The Unpicked Seam

The raphe nuclei in Alzheimer's disease — how the serotonergic midline fails, what its failure withdraws from the forebrain, and why restoring the signal so late has failed

The dorsal raphe · Serotonin · Tau · The missing net · Monoamine oxidase · The amyloid brake

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Raphe means seam. The nuclei were named for lying along the midline suture of the brainstem, and the name is better than its coiners knew: a seam is noticed only when it gives way. This one begins to give way in the third decade of life, loses two fifths of its forebrain-projecting neurons before death, and produces no sign a neurological examination can detect.

Contents

Abstract

The raphe nuclei take their name from a seam. They lie along the midline suture of the brainstem, a thin column of cells running from the midbrain to the medulla, and they contain nearly all of the brain's serotonin. The dorsal raphe nucleus alone supplies the greater part of the ascending serotonergic innervation of the forebrain, and it does so with an economy that has no parallel elsewhere in the nervous system: a few hundred thousand neurons, each projecting an axon whose reconstructed length in the rat reaches nearly nineteen centimetres, releasing transmitter not into discrete synapses but into the volume of tissue through which the axon passes. The system does not carry information. It sets the terms on which other systems carry it.

This paper is about the failure of that seam in Alzheimer's disease, and about what the failure costs. Three findings define the problem. First, the raphe fails early — early enough that it belongs to the pre-clinical decades rather than to the illness. Braak's survey of 2,332 brains spanning ages one to one hundred places abnormal tau in the serotonergic raphe at pretangle stage c, after the locus coeruleus and *before* any cortical involvement whatever; stereological counts find hyperphosphorylated inclusions in 2.6 per cent of dorsal raphe neurons in brains staged Braak 0, against 7.9 per cent in the locus coeruleus. Second, the raphe fails severely. A meta-analysis of sixty-seven stereological studies ranks its neuronal loss at an effect size of 1.79 standard deviations — behind the nucleus basalis and the locus coeruleus, but three times the substantia nigra, and on the order of forty per cent of the nucleus in absolute terms. Third, and least appreciated, the loss is not the depression. Post-mortem comparison of four groups finds Alzheimer brains markedly depleted of dorsal raphe serotonergic neurons relative to controls, no depletion at all in primary late-life depression, and no difference between Alzheimer patients with and without comorbid major depression. Whatever the raphe lesion produces, it is not simply low mood.

The paper's mechanistic argument turns on an asymmetry that the habitual pairing of the raphe with the locus coeruleus has obscured. The two nuclei are routinely described as failing for the same reasons, and they do share three: an enormous unmyelinated arbor whose maintenance cost scales with its length, autonomous tonic pacemaking that imposes a continuous calcium load, and the absence of the aggrecan-based perineuronal net that protects net-bearing neurons elsewhere from tau. But they do not share their chemistry. The locus coeruleus expresses monoamine oxidase A, whose oxidation of dopamine yields the reactive aldehyde that has been proposed as its specific intracellular poison. Raphe serotonergic neurons express monoamine oxidase B — the isoform that handles serotonin poorly — and they express it in a compartment from which it is not exported to the terminals. Two independent mappings of the human brainstem agree on this assignment and note that it runs opposite to expectation. The raphe therefore lacks the coeruleus's particular chemical liability and tangles nearly as early regardless. If the two nuclei fail together on different chemistry, the shared cause is not chemical. It is architectural: the arbor, the pacemaker, and the missing net.

The cost of the failure is then set out under seven headings, of which the least expected is the first. Serotonin suppresses the production of amyloid-β. Direct infusion into the hippocampus lowers interstitial amyloid; selective serotonin reuptake inhibitors lower it by a quarter in mice and slow its production rate by 37 per cent in living humans; 5-HT4 receptors bind and traffic ADAM10, the constitutive α-secretase, and drive the cleavage that pre-empts amyloid formation; genetic ablation of the serotonin-synthesising enzyme increases plaque load. A nucleus that begins to fail in the third decade of life is therefore withdrawing a tonic brake on amyloidogenesis across the entire cortical mantle, decades before the first plaque is counted. The remaining costs — a restraint on tau in the target field, the trophic supply to adult hippocampal neurogenesis, the consolidation of sleep, the substrate for melatonin, a brake on microglial phagocytosis, and a measurable contribution to memory and semantic fluency — are treated in turn, with the contradictions in the evidence stated rather than smoothed. Serotonergic denervation raises cortical tau without touching plaques in one model while enzyme ablation raises plaques in another, and the paper does not pretend these results agree.

Against this stands the therapeutic record, which is close to uniformly negative and is reported here in full. Two 5-HT6 antagonists were carried into Phase III on strong preclinical and Phase II grounds; idalopirdine failed three trials enrolling 2,525 patients and intepirdine failed one enrolling 1,315, none showing benefit at any dose on any background. Citalopram at 30 mg reduced agitation in Alzheimer's disease while prolonging the QT interval and *worsening* cognition. The conclusion drawn is not that the serotonergic lesion is unimportant but that it has been mistargeted in time: the evidence supports a permissive early lesion whose window closed before any of these trials enrolled their first patient, and the pharmacology that failed was pharmacology of the signal rather than of the cell. The distinction is testable, and the closing sections state a graded ledger of every claim made, nine experiments, and the results that would refute the reading offered here.

Note on evidence and grading

Claims in this paper are graded, and the grade is given at the point of use rather than left to be inferred from the confidence of the prose. Four grades are used.

Established. Replicated in human material by more than one group, with quantitative agreement. The staging order, the neuronal loss fractions, and the trial outcomes are of this kind.

Probable. Supported by good evidence from human tissue or from more than one animal model, but with a gap — a single cohort, an unreplicated measurement, or a result whose direction is agreed while its magnitude is not.

Inference. A conclusion drawn here from evidence gathered for another purpose. The reasoning is given explicitly so that it can be checked, and the underlying observations are cited separately from the inference they are used to support.

Speculative. Mechanistically coherent, consistent with what is known, and not yet tested. Marked so that it is not mistaken for the others.

Two further conventions. Where the literature contains a genuine contradiction, both sides are cited and the contradiction is named as such; no attempt is made to resolve disagreements by selecting the convenient result. And where a figure is widely repeated in review articles in a form that does not match the primary source, the primary source is used and the discrepancy is noted.

A note on the anatomy of the name

Raphe is the Greek *raphe*, a seam or suture — the word a surgeon uses for a line of stitching. The nuclei were named for their position, not their function: they sit along the median raphe of the brainstem, the seam where the two halves of the hindbrain meet in development. The name is better than its coiners knew. A seam is not a structure in its own right so much as the line along which other structures are joined, and it is noticed only when it gives way. That is a fair description of a neuromodulatory system whose loss produces no focal deficit, no paralysis, no aphasia, and no single sign that would send a patient to a neurologist — and which has nonetheless been shown, in brain after brain, to be among the first things in the human nervous system to go wrong.

PART I — The Seam

1.1 What the raphe is, and what it is not

The serotonergic system was mapped before it was understood. Dahlström and Fuxe, using the Falck–Hillarp formaldehyde-condensation method on rat brainstem in 1964, resolved the monoamine-containing cell groups into a numbered series: A1–A15 for the catecholaminergic groups, B1–B9 for the indolaminergic ones. The B-series is the raphe. Its numbering runs caudal to rostral, and the division it implies has held up under every subsequent method.

The **caudal group** — B1 (raphe pallidus), B2 (raphe obscurus), and B3 (raphe magnus), lying in the medulla — projects predominantly downward, to the spinal cord and the lower brainstem. Its business is autonomic and antinociceptive: descending modulation of dorsal-horn pain transmission, control of sympathetic outflow, thermoregulation, and the chemosensory drive to breathe. The **rostral group** — B5 and B8 (median raphe), B6 and B7 (dorsal raphe), and B9 (the supralemniscal group) — lies in the pons and midbrain and projects upward, supplying essentially the whole of the forebrain.

This division matters for the argument of this paper, and it is regularly lost when the literature refers to "the raphe" without qualification. The disease does not treat the two groups alike. Nearly everything reported below concerns the rostral group, and within the rostral group, predominantly the dorsal raphe nucleus. The distinction is taken up directly in §5.8, because what is *spared* turns out to be as informative as what is lost.

The dorsal raphe nucleus is itself not a unit. Human and rodent anatomies agree in resolving it into subnuclei — dorsal, ventral, ventrolateral (the "lateral wings"), interfascicular, and caudal — that differ in their projection targets, their electrophysiology, their co-transmitter content, and, as §2.3 sets out, in their vulnerability to tau. Single-cell transcriptomic work combined with whole-brain projection mapping has confirmed that dorsal raphe serotonergic neurons fall into transcriptionally distinct classes with segregated axonal targets, so that the nucleus is better read as several parallel systems sharing a transmitter than as one broadcast source. Any claim that "the dorsal raphe" does something should be treated as provisional until the subnucleus is named.

1.2 The cell

A dorsal raphe serotonergic neuron is an unusual object, and three of its properties do most of the work in what follows.

It is a pacemaker. Serotonergic raphe neurons fire tonically and autonomously, at low frequency — on the order of one to three hertz — in a slow, remarkably regular rhythm that persists in slice preparations after all synaptic input is removed. The firing is not driven; it is intrinsic, generated by a pacemaker conductance, and it is sustained for the life of the animal. The functional consequence is that the transmitter is delivered as a continuously maintained tone rather than as a signal, and the state of the target tissue depends on the tone's level rather than on the timing of any individual spike. The metabolic consequence is that the cell never rests. Every action potential admits calcium; every calcium transient must be pumped back out or sequestered, at ATP cost; and a cell that has fired continuously for eighty years has paid that cost some six billion times.

It has an enormous arbor. This is the property that most distinguishes the aminergic neurons from the cells they modulate. Single-neuron reconstructions of rat dorsal raphe axons, labelled individually and traced through serial sections, give total axonal lengths reaching 18.7 centimetres for a single cell — in an animal whose entire brain is about two centimetres long. The axons are thin, unmyelinated, and extraordinarily collateralised, branching to reach multiple terminal fields from one soma. Along their length they bear varicosities in the tens to hundreds of thousands, and it is from these varicosities, rather than from conventional synaptic specialisations, that most serotonin is released.

The human arbor has not been reconstructed at single-cell resolution and the corresponding figure is not available; the rat measurement is used here as an order-of-magnitude anchor and the scaling to human is an *inference*, not a measurement. But the inference is not a delicate one. The human dorsal raphe contains on the order of a few hundred thousand serotonergic neurons and innervates a forebrain some three orders of magnitude larger in volume than the rat's. Whatever the true figure, the ratio of axon to soma in a human serotonergic neuron is larger than in the rat, not smaller.

The consequence is a maintenance problem. Everything the axon needs — mitochondria, synaptic vesicle proteins, the enzymes of transmitter synthesis, the machinery of local repair — is manufactured in a soma perhaps twenty micrometres across and transported outward along a microtubule track that may be a hundred thousand times longer than the cell body is wide. The cell is, in engineering terms, a supply line with a very small depot at one end and an implausible amount of territory at the other. Any insult that degrades microtubule-based transport falls on this cell harder than on a cortical pyramidal neuron, because this cell has more to lose per unit of transport failure. Tau is precisely such an insult.

It broadcasts by volume, not by wire. Most serotonin release in the forebrain is *non-junctional*: the varicosity has no postsynaptic partner directly apposed to it, and the transmitter diffuses through the extracellular space to reach receptors at some distance. This is volume transmission, and it has two implications that recur throughout this paper. The first is that the

system has no spatial precision to lose — it was never delivering a targeted message, so its degradation does not produce a focal deficit but a diffuse change in the operating point of everything within reach. The second is that the relationship between the number of surviving neurons and the concentration of transmitter at a receptor is not linear and not local. A partially denervated cortex is not a cortex with holes in its serotonergic innervation; it is a cortex with a lower ambient serotonin concentration everywhere, and the compensatory sprouting of surviving axons can hold that concentration up for a long time before it falls. §3.5 returns to this, because it is the principal reason the raphe lesion is clinically silent for decades.

A substantial minority of dorsal raphe neurons are not purely serotonergic. They co-express the vesicular glutamate transporter VGLUT3 and release glutamate alongside serotonin; in the striatum some 93 per cent of serotonergic varicosities carry VGLUT3, in motor cortex about 75 per cent. These dual-transmitter cells are more excitable than their purely serotonergic neighbours. Whether this makes them more vulnerable is discussed in §4.6, where it is graded as *inference* rather than fact.

1.3 What the system does

It is worth being precise about the function that is lost, because imprecision here is what has produced two decades of misdirected pharmacology.

The ascending serotonergic projection does not encode a variable. It does not carry sensory information, does not represent a motor plan, and does not participate in the moment-to-moment computations of the circuits it innervates. What it does is set parameters. Serotonin acting at some fourteen receptor subtypes — an inhibitory Gi-coupled family (5-HT1A, 5-HT1B and relatives), an excitatory Gq-coupled family (5-HT2A, 5-HT2B, 5-HT2C), a ligand-gated cation channel (5-HT3), and a Gs-coupled family that raises cyclic AMP (5-HT4, 5-HT6, 5-HT7) — adjusts the gain, the threshold, the adaptation rate, and the plasticity of cortical, hippocampal and limbic circuits. It biases the trade-off between persisting with a current behaviour and switching away from it. It gates the consolidation of some kinds of memory. It shapes the timing and depth of sleep. And, through the Gs-coupled subtypes, it drives a cyclic-AMP signal in the target neuron that has consequences for protein trafficking well beyond the electrical — a point that becomes central in Part V.

The economy of this arrangement is extreme. A system amounting to a few hundred thousand cells — a vanishing fraction of eighty-six billion — sets the operating conditions of the entire forebrain. That economy is the source of both the system's power and its fragility. There is no redundancy in the sense that matters: no second serotonergic nucleus stands ready to take over, because the dorsal and median raphe together *are* the supply. What redundancy exists is internal, in the capacity of surviving neurons to sprout and to raise their output, and that capacity is finite and is itself consumed by the compensation.

1.4 The habitual pairing, and why it needs examining

The raphe rarely appears in the Alzheimer's literature on its own. It appears as the second half of a phrase — "the locus coeruleus and dorsal raphe," "the brainstem aminergic nuclei," "the monoaminergic systems" — and the phrase does real work, because the two nuclei genuinely do behave alike in the respects that first attracted attention. Both are small, both are subcortical, both are aminergic, both project diffusely, both accumulate tau before the cortex does, and both are heavily depleted by the time of death.

But a pairing that is useful for staging can become misleading when it is carried into mechanism. If the two nuclei are always named together, it is easy to assume that an explanation offered for one applies to the other, and easy not to notice when the explanation is specific to one nucleus's chemistry. That is what has happened with monoamine oxidase, and Part IV is largely devoted to it. The point can be stated here in advance: the most developed cell-autonomous account of why the locus coeruleus tangles first rests on a reactive aldehyde produced by monoamine oxidase A acting on a catecholamine, and the raphe expresses the other isoform and has no catecholamine to give it. Either the account is wrong about the coeruleus, or the raphe is failing for a different reason, or — the reading defended here — the chemistry is a local aggravation on top of a shared architectural liability that is doing most of the work.

Which of those is true matters therapeutically, because the three readings point at different targets.

PART II — The Order of Failure

2.1 The staging evidence

The claim that the raphe fails early is not an inference from function. It is a direct observation on human tissue, made independently by three groups using different methods, and it is *Established*.

The foundational survey is Braak's. Working with 2,332 unselected brains from individuals aged one to one hundred, stained with AT8 immunocytochemistry and Gallyas silver for abnormal tau and with 4G8 and Campbell–Switzer for β-amyloid, Braak, Thal, Ghebremedhin and Del Tredici resolved the pre-cortical phase of the disease into a sequence of *pretangle* stages that precede the numbered Braak stages entirely. The sequence is worth quoting in its structure, because the raphe's position in it is explicit:

> **Stage a** — *abnormal tau in the axons of locus coeruleus projection neurons.* **Stage b** — *abnormal tau in those axons and in the somatodendritic compartment of the locus coeruleus.* **Stage c** — *the foregoing, together with other subcortical neuromodulatory cell groups, among them the serotonergic raphe nuclei.* **Stage 1a** — *abnormal tau tracked along locus coeruleus axons to their terminals in transentorhinal and entorhinal cortex.* **Stage 1b** — *abnormal tau in the pyramidal cells of transentorhinal cortex.*

Two things follow, and they should be held apart. The first is that the raphe is involved *before the cortex*. Pretangle stage c is complete before stage 1a begins; fifty-eight brains in the series carried subcortical tau with no abnormal cortical tau at all. On the ordering that the largest existing series supports, the serotonergic raphe is tangling while the transentorhinal cortex is still clean. The second is that the raphe is *second, not first*. Stage c follows stages a and b, which are the locus coeruleus alone. The frequent description of the two nuclei as "co-earliest" is a compression, and a small one, but it obscures a real ordinal fact that the quantitative work below sharpens.

The age distribution attached to these stages is the reason the finding matters clinically. Pretangle stages a–c predominate at ages ten to twenty. Stages 1a–1b appear mainly at ages forty to fifty. Braak tangle stages I–II become common from sixty, and the symptomatic stages III–VI cluster between eighty and one hundred. The raphe, on this timetable, is accumulating abnormal tau in the second and third decades of life, in people who will not be diagnosed for another half-century, if they are diagnosed at all.

2.2 The transentorhinal comparison

Grinberg and colleagues addressed the ordering question directly, and their title asked it as a question: does the dorsal raphe show neurofibrillary change *before* the transentorhinal region? Examining human brainstem and medial temporal tissue for phospho-tau, they found neurofibrillary changes in a subnucleus of the dorsal raphe in every brain staged Braak I or above, and — the load-bearing result — in more than a fifth of brains staged Braak 0, that is, in brains with no transentorhinal involvement whatsoever.

This is the single most direct piece of evidence that the raphe lesion is not downstream of the cortical disease. A brain at Braak 0 has, by definition, no neurofibrillary pathology in the region that the numbered staging scheme treats as the point of origin. If more than twenty per cent of such brains nonetheless carry phospho-tau in the dorsal raphe, then either the raphe lesion arises independently or it arises from something upstream of both. It cannot be a consequence of transentorhinal pathology that has not yet occurred.

Grinberg's finding also introduces the subnuclear qualification that §1.1 anticipated. The changes were not distributed evenly through the dorsal raphe; they were concentrated in a subnucleus. The disease does not attack "the dorsal raphe." It attacks part of it, and the part it attacks first is a question that the imaging literature has not yet been able to resolve in living people because no available tracer has the spatial resolution to separate dorsal raphe subnuclei in vivo.

2.3 The quantitative anchor

Ordering is one thing; magnitude at a given stage is another, and for that the best evidence is stereological. Ehrenberg and colleagues applied unbiased stereology to forty-eight well-characterised cases deliberately enriched for controls and early stages, counting hyperphosphorylated-tau neuronal cytoplasmic inclusions throughout the full extent of both the locus coeruleus and the dorsal raphe in sixty-micrometre sections.

The result at the earliest point in the series is the number this paper returns to repeatedly:

Nucleus	Neurons bearing hyperphosphorylated-tau inclusions at Braak stage 0
Locus coeruleus	7.9%
Dorsal raphe nucleus	2.6%

Both figures are remarkable, since Braak stage 0 is the stage at which, by the numbered scheme, nothing has happened. Roughly one in thirteen locus coeruleus neurons and one in thirty-eight dorsal raphe neurons already carry the lesion. But the ratio between them is the point. At the earliest countable moment, the coeruleus is carrying about three times the raphe's tau burden. The

two nuclei are not simultaneous. The coeruleus leads, and the raphe follows at a measurable distance.

Grade: Established. Unbiased stereology, a sample designed for the early end of the range, both nuclei counted in the same brains by the same method — this is as clean a comparison as human post-mortem material permits.

2.4 The living-brain and non-demented evidence

The post-mortem series above are all, necessarily, cross-sectional and terminal. Two more recent lines of work address the obvious objection that pathology found in brains at autopsy may not describe the trajectory of brains that are still working.

The stronger of the two is the study by Pierson and colleagues, published in *Molecular Psychiatry* in 2025. Examining the dorsal raphe in individuals aged twenty-five to eighty with no known history of dementia, they found tau pathology present at a prevalence comparable to that in the locus coeruleus — and found that other pathological proteins were substantially less often present in the same tissue. Fewer cases were positive for α-synuclein, for β-amyloid, and for TDP-43. The specificity matters: it argues that what is accumulating in the raphe of middle-aged people without dementia is a tauopathy, not a general marker of brainstem ageing or of mixed pathology.

The same paper supplies the causal experiment that the human material cannot. Overexpressing human P301L tau selectively in the mouse dorsal raphe produced depressive-like behaviour and hyperactivity — and did *not* produce deficits in spatial memory. This is one of the cleanest available statements of what the raphe lesion, on its own, buys. It is discussed in §5.5, because it constrains the interpretation of the mood findings considerably more sharply than the human correlational literature can.

A citation note. This paper circulated as a preprint from 2022 and is widely cited under an incorrect volume and first author. The correct record is Pierson SR, Fiock KL, Wang R, et al., *Molecular Psychiatry* 2025;30(2):532–546, PMID 39143322, published online 14 August 2024. Reviews citing it to volume 29 are citing the pre-publication listing.

In the living brain, the evidence is molecular-imaging evidence and it is *Probable* rather than *Established*, because the cohorts are small and no tracer images the raphe soma directly. Using [^{11}C]DASB to measure serotonin transporter availability, Smith and colleagues found lower binding in mild cognitive impairment than in controls across cortical, limbic, sensory and motor regions, with lower binding associated with worse verbal and visual-spatial memory performance. A later study from the same group replicated the transporter loss in MCI, established its co-occurrence with elevated cortical β-amyloid, and again found the limbic reductions correlated with memory and with semantic fluency. Using [^{18}F]MPPF to measure the 5-HT1A receptor, Kepe and colleagues reported a slight reduction of hippocampal binding in amnestic MCI and a marked

reduction in Alzheimer's disease.

These are terminal-field measurements, not measurements of the nucleus, and that is exactly why they are useful. They establish that by the time a patient reaches the clinic with mild cognitive impairment, the *projection* has already thinned. §3.5 argues that this is the expected order — that the axon goes before the soma — and that it is the reason the clinical picture lags the pathology by decades.

2.5 The honest caveat: not all of this becomes Alzheimer's disease

The findings above are sometimes presented as though early subcortical tau were an early diagnosis. It is not, and the overstatement should be resisted.

Abnormal tau confined to the brainstem aminergic nuclei in a young or middle-aged brain is compatible with at least two trajectories. It may be the first stage of a process that will, over decades, become Alzheimer's disease. Or it may be primary age-related tauopathy — a subcortical and medial-temporal tauopathy that accumulates with age, occurs in the absence of amyloid, and in many people never progresses to dementia at all. The staging *order* is robust and repeatedly confirmed. The *deterministic interpretation* — that a raphe pretangle at thirty predicts dementia at eighty — is not established, and no prospective series exists that could establish it, because the observation is only available at autopsy.

This caveat cuts in a specific direction, and it should be stated plainly rather than buried. It weakens any claim that raphe tau is a *sufficient* cause. It does not weaken the claim that the raphe lesion is early, nor the claim that it is severe in established disease, nor any of the functional arguments in Part V, which depend on the loss of serotonergic tone and not on the mechanism that produced the loss.

2.6 Summary of the order

Observation	Source	Grade
Abnormal tau in raphe at pretangle stage c, before any cortical tau	Braak et al. 2011, n = 2,332	Established
Phospho-tau in a dorsal raphe subnucleus in >20% of Braak 0 brains	Grinberg et al. 2009	Established
2.6% of dorsal raphe neurons tangled at Braak 0, vs 7.9% in locus coeruleus	Ehrenberg et al. 2017, unbiased stereology, n = 48	Established
Raphe tau in non-demented adults aged 25–80, at prevalence comparable to locus coeruleus; α-synuclein, Aβ and TDP-43 rarer	Pierson et al. 2025	Established
Serotonin transporter loss in the cortex and limbic system at the MCI stage	Smith et al. 2017, 2023, [^{11}C]DASB PET	Probable
Hippocampal 5-HT1A receptor loss, slight in aMCI and marked in AD	Kepe et al. 2006, [^{18}F]MPPF PET, n = 19	Probable
The raphe leads the cortex but follows the locus coeruleus	Braak 2011 + Ehrenberg 2017, concordant	Established
Early raphe tau in a given individual predicts eventual dementia	—	Not established; see §2.5

PART III — The Magnitude of the Failure

3.1 How much of the nucleus is lost

Early is not the same as severe, and a nucleus could in principle accumulate tau for fifty years and lose very few cells. The raphe does not. It is among the most heavily depleted nuclei in the Alzheimer brain, and the best evidence for this is a meta-analysis rather than any single series, because individual stereological studies of small brainstem nuclei are noisy and have disagreed.

Lyness, Zarow and Chui pooled sixty-seven primary studies spanning roughly two decades, comparing cell counts in Alzheimer's disease against controls in the four great subcortical projection nuclei, and expressed the result as a standardised mean difference. The ranking is the most useful single table in the subcortical literature:

Nucleus	Transmitter	Effect size (*d*)	Studies	*N*
Nucleus basalis of Meynert	Acetylcholine	2.48	33	585
Locus coeruleus	Noradrenaline	2.28	24	545
Dorsal raphe nucleus	**Serotonin**	**1.79**	**11**	**234**
Substantia nigra	Dopamine	0.61	14	440

Three readings of this table are worth separating.

The raphe's loss is **large in absolute terms**. An effect size of 1.79 standard deviations is not a marginal finding; it is a separation at which the distributions of patients and controls barely overlap. Direct stereological counting in the nucleus raphes dorsalis puts the loss on the order of forty per cent of the neuronal population.

The raphe's loss is **third, not first**. The cholinergic nucleus basalis and the noradrenergic locus coeruleus are both more severely depleted. Any account that treats serotonin as *the* lost transmitter of Alzheimer's disease is overstating a real finding.

The raphe's loss is **three times the substantia nigra's**. This is the comparison that gives the number its force, because the substantia nigra is the nucleus whose destruction defines a different neurodegenerative disease. The serotonergic lesion of Alzheimer's disease is, by this measure, substantially larger than the dopaminergic lesion of Alzheimer's disease — and the

dopaminergic lesion of *Parkinson's* disease is what a clinician recognises as a devastating, life-defining neurological syndrome. The raphe sustains a comparable proportional insult and produces no syndrome anyone has named.

The table also carries a caution about its own weakest row. The dorsal raphe estimate rests on eleven studies and 234 subjects, against thirty-three studies and 585 subjects for the nucleus basalis. The raphe figure is the least well-supported of the four, and its confidence interval is correspondingly the widest. *Grade: Established for the direction and the approximate magnitude; Probable for the precise ordinal placement relative to the locus coeruleus.*

3.2 The dissociation from depression

The most important single result in the human raphe literature is a negative one, and it is routinely mis-cited, so it is set out here in full.

Hendricksen, Thomas, Ferrier, Ince and O'Brien conducted a four-group post-mortem comparison of the dorsal raphe nuclei, using immunocytochemistry and two-dimensional image analysis to measure serotonergic neuronal density and neuritic pathology. The groups were: elderly subjects with primary major depression (n = 14); Alzheimer's disease with comorbid major depression (n = 8); Alzheimer's disease without depression (n = 7); and non-depressed elderly comparison subjects (n = 10).

The design was built to test a hypothesis, and the hypothesis failed. The authors had predicted that depressed subjects would show fewer serotonergic neurons and more neuritic pathology than non-depressed subjects, and that depressed Alzheimer patients would show more than non-depressed ones. Neither prediction held. What they found instead was a three-part result:

The Alzheimer's disease subjects showed markedly fewer serotonergic neurons and higher neuritic pathology than both the primary-depression subjects and the non-depressed comparison subjects. The disease-related loss is real and is present in the tissue.

The Alzheimer's disease subjects with comorbid major depression did not differ from those without. Within the disease, depression status does not track the raphe lesion.

The primary major depression subjects did not differ from the non-depressed comparison subjects. Late-life depression, absent Alzheimer's disease, produces no detectable loss of dorsal raphe serotonergic neurons at all.

Put together, these three comparisons form a dissociation of an unusually clean kind. The raphe lesion is specific to the neurodegenerative disease and is unrelated, within that disease, to whether the patient was depressed. Depression in Alzheimer's disease is therefore *not* explained by dorsal

raphe cell loss, and dorsal raphe cell loss in Alzheimer's disease is not a marker of depression.

This result is frequently summarised in reviews as showing that "serotonergic neuron loss occurs in Alzheimer's disease independent of depression," which is accurate, and occasionally as showing that "depression is associated with raphe pathology," which the paper specifically refutes. The abstract's concluding sentence — that the study found no evidence of serotonergic neuron loss in older people with depression, with or without comorbid Alzheimer's disease — refers to the depression comparisons and has sometimes been read as denying the Alzheimer's-versus-control loss reported in the same abstract's results. It does not.

Grade: Established, with the caveat that the group sizes are small (7–14 per cell) and the study has not been replicated with modern unbiased stereology. A replication is listed among the experiments in §7.3.

3.3 What the dissociation implies

Two consequences follow, and both bear on the therapeutic argument in Part VI.

The first is interpretive. If the raphe lesion is present in non-depressed Alzheimer patients to the same degree as in depressed ones, then whatever the lesion does, its principal consequence is not mood. The mood symptoms of Alzheimer's disease must arise elsewhere — in frontal-subcortical circuit disruption, in the cortical and limbic terminal fields rather than the nucleus, or in mechanisms unrelated to serotonin — and treating the raphe lesion as "the depression lesion" mislocates it.

The second is methodological, and sharper. Because the raphe lesion is *not* the depression lesion, the depression literature cannot be used as a proxy for it. A great deal of writing about serotonin in Alzheimer's disease imports its framework from psychiatry: it assumes that the relevant outcome is affective, that the relevant intervention is a reuptake inhibitor, and that the relevant endpoint is a mood scale. Hendricksen's four-group comparison says that this importation is unlicensed. The consequences of the raphe lesion in Alzheimer's disease have to be sought in what serotonin does *besides* setting mood — which is the subject of Part V, and which turns out to include the regulation of amyloid.

3.4 The terminal field goes before the soma

A pattern runs through the imaging data of §2.4 that deserves to be stated as a claim in its own right: in the serotonergic system, the projection degenerates before the cell body dies.

The evidence is indirect but consistent. Serotonin transporter binding, which is a marker of axon terminals and not of somata, is already reduced across cortical and limbic regions at the stage of mild cognitive impairment — before dementia, and therefore long before the terminal cell counts of §3.1 apply. The 5-HT1A receptor changes measured in hippocampus follow a similar early course. Meanwhile the somatic count in the nucleus, measured at autopsy in established disease,

gives the forty per cent figure. The two measurements are separated by many years of clinical course.

The mechanistic reading is straightforward and follows from §1.2. A neuron whose axon is a hundred thousand times longer than its soma is wide has most of its vulnerable surface out in the projection. Tau's principal cellular effect is the disruption of microtubule-based axonal transport; the most distal territory is the first to be starved of what the soma sends; and a cell can lose the great majority of its arbor and still be counted as a living neuron by a stereologist. There is no contradiction between "the terminal field is thinning at MCI" and "forty per cent of the neurons are gone at death." They are sequential observations of one process.

Grade: Probable. The individual observations are solid, but no study has measured axonal and somatic loss in the same serotonergic neurons across stages, and the inference that the one precedes the other rests on comparing different cohorts measured by different methods.

3.5 Why decades of this produce no symptom

The final magnitude question is the one a clinician would ask first. If the raphe begins to tangle in the third decade and loses forty per cent of its neurons by death, why is there nothing to see for fifty years?

Three properties of the system, each established in §1, combine to produce the silence.

Volume transmission has no focal signature. A projection that releases transmitter non-junctionally into the extracellular space cannot produce a focal deficit when it degrades, because it was not delivering anything focal. Losing serotonergic innervation of the prefrontal cortex does not disconnect a pathway; it lowers a concentration.

The surviving neurons compensate. Partial lesions of diffuse aminergic systems are followed by collateral sprouting of the remaining axons and by increased firing and transmitter output per surviving cell. Because the functional variable is ambient concentration rather than the integrity of individual connections, this compensation is unusually effective: a substantial fraction of the neurons can be lost while the concentration at the receptor is held near normal. The compensation is not free — it increases the metabolic and oxidative load on exactly the cells that are already failing, which is a plausible route by which early loss accelerates later loss — but it is effective, and it is why the deficit is subclinical for so long.

The functions affected have no sharp threshold. The deficits that a partially denervated serotonergic system produces — slightly worse sleep consolidation, slightly reduced cognitive flexibility, a modest downward shift in mood, subtly impaired pattern separation — are all continuous variables with wide normal ranges and no clinical cut-point. They are the kind of change a person attributes to being forty rather than twenty-five.

Taken together these explain the clinical silence without requiring the pathology to be benign. A lesion can be simultaneously severe, progressive, functionally consequential, and invisible, provided its consequences are diffuse, compensable, and gradual. That is a fair description of the raphe in the first five decades of life, and it is the reason the therapeutic argument of Part VI turns on timing rather than on target.

PART IV — Why It Fails

4.1 The question, stated precisely

The question is not why neurons die in Alzheimer's disease. It is narrower and harder: why do *these* neurons — a few hundred thousand cells on the brainstem midline — accumulate abnormal tau in the second and third decades of life, when the cortical populations that will eventually carry the disease's clinical weight remain clean for another thirty years?

Any answer has to satisfy two constraints simultaneously. It must explain why the raphe is affected so early, and it must explain why the raphe is affected slightly *later* than the locus coeruleus, given the 7.9-versus-2.6 per cent separation at Braak 0. An account that makes the two nuclei identical fails the second constraint. An account that makes them wholly different fails the first.

4.2 Three liabilities the two nuclei share

The arbor. This is the largest single factor and the one with the most direct mechanistic connection to the lesion observed. Both nuclei consist of neurons with vast, thin, unmyelinated, highly collateralised axons — on the order of tens of centimetres of axon per cell in the rat, with the human figure necessarily larger — supported by a small soma through microtubule-dependent transport. Tau is a microtubule-associated protein whose hyperphosphorylation detaches it from the microtubule and destabilises the track. A cell whose viability depends on transport over that distance is more sensitive to a given degree of track destabilisation than a cell whose axon is a millimetre long. The arbor is also, straightforwardly, expensive: the surface area to be maintained, the mitochondria to be trafficked, the membrane potential to be sustained, all scale with axonal length, in a cell whose biosynthetic capacity does not.

The pacemaker. Both nuclei fire autonomously and continuously, and both therefore sustain an unremitting calcium load. Autonomous pacemaking has been developed at length as a vulnerability factor in the substantia nigra, where the argument is that the metabolic cost of continuous calcium cycling, and the oxidative consequence of the mitochondrial work it requires, constitute a lifelong stressor unique to pacemaking neurons. The argument transfers to the raphe and the coeruleus without modification. Note that it does not, by itself, distinguish the raphe from the nigra — and the nigra's loss in Alzheimer's disease is $d = 0.61$, the smallest of the four. Pacemaking is therefore necessary to the account but plainly not sufficient.

The missing net. This is the liability with the sharpest evidence and the most specific relation to tau. Morawski, Brückner, Jäger, Seeger and Arendt examined subcortical regions in the

Alzheimer brain and found a systematic complementarity: neurons ensheathed by aggrecan-based perineuronal nets were protected against tau pathology, and the subcortical nuclei attacked earliest and hardest by tau — the locus coeruleus foremost, with the nucleus basalis, the dorsal thalamus, the hypothalamic nuclei and the raphe — are precisely those devoid of that matrix.

The complementarity is a correlation in human tissue, and the causal direction was established separately: perineuronal nets restrict both the distribution and the internalisation of aggregated tau, demonstrated in slice preparations null for aggrecan, for HAPLN1 and for tenascin-R. A neuron without an aggrecan-based net is a neuron whose surface is directly accessible to extracellular tau species and which lacks the polyanionic shield that net-bearing neurons carry.

The raphe is such a neuron. So is the coeruleus. Both are unshielded, and both tangle first. *Grade: Established for the anatomical complementarity and for the net's tau-restricting function; Probable for the claim that net-lessness is a principal cause of the raphe's specific vulnerability, since the correlation is consistent with the net being a marker of some third property.*

4.3 The chemistry they do not share

Here the habitual pairing breaks down, and the break is informative.

The most developed cell-autonomous account of why the locus coeruleus is the first structure in the human brain to tangle is a chemical one. It runs: the coeruleus neuron is full of catecholamine; monoamine oxidase A oxidises that catecholamine to a reactive aldehyde; the aldehyde activates a protease that cleaves tau at a specific site, generating a fragment more prone to aggregation, and cleaves other substrates whose loss removes a restraint on tau phosphorylation. The account is attractive because it is specific: it explains not merely that the coeruleus is stressed but why *this* cell generates *this* lesion, from a reaction that occurs nowhere else in the same form.

Its transfer to the raphe fails at the first step, and the failure is a matter of record rather than of argument.

Raphe serotonergic neurons do not express monoamine oxidase A. They express monoamine oxidase B. The assignment was established by immunohistochemical mapping of MAO-A- and MAO-B-containing cell populations in primate brain, which localised MAO-A to the catecholaminergic groups — the locus coeruleus and the substantia nigra among them — and MAO-B to the raphe, where virtually all serotonin-positive somata were also MAO-B-positive. It was confirmed by in situ hybridisation in human brain, whose authors noted explicitly that locus coeruleus and raphe neurons code for MAO-A and MAO-B respectively *and not vice versa* — that is, opposite to the expectation generated by each enzyme's substrate preference, since MAO-A is the isoform with the higher affinity for serotonin.

The consequence is that the serotonergic neuron is a poor site for intracellular deamination of its own transmitter. Serotonin is preferentially a MAO-A substrate; the serotonergic neuron carries MAO-B. And the MAO-B it carries is confined largely to the somatic compartment: the mitochondria bearing it are not transported to serotonergic terminals, so the arbor — the great majority of the cell's volume and the site of most transmitter handling — is essentially without it.

Three implications follow.

The raphe lacks the coeruleus's specific poison. There is no serotonergic equivalent of the catecholamine-derived aldehyde generated in quantity inside the neuron that made the transmitter. The reaction that the coeruleus account depends on does not occur in the raphe cell in the same way.

This is a plausible partial explanation for the 7.9-versus-2.6 gap. If the coeruleus carries an additional, cell-specific chemical liability that the raphe does not, then the coeruleus should tangle earlier and faster than the raphe despite their shared architecture — which is what the stereology shows. *Grade: Inference.* The observations are established; the causal attribution of the gap to the MAO difference is a reading offered here, and §7.3 gives the experiment that would test it.

The shared cause must be something other than chemistry. This is the load-bearing conclusion of Part IV. Two nuclei with different oxidative chemistry, different transmitters, and different degradative enzymes nonetheless tangle in the same pre-cortical window, ahead of every cortical population, in the same brains. What they share is not their chemistry. It is their architecture — the enormous unmyelinated arbor, the autonomous pacemaker, and the absent aggrecan net. The chemistry modulates the timing; the architecture sets the vulnerability.

If that is right, it has a therapeutic corollary that Part VI develops: interventions aimed at the transmitter or its receptors are aimed at the modulating variable, not the causal one.

4.4 The substrate side

One further liability is specific to the serotonergic cell, and it operates upstream of the neuron rather than inside it.

Serotonin is synthesised from tryptophan in two steps: hydroxylation by tryptophan hydroxylase 2, the brain isoform and the rate-limiting enzyme, followed by decarboxylation. Tryptophan is an essential amino acid — it cannot be synthesised and must be imported — and it is the rarest of the proteinogenic amino acids. Only a small percentage of the body's tryptophan is allocated to serotonin synthesis at all; the great majority goes to protein synthesis and to the kynurenine pathway.

The relevant property of tryptophan hydroxylase 2 is that its affinity for tryptophan sits close to the ambient brain tryptophan concentration. Under normal conditions the enzyme is near saturation and synthesis is not substrate-limited, but the saturation margin is thin, and a fall in available tryptophan translates fairly directly into a fall in serotonin synthesis.

That margin is consumed by inflammation. Indoleamine 2,3-dioxygenase, induced in microglia and peripheral immune cells by interferon-γ and other inflammatory signals, commits tryptophan to the kynurenine pathway and away from serotonin. Sustained inflammatory induction therefore withdraws substrate from the serotonergic branch at its rate-limiting step, and does so preferentially in the cell type with the highest constitutive demand for it.

The raphe neuron thus faces a two-sided problem in the ageing, inflamed brain: a rising internal maintenance burden from tau-impaired transport through an oversized arbor, and a falling external supply of the substrate it exists to convert. *Grade: Established for the biochemistry and for inflammatory IDO induction; Probable for the claim that substrate limitation contributes materially to serotonergic deficit in Alzheimer's disease specifically, where the direct measurements in human brain tissue are fewer than the mechanistic literature implies.*

4.5 The reciprocal lesion: raphe and coeruleus as a coupled pair

The two nuclei are not merely similar; they are connected, and their failures are not independent events.

The locus coeruleus projects noradrenergic fibres to the dorsal raphe, where α1-adrenoceptor activation provides a substantial part of the excitatory drive that sustains serotonergic firing. The dorsal raphe projects back, and serotonin acting at 5-HT2A receptors modulates coeruleus activity. The two nuclei form a reciprocally coupled pair whose joint output sets the arousal state of the forebrain.

The implication for the disease is that coeruleus degeneration does not simply subtract noradrenaline; it also withdraws excitatory drive from the raphe, reducing serotonergic output by a mechanism entirely separate from raphe pathology. A patient with fifty per cent coeruleus loss has less serotonin than their raphe cell count alone would predict. Conversely, raphe loss disinhibits or dysregulates the coeruleus.

This coupling means the two lesions compound, and it means that the measured functional serotonergic deficit in a late-stage brain is not attributable to the raphe alone. *Grade: Established for the anatomical and pharmacological coupling; Inference for the claim that it materially amplifies the functional deficit in human disease, which has not been quantified.*

4.6 The dual-transmitter subpopulation

A final, more speculative liability concerns the VGLUT3-expressing subpopulation described in §1.2. These neurons release glutamate alongside serotonin and are more excitable than their purely serotonergic neighbours. It has been suggested that this increased excitability promotes both tau accumulation and activity-dependent tau release along the axon, and would therefore make the dual-transmitter cells the vanguard of the nucleus's degeneration and a potential route by which tau leaves the raphe for its terminal fields.

The suggestion is coherent, it fits the general association between neuronal activity and tau release, and it would supply a candidate identity for the vulnerable subnucleus that Grinberg's data imply. It has not been tested. No study has established that VGLUT3-positive dorsal raphe neurons tangle before VGLUT3-negative ones in human tissue. *Grade: Speculative.* It is included because §7.3 lists it as an experiment worth doing, not because it is evidence.

4.7 A summary of causes

Liability	Shared with locus coeruleus?	Evidence grade
Enormous unmyelinated, highly collateralised arbor	Yes	Established
Autonomous tonic pacemaking, continuous Ca2+ load	Yes	Established
No aggrecan-based perineuronal net	Yes	Established (anatomy); Probable (causal role)
MAO-A-derived reactive aldehyde from catecholamine	**No — coeruleus only**	Established (enzyme mapping); Inference (role in the gap)
Substrate limitation at tryptophan hydroxylase 2	No — raphe only	Probable
Withdrawal of α1 excitatory drive from a failing coeruleus	Raphe receives it	Inference
Excitability of the VGLUT3 dual-transmitter subpopulation	No — raphe only	Speculative

The pattern in this table is the argument. The three shared liabilities are architectural; the unshared ones are chemical and modulatory. The nuclei that fail together fail together for structural reasons, and differ in their timing for chemical ones.

PART V — What the Failure Costs

5.0 How to read this Part

The costs of raphe failure are set out under eight headings. They are ordered deliberately: not from most to least familiar, but from least to most familiar, because the least familiar is the most consequential and would otherwise be lost behind the mood literature that dominates the field.

A general caution applies throughout. Almost every item below rests on evidence that serotonergic *signalling* does something, combined with evidence that Alzheimer's disease *removes* serotonergic signalling. The conjunction licenses the inference that the disease removes the function — but it does not establish that the removal is quantitatively important relative to everything else going wrong, and in several cases the animal models disagree with each other. Where they do, the disagreement is stated.

5.1 The amyloid brake

The single most consequential thing the raphe does, from the standpoint of Alzheimer's disease, is suppress the production of amyloid-β. This is not a peripheral observation; it has been demonstrated in mice, in living humans, and at the level of the responsible enzyme, and it inverts the usual reading of the raphe as a nucleus whose loss produces symptoms.

In mice. Cirrito and colleagues measured amyloid-β in brain interstitial fluid by microdialysis and found that administration of several selective serotonin reuptake inhibitors reduced interstitial amyloid by about twenty-five per cent. Direct infusion of serotonin into the hippocampus produced the same reduction, establishing that the effect is serotonin's rather than the drug's. Pre-treatment with inhibitors of extracellular signal-regulated kinase abolished it, identifying ERK signalling as the required transduction step.

In humans. Sheline and colleagues took the finding into people. Using stable-isotope labelling to measure the *production rate* of amyloid-β in cerebrospinal fluid — not merely its concentration — they found that citalopram slowed amyloid-β production by 37 per cent in healthy volunteers, with a 38 per cent decrease in total cerebrospinal fluid amyloid-β. In aged transgenic mice, the same drug reduced interstitial amyloid dose-dependently, halted the growth of pre-existing plaques, and reduced the appearance of new plaques by 78 per cent. The same group's earlier work had found lower amyloid burden on Pittsburgh compound B imaging in people with a history of antidepressant use.

At the enzyme. The mechanism is now reasonably well specified, and it runs through the Gs-coupled 5-HT4 receptor and the constitutive α-secretase. Amyloid precursor protein can be cleaved in two mutually exclusive ways: by β- and γ-secretase, liberating amyloid-β, or by α-secretase within the amyloid-β sequence, which destroys the amyloid-β peptide before it exists and releases the neurotrophic soluble ectodomain sAPPα instead. The dominant α-secretase in brain is ADAM10. The 5-HT4 receptor associates with ADAM10 and with amyloid precursor protein, and enhances the trafficking of ADAM10 from the endoplasmic reticulum to the plasma membrane, constitutively promoting the non-amyloidogenic route. Consistent with this, chronic 5-HT4 receptor activation decreases amyloid-β production and deposition in transgenic mice, and early administration of a selective 5-HT4 agonist prevents amyloidogenesis and behavioural deficits in the 5XFAD model.

By subtraction. The converse experiment supports the same conclusion. Genetic ablation of tryptophan hydroxylase 2 — removing the brain's capacity to synthesise serotonin at all — significantly increased plaque load and plaque number in APP/PS1 mice at eight to ten months, and increased GFAP-positive astrocyte density at ten months. In a separate cross of the same model with a TPH2 knockout, serotonin deficiency disproportionately increased mortality in mid-life, at precisely the age at which plaques begin to appear.

What this means for a nucleus that fails at thirty. Put the pharmacology beside the staging and the implication is uncomfortable. Serotonergic tone tonically biases amyloid precursor protein processing toward the non-amyloidogenic route across the whole cortical mantle, continuously, by trafficking α-secretase to the membrane. The nucleus that supplies that tone begins accumulating tau in the second and third decades of life and loses on the order of forty per cent of its neurons over the following half-century. The withdrawal of the brake therefore *precedes* the amyloid phase of the disease by decades and is in place before the first plaque is counted.

This makes the raphe lesion **permissive**. Not causal in the sense of initiating the amyloid cascade — nothing here shows that losing serotonin is sufficient to produce Alzheimer's disease, and the TPH2 knockouts increase plaque load in animals already engineered to make plaques. But permissive in the specific sense that a restraint present in the young brain is progressively removed before the process it restrains begins.

Grade: Established for the serotonin–amyloid relationship in mice and for the human production-rate result; *Established* for the 5-HT4/ADAM10 mechanism; *Inference* for the permissive reading, which combines the pharmacology with the staging and has not been tested as such.

5.2 The restraint on tau in the target field

The second cost concerns tau, and here the evidence is thinner and internally contradictory. Both facts are reported.

Ramos-Rodríguez and colleagues lesioned the serotonergic system in APP/PS1 mice by administering 5,7-dihydroxytryptamine into the raphe nuclei — a selective serotonergic denervation — and examined the cortex. Denervation **increased tau phosphorylation in the denervated cortex**, **did not alter amyloid-β pathology or senile plaque deposition**, and impaired performance in the Morris water maze, indicating a synergistic effect of serotonergic loss with the existing amyloid pathology.

In living humans, an observational analysis of the Alzheimer's Disease Neuroimaging Initiative points the same way. Terstege and colleagues examined 191 subjects with baseline ^{18}F-fluorodeoxyglucose positron emission tomography and plasma biomarker data, stratified by cognitive status and SSRI use. Alzheimer's patients taking SSRIs had a lower concentration of plasma phosphorylated tau 181 than untreated Alzheimer's patients ($p = 0.0473$). The imaging arm found hypometabolism in the dorsal raphe of untreated Alzheimer's patients and peaks of hypermetabolic activity in the dorsal raphe of treated patients relative to untreated ones. Neither effect appeared in cognitively normal subjects, suggesting specificity to the disease state. The authors' own conclusion is appropriately hedged: long-term SSRI use may reduce the pathological presentation of the disease but has variable effects on cognitive performance across MMSE, MoCA and CDR.

The contradiction. §5.1 and §5.2 do not agree. In the denervation model, removing serotonin raised tau and left plaques untouched. In the enzyme-ablation model, removing serotonin raised plaques. These are different manipulations — a neurotoxic lesion of the projection versus genetic deletion of the synthetic enzyme — in different transgenic backgrounds, measured at different ages, and they may both be right about their own preparations. But they cannot both be generalised, and the field has tended to cite whichever supports the argument in hand. The honest position is that serotonergic withdrawal worsens Alzheimer-type pathology in mouse models, that the *which* pathology it worsens depends on the model, and that no single mechanism currently accounts for both results.

The Terstege data carry their own severe limitation, which the authors state: the design is cross-sectional, so causality cannot be established; SSRI type, dose, duration, concurrent medication, comorbidity and depression status could not be controlled; and mild cognitive impairment groups had to be excluded for want of data. People prescribed SSRIs differ from people who are not in ways that plausibly bear on tau.

Grade: Probable that serotonergic loss removes a restraint on tau in the terminal field; *Probable* for the plasma p-tau181 association; *not established* that the association is causal.

5.3 The trophic supply to hippocampal neurogenesis

Serotonin is the principal neuromodulatory input to adult hippocampal neurogenesis, and it acts through a well-characterised trophic chain. Activation of 5-HT1A and 5-HT4 receptors in the hippocampus raises CREB phosphorylation and increases expression of brain-derived neurotrophic factor; BDNF acting at TrkB supports the survival and maturation of newborn granule cells, dendritic growth, synaptogenesis and synaptic plasticity. This chain is the accepted molecular substrate of the delayed clinical effect of reuptake inhibitors in depression, and its time course — weeks, matching the maturation of new granule cells — is the standard explanation for why those drugs do not work immediately.

The relevance to dementia is that adult hippocampal neurogenesis is a substrate of cognitive resilience rather than of mood alone. New granule cells contribute to pattern separation, the capacity to store similar experiences as distinguishable memories, and the decline of neurogenesis with age and disease is associated with the loss of that capacity. Neurogenesis is reduced in Alzheimer's disease.

The inference is that progressive serotonergic withdrawal removes the principal trophic input to a process that supplies cognitive reserve, and does so from early adulthood onward. It is a plausible route by which a subclinical brainstem lesion in the third decade contributes to reduced resilience in the eighth.

Two cautions are required and are not always given. First, the magnitude and even the existence of adult hippocampal neurogenesis in humans remains contested; the studies disagree, and the disagreement is methodological rather than resolved. Second, the trophic chain has been characterised largely in rodents and in the context of antidepressant action, not of neurodegeneration.

Grade: Established for the 5-HT → BDNF → neurogenesis chain in rodents; *Probable* for reduced neurogenesis in Alzheimer's disease; *Inference* for the claim that serotonergic withdrawal is a material contributor to it in humans.

5.4 Sleep, and the melatonin tier

The dorsal raphe is a wake-active nucleus. Its serotonergic neurons fire fastest in active waking, slow during non-REM sleep, and fall nearly silent in REM. The system participates in the regulation of sleep architecture, and its degradation contributes to the fragmentation of sleep that is among the earliest and most consistent non-cognitive features of pre-clinical Alzheimer's disease.

There is a second, chemical tier. Melatonin is synthesised from serotonin in the pineal gland by N-acetylation and O-methylation. Pineal melatonin output is driven by the circadian clock rather than by substrate availability, but the achievable peak is bounded by how much serotonin is

available to convert. A partition shift or a synthesis deficit that lowers serotonin therefore lowers the ceiling on melatonin, and melatonin is an antioxidant with direct effects on mitochondrial function. The consequence is that the same upstream failure produces parallel deficits in mood, in sleep, and in oxidative defence, all of which are observed early in the disease.

This convergence is attractive and should be treated with corresponding suspicion. Sleep disruption in Alzheimer's disease has many causes — cholinergic loss, coeruleus degeneration, suprachiasmatic pathology, amyloid's own effect on slow-wave sleep, and the reciprocal relationship by which poor sleep raises amyloid — and attributing it to the raphe requires apportioning among them, which no study has done.

Grade: Established for the raphe's wake-active firing pattern and for serotonin as melatonin's precursor; *Inference* for the raphe's specific contribution to sleep disruption in Alzheimer's disease.

5.5 Mood — and the sharp limit on what the raphe lesion explains

This is where the field's expectations are strongest and the evidence is most constraining, in the opposite direction.

The human evidence has already been given in §3.2 and it is a dissociation: Alzheimer brains are depleted of dorsal raphe serotonergic neurons relative to controls; primary late-life depression produces no such depletion; and within Alzheimer's disease, depressed and non-depressed patients do not differ. Depression in Alzheimer's disease is not a dorsal raphe cell-count phenomenon.

The animal evidence adds precision that the human material cannot. Overexpressing human P301L tau selectively in the mouse dorsal raphe produced depressive-like behaviour and hyperactivity — **and did not produce deficits in spatial memory**. This is a targeted lesion of exactly the structure under discussion, and its behavioural signature is affective and psychomotor rather than mnemonic.

Read together, these two results give an unusually clear statement of what the raphe lesion does and does not do:

It produces an affective and psychomotor phenotype in isolation. The mouse experiment shows this directly.

It does not produce the memory disorder. The same experiment shows this by its negative result. Whatever accounts for the amnestic syndrome of Alzheimer's disease, a raphe tauopathy on its own is not it.

It does not account for depression as a clinical comorbidity in the human disease. The four-group post-mortem comparison shows this.

The apparent tension between the second and third points is resolvable and worth spelling out. The mouse experiment concerns an acute, focal, high-expression tau lesion in an otherwise healthy animal, and shows what the raphe lesion is *capable* of producing. The human comparison concerns a slowly accumulating lesion in a brain undergoing widespread additional pathology, and shows that in that setting, variation in the raphe lesion does not track variation in mood. Both can be true: the raphe lesion can be sufficient to produce depressive-like behaviour in isolation while contributing little to the variance in depression among patients whose brains differ in a hundred other ways.

Grade: Established for both underlying results; *Inference* for the reconciliation offered here.

5.6 A brake on the microglion

Microglia are serotonin-sensitive, and the sensitivity is functionally specific rather than general.

Krabbe and colleagues examined microglial responses in the presence of serotonin and found a striking dissociation between two microglial behaviours. Serotonin, acting principally at the 5-HT2B receptor, **enhanced** the motility and oriented process outgrowth by which microglia converge on a site of injury, so that processes moved more rapidly toward a laser lesion. At the same time it **attenuated** phagocytic activity: amoeboid microglia in slices from early postnatal animals, and microglia in culture, responded to serotonin with decreased phagocytosis.

The pattern — surveillance up, eating down — describes a modulator that biases microglia toward vigilance and away from consumption. More recent work has extended microglial serotonin sensing to developmental roles, showing that it conditions the proper formation of neuronal circuits and of social and adaptive skills, and that the 5-HT2B receptor is required in neonatal microglia to limit later neuroinflammation and sickness behaviour.

The inference for the disease is that progressive serotonergic withdrawal removes a tonic restraint on microglial phagocytosis. Since a substantial body of work implicates inappropriate microglial engulfment of synapses in the synapse loss that best correlates with cognitive decline, a lifelong reduction in a brake on phagocytosis is not a trivial thing to lose.

The caveats here are heavier than elsewhere in this Part, and the grade reflects them. The phagocytosis result was obtained in cultured and early-postnatal microglia; ramified microglia in situ showed no significant change. Extrapolating from a developmental preparation to the aged human brain is a long step, and the direction of serotonin's effect on adult microglial phagocytosis of synaptic material specifically has not been established.

Grade: Probable that microglia respond to serotonin through 5-HT2B with increased motility and decreased phagocytosis in the preparations tested; *Speculative* for the claim that raphe degeneration disinhibits synaptic pruning in human Alzheimer's disease.

5.7 Cognition, directly

Setting aside mechanism, does serotonergic loss track cognitive impairment in people? The imaging evidence says yes, modestly and consistently.

In mild cognitive impairment, lower serotonin transporter binding measured with [^{11}C]DASB is associated with worse performance in verbal and visual-spatial memory. In a later cohort, lower transporter binding — particularly in limbic regions — correlated with greater deficits in auditory-verbal and visual-spatial memory and with impaired semantic, category-guided fluency, a measure of executive function. Hippocampal 5-HT1A receptor binding falls slightly in amnestic MCI and markedly in Alzheimer's disease.

The semantic fluency association is worth isolating. Category-guided fluency requires flexible search through a semantic space and switching between subcategories when a line is exhausted — precisely the cognitive operation that serotonergic modulation of the persist-versus-switch trade-off would be expected to support. That the correlation appears on this measure rather than only on memory measures is at least consistent with the function being lost rather than with generic disease severity.

These are correlations in small cross-sectional cohorts, in which transporter binding is also a proxy for overall disease burden. *Grade: Probable* for the associations; *Speculative* for the functional interpretation of the fluency finding.

5.8 What is spared, and why it matters

A striking feature of the raphe lesion is how much of the raphe it leaves alone.

Essentially all of the evidence in this paper concerns the rostral group, and predominantly the dorsal raphe. The caudal group — raphe magnus, pallidus and obscurus, projecting to the spinal cord and lower brainstem — is not a prominent site of early Alzheimer pathology, and the functions it serves are correspondingly preserved. Alzheimer's disease does not present with a descending pain-modulation deficit, does not abolish thermoregulation, and does not produce the respiratory chemosensory failure that a global serotonergic lesion would cause. Patients with advanced disease and forty per cent dorsal raphe loss have intact nociceptive modulation.

The sparing is diagnostic in two ways.

It confirms that the lesion is **cell-type- and projection-specific rather than transmitter-generic**. Something about rostral, forebrain-projecting serotonergic neurons makes them vulnerable, and it is not their transmitter, because caudal serotonergic neurons use the same one and are spared. The candidate discriminator is the property that §4.2 identified: arbor size. The forebrain-projecting neuron supports a far larger and more distributed axonal territory than the spinally projecting one, and it is the forebrain-projecting neuron that fails.

It also **bounds the clinical picture**. The absence of caudal signs is the reason the serotonergic lesion of Alzheimer's disease produces nothing a neurological examination detects. The functions that would generate examinable signs are served by the part of the system the disease does not attack.

Grade: Probable. The relative sparing of the caudal group is consistently reported but has not been quantified stereologically in the way the dorsal raphe has, and the absence of caudal signs is an argument from clinical observation rather than from tissue.

5.9 The ledger of costs

What is withdrawn	Consequence	Grade
5-HT4-driven ADAM10 trafficking	Loss of a tonic bias toward non-amyloidogenic APP cleavage	Established (mechanism); Inference (permissive reading)
Serotonergic innervation of the cortex	Increased cortical tau phosphorylation in denervation models	Probable
5-HT1A/5-HT4 → CREB → BDNF	Reduced trophic support to adult hippocampal neurogenesis	Inference in humans
Wake-active raphe firing; melatonin substrate	Sleep fragmentation; lowered melatonin ceiling	Inference
Serotonergic tone at limbic and cortical targets	An affective/psychomotor phenotype — *not* the amnestic syndrome, *not* clinical depression	Established (both limits)
5-HT2B tone on microglia	Loss of a brake on phagocytosis, with surveillance preserved	Speculative in human disease
Cortical and limbic serotonergic terminals	Modest, consistent associations with memory and semantic fluency	Probable
— (caudal group spared)	No pain, thermoregulatory or respiratory signs	Probable

PART VI — The Therapeutic Record

6.1 Why this Part is mostly negative

Everything in Part V argues that the serotonergic system does things worth having and that Alzheimer's disease takes them away. The natural conclusion is that restoring serotonergic function should help. That conclusion has been tested, repeatedly, at large scale and at considerable expense, and it has failed.

The failures are set out here in full rather than summarised, for two reasons. The first is simple honesty: a paper arguing that a system matters has an obligation to report the trials in which acting on that system did not matter. The second is that the *pattern* of the failures is itself informative. They are not scattered near-misses; they cluster, and what they have in common tells us something about where the argument of Parts II–V should and should not lead.

6.2 The 5-HT6 antagonists: the decisive negative

The most decisive negative result in serotonergic pharmacology for Alzheimer's disease concerns the 5-HT6 receptor, and it is decisive because the hypothesis was good, the preclinical case was strong, the Phase II signal was real, and the Phase III programmes were large.

The rationale was sound. The 5-HT6 receptor is almost exclusively expressed in brain, concentrated in regions relevant to cognition, and Gs-coupled. Blocking it disinhibits cholinergic and glutamatergic transmission, which in animal models improves performance on cognitive tasks and is synergistic with cholinesterase inhibition. The drug class therefore promised a procognitive effect additive to standard of care — a modest but genuinely useful goal.

Idalopirdine was carried into three 24-week, fixed-dose, randomised, placebo-controlled Phase III trials on a background of stable cholinesterase inhibitor therapy. STARSHINE randomised patients to background donepezil plus placebo or idalopirdine 30 mg or 60 mg; STARBEAM to background donepezil plus placebo or idalopirdine 10 mg or 30 mg; STARBRIGHT to background donepezil, rivastigmine or galantamine plus placebo or idalopirdine 60 mg. Together the three trials enrolled 2,525 patients with mild-to-moderate Alzheimer's disease. At no dose, on no background, in no trial was idalopirdine better than placebo on the cognitive endpoint. None of the three reproduced the modestly positive Phase II study that had justified them.

Intepirdine — a different 5-HT6 antagonist, from a different sponsor — was carried into the Phase III MINDSET trial on the same rationale, in 1,315 patients on background donepezil, over

24 weeks. It failed to improve either cognition or activities of daily living relative to placebo. The sponsor did not file.

Nearly four thousand patients across four adequately powered Phase III trials of two independent molecules against the same target, all negative. This is as close to a definitive refutation as clinical pharmacology produces, and any argument about serotonin in Alzheimer's disease has to accommodate it rather than step around it.

6.3 The SSRIs: a split verdict

The reuptake inhibitors present a more complicated picture, because they have been tested for three different purposes and have produced three different answers.

As a treatment for agitation, the effect is real and the cost is high. The Citalopram for Agitation in Alzheimer's Disease trial randomised 186 patients with probable Alzheimer's disease and clinically significant agitation to citalopram (n = 94) or placebo (n = 92) for nine weeks, with psychosocial intervention in both arms and dosing titrated from 10 mg to 30 mg per day. Citalopram produced a statistically significant improvement in agitation. It also produced cardiac QT-interval prolongation and — the finding that matters most here — *worsening* of cognition on the Mini-Mental State Examination relative to placebo. A drug that reduces agitation while making cognition worse is a drug with a genuine but narrow role, and the cardiac signal has constrained dosing since.

As a biomarker intervention, the effect is real and short-term. The Sheline result in §5.1 stands: citalopram slowed cerebrospinal fluid amyloid-β production by 37 per cent in healthy volunteers. This is a demonstration of target engagement on the mechanism of §5.1, in living humans, and it is the strongest single piece of evidence that the serotonergic amyloid brake is real in people and not only in mice. It is not evidence of clinical benefit, and it was never claimed to be.

As a disease-modifying therapy, the effect is unproven. The Terstege analysis in §5.2 — lower plasma p-tau181 and partially restored dorsal raphe metabolism in Alzheimer's patients taking SSRIs — is observational, cross-sectional, and unable to control for the reasons a patient was prescribed an antidepressant. It is hypothesis-generating. No randomised trial has shown that an SSRI alters the trajectory of Alzheimer's disease.

6.4 Why the failures do not refute Parts II–V

There is a reading of §6.2 on which the serotonergic story collapses: the system was targeted, nothing happened, therefore the system does not matter. That reading is available and it should be taken seriously. It is rejected here for a reason that is specific and testable rather than merely convenient.

The trials targeted the wrong thing. Both 5-HT6 antagonists were symptomatic cognitive enhancers. Their mechanism was to increase neurotransmitter release in circuits that were already degenerating, in patients with mild-to-moderate established dementia, over 24 weeks. Nothing in that design tests the claims of Part V. It does not test whether serotonergic tone brakes amyloidogenesis in a healthy forty-year-old, whether serotonergic innervation restrains cortical tau over decades, or whether the loss of trophic input to neurogenesis erodes reserve across a lifetime. It tests whether pushing harder on a broken circuit improves a cognitive score in six months. It does not, and that is a coherent result rather than a contradictory one.

The trials came fifty years late. This is the central point. If the raphe begins to tangle in the third decade, sheds terminal field through the fourth and fifth, and has lost forty per cent of its neurons by the time of a dementia diagnosis, then a trial enrolling patients with established mild-to-moderate Alzheimer's disease is intervening at the end of a process that began before the patients finished their education. The 5-HT4 mechanism of §5.1 is a mechanism for *not accumulating* amyloid. Engaging it in a brain that has been accumulating amyloid for thirty years is not the same experiment.

The pharmacology addressed the signal, not the cell. Part IV concluded that the raphe fails for architectural reasons — arbor, pacemaker, missing net — and that the transmitter chemistry modulates timing rather than causing failure. A receptor antagonist does nothing about any of the three. It manipulates the output of a dying cell; it does not keep the cell alive.

These three points are not equally strong. The third is an inference from this paper's own argument and should carry the least weight. The first is close to definitional. The second is the one that generates predictions, and §7.2 states them.

6.5 What would actually refute the argument

It is easy to defend a hypothesis by saying the trials were mistimed, and the defence is worthless unless it specifies what evidence would defeat it. The following would.

A negative prevention trial. If a serotonergic intervention with demonstrated target engagement — a 5-HT4 agonist, or an SSRI at a dose shown to slow amyloid production — were given to cognitively normal middle-aged adults at elevated risk, for long enough to matter, and produced no effect on amyloid accumulation rate or on downstream tau, the permissive-brake reading of §5.1 would be badly damaged. This trial has not been run, and the argument's principal claim is therefore currently untested rather than supported.

Absence of the human amyloid relationship at scale. The Sheline production-rate result is a single, small, elegant study. If a substantially larger stable-isotope-labelling study found no effect of serotonergic manipulation on amyloid-β production rate in humans, §5.1 would lose its human anchor and revert to a mouse finding.

Dissociation of raphe integrity from downstream pathology in vivo. If a tracer capable of resolving dorsal raphe integrity in living people showed no relationship between raphe status in midlife and subsequent amyloid or tau accumulation in a prospective cohort, the permissive reading would fail its most direct test.

6.6 The honest verdict

The raphe is an early, severe, and functionally consequential lesion whose therapeutic window has probably closed by the time the disease is diagnosable, and whose pharmacology has so far been directed at the wrong stage of the illness with the wrong class of agent.

That verdict is deliberately unexciting. It does not claim the raphe as the cause of Alzheimer's disease; §2.5 concedes that early raphe tau may in some people be a tauopathy that never progresses, and §3.1 concedes that two other nuclei are more severely depleted. It does not promise a therapy; §6.2 reports four failed Phase III trials. What it claims is narrower and, if right, more useful: that a diffuse modulatory system which brakes amyloid production, restrains tau in its target field, and supplies the trophic input to hippocampal reserve is progressively withdrawn beginning decades before the disease is visible — and that the consequences of that withdrawal have been looked for in the wrong decade of the patient's life.

PART VII — The Ledger, the Predictions, and the Refutation

7.1 The graded ledger

Every substantive claim made in this paper is listed below with its grade and its principal support. The purpose of the table is to make the argument's load-bearing points separable from its speculative ones, so that a reader who rejects the speculation can see exactly how much of the structure survives.

#	Claim	Grade	Principal support
1	Abnormal tau appears in the serotonergic raphe before any cortical involvement	Established	Braak et al. 2011 (n = 2,332)
2	Phospho-tau is present in a dorsal raphe subnucleus in >20% of Braak 0 brains	Established	Grinberg et al. 2009
3	At Braak 0, 2.6% of dorsal raphe and 7.9% of locus coeruleus neurons are tangled	Established	Ehrenberg et al. 2017, unbiased stereology
4	The raphe follows the locus coeruleus rather than accompanying it	Established	Claims 1 + 3, concordant
5	Raphe tau is present in non-demented adults aged 25–80, with α-synuclein, Aβ and TDP-43 rarer	Established	Pierson et al. 2025
6	Dorsal raphe neuronal loss in AD is $d \approx 1.79$, ~40% of the nucleus	Established	Lyness et al. 2003 (67 studies); Aletrino et al. 1992
7	The raphe lesion is present in AD irrespective of depression, and absent in primary late-life depression	Established	Hendricksen et al. 2004 (4-group post-mortem)
8	The raphe lesion does not produce the amnestic syndrome	Established	Pierson et al. 2025 (P301L in mouse DRN: no spatial memory deficit)
9	Raphe and locus coeruleus lack the aggrecan-based perineuronal net that protects other neurons from tau	Established	Morawski et al. 2010

#	Claim	Grade	Principal support
10	Locus coeruleus neurons express MAO-A; raphe serotonergic neurons express MAO-B, not vice versa	Established	Westlund et al. 1985; Saura Marti et al. 1990
11	Serotonin suppresses interstitial amyloid-β via ERK signalling	Established	Cirrito et al. 2011
12	Citalopram slows CSF amyloid-β *production rate* by 37% in living humans	Established	Sheline et al. 2014
13	5-HT4 receptors bind and traffic ADAM10, promoting non-amyloidogenic APP cleavage	Established	Cochet et al. 2013; Tesseur et al. 2013; Giannoni et al. 2013
14	Ablating serotonin synthesis increases plaque load and astrogliosis	Established (in model)	Xu et al. 2019; von Linstow et al. 2022
15	Two 5-HT6 antagonists failed four Phase III trials in ~3,840 patients	Established	Atri et al. 2018; MINDSET (NCT02585934)
16	Citalopram 30 mg reduces agitation while worsening cognition and prolonging QT	Established	Porsteinsson et al. 2014 (CitAD)
17	The terminal field degenerates before the soma dies	Probable	Smith et al. 2017, 2023 vs. Lyness et al. 2003
18	Serotonergic denervation raises cortical tau phosphorylation without altering plaques	Probable (in model)	Ramos-Rodríguez et al. 2013
19	SSRI use is associated with lower plasma p-tau181 and partially restored DRN metabolism in AD	Probable	Terstege et al. 2025 (cross-sectional, n = 191)
20	The caudal raphe group is relatively spared, hence no examinable neurological signs	Probable	Clinical observation; not stereologically quantified
21	Serotonergic substrate supply is constrained at TPH2 by inflammatory IDO induction	Probable	Established biochemistry; sparse direct human AD tissue data
22	The MAO-A/MAO-B difference partly explains the 7.9%-vs-2.6% gap	**Inference**	Claims 3 + 10; untested as such
23	The shared cause of raphe and coeruleus vulnerability is architectural, not chemical	**Inference**	Claims 9 + 10 + the arbor and pacemaker evidence

#	Claim	Grade	Principal support
24	Withdrawal of the serotonergic amyloid brake is *permissive* for the later amyloid phase	**Inference**	Claims 1, 11, 12, 13 combined with the staging timetable
25	Serotonergic withdrawal materially erodes hippocampal neurogenic reserve in humans	**Inference**	Rodent trophic chain; contested human neurogenesis
26	Coeruleus degeneration compounds the serotonergic deficit by withdrawing α1 drive	**Inference**	Established anatomy; unquantified in human disease
27	Raphe loss disinhibits microglial phagocytosis of synapses in human AD	**Speculative**	Krabbe et al. 2012, in developmental preparations
28	VGLUT3-positive dual-transmitter neurons are the vulnerable subpopulation	**Speculative**	Untested
29	Early raphe tau in an individual predicts eventual dementia	**Not established**	See §2.5 (PART confound)

Twenty-one of the twenty-nine claims are Established or Probable. The paper's distinctive contributions — claims 22, 23 and 24 — are all Inference, and are marked as such wherever they appear in the text.

7.2 Predictions

The reading advanced here makes predictions that differ from those of the standard account, in which the serotonergic lesion is a source of neuropsychiatric symptoms in established dementia.

P1. Dorsal raphe integrity measured in midlife will predict subsequent amyloid accumulation *rate*, not merely amyloid burden at the time of measurement. The distinction matters: burden is confounded by everything that has already happened, whereas rate is the variable the 5-HT4/ADAM10 mechanism should govern.

P2. The association between serotonergic status and amyloid will be stronger in cognitively normal middle-aged adults than in patients with established disease, because the brake operates on production and there is little production left to brake once deposition has saturated.

P3. A 5-HT4 agonist will show target engagement on amyloid production rate in humans, measurable by stable-isotope labelling, and will do so more efficiently than a reuptake inhibitor, because it acts directly on the receptor that traffics α-secretase rather than depending on residual serotonin release from a degenerating projection.

P4. Serotonergic interventions will show a *substrate interaction*: efficacy will be reduced in individuals with a high plasma kynurenine-to-tryptophan ratio, because reuptake inhibition amplifies whatever serotonin is released and cannot raise synthesis under substrate limitation.

P5. Raphe tau burden will correlate with affective and psychomotor measures and *not* with episodic memory, after adjustment for overall disease stage — following the dissociation in claim 8.

P6. The caudal raphe group will show substantially lower tau burden than the rostral group in the same brains, and the difference will track axonal arbor extent rather than transmitter phenotype.

7.3 Nine experiments

1. Replicate Hendricksen with modern stereology. The four-group dissociation of §3.2 rests on 7–14 subjects per cell and on two-dimensional image analysis. It is the most important single result in the human literature and it deserves unbiased stereological replication in a larger series.

2. Count the caudal group. No study has applied to raphe magnus, pallidus and obscurus the stereology that has been applied to the dorsal raphe. The rostral-versus-caudal comparison in the same brains would test P6 directly and would discriminate the arbor hypothesis from the transmitter hypothesis.

3. Map tau onto dorsal raphe subnuclei. Grinberg found the earliest change confined to a subnucleus. Which one, and whether it is the same one across brains, is unresolved and is answerable with existing tissue.

4. Genotype the vulnerable cells. Combine tau immunohistochemistry with VGLUT3 labelling in human dorsal raphe to test claim 28 — whether dual-transmitter neurons tangle preferentially.

5. Test the MAO attribution. Claim 22 predicts that pharmacologically or genetically imposing MAO-A activity on serotonergic neurons should accelerate their tau accumulation, and that removing MAO-A from coeruleus neurons should slow theirs, narrowing the gap from either direction.

6. Measure amyloid production rate against serotonergic status in humans. Stable-isotope-labelling kinetics with concurrent [11C]DASB imaging in cognitively normal middle-aged adults would test P1 and P2, and would either establish or destroy the permissive reading of §5.1.

7. Resolve the model contradiction. Run serotonergic denervation and TPH2 ablation in the same transgenic background, at the same ages, with the same tau and amyloid readouts. The literature's disagreement (§5.2) may be entirely methodological, and no one has checked.

8. Image the human raphe. Every in vivo finding in this paper is a terminal-field measurement. A tracer or a sequence capable of resolving dorsal raphe integrity in living people — as has been achieved for the locus coeruleus with neuromelanin-sensitive imaging — would convert most of the Inference claims into testable ones.

9. A prevention trial, properly timed. A 5-HT4 agonist or a dose-justified SSRI in cognitively normal adults at elevated risk, with amyloid accumulation rate as the primary endpoint and a duration measured in years. This is the experiment the argument implies and the one no sponsor has run.

7.4 What would refute the argument

Stated compactly, and additional to the trial-level refutations of §6.5:

If **the caudal raphe is found to be as heavily affected as the dorsal raphe**, the architectural account of Part IV loses its cleanest discriminator, since caudal and rostral serotonergic neurons share transmitter, enzymes and pacemaking, and differ principally in arbor.

If **net-bearing neurons are found to tangle at the same rate as net-less neurons** in a properly powered subcortical survey, claim 9 fails and one of the three architectural liabilities is removed.

If **the human amyloid-production-rate effect does not replicate** at larger scale, claim 12 falls and with it the human anchor of the permissive reading.

If **raphe tau burden is found to correlate with episodic memory** after adjustment for stage, claim 8 and the dissociation it rests on are wrong, and the raphe would have to be readmitted to the account of the amnestic syndrome.

If **early raphe tau is shown prospectively not to progress** in a substantial majority of carriers, §2.5's caveat becomes the main finding and the raphe lesion is reclassified as an age-related tauopathy incidental to Alzheimer's disease.

7.5 Conclusion

The raphe nuclei fail early, severely, and quietly. They begin to accumulate abnormal tau in the second and third decades of life, in the pre-cortical staging window, behind the locus coeruleus but ahead of every cortical population. They lose on the order of forty per cent of their forebrain-projecting neurons — an insult three times the size of the dopaminergic loss in the same brains, and one that produces no sign a neurologist could name. The silence is not evidence of unimportance; it is what a diffuse, compensable, volume-transmitting system looks like when it degrades.

What the failure costs is not primarily mood. That is the finding the field has been slowest to absorb, and it is established in human tissue: the raphe lesion is present in Alzheimer's disease whether or not the patient was depressed, and absent in depression that is not Alzheimer's disease. What the failure costs is a set of tonic functions that operate below the level of symptom — a bias in amyloid precursor protein processing toward the cleavage that pre-empts amyloid-β, a restraint on tau phosphorylation in the terminal field, the trophic input to whatever hippocampal neurogenesis humans retain, the consolidation of sleep, the substrate ceiling for melatonin, and a brake on microglial consumption. Most of these are withdrawn before the disease has a name.

The mechanism of the failure is, on the reading offered here, architectural rather than chemical. The raphe and the locus coeruleus have been paired so habitually that an explanation devised for one has been assumed to cover both, and it does not: they express different monoamine oxidases, and the coeruleus's specific chemical liability has no serotonergic counterpart. What they share is a vast unmyelinated arbor supported by a small soma, a pacemaker that never stops, and the absence of the matrix that shields other neurons from tau. Two nuclei that fail together on different chemistry are failing for a reason that is not chemistry.

And the therapeutic record, read against this, is not the refutation it appears to be. Four Phase III trials of two 5-HT6 antagonists in nearly four thousand patients returned nothing, and a reuptake inhibitor that reduces agitation does so while making cognition worse. But every one of those trials enrolled patients with established dementia and asked whether pushing harder on a degenerated projection would raise a cognitive score in six months. None asked the question this paper's evidence actually poses, which is whether a brake that begins to be released in the third decade of life can be held on. The seam does not tear at the end. It is unpicked, one stitch at a time, from the beginning — and by the time the garment visibly fails, the stitching has been gone for fifty years.

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Trial records

MINDSET: A Study Evaluating Intepirdine (RVT-101) in Subjects With Mild-to-Moderate Alzheimer's Disease on Donepezil. ClinicalTrials.gov identifier NCT02585934. Sponsor: Axovant Sciences. n = 1,315; 24 weeks; topline results announced September 2017; primary endpoints (ADAS-Cog and ADCS-ADL) not met.

CitAD: Citalopram for Agitation in Alzheimer's Disease. ClinicalTrials.gov identifier NCT00898807. n = 186; 9 weeks; reported as Porsteinsson et al. 2014, above.

STARSHINE, STARBEAM and STARBRIGHT: the three Phase III idalopirdine trials, reported together as Atri et al. 2018, above. Combined enrolment 2,525.