# The Hand and the Address

*LilrB2/PirB, Complement C4d, Ephexin5, Kalirin-7, Tiam1 and Reelin — Two Accounts of How the Alzheimer Synapse Is Lost, the Single Unmeasured Limb That Joins Them, and the Opposite Signs They Predict There*

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*Neither account of the failing synapse contains a broken molecule. One describes a restraint pressed by the wrong hand; the other, an apparatus competent but at the wrong address. Where they are joined, they predict opposite signs at the same two residues — and nobody has measured them.*

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# Abstract

Synapse loss remains the strongest structural correlate of cognitive impairment in Alzheimer's disease, and two bodies of work now offer mechanistic accounts of it that are specific enough to be wrong. The first is a receptor account. A single neuronal receptor — LilrB2 in the human, PirB in the mouse — restrains plasticity and eliminates synapses throughout life; in disease it is engaged by soluble amyloid-β oligomers and by C4d, the durable covalently tethered terminal fragment of complement component 4, and the neuron disassembles its own dendritic spine. The second is an exchange-factor account. Amyloid strips the receptor tyrosine kinase EphB2 from the postsynaptic membrane; the Rho-family exchange factor Ephexin5 accumulates because EphB2 no longer licenses its destruction; Rac1 loading fails as Kalirin-7 leaves the spine and Tiam1 is cleaved; the branched actin network is not rebuilt; and the spine retracts. A third proposition sits across both: that in this pathway the disease-relevant change is one of location and covalent state rather than of amount, so that the field's dominant measurement — homogenise, blot, report a mean — is systematically blind to the lesion.

This paper tests the three against one another and against the primary record for every molecule they name. Four results are reported.

**The two accounts are joined by one limb, and that limb has never been pointed at any of its assigned substrates.** The receptor's cytoplasmic tail carries immunoreceptor tyrosine-based inhibitory motifs that recruit the phosphatases SHP-1 and SHP-2; this was reported in the founding paper in a single clause and has one demonstrated neuronal consequence — dephosphorylation of a third-party receptor tyrosine kinase, Trk, in the same membrane, with a structural effect reversed when the phosphatases are removed. An indexed literature search pairing the receptor or its phosphatases with EphB2, with Ephexin5, with Disabled-1 or with the assigned glutamate-receptor substrate returns no study on point, and the glutamate-receptor assignment is superseded by an established alternative enzyme.

**Where the two accounts are joined they predict opposite signs at the same two residues.** The exchange-factor account, extended through the Src-family kinase Fyn, whose postsynaptic mistargeting in disease is tau-dependent, predicts that phospho-occupancy rises at the licensing tyrosines of Ephexin5 and of Tiam1. The receptor account, by its own inhibitory architecture, predicts that postsynaptic tyrosine-phosphatase activity rises and that the same occupancies fall. Both cannot be the net. The collision is not rhetorical: the two readings place the disease on opposite arms of the structural balance and recommend opposite drugs, and the single measurement that separates them — the phosphorylated fraction of Ephexin5 at tyrosine 361 and of Tiam1 at tyrosine 384, reported jointly across single synapses — has not been attempted in disease tissue although the discriminating reagent has existed, validated against a phosphomutant and a genetic null, for sixteen years.

**A functional collision runs alongside the biochemical one.** The restraint receptor is required for activity-dependent spine shrinkage: animals lacking it form more spines, stabilise newly formed spines, and learn a skilled reaching task faster than their littermates. The exchange factor placed downstream of it is required for activity-driven spine growth, and the covalent state that the receptor's phosphatase limb would produce is the state that biases that enzyme towards growth. The route as drawn therefore asks a growth-requiring enzyme, pushed into its growth-biased state, to execute a shrinkage instruction.

**The address claim survives in three of four regulators and fails in the fourth, and the failure is informative.** Two studies from 2007, absent from every account of this pathway examined here, measured Kalirin-7 in nineteen Alzheimer hippocampi against fifteen controls and found it reduced at both transcript and protein, with the cerebellum of the same cases spared and housekeeping transcripts unaffected. That is the human abundance measurement the exchange-factor account needs, it exists, and it divides the Kalirin-7 lesion into two

mechanisms rather than one: acute displacement from the spine, and chronic under-expression. The generalisation that survives is weaker than a slogan and stronger than nothing — abundance has been measured in two of four cases, and in the two where it has not been measured the method could not have seen what moved.

A fifth result is a reconciliation. The literature on the terminal effector, cofilin, reports both directions in human Alzheimer cortex, and the disagreement survives restriction to one cell type and one disease stage. The two accounts jointly supply a resolution that neither states: phosphorylation stabilises filaments and arrests turnover, and dephosphorylation past the physiological range saturates and bundles filaments into cofilin–actin rods that arrest transport. Opposite signs at one residue, one functional endpoint. The quantity that distinguishes a population under one instruction from a population under several is the joint distribution of the marks across single synapses, not any mean.

The conclusion is a claim about the shape of the lesion. Neither account contains a broken molecule. In one, a restraint operates exactly as designed, pressed by ligands that accumulate; in the other, an apparatus remains intact and competent but is no longer where its substrate is. Both are failures of direction rather than of integrity, and that has a therapeutic consequence the field has not drawn: of the four interventions these accounts generate, every one removes a restraint, and none restores an address.

**Keywords:** Alzheimer's disease; dendritic spine; synaptic pruning; LilrB2; PirB; complement C4d; MHC class I; Ephexin5; ARHGEF15; EphB2; Kalirin-7; Tiam1; Rho GTPase; cofilin; SHP-1; SHP-2; Fyn; reelin; Disabled-1; array tomography

*Part I*

# Two Accounts of One Event

*A receptor programme that began by asking why a critical period closes, and an exchange-factor economy inside the spine. Both describe the loss of the same structure. Each is stated here in the terms its proponents use, before either is tested, and the conventions by which both will be graded are set out in advance.*

# 1. Introduction: one synapse, two descriptions

## 1.1 The variable that needs explaining

The dendritic spine is the smallest structure in the brain that can be said to hold a memory, and its disappearance is what tracks the clinic. In the post-mortem series that established the point, measures of synaptic density in frontal and parietal cortex accounted for the majority of the variance in cognitive scores, outperforming plaque counts, tangle counts and neuron counts taken separately [50]. The finding has been reproduced in substance for three decades, and it sets the problem for any mechanistic account of this disease: a theory that explains deposition but not synaptic attrition explains the wrong variable.

Two programmes have supplied candidate mechanisms with unusual specificity, and they are usually read as belonging to separate literatures. One is about an immune receptor on the surface of a neuron. The other is about the enzymes that load small GTPases inside the spine. One is executed over a critical period in the visual cortex of a mouse; the other over seconds, in a single spine head, during the structural plasticity that accompanies potentiation. A paper that put them together merely because both concern synapses would be a paper about nothing in particular.

They belong together for a specific reason. Each is incomplete in exactly the place the other is detailed. The receptor account names a ligand, a receptor, a cell type and a structural outcome, and has almost nothing to say about which enzyme inside the spine does the dismantling. The exchange-factor account names the enzymes in detail and has no upstream — nothing that explains why the apparatus should be disturbed in this disease and not in others, and nothing that supplies a stopping rule. In the published literature the two have begun to be welded together into a single route. This paper is about the weld.

## 1.2 The restraint account, stated as its proponents state it

In 1998 a screen designed to find genes that developing circuits use to refine themselves returned an answer the field did not want. The messenger RNAs whose levels fell when neural activity was blocked encoded class I major histocompatibility complex molecules — the proteins by which the immune system displays a cell's contents for inspection, and which the brain was believed not to express [16]. Mice unable to present class I MHC at the cell surface failed to refine their retinal projections and showed distorted synaptic learning rules, with enhanced potentiation and absent depression [29]. Two of the fifty-odd class I MHC genes accounted for most of the phenotype [18], and one of them is necessary and sufficient for synapse elimination in the retinogeniculate pathway [34].

A receptor for these molecules was then identified on cortical neurons. Paired immunoglobulin-like receptor B is expressed in subsets of neurons throughout the brain, is associated with synapses, forms complexes with the phosphatases SHP-1 and SHP-2, and binds cortical neurons in an MHC-class-I-dependent manner; in mice lacking functional PirB, ocular-dominance plasticity is more robust at every age tested [49]. The receptor restricts the structural substrate as well as the functional one: spine density is held down, and the restraint is cell-autonomous, since sparse deletion in otherwise wild-type cortex raises spine density in the deleted cells alone [20,52]. Acute disruption in an adult, by minipump infusion of a soluble receptor ectodomain, increases

spine density and miniature excitatory postsynaptic current frequency and permits recovery from amblyopia established months earlier [7].

Only with that structure in place did the programme turn to disease. In 2013 it reported that PirB and its human orthologue LilrB2 bind soluble amyloid-β oligomers at nanomolar affinity through the same two immunoglobulin domains that bind MHC class I, that engagement leads to enhanced cofilin signalling seen also in human Alzheimer brain, and that deleting the receptor rescues both long-term potentiation and memory in an amyloid-overexpressing mouse [32]. In 2025 it reported a second ligand for the same domains: C4d, the terminal cleavage fragment of complement component 4, which remains covalently attached to the surface where the cascade fired. C4d binds the receptor at nanomolar affinity, colocalises with LilrB2 at excitatory synapses in human cortex, rises with age and further in Alzheimer's disease, and — infused into the cortex of an adult mouse carrying no transgene — removes dendritic spines, with the loss completely prevented by deletion of the receptor [11].

Stated at its own strength, the account is this. **The machinery that eliminates synapses in development is never dismantled. It is held in reserve, under the control of ligands whose concentrations rise with age; in Alzheimer's disease two such ligands accumulate at the same receptor, and the normal elimination programme runs without a stopping rule.** Nothing in the account breaks. There is no loss of function anywhere in its core. The brake is not faulty; the brake is being pressed by the wrong hand.

### 1.3 The exchange-factor account, stated as its proponents state it

The second account begins inside the spine and is just as clean.

Soluble amyloid-β oligomers bind the fibronectin-repeat domain of the receptor tyrosine kinase EphB2 and trigger its degradation in the proteasome; knockdown of EphB2 reduces N-methyl-D-aspartate-receptor currents and impairs potentiation in the dentate gyrus, and increasing EphB2 expression in an amyloid-precursor-protein transgenic mouse reverses deficits in potentiation and memory [15]. EphB2 is the kinase that phosphorylates the Rho-family exchange factor Ephexin5 on a single tyrosine, Y361, thereby recruiting the ubiquitin ligase Ube3A and consigning the exchange factor to the proteasome [36]. With the licensing kinase gone, Ephexin5 accumulates; amyloid-β also raises it directly in mature hippocampal neurons and in transgenic mice, and it is reported elevated in the hippocampi of human Alzheimer patients [46]. Accumulated Ephexin5 loads RhoA, RhoA signals through Rho-associated kinase to LIM-domain kinase and cofilin, and the spine's actin scaffold fails. On the other side of the balance, amyloid-derived oligomers deplete Kalirin-7 from spines [54] and calcium-activated calpain is said to cleave Tiam1, so that Rac1 cannot be loaded and the branched actin network that holds the spine head open is not rebuilt. Genetic removal of Ephexin5 from the transgenic line eliminates hippocampal spine loss and rescues behaviour, and short-hairpin reduction in presymptomatic animals prevents later impairment [46].

Stated at its own strength: **the spine is maintained by a balance between enzymes that build and enzymes that restrict, and amyloid tips that balance by removing a licensing kinase on one side and the building enzymes on the other.**

### 1.4 The third proposition: address rather than abundance

A third claim runs across both accounts and is the reason this paper exists.

Both are told as arithmetic. Ephexin5 went up; RhoA went up; Kalirin-7 went down; reelin went down. Each is a statement that a quantity moved and that the sign of the disease follows from the sign of the movement. Expressed that way the accounts are easy to teach, easy to draw and easy to test — and when one tests them molecule by molecule against the measurements actually made in human Alzheimer tissue, the arithmetic does not hold cleanly.

The only published survey to measure RhoA, Rac1 and Cdc42 together in human Alzheimer brain reports total RhoA *reduced* in hippocampus and redistributed out of the neuropil into tangle-bearing somata, with no major change in Rac1, Cdc42 or p21-activated kinase [28]. Reelin messenger RNA and protein *rise* in Alzheimer frontal cortex at late Braak stages while reelin-dependent Disabled-1 phosphorylation falls, because the secreted ligand is trapped with amyloid aggregates in the compartment it must cross [17]. Kalirin-7 is depleted from spines by oligomers, which is a change of location, not a demonstration of destruction [54]. And Ephexin5 rises as total protein while the single covalent mark that decides whether it loads the restrictive GTPase or the constructive one has never been measured in disease tissue [46,41].

From those four observations a proposition has been drawn: that the disease-relevant change in this pathway is of address or of covalent state rather than of amount, and that the field's dominant measurement is blind to exactly that class of lesion. This paper states that proposition in full, tests it, and finds that it holds in three of four regulators and fails in the fourth — and that the failure, when it is examined, is more informative than the rule.

### 1.5 What this paper does

Three questions organise it.

**Where are the two accounts joined, and what is the joint made of?** Part III answers this. The joint is a phosphatase limb with one demonstrated neuronal precedent and four unevidenced substrate assignments, and an audit of it is the single most consequential thing in this paper.

**Do the two accounts agree about the sign of the lesion?** They do not. Section 6 shows that where they meet they predict opposite phospho-occupancies at the same two residues and opposite structural outcomes from the same covalent state, and Section 7 shows that at the terminal effector the published human record already reports both directions.

**What kind of lesion do they jointly describe?** Part IV answers this. The answer is that neither contains a broken molecule, that both describe misdirection rather than damage, and that this is a substantive claim with testable consequences rather than a figure of speech.

### 1.6 What this paper does not do

It does not adjudicate the cause of Alzheimer's disease. Both accounts are amyloid-gated or complement-gated: the first requires enough ligand at the receptor, the second enough soluble oligomer to deplete EphB2. Both therefore describe mechanisms of execution in the symptomatic and immediately prodromal period. They execute; they do not initiate, and nothing here bears on what starts the disease.

It does not concern inhibitory synapses, glial engulfment as a separate arm, or the brainstem nuclei in which tau pathology appears earliest. Its subject is the excitatory synapse on a cortical or hippocampal pyramidal neuron.

And it reports no measurement of its own. Every claim below is a reading of published work, and the central positive proposals in Sections 6.5 and 17 are predictions that have never been tested.

# 2. Conventions

### 2.1 Direction is always stated

Where a paper reports that something increased or decreased, the direction is given explicitly rather than implied by a verb such as "altered" or "dysregulated." Most of the contradictions this paper identifies are invisible in prose that does not commit to a sign, and at least one of them — the four-way disagreement at cofilin serine 3 — has survived two decades partly because reviews of it do not commit.

### 2.2 Strength of evidence

Each claim is graded on what kind of thing has been shown, not on how important it is.

| Grade | What it means |
|---|---|
| **Established** | Replicated across independent laboratories, or demonstrated with genetic controls that exclude the obvious alternatives |
| **Supported** | Demonstrated once, with adequate internal controls, by one laboratory |
| **Inference** | Two sound findings joined by a step that has not been measured in the system at issue |
| **Unevidenced** | An indexed literature search returns no study on point; the claim is neither supported nor refuted, it is unaddressed |
| **Contradicted** | The published result on which the step rests reports the opposite direction from the one the claim requires |

A grade of Unevidenced is not a dismissal, and a grade of Established is not a guarantee. The point of the scale is to make visible the moment at which an argument crosses from one kind of claim to another without saying so.

Alongside the grade, each claim is marked for the system in which it was shown: **H** for human tissue or human genetics, **A** for an intact animal, **N** for neurons in culture, **X** for a non-neuronal cell or a reconstitution. A transposition is not an error; an untested transposition reported as a mechanism is.

### 2.3 The three questions asked of every junction

Because both accounts are chains, and because a chain assembled from true links is not thereby true, every junction below is put to three questions.

**Is the link measured?** Not whether the two things it connects have been measured — whether the claim that *in this tissue, at this stage, this quantity changes because that one did* has been measured.

**Is its sign fixed?** A chain can arrive at a conclusion its own stated mechanism forbids, and the contradiction will not be felt by anyone reading forwards, because each step is locally consistent with the one before it. Reading the chain backwards is a cheap and surprisingly productive exercise.

**In which compartment?** A dendritic spine is a fraction of a micron across. A postsynaptic-enriched fraction of cortex is not a cell type, and a cortical homogenate is not a compartment. Where a claim's content depends on locality — and in this pathway most do — the grade records the resolution at which it was shown.

*Part II*

# What Each Account Establishes

*Taken separately and at full strength, before either is tested against the other. The receptor account is graded on its developmental foundation, its two ligands, and the two unanswered negative results it carries — one in each ligand arm. The exchange-factor account is graded on its physiology, its single human anchor, and the survey that measured the three GTPases together.*

## 3. The restraint receptor: what is secure

### 3.1 A brake found by asking a different question

The order of discovery matters here, because the meaning of the disease work depends entirely on what was already known when it arrived.

Between 1998 and 2014 the restraint programme established, in the healthy brain and with no reference to disease, that a set of immune molecules sets how much a cortical circuit is allowed to change. Class I MHC transcripts are present in neurons and are up- and down-regulated by activity [16]. Mice that cannot present class I MHC fail to refine retinal projections and show enhanced potentiation with absent depression [29]. Two of the class I genes carry most of the phenotype [18], and one of them is necessary and sufficient for retinogeniculate synapse elimination [34]. A neuronal receptor for them holds visual-cortical plasticity down at every age [49].

*Grade: Established, tiers A and N, multiple alleles across more than one laboratory.*

The receptor was not discovered by looking for something that amyloid binds. It was discovered by asking why a critical period closes. That gives the disease claim a prior that most candidate amyloid receptors do not have: a molecule already known to subtract synapses on purpose turned out to be engaged by a molecule that accumulates in the disease. Whether or not the amyloid-binding claim survives — and Section 3.5 reports that it is contested — the receptor's day job is not in doubt.

### 3.2 The structural substrate, and cell autonomy

Three results fix the receptor's physiological effect on structure rather than on physiology alone.

Spine density on cortical pyramidal neurons is higher in animals lacking the receptor, and the receptor regulates a structural substrate for cortical plasticity [20]. The effect is cell-autonomous: sparse deletion in an otherwise wild-type cortex raises spine density in the deleted neurons alone, which excludes a circuit-level or non-neuronal explanation [52]. And acute disruption in the adult, by infusion of a soluble ectodomain for one week, increases spine density and the frequency of miniature excitatory postsynaptic currents, unlocks ocular-dominance plasticity, and permits recovery of visual acuity and spine density after long-term monocular deprivation [7].

*Grade: Established, tier A, with a genetic and a pharmacological arm and a cell-autonomy control.*

The third of these is the most consequential for anything therapeutic, because it demonstrates that the restraint is active and reversible in an adult brain. It is also the result that makes the therapeutic reading in Section 13 uncomfortable, for reasons given there.

### 3.3 The receptor is required for activity-dependent spine shrinkage

One result deserves separation from the rest, because it fixes what the receptor *does* mechanically rather than what its absence permits, and because it bears directly on the weld examined in Part III.

Pyramidal neurons in animals lacking the receptor show increased spine formation rates and increased density. Those animals learn a skilled reaching task *faster* than their wild-type littermates, which is an exception to the usual rule that altered spine dynamics impair performance. The stabilisation of learning-induced spines is elevated, and the degree of survival of newly formed spines correlates with performance. Single-spine glutamate uncaging indicates that the receptor is required for N-methyl-D-aspartate-receptor-dependent spine *shrinkage*. Acute inhibition of receptor function in the motor cortex of an adult wild-type animal increases the survival of learning-induced spines and enhances motor learning [1].

*Grade: Established, tier A, genetic plus acute pharmacological, with a single-spine mechanistic arm.*

Read exactly, this says that the receptor is the surface molecule through which a neuron executes the structural half of synaptic depression. It is not a general brake on everything; it is the permission step for selective removal. That is a more specific and more useful claim than "restrains plasticity", and Section 6.4 shows that it is incompatible with the way the receptor has been wired to the exchange-factor economy downstream.

### 3.4 The second ligand, and a pruning arm that needs no phagocyte

The literature on synapse elimination in this disease has, since 2007, been largely a literature about microglia. The classical complement protein C1q is expressed by postnatal neurons and tags synapses for elimination in the developing visual system [48]. In Alzheimer mouse models C1q is increased and associated with synapses before overt deposition, inhibiting C1q, C3 or the microglial complement receptor reduces early synapse loss, and microglia engulf synaptic material in a complement-receptor-dependent manner when exposed to soluble oligomers [27].

That account has a structural feature worth naming: every step of it requires a microglion. The opsonin is a flag; the flag must be read by a phagocyte; the phagocyte does the removing.

The 2025 result adds a second arm with a different topology [11]. C4d — generated by factor I cleavage of surface-deposited C4b, and remaining covalently attached to the surface where the cascade fired — binds LilrB2 at nanomolar affinity in a cell-free measurement, with no detectable binding to the closely related family member LilrB1. Binding maps to the N-terminal immunoglobulin domains: the same module that binds class I MHC. In human cortex, array tomography places C4 or LilrB2 immunoreactivity at the majority of excitatory synapses and explicit C4d signal at roughly a third of them, with colocalisation at inhibitory synapses several-fold lower. C4 and C4d rise with age and rise further in Alzheimer cortex, and C4d–LilrB2 colocalisation is enriched near plaques. And recombinant C4d infused by osmotic minipump into the cortex of an adult wild-type mouse — no transgene, no amyloid, no tau, no inflammation — reduced dendritic spine density on layer 5 pyramidal neurons, while in receptor-null animals spine density after the same infusion was indistinguishable from control.

*Grade: Supported, single laboratory, tiers H and A, with a clean genetic necessity test.*

Read precisely, the result has four properties and all four matter. It establishes **sufficiency**: one complement fragment, applied to an otherwise normal adult brain, removes spines. It establishes **necessity of the receptor**: the effect is not attenuated in the null, it is abolished. It is **adult**, not a developmental-window phenomenon. And it is **local**, confined to roughly the diffusion field of the infusion, which is what one expects of a ligand acting where it lands.

Three consequences follow. The pruning debate acquires a neuron-intrinsic arm that requires no phagocyte, which resolves an evidentiary awkwardness, because colocalisation in fixed human tissue is more tractable than catching a phagocyte in the act. The therapeutic arithmetic changes: agents directed at the glial arm leave the neuronal arm running, and agents directed at the receptor leave the opsonin in place. And complement's role is reframed from a tagging system into a signalling system with a durable covalently tethered ligand and a neuronal receptor — a considerably more consequential position for it to occupy, and one with a precedent in the genetics of a different disorder, where complement component 4 structural variation is the strongest common-variant signal and is read through synaptic pruning [45].

### 3.5 Two unanswered negatives, one in each ligand arm

The receptor layer is the best-evidenced part of either account. It also contains two negative results, each obtained with the physiological rather than the recombinant form of a ligand, and neither has been answered.

**The amyloid arm.** A study designed to remove between-laboratory variability compared reported amyloid-β receptors under standardised conditions — the same assays, the same preparations, cell-surface expression confirmed and controlled. Of fifteen candidates, three bound synaptotoxic assemblies of *synthetic* amyloid: cellular prion protein, Nogo receptor 1, and LilrB2. That is a genuine partial vindication and is rarely reported as one. But binding studies with soluble oligomers extracted from human Alzheimer brain revealed strong affinity for prion protein, weak affinity for Nogo receptor 1, and **no detectable affinity for LilrB2**; and hippocampal neurons lacking both Nogo receptor 1 and LilrB2 showed a smaller reduction in oligomer binding than neurons lacking prion protein alone [47].

*Grade of the amyloid-binding claim: Contested, unresolved. Tier N and X with human-derived material.*

Three things should be said about how to read this. It is a serious and well-designed challenge whose whole point was to remove the variability that makes this field difficult, and its result on human-derived material contradicts the claim that most needs human support. Its scope is narrower than it first appears: it tested

binding of amyloid species to receptors, it says nothing about C4d, it says nothing about the developmental work, and it does not address whether receptor engagement, however achieved, produces the downstream phenotypes — which independent work with an endogenous antagonist supports [31]. And the competing-interest structure of the comparison is disclosed in the paper and is a reason to want independent replication rather than a reason to discount a controlled negative. No independent replication of the comparison has appeared in the seven years since.

**The complement arm has a negative of the same shape, and it is older.** The same receptor, under its immunological name, was identified as a cellular receptor for soluble complement fragment C4d nine years before the 2025 report. Three findings in that paper bear on the present claim. Cell-surface receptor engaging *soluble monomeric* C4d results in endocytosis of the ligand, and the authors characterise the receptor as a scavenger-type endocytotic complement receptor. **Binding of the soluble receptor to C4d covalently immobilised on a cell surface by classical complement activation could not be detected.** Thioester-immobilised C4d is nonetheless bioactive, suppressing monocyte cytokine secretion by up to half, an activity the authors attribute to a yet-to-be-identified receptor. And the receptor also binds C3d, C4b, C3b and iC3b, so it is not selective across the split-product family [26].

> *Grade of the complement-binding claim in its physiologically deposited form: Unevidenced, with one prior negative result on the immobilised pool.*

The relevance is a mismatch of ligand species rather than a flat contradiction. The 2025 binding measurements and the causal minipump demonstration all use **soluble** recombinant C4d; the human anatomy, by array tomography, reports the **deposited** pool. The 2016 result says those two pools are not interchangeable for this receptor. This does not overturn the complement arm. It qualifies it in precisely the way the 2019 comparison qualifies the amyloid arm, and it identifies the cheapest experiment in this paper: repeat the surface-plasmon measurement with C4d immobilised through its own thioester rather than through a tag.

### 3.6 What the receptor account establishes

Four things, and they are not small.

A receptor expressed on cortical and hippocampal pyramidal neurons holds spine density and plasticity down throughout life, cell-autonomously, and its acute blockade in an adult restores both. It is required for activity-dependent spine shrinkage. At least one ligand that rises in human Alzheimer cortex engages it, and infusion of that ligand into a normal adult brain removes spines in a manner abolished by deletion of the receptor. And the receptor's cytoplasmic tail carries an inhibitory phosphatase-recruiting architecture that, in neurons, has one demonstrated structural consequence.

What the account does not establish is anything about the enzymes inside the spine. The receptor arrives at the cytoskeleton by a single named step — cofilin — and the step between the phosphatases and cofilin is not in the account at all. That is the gap the second account has been recruited to fill.

# 4. The exchange-factor economy: what is secure

### 4.1 A spine is a steady state, and there are two ways to lose one

A dendritic spine is a membrane-bounded protrusion a fraction of a micron across whose shape is maintained by a dense, short-filament actin network. It has no microtubule core and no rigid skeleton. Its volume is a running balance between nucleation and elongation, which push the membrane out, and severing and depolymerisation, which let it fall back. The balance turns over on a timescale of seconds to minutes. A spine's size at any moment is a steady state, not a structure.

This has a consequence that governs everything in this part of the paper. A spine can shrink in two mechanically distinct ways: severing can increase, or nucleation can decrease. The two produce the same end state and are not distinguishable by measuring spine density, which is the measurement the field almost always makes. They are distinguishable by measuring which arm of the regulatory machinery moved, which is the measurement the field almost never makes in human tissue. Much of the confusion documented below follows from reading a density change as evidence for one of the two mechanisms when it is evidence for neither in particular — and it is worth noting at the outset that the receptor account's central structural measurement, in both the physiological and the disease work, is spine density.

### 4.2 Three GTPases, and what "antagonism" does and does not mean

Rho-family small GTPases are binary switches cycling between a GTP-bound active state and a GDP-bound inactive one. Guanine-nucleotide exchange factors load GTP; GTPase-activating proteins accelerate hydrolysis. Three members dominate spine biology. **RhoA**, when active, signals through Rho-associated kinase and the formin mDia; its canonical structural effect in a spine is restrictive. **Rac1** signals through p21-activated kinase and the WAVE complex to Arp2/3, which nucleates the branched filaments that hold a mature spine head open. **Cdc42** signals through WASP-family proteins to Arp2/3 as well, and is associated with the initiation of new protrusions and the stabilisation of potentiated spines.

These assignments are sound as first approximations and are the basis of the "seesaw" picture, in which RhoA opposes Rac1 and Cdc42 and the spine's fate is set by which side is up. As a statement about disease the picture is a trap, for two reasons.

Mutual antagonism among Rho-family GTPases is demonstrated in specific cells, in specific compartments, on specific timescales — most robustly in migrating cells, where a leading edge and a trailing edge must do opposite things. Within a single spine the three are co-active, segregated on a sub-micron scale, and cycled over seconds during plasticity. The inference that bulk active RhoA in a brain homogenate reports the state of a spine's seesaw crosses four orders of magnitude of spatial scale, and it is not safe.

And antagonism makes a specific, testable prediction about disease that has rarely been tested as such. If the lesion is a tipped seesaw, the two sides must move in opposite directions in the same tissue. If both fail to move, or if one moves the wrong way, the model is not merely unsupported — it is the wrong shape of model. Section 4.6 reports what happened when the three were measured together in human Alzheimer brain.

### 4.3 Ephexin5: the brake that turned out to be a switch

Excitatory synapse formation in the developing brain is restrained as well as promoted. Ephexin5 was identified in 2010 as the restraint: a Rho-family exchange factor highly expressed in immature neurons which negatively regulates excitatory synapse development until ephrin-B binding to the EphB receptor triggers its phosphorylation, ubiquitination and degradation. The mechanism turns on a single residue: EphB2 signalling phosphorylates tyrosine 361, the phosphate recruits the ubiquitin ligase Ube3A, and the proteasome destroys the enzyme [36]. A second regulatory input was later found — protein kinase C epsilon reduces spine number in immature neurons by phosphorylating Ephexin5 at sites distinct from Y361 [44] — so the enzyme is set by at least two kinases with different developmental profiles, one of which marks it for destruction and one of which does not.

*Grade: Established, tiers A and N, single laboratory but internally well controlled.*

The proposition that the enzyme is RhoA-selective was already under strain in 2017, in work co-authored by the laboratories that described it: reducing it increased overall spine outgrowth and increasing it decreased outgrowth, consistent with a brake — but tagged Ephexin5 was found *elevated* on the dendritic shaft at the sites of future new spines before those spines appeared, and lowering it *inhibited* new spine outgrowth in response to both global and local stimulation, so the authors concluded a dual role [23].

The mechanism of the duality was established in 2025 [41]. Ephexin5 activates both RhoA and Cdc42; in knockout brain the activated pools of both fall by roughly seventy per cent. Live imaging of resonance-energy-transfer biosensors at single spines showed that during plasticity induced by high-frequency glutamate uncaging the enzyme regulates the activation of Cdc42 but not of RhoA: knockout neurons showed about half the normal Cdc42 activation, with RhoA activation unchanged. **Substrate selection is not a fixed property of the enzyme but a property of its covalent state**, and the state is set by tyrosine phosphorylation: phospho-tyrosine Ephexin5 falls developmentally, chemical long-term potentiation reduces it by about three-quarters within minutes, and that reduction is dephosphorylation rather than degradation, since it survives proteasome inhibition. The non-phosphorylatable Y361F mutant enhances Cdc42 activation while leaving RhoA activation unchanged.

The functional consequence is the part that matters here. Spines in knockout neurons failed to sustain activity-driven growth: stimulated spines returned to approximately baseline volume against roughly twofold sustained growth in wild type. Re-expressing full-length enzyme restored growth; re-expressing a catalytically dead exchange factor did not. The Y361F phosphomutant rescued growth.

*Grade: Supported, tier N, single laboratory, not independently replicated, but with the internal controls — catalytic-dead rescue and phosphomutant rescue — that distinguish a mechanism from a correlation.*

Ephexin5 is therefore **required** for the structural plasticity that accompanies potentiation. It is not a brake the adult brain has switched off. It is a switch the adult brain keeps and throws, and the throwing is what learning-associated spine growth depends on.

### 4.4 Kalirin-7 and Tiam1: the building side

**Kalirin-7** is the brain-specific isoform predominant in adult forebrain, localised to the postsynaptic density, a Rac1-specific exchange factor and a principal regulator of spine morphogenesis and maintenance. On the seesaw it is the molecular identity of the building side. One study bears directly on it in this disease, and the result should be read precisely because the paraphrase in circulation differs from it in a way that matters. Amyloid-β-derived diffusible ligands applied to cultured mature cortical pyramidal neurons bound the neurons and induced spine dysgenesis; treatment induced the **rapid depletion of Kalirin-7 from spines**; and overexpression of Kalirin-7 prevented the oligomer-induced spine degeneration [54].

*Grade: Supported, tier N, single laboratory, with a gain-of-function rescue.*

The rescue arm is the valuable part: it establishes that the availability of this exchange factor is rate-limiting for the spine's survival under this insult. What the experiment establishes about mechanism is narrower than the account states. The account says amyloid evicts Kalirin-7 and sends it to the proteasome. The eviction is measured. The proteasomal destination is an inference, and the distinction is not cosmetic — eviction is a trafficking lesion, reversible in principle by restoring an anchoring interaction; destruction is a turnover lesion requiring synthesis. Section 8 returns to this, because the human measurement that bears on it has been overlooked for nineteen years.

**Tiam1** is a Rac1-specific exchange factor present in dendrites and spines, required for their development, which interacts with the N-methyl-D-aspartate receptor and is phosphorylated in a calcium-dependent manner on receptor stimulation; blockade by RNA interference and dominant interfering mutants showed that it mediates the receptor's effects on dendritic development by inducing Rac1-dependent actin remodelling and protein synthesis [51].

*Grade: Established, tier N.*

Two features of that result are usually dropped and both matter. Tiam1 is the molecular coupling between glutamatergic activity and structural growth — it is how a synapse that has been used gets rebuilt. And the measured response to receptor activation was **phosphorylation**, with the functional consequence being a requirement for growth. Tiam1 is a builder recruited by activity, not a passive substrate awaiting proteolysis.

The disease claim about it is different in kind. The account states that amyloid-driven receptor dysfunction causes excessive calcium entry, calcium activates calpain, and calpain cleaves Tiam1. Each step is individually plausible, and the sentence as a whole has, so far as we can determine, never been tested in a neuron. An indexed search for calpain-dependent cleavage of Tiam1 returns no studies in neurons and none in any Alzheimer model. It returns two substantive findings, both outside the nervous system: stress-induced senescence decreases fibroblast Tiam1, and senescent fibroblast lysates induce its degradation in a calcium- and calpain-dependent fashion, in a cancer-microenvironment context [35]; and, in epithelial cells, Tiam1 is phosphorylated on tyrosine 384 by Src predominantly at adherens junctions, the phosphorylation is required for Src-induced junction disassembly, it creates a docking site for the adaptor Grb2, and recruitment of Grb2–Sos1 activates extracellular-signal-regulated kinase, which triggers the localised degradation of Tiam1, **likely involving calpain proteases**; in human tumours, tyrosine-384 phosphorylation correlated positively with Src activity and inversely with total Tiam1 [53].

*Grade of the neuronal calpain claim: Unevidenced. Grade of the architecture in its own system: Established, tier X.*

The defensible statement is narrower and more useful. In non-neuronal cells Tiam1's local abundance is controlled by a Src-family kinase writing a single tyrosine that recruits an adaptor and licenses local proteolysis. Whether that architecture operates at the postsynaptic density, and whether it is engaged in this disease, is unknown and is testable with existing reagents. That formulation loses the closure of the cascade diagram and gains the property of being checkable.

### 4.5 One design, three regulators

Set the two licensing architectures side by side. The parallel is architectural rather than sequence-based; no claim of homology is made here, and we are not aware of evidence for one.

| | Ephexin5 (ARHGEF15) | Tiam1 |
|---|---|---|
| **GTPase loaded** | RhoA and Cdc42 | Rac1 |
| **Structural effect of its output** | Restrictive (RhoA) or constructive (Cdc42) | Constructive |
| **Licensing residue** | Y361 | Y384 |
| **Kinase that writes it** | EphB2, a receptor tyrosine kinase | Src, a non-receptor tyrosine kinase |
| **What the phosphate recruits** | Ube3A, a ubiquitin ligase | Grb2, an adaptor, then Sos1 and ERK |
| **Terminal protease** | Proteasome | Probably calpain, locally |
| **Second consequence of the same phosphate** | Biases substrate choice toward RhoA | None reported |
| **Shown in neurons** | Yes | No |

What is shared is a design principle: a single tyrosine, phosphorylated by a kinase resident in an adhesive signalling platform, creates a docking site for the machinery of the exchange factor's own removal. Phosphorylation is the licence; the protein's **local concentration** is the regulated variable; and the kinase, not the protease, is the specificity-determining step. The same design governs a third regulator of the same spine: reelin-induced tyrosine phosphorylation of the adaptor Disabled-1 both transduces the reelin signal and licenses that adaptor's own proteasomal destruction [9].

Three regulators of one spine, built to the same self-terminating plan. If the design is general, then the governing variable throughout this pathway is phospho-occupancy rather than abundance — and that is the claim Part III puts to the test, because it is also the claim at which the two accounts collide.

**The asymmetry inside the design fixes a sign.** On Tiam1, phosphorylation of the licensing tyrosine has one documented consequence: destruction. More kinase activity means less Tiam1, less Rac1 loading, less branched nucleation. The sign is unambiguous; it is a one-way street. On Ephexin5, phosphorylation has two consequences that pull in opposite directions for the spine: it marks the protein for destruction, which removes both of its outputs, and it biases the surviving pool towards RhoA, which is the restrictive output. More kinase activity therefore means less Ephexin5 but a more RhoA-biased remainder, and whether net RhoA signalling rises or falls depends on the relative rates of re-biasing and degradation — two rates nobody has measured in any tissue, let alone a diseased one. It is a two-way switch.

The consequence is a conclusion about the shape of the lesion that neither account reaches. If postsynaptic tyrosine-kinase activity rises, the Rac1 arm falls for a reason whose sign is determined, while the RhoA arm moves in a direction that is genuinely undetermined by present evidence. The seesaw does not tip because one side is pushed down. It tips because one side is unsupported. Section 6 shows that the receptor account's own architecture predicts the opposite kinase balance, which is why that conclusion cannot yet be drawn.

### 4.6 What the human Alzheimer brain shows about the three GTPases

One published study measured the expression, activity and localisation of RhoA, Rac1 and Cdc42, together with p21-activated kinase, in an amyloid-precursor-protein transgenic mouse and in human Alzheimer brain, with Pick's disease as a tauopathy comparison [28]. Because it is the only survey of its kind we could identify, and because its result is almost never quoted in accounts of this pathway, it is worth reporting in full.

Immunohistochemistry revealed a distinct localisation for each GTPase — in synapses, dendrite shafts, neuronal bodies or astrocytes — and the association of RhoA with synapses and dendritic microtubules was confirmed by electron microscopy. In the transgenic mouse, RhoA expression **decreased in synapses** and **increased in dystrophic neurites**, which the authors read as altered subcellular targeting. In human Alzheimer brain, RhoA immunostaining **decreased in the neuropil** and **markedly increased in neurons**, colocalising with hyperphosphorylated tau inclusions as though the GTPase were being sequestered by tangles. **Total RhoA protein was lower in the Alzheimer hippocampus**, which the authors attributed to loss of the membrane-bound and presumably active pool. RhoA also colocalised with hyperphosphorylated tau in Pick's disease, suggesting the mislocalisation tracks neurodegeneration rather than amyloid specifically. And **no major immunohistochemical changes were observed for Rac1, Cdc42 or p21-activated kinase**. The authors' concluding sentence identifies RhoA among the Rho GTPases as a possible therapeutic target on exactly that basis — that it is the one that changes.

*Grade: Supported, tier H with tier-A and second-tauopathy comparison; single laboratory; immunohistochemical with one quantitative immunoblot arm, not an activity-state measurement.*

Set that against what the exchange-factor account requires. The account requires RhoA signalling **up**: the measurement finds total RhoA **down** in hippocampus with the membrane pool specifically lost. The account requires Rac1 and Cdc42 signalling **down**: the measurement finds **no major change** in either, nor in the shared effector immediately downstream of both.

*Grade of the seesaw as a quantity claim in human tissue: Contradicted.*

Three objections must be entered and none rescues the quantity claim. **The measurement is of protein, not of activity** — true and important, since a GTPase's output is set by GTP-loading; but the objection cuts against the account as much as for it, because the account's own evidence for increased RhoA signalling is not an activity measurement either, it is an inference from the abundance of an exchange factor, and one cannot invoke the activity-versus-abundance distinction to protect a claim that rests on abundance. **Immunohistochemistry is semi-quantitative** — also true, which is why the hippocampal immunoblot matters: it is the quantitative arm and it reports a decrease. **End-stage autopsy tissue may not reflect the period when the lesion operates** — this is the strongest objection and it is substantially correct; sequestration into tangles is plainly late, and a transient early rise in active RhoA in the neuropil would be invisible in a brain whose tangle-bearing neurons have been accumulating protein for years. We accept it. What it establishes is that the human data cannot *confirm* the seesaw either, and that the seesaw is at present an

entirely preclinical model whose human arm, when attempted, returned the wrong sign. The field's confidence in it is not calibrated to that.

What the survey reports positively is a change of **place**. The protein is present. It is in the wrong compartment, and the compartment it has left is the one where it does its structural work.

*Part III*

# The Joint

*Where the two accounts are welded together, what the weld is made of, and what happens when the arithmetic across it is done. One limb carries the whole junction, it has never been pointed at any of its assigned substrates, and the two accounts predict opposite signs at the same two residues on either side of it.*

## 5. The weld, and what it is made of

### 5.1 How the two accounts have been joined

In reviews, teaching summaries and grant narratives the two accounts now circulate as one route. It runs as follows, and it is set out here in the vocabulary in which it circulates, because the composition rather than any individual study is the object of this section.

Soluble amyloid-β oligomers and the complement fragment C4d converge on LilrB2/PirB. The receptor's immunoreceptor tyrosine-based inhibitory motifs are phosphorylated and recruit the phosphatases SHP-1 and SHP-2 to the postsynaptic compartment. Those phosphatases strip the activating phosphate from EphB2, destabilising it at the surface; they strip it from the reelin adaptor Disabled-1, severing the protective pathway's internal relay; and they strip tyrosine phosphates from the GluN2B subunit of the N-methyl-D-aspartate receptor, unmooring it from the synapse. Amyloid, in parallel, binds EphB2 directly and triggers its proteasomal degradation. Because phosphorylation of Ephexin5 by EphB2 is what licenses its destruction, loss of EphB2 by either route removes the brake, and Ephexin5 accumulates. Accumulated Ephexin5 loads RhoA; RhoA drives Rho-associated kinase and LIM-domain kinase; cofilin is phosphorylated and inhibited; the branched network is not recycled. On the constructive side amyloid evicts Kalirin-7 and calpain cleaves Tiam1, so Rac1 cannot load. Loss of reelin signalling removes the one input that inhibits cofilin, so cofilin remains active and shreds what remains. The spine retracts.

Every arrow joins two measured things. Nearly every arrow can be traced to a real study whose real result is approximately as described. And the route has the properties for which mechanistic accounts are prized: it names molecules, assigns each a direction, converges on a single structural outcome, and generates a drug target at every level.

It also contains no gaps, and that is the reason to look at it closely. A real pathway in a real tissue, measured at the resolution available today, has gaps everywhere. An account with no gaps has either solved an unusual number of problems at once, or has filled them with prose.

### 5.2 The phosphatase limb: one precedent, four assignments

The cytoplasmic tail of the receptor carries immunoreceptor tyrosine-based inhibitory motifs. In immune cells the canonical sequence is textbook: ligand engagement phosphorylates the motifs, the phosphorylated motifs recruit SHP-1 and SHP-2 through their SH2 domains, and the recruited phosphatases dephosphorylate substrates within reach, raising the activation threshold of the cell. That the neuronal receptor has the same limb was reported in the founding paper, in one clause: neuronal PirB protein is associated with synapses and forms complexes with SHP-1 and SHP-2 [49].

*Grade of the association: Established, tier A.*

The clause has not been followed up in the disease context, and the obvious question has not been asked: **which substrates?**

It is not an idle question, because in one case the answer is known, and it is not the receptor's own tail. Myelin-associated glycoprotein binding to PIR-B leads to association of the receptor with Trk neurotrophin receptors; SHP-1 and SHP-2, recruited on ligand binding, function as **Trk tyrosine phosphatases**; inhibition of the two phosphatases reduced ligand-induced dephosphorylation of Trk and abolished the inhibitory effect on neurite growth; and small interfering RNA against either phosphatase, delivered in vivo, induced axonal regeneration after optic nerve injury [21]. A companion study established that p75 is required both for signal transduction through the receptor and for the phosphatase activation that ligand binding induces [22].

*Grade: Established, tiers A and N, for this receptor's phosphatase limb acting on a third-party receptor tyrosine kinase in the same membrane, with a structural consequence reversed when the phosphatases are removed.*

That should be stated at full strength rather than hedged. The mechanism class is not hypothetical. It is a demonstrated operation of this receptor, in neurons, on a substrate of exactly the kind at issue.

It licenses a strong hypothesis and it does not license a finding, and three differences between the precedent and the disease claim must be held in view. **The ligand differs**: myelin-associated glycoprotein is membrane-presented and engages the receptor in trans across an axon–glia interface, whereas the disease ligands are soluble and in cis at a synapse, and the geometry of engagement sets which substrates fall within the phosphatases' reach — and reach is the whole question. **The compartment differs**: the precedent concerns a growth cone on a regenerating axon; the claim concerns a dendritic spine. Both are actin-rich protrusions with receptor tyrosine kinases in the membrane, which is what makes the transposition tempting, but the composition of a postsynaptic density is not the composition of a growth cone. **The co-receptor requirement is specific**: in the precedent p75 is required, and whether p75 is present and engaged at the synapse under the conditions the route assumes has not been examined.

### 5.3 The four substrates, graded

The route assigns four substrates to this limb. None has been tested against the receptor. The emptiness should be stated precisely rather than loosely, because an assertion of emptiness is falsifiable and should be. An indexed search pairing PirB or LILRB2 with Ephexin5, with *ARHGEF15*, with reelin, with ApoER2 or with Disabled-1 returns no record whatever. Pairing the receptor with EphB2 returns a small number of records, none of which tests an interaction: a review listing both among candidate amyloid receptors, an unrelated expression panel, and the standardised receptor comparison in which the two appear as separate entries on one list. Pairing either phosphatase with Disabled-1, with dephosphorylation of EphB2, or with GluN2B returns nothing.

**EphB2 — the plausible one.** EphB2 is a receptor tyrosine kinase, the same class as the demonstrated substrate in the precedent, on the dendrites of the neurons at issue, and it is the kinase whose signalling writes the licensing tyrosine of Ephexin5 [36]. The established route by which amyloid removes it is proteasomal [15]. If the receptor's phosphatase limb also dephosphorylates EphB2, the disease has **two** routes to the same end — one that removes the kinase and one that silences it — and the second requires no proteasomal step at all. This is the single most testable prediction in the route, and the assay already exists: it is the one built for the Trk precedent. *Grade: Unevidenced as to the specific assignment; Established as to the mechanism class.*

**Ephexin5 tyrosine 361 — a phosphatase exists and is unnamed.** The residue both selects which GTPase the enzyme loads and licenses its destruction [36,41]. Whether a tyrosine phosphatase acts on it in the dendrite was an open question until the 2025 work settled half of it inadvertently: chemical long-term potentiation lowers phospho-tyrosine Ephexin5 sharply within minutes and the fall survives proteasome inhibition, so the mechanism is dephosphorylation rather than degradation [41]. A phosphatase acting on this residue therefore certainly exists in the dendrite and has not been named. The route assigns the job to SHP-1 and SHP-2 without evidence. The assignment is a guess; it is a reasonable guess; and it is cheap to test and expensive to leave untested, because the identity of this phosphatase determines whether the residue is under receptor control at all. *Grade: Unevidenced as to identity; Established, tier N, as to the existence of an unnamed dendritic phosphatase on this residue.*

**Disabled-1 — nothing, and a retrodiction.** Disabled-1 is the adaptor whose reelin-induced tyrosine phosphorylation both transduces the reelin signal and licenses its own proteasomal destruction [25,9], and its kinase is a Src-family kinase [8,2]. No phosphatase acting on it has been identified. *Grade: Unevidenced.*

The reason to take the possibility seriously is that it accounts for a human observation that currently has no proposed mechanism, and an audit should record retrodictions as well as predictions while marking them as the weaker currency. Suppose the limb does dephosphorylate Disabled-1. Chronic receptor occupancy then produces a peculiar state: the protective signal is not transduced, because the phosphorylation that transduces it has been removed; *and* the adaptor is not cleared, because that same phosphorylation is what licenses its degradation. Disabled-1 should accumulate, unsignalled, exactly where the receptor is engaged. That is what is found: in post-mortem specimens across the clinical range, apolipoprotein E, lipid-aldehyde-modified apolipoprotein E, reelin, ApoER2, **Disabled-1** and downstream phospho-markers accumulate together near neuritic plaques in the terminal zones of the entorhinal–hippocampal perforant path, with several markers higher in Alzheimer cases and correlating with histological progression and inversely with cognition [42]. A competing explanation exists in the same paper and is at least as strong — peroxidation chemistry that covalently crosslinks apolipoprotein E to the ligand-binding modules of ApoER2 would silence the arm upstream without any phosphatase. Both mechanisms predict accumulation. They differ in the phospho-state

of the accumulated adaptor: crosslinking predicts an adaptor that never received the signal, dephosphorylation predicts one that received it and lost it.

**GluN2B — the substrate assigned to the wrong enzyme.** The route's fourth assignment is that the receptor's phosphatases strip tyrosine phosphates from GluN2B, breaking its anchor. The phenomenon is real and well evidenced, and it has a characterised phosphatase mechanism — but the enzyme is STEP61, the striatal-enriched protein tyrosine phosphatase, whose level rises in the disease through impaired ubiquitination, with GluN2B among its established substrates [33]. It is not SHP-1 and it is not SHP-2, and nothing connects it to the receptor under audit. *Grade: Unevidenced, and superseded by an established alternative enzyme.*

This is the most instructive error in the whole route, because it is not a fabrication. Every component is real, the phenomenon is real, and the attribution is wrong in exactly the direction that increases the apparent unity of the mechanism.

**Table 1 — The phosphatase limb, substrate by substrate.**

| Assigned substrate | Mechanism class | Specific assignment | Records testing the pairing | Established alternative |
|---|---|---|---|---|
| **Trk receptors** | — | **Established**, tiers A/N | Direct demonstration | — |
| **EphB2** | Established | **Unevidenced** | None that test an interaction | Proteasomal removal by amyloid |
| **Ephexin5 Y361** | Established | **Unevidenced** | None | An unnamed dendritic phosphatase |
| **Disabled-1** | Established | **Unevidenced** | None | ApoE–ApoER2 crosslinking |
| **GluN2B** | Established | **Unevidenced** | None | STEP61 |

## 5.4 What the limb would explain, and what it makes worse

The phosphatase hypothesis is attractive on the protective arm, for the reason just given. On the destructive arm it does the opposite of tidying up, and that is the most useful thing about it.

If the limb dephosphorylates EphB2, EphB2's kinase output falls. EphB2's kinase output is what writes the licensing phosphate on Ephexin5 tyrosine 361. Less kinase output means **less** phospho-Ephexin5. And the phosphorylated state is what biases Ephexin5 towards RhoA; the dephosphorylated state biases it towards Cdc42 and supports spine growth [41].

So the limb, if real, predicts an Ephexin5 pool that is **more abundant and less RhoA-directed**. The route requires a pool that is more abundant and *more* RhoA-directed. The hypothesis recruited to unify the two accounts simultaneously inverts the sign of the arm it was recruited to strengthen.

This is not a reason to discard the hypothesis. It is a reason to notice that nobody has done the arithmetic — and the arithmetic, once done, turns out to be the central result of this paper.

# 6. The sign collision at the licensing tyrosines

## 6.1 The arithmetic, stated plainly

The route's own premise is that amyloid depletes EphB2. EphB2 is the kinase that writes Y361. Losing the kinase must **lower** the phosphorylated fraction. Lowering the phosphorylated fraction explains the accumulation of total Ephexin5 elegantly, because the phospho-form is the degraded form. But on the 2025 model, lowering the phosphorylated fraction also yields a **Cdc42-biased** pool, and the Cdc42 arm drives spine growth and stabilisation. The route's premise therefore predicts, through its own mechanism, a growth-promoting pool of Ephexin5. The observed phenotype is spine loss.

There are at least four ways out and none has been tested.

- A **second kinase** writes Y361 in disease without coupling to Ube3A recruitment, so the accumulated pool is both abundant and RhoA-locked.
- The accumulated pool is phosphorylated at **other residues** with different consequences — protein kinase C epsilon being a documented example [44].
- The **Cdc42 arm is blocked downstream**, so a growth-biased exchange factor produces no growth.
- The accumulation is a **failed repair response** rather than a cause, in which case genetic deletion rescues by removing a costly and ineffective compensation.

The fourth deserves to be stated plainly, because it inverts the therapeutic reading entirely. If Ephexin5 accumulation is a growth-directed response to synaptic loss, then deleting it removes an attempt at repair whose collateral cost exceeds its benefit, and the rescue in the mouse is real but means the opposite of what it has been taken to mean.

## 6.2 The kinase reading predicts the occupancy rises

The first way out is the one the exchange-factor account has developed, and it has a specific candidate rather than a generic one.

Fyn, a Src-family kinase, is mistargeted to the postsynaptic density in a tau-dependent manner in Alzheimer models; missorting of tau and absence of tau both disrupt its postsynaptic targeting, and uncoupling the Fyn-mediated interaction of the N-methyl-D-aspartate receptor with PSD-95 rescues the phenotype [30]. That places an **active Src-family tyrosine kinase at exactly the compartment in question, in a disease-dependent way**. In the non-neuronal Tiam1 work the kinase that writes Y384 is Src itself [53]. And a Src-family kinase is a plausible, though undemonstrated, alternative writer of Ephexin5 Y361 — which is the first of the four ways out, and the one that would restore the RhoA bias that the loss of EphB2 removes.

The reading therefore predicts: **phospho-occupancy at Y361 and at Y384 both rise**, Tiam1 abundance falls for a reason whose sign is determined, Rac1 loading falls, and RhoA moves in a direction that is undetermined because the two consequences of the Y361 phosphate pull against each other.

*Grade: Inference. The components are Established or Supported; the composition has not been measured.*

### 6.3 The receptor reading predicts the occupancy falls

The second account's architecture makes the opposite prediction at the same residues, and it makes it by its own definition rather than by extension.

An immunoreceptor tyrosine-based inhibitory motif is, by construction, a device for raising local phosphatase activity. The receptor's two phosphatases are recruited to the compartment on ligand binding [49], and in the one neuronal case where their substrate is known they act on a third-party receptor tyrosine kinase in the same membrane and lower its phospho-occupancy [21]. In disease, by the route's own account, two ligands at nanomolar affinity occupy this receptor chronically at a third of human excitatory synapses [11,32].

The reading therefore predicts: **net postsynaptic tyrosine-phosphatase activity rises, phospho-occupancy at the licensing tyrosines falls**, Ephexin5 accumulates because the mark that licenses its destruction is being erased, the accumulated pool is Cdc42-biased, and Tiam1 is *protected* from the degradation its own licence would otherwise trigger.

*Grade: Inference. The components are Established; the composition has not been measured.*

### 6.4 Both cannot be the net, and the collision is functional as well as biochemical

Put the two side by side.

**Table 2 — Opposite predictions at the same residues.**

| Variable | Kinase reading (Fyn mistargeted) | Receptor reading (SHP recruited) |
|---|---|---|
| **Net postsynaptic tyrosine-kinase/phosphatase balance** | Kinase-shifted | Phosphatase-shifted |
| **Phospho-Y361 fraction of Ephexin5** | Up | Down |
| **Substrate bias of the surviving Ephexin5 pool** | RhoA | Cdc42 |
| **Total Ephexin5** | Down, or flat if a second kinase uncouples the licence | Up |
| **Phospho-Y384 fraction of Tiam1** | Up | Down |
| **Total Tiam1** | Down | Up or flat |
| **Active Rac1** | Down | Flat or up |
| **Active RhoA** | Undetermined | Flat or down |
| **Where the lesion sits** | Construction withdrawn | Neither arm, at this level |

Only one of these columns can describe the postsynaptic density of an Alzheimer neuron. Both readings are currently held by people who cite each other's work, and neither has noticed that they disagree, because each has measured a different variable: the kinase reading rests on a localisation result for Fyn, the receptor reading on a co-immunoprecipitation clause and an immunological architecture, and neither has measured an occupancy.

A functional collision runs alongside the biochemical one, and it is harder to dismiss.

The receptor is **required for activity-dependent spine shrinkage**: deleting it raises spine formation, stabilises new spines and accelerates learning, and single-spine uncaging locates the requirement at the shrinkage step [1]. The exchange factor placed downstream of it is **required for activity-driven spine growth**, with catalytic-dead and phosphomutant rescues to prove the mechanism [41]. And the covalent state the receptor's phosphatase limb would produce at Y361 — dephosphorylated — is precisely the state that biases that enzyme towards Cdc42 and growth.

Drawn as a route, the account therefore asks a growth-requiring enzyme, pushed by the upstream receptor into its growth-biased state, to execute a shrinkage instruction. Each link is real. The composition contradicts the function of both molecules at once.

### 6.5 Three resolutions, and how to tell them apart

The collision has three candidate resolutions. They are experimentally separable, and separating them is the most valuable thing that could be done in this pathway.

**The limb is not real at these substrates.** The phosphatases are recruited, but nothing in the dendritic spine that the exchange-factor account names falls within their reach; the Trk precedent is a growth-cone result and does not transpose. The receptor then reaches the cytoskeleton by some other route — cofilin dephosphorylation, as originally reported, by an enzyme not yet identified — and the exchange-factor economy is downstream of amyloid but not downstream of the receptor. *Predicts:* receptor engagement in mature neurons alters neither phospho-EphB2 nor phospho-tyrosine Ephexin5; the Fyn reading is unopposed; Y361 and Y384 occupancies rise in disease.

**The limb is real and the kinase reading is wrong.** Receptor-driven phosphatase activity dominates. *Predicts:* receptor engagement lowers phospho-EphB2 and phospho-tyrosine Ephexin5 in mature neurons, in a manner blocked by phosphatase inhibition; occupancies fall in disease; total Ephexin5 rises, as reported; and the accumulated pool is Cdc42-biased, which makes it a failed repair response and inverts the therapeutic reading.

**Both are real and segregated in space.** The two enzymes act in different nanodomains of the same spine — the kinase at the postsynaptic density proper, where tau delivers it, and the phosphatases within the reach of the receptor, wherever in the membrane it sits — so that no population mean describes either. *Predicts:* the mean occupancy at each residue is unremarkable while its **dispersion across single synapses rises**, and the covariance between the two marks falls; and the dispersion resolves into receptor-associated and receptor-free modes rather than one broadened population.

The third is the one this paper favours, for reasons developed in Section 7 and Part IV, and it is the only one of the three that explains why two decades of bulk measurement have produced a literature that reports both directions for the same residue in the same disease.

**The measurement that separates them.** Quantify, in the same human cortical tissue, the **phosphorylated fraction** of Ephexin5 at Y361 and of Tiam1 at Y384 — not the total level of either — stratified by disease stage, with parallel pulldown of active RhoA, active Rac1 and active Cdc42, and with cell-type resolution sufficient to separate neuronal from endothelial pools; and report the **joint distribution across single synapses** rather than three means.

A note on feasibility, because the claim that this is blocked at step one has been made and is not correct. A site-specific antibody against phospho-Y361 was generated by the laboratory that identified the residue and

validated in two independent ways — against the non-phosphorylatable Y361F mutant and against Ephexin5-knockout hippocampus, with signal in wild-type tissue and none in null — and applied by both immunoblot and dendritic immunofluorescence [36]. Validation against both a phosphomutant and a genetic null places that reagent above most phospho-specific antibodies in general use. What is genuinely unknown is narrower and concerns tissue rather than chemistry: the reagent has never been reported on human post-mortem cortex, where phospho-epitope lability and the antigen retrieval that array tomography demands are the real obstacles, and whether usable serum survives sixteen years is not known to us. The 2025 characterisation measured by immunoprecipitation and pan-phosphotyrosine blot with residue attribution resting on the Y361F mutant [41], which says nothing about the antibody either way. The measurement is **unattempted in disease tissue rather than impossible**, and that distinction is the difference between a question to put to a laboratory and a programme to fund.

The post-mortem caveat is real and cuts in a specific direction. Phosphorylation states change rapidly after death and do so unequally across proteins and sites. Any negative result in autopsy tissue is biased toward the null, and a study finding no difference in occupancy has not excluded one. That is an argument for running a perfusion-fixed animal arm first — in which the receptor can be engaged acutely with a defined ligand, which no human study can do — and not for abandoning the human measurement.

# 7. The sign collision at cofilin serine 3

### 7.1 The residue both accounts write to

Downstream of the GTPases the pathways narrow sharply, which is why the field keeps arriving at the same molecules from different directions. The RhoA-to-Rho-associated-kinase arm reaches actin by at least two routes: it phosphorylates myosin light chain, raising actomyosin tension, and it activates LIM-domain kinase, which phosphorylates cofilin on serine 3. The direction here is routinely reversed in secondary accounts and is worth stating with care: **phosphorylation inactivates cofilin and therefore stabilises filaments; dephosphorylation activates cofilin and permits severing.**

Two further facts make this residue the natural terminus of both accounts. The Rac1 and Cdc42 arms converge on Arp2/3 through p21-activated kinase, which also phosphorylates LIM-domain kinase — so the Rac1 arm too can raise phospho-cofilin, and the claim that the two sides of the seesaw have opposite effects at serine 3 is not correct as biochemistry. What differs is the spatial and temporal pattern, not the sign at that residue. And the restraint receptor's own disease claim terminates here: ligand engagement leads to enhanced cofilin signalling, reported also in human Alzheimer brain [32].

### 7.2 What the human record actually says, and an arithmetic correction

The literature at this residue is usually described as a four-way disagreement. It is worth counting it exactly, because the count that circulates is wrong in both directions and the error is instructive.

**Reelin adds the phosphate, and this stabilises.** Reelin signalling leads to serine-3 phosphorylation of n-cofilin through ApoER2, Disabled-1, Src-family kinases and phosphatidylinositol 3-kinase; the phosphorylated protein cannot depolymerise filamentous actin; and the effect was localised to the leading

processes of migrating neurons as they approached the reelin-rich marginal zone [13]. *Direction: phosphorylation up. Consequence: stabilisation. Grade: Supported, tier N.*

**Amyloid removes the phosphate, through the restraint receptor.** Engagement of PirB and LilrB2 by soluble oligomers leads to enhanced cofilin signalling — the active, dephosphorylated species — also seen in human Alzheimer brains; in human tissue, phospho-serine-3 normalised to total cofilin was about 38 per cent lower in Alzheimer brain than in controls [32]. *Direction: phosphorylation down. Consequence: destabilisation. Grade: Supported, tier A for the mechanism; tier H for the measurement, single series.*

**An independent laboratory reproduced the dephosphorylation.** Working on an unrelated problem — an endogenous antagonist of the same receptor — a different group showed that blocking amyloid binding at the receptor suppressed amyloid-induced **dephosphorylation** of cofilin and the associated loss of PSD-95 and spine density, and that the human orthologue behaved the same way [31]. *Direction: phosphorylation down. Grade: Supported, tier N, corroborating a direction with a different tool in a different country.*

**And an independent laboratory reported the opposite, in human cortex.** Elevated phospho-cofilin-1 was found in the postsynaptic-enriched synaptosomal fraction from cortical samples of APP/PS1 mice **and of human Alzheimer cases**. In primary cortical neurons, oligomers induced rapid actin **stabilisation** and **increased** phospho-cofilin within thirty minutes; experiments with active and inactive cofilin mutants indicated that cofilin phosphorylation is necessary and sufficient for amyloid-induced synaptic impairment via actin stabilisation, occurring *before* cofilin–actin rods form; and the Rho-associated-kinase inhibitor fasudil prevented the stabilisation, the impairment and the synaptic loss by blocking cofilin phosphorylation [43]. *Direction: phosphorylation up. Consequence: stabilisation, and that stabilisation is itself the lesion. Grade: Supported, tier H for the human measurement.* The paper's own framing is notable: it describes how amyloid affects the actin cytoskeleton as remaining unknown and contentious.

**And the rods require the phosphate to be absent.** Rod-like inclusions of cofilin and actin form spontaneously in neurons overexpressing **active** cofilin, so dephosphorylation is sufficient to induce them, and persistent rods span the neurite, disrupt microtubules and degenerate the distal process without killing the neuron [38]. *Grade: Established, tier N.*

Now the arithmetic. **There is one human cortical series reporting phospho-cofilin down and one reporting it up.** The mouse mechanisms divide two against two. Accounts of this disagreement in circulation variously describe it as "two human series up against one down" and as "one up against two down"; both tallies overcount in opposite directions, because one of the two reports usually counted as a human series is a cultured-neuron study with a human-orthologue binding arm, not a human tissue measurement [31]. The contradiction is real. The arithmetic describing it is not, and the slippage matters, because a reader deciding how much weight to put on either direction is being told that one of them has twice the human support it has.

*Grade of the direction of cofilin serine-3 phosphorylation in human Alzheimer cortex: Contradicted — two human series, opposite signs, neither replicated.*

### 7.3 The effector's own dose–response reverses sign

One property of cofilin is often omitted and dissolves part of the apparent contradiction while creating a real one. Its effect on filaments is not monotonic in its own concentration. Cofilin binds cooperatively along ADP-actin subunits; it **severs** filaments at low cofilin-to-actin ratios and **stabilises and bundles** them at high ratios, and in the bundled state it forms a one-to-one cofilin–actin lattice [4].

An effector whose output reverses sign as its occupancy rises cannot be assigned a direction from a bulk measurement of how much of it is active. That single fact means that "active cofilin is up" and "active cofilin is down" are not, by themselves, statements about what happened to the filaments.

### 7.4 The reconciliation the two accounts jointly supply

Neither account states the following, and it falls out of putting them together.

The restraint account's mechanistic claim is not merely that the receptor dephosphorylates cofilin. It is that engagement drives dephosphorylation **past the physiological range** — out of the severing regime and into the saturating, bundling regime where cofilin–actin rods form. A rod occupying a spine neck occludes it: cargo cannot pass, receptors and mitochondria cannot be delivered, and the spine is functionally disconnected before it is anatomically absent [38,4].

The competing human series reports the opposite phospho-sign and the *same* structural consequence. Amyloid oligomers induce rapid actin stabilisation with increased phospho-cofilin, and the stabilisation is itself the lesion [43].

Set them together and the disagreement changes shape. **Both routes arrest turnover.** Phosphorylation arrests it by removing the severing enzyme from play. Dephosphorylation past saturation arrests it by converting the severing enzyme into a cross-linker. The spine's actin network is a steady state maintained by continuous severing and renucleation; either excursion from the physiological band removes the turnover, and a spine that cannot turn over cannot grow, cannot shrink on instruction, and cannot deliver cargo through its neck.

On that reading the two human series are not reporting contradictory lesions. They are reporting the two ends of one non-monotonic variable, and the quantity that is actually abnormal in both is neither the phosphate nor the activity but the **rate of filament turnover**, which neither study measured.

*Grade: Inference. The components are Established or Supported; the synthesis is proposed here and is tested by prediction 5 in Section 17.*

This has a consequence that should be stated plainly because it bears on a drug. The rationale for inhibiting Rho-associated kinase in this disease rests on the finding that phospho-cofilin is elevated and that the elevation is the lesion [43]. If the turnover reading is right, then lowering phospho-cofilin helps a synapse sitting on the high side of the band and pushes a synapse sitting on the low side further towards rods. A drug whose mechanism is to lower phospho-cofilin, given to a population in which the direction of the abnormality is contested and may be mixed *within a single cortex*, is a drug that may help one subgroup and harm another, with no stratifying biomarker. That is not a reason not to develop it. It is a reason to measure the distribution before, not after, it reaches a patient.

### 7.5 Two reconciliations, one discriminator

There are now two candidate explanations of the serine-3 contradiction and they are distinguishable.

**Dispersion.** A non-monotonic effector under lost governance will show increased variance rather than a consistent shift in mean, and bulk measurements of different cortical regions at different stages will sample opposite tails. If this is right, the correct measurement is not the mean of phospho-cofilin but its dispersion across single synapses, and every study above measured the wrong statistic.

**Stage.** The excursion runs in one direction and then the other: early phosphorylation and over-stabilisation, as reported in the thirty-minute oligomer exposure and in the postsynaptic fraction of a transgenic cortex; later dephosphorylation into the rod-forming regime, as reported in end-stage human tissue and in the rod literature. The two human series would then be sampling different points on one trajectory. The first study's own observation that the stabilisation it describes occurs *before* rods form is consistent with this and is, so far as we can find, the only published statement that orders the two states in time.

The two make different predictions and can be told apart in one experiment. Dispersion predicts increased variance at every stage, with the mean uninformative throughout. Stage predicts a mean that moves, with the sign flipping between early and late disease, and rods confined to the late window. A staged human series with per-synapse resolution — Braak-stratified, array tomography, phospho-serine-3 normalised to total cofilin at each synapse, with rod burden scored in the same specimens — separates them, and the answer changes what a cofilin-directed drug should be aimed at.

# 8. Kalirin-7: where the address claim fails, and what the failure teaches

## 8.1 The human measurement nobody cites

The exchange-factor account holds that Rac1 signalling falls. For one of the two Rac1 exchange factors it names, the human abundance measurement exists, and it has been absent from the literature that needs it most for nineteen years.

A transcriptional survey of hippocampus and cerebellum from nineteen Alzheimer patients and fifteen age- and sex-matched controls found *kalirin* to be the most consistently under-expressed gene in Alzheimer hippocampus, verified by semi-quantitative and real-time polymerase chain reaction. The change was regionally specific: it was absent from the cerebellum of the same cases, and housekeeping transcripts were unaffected [55]. A companion study pursued the protein: Kalirin-7 was significantly diminished at both messenger RNA **and protein** level in hippocampal specimens from the same cohort; less Kalirin-7 was complexed with inducible nitric oxide synthase in Alzheimer extracts; and inducible nitric oxide synthase activity was considerably higher in Alzheimer hippocampus [56].

*Grade: Supported, tier H, single laboratory, two publications, with a regional internal control.*

This is, so far as we can determine, the only human measurement of a Rho-family exchange factor's abundance in Alzheimer tissue at both transcript and protein level, in a cohort of reasonable size with an internal anatomical control.

## 8.2 Eviction and under-expression are two lesions

The oligomer result and the human result are not the same lesion and should not be merged.

In cultured neurons under acute oligomer exposure, Kalirin-7 leaves the spine; total protein is not the measured variable [54]. In human hippocampus at autopsy, both the transcript and the protein are reduced [55,56]. Three readings are available and they are not equally well supported.

**Two stages of one process.** Acute redistribution out of the spine precedes, and perhaps causes, chronic transcriptional down-regulation. Plausible, and it predicts that redistribution should be detectable before the abundance change in a staged series. Untested.

**Two independent lesions.** The acute trafficking effect and the chronic transcriptional deficit have different causes, and the human finding is not amyloid-driven at all. The regional specificity is consistent with this: the cerebellum of the same cases was spared, and the cerebellum is relatively spared of the disease process generally, so the finding tracks regional pathology rather than naming its cause.

**Cell loss.** A hippocampal homogenate from an Alzheimer brain contains fewer pyramidal neurons, and a transcript enriched in those neurons will fall for that reason alone. The internal control partly addresses this — housekeeping transcripts were unaffected and the cerebellum was spared — but it does not exclude it, and no study has normalised this measurement to a neuron-specific denominator.

The third is the most serious and the least discussed. It applies to every bulk abundance measurement in this field, and it is why the replication worth funding is cell-type-resolved rather than larger.

### 8.3 What this costs the address claim, exactly

It costs it the status of a rule, and the honest accounting is worth setting out rather than glossing.

| Regulator | What the account says changed | What the human or primary measurement shows | Class of lesion |
|---|---|---|---|
| **RhoA** | Activity rises | Total protein lower in hippocampus; redistributed out of neuropil into tangle-bearing somata; Rac1, Cdc42, PAK unchanged | Address |
| **Kalirin-7** | Destroyed by the proteasome | Depleted from spines acutely; **and reduced at transcript and protein in human hippocampus** | Address **and** abundance |
| **Ephexin5** | Rises, driving RhoA | Total protein rises; the tyrosine that decides which GTPase it loads never measured in disease | State |
| **Tiam1** | Cleaved by calpain at the synapse | Calpain-dependent degradation documented only in fibroblasts and epithelial junctions, downstream of a Src-written tyrosine; untested in neurons | State, if the architecture transposes |
| **Reelin** | Falls, or its neurons are lost | mRNA and protein **rise** at late Braak stages in frontal cortex; receptor unchanged; Disabled-1 phosphorylation falls; ligand trapped with amyloid | Address |

Four of the five rows are changes of address or covalent state. One is both. The generalisation that survives is therefore weaker than a slogan and stronger than nothing: **abundance has been measured in human tissue in two of these cases, and in the two where it has not been measured the dominant method could not have seen what moved.** The Kalirin-7 exception is instructive precisely because it is an exception — when somebody did measure abundance in human tissue with an anatomical control, they found

a change. The claim that survives is not that abundance never moves. It is that the field has systematically measured the variable it can measure rather than the variable the disease moves, and that where both have been measured the two tell different stories about the same molecule.

There is a second cost and it should be paid openly. The address reading, in the form in which it circulates, treats the absence of a published measurement as evidence that the measurement would be null. It is not. The Kalirin-7 case shows what happens when the search is done properly: the measurement existed, it had been made nineteen years earlier in a journal the pathway's literature does not read, and it goes the other way.

# 9. Reelin: an arm that does not reach where it is drawn



## 9.1 The cell that writes is not the cell that reads

Reelin binds the ectodomains of the very-low-density-lipoprotein receptor and apolipoprotein E receptor 2; blockade of those receptors abolishes reelin-induced tyrosine phosphorylation of Disabled-1; and mice lacking either reelin or both receptors show hyperphosphorylation of tau [25]. Binding stimulates phosphatidylinositol 3-kinase and protein kinase B and thereby **inhibits** glycogen-synthase kinase-3β, with genetic dependency on the receptors and the adaptor [5]; and mice lacking reelin, its receptors, or the adaptor all display increased phosphorylated tau, with glycogen-synthase kinase-3β activity rising and phosphatase activity unchanged across conditions, so the increase reflects kinase gain rather than erasure loss [39]. The kinases that phosphorylate the adaptor are the Src family, and the adaptor is both their substrate and, once phosphorylated, their activator [8,2].

*Grade: Established, tier A, across multiple genotypes in several laboratories.*

One structural fact about this system is routinely lost and matters for every therapeutic proposal made on it. **The cell that makes reelin is not the cell that responds to it.** In adult cortex reelin is expressed principally by a restricted subset of GABAergic interneurons and secreted extrasynaptically into the perineuronal matrix, while Disabled-1, through which the signal is read, is expressed predominantly in pyramidal neurons [40]. The signal is written by one interneuron class into a shared extracellular compartment and read by a different, excitatory cell. Anything that interferes with the compartment — and amyloid is deposited in exactly that compartment — can break the pathway without altering either cell.

## 9.2 Reelin rises, and the signal falls

The measurement that matters most for the arithmetic was made in human frontal cortex and goes the other way from the account.

Reelin and amyloid-β oligomers co-immunoprecipitated from human brain extracts and were present in the same size-exclusion fractions. Amyloid treatment of cells **increased** reelin expression, but the secreted reelin was trapped together with amyloid aggregates. In frontal cortex extracts, an **increase** in reelin messenger RNA and in both soluble and guanidine-extractable reelin protein was associated with late Braak stages, while expression of ApoER2 did not change. Yet reelin-dependent induction of Disabled-1

phosphorylation appeared **reduced** in Alzheimer tissue. In cells, amyloid reduced reelin's capacity to induce internalisation and processing of ApoER2, and soluble ApoER2 fragments generated after reelin binding, detectable in cerebrospinal fluid, correlated with reelin levels in control subjects but not in patients, where they diminished [17].

*Grade: Supported, tier H with cerebrospinal-fluid and cell-biological arms, single laboratory.*

Set beside it the observation that a specific glutamatergic population loses reelin expression — reelin-expressing pyramidal cells are reduced in the entorhinal cortex of transgenic mice with corresponding reductions of hippocampal reelin protein, with qualitatively similar reductions in human Alzheimer brains, while the number of reelin-expressing **GABAergic interneurons was not altered** [14] — and the picture is specific rather than general. A particular excitatory population in a particular region loses expression; the interneuron population that supplies most of the adult cortical reelin signal does not.

Three consequences follow and they are practical. "Reelin is depleted in Alzheimer's disease" is not an accurate summary of the human data, and any argument that depends on it — including arguments for reelin replacement — needs restating: the deficit is in transduction at the receptor, not in ligand supply, and supplying more ligand into a compartment that sequesters ligand is unlikely to help. The right pharmacodynamic endpoint is therefore not reelin but the soluble ApoER2 fragment generated by reelin binding, whose correlation with ligand level is lost in disease — a measurement of whether the signal was *received*. And a gain-of-function ligand is, in principle, the right class of countermeasure for a transduction deficit, which is the strongest theoretical reason to take human resilience variants in this pathway seriously.

### 9.3 The EphB arm, and a ligand that consumes its receptor

The route under audit joins reelin to the exchange-factor economy through EphB2: reelin binds EphB2, so when amyloid or the phosphatase limb removes EphB2 the neuron becomes deaf to reelin. The first clause is correct. The rest is not what the cited work reports.

Reelin binds the extracellular domains of EphB transmembrane proteins, inducing receptor clustering and activation of EphB forward signalling in neurons — **independently of ApoER2 and VLDLR**; and mice lacking EphB1 and EphB2 show a positioning defect of CA3 pyramidal neurons similar to that in reelin-deficient mice, dependent on EphB kinase activity [10]. Three further findings in the same paper bear on the account and all three run against it. **Reelin destroys the receptor**: treatment induces autophosphorylation and subsequent proteolytic degradation of EphB proteins, with a carboxy-terminal fragment accumulating under proteasome inhibition and a larger fragment under combined γ-secretase inhibition, consistent with ligand-induced degradation of the kind ephrin B1 produces. **The degradation does not require the canonical reelin receptors**: it persists when lipoprotein receptors are blocked and in neurons from embryos lacking both. And **the cellular consequence is retraction**: stimulation of endogenous EphB2 with reelin led to cell retraction and rounding, the deadhesive change expected on EphB activation.

*Grade of the bridge as the route states it: Contradicted.*

The nature of the error is instructive rather than careless. A true sentence — reelin binds EphB2 and activates forward signalling — was read, and the valence was supplied from context: reelin is the protective molecule, therefore its action on a receptor must be protective, therefore it stabilises what it binds. The

inference is natural and wrong, because activation of a receptor tyrosine kinase by its ligand is, in this family, the beginning of the receptor's destruction.

The structural consequence is more serious than a mis-stated valence. EphB2 kinase output writes the phosphate on Ephexin5 Y361 [36]; reelin activates EphB2 [10]; therefore reelin should **lower** Ephexin5, transiently, through the kinase, before the receptor itself is consumed. Nobody has tested that, and it is a good test because either answer is informative. But in the account reelin and amyloid are antagonists meeting at EphB2 from opposite sides — amyloid removes the receptor, reelin needs it. On the published record they are not antagonists at that node at all. **They both consume the receptor**, and the transient kinase pulse that precedes consumption is the only part of the story in which they differ.

The honest version is less satisfying. EphB2 is a receptor with a short signalling life, consumed by every ligand that activates it, whose steady-state surface level is set by the balance of synthesis against ligand-driven destruction, and whose licensing function therefore depends on the **rate** at which it is replenished rather than on which ligand is present. That is a third kind of variable — neither abundance nor address but turnover — and it is the variable nobody in this pathway measures.

### 9.4 Where reelin and the restraint receptor actually meet

They meet at cofilin serine 3, and the meeting is an inference rather than an observation.

Reelin signalling, through the same receptors, the same adaptor, the same Src-family kinases and the same phosphatidylinositol 3-kinase, phosphorylates n-cofilin at serine 3 and thereby stabilises filamentous actin [13]. Amyloid, through the restraint receptor, dephosphorylates it [32,31]. Two receptor systems on the same neuron therefore write to the same residue with opposite signs — one a guardian of structure, the other an instruction to dismantle.

This has not been demonstrated in a single experiment and should be carried as an inference. It is also weaker than it looks under a cell-type restriction, and the weakness should be stated. The weight of the reelin literature bears on developing cortex, on layer II entorhinal neurons and on interneurons, not on the mature cortical pyramidal population in which the restraint receptor's effects are established. The claim that the two arms meet on the dendrites of the same mature pyramidal neurons is a reasonable inference. It is not a finding, and it has never been tested in one system.

There is a further and uncomfortable consequence. Reelin pushes phospho-cofilin **up**. One of the two human series says phospho-cofilin is already up in Alzheimer cortex and that the elevation is itself the lesion [43]. On that reading, enhancing reelin signalling pushes a pathological variable further in its pathological direction. We do not assert this; we assert that the field has not confronted it, and that on the turnover reading of Section 7.4 the question is sharper still — because a systemic, chronic reelin-mimetic delivers everywhere and continuously a signal whose documented physiological value is that it is local and transient.

And there is a structural reason, not an empirical one, why the sign of "mimic reelin" cannot be read off its physiology. Reelin's transduction runs through the Src family [8]. A Src-family kinase licenses Tiam1's destruction in the system where that licence is characterised [53]. And a Src-family kinase is mistargeted to the postsynaptic density in this disease [30]. The protective signal of one account and the destructive licence of the other are carried by one set of enzymes. A signal whose transduction runs through a kinase family with substrates on both sides of the structural balance cannot have its therapeutic sign assigned from its physiological description — which is the same structural reason that "lower Ephexin5" has an undetermined net sign at

RhoA. In both cases the ambiguity is created by one molecule having two jobs, and in both cases the field has assigned the sign from the job it noticed first.

*Part IV*

# The Shape of the Lesion

*What the two accounts jointly describe, once the weld between them has been audited. Neither contains a broken molecule. Both describe an intact apparatus operating in the wrong place or at the wrong time, and that is a substantive claim with consequences for what counts as a therapy.*

## 10. Nothing in either account is broken

### 10.1 Two intact systems, two kinds of misdirection

Mechanistic accounts of Alzheimer's disease are almost always accounts of something breaking. A clearance system fails; an enzyme is lost; a barrier leaks; a cell type turns hostile. The implicit grammar is of a protective apparatus giving way.

Neither account examined here has that grammar, and this is the single most important thing the two have in common.

In the restraint account, nothing breaks. The molecules do in disease exactly what they do in health — restrain plasticity and eliminate synapses — and the pathology consists in their doing it at the wrong time, in the wrong place, and without a stopping rule. There is no loss of function anywhere in the core mechanism. The receptor is intact, its ligands are the ligands it evolved to read or close relatives of them, its phosphatases are recruited as designed, and its structural output is the output it produces during development and during ordinary learning. The brake is not faulty. The brake is being pressed by the wrong hand.

In the exchange-factor account, nothing breaks either, although this is less often said. Ephexin5 is not mutated, truncated or inactivated; it is abundant and catalytically competent, and the question is only which GTPase it is loading. Kalirin-7 is not destroyed; it is displaced, and the human evidence shows it also under-expressed — but the molecules remaining are the same molecules. RhoA is present in the Alzheimer hippocampus; it is in the soma rather than the neuropil. Reelin is present in Alzheimer frontal cortex in *greater* quantity than in control; it is bound up with amyloid in the compartment it must cross. Nothing in the list has failed as a protein. The architect is not missing. The architect has been locked out of the building.

Two misdirections, then, and they are of different types. One is a misdirection of **instruction**: a competent effector receiving a command it should not be receiving, from ligands that have accumulated. The

other is a misdirection of **address**: a competent effector no longer in the compartment where its substrate is, or no longer carrying the mark that tells it which substrate to act on.

### 10.2 Why this is a substantive claim and not a figure of speech

Four things follow from it that would not follow from a damage account, and each is checkable.

**It predicts that total abundance is the wrong measurement, and says when.** A damage account predicts that the damaged species falls, that the fall tracks severity, and that the fall is visible in a homogenate. A misdirection account predicts that the total may be flat, may rise, or may fall for an unrelated reason such as cell loss, and that the informative variable is a ratio — occupancy over total, synaptic over somatic, received signal over ligand. This is not a retreat into unfalsifiability; it is a specification of which measurement decides. Of the five regulators tabulated in Section 8.3, abundance has been measured in human tissue in two, and in one of those two it moved in the direction the account did not predict.

**It predicts that the lesion is cheap, and therefore early.** Destroying a protein costs a cell transcription, translation and transport to replace it, and leaves a transcriptional signature. Moving a protein out of a compartment costs nothing and leaves none. A spine is a steady state maintained by enzymes that must be *in* it; any mechanism that removes an enzyme from the compartment produces the same functional deficit as destroying it, at a fraction of the cost. Evolution will reach for the cheap mechanism when it builds a regulated process, and a disease that corrupts a regulated process will inherit the cheap mechanism. That predicts that the earliest detectable lesions in this pathway are redistributions, and that they precede any measurable change in amount.

**It predicts that reversibility is a trafficking question, not a synthesis question.** A destroyed protein must be resynthesised, which takes hours. A displaced protein is still present and still catalytically competent, and its return requires only that whatever anchors it be restored. The two call for different drugs, and the difference is testable: on the eviction reading, the rescue by overexpression of Kalirin-7 works by mass action and should show a steep threshold dose–response, with modest increases doing nothing.

**It predicts a class of therapeutic that does not currently exist.** Section 13 develops this. Every intervention generated by either account is a subtraction — lower an enzyme, inhibit a kinase, block a receptor — and none of them restores an address.

### 10.3 Why the field measures the wrong thing, and it is not carelessness

It is worth asking why the quantity narrative dominates, because the answer is not that the field is careless.

**Quantity is what the dominant methods measure.** Homogenise tissue, run a blot, compare band intensities: the output is a number per protein per sample, and the question it answers is "how much." Localisation requires imaging at a resolution that resolves a spine from a dendritic shaft, which in human post-mortem tissue means array tomography or an equivalent, which is laborious and rarely done. Covalent state requires a validated site-specific antibody that survives post-mortem delay. Received-signal assays require a cleavage product or a reporter. All three are harder than a blot, and the easy measurement answers a question the disease may not be asking.

**Quantity is what a diagram can show.** An arrow from one box to another with "up" or "down" beside it is a complete statement in the visual grammar of a pathway figure. "Present but in the wrong compartment" has no arrow. "Abundant but hypo-phosphorylated at one residue, and therefore loading a

different GTPase" has no arrow. The figure format selects for the quantity story, and the figure is what is remembered.

**And quantity licenses a prescription.** If a protein is too high, lower it; if too low, raise it. That is the form a drug-discovery programme can act on, and the two prescriptions these accounts have generated — lower Ephexin5, mimic reelin — are both of exactly this form.

The mismatch is not a failure of any individual study. It is a systematic bias of the method against the kind of lesion this disease appears to have. The remedy is not to distrust blots; it is to notice that a blot and a spine answer different questions, and to say which question is being asked.

# 11. What each account cannot do without the other

### 11.1 The restraint account has no execution step inside the spine

The receptor account reaches the cytoskeleton in one move. Ligand engages receptor; phosphatases are recruited; cofilin is dephosphorylated; the spine fails. Between the phosphatases and cofilin there is, in the account as published, nothing at all — no exchange factor, no GTPase, no kinase. The one neuronal substrate the limb has been shown to act on is a neurotrophin receptor in a growth cone [21].

That is not a criticism of the laboratory; it is a description of where the work has got to. But it means the receptor account cannot, on its own, say which of the two mechanically distinct routes to a smaller spine it is using. Severing increased, or nucleation decreased? The account's own structural measurement is density, and density cannot distinguish them. A receptor that removes spines by withdrawing construction and a receptor that removes them by activating demolition are different drug targets with different windows, and nothing in the receptor literature decides between them.

### 11.2 The exchange-factor account has no upstream, and no stopping rule

The exchange-factor account has the opposite problem. It specifies the enzymes with unusual precision and cannot say why they should be disturbed in this disease and not in others, or why the disturbance should persist.

Its named upstream event is the amyloid-driven depletion of EphB2, which is well evidenced and has the unusual strength of bidirectional manipulation within one study [15]. But EphB2 depletion is a statement about a ligand concentration, not about a regulated process, and it supplies no mechanism by which the loss becomes self-sustaining. The restraint account supplies exactly that: a receptor whose occupancy rises with age across cortex from the mid-twenties, whose ligands are generated by a cascade that amplifies, and whose output is a programme with no built-in termination once the ligand remains.

The two deficits are complementary in a way that explains why they have been welded. One account has a stopping-rule problem and no executor; the other has an executor and no stopping-rule problem. The weld is attractive because it solves both at once. That is also the reason to be careful of it: a joint that resolves two difficulties simultaneously is a joint that will be accepted on less evidence than either difficulty warranted.

### 11.3 Four absences the two accounts share

Welding them does not remove these, and they should be named.

**Tau.** Neither account has much to say about the pathology that tracks the topography and timing of neurodegeneration most closely. The one connection either makes is indirect and runs in the direction the exchange-factor account needs: the Src-family kinase whose mistargeting to the postsynaptic density supplies the kinase reading of Section 6.2 is mistargeted in a *tau-dependent* manner [30]. And a recent result from an unrelated laboratory reports that inhibiting cleavage of the restraint receptor reduces tau phosphorylation as well as plaque burden [24]. Neither is a theory of tau; both are places where one could be attached.

**Regional selectivity.** Nothing in either mechanism explains why entorhinal cortex and hippocampus fail first. Receptor and ligand expression rise with age across cortex. A mechanism with a uniform substrate does not by itself produce a regionally ordered disease. The one regionally specific finding in this paper — the sparing of cerebellum in the Kalirin-7 series [55] — tracks the disease's topography rather than explaining it.

**The age curve.** Receptor and complement expression rise through early adult life and are elevated long before any symptom [11]. Symptomatic disease begins decades later. Whatever converts a long-standing elevated ligand-and-receptor state into synapse loss is in neither account.

**Excitatory–inhibitory balance.** The ligand's synaptic colocalisation is several-fold lower at inhibitory synapses [11]. If the mechanism preferentially removes excitatory contacts it should shift the cortex's excitatory–inhibitory balance in a specific direction, with network consequences that are measurable and have not been measured.

### 11.4 A receptor with more addresses than the account claims

One further caution belongs here, because it bears on how much weight the weld can carry.

Independent laboratories have placed the same receptor in places the restraint programme never claimed. It inhibits TREM2 signalling on microglia; it suppresses glutamate transporters on astrocytes via mTOR, with suppression of the astrocytic pool alleviating amyloid-induced excitotoxicity [57]; a cleaved cytoplasmic fragment accumulates in the Golgi, binds the GAT domain of GGA3, disrupts Golgi transport and lysosomal maturation, and inhibiting the cleavage reduces plaque burden, tau phosphorylation and memory deficits [24]; it mediates myelin inhibition of axonal regeneration [3]; and a non-classical class I ligand expressed in layer 6 neurons regulates plasticity through a *microglial* receptor rather than a neuronal one [37]. A receptor that appears in every cell type, binds every ligand tried, and whose blockade improves every outcome measured is a receptor whose literature is running ahead of its evidence.

The generous reading is that an inhibitory receptor family evolved to hold immune effectors in check has been recruited by several cell types to hold their effector functions in check, and one antagonist would release all of them. The sceptical reading is that fields reporting only positive findings for a single target tend to be corrected later. Both readings are available and this paper takes neither. What follows for the weld is narrower and is not in doubt: if the receptor has several addresses, then a result obtained by deleting it globally cannot be assigned to the neuronal address without a conditional, and much of the disease work is global.

The genetics are consistent with the locus mattering and are not consistent with it mattering greatly or simply. The gene cluster does not appear as a large-effect locus in large European association studies [6], and the most informative recent work establishes an ancestry-specific effect in which the lead variant is associated with

decreased risk in Europeans and increased risk in East Asians, with a different family member's expression tracking the variant in each [12]. A locus whose effect direction inverts between populations is not a locus that establishes a mechanism. The same caution applies, in a different form, to the other account: the gene encoding Ephexin5 is not an established Alzheimer risk locus either, and its only Mendelian human phenotype points away from the prescription the account generates [19].

# 12. One measurement, three readings

It is worth collecting, in one place, what a single experiment would settle, because the convergence is unusual and it is the practical conclusion of this paper.

Array tomography on Braak-stratified human cortex, with per-synapse quantification of (i) the phosphorylated fraction of Ephexin5 at tyrosine 361, (ii) phospho-serine-3 cofilin normalised to total cofilin, (iii) receptor and ligand immunoreactivity at the same synapse, and (iv) rod burden in the same specimens — reported as a joint distribution and not as four means — decides the following.

**It decides the sign at the licensing tyrosine**, and therefore which of the two readings in Section 6 describes the Alzheimer dendrite. If occupancy is elevated, the kinase reading holds, the Rac1 arm is the determined one, and the therapeutic object becomes the kinase. If reduced, the receptor reading holds, Ephexin5 accumulation should be reinterpreted as a Cdc42-biased and plausibly compensatory response, and the prescription that follows from the exchange-factor account is inverted rather than weakened.

**It decides whether the cofilin contradiction is a sampling artefact or a real disagreement about direction.** Increased dispersion with an uninformative mean supports the first; a mean that moves, with sign flipping between early and late disease and rods confined to the late window, supports the second.

**It decides whether receptor occupancy and cytoskeletal state are coupled at the level of the individual synapse.** This is the question the weld asserts and nobody has asked. If synapses bearing ligand and receptor are not cytoskeletally distinguishable from those that do not, the weld fails at its most basic prediction, and no amount of biochemistry at the level of the homogenate will have told anybody.

Two reagent facts make this tractable rather than aspirational. The site-specific antibody against the licensing tyrosine exists and was validated against both a phosphomutant and a genetic null [36]. And array tomography has already been applied to this exact question in human cortex, by the laboratory that reported the complement ligand, with one of the technique's developers as an author [11]. What has not been done is to point the second method at the first reagent.

A perfusion-fixed animal arm should run first, for a reason that is about bias rather than convenience. Post-mortem phospho-lability biases a human negative towards the null, so a human study that finds no difference has not excluded one; and only an animal arm permits the receptor to be engaged acutely with a defined ligand while the two occupancies are read out, which is the manipulation that converts a correlation into a direction.

*Part V*

# Consequences

*What follows for therapy, for the shape of a defensible programme, and for the two accounts themselves. Every intervention either account generates removes a restraint; none restores an address. The evidence is tabulated, the predictions are stated so that a result can contradict them, and the conditions under which this paper's own argument fails are given.*

## 13. Every proposal on the table removes a restraint

### 13.1 The pattern, stated first

Four interventions follow from the two accounts, and they are usually discussed as alternatives with different risks. They have a property in common that is not usually noticed and that follows directly from Section 10: **each of them works, if it works, by removing a restraint.**

Lower Ephexin5 — remove a synapse-restricting exchange factor. Inhibit Rho-associated kinase — remove the effector arm that restricts spine growth. Block the receptor — remove the brake that restrains plasticity and licenses spine shrinkage. Mimic reelin — add a ligand whose documented structural action is to stop a process, delivered chronically instead of transiently.

If both accounts are accounts of an intact restraint system running without a stopping rule, then this is not a coincidence. It is what the accounts entail. And it has a consequence: none of the four restores a molecule to a place, a mark to a residue, or a signal to a receptor that cannot read it. They are all subtractions from a system whose problem, on the reading developed here, is misdirection rather than excess.

### 13.2 "Lower Ephexin5" cannot be prescribed in that form

The therapeutic reading drawn from the exchange-factor account was stated by its authors: because the enzyme is minimally expressed in healthy adult brain, because its pathological elevation drives amyloid-induced memory impairment, and because deleting it rescues spines and behaviour, strategies aimed at reducing it may be effective [46]. The logic is clean and the window looks wide. Three independent findings have made the inference untenable in that form, any one of which would suffice.

**Reducing the protein removes the growth arm with the collapse arm.** Knockout neurons cannot sustain activity-driven spine growth, and the deficit is rescued only by a catalytically competent enzyme [41]. A drug that lowers the enzyme in a patient whose remaining synapses depend on activity-driven structural plasticity removes part of the repair capacity along with part of the damage.

**Chronic reduction has a human phenotype, and it is a dementing one.** Heterozygous loss-of-function mutations in the gene encoding Ephexin5 cause autosomal-dominant hereditary cerebral small-vessel disease with severe osteoporosis and osteoporotic fracture. One non-synonymous mutation co-segregated completely in two families, a further non-synonymous and a stop-gain mutation were found in sporadic cases, every carrier had severe osteoporosis, and a transgenic mouse carrying one variant developed small-vessel pathology, behavioural phenotypes and osteoporosis. The in vitro mechanism is **RhoA/ROCK2 inactivation** with consequent F-actin disorganisation in vascular smooth-muscle and endothelial cells [19].

*Grade: Established, tier H, complete co-segregation in two families plus sporadic cases plus an animal model.*

The direction is the detail worth dwelling on. In neurons, the proposal casts RhoA activation by this enzyme as destructive and its suppression as therapeutic. In vessels and bone, loss of function causes disease *through* RhoA/ROCK2 inactivation. The same axis, suppressed, is pathogenic in one tissue and proposed as protective in another. There is nothing incoherent in that — Rho signalling is context-specific, and a set-point too high in a spine may be load-bearing in a smooth-muscle cell. But it removes the argument that made the enzyme attractive: it is not dispensable in the adult human, and one functional copy is not enough. The difference between this and ordinary pleiotropy is the difference between a hypothetical off-target effect and a documented on-target one.

**And the sign of the neuronal effect is not secure** (Sections 6.1–6.4).

What replaces the prescription is narrower and harder. If the lesion is a switch stuck in one position rather than an excess of protein, the object of intervention is the switch: an agent biasing substrate selection without depleting the enzyme — functionally a small-molecule mimic of the Y361F state — or an agent restoring the activity-driven dephosphorylation that potentiation normally produces. Neither exists. Both would require structural work on the interface the phosphomutant studies localise, which is at least a defined starting point. Neuron-restricted targeted degradation solves the tissue-selectivity problem created by the vascular phenotype but not the intraneuronal problem created by the growth arm, because it removes the protein rather than biasing it.

## 13.3 Inhibiting Rho-associated kinase inherits the problem twice

Blocking the effector rather than the exchange factor is the obvious alternative and has independent motivation: a Rho-associated-kinase inhibitor prevented amyloid-induced actin stabilisation, synaptic impairment and synaptic loss in culture by blocking cofilin phosphorylation [43].

Two difficulties follow from this paper rather than from the drug's own literature.

The first is a symmetry that is not generally remarked upon. The human Mendelian syndrome produced by loss of the upstream exchange factor is caused by RhoA/ROCK2 **inactivation** in vascular cells [19]. A systemic Rho-associated-kinase inhibitor reproduces, pharmacologically and in the same tissue, the biochemical state that the Mendelian disease produces genetically. That is a specific, mechanistically grounded reason to expect cerebrovascular liability from chronic inhibition in this indication, and it is stronger than a generic caution about a pleiotropic kinase. It argues for isoform selectivity, for central restriction, and for deliberate vascular monitoring in any trial.

The second is the one Section 7 creates. The rationale rests on phospho-cofilin being elevated and that elevation being the lesion. The other human series reports it reduced, and on the turnover reading both are lesions. A drug whose mechanism is to lower phospho-cofilin, given to a cortex that may contain synapses on

both sides of a non-monotonic band, needs a stratifying measurement that does not exist. This is not a reason not to develop it. It is a reason to run the dispersion measurement of Section 12 first.

### 13.4 Blocking the receptor: the best-precedented and the bluntest

The receptor account reaches its therapeutic section with two pieces of prior evidence that are rare in combination. Blocking the receptor acutely in an adult mammalian brain, with a soluble decoy delivered locally for one week, restored a structural and functional deficit established months earlier [7]. And antagonist antibodies against the human receptor exist and have been through clinical trials in oncology, where it is an immune checkpoint on tumour-associated macrophages, so the toxicology, manufacture and human pharmacology of blocking it systemically are partly known.

Four reasons the oncology programme is not a neurology programme. The antibodies were designed to act on peripheral myeloid cells, and no meaningful brain exposure should be assumed. The oncology intent is to activate immunity by removing a myeloid checkpoint; in the brain the intent is partly the same and partly different, and these are not the same pharmacology. Chronic dosing in an ageing population is a different safety problem from time-limited dosing in advanced cancer, and removing an inhibitory checkpoint chronically is the mechanism by which checkpoint inhibitors cause autoimmune toxicity. And the epitope may matter: an antibody that blocks class I MHC binding will not necessarily block the complement fragment or amyloid, even though all three engage the same immunoglobulin domains, and which ligands a given antagonist displaces has not been asked for any existing molecule.

Then there is the double edge, which the programme's own data make unavoidable. Every reported consequence of blocking this receptor is an increase in plasticity: more spines, more functional synapses, larger potentiation, deficient depression, faster learning, an unclosing critical period [7,1,49]. In a young mouse over weeks this reads as benefit. In an ageing human over years it might read as something else — a cortex that cannot stop changing, that cannot weaken a synapse, that cannot consolidate. Long-term depression is not a defect; it is half of the mechanism by which a circuit stores anything, and every manipulation in this programme impairs it. The result that the receptor is specifically required for activity-dependent spine shrinkage [1] sharpens this rather than softening it: the drug would not be blunting a general restraint, it would be removing the cell's ability to carry out the structural half of depression.

Blocking a brake to treat a disease of excessive braking is coherent, and it is not the same as repairing something broken. The therapeutic window lies between too little restraint and too much, and nobody has measured either edge.

### 13.5 Two routes that are not subtractions from the neuron

Two of the available routes differ in kind from the other four, and both deserve more attention than they have had.

**Ligand blockade rather than receptor blockade.** Prevent the disease ligands from reaching the binding site while leaving the physiological ligand's engagement intact. The endogenous antagonist work is the proof of principle that this is chemically possible and that it produces the expected downstream rescue [31]. It is the route that preserves the brake, and it is the least developed.

**Upstream complement modulation.** If the complement fragment is the ligand that matters, then reducing classical-pathway activation reduces the ligand without touching the receptor at all — and it would act on the glial and the neuronal arms simultaneously, which nothing else on this list does. The relevant point

from Section 3.5 is that this route's target is the *deposited* pool, which is the pool the 2016 negative result says the receptor may not bind; so the route and the objection bear on each other, and the binding experiment with thioester-immobilised ligand decides both.

### 13.6 The class that does not exist

On the reading developed in Part IV, the therapeutic question that neither account asks is this: **what would it take to put a competent enzyme back where its substrate is?**

For Kalirin-7 the answer would be an agent acting on the anchoring interaction rather than on expression, and on the eviction reading an agent that raised expression modestly would do nothing [54]. For Ephexin5 it would be an agent biasing substrate choice rather than lowering abundance. For reelin it would be an agent that frees the ligand from sequestration or improves transduction per unit ligand, not one that supplies more ligand into a sequestering compartment [17]. For RhoA it would be an agent that prevents sequestration into somatic inclusions, which is a tau problem rather than a Rho problem [28].

These are harder targets than a kinase, and three of the four have no precedent in any indication. We state them not because any is near a molecule, but because a field that has produced four subtraction strategies and no re-addressing strategy should notice that the asymmetry follows from its measurements rather than from the biology. You cannot design a drug against a variable you do not measure.

## 14. What a defensible programme looks like

Three steps, in order, and the first two are measurements rather than molecules.

**Establish the sign before choosing a direction.** Measure the licensing-tyrosine occupancies and the GTPase activity states in the same tissue, with per-synapse resolution, and measure the dispersion of phospho-cofilin rather than its mean (Section 12). Both are achievable with existing reagents and neither has been done. Until they have, every prescription on this pathway is a bet on an unmeasured sign, and the two bets currently on the table are placed on the arm where the sign cannot be known.

**Measure received signal, not ligand.** For the reelin arm the soluble ApoER2 fragment in cerebrospinal fluid is an assay of whether the signal arrived, and it decouples from ligand level in disease [17]. It should be the primary pharmacodynamic endpoint of any reelin-directed agent, and it can be validated in an unselected population now. For the receptor arm the equivalent assay does not exist and should be built: a measure of receptor engagement at the synapse rather than of ligand abundance in the tissue. The covalently tethered ligand is an unusually good substrate for this, because it stays where it was generated — which also means that a fluid assay for it may be measuring the wrong pool.

**Prefer targets whose human loss-of-function phenotype is known and favourable.** The single most useful fact in this paper for a drug-discovery decision is that haploinsufficiency of the gene encoding Ephexin5 causes a dementing vasculopathy with skeletal fragility [19]. That fact was available from 2023 and it retires a target. The corresponding question has not been asked for *KALRN*, for *TIAM1* or, in a form that settles anything, for the receptor gene cluster, where the association evidence points in opposite directions in different ancestries [12]. It should be asked before, not after, a programme commits.

A fourth step belongs with these and is cheaper than any of them. **Do the binding experiments with the physiological form of each ligand.** Both ligand arms of the receptor account carry an unanswered negative obtained with the physiological rather than the recombinant species [47],[26]. Neither negative is fatal; both are cheap to address; and a programme that proceeds to the clinic without addressing them is carrying a risk it did not need to carry.

# 15. Strength of evidence

**Table 3 — Every claim this paper relies on, with its grade.**

| Claim | Source | Grade | Note |
|---|---|---|---|
| **Synapse loss is the strongest structural correlate of cognitive impairment** | Terry et al., 1991 | Established, H | Sets the variable to be explained |
| **Class I MHC molecules are expressed by neurons under activity control and are required for circuit refinement** | Corriveau 1998; Huh 2000; Datwani 2009; Lee 2014 | Established, A | Multiple alleles, more than one laboratory |
| **PirB/LilrB2 is a lifelong brake on cortical plasticity and spine density, cell-autonomously** | Syken 2006; Djurisic 2013; Vidal 2016 | Established, A | Sparse deletion controls for circuit effects |
| **Acute blockade in an adult raises spines and functional synapses and permits recovery from amblyopia** | Bochner 2014 | Established, A | Pharmacological, adult, reversible |
| **The receptor is required for activity-dependent spine shrinkage; its loss accelerates motor learning** | Albarran 2021 | Established, A | Genetic plus acute, with single-spine mechanism |
| **The receptor's tail recruits SHP-1 and SHP-2** | Syken 2006 | Established, A | Association only; substrates not identified |
| **The receptor's phosphatases dephosphorylate a third-party receptor tyrosine kinase in neurons** | Fujita 2011a, 2011b | Established, A/N | Growth cone, myelin ligand, p75-dependent |
| **LilrB2/PirB binds synthetic Aβ oligomers** | Kim 2013; Smith 2019 | Supported, N/X | One of three survivors of fifteen candidates |
| **LilrB2 binds Aβ oligomers extracted from human brain** | Smith 2019 | **Contradicted** | No detectable affinity; unanswered since 2019 |

| Claim | Source | Grade | Note |
|---|---|---|---|
| **C4d binds LilrB2/PirB at nanomolar affinity (soluble recombinant)** | Brott 2025 | Supported, X | Selective within the family |
| **Soluble receptor binds C4d immobilised by complement activation** | Hofer 2016 | **Contradicted** | Not detected; the deposited pool is the human pool |
| **C4d marks ~30% of human excitatory synapses and rises with age and disease** | Brott 2025 | Supported, H | Array tomography, small specimen numbers |
| **C4d infusion removes spines in adult wild-type cortex, abolished in PirB-null** | Brott 2025 | Supported, A | Sufficiency plus genetic necessity; supraphysiological dose |
| **The receptor's phosphatases act on EphB2, Ephexin5 Y361 or Disabled-1** | — | **Unevidenced** | No indexed record pairs them |
| **GluN2B tyrosine dephosphorylation in disease is mediated by SHP-1/2** | — | **Unevidenced**, superseded | The established enzyme is STEP61 (Kurup 2010) |
| **Aβ binds EphB2 and drives its proteasomal degradation; restoring EphB2 rescues potentiation and memory** | Cissé 2011 | Supported, A | Bidirectional manipulation within one study |
| **EphB2 writes Ephexin5 Y361; the phosphate recruits Ube3A and licenses destruction** | Margolis 2010 | Established, A/N | Antibody validated against phosphomutant and null |
| **Ephexin5 is elevated in human Alzheimer hippocampus** | Sell 2017 | Supported, H | The account's only direct human anchor; unreplicated |
| **Deleting Ephexin5 rescues spines and behaviour in hAPP mice** | Sell 2017 | Supported, A | One line, one laboratory, with a presymptomatic arm |
| **Ephexin5 loads RhoA and Cdc42; Y361 sets the choice; the enzyme is required for activity-driven growth** | Petshow 2025 | Supported, N | Catalytic-dead and phosphomutant rescues |
| **A dendritic phosphatase acts on Ephexin5 phosphotyrosine** | Petshow 2025 | Established, N | Exists; unnamed |
| **Heterozygous *ARHGEF15* loss of function causes cerebral small-vessel disease with osteoporosis, via RhoA/ROCK2 inactivation** | Ding 2023 | Established, H | Two families with complete co-segregation plus a mouse |

| Claim | Source | Grade | Note |
|---|---|---|---|
| **Aβ oligomers deplete Kalirin-7 from spines; overexpression rescues** | Xie 2019 | Supported, N | Eviction shown; destruction not shown |
| **Kalirin and Kalirin-7 are reduced at transcript and protein in human Alzheimer hippocampus** | Youn 2007a, 2007b | Supported, H | 19 vs 15, cerebellum spared; denominator unresolved |
| **Tiam1 couples the NMDA receptor to Rac1-dependent growth and is phosphorylated on stimulation** | Tolias 2005 | Established, N | A builder recruited by activity |
| **Src writes Tiam1 Y384, creating a Grb2 site; ERK then licenses local degradation, probably via calpain** | Woodcock 2009 | Established, X | Authors' own hedge on the protease |
| **Calpain cleaves Tiam1 in neurons or in Alzheimer's disease** | — | **Unevidenced** | No indexed study found |
| **Total RhoA is lower in Alzheimer hippocampus and redistributed into tangle-bearing somata; Rac1, Cdc42, PAK unchanged** | Huesa 2010 | Supported, H | The only survey of all three in human tissue |
| **Phospho-cofilin serine 3 is lower in human Alzheimer brain** | Kim 2013 | Supported, H | ~38% lower, normalised to total cofilin |
| **Phospho-cofilin serine 3 is higher in human Alzheimer cortex, and the elevation is the lesion** | Rush 2018 | Supported, H | Independent group; ROCK-inhibitor-reversible |
| **Cofilin severs at low occupancy and stabilises and bundles at high occupancy** | Bamburg & Bernstein 2016 | Established | Forbids assigning direction from a bulk mean |
| **Dephosphorylation is sufficient to induce cofilin–actin rods, which occlude neurites** | Minamide 2000 | Established, N | The structural endpoint of the low-phosphate excursion |
| **Reelin binds VLDLR and ApoER2, induces Disabled-1 phosphorylation; loss of any component raises phospho-tau** | Hiesberger 1999; Ohkubo 2003 | Established, A | Phosphatase control attributes the rise to kinase gain |
| **Reelin inhibits GSK-3β through PI3K and PKB** | Beffert 2002 | Supported, N | Genetic dependency controls |

| Claim | Source | Grade | Note |
|---|---|---|---|
| **Reelin activates Src-family kinases; Disabled-1 is both substrate and activator** | Bock & Herz 2003; Arnaud 2003 | Established, A | The enzymes shared by both accounts |
| **Reelin phosphorylates cofilin serine 3 and stabilises actin** | Chai 2009 | Supported, N | The pathway's second limb, usually omitted |
| **Reelin is written by a restricted interneuron subset and read by pyramidal neurons** | Pesold 1999 | Supported, A | Writer and reader are different cells |
| **Reelin mRNA and protein rise at late Braak stages while Disabled-1 phosphorylation falls; ligand trapped with amyloid** | Cuchillo-Ibáñez 2016 | Supported, H | Independent group; CSF arm |
| **Reelin-expressing entorhinal pyramidal cells are reduced; reelin-positive interneurons are not** | Chin 2007 | Supported, A/H | The loss is population-specific |
| **Reelin activates EphB2 independently of ApoER2/VLDLR, then drives its degradation; the cellular consequence is retraction** | Bouché 2013 | Established, N | Three genetic and pharmacological controls |
| **Fyn is mistargeted to the postsynaptic density in a tau-dependent manner** | Ittner 2010 | Supported, A | Supplies the kinase reading of Section 6.2 |
| **Postsynaptic tyrosine-kinase activity rises in disease and raises occupancy at both licensing tyrosines** | This paper | **Inference** | Prediction 1 |
| **Receptor-recruited phosphatase activity rises in disease and lowers the same occupancies** | This paper | **Inference** | Prediction 2; opposite sign to the above |
| **The two readings are both true and spatially segregated within the spine** | This paper | **Inference** | Prediction 3; favoured here |
| **Both excursions at cofilin serine 3 arrest filament turnover, and turnover is the abnormal variable** | This paper | **Inference** | Prediction 5 |
| **The disease-relevant change in this pathway is predominantly of address or covalent state** | This paper | **Supported in 4 of 5 regulators; one exception** | Section 8.3 |

# 16. The shorter account that survives

Stripped of everything this paper has graded Unevidenced or Contradicted, the following remains. It is shorter than either account and it is stated with its holes left open rather than filled.

A receptor expressed on cortical and hippocampal pyramidal neurons restrains spine density and plasticity throughout life, cell-autonomously, and is required for the structural half of activity-dependent synaptic depression. Its acute blockade in an adult restores spines and function. Its cytoplasmic tail recruits two tyrosine phosphatases, which in neurons have been shown once to act on a third-party receptor tyrosine kinase in the same membrane with a structural consequence. Which substrates they reach in a dendritic spine is unknown.

At least one ligand that rises in human cortex with age and further with Alzheimer's disease engages that receptor, marks roughly a third of human excitatory synapses, and — in soluble recombinant form, at a supraphysiological dose — removes spines from a normal adult cortex in a manner abolished by deletion of the receptor. Whether the physiologically deposited form of that ligand engages the receptor has not been shown and one prior study failed to detect it. Whether the other proposed ligand engages the receptor in the form found in human brain has been tested once, under standardised conditions, and was not detected.

Inside the spine, three exchange factors govern the actin machine. One of them is not a brake but a switch, whose choice between a restrictive and a constructive GTPase is set by the occupancy of a single tyrosine, and which is required for activity-driven growth. A second is displaced from spines by amyloid oligomers and is also under-expressed in human Alzheimer hippocampus. A third is governed, in non-neuronal cells, by an architecture of the same design, which has never been looked for in a neuron. In the only human survey to measure the three GTPases together, the restrictive one is reduced in total and redistributed out of the neuropil and the other two do not move.

The terminal effector is a non-monotonic protein whose direction of change in human Alzheimer cortex is reported both ways by two independent series, neither replicated, and whose two excursions have the same functional consequence: a cytoskeleton that cannot turn over.

Between the receptor and the exchange factors there is one proposed limb. It has a demonstrated mechanism class, no demonstrated substrate in this compartment, and — if it operates as proposed — it predicts the opposite covalent state from the one the account below it requires.

That is the account. It is less satisfying than either of the two it replaces, and every one of its holes is a measurement rather than a mystery.

# 17. Predictions

Each is stated so that a result can contradict it.

**1. The paired licensing-tyrosine measurement.** In human Alzheimer and age-matched control cortex, the phosphorylated fraction of Ephexin5 at Y361 and of Tiam1 at Y384 will both be elevated relative to total protein, with active Rac1 reduced and active RhoA not reliably changed. *Refuted if* the two fractions move in opposite directions, or if active Rac1 is unchanged while active RhoA rises. This is the primary falsifier of the kinase reading and it requires no new reagent for the Ephexin5 arm. The perfusion-fixed animal arm should run first, because post-mortem phospho-lability biases a human negative towards the null.

**2. The receptor's phosphatase limb reaches EphB2 and the licensing tyrosine.** In mature hippocampal neurons, engaging the receptor with either ligand will lower phospho-EphB2 and lower phospho-tyrosine Ephexin5, and both effects will be prevented by inhibition of SHP-1 and SHP-2. This is the 2011 Trk assay, re-pointed. *Refuted if* neither moves, in which case the weld between the two accounts has no biochemical basis in this compartment and the exchange-factor economy is downstream of amyloid but not of the receptor.

**3. The two enzymes are segregated within the spine.** Across single excitatory synapses in human Alzheimer cortex, the dispersion of the phospho-Y361 fraction will be increased relative to control while its mean is not reliably shifted, and its covariance with phospho-serine-3 cofilin will be reduced. *Refuted if* dispersion is unchanged while the mean shifts consistently.

**4. Receptor occupancy and cytoskeletal state are coupled at the single synapse.** Within one Alzheimer cortex, synapses bearing receptor-and-ligand immunoreactivity will differ in phospho-serine-3 cofilin from synapses in the same section that do not, and the difference will exceed the between-case variance. *Refuted if* ligand-bearing and ligand-free synapses are cytoskeletally indistinguishable — which would falsify the weld at its most basic prediction and would not be visible in any homogenate.

**5. Both excursions at serine 3 arrest turnover.** In cortical neurons, photoactivation or photobleaching measurement of actin turnover in individual spines will show reduced turnover both when cofilin phosphorylation is raised and when it is lowered past the severing range, with a maximum of turnover in the physiological band. *Refuted if* turnover rises monotonically as phospho-cofilin falls.

**6. The sign at serine 3 flips with stage.** In a Braak-stratified human cortical series with per-synapse resolution, phospho-serine-3 normalised to total cofilin will be higher than control at early stages and lower at late stages, with rod burden confined to the late window. *Refuted if* the direction is constant across stages, which would support dispersion rather than stage as the explanation of the contradiction.

**7. Kalirin-7's two lesions are separable.** Subcellular fractionation or array tomography of human Alzheimer cortex will show Kalirin-7 reduced in the postsynaptic fraction by a larger factor than total cortical Kalirin-7 is reduced, and the total reduction will shrink when normalised to a neuron-specific denominator. *Refuted if* the synaptic and total reductions are proportionate and survive neuronal normalisation, which would make the lesion one of abundance alone.

**8. The deposited ligand engages the receptor.** Surface plasmon resonance or an equivalent with C4d immobilised through its own thioester, rather than through a tag, will show detectable binding to the soluble receptor ectodomain. *Refuted if* binding is undetectable, as it was in the one prior attempt — in which case the human anatomy and the mouse causality in the complement arm concern two different ligand pools and the arm needs rebuilding.

**9. The Tiam1 architecture operates at the synapse.** In cultured neurons, amyloid oligomers will increase phospho-Y384 Tiam1 and reduce total Tiam1, and the reduction will be prevented by Src-family inhibition and by calpain inhibition independently. *Refuted if* oligomers reduce Tiam1 without raising phospho-Y384, which would indicate a different degradation route.

**10. Reelin lowers Ephexin5 before it consumes its receptor.** In cortical neurons, recombinant reelin will produce a transient fall in total Ephexin5 preceded by a rise in phospho-tyrosine Ephexin5, both dependent on EphB kinase activity and both independent of ApoER2 and VLDLR. *Refuted if* reelin alters neither.

**11. Received signal, not ligand, tracks the disease.** In an unselected clinical population, the cerebrospinal-fluid soluble ApoER2 fragment will correlate with cognitive trajectory better than cerebrospinal-fluid or cortical reelin level does. *Refuted if* reelin level is the better predictor.

**12. The address lesions precede the abundance lesions.** In a staged animal series, redistribution of Kalirin-7 out of the postsynaptic fraction and of RhoA out of the neuropil will be detectable at a stage at which total cortical levels of both are indistinguishable from control. *Refuted if* the abundance change is detectable first or simultaneously.

# 18. What would refute this paper

The paper makes three claims of its own. Each has a defined failure condition.

**That the two accounts are joined by an unmeasured limb.** This fails the moment anybody measures it. If receptor engagement is shown to alter phospho-EphB2 or phospho-tyrosine Ephexin5 in a dendritic spine, the limb is no longer unevidenced and Section 5 is of historical interest only. We would regard that as the best outcome in this paper, and we note that the experiment is a re-pointing of an assay that has existed since 2011.

**That the two accounts predict opposite signs at the licensing tyrosines.** This fails if the readings turn out not to be in competition — for example if the receptor's phosphatases prove to be excluded from the postsynaptic density proper by a scaffold that holds them at the periphery, so that the two enzymes never contest the same substrate pool. That would not make either reading wrong; it would make the collision an artefact of treating the spine as one compartment, which is precisely the error this paper accuses others of. The honest position is that prediction 3 is as much a test of our framing as of the biology.

**That the lesion is misdirection rather than damage.** This fails if abundance, correctly measured, turns out to move in the direction each account requires. The most likely single result to go against us is the one named in prediction 1: if active RhoA measured by pulldown in human Alzheimer cortex, with the neuropil

fraction separated from the somatic, is elevated while active Rac1 is reduced, the seesaw is a quantity lesion after all and Section 4.6 is wrong. We regard this as the most likely of the three to come out against us, because the existing human survey measured protein rather than activity state and did so in end-stage tissue. It would also fail if the Kalirin-7 result generalises — if total cortical levels of Ephexin5's phospho-fraction, of Tiam1 and of the GTPases prove, on proper measurement, to move in the predicted directions, then the exception reported in Section 8 was the rule and the generalisation was an artefact of which molecules happened to have been measured.

A fourth failure condition applies to the synthesis rather than to any claim. If the restraint receptor's amyloid arm is abandoned — if the 2019 negative result is confirmed and the receptor proves not to bind brain-derived oligomers — then one of the two ligand arms is lost and the weld becomes a weld between the complement arm and the exchange-factor economy. Nothing in Part III would change, because the phosphatase limb is the same limb whichever ligand occupies the receptor. But the paper's framing, in which two amyloid-initiated accounts are welded at a joint, would need restating as a single complement-initiated account joined to an amyloid-initiated one, and the question of why they should share an executor would become sharper rather than easier.

# 19. Limitations

**This paper reports no measurement.** It is a reading of published work. Its three original claims — the opposite-sign collision at the licensing tyrosines, the turnover reconciliation at serine 3, and the characterisation of both accounts as misdirection — are inferences from literatures that do not cite one another, and each is labelled as such in Table 3.

**The central human measurement on which Section 4.6 depends is old and semi-quantitative.** The GTPase survey dates from 2010, measures protein rather than GTP-loading, and is immunohistochemical with one quantitative immunoblot arm. We have leaned on it heavily because it is the only study of its kind we could identify, and the field would be better served by a modern replication with activity-state readouts and stage stratification than by any argument here.

**The licensing-tyrosine parallel is architectural and therefore weaker than it looks.** No claim of sequence homology between the Ephexin5 and Tiam1 motifs is made and we are not aware of evidence for one. What is claimed is a shared regulatory design assembled from two literatures that do not cite one another, and the Tiam1 half has never been shown in a neuron. If the architecture does not operate at the postsynaptic density, Section 6's collision loses one of its two residues, although not the collision itself, which stands on the Ephexin5 residue alone.

**The transposition criticisms cut both ways.** This paper criticises the calpain–Tiam1 claim as an untested transposition from epithelial cells, and then proposes, on the strength of the same epithelial papers, that the architecture may operate at the synapse. The difference is that ours is labelled a hypothesis and given an experiment that tests it. A reader who finds the distinction too convenient is entitled to apply prediction 9 against us.

**The weld is a composition, and compositions have no single author.** The route audited in Section 5 is assembled from reviews, teaching summaries and the connective prose of papers whose primary results are not in dispute. We have cited the primary work at every node and have tried not to attribute the composition to anyone. Where a step appears only in secondary presentation, we have said so; where we could not determine whether a step is held by the laboratories whose results it joins, we have graded the step rather than the laboratory.

**Scope.** This paper concerns excitatory synapses on cortical and hippocampal pyramidal neurons. It says nothing about inhibitory synapses, about the interneuron populations that supply the reelin signal, about microglial engulfment as a parallel arm beyond what Section 3.4 requires, or about the brainstem nuclei where tau pathology appears earliest.

**Timing.** Both accounts are ligand-gated and therefore describe the symptomatic and immediately prodromal period. They execute; they do not initiate. Nothing here bears on what starts the disease, and the four absences in Section 11.3 — tau, regional order, the age curve, and excitatory–inhibitory balance — are absences in both.

# 20. Conclusion

Two accounts of the failing Alzheimer synapse were set against one another and against the primary record for every molecule they name.

The receptor account is the better-evidenced of the two in the compartment it describes, and its strongest single result is a genetic one: a complement fragment applied to a normal adult cortex removes dendritic spines, and deletion of one receptor abolishes the loss entirely. Its weakest points are both at the same place — in each of its two ligand arms, the binding has been tested with the physiological form of the ligand and was not detected, once in 2016 and once in 2019, and neither negative has been answered.

The exchange-factor account is the more detailed of the two and the more exposed. Its central human anchor is a single unreplicated report of elevated protein; the gene concerned has a Mendelian human phenotype that points the opposite way from the therapy it recommends; the enzyme it casts as a brake has turned out to be a switch required for growth; the one survey that measured its three GTPases together in human tissue returned the wrong sign on one and no change on the other two; and the step by which it removes the second of its two building enzymes has never been tested in a neuron.

Between them there is one limb. The receptor recruits two phosphatases — this is established — and in neurons those phosphatases have been shown once to strip the activating phosphate from a receptor tyrosine kinase in the same membrane, with a structural consequence that reverses when they are removed. On that precedent the field has assigned them four substrates in the dendritic spine. An indexed search pairing the receptor or its phosphatases with any of the four returns nothing, and the fourth belongs to a different enzyme entirely.

Doing the arithmetic across that limb produces the paper's central result. The exchange-factor account, extended through the kinase whose postsynaptic mistargeting in this disease is tau-dependent, predicts that phospho-occupancy at the two licensing tyrosines rises. The receptor account, by the definition of an inhibitory

motif, predicts that it falls. The same covalent state that one reading makes the mark of destruction, the other makes the mark of growth; and the receptor whose deletion accelerates learning and whose function is to permit spine shrinkage is drawn as acting through an enzyme that is required for spine growth and that the receptor's own limb would push into its growth-biased state. Only one of these can describe an Alzheimer dendrite, and the measurement that decides — the joint distribution of two phospho-fractions across single synapses — has never been attempted in disease tissue, although the discriminating antibody has existed, validated against a phosphomutant and a genetic null, for sixteen years.

At the terminal effector the two accounts disagree about sign and agree about outcome, and that is the paper's second result. One human series reports the phosphate on cofilin serine 3 elevated in Alzheimer cortex and the elevation to be the lesion; another reports it reduced, and the receptor account's own mechanism requires the reduction to run past the severing range into the regime where cofilin saturates and bundles actin into rods. Both excursions arrest turnover. The variable that is actually abnormal is neither the phosphate nor the activity but the rate at which the spine's filaments are renewed, and nobody has measured it.

The address claim survives in four of five regulators and fails in the fifth, and the failure is the most useful thing in it. When somebody did measure abundance in human tissue with a regional control, in nineteen hippocampi against fifteen, they found Kalirin-7 reduced at transcript and protein — and the pathway's literature has not cited it in nineteen years. The claim that survives is therefore not that abundance never moves. It is that the field has measured the variable it can measure rather than the variable the disease moves, and that where both have been measured in the same molecule they tell different stories.

What the two accounts jointly describe, when the weld between them is set aside, is a disease with no broken molecule in it. The receptor is intact and doing its job; its ligands have accumulated. The exchange factors are intact and catalytically competent; they are in the wrong compartment, or carrying the wrong mark, or reading a ligand their receptor can no longer transduce. Two kinds of misdirection — a restraint pressed by the wrong hand, and an apparatus at the wrong address — and not a single loss of function between them.

That is not a rhetorical flourish; it has a cost attached. Of the interventions these two accounts have generated, every one is a subtraction — lower the exchange factor, inhibit the kinase, block the receptor, supply more of a ligand that is already in surplus and already trapped. Each removes a restraint from a system whose difficulty, on the reading assembled here, is not that it restrains too much but that it restrains the wrong things in the wrong places. Nothing in the pipeline puts an enzyme back where its substrate is, and nothing can, because nobody is measuring where the enzymes are.

What should be done next is a measurement, not a molecule. Quantify the two licensing tyrosines and the three GTPase activity states in the same human tissue; report the joint distribution across single synapses rather than a set of means; and score, at each of those synapses, whether the restraint receptor is occupied. One of the two antibodies required has been validated against both a phosphomutant and a genetic null for sixteen years and has never been pointed at a diseased human brain. The imaging method required has already been applied to this exact cortex by the laboratory that found the ligand. Until the two are put together, every prescription on this pathway is a wager on an unmeasured sign, and the wagers currently on the table are placed on the arm where the sign cannot be known.

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*Bibliographic details for every entry below were verified against the PubMed record — title, authorship, year, journal, volume, pages, and the direction of the reported effect — rather than recalled. Two of this paper's central observations turn on the precise wording of published results, including two negative results and a pair of human series that report opposite signs, and a citation reconstructed from memory would have silently resolved disagreements the literature has not resolved. Where PubMed returned no DOI, the PubMed record is linked instead.*


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