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THE NET THAT WAS NEVER WOVEN

*The Perineuronal Matrix, the Cortical Immature Neuron,
and Why the Failure of a Net Is the Wrong Account
of Layer Two*

*Netting as the Terminal Act of Maturation, the Antagonism of Sugars,
the Second Vacancy, and Degradation Read as Permission*

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the disease begins where the net was never issued, and degradation is a permission rather than an exposure

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Abstract

Two literatures have converged on cortical layer two without meeting. The first holds that a population of prenatally generated neurons persists there in arrested maturation across the human lifespan, and that its distribution reproduces the topography in which tau pathology begins. The second holds that the perineuronal net — the lattice of chondroitin sulphate proteoglycans that closes the developmental critical period and thereafter shields the neuron it surrounds — is degraded in Alzheimer's disease by activated microglia and by upregulated matrix metalloproteinases, and that its loss exposes the neurons beneath it. The natural way to join the two is to say that layer two immature neurons lose their net and are thereby exposed. This paper argues that the natural joining is wrong, that it is wrong in an instructive direction, and that the correction produces a sharper hypothesis than the one it replaces.

The correction rests on three observations that are each established and have not, to our knowledge, been set beside one another. The first is that netting is not a service supplied to a finished neuron but the terminal act of its maturation: the condensation of proteoglycans into a net coincides with the end of the critical period, the sulphation ratio that permits condensation is required for the functional maturation of the cell it condenses around, and enzymatic removal of the net returns the adult cortex to a juvenile permissive state. A cell arrested short of maturity is, by the definition of the arrest, a cell arrested short of its net. The second is molecular and it is the hinge of the argument: polysialic acid, the modification that gives the immature neuron its defining marker, is the modification that prevents the net protein neurocan from binding the neural cell adhesion molecule. The immature cell does not merely lack a net; it carries on its surface the sugar that refuses one. The third is topographic and it is the most surprising: in the human entorhinal cortex, net-associated pyramidal cells are extremely rare, and the subcortical nuclei that take tau earliest — the locus coeruleus, the raphe, the basal nucleus of Meynert — are devoid of an aggrecan-based matrix altogether. The territories where the disease begins are not territories whose nets were stripped. They are territories that were never substantially netted.

We document, by systematic search, a second vacancy to set beside the molecular vacancy of the parent paper. The indexed literature contains no study combining the phrase "cortical immature neurons" with the perineuronal net, no study combining the paralamina nucleus with the perineuronal net in title or abstract, two studies combining doublecortin with the perineuronal net of which neither concerns the question, and ten combining polysialylated adhesion molecule with the perineuronal net of which none examines the cortical reservoir. Whether these cells bear nets, whether their neighbours' nets constrain them, and what becomes of them when the surrounding matrix is degraded are three questions that have not been asked.

We then develop the inversion that the correction makes available. If the adult matrix rich in chondroitin sulphate proteoglycans is, as the parent paper argued, the principal tissue obstacle

to a dormant cell's late maturation, then matrix degradation is not an exposure of the reservoir but a permission granted to it — and the disease supplies that permission at scale, by microglial engulfment and by protease activation, in the tissue where the reservoir lives. This converts the parent paper's largest acknowledged gap, the unknown trigger of awakening, into a named and testable candidate. We add a second route to the same compartment: the reversal of established maturation, or dematuration, which is a documented phenomenon in adult granule cells and cortical interneurons, and in which net loss and the loss of mature markers occur together. Two doors, then, into one immature compartment — the cell that never matured and the cell that reverted — and a disease that plausibly opens both. We are careful to say that the causal step in the second route is unproven: in the dematuration literature net loss accompanies the immature phenotype and has not been shown to cause it.

The paper closes with what this reframing predicts and what would refute it. It predicts that the reservoir cell should be net-negative in health, that the protection-by-net finding which spares aggrecan-coated neurons from tangles should be uninformative rather than reassuring for a cell that never had a coat, that enzymatic matrix degradation should increase the rate of reservoir awakening, and that the earliest-affected cortical fields should be net-poor before they are diseased. Each prediction is addressable with antibodies and tissue that already exist. The verdict is that the net's relationship to the disease's first cells is better described by absence than by loss, and that a field which has spent fifteen years asking what removes the net has not asked what happens where there was never one to remove.

I. Introduction

1.1 The Question and Its Obvious Answer

The parent paper to this one argued that the cellular inventory of Alzheimer's disease is missing an entry: a population of prenatally generated neurons, held in arrested maturation for decades, concentrated in cortical layer two and in the paralaminar nucleus of the amygdala, spent slowly across the lifespan and never replenished (Truchard and Gustafsson, 2026). It argued that the distribution of these cells reproduces the topography in which tau pathology begins, that the molecular question on which the correspondence turns — what tau these cells contain — has never been asked in any species, and that the experiment which would settle the matter is a stain.

One question that paper did not take up is what these cells' relationship is to the extracellular matrix in which they sit. It mentioned the matrix three times, always as background: as the tissue through which chains of immature cells move, as the adult environment whose chondroitin sulphate proteoglycans make late maturation difficult, and as one of several variables a spatial-transcriptomic

study might measure. The matrix was scenery.

It should not have been. A second literature, developed largely in parallel and with almost no traffic between them, holds that the perineuronal net — the condensed lattice of chondroitin sulphate proteoglycans that ensheathes the somatodendritic surface of certain neurons (Fawcett et al., 2019) — is a principal determinant of what a cortical neuron can and cannot do, and that it is degraded in Alzheimer's disease. That literature is now substantial. Nets close the critical period for experience-dependent plasticity, and their enzymatic removal reopens it (Pizzorusso et al., 2002). They protect the fast-spiking interneuron against oxidative stress (Cabungcal et al., 2013) and position the ion channels and receptors on which its physiology depends (Favuzzi et al., 2017). They are lost in the Alzheimer brain in proportion to plaque burden, engulfed by activated microglia, and their loss is prevented by eliminating those microglia (Crapser et al., 2020). Their principal protease regulator, matrix metalloproteinase-9, is differentially regulated across a range of conditions in which cortical excitability is disturbed, Alzheimer's disease among them (Wen et al., 2018).

Set the two literatures side by side and an answer to the question suggests itself immediately. Layer two holds immature neurons. Layer two is where the disease starts. Nets protect neurons. Nets are degraded in the disease. Therefore the immature neurons of layer two lose their protective nets to microglial degradation and are thereby exposed to the pathology that follows. The proposition is clean, it joins two well-evidenced bodies of work, and it converts a coincidence of address into a mechanism.

We think it is wrong. Not wrong in the sense of being unsupported — it is unsupported, but so are many things worth proposing — but wrong in its causal grammar, and wrong in a way that conceals a better hypothesis underneath it.

1.2 What Is Wrong With It

Three things.

The first is that netting is not a service rendered to an existing neuron. It is the last thing a neuron does on its way to being finished. The condensation of proteoglycans into a perineuronal net coincides with the closure of the critical period rather than following it at some later date (Pizzorusso et al., 2002), the developmental change in chondroitin sulphate sulphation that permits condensation is itself required for the functional maturation of the cells being condensed around (Miyata et al., 2012), and the same sulphation program governs the opening of the plasticity window as well as its closing (Hou et al., 2017). To be netted is to be mature; to be unnetted is to be, in the relevant sense, not yet finished. A cell defined by arrested maturation is therefore a cell one should expect to find without a net — not because something took it, but because the cell never reached the point in its own program at which the net is laid down.

The second is molecular, and it is the observation on which this paper principally turns. Polysialic acid — the modification of the neural cell adhesion molecule that supplies the immature neuron with its second defining marker, and which the reservoir literature uses to identify these cells — is not merely a correlate of the immature state. It is an active impediment to net assembly at the cell surface. Neurocan, a core component of the perineuronal net, binds the neural cell adhesion molecule directly, at a defined site within the molecule's second immunoglobulin domain; and the polysialylated form of that molecule binds neurocan less efficiently than the mature, non-polysialylated form (Sullivan et al., 2018). The sugar that marks the cell as immature is, in a literal biochemical sense, the sugar that keeps the net off it.

The third is topographic, and it is the observation that ought to have prevented the obvious answer from being attractive in the first place. In a survey of human cortex across motor, auditory, prefrontal and temporal areas, net-associated pyramidal cells were most numerous in primary motor and primary auditory cortex and declined through association areas — and in the entorhinal cortex they were *extremely rare* (Brückner et al., 1999). In a parallel subcortical survey, the structures preferentially affected by tau in Alzheimer's disease — the basal nucleus of Meynert, the dorsal thalamus, hypothalamic nuclei, the raphe nuclei and the locus coeruleus — were found to be devoid of a characteristic aggrecan-based matrix (Morawski et al., 2010). And in the human hippocampus specifically, the perineuronal protomap departs from that of other mammals: pyramidal cells there lack perineuronal nets altogether (Lendvai et al., 2013).

The regions where Alzheimer's disease begins, in other words, are not regions whose nets the disease removed. They are regions that carry few nets in the healthy brain and, in the case of the entorhinal principal cells, almost none. The protection whose loss the obvious answer invokes was never issued.

1.3 What This Paper Proposes Instead

If the net was never there, then two questions replace the one the obvious answer was trying to settle.

The first is what it means, for a cell and for a tissue, to be unnetted by constitution rather than by injury. Here the argument runs toward vulnerability, and it is the argument that the existing literature on net-poor nuclei already makes: a neuron without an aggrecan coat lacks the antioxidant shield that coat provides, lacks the physical barrier that a microglial process must breach before it reaches the somatodendritic surface, and lacks whatever contribution the coat makes to the striking resistance of netted neurons to neurofibrillary change (Brückner et al., 1999; Morawski et al., 2010). Applied to the reservoir, this says the dormant cell sits in the cortex for decades with none of the protections its netted neighbours carry.

The second question is the one we think is more interesting, and it runs in the opposite direction. The parent paper's second candidate conversion mechanism held that the danger in a dormant cell lies not in its dormancy but in its exit from it — that maturation attempted at eighty, in a tissue configured for stability rather than growth, is a project of sustained cytoskeletal and proteostatic strain. Among the features of that hostile tissue it named the adult extracellular matrix rich in chondroitin sulphate proteoglycans. And it conceded, as the largest hole in the account, that nobody knows what triggers awakening, and therefore that nobody can say whether the disease increases it.

The matrix literature supplies a candidate for exactly that missing trigger, and it does so by pointing in the direction the obvious answer got backwards. If the condensed adult matrix is what holds the tissue in a non-permissive state, then degrading it is not an exposure. It is a permission. Enzymatic removal of chondroitin sulphate proteoglycans in the adult cortex reopens experience-dependent plasticity (Pizzorusso et al., 2002); in the adult amygdala it returns fear memory to the erasable condition of the juvenile animal (Gogolla et al., 2009); it unlocks juvenile plasticity through decreased inhibition and increased gamma activity (Lensjø et al., 2017). These are experiments in which removing the net makes adult cortex behave like young cortex. The Alzheimer brain performs the same manipulation, chronically, regionally, and without an experimenter: microglia engulf the matrix in proportion to plaque burden (Crapser et al., 2020), and the protease system that cuts the lattice is dysregulated (Wen et al., 2018).

The hypothesis this paper develops is therefore that matrix degradation in Alzheimer's disease acts on the reservoir not by stripping it of a protection it never had, but by removing the tissue-level restraint that kept it dormant — and that the pathological consequence, if there is one, follows from awakening into a ruined tissue rather than from exposure within an intact one.

1.4 The Second Door

There is a further consequence, and it is the one that most directly answers the question that prompted this paper: what happens to immature neurons when the net fails.

The literature contains a phenomenon called dematuration: the reversal of established neuronal maturation in adult cells. Chronic treatment with a serotonergic antidepressant strongly reduces expression of the mature granule cell marker calbindin in the adult dentate gyrus, induces somatic membrane properties resembling those of immature granule cells, and abolishes the synaptic facilitation that characterizes mature dentate-to-CA3 transmission — changes that cannot be explained by the addition of newly generated cells and are best described as the dematuration of cells that were already mature (Kobayashi et al., 2010). The same treatment in the medial frontal cortex reduces the number of parvalbumin-positive cells and of net-bearing parvalbumin cells without inducing cell death, which the authors read as dematuration of that interneuron class (Ohira et al., 2013); in the marmoset it reduces parvalbumin expression and the number of

net-bearing neurons in the hippocampus while increasing immature markers (Ohira et al., 2019). The framework that has grown up around these findings treats the induced state as a return of juvenile-like plasticity to the adult brain, with net loss among its defining features (Umemori et al., 2018).

Dematuration therefore describes a second route into the immature compartment. The reservoir supplies cells that never matured. Dematuration supplies cells that matured and went back. If net loss is a component of the dematured phenotype — and in every study cited above it is — then a disease that degrades nets at scale is a disease that may be manufacturing immature-phenotype neurons in tissue that also contains a standing population of them.

We state immediately the limit of this. In the dematuration literature, net loss and the loss of mature markers are observed together, in animals treated with a drug that does many things. No experiment in that literature removes the net and asks whether dematuration follows. The causal claim — that disrupting the net flips a switch which returns mature neurons to an immature molecular phenotype — is a reasonable reading of an association and is not, on the published record, a demonstrated result. Section 8 treats this carefully, because it is the point at which an attractive synthesis is most likely to overreach.

1.5 The Shape of the Argument

Section 2 establishes what netting is and why it should be read as the terminal act of maturation rather than as a protective service. Section 3 develops the antagonism of sugars and what it implies about whether the reservoir cell can be netted at all. Section 4 documents the second vacancy, stating the searches and their returns. Section 5 sets out the topographic correction: the fields where the disease begins are net-poor by constitution. Section 6 states, fairly, where the stripping account is right, since it is right about a great deal. Section 7 develops the inversion — degradation as permission — and connects it to the parent paper's missing trigger. Section 8 treats dematuration and the limits of the causal claim. Section 9 asks what the reframing predicts about tau specifically, which is the question the parent paper exists to raise. Section 10 returns to the addressing problem with one speculative observation about net-poor cortical territory. Section 11 specifies the experimental programme. Section 12 states what would refute the account. Section 13 gives the verdict and section 14 the validity ledger.

2. Netting as the Terminal Act of Maturation

2.1 The Coincidence That Named the Function

The perineuronal net entered modern neuroscience through a coincidence of timing. In the rodent visual cortex, the organization of chondroitin sulphate proteoglycans into perineuronal nets coincides with the end of the critical period for ocular dominance plasticity, and dark rearing — which delays the critical period — delays the condensation as well. Degrading the proteoglycans with chondroitinase ABC in adult rats restores an ocular dominance shift to monocular deprivation that the adult cortex does not otherwise show (Pizzorusso et al., 2002). The mature matrix, on this evidence, is inhibitory for experience-dependent plasticity, and its removal reactivates it.

The same relationship was then found in a second system with a different behavioural read-out. In the amygdala, the organization of proteoglycans into nets coincides with the developmental switch after which conditioned fear becomes resistant to erasure by extinction; in adults, degrading the nets renders subsequently acquired fear memories susceptible to erasure again (Gogolla et al., 2009). And the electrophysiological correlates have been characterized: net removal in adult cortex unlocks juvenile plasticity through decreased inhibition and increased gamma activity (Lensjø et al., 2017).

Three systems, three read-outs, one relationship. The net is not merely present in the mature brain. It is what makes the brain mature, in the specific sense of closing the window during which experience can restructure a circuit.

2.2 The Sulphation Code and the Maturation of the Cell Beneath

The stronger form of the claim — that netting is constitutive of maturation rather than consequent upon it — comes from the biochemistry of the sugar rather than from the timing of the lattice.

Chondroitin sulphate chains are sulphated at either the fourth or the sixth carbon, and the ratio between the two changes developmentally. A developmental increase in the four-sulphation to six-sulphation ratio terminates the critical period for ocular dominance plasticity. Cell-autonomous overexpression of six-sulphation, which holds the ratio low, prevents the condensation of proteoglycans into nets around parvalbumin interneurons. And — this is the part that matters here — the increase in the ratio is required both for the accumulation of Otx2, the homeoprotein that drives parvalbumin cell development, and for the functional electrophysiological maturation of those cells (Miyata et al., 2012). The sulphation state of the matrix does not merely surround a mature cell. It is a condition of the cell becoming mature.

The relationship runs in both directions. Mice lacking a key chondroitin sulphate synthesizing enzyme show impaired *initiation* of ocular dominance plasticity, with weaker visual responses in parvalbumin cells; plasticity onset is restored by Otx2, which binds aggrecan and promotes its further expression; and continuous accumulation of chondroitin sulphate together with Otx2 contributes bidirectionally to both the onset and the offset of the critical period by promoting

parvalbumin cell function across the lifetime (Hou et al., 2017). The matrix is a developmental instrument that operates at both ends of the window.

2.3 The Consequence for a Cell in Arrest

The consequence for the population at issue in this paper is direct, and we state it as an expectation rather than as a finding, because it has not been measured.

A cortical immature neuron is defined by arrested maturation: it expresses doublecortin and polysialylated neural cell adhesion molecule into adult life, lacks the markers of mature neurons and of the principal interneuron classes, is largely without synapses, and is capable of resuming maturation at any point across the lifespan (Gómez-Climent et al., 2008; Rotheneichner et al., 2018; Benedetti et al., 2023). If netting is the terminal act of maturation, and if the net's assembly is entangled with the functional maturation of the cell it forms around, then a cell held short of maturity should be held short of its net. The expected phenotype of a reservoir cell is net-negative.

This is an expectation of the kind that is easy to state and easy to be wrong about, which is why section 4 documents that nobody has looked. We note only that the expectation is not a bare inference from timing. It has a molecular basis, which is the subject of the next section.

3. The Antagonism of Sugars

3.1 What Polysialic Acid Does

Polysialic acid is a large, negatively charged carbohydrate polymer attached to the neural cell adhesion molecule. Its conventional description in the immature-neuron literature is anti-adhesive: by increasing the hydrated volume of the molecule and interposing charge, it reduces cell–cell adhesion and permits the structural remodelling that migration and process extension require. That description is correct and is the reason the modification marks cells that are still moving and still growing.

It is also incomplete in a way that matters here. The reduction in adhesion is not indiscriminate. It has specific molecular consequences at specific binding sites, and one of those sites is occupied by a perineuronal net protein.

3.2 Neurocan Binds the Adhesion Molecule, and Polysialylation Interferes

Neurocan is one of the lecticans — aggrecan, brevican, neurocan, versican — that form the proteoglycan lattice of the net. Work on the postnatal remodelling of inhibitory perisomatic synapses established that neurocan binds the neural cell adhesion molecule directly, at the EphA3 binding site within the molecule's second immunoglobulin domain, and that through this binding it inhibits the association of the adhesion molecule with EphA3, the membrane clustering of the complex in interneuron axons, EphA3 kinase activation, and ephrin-induced growth cone collapse. In the course of establishing this, the same work reported the observation relevant to the present argument: the polysialylated form of the adhesion molecule binds neurocan *less efficiently* than the mature, non-polysialylated form (Sullivan et al., 2018).

The finding was made in the service of a different question — how the net terminates axonal remodelling and stabilizes perisomatic synapses — and its implication for immature neurons was not the authors' subject. But the implication is plain. The molecule the field uses as a marker of neuronal immaturity, in the glycosylation state that defines that marker, is a molecule that a perineuronal net protein binds poorly. The immature phenotype and the netted phenotype are not merely sequential states of the same cell. They are, at one identified molecular interface, mutually resistant.

3.3 What This Does and Does Not Establish

It does not establish that reservoir cells lack nets. It establishes that there is a molecular reason to expect them to, and that the reason is independent of the developmental-timing argument of section 2. Two independent lines pointing at the same expectation is better than one, and it is still not a measurement.

It also does not establish the converse claim — that polysialylation is sufficient to prevent net formation around a cell. Neurocan is one lectican among four; aggrecan is the component whose presence is most reliably associated with the protective phenotype in human tissue; and the net binds many partners besides the adhesion molecule. A cell could in principle carry polysialylated adhesion molecule and a partial aggrecan-based coat at once. Whether any cell does is, again, unmeasured.

What the finding does establish is that the question is a real one with a real molecular substrate, rather than a terminological coincidence between two fields that both happen to talk about layer two.

3.4 A Human Observation That Cuts Both Ways

One human dataset bears on the polysialylation side of this and it is worth stating because it complicates the picture in the honest direction. Polysialylated adhesion molecule immunoreactivity is significantly reduced in the entorhinal cortex in Alzheimer's disease, and the reduction correlates inversely with hyperphosphorylated tau load (Murray et al., 2016). The follow-up quantification found the decrease in polysialylated-marker-positive cells in layers two and five to be driven by cells that co-label for a mature neuronal marker and for parvalbumin — that is, by interneurons — rather than by the immature population (Murray et al., 2018).

For the present argument this is a two-sided result. It says that a substantial part of the polysialylation signal in human entorhinal layer two belongs to parvalbumin interneurons, which are the archetypal net-bearing class, and therefore that polysialylation and netting are not simply exclusive at the level of cell identity. It also says, since these are the cells being lost, that the population whose polysialylation is disappearing in the disease is a population defined by both markers at once — which is exactly the intersection that section 2's argument says should not be stable. Neither study set out to characterize the reservoir, and neither reports the net status of the cells it counted. The result is a reason to do the triple stain rather than a reason to believe any particular outcome of it.



4. The Second Vacancy

4.1 Why the Search Is Reported

The parent paper's central empirical claim was a claim about absence: that no study has measured tau isoform composition or phosphorylation in cortical layer two immature neurons, in any species, in health or disease. Because a claim of absence is only as good as the search that produced it, that paper set out its queries. We do the same here, for the same reason, and with the same caveat that a negative search is a weak instrument.

4.2 The Queries and Their Returns

We queried the indexed biomedical literature for the intersection of the perineuronal net with each of the terms by which this population is known and with each of its anatomical locations.

The exact phrase "cortical immature neurons" in combination with "perineuronal" returns **no records**.

The term "paralaminar" in combination with "perineuronal", restricted to title and abstract, returns **no records**.

"Doublecortin" in combination with "perineuronal net" returns **two records** in the entire indexed literature. Neither concerns the question. One is a study of noise-induced hearing loss in young rats in which perineuronal nets and doublecortin appear as two among several histological markers of circuit maturation, and in which no relationship between them is reported or examined (Jagersma et al., 2024). The other is the marmoset antidepressant study discussed in section 8, in which both markers change under a drug treatment in different regions (Ohira et al., 2019).

"PSA-NCAM" in combination with "perineuronal net" returns **ten records**. All ten concern the two molecules as parallel markers of interneuronal plasticity — in schizophrenia models, chronic stress, peripubertal stress, dark exposure, antidepressant treatment, erythropoietin treatment — and none examines the cortical immature neuron population. The single record that addresses a direct molecular relationship between the two is the neurocan binding study of section 3 (Sullivan et al., 2018).

"Chondroitinase ABC" in combination with "doublecortin" and "neurogenesis" returns **no records**, which means that even the coarse question — does enzymatic matrix degradation alter the immature-neuron compartment — has not been asked in that form.

4.3 What the Vacancy Is and Is Not

The vacancy is not that nobody has studied perineuronal nets in Alzheimer's disease; that literature is large and is summarized in section 6. Nor is it that nobody has studied immature neurons in the adult cortex; that literature is two decades old. The vacancy is at the intersection, and it is close to total.

We therefore do not know whether cortical immature neurons bear perineuronal nets. We do not know whether they sit inside, beside, or outside the nets of their neighbours. We do not know what their net status is in the aged human brain, in the Alzheimer brain, or at any point along the maturation gradient that the human material describes. And we do not know whether degrading the matrix changes their number, their state, or their rate of awakening.

Each of these is answerable with a double stain on tissue that exists. The first — *Wisteria floribunda* agglutinin or an anti-aggrecan antibody together with doublecortin or the polysialylated adhesion molecule, on human layer two — could be run this week by any laboratory that holds both antibodies, and most laboratories in either field hold one of them.



5. The Territory That Was Never Netted

5.1 The Entorhinal Finding

The observation that most directly contradicts the stripping account has been in the literature since 1999 and has not, to our knowledge, been brought into contact with the reservoir question.

A survey of perineuronal nets across human cortical areas — primary motor, primary auditory, several prefrontal and temporal association areas, and the entorhinal cortex — in normal brains and in brains meeting neuropathological criteria for Alzheimer's disease found net-associated neurons most numerous in primary motor cortex, at approximately ten per cent of neurons in Brodmann's area four, and in primary auditory cortex; their number was lower in secondary and higher-order association areas; and in the entorhinal cortex, net-associated pyramidal cells were *extremely rare*. In the Alzheimer brains, the characteristic distribution of hyperphosphorylated tau stained with AT8 largely excluded the zones abundant in nets and in neuropil-associated proteoglycans, and in double-stained sections, neurons ensheathed by nets were virtually unaffected by neurofibrillary change even in severely damaged regions (Brückner et al., 1999).

Two findings, therefore, in one study: netted neurons resist tangles, and the region where tangles start has almost no netted pyramidal cells.

5.2 The Subcortical Parallel

The same relationship holds below the cortex, and there it is cleaner because the anatomy is nuclear rather than laminar. Neurons associated with aggrecan-based nets are relatively protected against tau pathology; and the structures preferentially affected by tau in Alzheimer's disease — the basal nucleus of Meynert, the dorsal thalamus, hypothalamic nuclei, the raphe nuclei, the locus coeruleus — are devoid of a characteristic aggrecan-based matrix, with the distribution of tangles and of nets largely complementary within structures of mixed vulnerability (Morawski et al., 2010).

The locus coeruleus is the extreme case and it is instructive. It carries pretangle tau in early adult life, before any cortical involvement, in the majority of brains examined (Braak and Del Tredici, 2011). It is also, by the survey above, a nucleus without an aggrecan-based net. Whatever else explains its priority — and its long unmyelinated axons, catecholamine chemistry and metabolic load are all candidates — it did not lose a net. It never had one.

5.3 The Human Hippocampal Departure

A third observation makes the point specific to the species in which the disease occurs. In a neurochemical mapping of the human hippocampus, the perineuronal protomap proved distinct from that of other mammals: human hippocampal pyramidal cells lack perineuronal nets, while calretinin- and calbindin-positive interneurons carry a matrix that does not correspond to the rodent pattern. What the human hippocampus does carry is a different structure — isolated perisynaptic coats built of cartilage link protein-1 and brevican, engulfing individual excitatory and inhibitory terminals, contributed to by presynaptic neurons via axonal transport. And these axonal coats were found preserved in distribution and turnover around functional synapses in Alzheimer patients and transgenic mice, including along dendrite segments containing hyperphosphorylated tau and within amyloid-laden microdomains (Lendvai et al., 2013).

Three consequences follow. The rodent net map is not the human net map, so inferences about human medial temporal vulnerability drawn from mouse net biology should be made cautiously. The perisynaptic matrix is a distinct compartment from the perineuronal net and behaves differently in disease — it persists where the net is lost. And the principal cells of the human hippocampal formation belong, with respect to the net, to the same category as the locus coeruleus: unnetted from the start.

5.4 The Correction, Stated Plainly

The stripping account holds that layer two neurons lose a defensive barrier when microglia degrade it. In the territory where the disease begins, the barrier is largely absent in the healthy brain. Entorhinal net-associated pyramidal cells are extremely rare (Brückner et al., 1999); human hippocampal pyramidal cells lack nets (Lendvai et al., 2013); the subcortical nuclei that tangle first lack aggrecan-based matrix (Morawski et al., 2010).

This does not make the protective finding wrong. It makes it work differently than the stripping account supposes. Netted neurons resist tangles, and the disease starts in places with few netted neurons — which is a statement about the distribution of a protection, not about its removal. The right causal grammar is not *the net was taken and the neuron was exposed*. It is *the protection was never issued in this territory, and the disease begins where it was not issued*.

For the reservoir cell, that grammar is exact twice over: it sits in a net-poor territory, and it is, by its own developmental arrest and by the antagonism of section 3, a cell that should not be netted itself.



6. Where the Stripping Account Is Right

A correction of this kind is worth less if it is unfair to what it corrects, and the evidence for matrix degradation in Alzheimer's disease is strong. We set it out.

6.1 The Degradation Is Real

Histological analysis across a transgenic amyloid model at four, eight, twelve and eighteen months, validated in post-mortem human tissue, showed that perineuronal nets are lost extensively and in proportion to plaque burden; that activated microglia closely associate with and engulf damaged nets; that inclusions of net material are visible inside microglia in both species; that aggrecan is deposited within human dense-core plaques; and that eliminating microglia pharmacologically prevented or rescued the disease-related reduction in nets in two independent models (Crapser et al., 2020). The protease side is consistent: matrix metalloproteinase-9, the principal enzymatic regulator of the net, is differentially regulated across the disorders in which net abnormalities are found, including Alzheimer's disease (Wen et al., 2018). And the net's principal component is not a bystander: aggrecan protects against plaque accumulation and is required for proper microglial responses to plaques (Barahona et al., 2025).

None of this is in dispute here. Nets are degraded in the Alzheimer brain, microglia do it, and proteases participate.

6.2 The Degradation Is Not Only Pathological

What the disease-facing account tends to omit is that microglial engulfment of matrix is a normal function. Neuronal interleukin-33, expressed in an experience-dependent manner by adult hippocampal neurons, instructs microglial engulfment of extracellular matrix; loss of the cytokine or of its microglial receptor impairs spine plasticity, reduces newborn-neuron integration and degrades the precision of remote memories, with matrix proteins accumulating in contact with synapses (Nguyen et al., 2020). Microglial matrix engulfment is, in the healthy hippocampus, a mechanism of experience-dependent remodelling.

This matters for the present argument because it means the disease does not introduce a new process. It runs an existing one without its normal restraint — which is precisely the condition under which a physiological permission becomes a pathological one.

6.3 The Resilience Result That Complicates Everything

One human finding should be set against any account, including ours, that treats net integrity as straightforwardly good. In a comparison of control, Alzheimer, and cognitively resilient donors — individuals with the neuropathological hallmarks of the disease but intact cognition — aggrecan expression around parvalbumin neurons was decreased in both the Alzheimer and the resilient groups, while the net's sugar chains detected by *Wisteria floribunda* agglutinin were reduced only in the resilient subjects; genes related to net degradation were upregulated in the disease, and fewer presynaptic terminals were found on parvalbumin neurons (de Vries et al., 2025).

The humans who escape the clinical disease are not the ones whose matrix is most intact. They are the ones whose matrix is most differently remodelled. Any simple mapping from net loss to harm has to account for this, and ours does not attempt one: the account developed in section 7 is that net loss changes what the tissue permits, and permission is not the same as damage.

6.4 The Scope of the Correction

The correction of section 5 is therefore narrow and specific. It does not say that nets are not degraded in Alzheimer's disease. It says that in the particular territory where the disease begins, and for the particular cell population this paper is about, the degradation account cannot be the mechanism of first injury, because the structure it invokes is largely absent before the disease starts. Where nets are abundant — motor cortex, auditory cortex, the parvalbumin population throughout — the degradation account applies and is well supported. Those are also, conspicuously, the territories the disease reaches late.



7. The Inversion: Degradation as Permission

7.1 The Obstacle the Parent Paper Named

The parent paper's second candidate mechanism for converting a dormant cell into a pathological one located the danger in the exit from dormancy rather than in dormancy itself. Maturation is the most demanding thing a neuron does; in development it occurs in a tissue configured to support it, with permissive extracellular matrix, abundant trophic factor, immature inhibitory architecture and synchronous neighbours; a cell awakening at eighty attempts the same program in a tissue configured for stability, and the first item on that paper's list of what makes the adult tissue hostile was *an adult extracellular matrix rich in chondroitin sulphate proteoglycans*.

The paper then conceded the gap: we do not know what triggers awakening, and therefore cannot say whether the disease increases it. The dedicated review of these cells lists the molecular program sustaining immaturity and the triggers of differentiation among the field's three critical unknowns (Riga et al., 2026).

7.2 The Obstacle Is Exactly What the Disease Removes

Set the two literatures together and the gap has a candidate filler.

If the condensed chondroitin sulphate matrix is the principal tissue-level restraint on structural change in the adult cortex — which is the content of the critical-period literature summarized in section 2 — and if Alzheimer's disease degrades that matrix regionally and progressively through microglial engulfment and protease activation — which is the content of section 6 — then the disease is performing, chronically and without an experimenter, the manipulation that the plasticity literature performs acutely with chondroitinase.

The experimental manipulation has known consequences. It restores ocular dominance plasticity to adult cortex (Pizzorusso et al., 2002). It restores erasability to adult fear memory (Gogolla et al., 2009). It decreases inhibition and increases gamma activity, which the authors describe as unlocking juvenile plasticity (Lensjø et al., 2017). These are descriptions of an adult tissue made permissive.

A dormant cell in a permissive tissue is a cell whose principal obstacle to maturation has been removed.

7.3 The Hypothesis

We state it in the form in which it should be tested.

The restraint claim. The condensed chondroitin sulphate proteoglycan matrix of adult cortex contributes to holding cortical immature neurons in arrest, such that its local degradation increases the rate at which they resume maturation.

The disease claim. Alzheimer's disease degrades that matrix in the territories where the reservoir resides, by microglial engulfment and protease activation, and therefore increases the rate of reservoir awakening in those territories.

The conversion claim. Cells awakening under these conditions attempt maturation in a tissue that is permissive with respect to matrix but hostile with respect to trophic support, metabolic supply and proteostatic capacity, and it is that combination — permission without support — that converts a physiological process into a pathological one.

The corollary. The reservoir should be depleted faster in Alzheimer's disease than in normal ageing, and the depletion should be regionally correlated with matrix degradation rather than with plaque or tangle burden per se.

The restraint claim is untested and is the load-bearing one. The disease claim follows from two established bodies of evidence and one untested joint. The conversion claim inherits the parent paper's difficulties intact. The corollary is the cheapest thing on the list to measure and is specified as an experiment in section 11.

7.4 Why This Is Better Than the Account It Replaces

Three reasons.

It uses the evidence in the direction the evidence runs. The plasticity literature's central demonstration is that removing the matrix makes adult tissue behave like young tissue. An account that reads matrix removal as *permission for developmental behaviour* is reading that demonstration straightforwardly. An account that reads it as *exposure of a vulnerable cell* is borrowing a different literature's framing and applying it to a cell for which the protective structure was never documented.

It supplies the missing trigger. The parent paper's honest statement of its largest hole was that nobody knows what wakes these cells. This account names a candidate that is present in the diseased brain, absent in the healthy one, regionally graded, experimentally manipulable in both directions, and already measured in the tissue of interest.

It makes a prediction that separates it from its rival. The stripping account predicts that reservoir cells in diseased tissue should be found damaged in place — net-stripped, pathology-bearing, still immature. The permission account predicts that they should be found *converting*: reduced in number, with the residue showing intermediate marker profiles and enlarged somata, in regions where the matrix has gone. These are different sections under the same microscope.



8. The Second Door: Dematuration, and What It Does Not Show

8.1 The Phenomenon

Adult neurons can lose the mature phenotype. Chronic treatment of adult mice with the serotonergic antidepressant fluoxetine strongly reduces expression of the mature granule cell marker calbindin in the dentate gyrus, induces active somatic membrane properties resembling those of immature granule cells, and markedly reduces the synaptic facilitation that characterizes mature dentate-to-CA3 transmission. The authors were explicit that these changes cannot be explained by the addition of newly generated immature neurons and are best characterized as the dematuration of cells that were already mature; the effect developed alongside increased serotonin efficacy and was attenuated in mice lacking the 5-HT₄ receptor (Kobayashi et al., 2010). The dematured state persists after drug withdrawal and correlates with a distinct behavioural signature (Kobayashi et al., 2011).

The phenomenon is not confined to granule cells. In the medial frontal cortex, the same treatment reduced the number of parvalbumin-positive cells and of net-bearing parvalbumin cells, with no change in total GABAergic cell number and no increase in apoptosis — indicating reduced expression of a maturity marker rather than loss of the cells — which the authors read as dematuration of fast-spiking interneurons and as the induction of a juvenile-like state (Ohira et al., 2013). In the common marmoset, the treatment increased doublecortin and calretinin in the dentate gyrus and reduced parvalbumin expression and the number of net-bearing neurons in the hippocampus (Ohira et al., 2019). The framework that has grown around these results treats the induced condition as juvenile-like plasticity restored to the adult brain, with dematuration of net-enwrapped parvalbumin interneurons among its cardinal features (Umemori et al., 2018).

8.2 Two Routes Into One Compartment

The relevance to the reservoir argument is structural rather than pharmacological. The immature compartment of the adult cortex has, on this evidence, two possible sources. One is the standing population of cells that never matured — the reservoir. The other is the flux of cells that matured and reverted.

If a disease degrades nets at scale, and if net loss belongs to the dematured phenotype, then the disease may be enlarging the immature compartment from both directions at once: permitting the dormant cells to leave it and pushing mature cells into it. The compartment would then be a bidirectional pool rather than a draining reservoir, and its size at any moment would be uninformative about either flow.

This has an immediate methodological consequence that bears on the parent paper. If both routes operate, then a count of doublecortin- or polysialylation-positive cells in diseased cortex cannot distinguish a preserved reservoir from a depleted reservoir replenished by dematured neighbours. Any study that scores these markers in Alzheimer tissue — and the field's instinct will be

to score them — must therefore include a means of distinguishing the two origins. Section 11 specifies one.

8.3 The Causal Claim, and Why We Do Not Make It

It is tempting to complete the synthesis by asserting that net disruption causes dematuration — that removing the net flips a switch and returns the mature neuron to an immature molecular phenotype. The assertion is circulating, it is plausible, and the published record does not support it.

What the record contains is co-occurrence under a drug. In every study cited above, the animals received chronic fluoxetine, which alters serotonergic signalling, brain-derived neurotrophic factor expression, neurogenesis and inhibitory circuit function simultaneously. Net loss and loss of mature markers were both observed. Neither was shown to produce the other. The most economical reading is that both are downstream of the same induced state, and indeed the framework's own logic makes net loss a *feature* of the juvenile-like condition rather than its cause (Umemori et al., 2018).

What would establish the causal claim is the experiment nobody has published: degrade the matrix enzymatically in adult cortex, without a drug, and score mature-marker expression, membrane properties and immature-marker expression in the neurons beneath and beside the degraded region. The materials for it have existed since 2002. The search reported in section 4 returned no record of it having been done with immature-neuron markers as the read-out.

We therefore treat the causal step as a named, testable, currently unsupported conjecture, and we flag it here rather than in a footnote because it is the point at which this paper's synthesis is most likely to be repeated in a stronger form than it deserves.

8.4 What Follows If It Is False

If net degradation does not cause dematuration, the argument of section 7 is unaffected. The permission account concerns the tissue-level restraint on a dormant cell's maturation, not the reversion of mature cells, and it stands or falls on the restraint claim alone. The second door would simply close, leaving one route into the immature compartment instead of two, and leaving the methodological problem of section 8.2 solved rather than open. This is worth saying because the two claims are often bundled and they have different evidential standing: the first is an inference from a robust experimental literature, the second is a conjecture from an association.



9. What This Predicts About Tau

9.1 The Protection Finding Reconsidered

The parent paper's organizing question is what tau these cells contain. The matrix literature bears on it through one finding that looks, at first, like a reason for optimism and is on closer reading a reason for nothing at all.

Neurons ensheathed by aggrecan-based nets are strikingly resistant to neurofibrillary change — virtually unaffected even in severely damaged regions (Brückner et al., 1999) — and the structures that tangle first lack such nets (Morawski et al., 2010). If reservoir cells are net-negative, as sections 2 and 3 lead us to expect, then they fall on the unprotected side of this dichotomy, and the finding might be read as predicting that they should tangle.

It does not predict that, and the reason is worth stating because it is the kind of inference this literature invites. The protection finding is an association between two distributions in human tissue. It is equally consistent with the net protecting the neuron and with the net marking a cell class that is protected for other reasons — parvalbumin interneurons differ from pyramidal cells in far more than their coats. Applied to a cell type that was never examined, in a layer whose principal cells are net-poor regardless, the association predicts nothing. What it does is remove a reassurance: one cannot argue that reservoir cells are protected by the mechanism that protects netted neurons, because on the expectation developed here they do not have the structure that mechanism requires.

9.2 The Prediction That the Reframing Does Make

The permission account of section 7 makes a prediction about tau that the stripping account does not, and it concerns timing rather than presence.

On the parent paper's second conversion candidate, a cell attempting late maturation is a cell in sustained cytoskeletal and proteostatic strain, continuously producing a labile hyperphosphorylated tau species under conditions of impaired disposal. If matrix degradation is the trigger for awakening, then tau accumulation in these cells should be *downstream of local matrix loss and not of local tangle burden* — it should appear in cells adjacent to degraded matrix, in regions where degradation has occurred, at a stage that precedes rather than follows the regional tangle count.

This is a scoreable prediction on human tissue. It requires a matrix stain, an immature-marker stain, a phospho-tau stain, and a regional gradient — which the disease supplies for free.

9.3 The Pretangle Complication Persists

The parent paper identified the strongest single objection to the whole family of developmental-tau theories: pretangles label for four-repeat tau and not three-repeat, with the three-repeat signature that a developmental account reads as the signature of origin appearing instead at the end, in ghost tangles.

Nothing in the present paper addresses that objection, and it would be dishonest to imply otherwise. The matrix argument concerns what wakes a cell and what tissue it wakes into. It says nothing about which tau isoform the cell carries, and the isoform sequence remains a genuine problem that the reservoir hypothesis inherits. The only legitimate response remains the one the parent paper gave: the sequence was established in pyramidal populations, not in immature cells, and has not been examined in the latter.

10. The Addressing Problem, and One Speculation

10.1 What the Parent Paper Conceded

The parent paper conceded that the reservoir constrains the topography of the disease without explaining it: the map of immature cells is a map of possible starting points, and within that territory nothing in the hypothesis says why this patient's posterior parietal cortex and that patient's left perisylvian cortex.

The matrix adds a variable to that problem, and we want to be careful about how much weight to put on it, because it is exactly the kind of observation that invites overreach.

10.2 The Observation

Perineuronal net density is not uniform across the cortex. In the human survey, netted neurons were most numerous in primary motor and primary auditory cortex and declined through secondary and higher-order association areas, reaching near-absence in entorhinal cortex (Brückner et al., 1999). The gradient runs from primary sensorimotor to association territory — which is, coarsely, the inverse of the gradient along which Alzheimer's disease progresses.

A second observation, in the mouse, links net-poverty to persistent immaturity in the same tissue. The postnatal cortical domain of highest retinoic acid action, distinguished by the synthesizing enzyme RALDH3, constricts to a band that includes the dorsal visual stream and the attentional and executive networks; and that band shows suppressed parvalbumin, neurofilament,

cytochrome oxidase and perineuronal net maturation, together with persistence of the embryonic polysialylated form of the neural cell adhesion molecule, relative to surrounding cortex (Wagner et al., 2006).

A cortical territory, that is, defined by delayed maturation, low net density and persistent polysialylation — the three properties this paper has been treating as one state.

10.3 The Speculation, and Its Weakness

The territories named in that mouse study — dorsal visual stream, attentional and executive networks — correspond, loosely, to the territories of the atypical Alzheimer variants: posterior cortical atrophy takes posterior parietal and occipital cortex, and the dysexecutive and behavioural variants take frontal territory. The speculation is that variant topography follows a map of maturational lag, and that net density is the readable correlate of that map.

The weaknesses are severe and we list them rather than qualify them. The study is in mouse, and section 5.3 established that the human net map departs from the rodent one in exactly the region that matters most. The correspondence between a rodent *RALDH3* band and human variant topography is an analogy between coarse anatomical descriptions, not a measured overlap. The variants are defined clinically and their anatomical boundaries are not sharp. And a territory that is net-poor is also, by the cited gradient, a territory that is associative, which is a property that independently predicts late-life vulnerability on several other accounts.

We include the speculation because it is testable and cheap to test — net density is quantifiable in post-mortem cortex, variant topography is known, and the comparison has not been made — and because the alternative to stating a weak hypothesis clearly is leaving it implicit. It should be read as a proposal for a measurement, not as a claim about the disease.

II. The Experimental Programme

Every experiment below uses existing antibodies and, with two exceptions, existing tissue.

11.1 The Net Status of the Reservoir Cell

Double immunofluorescence on human cortex — entorhinal, temporal, and a net-rich control region such as primary motor cortex — using *Wisteria floribunda* agglutinin or an anti-aggrecan antibody for the net, and doublecortin or anti-polysialylated adhesion molecule for the cell. Score: the proportion of immature-marker-positive cells bearing any detectable net, partial net, or none; and the spatial relationship between immature cells and the nets of their neighbours.

This is the foundational experiment and everything else depends on its outcome. If reservoir cells prove to be net-negative, the account of sections 2, 3 and 7 is on its expected footing. If a substantial fraction prove net-positive, the developmental-timing argument of section 2 is wrong in an important way and should be abandoned rather than rescued.

11.2 The Maturation Gradient

The same stain, scored along the morphological maturation gradient that the human material describes — cells with small somata and strong doublecortin reactivity at one end, larger somata with weaker reactivity at the other (Li et al., 2023). The prediction of section 2 is that net acquisition should track position on that gradient, with nets appearing late. A demonstration that netting is the terminal step of a reservoir cell's own maturation would convert the argument of section 2 from an inference about critical periods into a direct observation about this population.

11.3 The Degradation Experiment

The one experiment here that requires an animal. Inject chondroitinase ABC unilaterally into the adult rodent piriform cortex — the only cortical region in which rodents hold a substantial reservoir, and conveniently among the earliest regions affected in human disease — with vehicle contralaterally. Score at intervals: immature-marker-positive cell number, the proportion showing intermediate maturation markers, and mature-neuron marker acquisition.

The restraint claim of section 7.3 predicts that matrix degradation accelerates awakening: fewer immature cells, more cells in transition, on the treated side. A null result would falsify the load-bearing claim of this paper directly. The search of section 4.2 indicates this experiment has not been done.

11.4 The Dematuration Test

The same preparation, scored for the converse question. Following enzymatic degradation, assess mature-marker expression — calbindin in granule cells, parvalbumin in cortical interneurons — and immature-marker expression in cells that were mature before treatment, using a lineage or birth-dating label to establish that the cells scored were not immature to begin with.

This is the experiment that would decide the causal claim of section 8.3, and its design must distinguish the two routes of section 8.2. Without the birth-dating label, the result is uninterpretable, because an increase in immature-marker-positive cells is equally consistent with dematuration and with the reservoir being held rather than spent.

11.5 The Human Disease Series

Immature-marker density in human layer two across Braak stages, scored together with local matrix integrity in the same sections. The corollary of section 7.3 predicts that reservoir depletion correlates regionally with matrix loss rather than with local tangle burden — a dissociation that is measurable because the two do not perfectly co-vary.

11.6 The Variant Comparison

Quantification of net density across cortical fields in post-mortem tissue from typical amnesic, posterior cortical atrophy, and logopenic or dysexecutive cases. The speculation of section 10 predicts that the affected field in each variant is net-poorer than the corresponding field in cases where it is spared. This is the weakest hypothesis in the paper and the comparison is the one most likely to return a clean negative, which is a reason to run it rather than a reason not to.

11.7 The Combined Stain

For any laboratory prepared to run one section rather than a programme: net marker, immature-neuron marker, and phospho-tau, on human entorhinal cortex from a mid-Braak case. It answers, in a single field of view, whether reservoir cells in diseased tissue are netted, whether they carry tau, and whether the two properties are related — which are the three questions this paper and its parent have between them been unable to answer from the literature.



12. What Would Refute This

The account has four separable claims and each fails independently.

If cortical immature neurons prove to bear perineuronal nets in healthy human cortex, the developmental-timing argument of section 2 and the molecular argument of section 3 are both wrong, the stripping account becomes available again for these cells, and this paper's central correction collapses. The experiment is section 11.1.

If enzymatic matrix degradation does not increase the rate at which dormant cells resume maturation, the restraint claim fails and with it the permission account of section 7, including its claim to supply the parent paper's missing trigger. The experiment is section 11.3.

If reservoir depletion in human disease tracks tangle burden rather than local matrix loss, the disease claim of section 7.3 is not supported, and matrix degradation becomes a correlate of the process rather than a step in it. The experiment is section 11.5.

If enzymatic degradation without a drug produces no change in mature-marker expression, the causal step in the dematuration argument is dead. We have already declined to assert it, so this is a refutation of a conjecture rather than of a claim — but it would close the second door of section 8.2 and simplify the picture.

One further outcome would be awkward without being fatal. If reservoir cells prove net-negative but their number is *unchanged* across Braak stages while matrix around them is lost, then the cells are neither protected by nets nor mobilized by their removal, and the population becomes a bystander to the matrix story — present, unnetted, and irrelevant. That is a real possibility and we can see no argument that excludes it in advance.



13. Verdict

The question that prompted this work was what happens to the immature neurons of cortical layer two when the perineuronal net fails. The answer we arrive at is that the question contains a premise which the human tissue does not support, and that correcting the premise is more productive than answering the question as posed.

In the territory where Alzheimer's disease begins, there is very little net to fail. Net-associated pyramidal cells are extremely rare in the human entorhinal cortex; human hippocampal pyramidal cells lack nets entirely, unlike their counterparts in other mammals; and the subcortical nuclei that take tau earliest are devoid of aggrecan-based matrix. The relationship between the net and the disease's first cells is better described by absence than by loss. And the cell this paper is about should, on two independent arguments, be unnetted by constitution: because netting is the terminal act of a maturation these cells never completed, and because the polysialylated adhesion molecule that defines their immature state is bound poorly by the net protein neurocan. The immature cell does not lose its net. It carries the sugar that declines one.

What the failure of the net does to such a cell is therefore not exposure. It is permission. The condensed adult matrix is the principal tissue-level restraint on structural change in the mature cortex — this is the content of two decades of critical-period work, in which enzymatic removal of that matrix returns adult cortex to a juvenile state in visual plasticity, in fear memory and in cortical rhythm. The parent paper named that same matrix as the first obstacle facing a dormant cell attempting to mature at eighty, and conceded that it could not say what triggers awakening. Alzheimer's disease degrades the matrix regionally, progressively, and by a mechanism that has been characterized in both mouse and human tissue. The disease performs, chronically, the manipulation the plasticity literature performs acutely. That is a candidate trigger, it is testable in both directions, and it converts the parent paper's largest acknowledged hole into an experiment.

The strength of this account is that it reads the evidence in the direction the evidence runs and makes a prediction that separates it from its rival: the stripping account predicts damaged cells found in place, the permission account predicts converting cells found in transition, and these are different sections under the same microscope. Its weaknesses are three. The restraint claim on which everything rests is untested. The dematuration route, which would double the argument's reach, rests on a co-occurrence under a drug and not on a causal experiment, and we have declined to assert it. And the net status of the reservoir cell — the single measurement on which the whole reframing depends — has never been made, in any species, in health or in disease.

That last absence is the more striking for how small it is. The indexed literature contains no study combining the phrase "cortical immature neurons" with the perineuronal net, none combining the paralaminar nucleus with it in title or abstract, and two combining doublecortin with it, neither of which concerns the question. Two fields have worked on the same cortical layer for two decades without running the double stain that would tell either of them whether the other's structure is present on the other's cell. It requires one section, two antibodies, and an afternoon.

14. The Validity Ledger

``ledger - tier: strong basis: "multiple systems, multiple laboratories, causal manipulation" claim: "The condensation of chondroitin sulphate proteoglycans into perineuronal nets closes the critical period for experience-dependent plasticity, and enzymatic degradation of that matrix reopens a juvenile-like permissive state in the adult brain." why: "Demonstrated in visual cortex with ocular dominance as read-out, in amygdala with fear-memory erasability as read-out, and in cortical network physiology with inhibition and gamma as read-outs. The manipulation is causal and bidirectional in effect."

- tier: strong basis: "developmental biochemistry with cell-autonomous manipulation" claim: "Net assembly is entangled with the functional maturation of the cell it forms around, not merely consequent upon it." why: "The developmental change in chondroitin 4-/6-sulphation ratio that permits net condensation is itself required for Otx2 accumulation and for the electrophysiological maturation of parvalbumin cells; the same sulphation program governs critical-period onset as well as offset. Established in rodent visual cortex."

- tier: moderate basis: "one binding study, made for another purpose" claim: "Polysialylation of the neural cell adhesion molecule impedes its binding by the net protein neurocan, making the immature marker molecularly antagonistic to net assembly." why: "A direct biochemical result with a mapped binding site, but reported incidentally within a study of perisomatic synapse stabilization, not replicated for this purpose, and concerning one lectican of four. It supports an expectation; it does not establish that reservoir cells are net-free." settles: "The double stain of section 11.1."

- tier: strong basis: "two independent human surveys, one cortical and one subcortical" claim: "The territories in which Alzheimer's disease begins are net-poor in the healthy brain: entorhinal net-associated pyramidal cells are extremely rare, and the earliest-affected subcortical nuclei lack aggrecan-based matrix." why: "Systematic mapping across cortical areas and across subcortical structures, in normal and Alzheimer tissue, with the tau distribution scored in the same material. The human hippocampal departure from the mammalian net protomap is an independent third observation."

- tier: absent basis: "systematic search returning near-zero" claim: "Whether cortical immature neurons bear perineuronal nets, in any species, in health or disease, is unknown." why: "The exact phrase 'cortical immature neurons' with 'perineuronal' returns no records; 'paralamina' with 'perineuronal' in title or abstract returns none; 'doublecortin' with 'perineuronal net' returns two, neither on the question; 'PSA-NCAM' with 'perineuronal net' returns ten, none examining this population. A negative search is weak evidence of absence, but the consistency across formulations and the total absence at the exact-phrase level make the gap likely real. This is the paper's central vacancy." settles: "Section 11.1."

- tier: strong basis: "transgenic and human tissue, with pharmacological rescue" claim: "Perineuronal nets are degraded in the Alzheimer brain, microglia perform the degradation, and eliminating microglia prevents it." why: "Loss in proportion to plaque burden across four ages, microglial engulfment documented in both mouse and human, net material visible inside microglia, aggrecan deposited in human dense-core plaques, and prevention or rescue by microglial elimination in two models. Not in dispute here; the correction concerns where this mechanism can act first, not whether it acts."

- tier: weak basis: "inference joining two established literatures at an untested joint" claim: "Matrix degradation acts on the dormant reservoir as a permission to mature rather than as an

exposure, and therefore constitutes a candidate trigger for awakening." why: "Both premises are well evidenced — the matrix restrains structural change; the disease degrades the matrix — and the joint between them has never been tested. No experiment has degraded the matrix and scored the immature-neuron compartment. This is the paper's load-bearing claim and it is currently an inference." settles: "Section 11.3."

- tier: contested basis: "co-occurrence under a pharmacological treatment" claim: "Disruption of the perineuronal net causes mature neurons to revert to an immature molecular phenotype." why: "Dematuration is a real and replicated phenomenon in adult granule cells and cortical interneurons, and net loss is among its cardinal features. But in every study the state is induced by chronic fluoxetine, which alters serotonergic signalling, neurotrophin expression and inhibitory circuit function simultaneously; net loss and mature-marker loss are observed together and neither has been shown to produce the other. On the framework's own logic net loss is a feature of the induced state, not its cause. We decline to assert the causal claim." settles: "Section 11.4, with birth-dating to exclude the reservoir as the source."

- tier: moderate basis: "established human association, misapplied if read as prediction" claim: "The resistance of net-bearing neurons to neurofibrillary change predicts that net-free reservoir cells should tangle." why: "The protection finding is robust and reproducible, and it is an association between two distributions. It is equally consistent with the net protecting the cell and with the net marking a cell class protected for other reasons. Applied to a population never examined, it predicts nothing; what it removes is a reassurance, not an uncertainty."

- tier: weak basis: "cross-species analogy between coarse anatomical descriptions" claim: "Variant Alzheimer topography follows a map of cortical maturational lag for which net density is the readable correlate." why: "Rests on a mouse study of a retinoic-acid-defined cortical band showing suppressed net maturation and persistent polysialylation, whose named territories correspond loosely to the fields taken by posterior cortical atrophy and the dysexecutive variants. The human net map departs from the rodent map in the region that matters most; the correspondence is not a measured overlap; and net-poor territory is also associative territory, which independently predicts late-life vulnerability. Stated as a proposal for a measurement." settles: "Section 11.6."

- tier: absent basis: "acknowledged, inherited, unaddressed" claim: "This account resolves the pretangle isoform objection to developmental-tau theories." why: "It does not, and does not bear on it. Pretangles label for four-repeat and not three-repeat tau, with the three-repeat signature appearing at the end of lesion evolution. The matrix argument concerns what wakes a cell and what tissue it wakes into, and says nothing about isoform. The objection stands where the parent paper left it." ```

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