
The Price of the Net

*Microglial Suppression Restores the Perineuronal Matrix and Destabilises the
Rhythm It Supports — Why Colony-Stimulating Factor 1 Receptor Inhibition and
Forty-Hertz Entrainment Are Not Complementary Interventions, and the Experiment
That Would Decide*

A review and adjudication of conflicting interventional evidence

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*The same cell that digests the scaffold also damps the circuit. Remove it and you are given back the structure, less the function
the structure was preserved for.*

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Abstract

Two lines of interventional evidence in Alzheimer's disease have been read as pointing the same way. The first is matrix-directed: perineuronal nets — the condensed chondroitin-sulfate sheaths that enwrap fast-spiking parvalbumin interneurons — are lost in proportion to plaque burden in the 5xFAD brain and in human cortex, microglia engulf them, and eliminating microglia with a colony-stimulating factor 1 receptor inhibitor restores them. The second is rhythm-directed: forty-hertz sensory entrainment drives gamma-frequency activity, attenuates amyloid and phosphorylated tau in several mouse models, and has reached controlled trials in patients. Because the perineuronal net is the structure that permits the parvalbumin cell to fire fast, and because the parvalbumin cell is the generator of the gamma rhythm, the two interventions have been treated as one intervention in two parts: preserve the substrate, then exercise it.

This paper argues that the pairing does not survive the evidence, and that the strongest reason is now a direct experiment. In APP/PS1 mice, the colony-stimulating factor 1 receptor inhibitor GW2580 reduced microglial proliferation, attenuated the disease-associated microglial phenotype, reduced CD68 expression and synaptic engulfment, and partially rescued synaptic loss — and, in the same animals, exacerbated gamma-oscillation disruption, increased network hyperactivity, and increased the incidence and complexity of epileptiform discharge, with no effect on plaque burden or cognition. The manipulation offered as the way to preserve the matrix degrades the rhythm the matrix is held to serve.

We argue that this is not an anomaly but the predictable consequence of a conflation. Matrix integrity, the intrinsic excitability of the fast-spiking cell, and the excitatory/inhibitory set-point of the surrounding network are three variables, and only the first is improved by removing microglia. Microglia are not merely the agent that digests the net; they are also a homeostatic brake on network activity in their own right, catabolising extracellular adenosine triphosphate to adenosine and suppressing neuronal firing, such that their ablation amplifies and synchronises activity and produces seizures in otherwise normal animals. They are additionally required for the correct construction of inhibitory circuitry, sculpting inhibitory synapses through a receptor-gated programme that does not touch excitatory ones. Remove them and the net returns; so does the seizure.

We show further that the causal arrow between net and microglia runs in both directions, which forecloses the simple reading in which the net is a passive casualty. Genetic ablation of aggrecan in 5xFAD mice — abolishing the net — increases plaque deposition, reduces plaque sphericity, impairs microglia-plaque association and enhances the disease-associated microglial signature. The net organises the microglial response as much as the microglion degrades the net. Eliminating microglia in a Huntington model likewise raised perineuronal net abundance above that of untreated littermates, indicating that microglia set net abundance in the healthy brain and not only in disease.

We separate three candidate relations between the two interventions — antagonism, sequence, and mis-pairing — and argue that the evidence favours mis-pairing: the interventions act on different variables, on different timescales, with different signs on the excitability axis, and were joined by an

inference about shared substrate rather than by any experiment that ran them together. We further note that the direction of parvalbumin-cell failure is itself unsettled, with one literature reporting a sodium-channel-dependent loss of fast-spiking capacity and another reporting early hyperexcitability whose chemogenetic suppression reduces amyloid and improves cognition; an entrainment protocol that drives the fast-spiking population is a different intervention under each account.

We grade every claim on a four-tier scale and state the four negatives that would refute the argument. We propose one decisive experiment: a two-by-two factorial of sustained microglial suppression against forty-hertz multisensory entrainment in a single amyloid cohort, with perineuronal net integrity, parvalbumin-cell intrinsic excitability, gamma power and epileptiform burden measured as four separate endpoints in the same animals, in vivo rather than in slice, and with electrographic seizure as a pre-specified safety endpoint rather than an incidental finding. Until that experiment is run, the two interventions should not be described as complementary, and a combination trial that assumes they are would be assuming the point at issue.

1. Introduction

A therapeutic argument can be constructed out of two results that are each secure, joined by an inference that is never tested. The joint is usually a shared noun. If one result concerns a structure and the other concerns a function that the structure is known to support, then an intervention that preserves the structure and an intervention that exercises the function will look like partners. They will be written into the same combination protocol, and the protocol will inherit a confidence that neither result earned on its own.

This paper is about one such joint in Alzheimer's disease, and about a recent experiment that pulls it apart.

The structure is the perineuronal net: a condensed, lattice-like specialisation of the extracellular matrix, built on a hyaluronan backbone cross-linked by link proteins and tenascin-R and studded with chondroitin sulfate proteoglycans of the lectican family, of which aggrecan is the obligatory component[1]. Perineuronal nets preferentially envelop fast-spiking, parvalbumin-expressing inhibitory interneurons. They appear as the critical period of developmental plasticity closes, and their enzymatic removal reopens it[1]. They are lost in the Alzheimer brain, and microglia have been shown to engulf them: in 5xFAD mice, perineuronal nets are lost in proportion to plaque burden, activated microglia associate with and engulf damaged nets, inclusions of net material are found inside microglia in both mouse and human tissue, and aggrecan is deposited within human dense-core plaques[2]. Crucially — and this is the result that made the observation therapeutic rather than merely descriptive — treating 5xFAD and 3xTg-AD mice with the colony-stimulating factor 1 receptor inhibitor PLX5622, which eliminates microglia, prevented or rescued the disease-related reduction in nets[2].

The function is the gamma rhythm. Parvalbumin interneurons are the generators of cortical gamma oscillations: optogenetic inhibition of these cells suppresses gamma in vivo, while driving them is sufficient to generate emergent gamma-frequency rhythmicity[3], and selective activation of fast-spiking interneurons across a range of driving frequencies amplifies gamma specifically, a cell-type-specific double dissociation from pyramidal-cell drive[4]. Gamma-band activity is reduced in Alzheimer models and patients. A decade of work has shown that non-invasive forty-hertz sensory stimulation reduces amyloid burden and modifies microglial morphology in 5xFAD visual cortex[5], extends across sensory modalities to reduce amyloid in auditory cortex and hippocampus and phosphorylated tau in a tauopathy model[6], preserves neuronal and synaptic density in two models of neurodegeneration[7], and recruits glymphatic clearance through vasoactive-intestinal-peptide-interneuron-dependent regulation of arterial pulsatility[8].

The joint is the parvalbumin cell. The net is what allows the fast-spiking cell to fire fast; the fast-spiking cell is what generates the rhythm; therefore — the inference runs — preserving the net preserves the substrate on which the rhythm protocol must operate, and the two interventions are complementary: one protects the instrument, the other plays it. Stated that way it is close to irresistible, and therapeutic proposals have been built on it, pairing microglial-tone modulation with forty-hertz entrainment as sequential components of a single stack.

The inference has now been tested, in the only way that matters, by running one of the two interventions and measuring the other's endpoint. In APP/PS1 mice at nine to eleven months, gamma oscillations recorded in acute brain slices showed reduced amplitude, increased beta-band power and heightened epileptiform activity, accompanied by reductions in parvalbumin- and somatostatin-positive interneuron populations and increased network sensitivity to the GABA-A antagonist gabazine — a coherent picture of compromised inhibitory control[9]. Because these oscillatory changes correlated with microglial reactivity, the investigators tested a causal role for microglia by administering the colony-stimulating factor 1 receptor inhibitor GW2580. The drug did what the matrix argument would predict of it upstream: it reduced microglial proliferation, attenuated development of the disease-associated microglial phenotype, reduced CD68 expression and the engulfment of synaptic elements, and partially rescued synaptic loss. It had no significant effect on amyloid plaque burden or on cognitive deficits. And it made the electrophysiology worse: it exacerbated the disruption of gamma oscillations and induced network hyperexcitability, increasing both the incidence and the complexity of epileptiform activity[9].

The authors' own conclusion is a caution against indiscriminate suppression of microglial activity, on the grounds that microglia regulate network homeostasis and that therapeutic strategies targeting them must account for that function[9]. The present paper takes that conclusion as a starting point and asks the question it raises for the combined protocol: if the manipulation that restores the net destabilises the rhythm, what exactly was the relation between the net and the rhythm, and what is the relation between the two interventions?

We argue for four claims.

First, that matrix integrity, the intrinsic excitability of the parvalbumin cell, and the excitatory/inhibitory set-point of the surrounding network are three dissociable variables that the shared-substrate inference treats as one, and that microglial suppression moves them in different directions.

Second, that microglia are not only the agent of net degradation but an independent homeostatic brake on network excitability and a required participant in the construction of inhibitory circuitry, so that their removal has a direct disinhibitory consequence that has nothing to do with the matrix at all.

Third, that the causal arrow between net and microglia runs in both directions — the net organises the microglial response to plaques, and microglia set net abundance in the healthy brain — which forecloses any account in which the net is a passive casualty and microglial removal is a clean subtraction.

Fourth, that the two interventions are best described not as antagonistic and not as sequential but as mis-paired: joined by an inference about shared substrate rather than by any experiment, acting on different variables with different signs, and requiring a factorial design to separate.

We close with that design, with four predictions that would be falsified by it, and with an explicit statement of what in this argument is established and what is inference.

2. What the Perineuronal Net Does for the Fast-Spiking Cell

The inference under examination rests on a premise about what the net contributes to the cell it surrounds. That premise needs to be stated precisely, because the usual shorthand — that the net is what lets the parvalbumin cell fire fast — compresses several distinct functions, and the compression is where the trouble begins.

2.1 A protective shield against oxidation

The clearest established function is protective. Fast-spiking parvalbumin interneurons are metabolically extreme cells. The interneuron energy hypothesis holds that their action-potential kinetics and ion conductances impose an energy expenditure reflected in enrichment for mitochondria and cytochrome c oxidase and a corresponding dependence on oxidative phosphorylation, and that this renders them a vulnerable target for metabolic and oxidative stress[10]. The dependence is not merely correlative: loss of the mitochondrial calcium uniporter reduces hippocampal gamma power by more than forty per cent, degrades synchrony and the precision of spike generation, and eliminates activity-dependent pyruvate dehydrogenase dephosphorylation[11]. Fast network rhythms are an oxidative-phosphorylation-dependent output.

Against that background, the perineuronal net functions as an antioxidant shield. In mice carrying a genetic redox imbalance, the robustness of the net around a parvalbumin cell varies inversely with the intracellular oxidative stress that cell displays; enzymatic degradation of the net renders mature parvalbumin cells — and fast rhythmic neuronal synchrony — more susceptible to oxidative stress; and the nets are themselves sensitive to oxidative load, so that protection reflects a balance between the burden on net degradation and the capacity to maintain the net[12].

2.2 A regulator of ion channels and receptors

The second function is regulatory rather than protective, and it is more specific than the shorthand suggests. The net protein brevican controls parvalbumin-cell physiology by regulating the localisation of potassium channels and of AMPA receptors, gating cellular and synaptic plasticity in these cells simultaneously; experience modulates brevican levels, and the resulting molecular and cellular changes in parvalbumin cells are required for learning and memory[13]. The net, on this evidence, is not a passive sleeve. It positions the machinery of fast spiking.

2.3 A marker of relative protection in the human brain

The third function is inferred from human tissue rather than demonstrated. Neurons associated with aggrecan-based perineuronal nets are relatively protected against tau pathology, a relationship first established in cortex and then extended to subcortical regions: many of 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, and within structures of mixed vulnerability the distribution of neurofibrillary tangles and of nets is largely complementary[14].

This is a real and reproducible association. It is also, and this matters for the therapeutic argument, an association and not an intervention. The complementary distribution is equally consistent with the net protecting the neuron and with the net being a marker of a cell class that is protected for other reasons.

Recent human work complicates it further in a direction that the simple "preserve the net" programme has not absorbed. 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 Alzheimer's disease, and fewer presynaptic terminals were found on parvalbumin neurons[15]. The resilient brain, on this evidence, does not have more nets. It has differently remodelled ones. Any therapeutic slogan of the form "restore the matrix" has to reckon with the observation that the humans who escape the clinical disease are not the ones whose matrix is most intact.

2.4 What the net is not

Nothing in the foregoing establishes that the net generates, sets the frequency of, or is required for the gamma rhythm. It protects the cell, positions its channels, and marks a population of relatively spared neurons. The rhythm is generated by the cell's firing and by the reciprocal inhibitory and excitatory connectivity in which that firing is embedded[3,4]. The distance between "the net supports the health of the generator" and "the net is required for the rhythm" is the distance the shared-substrate inference silently crosses.

That distance is not rhetorical. It is measurable, and it has been measured, in the wrong direction for the inference — a point we return to in Section 6.

3. The Microglial Account of Net Loss

3.1 The observation and the intervention

The case that microglia degrade perineuronal nets in Alzheimer's disease is strong and was built carefully. Histological analysis across 5xFAD mice at four, eight, twelve and eighteen months, validated in post-mortem human tissue, showed that 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 evident inside microglia in both species; and that aggrecan is deposited within human dense-core plaques[2]. Lipopolysaccharide treatment was used to test whether neuroinflammation alone suffices to drive net loss. And microglial elimination with PLX5622 prevented or rescued the disease-related reduction in nets in two independent models[2].

A companion result in a different disease strengthens the general claim and, in doing so, introduces the complication this paper is concerned with. In the R6/2 model of Huntington's disease, microglial elimination with the colony-stimulating factor 1 receptor inhibitor pexidartinib reduced or prevented grip-strength and object-recognition deficits, mutant huntingtin accumulation, astrogliosis and striatal volume loss — and prevented the loss of proteoglycan-containing perineuronal nets. The finding that matters here is the one obtained in the controls: microglial elimination *strikingly increased* perineuronal nets in the brains of naive littermates, which the authors read as evidence for a role for microglia as homeostatic regulators of perineuronal net formation and integrity[16].

That is a different claim from "microglia destroy the net in disease." It says that microglia set the steady-state abundance of the net in the normal brain. Removing them does not restore a baseline; it pushes the matrix above baseline. We return to what follows from this in Section 7.

3.2 What the pharmacology actually does

The colony-stimulating factor 1 receptor is required for microglial survival in the adult brain. Extended treatment of adult mice with selective inhibitors eliminates approximately ninety-nine per cent of microglia brain-wide; the depleted mice show no behavioural or cognitive abnormalities on the tasks examined, and the brain repopulates fully within a week of inhibitor withdrawal[17]. Sustained depletion from before pathology onset in 5xFAD mice prevents parenchymal plaque formation except in regions containing surviving microglia, redirecting deposition into cortical blood vessels in a pattern reminiscent of cerebral amyloid angiopathy, and reverses the disease-associated hippocampal transcriptional signature[18].

It is important for what follows that "colony-stimulating factor 1 receptor inhibition" does not name a single manipulation. PLX5622 and pexidartinib, at the exposures used in the net studies, eliminate the population. GW2580 — the compound used in the experiment that motivates this paper — is characteristically deployed as an inhibitor of microglial *proliferation* rather than as a depleting agent: in a prion model of chronic neurodegeneration, selective inhibition of microglial proliferation with GW2580 reduced the expansion of the microglial population and, with it, the neurogenic response, revealing a pro-neurogenic role for microglia that the depletion framing had obscured[19]. The distinction between blocking the expansion of a population and removing the population is a distinction between two different brains, and it is one of the axes along which the results discussed below could in principle be reconciled. It is also, notably, an axis along which the therapeutic proposals have been imprecise: "microglial-tone modulation" is offered as though sub-depleting dosing were a known safe harbour, when the compound that most closely realises sub-depleting modulation is the one that produced the adverse electrophysiology.

3.3 Microglia remodel the matrix in health, too

The disease-facing account has a developmental and homeostatic counterpart that is easy to miss and that bears directly on the therapeutic reading. 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 fear memories, and is accompanied by an accumulation of matrix proteins in contact with synapses[20]. Microglial engulfment of extracellular matrix is, in the healthy hippocampus, a mechanism of experience-dependent synaptic remodelling and memory consolidation.

This means the engulfment of matrix by microglia is not intrinsically pathological. It is a normal function that, in disease, operates on a matrix that is already compromised and in a cell whose regulatory state has changed. A therapy that abolishes the function abolishes the physiological version along with the pathological one — and the physiological version, on this evidence, is required for plasticity and for memory.

4. The Result That Breaks the Pairing

We can now state the primary finding precisely.

In APP/PS1 mice aged nine to eleven months, acute brain slices showed gamma oscillations of significantly reduced amplitude together with increased beta-frequency power and heightened epileptiform activity — a slowing of network rhythms consistent with the neurophysiological signature reported in patients. Immunohistochemistry showed reductions in both parvalbumin- and somatostatin-positive inhibitory interneuron populations. Application of gabazine revealed increased network sensitivity to GABA-A receptor antagonism, indicating that inhibitory control was already compromised. These oscillatory abnormalities correlated with microglial reactivity, which motivated a test of microglial causality[9].

Administration of GW2580 produced a dissociation across endpoints:

- **Microglial endpoints, improved.** Reduced microglial proliferation; attenuated development of the disease-associated microglial phenotype; reduced CD68 expression; decreased engulfment of synaptic elements.
- **Synaptic endpoint, partially improved.** Synaptic loss was partially rescued.
- **Amyloid endpoint, unchanged.** No significant impact on plaque burden.
- **Cognitive endpoint, unchanged.** No significant impact on cognitive deficits.
- **Network endpoints, worsened.** Exacerbated disruption of gamma oscillations; induced network hyperactivity; increased incidence and complexity of epileptiform activity[9].

Three features of this pattern deserve emphasis before we interpret it.

The first is that the improved and worsened endpoints were measured in the same animals under the same protocol. This is not a discrepancy between laboratories or between models; it is a dissociation within an experiment. Whatever reconciles it has to reconcile it at the level of mechanism, not of methodology.

The second is that the amyloid endpoint did not move. In the sustained-depletion paradigm begun before pathology onset, plaque formation is prevented[18]; here, with a proliferation-directed compound given to animals with established pathology, it is not. The plaque-facing rationale for microglial suppression, whatever its merits in prevention, did not operate in this treatment window.

The third is that cognition did not move either — in either direction. The exacerbated epileptiform activity did not translate into measurable cognitive worsening on the tasks used, and the partially rescued synapses did not translate into improvement. This is a genuine limit on what the experiment licenses. It establishes a physiological cost and leaves the behavioural consequence of that cost undetermined.

The authors' stated interpretation of the hyperexcitability is that reduced CD68 expression and decreased engulfment of synaptic elements potentially facilitated the persistence of hyperexcitable synapses[9]. That is the natural reading if one assumes that what microglia remove is, on balance, excitatory. Section 5.2 argues that the best available evidence on what microglia preferentially remove points the other way, and that a different mechanism fits the result better.

5. What Microglial Suppression Does to Excitation and Inhibition

The result above is surprising only if one holds the view that microglia contribute to network physiology exclusively through what they eat. They do not. Three independent mechanisms connect microglial presence to network excitability, and only one of them concerns phagocytosis.

5.1 Microglia are a direct homeostatic brake on neuronal activity

The most direct evidence is also the most consequential for this argument. Microglia respond to neuronal activation by suppressing neuronal activity, and ablation of microglia amplifies and synchronises the activity of neurons, leading to seizures. The suppression is region-specific and depends on the capacity of microglia to sense and catabolise extracellular adenosine triphosphate, which is released on neuronal activation by neurons and astrocytes: adenosine triphosphate recruits microglial protrusions and is converted by the microglial ectoenzyme CD39 into adenosine monophosphate, which CD73 converts to adenosine, which suppresses neuronal responses through the adenosine receptor. Microglial sensing of adenosine triphosphate, the ensuing microglia-dependent production of adenosine, and adenosine-mediated suppression of neuronal responses are together essential for the regulation of neuronal activity and of behaviour; the authors describe this as a negative-feedback mechanism operating similarly to inhibitory neurons and essential for protecting the brain from excessive activation[21].

This is, on its own, sufficient to predict the finding of Section 4. Remove the microglia and you remove an activity-dependent brake whose output is an inhibitory neuromodulator. The network becomes more excitable and more synchronous. That is what was observed[9]. It requires no

assumption about which synapses were or were not engulfed, and it operates on a timescale of seconds to minutes rather than of structural remodelling.

It is worth stating plainly how awkward this is for the matrix programme. The cell whose removal restores the net is the same cell whose removal produces seizures in an otherwise healthy animal.

5.2 The engulfment explanation does not survive the preferential-target data

The alternative explanation offered for the hyperexcitability — that reduced engulfment permits hyperexcitable synapses to persist[9] — requires that the synapses microglia preferentially remove be excitatory ones. The evidence runs the other way.

Microglia prune synapses during development, engulfing synaptic material in a process necessary for normal brain wiring[22], and complement-dependent, microglia-mediated synapse elimination is reactivated inappropriately in Alzheimer models, where inhibition of C1q, C3 or the microglial complement receptor CR3 reduces the number of phagocytic microglia and the extent of early synapse loss[23]. But when the engulfed material has been resolved by synapse type, the preference is not the one the persistence argument needs. In tau-transgenic hippocampus, astrocytic lysosomes contained relatively more excitatory synapse marker proteins, whereas *microglial lysosomes contained more inhibitory synapse material*; C1q deletion reduced engulfment by both cell types and rescued synapse density; and in a combined amyloid-and-tau model, deletion of *Trem2* impaired microglial phagocytosis of synapses while astrocytes engulfed more inhibitory synapses around plaques[24].

If microglia preferentially engulf inhibitory synaptic material, then reducing microglial engulfment should, other things being equal, *spare* inhibitory synapses and *reduce* excitability. The observed direction is the opposite. The persistence-of-hyperexcitable-synapses account therefore requires either that the preference reverses in the APP/PS1 model at nine to eleven months, or that the engulfment-mediated contribution is swamped by a larger effect of opposite sign. The adenosinergic brake of Section 5.1 is the obvious candidate for that larger effect, and it makes the correct prediction without further assumption.

We put this forward as an argument about which mechanism is better supported, not as a refutation of the primary result, which stands on its measurements rather than on its interpretation. But the distinction is not academic: the two mechanisms imply different therapeutic remedies. If the lesion is unengulfed excitatory synapses, the answer is to restore selective pruning. If the lesion is a withdrawn adenosinergic brake, pruning is beside the point and the answer is not to withdraw the brake.

5.3 Microglia are required to build inhibitory circuitry

A third mechanism concerns construction rather than moment-to-moment regulation. A specialised subset of microglia expressing GABA-B receptors selectively interacts with inhibitory cortical synapses during a critical window of postnatal development; GABA initiates a transcriptional remodelling programme within these cells, which sculpt inhibitory connectivity *without impacting excitatory synapses*; and ablation of GABA-B receptors within microglia impairs the process and produces behavioural abnormalities[25].

The direct relevance is to the developing brain, and we do not claim the adult equivalent has been demonstrated. The bearing on the present argument is nonetheless real: it establishes that microglia possess a dedicated, receptor-gated programme whose output is correctly wired inhibition. A therapy that removes microglia removes a cell type with a demonstrated inhibitory-circuit-specific function, in a disease whose electrophysiological signature is a deficit of inhibitory control[26].

5.4 The convergent expectation

Taken together, the three mechanisms produce a convergent expectation that microglial removal should shift the excitatory/inhibitory balance toward excitation: it withdraws an activity-dependent adenosinergic brake[21], it removes a cell with an inhibitory-circuit-specific developmental programme[25], and — if the engulfment preference is toward inhibitory material[24] — the pruning contribution is either neutral or of the same sign. Against this, the matrix argument predicts improvement, on the grounds that the restored net should restore fast-spiking function[2].

The experiment ran, and excitability won[9]. This is the substance of the claim that the net and the rhythm come apart.

6. Why Net Preservation and Rhythm Preservation Come Apart

6.1 Removing the net does not silence the rhythm

The most direct evidence against the premise that the net is required for gamma is an experiment in which the net was removed and the rhythm measured. In adult rat visual cortex, enzymatic removal of perineuronal nets altered the balance between inhibitory and excitatory spiking directly: inhibitory activity was *reduced*, spiking variability *increased*, and the network was reset toward an immature, juvenile state. When activity-dependent plasticity was then induced by monocular deprivation, both net-depleted adults and juveniles showed an *immediate potentiation of gamma activity*[27].

Nothing in this is compatible with the simple reading in which net loss silences the fast-spiking cell and collapses the rhythm. Removing the net reduced inhibition and, under plasticity induction, raised gamma. The net stabilises the network; it is not the engine of the oscillation.

This result deserves more weight in the therapeutic discussion than it has received, because it converts the matrix–rhythm relation from a dependency into something more like a coupling with sign that depends on state. If net removal reduces inhibition and raises gamma power in the healthy adult cortex, then net restoration cannot be assumed to raise gamma in the diseased one, and the finding that microglial suppression restored nets while degrading gamma[9,2] stops being paradoxical.

6.2 Three variables, not one

The shared-substrate inference treats as a single quantity what is in fact three:

Matrix integrity — the abundance and composition of the perineuronal net. Reduced in Alzheimer tissue in proportion to plaque burden[2]; remodelled rather than simply preserved in cognitively resilient humans[15]; increased above baseline by microglial elimination even in naive animals[16].

Intrinsic excitability of the fast-spiking cell — the capacity of the parvalbumin neuron to fire at high frequency. Depends on sodium-channel availability[28], on potassium-channel and AMPA-receptor localisation gated by net proteins[13], and on oxidative-phosphorylation capacity[10,11].

Network excitatory/inhibitory set-point — the balance of drive in the circuit the cell inhabits. Set by inhibitory synapse number and strength[24], by glutamate reuptake[29], and by the microglial adenosinergic brake[21].

Microglial suppression raises the first, leaves the second largely untouched, and moves the third toward excitation. Forty-hertz entrainment drives the second and, through it, transiently imposes structure on the third, while doing nothing directly to the first. The two interventions do not act on

the same variable in the same direction, and the appearance that they do is an artefact of describing all three with the word "gamma."

6.3 What "partially rescued synaptic loss" does and does not buy

The GW2580 experiment did partially rescue synaptic loss[9], and it is worth being clear why that is not the vindication of the structural programme it might appear to be. A rescued synapse count is a static measure. The endpoints that worsened — oscillation amplitude, hyperactivity, epileptiform incidence and complexity — are dynamic measures of how the surviving elements are coordinated. The experiment therefore reports, in a single cohort, that structure was preserved and coordination was degraded. That is the paper's central lesson in one sentence, and it generalises beyond this compound: interventions that protect structural endpoints cannot be assumed to protect functional ones, and in a disease whose clinical expression is a failure of coordination, the functional endpoint is the one that matters.

7. The Reversed Arrow

A further difficulty for the sequential reading is that the causal relation between net and microglion is not one-directional.

Conditional knockout of *Acan* — the gene encoding aggrecan, the obligatory net component — in nestin-expressing cells of 5xFAD mice results in loss of aggrecan and ablation of the perineuronal net structure. The consequences are not confined to the neurons the net used to surround. In 5xFAD animals, aggrecan conditional knockout produced *increased* plaque deposition, *reduced* plaque sphericity, and *impaired* microglia–plaque association; single-cell spatial transcriptomics identified an *enhanced* disease-associated microglial phenotype in the knockouts, accompanied by *decreased* dystrophic neurite formation. The authors conclude that perineuronal nets may play a crucial role in mediating the microglial response to plaques[30].

Three things follow.

First, the net is not a passive casualty. It is an organiser of the microglial response to amyloid, and its absence degrades the compaction of plaques and the association of microglia with them — the very containment function by which microglia are held to limit the toxicity of the deposit.

Second, net status and microglial state are coupled in a loop rather than a chain. Microglia engulf nets[2] and set net abundance in the healthy brain[16]; nets in turn condition the microglial phenotype and its engagement with plaques[30]. An intervention that enters this loop at the microglial node does not subtract a single term.

Third — and this is the most awkward consequence for the combined protocol — the two experiments are, in a limited sense, mutually confirming in the wrong direction. Ablating the net enhanced the disease-associated microglial signature[30]; suppressing microglia attenuated it and degraded the rhythm[9]. Both results place the disease-associated phenotype and the net on the same axis, and both indicate that intervening on that axis has consequences elsewhere that the intervention was not designed to produce.

We flag one caveat that limits how far the reversed arrow can be pressed. The aggrecan knockout is constitutive within the nestin lineage and therefore removes the net through development, not in the adult; developmental absence of a structure is not the same manipulation as its degradation in an aged brain, and compensations available to the former are not available to the latter. The result establishes that the net conditions the microglial response; it does not establish that adult net restoration would normalise it.

8. The Direction-of-Failure Problem

An unresolved question sits underneath both interventions and is rarely stated: in which direction does the parvalbumin cell fail?

One literature says the cell loses its capacity to fire. In human amyloid precursor protein transgenic mice, spontaneous epileptiform discharges occurred primarily during reduced gamma oscillatory activity; both the mice and Alzheimer patients showed decreased levels of the interneuron-specific, parvalbumin-predominant voltage-gated sodium channel subunit Nav1.1; and restoring Nav1.1 increased inhibitory synaptic activity and gamma oscillations while reducing hypersynchrony, memory deficits and premature mortality[28]. On this account the cell is hypofunctional, the rhythm fails for want of a generator, and an intervention that drives the fast-spiking population is compensating for a deficit.

A second literature says the cell is, at least early, overactive — and that this is causal. In APP/PS1 mice, hippocampal parvalbumin interneurons become hyperexcitable at around sixteen weeks of age, before any change in the intrinsic properties of pyramidal cells; the hyperexcitable state coincides with increased inhibitory transmission onto pyramidal neurons and with spatial memory deficits; and treatment aimed at preventing parvalbumin interneurons from becoming hyperexcitable restored their properties, reduced inhibitory input onto pyramidal cells, rescued memory, and reduced amyloid plaque deposition[31]. The same group showed that prolonged chemogenetic activation of parvalbumin neurons induces long-term hyperexcitability, disrupts synaptic transmission and causes spatial memory deficits, and that this state renders hippocampal function vulnerable to a subsequent low dose of amyloid- β [32].

An independent group has now extended the finding to the earliest affected hippocampal subfield. In 5xFAD mice, amyloid- β accumulates in the subiculum at early disease stages; laser microdissection with proteomics identified early dysregulation of GABAergic neurons there; parvalbumin interneurons exhibited pronounced hyperactivity during initial stages; and targeted inhibition of that hyperactivity, by chemogenetic approaches and by parvalbumin downregulation, significantly reduced amyloid- β accumulation in the subiculum and connected regions while enhancing cognitive function. Single-nucleus RNA sequencing from human Alzheimer donors showed parvalbumin expression in GABAergic neurons peaking at early disease stages, and parvalbumin-interneuron inhibition restored protein homeostasis by normalising GABAergic synapses, improving lysosomal function, and promoting amyloid precursor protein degradation through K63-linked ubiquitination[33].

Two laboratories, two models, two methods, converging on a claim that inverts the therapeutic sign: at the earliest stage, the parvalbumin cell is doing too much, and quieting it reduces amyloid and improves cognition.

We do not claim these literatures are irreconcilable. The obvious reconciliation is temporal — early hyperexcitability giving way to later failure, as the loss of parvalbumin-positive cells observed at nine to eleven months in APP/PS1[9] and the reductions reported in patients[28] would require. That reconciliation is plausible and is, in our view, the most likely resolution. But it is a hypothesis about staging that no study has established with the resolution the therapeutic question demands, and until it is established it makes a specific and uncomfortable point about forty-hertz entrainment.

Entrainment drives the fast-spiking population[4]. Under the hypofunction account that is replacement of a missing drive. Under the early-hyperactivity account, applied in the early window, it is amplification of the lesion — and the intervention that helped in that window was chemogenetic *inhibition* of the same cells[33,31]. The therapeutic sign of the rhythm intervention depends on a staging question that is unresolved, which means the confidence with which it is paired with anything else is overstated at the root.

9. The Network Already Seizes

The argument that microglial suppression is costly because it raises excitability depends on excitability already being a liability in this disease. It is, and the human evidence is stronger than the animal evidence on this specific point.

Network hypersynchrony is intrinsic to amyloid pathology in model systems: human amyloid precursor protein mice show spontaneous non-convulsive seizure activity in cortical and hippocampal networks with GABAergic sprouting and enhanced synaptic inhibition in the dentate gyrus, and blocking overexcitation prevents many of the downstream alterations[34]. Two-photon calcium imaging showed that the cortical disturbance is a redistribution rather than a uniform decrease: twenty-nine per cent of layer 2/3 neurons decreased activity while twenty-one per cent became hyperactive, with hyperactive neurons found exclusively near plaques and the hyperactivity attributable to a relative decrease in synaptic inhibition[35]. The initiating step has been traced to suppression of glutamate reuptake, with hyperactivation occurring in neurons that already had baseline activity and being sustained by Alzheimer brain extracts and purified amyloid- β dimers[29]. The synthesis of this literature treats interneuron dysfunction and network hypersynchrony as candidate mechanisms of cognitive dysfunction rather than as epiphenomena[26].

In patients, subclinical epileptiform activity was detected by overnight video-electroencephalography and magnetoencephalography in 42.4 per cent of Alzheimer patients with no seizure history, against 10.5 per cent of controls; patients with it were clinically indistinguishable at the time of monitoring but subsequently declined faster — 3.9 versus 1.6 Mini-Mental State Examination points per year, and faster in executive function[36]. Intracranial recording with foramen ovale electrodes in two patients without seizure history detected clinically silent hippocampal seizures and epileptiform spikes during sleep that were largely invisible to simultaneous scalp electroencephalography[37], which indicates that surface recording understates the prevalence.

The interventional evidence runs the same way, and with a specific and instructive pattern. In human amyloid precursor protein mice, levetiracetam alone among a panel of antiepileptic drugs reduced abnormal spike activity, and chronic treatment reversed hippocampal remodelling, behavioural abnormalities, synaptic dysfunction and learning and memory deficits[38]. In amnesic mild cognitive impairment, low-dose levetiracetam reduced elevated dentate gyrus/CA3 activation to control levels and improved memory performance in the scanning task, results the authors read as contradicting the view that greater hippocampal activation is beneficial[39]. In a phase 2a

randomised double-blind placebo-controlled crossover trial in thirty-four adults with Alzheimer's disease, levetiracetam did not change the primary executive-function outcome overall; but among the participants with epileptiform activity — 38.2 per cent of those enrolled — it improved performance on the Stroop interference naming subscale and on a virtual route learning test[40].

The pattern is that of a real mechanism operating in a subset. That is precisely the condition under which an intervention with a pro-excitatory side effect is dangerous: the effect will be diluted to invisibility in an unselected cohort while falling hardest on the fraction already at risk. An unselected trial of a microglia-suppressing agent could therefore report no safety signal and still be harming the forty per cent of patients with occult epileptiform activity[36,40].

10. What the Forty-Hertz Literature Does and Does Not Support

The rhythm arm of the pairing requires its own audit, because the pairing inherits whatever confidence is placed in it.

The positive preclinical series is substantial. Driving fast-spiking parvalbumin interneurons at forty hertz, but not at other frequencies, reduced amyloid- β levels; gene expression profiling showed induction of genes associated with microglial morphological transformation; and a non-invasive forty-hertz light-flicker regime reduced amyloid in the visual cortex of pre-depositing mice and mitigated plaque load in aged depositing mice[5]. Auditory stimulation driving gamma activity in auditory cortex and hippocampal CA1 improved spatial and recognition memory and reduced amyloid in those regions, reduced phosphorylated tau in a P301S model, and — combined with visual stimulation — produced microglial clustering responses and reduced amyloid in medial prefrontal cortex, with whole-brain analysis showing widespread plaque reduction[6]. Chronic daily stimulation in Tau P301S and CK-p25 mice preserved neuronal and synaptic density across multiple brain areas and modified cognitive performance[7]. A mechanism has been supplied: multisensory gamma stimulation promotes cerebrospinal fluid influx and interstitial fluid efflux, associated with increased aquaporin-4 polarisation and dilated meningeal lymphatic vessels; inhibiting glymphatic clearance abolished amyloid removal; and vasoactive intestinal peptide interneurons facilitate the clearance by regulating arterial pulsatility[8].

Three qualifications bear on the pairing.

The first is a replication failure of the entrainment premise itself. Using multisite silicon-probe recording in visual cortex, entorhinal cortex and hippocampus of APP/PS1 and 5xFAD mice, one

group found that forty-hertz flickering stimulation *did not engage native gamma oscillations* in these regions; spike responses in hippocampus were weak, suggesting that forty-hertz light does not effectively entrain deep structures; mice avoided the flickering light, with elevated hippocampal cholinergic activity; and no reliable changes were found in plaque count or microglial morphology by immunohistochemistry or in vivo two-photon imaging, nor reduced levels of amyloid- β 40/42[41]. This is a single laboratory and it is a negative result, with the interpretive limits that attach to both. It is nonetheless a direct challenge to the mechanism on which the pairing depends: if the protocol does not entrain the deep structures where the disease begins, then "exercising the preserved network" is not a description of what it does there.

The second is that the foundational report carries a published author correction to an extended-data figure and table[42]. This does not touch the principal claims and we note it for completeness rather than as an objection.

The third is that the human evidence, while now genuinely controlled, is not yet evidence about the endpoints at issue here. In a subset analysis of participants from a randomised controlled trial of daily combined visual and auditory forty-hertz stimulation over six months, active treatment was associated with reduced total and regional white matter atrophy and reduced myelin content loss relative to sham, with the most significant changes in the entorhinal region[43]. That is a positive controlled human imaging result. It is not a report of gamma-band restoration, of perineuronal net status, or of epileptiform burden, and it cannot bear on whether the rhythm protocol composes with a microglia-directed one.

We draw a narrow conclusion. The forty-hertz programme is a serious intervention with a mechanism, a preclinical series, a significant unreplicated premise, and early controlled human data on structural endpoints. It is not established to work by preserving or exploiting the perineuronal net, and no study has measured net status as an outcome or a moderator of it. The substrate claim that joins it to the matrix programme is, at present, unmeasured in both directions.

II. Antagonistic, Sequential, or Mis-Paired?

Three relations between the two interventions are compatible with the evidence. They are not equally supported, and they carry different consequences.

11.1 Antagonism

On this reading the interventions actively oppose one another: microglial suppression raises excitability[21,9], forty-hertz entrainment imposes rhythmic drive on an already destabilised network, and the combination is worse than either alone. The strong form of this claim would be that entrainment in a microglia-suppressed, hyperexcitable network provokes seizure.

We regard this as plausible and untested. The premises are individually supported — the hyperexcitability is measured[9], the epileptiform substrate in the disease is established[36,37], and rhythmic sensory drive is a recognised provocation in epileptology. But no experiment has applied both. We state it as a prediction in Section 12 rather than as a finding, and we note that it is the reading with the most immediate implication for trial safety: a factorial design must treat the combination cell as the one requiring monitoring, not as the cell expected to perform best.

11.2 Sequence

On this reading the interventions are not simultaneous partners but ordered ones: suppress microglia first to preserve the matrix, withdraw the suppression, then entrain. Microglial repopulation after inhibitor withdrawal is rapid and essentially complete within a week[17], which makes the sequence pharmacologically feasible.

The difficulty is that the sequence requires the matrix benefit to outlast the suppression while the excitability cost does not. Nothing establishes that asymmetry. The net gain is documented during depletion[2]; whether it persists after repopulation is, to our knowledge, unmeasured, and the observation that microglia set net abundance in the healthy brain[16] predicts that repopulation returns the net toward its previous set-point. A sequential protocol may therefore purchase a transient structural gain at the cost of a transient functional one and retain neither.

11.3 Mis-pairing

On this reading the two were never partners. They act on different variables (Section 6.2), on different timescales, with opposite signs on the excitability axis, and were joined not by an experiment but by an inference from a shared noun. The evidence favours this reading, on four grounds.

The interventions have never been run together, in any model, by any group. The pairing therefore has no empirical content.

The one experiment that ran one and measured the other's endpoint found a cost, not a synergy[9].

The premise that the net is required for the rhythm is contradicted by the direct experiment: removing the net reduced inhibition and, under plasticity induction, raised gamma[27].

And the causal relation between the two targets is a loop rather than a chain[30,16], so that entering it at one node does not leave the other node undisturbed.

We therefore conclude that the correct description is mis-pairing, with antagonism as an untested and clinically material possibility, and sequence as a design that would need to establish an asymmetry of persistence that has not been demonstrated.

11.4 The dose axis, which is the best remaining defence

The strongest defence of the pairing is that it never proposed depletion. Sub-depleting modulation of microglial tone — reducing phagocytic activation without abolishing the population — is a coherent aim, and the results discussed here were obtained with compounds and exposures at or near the depleting end.

We take this seriously and think it survives partly. It does not survive fully, for two reasons. The compound used in the experiment that produced the adverse electrophysiology is characteristically a proliferation inhibitor rather than a depleting agent[19], which places it nearer the sub-depleting end of the axis than the depletion studies[17,18], not further from it. And the adenosinergic brake is a function of microglial *presence and activity*[21]; a manipulation that reduces microglial activation without removing cells may attenuate that brake rather than sparing it. The dose defence converts the question into an empirical one — at what exposure does the matrix benefit appear and the excitability cost remain absent, and is that window non-empty? — which is exactly the question a dose-ranging arm of the factorial in Section 13 would answer.

12. Strength of Evidence

Each claim on which the argument depends is graded below. *Established* denotes a result replicated across laboratories or demonstrated in human tissue with an intervention. *Probable* denotes a result secure in its source but not independently replicated, or replicated only within a model system. *Inferred* denotes a conclusion we draw from established results that has not itself been tested. *Speculative* denotes a proposal offered for falsification.

Claim	Strength	Basis
Perineuronal nets are reduced in the Alzheimer brain and microglia engulf net material	Established	Mouse time course plus human post-mortem validation
Microglial elimination restores perineuronal nets in amyloid models	Probable	Two models, one laboratory, one compound class
Microglia set perineuronal net abundance in the healthy brain	Probable	Naive littermate controls in a Huntington model
Parvalbumin interneurons generate cortical gamma oscillations	Established	Reciprocal optogenetic gain and loss of function, independent groups
The perineuronal net protects fast-spiking cells from oxidative stress and positions their channels	Established	Genetic and enzymatic manipulation, two independent lines of work
The perineuronal net is required for the gamma rhythm	Refuted as stated	Net removal reduced inhibition and raised gamma under plasticity induction
CSF1R inhibition exacerbates gamma disruption and induces hyperexcitability in APP/PS1	Probable	Single report, single model, slice electrophysiology
Microglia suppress neuronal activity via CD39/CD73-derived adenosine; ablation causes seizures	Established	Mechanistic dissection with genetic and pharmacological confirmation
Microglial lysosomes preferentially contain inhibitory synaptic material	Probable	Single multi-omic study, tau and combined models
Microglia are required to sculpt inhibitory circuitry	Probable	Developmental cortex; adult equivalent not demonstrated

Claim	Strength	Basis
Perineuronal net loss enhances the disease-associated microglial phenotype and impairs microglia–plaque association	Probable	Single conditional-knockout study in 5xFAD
Network hyperexcitability and subclinical epileptiform activity are prevalent in Alzheimer's disease and predict faster decline	Established	Human EEG/MEG cohort plus intracranial confirmation
Anti-excitability treatment benefits the epileptiform-positive subset	Probable	Randomised crossover trial, prespecified subgroup, n = 34
Parvalbumin interneurons are hyperactive early and their inhibition reduces amyloid	Probable	Two laboratories, two models, converging methods
Parvalbumin cells lose Nav1.1-dependent fast-spiking capacity in disease	Established	Mouse and human tissue, with genetic rescue
Early hyperactivity and later hypofunction are successive stages of one trajectory	Inferred	Reconciles both literatures; not directly demonstrated
Forty-hertz entrainment reduces amyloid and tau pathology in mouse models	Probable	Extensive single-programme series with one direct negative replication
Forty-hertz entrainment acts through preservation or exploitation of the perineuronal net	Speculative	Net status has never been measured in any entrainment study
The two interventions are complementary	Unsupported	Never run together in any model
The two interventions are antagonistic in combination	Speculative	Mechanistically motivated; no experiment has tested it

13. Predictions

We state four predictions that would be tested by the design in Section 14, each with the result that would count against the argument.

Prediction 1 — the dissociation generalises beyond the compound. Microglial suppression achieved by a mechanistically distinct route — a depleting inhibitor rather than a proliferation inhibitor, or genetic ablation — will reproduce the pattern of restored perineuronal net with degraded gamma and increased epileptiform burden in an amyloid model. *Refuted if* a depleting agent restores nets and leaves gamma power and epileptiform incidence unchanged or improved, which would confine the finding to the proliferation-inhibition mechanism.

Prediction 2 — the excitability cost is adenosinergic, not phagocytic. Pharmacological or genetic interruption of the microglial CD39/CD73–adenosine pathway will reproduce most of the hyperexcitability produced by microglial suppression, without restoring perineuronal nets and without changing synaptic engulfment. *Refuted if* interrupting the adenosine pathway leaves network excitability substantially unchanged while blocking complement-dependent engulfment reproduces the hyperexcitability, which would support the persistence-of-hyperexcitable-synapses reading.

Prediction 3 — the combination is not additive and may be adverse. In a factorial design, the cell receiving both microglial suppression and forty-hertz entrainment will show epileptiform burden at least as high as microglial suppression alone, and cognitive outcome no better than the better single intervention. *Refuted if* the combination cell shows lower epileptiform burden than suppression alone together with a cognitive benefit exceeding either monotherapy, which would establish the complementarity the pairing assumes.

Prediction 4 — net status does not moderate the entrainment response. Entrainment efficacy, measured as induced gamma power and as pathological or cognitive outcome, will not correlate with baseline perineuronal net integrity across animals. *Refuted if* net integrity predicts entrainment response, which would vindicate the substrate argument and convert the pairing from an inference into a finding — and which, we note, is the single most informative measurement available, because no entrainment study has ever made it.

We regard Prediction 4 as the crux. The entire pairing rests on the proposition that the net is the substrate the rhythm protocol requires. That proposition has never been measured. It could have been measured in any of the entrainment studies at the cost of one additional stain.

14. The Experiment That Would Settle It

The design follows from the three-variable analysis. What is required is not a better version of either experiment but one experiment that runs both and measures all three variables in the same animals.

Design. A two-by-two factorial in a single amyloid cohort — APP/PS1 or 5xFAD — with factors *microglial suppression* (vehicle versus a colony-stimulating factor 1 receptor inhibitor) and *forty-hertz multisensory entrainment* (sham versus active). Four cells: control, suppression alone, entrainment alone, combination.

Dose-ranging on the suppression factor. Because the sub-depleting defence (Section 11.4) turns on whether a therapeutic window exists, the suppression factor should carry at least two exposures — one depleting, one explicitly sub-depleting with population size confirmed by stereology — so that the matrix benefit and the excitability cost can be plotted against the same dose axis. A window is demonstrated only if the two curves separate.

Recording in vivo, not in slice. The motivating experiment used acute slices[9]. Slices lack the intact thalamocortical and neuromodulatory afferents that sensory entrainment must recruit, and an entrainment factor cannot be delivered to a slice in any case. Chronically implanted multisite electrodes are required, and — given the report that forty-hertz flicker does not engage native gamma in deep structures[41] — recording must include hippocampus and entorhinal cortex, not visual cortex alone. Entrainment efficacy must be verified rather than assumed in every animal.

Four endpoint families, measured in every animal.

1. *Matrix.* Perineuronal net abundance and composition, with aggrecan and *Wisteria floribunda* agglutinin scored separately, since the human resilience data show these dissociate[15].
2. *Cellular.* Intrinsic excitability of parvalbumin interneurons, with Nav1.1 quantified[28], and a direct measure of firing rate in vivo, so that the hyperactivity-versus-hypofunction question[31,33] is settled within the cohort rather than assumed.
3. *Network.* Gamma power and coherence, beta power, and — pre-specified rather than incidental — epileptiform spike and seizure burden over continuous recording, since the human data show surface recording understates this[37].
4. *Outcome.* Plaque burden, plaque sphericity and microglia-plaque association[30], and cognition.

Timing as a third factor, if resources permit. Given the unresolved staging question of Section 8, the design gains substantially from running at two ages — one before and one after the age at which parvalbumin hyperactivity is reported[31] — because the sign of the entrainment effect may reverse across that boundary.

Pre-specified safety endpoint. Electrographic seizure incidence in the combination cell, treated as a stopping rule rather than as an outcome to be reported. This is the design element most likely to be omitted and the one this paper most wishes to insist upon.

What each outcome would mean. If nets improve and gamma degrades across both suppression doses, the mis-pairing conclusion stands and the combination should not proceed. If the curves separate at a sub-depleting exposure, the pairing is rescued in a specified window and the window is now known. If net integrity predicts entrainment response, the substrate argument is vindicated and the sequence reading becomes the right one. If entrainment efficacy is uncorrelated with net status, the joint that holds the two interventions together dissolves and they should be developed separately.

15. Limitations

We state the weaknesses of this argument plainly.

The primary result is a single report in a single model. The dissociation that motivates this paper comes from one laboratory, one mouse line, one compound, and slice electrophysiology[9]. It has not been independently replicated. Our argument is that it is *predicted* by three independent mechanisms and therefore should not be treated as an anomaly; that is an argument about prior plausibility, not a substitute for replication.

Our reading of that report rests on the abstract-level record. The article was not available in full text through the repositories we could access. We have been careful to attribute to it only endpoints and directions it states explicitly, and the reader should treat effect magnitudes as unquantified in our treatment.

The matrix and rhythm literatures are each substantially single-programme. The microglia–perineuronal net results derive predominantly from one laboratory and its collaborators[2,16,30], and the forty-hertz series predominantly from another[5,6,7,8]. Our criticism of the pairing should not be read as doubting either programme; concentration of provenance is a limitation on both sides of the argument, including ours.

The adenosinergic mechanism was established in wild-type animals, not in disease. The negative-feedback control of neuronal activity by microglia[21] was demonstrated outside an amyloid model. Whether the brake is intact, attenuated or already lost in aged APP/PS1 mice is unknown; if it is already lost, its withdrawal cannot explain the observed hyperexcitability and Prediction 2 will fail.

The inhibitory-preference result is a single study. The finding that microglial lysosomes preferentially contain inhibitory synaptic material[24] is the basis of our argument against the persistence reading. It is one multi-omic study, in tau and combined models rather than in APP/PS1, and synapse-type preference may be model- and stage-dependent.

The developmental evidence does not transfer automatically. GABA-receptive microglial sculpting of inhibitory circuits was established in a postnatal critical window[25]. We use it to establish that such a programme exists, not to claim it operates in the aged brain.

The aggrecan knockout is developmental. As noted in Section 7, ablating the net through development is not the same manipulation as degrading it in an aged brain[30].

Cognition did not change in the motivating experiment. The strongest deflationary reading of this paper is that a physiological cost with no measured behavioural consequence may not be a therapeutic cost at all. We accept the force of this, and note only that the human data attach real prognostic weight to subclinical epileptiform activity[36] and that rodent cognitive batteries are not sensitive instruments for the deficits that activity predicts.

We have not addressed the vascular arm. Sustained microglial depletion redirects amyloid deposition into cortical vessels in a pattern resembling cerebral amyloid angiopathy[18]. That is a further and independent hazard of the microglial arm which we note and do not analyse.

16. Conclusion

A perineuronal net and a gamma rhythm are not two names for one thing. The net protects a metabolically extreme cell from oxidation and positions the channels that let it fire; the rhythm is produced by that cell's firing within a circuit whose excitatory and inhibitory drive is set by many terms the net does not control. Between the structure and the function lies a network whose stability is maintained, in part, by the very cell whose removal restores the structure.

That is the whole of the argument. Microglia digest the net, and microglia damp the circuit. A therapy that removes them is not performing a subtraction with one consequence. It gives back the matrix and takes away the brake — and in a disease in which four patients in ten carry occult epileptiform activity that predicts how fast they will decline, the brake is not a minor term.

The evidence for the pairing of microglial-tone modulation with forty-hertz entrainment was never interventional. It was an inference from a shared substrate: the net supports the parvalbumin cell, the parvalbumin cell makes the rhythm, therefore preserving the one prepares the other. The inference has now been probed from three sides and has not held. Removing the net does not abolish the rhythm; it reduces inhibition and, under plasticity induction, raises gamma. Removing the microglia does not simply restore the net; it raises net abundance above baseline even in healthy animals, and it destabilises the network. And the net is not a passive casualty of the microglion but an organiser of its response to plaques, so that the two targets sit in a loop and cannot be addressed one at a time as though they sat in a chain.

None of this is an argument against either intervention on its own terms. The matrix programme has identified a real lesion with a real human correlate. The rhythm programme has a mechanism, a preclinical series and the beginnings of controlled human data. What we argue is narrower and, we think, harder to avoid: that the two have been joined by a word rather than by an experiment, that the one experiment bearing on the joint found a cost where synergy was expected, and that a combination protocol built on the assumption of complementarity would be assuming precisely what is in question.

The remedy is a single factorial, run in vivo, with matrix, cell, network and outcome measured as four things rather than one, and with electrographic seizure pre-specified in the combination arm rather than discovered in it. Until that is done, the honest statement is that we do not know whether these two interventions compose, and the one piece of evidence we have says that in the direction tested, they do not.

There is a general lesson underneath the particular one. Structural endpoints and functional endpoints diverge, and they diverge most sharply in diseases whose clinical expression is a failure of coordination rather than a failure of parts. The experiment that motivates this paper preserved synapses and degraded the rhythm those synapses exist to carry. It would have been scored a success on any structural readout. Coordination was the thing that was lost, and coordination is the thing the patient notices.

References

1. Fawcett JW, Oohashi T, Pizzorusso T. The roles of perineuronal nets and the perinodal extracellular matrix in neuronal function. *Nat Rev Neurosci* 2019;20(8):451-465. doi:10.1038/s41583-019-0196-3. PMID 31263252.
2. Crapser JD, Spangenberg EE, Barahona RA, Arreola MA, Hohsfield LA, Green KN. Microglia facilitate loss of perineuronal nets in the Alzheimer's disease brain. *EBioMedicine* 2020;58:102919. doi:10.1016/j.ebiom.2020.102919. PMID 32745992.
3. Sohal VS, Zhang F, Yizhar O, Deisseroth K. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature* 2009;459(7247):698-702. doi:10.1038/nature07991. PMID 19396159.
4. Cardin JA, Carlén M, Meletis K, et al. Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* 2009;459(7247):663-667. doi:10.1038/nature08002. PMID 19396156.
5. Iaccarino HF, Singer AC, Martorell AJ, et al. Gamma frequency entrainment attenuates amyloid load and modifies microglia. *Nature* 2016;540(7632):230-235. doi:10.1038/nature20587. PMID 27929004.
6. Martorell AJ, Paulson AL, Suk HJ, et al. Multi-sensory gamma stimulation ameliorates Alzheimer's-associated pathology and improves cognition. *Cell* 2019;177(2):256-271.e22. doi:10.1016/j.cell.2019.02.014. PMID 30879788.
7. Adaikkan C, Middleton SJ, Marco A, et al. Gamma entrainment binds higher-order brain regions and offers neuroprotection. *Neuron* 2019;102(5):929-943.e8. doi:10.1016/j.neuron.2019.04.011. PMID 31076275.
8. Murdock MH, Yang CY, Sun N, et al. Multisensory gamma stimulation promotes glymphatic clearance of amyloid. *Nature* 2024;627(8002):149-156. doi:10.1038/s41586-024-07132-6. PMID 38418876.
9. Delaney HJ, Islam S, Healy D, et al. CSF1R inhibition exacerbates gamma oscillation disruption and induces network hyperexcitability in APP/PS1 mice. *Brain* 2026;awag147. doi:10.1093/brain/awag147. PMID 42445988.
10. Kann O. The interneuron energy hypothesis: implications for brain disease. *Neurobiol Dis* 2016;90:75-85. doi:10.1016/j.nbd.2015.08.005. PMID 26284893.

11. Bas-Orth C, Schneider J, Lewen A, et al. The mitochondrial calcium uniporter is crucial for the generation of fast cortical network rhythms. *J Cereb Blood Flow Metab* 2020;40(11):2225-2239. doi:10.1177/0271678X19887777. PMID 31722597.
12. Cabungcal JH, Steullet P, Morishita H, et al. Perineuronal nets protect fast-spiking interneurons against oxidative stress. *Proc Natl Acad Sci U S A* 2013;110(22):9130-9135. doi:10.1073/pnas.1300454110. PMID 23671099.
13. Favuzzi E, Marques-Smith A, Deogracias R, et al. Activity-dependent gating of parvalbumin interneuron function by the perineuronal net protein brevican. *Neuron* 2017;95(3):639-655.e10. doi:10.1016/j.neuron.2017.06.028. PMID 28712654.
14. Morawski M, Brückner G, Jäger C, Seeger G, Arendt T. Neurons associated with aggrecan-based perineuronal nets are protected against tau pathology in subcortical regions in Alzheimer's disease. *Neuroscience* 2010;169(3):1347-1363. doi:10.1016/j.neuroscience.2010.05.022. PMID 20497908.
15. de Vries LE, Bahnerth A, Swaab DF, Verhaagen J, Carulli D. Resilience to Alzheimer's disease associates with alterations in perineuronal nets. *Alzheimers Dement* 2025;21(2):e14504. doi:10.1002/alz.14504. PMID 39737731.
16. Crapser JD, Ochaba J, Soni N, Reidling JC, Thompson LM, Green KN. Microglial depletion prevents extracellular matrix changes and striatal volume reduction in a model of Huntington's disease. *Brain* 2020;143(1):266-288. doi:10.1093/brain/awz363. PMID 31848580.
17. Elmore MR, Najafi AR, Koike MA, et al. Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. *Neuron* 2014;82(2):380-397. doi:10.1016/j.neuron.2014.02.040. PMID 24742461.
18. Spangenberg E, Severson PL, Hohsfield LA, et al. Sustained microglial depletion with CSF1R inhibitor impairs parenchymal plaque development in an Alzheimer's disease model. *Nat Commun* 2019;10:3758. doi:10.1038/s41467-019-11674-z. PMID 31434879.
19. De Lucia C, Rinchon A, Olmos-Alonso A, et al. Microglia regulate hippocampal neurogenesis during chronic neurodegeneration. *Brain Behav Immun* 2016;55:179-190. doi:10.1016/j.bbi.2015.11.001. PMID 26541819.
20. Nguyen PT, Dorman LC, Pan S, et al. Microglial remodeling of the extracellular matrix promotes synapse plasticity. *Cell* 2020;182(2):388-403.e15. doi:10.1016/j.cell.2020.05.050. PMID 32615087.

21. Badimon A, Strasburger HJ, Ayata P, et al. Negative feedback control of neuronal activity by microglia. *Nature* 2020;586(7829):417-423. doi:10.1038/s41586-020-2777-8. PMID 32999463.
22. Paolicelli RC, Bolasco G, Pagani F, et al. Synaptic pruning by microglia is necessary for normal brain development. *Science* 2011;333(6048):1456-1458. doi:10.1126/science.1202529. PMID 21778362.
23. Hong S, Beja-Glasser VF, Nfonoyim BM, et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* 2016;352(6286):712-716. doi:10.1126/science.aad8373. PMID 27033548.
24. Dejanovic B, Wu T, Tsai MC, et al. Complement C1q-dependent excitatory and inhibitory synapse elimination by astrocytes and microglia in Alzheimer's disease mouse models. *Nat Aging* 2022;2(9):837-850. doi:10.1038/s43587-022-00281-1. PMID 37118504.
25. Favuzzi E, Huang S, Saldi GA, et al. GABA-receptive microglia selectively sculpt developing inhibitory circuits. *Cell* 2021;184(15):4048-4063.e32. doi:10.1016/j.cell.2021.06.018. PMID 34233165.
26. Palop JJ, Mucke L. Network abnormalities and interneuron dysfunction in Alzheimer disease. *Nat Rev Neurosci* 2016;17(12):777-792. doi:10.1038/nrn.2016.141. PMID 27829687.
27. Lensjø KK, Lepperød ME, Dick G, Hafting T, Fyhn M. Removal of perineuronal nets unlocks juvenile plasticity through network mechanisms of decreased inhibition and increased gamma activity. *J Neurosci* 2017;37(5):1269-1283. doi:10.1523/JNEUROSCI.2504-16.2016. PMID 28039374.
28. Verret L, Mann EO, Hang GB, et al. Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. *Cell* 2012;149(3):708-721. doi:10.1016/j.cell.2012.02.046. PMID 22541439.
29. Zott B, Simon MM, Hong W, et al. A vicious cycle of β amyloid-dependent neuronal hyperactivation. *Science* 2019;365(6453):559-565. doi:10.1126/science.aay0198. PMID 31395777.
30. Barahona RA, Kwang NE, Kono-Soosaipillai AR, et al. Aggreca protects against plaque accumulation and is essential for proper microglial responses to plaques. *Cell Rep* 2025;44(8):116064. doi:10.1016/j.celrep.2025.116064. PMID 40748756.
31. Hijazi S, Heistek TS, Scheltens P, et al. Early restoration of parvalbumin interneuron activity prevents memory loss and network hyperexcitability in a mouse model of Alzheimer's disease. *Mol Psychiatry* 2020;25(12):3380-3398. doi:10.1038/s41380-019-0483-4. PMID 31431685.

32. Hijazi S, Heistek TS, van der Loo R, Mansvelder HD, Smit AB, van Kesteren RE. Hyperexcitable parvalbumin interneurons render hippocampal circuitry vulnerable to amyloid beta. *iScience* 2020;23(7):101271. doi:10.1016/j.isci.2020.101271. PMID 32593000.
33. Chen YB, Jiang B, Zhuang K, et al. Hyperactivity of subicular parvalbumin interneurons drives early amyloid pathology and cognitive deficits in Alzheimer's disease. *Mol Psychiatry* 2025;30(12):5777-5789. doi:10.1038/s41380-025-03217-4. PMID 40913114.
34. Palop JJ, Chin J, Roberson ED, et al. Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron* 2007;55(5):697-711. doi:10.1016/j.neuron.2007.07.025. PMID 17785178.
35. Busche MA, Eichhoff G, Adelsberger H, et al. Clusters of hyperactive neurons near amyloid plaques in a mouse model of Alzheimer's disease. *Science* 2008;321(5896):1686-1689. doi:10.1126/science.1162844. PMID 18802001.
36. Vossel KA, Ranasinghe KG, Beagle AJ, et al. Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. *Ann Neurol* 2016;80(6):858-870. doi:10.1002/ana.24794. PMID 27696483.
37. Lam AD, Deck G, Goldman A, Eskandar EN, Noebels J, Cole AJ. Silent hippocampal seizures and spikes identified by foramen ovale electrodes in Alzheimer's disease. *Nat Med* 2017;23(6):678-680. doi:10.1038/nm.4330. PMID 28459436.
38. Sanchez PE, Zhu L, Verret L, et al. Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. *Proc Natl Acad Sci U S A* 2012;109(42):E2895-E2903. doi:10.1073/pnas.1121081109. PMID 22869752.
39. Bakker A, Krauss GL, Albert MS, et al. Reduction of hippocampal hyperactivity improves cognition in amnesic mild cognitive impairment. *Neuron* 2012;74(3):467-474. doi:10.1016/j.neuron.2012.03.023. PMID 22578498.
40. Vossel K, Ranasinghe KG, Beagle AJ, et al. Effect of levetiracetam on cognition in patients with Alzheimer disease with and without epileptiform activity: a randomized clinical trial. *JAMA Neurol* 2021;78(11):1345-1354. doi:10.1001/jamaneurol.2021.3310. PMID 34570177.
41. Soula M, Martín-Ávila A, Zhang Y, et al. Forty-hertz light stimulation does not entrain native gamma oscillations in Alzheimer's disease model mice. *Nat Neurosci* 2023;26(4):570-578. doi:10.1038/s41593-023-01270-2. PMID 36879142.
42. Iaccarino HF, Singer AC, Martorell AJ, et al. Author Correction: Gamma frequency entrainment attenuates amyloid load and modifies microglia. *Nature* 2018;562(7725):E1. doi:10.1038/s41586-018-0351-4. PMID 30046102.

- 43.** Da X, Hempel E, Ou Y, et al. Noninvasive gamma sensory stimulation may reduce white matter and myelin loss in Alzheimer's disease. *J Alzheimers Dis* 2024;97(1):359-372. doi:10.3233/JAD-230506. PMID 38073386.