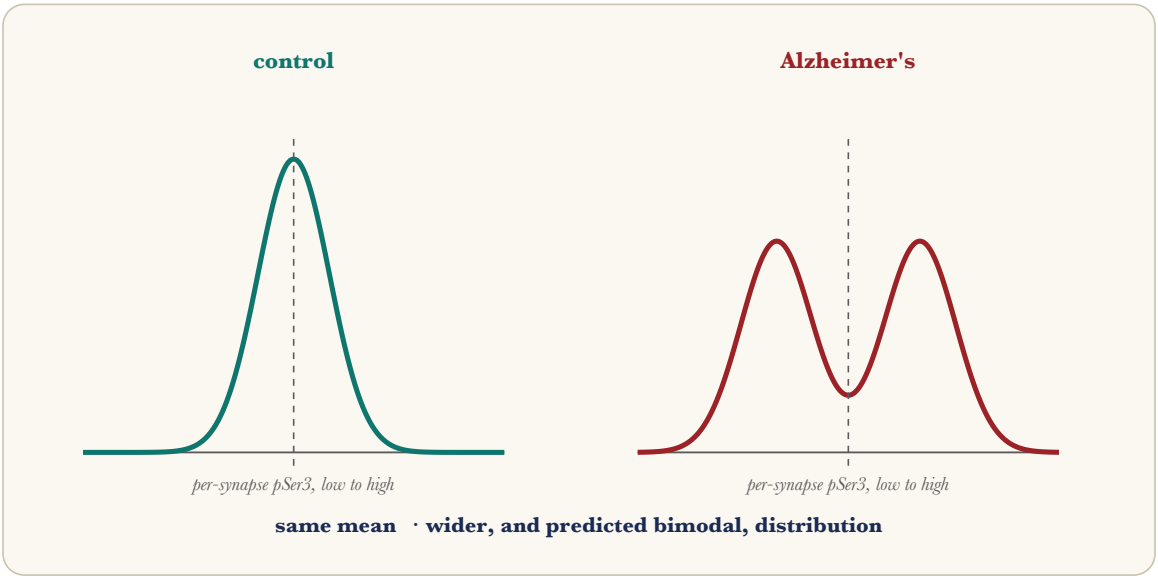
This reading has the property that a good reconciliation should have: it makes both disputing groups right about their own preparations, and both wrong about the disease.

VI. The prediction that distinguishes it

If the lesion were a displacement of the mean, the mean would move and the disagreement would be an artefact of technique. If the lesion is a loss of governance, then the mean may be normal while the **distribution across synapses is wider**.

The prediction: in Alzheimer cortex, the synapse-to-synapse variance of phospho-serine-3 cofilin is elevated relative to age-matched control, including in regions and stages where the mean is unchanged.

Figure 4 · The prediction: the variance moves where the mean does not



The claim is a loss of governed range, not a displacement of the mean — so per-synapse phospho-serine-3 should be more widely distributed in Alzheimer cortex than in age-matched control, including where the mean is unchanged, and should separate into a LilrB2-associated and a Rho-kinase-associated mode. None of the four accounts in Figure 1 predicts this; each predicts a mean shift and is silent about dispersion. Array tomography resolves single human excitatory synapses and has already been applied to these synapses, so the experiment is a re-analysis rather than a new programme.

This is a strong prediction in the useful sense. It is not implied by any of the four accounts above; each of them predicts a mean shift and is silent about variance. It is quantitative. It fails cleanly: if the variance is unchanged, or if it moves only where the mean moves, the proposal is wrong and one of the conventional resolutions in Section IV is right.

And it is measurable now, in accessible human tissue, by a method that has already been applied to these exact synapses. Array tomography resolves individual excitatory synapses in human

cortex and quantifies protein content synapse by synapse; it was used to establish the colocalisation of C4d and LilrB2 at human excitatory synapses in the 2025 study, whose author list includes the method's principal developer (Brott et al., 2025). The experiment is a re-analysis of the kind of material that laboratory already holds, with an antibody the field already uses.

Two secondary predictions sharpen it:

The variance should be bimodal, not merely broad, with a LilrB2-associated dephosphorylated mode and a Rho-kinase-associated phosphorylated mode. Co-staining for LilrB2 assigns each synapse to a mode; if the two modes do not separate on receptor content, the compartment explanation in Section V fails even if the variance result holds.

The variance should scale with local amyloid proximity rather than with global burden — the rods were reported to be most prominent in neurites contacting amyloid deposits (Minamide et al., 2000), and this reading predicts the same geometry for the dispersion.

And the dispersion should be lower on pyramidal neurons that still carry a perineuronal net. A minority of excitatory neurons bear the condensed matrix normally associated with parvalbumin interneurons, and in human frontal cortex those neurons are conspicuous: *"excitatory neurons bearing a PNN show low amounts of ptau"* (de Vries et al., 2024). The net restricts the lateral mobility of surface components and limits access to the neuronal membrane, which on this reading should buffer exactly the chronic, ungoverned ligand occupancy that pins the residue. **Net-bearing pyramidal neurons are therefore predicted to retain their dynamic range while their netless neighbours lose it.**

This costs one antibody. *Wisteria floribunda* agglutinin is the marker used in the human resilience work above, and adding it as a channel to the experiment in Section VII.1 tests the prediction in the same tissue and the same imaging run. If net-bearing excitatory neurons show the *same* dispersion as netless ones, the matrix plays no protective role at this residue and that arm should be dropped.

VII. The discriminating experiments

Five experiments, ranked by what they settle per unit of effort. Each is stated so that a specific result would count against the proposal.

1. Per-synapse dispersion in human cortex. Array tomography of Alzheimer and age-matched control cortex, staining phospho-serine-3 cofilin, total cofilin, a synaptic marker, LilrB2, and *Wisteria floribunda* agglutinin to identify net-bearing neurons. Report the *distribution*, not the mean, and report it separately for net-bearing and netless pyramidal neurons. **Refutes if** variance is unchanged where the mean is unchanged.

2. Co-manipulation at one residue in one system. Manipulate ApoER2/Dab1 signalling and LilrB2 signalling in the same neurons and measure serine-3 phosphorylation. The two pathways have never been run in the same preparation. **Refutes the convergence** if they do not interact at the residue, in which case the antagonism proposed here and elsewhere is coincidental and should be dropped.

3. The fasudil stratification. Rush and colleagues showed fasudil rescues the phosphorylation arm. This reading predicts fasudil will *worsen* synapses already on the dephosphorylated side. Test in tissue or animals stratified by LilrB2 engagement before any further clinical development of the class. **Refutes if** Rho-kinase inhibition is beneficial across the range.

4. Dynamic range rather than level. Measure the *excursion* of serine-3 phosphorylation in response to a plasticity-inducing stimulus in Alzheimer versus control tissue or model. The claim is that the excursion is reduced even where baseline is normal. **Refutes if** excursion is preserved.

5. Reelin as governor, not as agonist. The RELN-COLBOS allele is a gain-of-function variant with stronger Dab1 activation, carried by a man who remained cognitively intact to 67 despite a PSEN1-E280A mutation and a very high amyloid burden (Lopera et al., 2023). This reading predicts its benefit derives from restoring *responsiveness* at serine 3, not from raising phosphorylation tonically. **Refutes if** the knockin animal shows a tonic elevation of phospho-cofilin with no improvement in dynamic range.

6. Separate the two sugars. Reelin's staging requirement and the perineuronal net are routinely discussed as one matrix, and they are not the same polymer: the net is a **chondroitin**-sulfate structure, while reelin requires **N-sulfated heparan** sulfate to dimerise ApoER2 (Pan et al., 2025), and heparan-sulfate proteoglycans are components of the diffuse extracellular matrix surrounding all central nervous tissue rather than of the condensed net (Fawcett

et al., 2022). The two are therefore dissociable by enzyme. Treat with chondroitinase, which removes the net and leaves heparan sulfate, and separately with heparinase, which does the reverse; measure reelin-induced serine-3 phosphorylation after each. **Predicts** that reelin signalling survives chondroitinase and fails under heparinase — that is, that reelin does not require the perineuronal net at all. **Refutes the matrix arm** if chondroitinase abolishes the signal, in which case the net is doing something the sugar chemistry does not predict.

VIII. What this forbids

A claim that forbids nothing is not worth grading. This one forbids four things, and two of them are being done now.

It forbids bulk phospho-cofilin as an endpoint. Any trial or biomarker programme that reports mean phospho-serine-3 cofilin from homogenate is measuring a quantity this account predicts is uninformative and potentially sign-inverted relative to the synapses that matter. This is a live practice.

It forbids monotonic Rho-kinase therapy as a population treatment. If the residue is pinned in different directions at different synapses, a drug that pushes it one way will help one stratum and harm another. The expected clinical signature is a failed trial with a real positive subgroup — the pattern that has repeatedly been misread in this field as a dosing or staging problem. Rho-kinase inhibitors are in active development for this indication (Zheng et al., 2025).

It forbids the inference from "cofilin is dysregulated" to "inhibit cofilin." The most common therapeutic move at a dysregulated node is to block it. At a non-monotonic effector, blockade moves every synapse in one direction along a curve that turns over, and a subset will cross the turnover point.

And it forbids the assumption that the two literatures can be merged by staging alone. A temporal sequence would predict that variance and mean move together as disease advances. This account predicts they dissociate. That is a discriminable difference, and Section VII item 1 discriminates it.

IX. Scope: what portion of the disease this claims

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

What is claimed. This account applies to the *execution* of synaptic loss in Alzheimer's disease — the mechanical step by which an upstream cause becomes a lost spine. Synapse loss is the strongest structural correlate of cognitive impairment in the disease, with neocortical synapse density reaching a multivariate correlation of 0.96 against the Dementia Rating Scale, and plaque density contributing 26 per cent of that model's strength (Terry et al., 1991). To the extent that dementia in Alzheimer's disease is caused by synapse loss, this account claims the final common step.

What is not claimed, and these are large.

Initiation. Nothing here says what starts the disease. The account is compatible with amyloid-first, tau-first, vascular-first and inflammatory-first origins, which is a weakness as well as a strength: a claim compatible with every upstream story constrains none of them.

Selective vulnerability. It does not explain why particular neurons and particular circuits fail first. Cofilin is ubiquitous; the disease is not.

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

Dementia not attributable to Alzheimer pathology. A substantial minority of dementia in community-based series is not explained by the pathologies conventionally measured, and a substantial minority of people meeting neuropathological criteria for Alzheimer's disease are not demented at death. This account speaks to neither group. It is a theory of a mechanism, not a theory of a population.

The honest summary of scope: this is a claim about the last step of one route, that route being the one that most of the field's structural evidence points at, with no claim over who enters the route or why. A reader who wants a theory of the disease's cause will not find one here, and should not be told they have.

9.1 The inhibitory neuron, and why it is not forgotten

The most frequent objection to a spine-centred account of this disease is that the cortex's most conspicuously vulnerable inhibitory cell — the parvalbumin-positive fast-spiking interneuron — is aspiny, and that an argument about spine actin therefore misses it. The objection is correct as far as it goes, and it is accepted here rather than argued around. The mechanics set out above are the mechanics of a spine, and the parvalbumin cell does not have many.

That cell enters this account in one way only, and it is worth being exact about it because the alternative is a false unification. **The perineuronal net is a modifier of ligand exposure upstream of the residue, not the site of the lesion.** Its degradation removes the protection of the interneuron it sheathes; the resulting failure of inhibition raises the activity of the pyramidal population; heightened pyramidal activity increases the local production of the very ligands — amyloid-β, and with age and disease the complement fragment C4d — that occupy LilrB2 on pyramidal dendrites (Brott et al., 2025). The matrix is therefore upstream of the ligand, and the ligand is upstream of the residue.

Two things follow, and they are of opposite sign, which is why the relationship must be stated rather than assumed. The net is *indirectly* relevant to nearly every pyramidal neuron, through disinhibition. And it is *directly* relevant only to the minority of excitatory neurons that carry a net of their own — the neurons that in human frontal cortex carry conspicuously little phospho-tau (de Vries et al., 2024), and the only population in which spines, a net, and reelin-responsiveness are properties of a single cell. Section VI predicts that this minority is also the population that retains control of serine 3, and Section VII tests it.

No claim is made that the parvalbumin interneuron fails by the mechanism described in this paper. It probably does not.

X. What would refute this

Written before the argument was assembled, and unchanged by it.

1. **Per-synapse variance of phospho-serine-3 cofilin is not elevated in Alzheimer cortex** where the mean is unchanged. This is the primary falsifier and it is cheap.
2. **The two receptor systems do not interact at serine 3** when co-manipulated in one preparation. The convergence is then coincidental.
3. **Rho-kinase inhibition proves beneficial across the range of synaptic states**, including those with reduced phospho-cofilin. The non-monotonic argument then has no therapeutic consequence even if the biochemistry holds.
4. **The disputing measurements are shown to differ by stage alone**, with variance tracking the mean throughout. The conventional resolution then suffices and this proposal is unnecessary.
5. **Cofilin's turnover from severing to bundling is shown not to occur at concentrations reachable in a spine.** The reconciliation then rests on an in-vitro property with no cellular reality.

Any one of 1, 2 or 5 removes the paper's central proposal. Item 4 removes its necessity.

XI. Ledger of claims

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

claim	source	grade	note
Synapse loss is the strongest structural correlate of cognitive impairment	Terry et al., 1991	**M2**	$r = 0.96$ multivariate; plaques 26% of model strength; n = 15 + 9
Cofilin is switched at serine 3; phosphorylation prevents F-actin binding	Chai et al., 2009; Bamburg et al., 2021	**M5**	Established biochemistry
Reelin/ApoER2/Dab1 phosphorylates serine 3 and stabilises	Chai et al., 2009	**M4**	Developmental context; not shown in adult disease
LilrB2/PirB are Aβ-oligomer receptors at nanomolar affinity	Kim et al., 2013	**M3**	Human LilrB2 present in human brain
Aβ→LilrB2 enhances cofilin signalling (dephosphorylation), seen in human AD brain	Kim et al., 2013	**M2**	Human observation is of cofilin signalling, not a quantified per-synapse ratio
The dephosphorylation direction reproduces in an independent laboratory	Kawaguchi et al., 2022	**M4**	Antagonist rather than knockout; different tool, same direction
C4d binds LilrB2/PirB, is elevated in AD, and is sufficient to strip spines	Brott et al., 2025	**M2 / M3**	Human colocalisation and elevation M2; sufficiency in mouse M3, abolished in PirB-null
Aβ→ROCK increases phospho-cofilin in human AD cortex; necessary and sufficient for impairment	Rush et al., 2018	**M2**	Post-synaptic-enriched synaptosome fraction; opposite direction to the two rows above
Cofilin–actin rods are present in human AD brain and not normal brain	Minamide et al., 2000; Bamburg and Bernstein, 2016	**M2**	Most prominent in neurites contacting amyloid deposits

claim	source	grade	note
Rod formation requires active (dephosphorylated) cofilin	Minamide et al., 2000	**M4**	Overexpression of active cofilin is sufficient
Cofilin severs at low cofilin:actin ratio and stabilises at high ratio	Bamburg and Bernstein, 2016	**M5**	The non-monotonicity on which Section V turns
ROCK1/2 are elevated in AD and the inhibitor class is in development	Zheng et al., 2025	**M2 / review**	The review itself states "activation or inhibition" alters synaptic structure
A RELN gain-of-function allele confers resistance to autosomal-dominant AD	Lopera et al., 2023	**M2**	n = 1 index case; stronger Dab1 activation; reduced tau phosphorylation in knockin
Reelin is secreted by NPY/somatostatin interneurons and never by parvalbumin cells; Dab1 is predominantly pyramidal	Pesold et al., 1999	**M4**	Rat cortex; the source, the net-bearer and the responder are three different cells
Excitatory neurons bearing a perineuronal net carry low phospho-tau in human frontal cortex	de Vries et al., 2024	**M2**	Also: aggrecan falls in both AD and resilient; WFA sugars fall only in the resilient
N-sulfated heparan sulfate is an obligate co-receptor for reelin-induced ApoER2 dimerisation	Pan et al., 2025	**M5**	K_D 17 nM, COLBOS 10 nM; heparinase, NDST1-KO and free heparin all block; N-desulfated heparin does not
Heparan-sulfate proteoglycans are diffuse-ECM components; the perineuronal net is condensed chondroitin sulfate	Fawcett et al., 2022	**Review**	The basis for the claim that reelin does not require a net
All four literatures converge on the pyramidal dendritic spine without saying so	This paper	**Observation**	Verifiable from the four preparations; §2.1
The four literatures disagree in sign and have not addressed each other	This paper	**Observation**	Verifiable directly from the sources tabulated in §IV
Net-bearing pyramidal neurons retain dynamic range at serine 3	This paper	**Prediction**	One extra WFA channel in the experiment of §VII.1

claim	source	grade	note
The lesion is loss of range at serine 3, not displacement of the mean	This paper	**Inference (M5)**	The paper's original claim. Untested. §VI states its falsifier
Per-synapse variance of pSer3 is elevated in AD where the mean is not	This paper	**Prediction**	Never measured; method exists and has been applied to these synapses

Two entries in this table are the paper's own and neither has been tested. That is stated here rather than in a discussion section, and the two are the first things a reader should attack.

XII. What follows for treatment

The therapeutic reading of a non-monotonic node is unusual and worth stating carefully, because the obvious move is the wrong one.

If a node is dysregulated, the reflex is to block it. Here, blockade pushes every synapse in one direction along a curve that inverts, so it must convert some fraction of synapses from one failure mode into the other. That is not a theoretical worry: it is the most economical explanation available for a class of intervention that produces convincing rescue in one preparation and no population benefit.

The target implied by this reading is not the kinase and not the phosphatase. It is the **restoration of signal-driven control** — the capacity of the residue to be moved transiently and locally and then released. Two things follow.

The physiological system that does this is reelin through ApoER2. It is the only characterised pathway that delivers serine-3 phosphorylation as a *placed, transient signal* rather than as tone (Chai et al., 2009). It has a human genetic anchor of unusual quality: a gain-of-function RELN variant carried by a man who resisted an autosomal-dominant Alzheimer mutation into his late sixties, with a very high amyloid burden and limited entorhinal tangle burden, and with the variant shown to activate Dab1 more strongly and to reduce tau phosphorylation in a knockin mouse (Lopera et al., 2023). Whether that protection works through the cofilin residue is not known and is a stated experiment above.

But the route by which reelin is delivered is a sulfated sugar, and that creates a drug conflict nobody has named. Reelin cannot activate its receptor as a simple two-body ligand. It requires N-sulfated heparan sulfate as a co-receptor: full-length reelin binds heparan sulfate at 17 ± 5 nM — tightened to 10 ± 2 nM by the resilience-associated COLBOS variant — N-sulfation is the critical glycan determinant, and heparinase treatment or knockout of the N-sulfation enzyme NDST1 strips reelin from the cell surface. Decisively, **heparinase or free heparin in the medium reduces reelin-induced ApoER2 dimerisation, while N-desulfated heparin does not** (Pan et al., 2025).

Heparan-sulfate proteoglycans are also the route by which pathological tau enters neurons, which has made heparin mimetics an attractive strategy for blocking transcellular tau propagation. The two facts collide: **a heparin mimetic given to stop tau spreading would, by the same chemistry, silence the reelin signal that restrains it.** That is a specific, verified, drug-level contradiction, and it argues that the sulfated matrix must be modulated rather than blocked.

It also disposes of a common conflation. The perineuronal net is built from **chondroitin** sulfate; reelin's requirement is for **heparan** sulfate, a component of the diffuse matrix that surrounds all central nervous tissue rather than of the condensed net (Fawcett et al., 2022). **Reelin therefore does not require a perineuronal net in order to signal**, and the alternatives to the net for staging it are the ordinary heparan-sulfate proteoglycans of the neuronal surface — glypicans, syndecans, agrin — including those on the responding pyramidal dendrite itself, which needs no external depot at all. Section VII.6 separates the two experimentally with a pair of enzymes.

And the endpoint must change before the drug does. No programme at this node should proceed on a bulk phospho-cofilin readout. The per-synapse distribution is the quantity of interest, the method to measure it exists, and until it is measured the field is choosing the direction of a therapy by which laboratory it happens to believe.

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All references were verified against PubMed at the time of writing: title, authorship, year, journal and the direction of the reported effect were each checked against the source record rather than recalled. The directional disagreement reported in Section IV is the reason that check was necessary.