The Fifty-Year Prescription

One continuous mechanism from the gut lumen to the failed antidepressant trial — how an ecological drift in the intestine unpicks a brainstem seam, why the serotonergic brake on amyloid is released decades before the first plaque, and why a drug that engages the right target arrives half a century late and on the wrong side of the rate-limiting step

The ferment · The tryptophan fork · The vagal relay · The self-poisoning nucleus · The unpicked seam · The amyloid brake

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A fuse, not a switch. A switch is thrown and the consequence is immediate; a fuse is lit at one end and burns for a length of time set by its own construction, and the fire at the far end is the same event as the spark at the near one, separated only by the burning. The spark is ecological, the fuse is fifty years long, and the drug has been given, over and over, to the fire.

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Abstract

Selective serotonin reuptake inhibitors have one of the strangest evidence profiles in neurology. Given to a healthy volunteer, citalopram slows the production of amyloid-β in cerebrospinal fluid by 37 per cent — a demonstration of target engagement on the amyloid pathway more direct than most disease-modifying candidates ever achieve. Given to a patient with established Alzheimer's disease and agitation, the same drug improves the agitation while making cognition measurably worse. Given for more than four years to a person with mild cognitive impairment and a history of depression, it is associated with a delay in conversion to dementia of about three years — without producing any detectable difference in cerebrospinal-fluid biomarkers. Given for six months to patients with mild-to-moderate dementia, in the form of a receptor antagonist rather than a reuptake inhibitor, it does nothing at all: two 5-HT6 antagonists failed four adequately powered Phase III trials in nearly four thousand patients. The same pharmacological system appears, on this record, to be simultaneously a validated anti-amyloid target, a cognitive toxin, a slow disease-modifier, and a therapeutic dead end.

This paper argues that the profile is not a paradox but a signature, and that reading it requires following one mechanism the whole way from its origin to its clinical endpoint without breaking the chain. The chain runs: a lifelong drift in the ecology of the gut, away from fibre-fermenting butyrate producers and toward endotoxin-bearing taxa, which weakens the barrier that would contain it; the export of that ecology's chemistry — lipopolysaccharide, trimethylamine N-oxide, altered bile acids, bacterial amyloid, and a shifted allocation of dietary tryptophan — into the portal and systemic circulation; the transduction of that export at the vagal afferent terminal and, in parallel, at the area postrema, into a chronic afferent load delivered through an obligatory first station in the nucleus tractus solitarius and a dual medullary relay to the locus coeruleus; the bioenergetic and excitatory cost of that load to the one nucleus in the brain that manufactures its own poison, where norepinephrine escaping the synaptic vesicle is oxidised to a reactive aldehyde that activates a single protease with two substrates — cutting tau into a propagation-competent seed and cleaving the inhibitor that silences tau's phosphatase; the withdrawal, as that nucleus fails, of the α1-adrenergic drive that sustains firing in the serotonergic raphe, which is architecturally unguarded in the same three ways and tangles next in the same brains; and the consequent slow subtraction, across the whole cortical mantle and over five decades, of a serotonergic tone whose least-appreciated function is to hold amyloid precursor protein processing toward the cleavage that pre-empts amyloid-β.

The paper's own contributions are three, and each is marked as inference where it is one. The first is the **two-hit account of serotonergic withdrawal**: the forebrain loses serotonergic tone by two mechanically independent routes — the loss of the cells that make it, driven by arbor, pacemaker, and the absent aggrecan-based perineuronal net, and the loss of the *substrate* they make it from, driven by inflammatory induction of indoleamine 2,3-dioxygenase under the gut's control. The field has modelled the first and has largely ignored the second, and the two are

not interchangeable. The second is the **rate-limiting-step objection to reuptake inhibition**: a reuptake inhibitor redistributes serotonin that has already been synthesised and cannot raise the output of a pathway that is substrate-limited at tryptophan hydroxylase 2, whose Michaelis constant sits close to the ambient brain tryptophan concentration. It follows that the efficacy of serotonergic augmentation should be a lawful function of the plasma kynurenine-to-tryptophan ratio, and that the trial which has never been run is not "reuptake inhibitor versus placebo" but "reuptake inhibitor versus reuptake inhibitor plus partition restoration." The third is the **reading of the duration asymmetry**: the observation that long-term but not short-term reuptake inhibition delays conversion, without moving a cross-sectional biomarker, is the shape a slow permissive brake predicts and is not the shape a symptomatic agent predicts.

The failures are reported in full and not softened. The trial record is close to uniformly negative; the human cohort evidence for the gut origin is cross-sectional and cannot exclude reverse causation; the reconstructed vagal relay is dual and balance-dependent, so it does not deliver a fixed signal; the animal models of serotonergic subtraction disagree with each other about whether tau or plaque is the consequence; the raphe cell-count lesion is demonstrably *not* the depression lesion, which constrains the mood arm of the argument more sharply than most accounts admit; and the most upstream link in the whole chain — that the intestinal ecology is a cause rather than a companion of the brainstem lesion — remains the least established. The closing sections give a forty-one-claim graded ledger, eleven predictions, nine experiments, and the specific results that would break the chain, link by link, so that a reader who rejects one span can see precisely how much of the structure survives.

Note on evidence and grading

A paper that assembles a chain from many literatures has a particular obligation, because a chain of thirteen links each of which is "plausible" is not a plausible chain. Every substantive claim below is therefore graded at the point of use rather than left to be inferred from the confidence of the prose, and the grade is repeated in the ledger of §11.1. Four grades are used.

Established. Replicated in human material, or demonstrated causally in more than one model system by more than one group, with quantitative agreement. The staging order of brainstem tau, the cell-loss fractions, the enzymology of tryptophan catabolism, the identity of the protease and its two substrates, 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. The paper's three distinctive contributions are all of this kind.

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

Three further conventions govern the text. Where the literature contains a genuine contradiction, both sides are cited and the contradiction is named as such; no attempt is made to resolve a disagreement by selecting the convenient result, and §7.6, §8.2, §9.4 and §10.6 each report a result that damages the argument being made around it. 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. And where a link in the chain is carried by animal work alone, the species gap is stated at that link rather than absorbed silently into the summary.

One asymmetry in the evidence base deserves stating at the outset, because it recurs at every stage. The upstream half of the chain — the gut, the metabolome, the barrier, the nerve — is where causal manipulation is easy and human observation is weak: germ-free animals, faecal transplantation, and vagotomy give clean causal closure in rodents, while the human data are almost entirely cross-sectional. The downstream half — the brainstem nuclei, the staging, the cell counts, the trials — is where human observation is strong and causal manipulation is impossible: there are large, well-characterised autopsy series and adequately powered randomised trials, and no way to perturb the system in a living person. The chain is therefore best-evidenced at both ends and thinnest in the middle, and the middle is exactly where the causal claim has to hold.

A note on what "one mechanism" is being claimed

The title asserts a single continuous mechanism, and the assertion should be bounded before it is defended, because the most common failure of a synthesis of this kind is to claim that everything is one thing.

Four claims are **not** made here. It is not claimed that the gut microbiome is the sole cause of Alzheimer's disease, or that every case runs through this chain; brain-first phenotypes are well described in the neighbouring synucleinopathy literature and the analogous question in Alzheimer's disease is open. It is not claimed that the serotonergic lesion is *the* lesion; two other subcortical nuclei are more severely depleted in the same brains, and the amnestic syndrome that defines the disease clinically is demonstrably not produced by a raphe tauopathy in isolation. It is not claimed that early brainstem tau is a diagnosis; a substantial fraction of it is probably primary age-related tauopathy that never progresses, and no prospective series can currently distinguish the two. And it is not

claimed that reversing the chain would cure an established dementia; the argument's whole point is that the interval in which the chain is reversible closes long before the diagnosis is made.

What *is* claimed is narrower and, if right, more useful. It is that there exists a continuous, link-by-link specifiable path from an ecological variable outside the body to a pharmacological result inside a clinical trial; that every link on that path has been measured by someone, usually in isolation and usually without reference to the links on either side; that the path predicts the peculiar shape of the reuptake-inhibitor evidence better than any account which begins at the receptor; and that the two places where it is most tractable to intervene — the intestinal ecology and the tryptophan partition — are both upstream of everything the last thirty years of serotonergic pharmacology has targeted.

The organising image is a fuse rather than a switch. A switch is thrown and the consequence is immediate; a fuse is lit at one end and burns for a length of time set by its own construction, and the fire at the far end is the same event as the spark at the near one, separated only by the burning. On the reading offered here the spark is ecological, the fuse is fifty years long, and the drug has been given, over and over, to the fire.

PART I — The Chain, Stated Once

1.1 The problem that generates the paper

The evidence on serotonergic pharmacology in Alzheimer's disease does not form a pattern that any single-level account can absorb. Set the four principal results beside each other and the difficulty is immediate.

Result one. Sheline and colleagues, using stable-isotope labelling to measure the *production rate* of amyloid-β rather than its standing concentration, found that citalopram slowed amyloid-β production in the cerebrospinal fluid of healthy volunteers by 37 per cent, with a 38 per cent fall in total cerebrospinal-fluid amyloid-β; in aged transgenic mice the same drug reduced interstitial amyloid dose-dependently, arrested the growth of existing plaques, and cut the appearance of new plaques by 78 per cent. Whatever else is true, the serotonergic system is coupled to the production of the peptide the field has spent forty years trying to reduce, and the coupling is demonstrable in living people.

Result two. The Citalopram for Agitation in Alzheimer's Disease trial randomised 186 patients with probable Alzheimer's disease and clinically significant agitation to citalopram or placebo for nine weeks. Citalopram significantly improved agitation. It also prolonged the QT interval and *worsened* cognition on the Mini-Mental State Examination relative to placebo. In an established dementia the same drug class is, on the cognitive endpoint, harmful.

Result three. Two 5-HT6 receptor antagonists — idalopirdine and intepirdine, from different sponsors, on a shared and well-argued procognitive rationale — were carried into four Phase III trials enrolling 3,840 patients with mild-to-moderate disease. At no dose, on no cholinesterase-inhibitor background, in no trial, did either beat placebo. This is as close to definitive refutation as clinical pharmacology produces.

Result four. Bartels and colleagues examined 755 currently non-depressed participants in the Alzheimer's Disease Neuroimaging Initiative and found that, among patients with mild cognitive impairment and a history of depression, reuptake-inhibitor treatment lasting more than four years was associated with a delay of roughly three years in progression to Alzheimer's dementia — relative to short-term treatment, to other antidepressants, and to no treatment. No differences in cerebrospinal-fluid biomarkers were observed between the treatment groups.

An account pitched at the receptor cannot hold these together. If serotonergic signalling is procognitive, result three should not have happened. If it is irrelevant, results one and four should

not have happened. If it is disease-modifying, result four should have moved the biomarkers. And if it is symptomatic, the effect in result four should have appeared in the first months rather than requiring four years, while the effect in result two should not have gone the wrong way.

The claim of this paper is that all four results are the expected output of a single mechanism observed at four different points along a fifty-year time course, and that the mechanism cannot be seen at all if one begins at the receptor, because it begins in the gut.

1.2 The chain in thirteen links

The whole argument is given here in compressed form, so that the reader knows from the outset where every subsequent Part is going and can see which link each is defending. Each link is elaborated, evidenced and graded in the Part indicated.

L1 — The ecology drifts. The resident microbial community of the gut loses diversity and fibre-fermenting, butyrate-producing taxa with age, frailty and diet, and gains relatively pro-inflammatory, endotoxin-bearing ones. *(Part II; Established as an association, Probable as an age trajectory.)*

L2 — The barrier follows the ecology. Butyrate is the colonocyte's primary fuel and a signal for tight-junction assembly, so the same drift that lowers butyrate weakens the barrier that would otherwise contain its consequences. Barrier failure converts a contained compartment into an exporting one. *(Part II; Established mechanistically, Probable in the ageing human.)*

L3 — The export has a chemistry. What crosses is not "inflammation" in the abstract but a specifiable set of molecules: lipopolysaccharide, trimethylamine N-oxide, a shifted secondary bile-acid profile, and — in the seeding version of the argument — bacterial amyloid. *(Part II; Established for the associations, Probable for the seeding.)*

L4 — The gut controls the fork. Dietary tryptophan is partitioned among protein synthesis, the kynurenine pathway, serotonin synthesis, and direct microbial conversion to indoles. The microbiome controls that partition three ways: by consuming the substrate, by inducing the diverting enzyme through inflammatory tone, and — demonstrated by transplantation — by carrying the altered partition with it into a naïve host. *(Part III; Established.)*

L5 — The diverted fork starves the serotonergic branch at its rate-limiting step. Tryptophan hydroxylase 2 has a Michaelis constant close to the ambient brain tryptophan concentration. Under homeostatic conditions central serotonin synthesis is not substrate-limited; under sustained induction of indoleamine 2,3-dioxygenase it becomes so. *(Part III; Established for the biochemistry, Probable for its magnitude in human disease.)*

L6 — The same export loads the vagus. Vagal afferents sense the peripheral immune and metabolic state directly, and an enteroendocrine sensor forms a genuine fast glutamatergic synapse onto a vagal afferent, placing a luminal detector one synapse from the nerve. A humoral channel through the area postrema runs in parallel and converges on the same relay. *(Part IV; Established.)*

L7 — The relay is obligatory, indirect, and dual. All vagal afferent traffic passes through the nucleus tractus solitarius; from there the dominant route to the locus coeruleus is indirect, excitatory through the glutamatergic nucleus paragigantocellularis and inhibitory through the GABAergic nucleus prepositus hypoglossi, with a sparse direct projection and a parabrachial route in parallel. The net effect is therefore conditional, not fixed. *(Part IV; Established in rodent, inferred in human.)*

L8 — The receiver is the one nucleus that poisons itself. Chronic afferent drive and stress-induced internalisation of the α2A autoreceptor push norepinephrine out of the synaptic vesicle into the cytosol, where monoamine oxidase A oxidises it to 3,4-dihydroxyphenylglycolaldehyde — a reactive aldehyde produced exclusively in noradrenergic neurons. The commonest genetic risk factor for the disease accelerates the same step by inhibiting the vesicular transporter. *(Part V; Established.)*

L9 — One protease commits two crimes. That aldehyde activates asparagine endopeptidase, which cleaves tau at asparagine-368 into an aggregation- and propagation-competent seed, and separately cleaves the phosphatase inhibitor I2PP2A/SET at asparagine-175, whose fragments translocate to the cytoplasm and silence protein phosphatase 2A. The writer side and the eraser side of the tau balance are deranged together, by one activation. *(Part V; Established.)*

L10 — The coeruleus takes the raphe with it. The locus coeruleus supplies α1-adrenergic excitatory drive to the dorsal raphe; its degeneration therefore subtracts serotonergic output by a route entirely separate from raphe pathology. The raphe is independently vulnerable for three architectural reasons it shares with the coeruleus — an enormous unmyelinated arbor, autonomous pacemaking, and the absence of the aggrecan-based perineuronal net — but not for the coeruleus's chemical one, since it expresses the other monoamine oxidase. *(Part VI; Established for the anatomy and the enzyme assignment, Inference for the architectural conclusion.)*

L11 — The seam gives way quietly. Abnormal tau appears in the raphe at pretangle stage c, before any cortical involvement; 2.6 per cent of dorsal raphe neurons carry inclusions at Braak 0 against 7.9 per cent in the coeruleus; roughly forty per cent of the nucleus is lost by death, an effect size three times the substantia nigra's. None of this produces a sign a neurologist can name, for reasons that follow from volume transmission and compensation. *(Part VII; Established.)*

L12 — What is withdrawn includes a brake on amyloid. Serotonin acting at the Gs-coupled 5-HT4 receptor traffics ADAM10, the constitutive α-secretase, to the plasma membrane and biases amyloid precursor protein toward the cleavage that destroys amyloid-β before it exists. Ablating serotonin synthesis raises plaque load; serotonergic denervation raises cortical tau. The brake is released across the whole mantle, continuously, from early adulthood. *(Part VIII; Established for the mechanism, Inference for the permissive reading.)*

L13 — The drug arrives at the fire. Every trial of serotonergic augmentation in Alzheimer's disease has enrolled patients with established disease, has acted on the signal rather than the cell, and has attempted to amplify release on the wrong side of a rate-limiting step that L5 has already throttled. The one exposure long enough and early enough to matter — four or more years of reuptake inhibition at the mild-cognitive-impairment stage — is also the one associated with a change in trajectory. *(Parts IX–X; Established for the trial record, Inference for the explanation.)*

1.3 What the chain buys that the parts do not

Each of these thirteen links exists in the published literature, most of them securely. The contribution claimed here is not the discovery of a link but the load-bearing consequences of joining them, and there are four.

It supplies a cause for the timing. The most striking and least explained fact about Alzheimer's disease is the length of its prodrome. Abnormal tau is present in the brainstem in the second and third decades of life in people who will not be diagnosed for fifty years. Accounts that begin inside the brain have to treat this as a property of neurons — they are slow to die — which is true but not explanatory. The chain supplies an external variable with the right time constant: an ecological drift that is itself slow, cumulative, dose-dependent, and continuously reinforced by the behaviour its own downstream consequences produce.

It explains why the brainstem and not the cortex. The disease begins in two small aminergic nuclei rather than in the structures that will eventually carry its clinical weight. The chain gives a specific reason: those nuclei are the ones physically closest, in synapses, to the afferent load, they are constitutionally unshielded against tau, and one of them manufactures a poison that no other neuron can make. The cortex is not spared because it is robust; it is spared because it is downstream.

It converts the tryptophan partition from a metabolic curiosity into a therapeutic variable. The kynurenine literature and the serotonergic literature have developed almost separately, the first as immunometabolism, the second as psychiatry. Placing them on one chain makes the plasma kynurenine-to-tryptophan ratio a candidate *effect modifier* of serotonergic pharmacology rather than an epiphenomenon — which is a testable and, so far as this paper can determine, untested proposition about drugs that hundreds of millions of people take.

It makes the negative trial record informative rather than merely discouraging. A uniformly negative record is usually read as evidence that a target is wrong. The chain reads the same record as evidence about *when* the target is reachable, and it converts that reading into predictions that differ from the standard account's — most sharply, that serotonergic effect size should scale with exposure duration and inversely with the kynurenine-to-tryptophan ratio, and that the relevant endpoint is accumulation rate rather than cross-sectional burden.

1.4 The shape of the rest of the paper

Parts II through VIII walk the chain from the lumen to the cortex, one compartment at a time, and each closes with a table of what has been established at that link and what has not. Part IX takes up the first clinical reading of the chain — the depressive phenotype — and confronts the human post-mortem result that most sharply constrains it. Part X assembles the therapeutic record in full and gives the three-part explanation the chain supplies for it, including the paper's central claim about the rate-limiting step. Part XI is a graded ledger of every claim made. Part XII gives eleven predictions, nine experiments, and the results that would break the chain at each link.

A reader who wants the argument without the evidence can read §1.2, §10.5 and §11.1 and will have it. A reader who wants to attack the argument should go to §12.3, where the paper states what would defeat it.

PART II — The Ferment

2.1 An organ the disease model was built without

The intellectual difficulty of any gut-origin argument in neurodegeneration is historical before it is evidential. For the whole of the period in which the neuropathology of Alzheimer's disease was established — from the first descriptions through the staging schemes that still organise the field — the gut microbiome was unreadable. It could not be cultured comprehensively, it could not be sequenced affordably, and it was therefore, in practice, invisible to the disciplines that built the model. The model was not wrong to look inside the skull; it was, for technical reasons, unable to look anywhere else.

Culture-independent sequencing made the gut ecosystem legible, and what it revealed has the dimensions of an organ. The adult human gut harbours on the order of tens of trillions of microbial cells, several hundred to a thousand or more bacterial species in a typical adult, and a collective gene catalogue exceeding the human genome by roughly two orders of magnitude. It performs metabolic functions the host genome does not encode: the fermentation of indigestible dietary fibre into short-chain fatty acids, the synthesis of vitamins, the deconjugation and transformation of bile acids, and the metabolism of dietary amino acids — tryptophan among them — into a large repertoire of bioactive compounds.

The consequence for this paper is a prior, not a conclusion. An organ of that size, that metabolic activity, and that anatomical connection to the first-failing nucleus of the brainstem has a strong claim to relevance in a disease of that nucleus. The burden that the cephalocentric default has implicitly carried — that an organ of this consequence is irrelevant to a disease of the structure it is wired to — has never actually been discharged. It has simply not been raised.

2.2 What the healthy ecology supplies

In health the adult gut microbiome performs a set of services which are, almost item for item, the reverse of the mechanisms that appear later in this chain.

It ferments dietary fibre into short-chain fatty acids — acetate, propionate and especially butyrate — that fuel the colonocyte, restrain inflammation, and act as histone deacetylase inhibitors with broad epigenetic consequences. It maintains, through the same butyrate, the barrier that prevents translocation of its own endotoxin. It transforms bile acids and metabolises tryptophan along pathways from which the host benefits. It competitively excludes pathogens. And it tonically conditions the host immune system, including — as §2.6 develops — the microglia of the brain

itself, whose maturation and function are continuously controlled by the microbiota and are malformed in germ-free animals.

The healthy ecology is, in the terms of this paper, a continuous *suppressor* of the afferent load that Part IV will identify as the injurious input to the brainstem. What follows is the systematic reversal of that suppression.

2.3 The drift

The microbiome has a degenerative trajectory as well as a developmental one, and this is the fact that gives an ecological account the right time constant for a disease with a fifty-year prodrome.

Claesson and colleagues, in a cohort of older Irish adults stratified by residence setting, established that gut microbiota composition correlates with diet and with health in the elderly: the microbiota of older adults is more variable between individuals than that of younger ones, and in those who are frail or long-stay institutionalised it is characterised by reduced diversity and a shift away from the fibre-fermenting, butyrate-producing taxa of the healthy adult gut. Diet and community structure moved together with measures of frailty, inflammation and nutritional status. This age-associated drift — loss of diversity, loss of butyrate producers, relative expansion of pro-inflammatory Proteobacteria — is the ecological backdrop against which any disease-specific signature must be read.

Onto that backdrop the disease cohorts superimpose their own. Vogt and colleagues characterised the faecal bacterial composition of participants with and without a diagnosis of dementia due to Alzheimer's disease and found decreased microbial diversity and a compositionally distinct community in the patients: decreased Firmicutes, increased Bacteroidetes, decreased *Bifidobacterium*, with correlations between the abundance of differentially represented genera and cerebrospinal-fluid biomarkers of the disease. Cattaneo and colleagues made the sharper observation: in cognitively impaired elderly subjects, the abundance of a pro-inflammatory taxon (*Escherichia/Shigella*) correlated positively, and that of an anti-inflammatory butyrate producer (*Eubacterium rectale*) correlated negatively, with brain amyloid deposition measured by positron emission tomography and with peripheral inflammatory markers — linking, in one human dataset, the composition of the gut, the inflammatory state of the blood, and the amyloid burden of the brain.

The pattern that recurs across these studies is not any single taxon but a *direction*: loss of fibre-fermenting, barrier-supporting, butyrate-producing organisms and relative gain of endotoxin-bearing, pro-inflammatory ones. That direction is what this paper treats as the disease-relevant ecological variable.

Grade: Established for the existence and reproducibility of the association; *Probable* for the age trajectory as a general feature of human ageing; *not established* that the signature is causal in humans

— see §2.7.

2.4 The coupling that makes the drift self-amplifying

The drift is not a list of independent deficits. It is a coordinated reversal in which each failure produces the next, and the coupling is specific enough to name.

Butyrate is the colonocyte's primary energy source and a signal for tight-junction assembly. The epithelium that separates the densest microbial habitat on earth from the host's interior is therefore fuelled and maintained, in part, by the very organisms it contains. A dysbiotic loss of butyrate producers thus weakens the barrier that would otherwise contain the consequences of dysbiosis. The weakened barrier permits translocation of microbial products; the translocated products drive systemic inflammation; and inflammation further degrades barrier function and further disfavours the oxygen-intolerant obligate anaerobes that produce butyrate.

Cani and colleagues established the prototype of the downstream half of this loop in the metabolic context: a low-grade elevation of circulating lipopolysaccharide — "metabolic endotoxemia" — is sufficient to initiate the inflammatory and metabolic changes of diet-induced obesity and insulin resistance, and is produced by a high-fat diet acting through the gut microbiota. The finding matters here not for its metabolic conclusion but for its structure: it demonstrates that a dietary change, mediated by an ecological change, produces a *systemic inflammatory tone* by way of barrier failure, and that the tone is measurable in the blood.

The barrier is, in consequence, the gating variable of the entire chain. A perfectly dysbiotic gut behind a perfectly intact barrier would export very little. The disease-relevant condition is not dysbiosis alone but dysbiosis behind a failing barrier — and because butyrate fuels the barrier, the second follows near-automatically from the first.

Grade: Established for the butyrate–barrier coupling and for metabolic endotoxemia; *Probable* for the self-amplifying loop as a description of the ageing human gut.

2.5 What crosses

Naming what crosses matters, because "inflammation" is not a mechanism and a chain that passes through an abstraction has a hole in it. Four classes of molecule carry the export, and each has been measured in human Alzheimer's material.

Lipopolysaccharide. The endotoxin of the gram-negative outer membrane and the prototypical Toll-like-receptor-4 agonist. Beyond its role in metabolic endotoxemia, Zhao and Lukiw reported lipopolysaccharide accumulation in neocortical neurons of the Alzheimer brain, with impairment of transcription in human neuronal–glial primary co-cultures. Endotoxin is not merely a circulating signal in this disease; it is present in the target tissue.

Trimethylamine N-oxide. The hepatic oxidation product of microbial trimethylamine, itself derived from dietary choline and carnitine. Vogt and colleagues found trimethylamine N-oxide elevated in the cerebrospinal fluid of individuals with Alzheimer's disease and correlated with cerebrospinal-fluid biomarkers of neuronal injury and of tau pathology. This supplies a blood-borne microbial signal with a measurable central correlate in the compartment that matters.

The secondary bile acids. Host primary bile acids transformed by microbial enzymes. MahmoudianDehkordi and colleagues, in 1,464 subjects across the diagnostic spectrum of the Alzheimer's Disease Neuroimaging Initiative, found lower serum concentrations of the primary bile acid cholic acid in Alzheimer's disease and increased levels of the bacterially produced secondary bile acid deoxycholic acid and its conjugates. The ratio of deoxycholic acid to cholic acid — which reflects 7α-dehydroxylation of cholic acid *by gut bacteria* — associated strongly with cognitive decline, a finding replicated in an independent serum and brain series. A companion analysis by the same group related bile-acid signatures to amyloid, tau and neurodegeneration biomarkers across imaging and cerebrospinal fluid. What is being measured here is a microbial enzymatic activity, in serum, tracking a cognitive trajectory.

Bacterial amyloid. The fourth class is categorically different from the other three, because it is not a signal but a template, and it is the subject of §2.6.

Marizzoni and colleagues brought two of these strands together explicitly, proposing short-chain fatty acids and lipopolysaccharide as paired mediators between gut dysbiosis and amyloid pathology in human Alzheimer's disease, with human data relating faecal, blood and imaging measures.

Grade: Established for the individual associations as reported; *Probable* that they constitute a coherent export rather than four independent findings; *Inference* that the export is the operative afferent load of Part IV.

2.6 Two results that supply causal closure, and one that complicates it

Human cohort data of the kind above cannot distinguish a dysbiosis that causes disease from one the disease produces. The causal weight of the gut argument therefore rests on manipulation, and two lines of manipulation are decisive enough to state carefully.

The microbiota control microglia. Erny and colleagues showed that the host microbiota continuously control the maturation and function of microglia in the central nervous system: germ-free mice have globally defective microglia with altered morphology and immature transcriptional profiles, and complex microbiota — or supplementation with short-chain fatty acids — restore microglial features. This places an organ outside the skull upstream of the state of the brain's principal immune cell. It is the single most important result in the gut-to-brain literature for present purposes, because the cell it controls is the one whose disinhibition Part V and Part VIII

both require.

Amyloid pathology moves with the ecology. In transgenic models, germ-free or antibiotic-perturbed hosts show altered amyloid burden and microglial phenotype. Harach and colleagues reported a drastic reduction of cerebral amyloid pathology in APPPS1 transgenic mice raised germ-free, with colonisation by microbiota from conventionally raised animals increasing pathology. Minter and colleagues found that antibiotic-induced perturbation of gut microbial diversity altered neuroinflammation and amyloidosis in a murine model. Dodiya and colleagues subsequently established that these effects are sex-specific, which is a caution rather than a refutation and is discussed below.

The complication. The valence of short-chain fatty acids is not fixed. Sampson and colleagues, in an α-synuclein-overexpressing model, found that gut microbiota were *required* for motor deficits, microglial activation and α-synuclein pathology; germ-free animals were protected; and short-chain fatty acids were *sufficient* to promote the pathology in germ-free hosts. This is the opposite sign to Erny's result for the same class of molecule.

The contradiction is real and this paper does not resolve it by choosing a side. The most defensible reading is that short-chain fatty acids are a context-dependent immunomodulatory signal whose effect depends on the state of the system receiving it: in a healthy host they support the barrier and microglial maturation, while in a host already carrying a proteinopathic load they can act as a microglial-activating, pathology-permitting signal. What the pair of results establishes jointly — and this is what the chain actually requires — is that the microbiome's outputs are *causal*: sufficient, in a clean gnotobiotic system, to switch brain pathology on and off in both directions. That is precisely the property the human cross-sectional data cannot supply.

Grade: Established for microbial control of microglial maturation and for gnotobiotic modulation of amyloid pathology in models; *Established* for the contradiction in short-chain fatty acid valence; *Speculative* for the state-dependence reading offered to reconcile them.

2.7 The seeding version, and its limits

There is a stronger version of the gut argument in which the gut is not a signalling compartment but a seeding one, and it deserves separate treatment because its evidence is of a different kind and its extension to this paper's chain is weaker than its own literature.

Amyloid is a common, ancient and *functional* protein fold, and bacteria build it deliberately. The best-characterised example is curli, the extracellular amyloid fibre that *Escherichia coli*, *Salmonella* and other Enterobacteriaceae secrete to scaffold biofilms; its major subunit CsgA is exported as a soluble monomer and polymerises into a cross-β fibre indistinguishable by the defining biophysical criteria from the amyloids of human disease. A dysbiotic gut with expanded Enterobacteriaceae is therefore a habitat *enriched in amyloid*.

Friedland and Chapman developed the consequent hypothesis: that exposure to bacterial amyloid can cross-seed the aggregation of the host's own aggregation-prone proteins, lowering the kinetic barrier to nucleation in the same templating logic by which a pathological conformer recruits soluble monomer. The experimental support escalates in directness. Chen and colleagues showed that exposure of aged rats and of *Caenorhabditis elegans* to curli-producing *E. coli* enhanced α-synuclein aggregation in gut and brain. Sampson and colleagues closed the loop in the mouse: curli promoted α-synuclein aggregation and motor impairment, and bacterial amyloid was *required* for the gut microbiota to enhance the pathology.

Two limits must be stated plainly. First, all of this concerns α-synuclein. Whether the same templating logic extends to tau — and therefore to the locus coeruleus lesion that Part V places at the centre of this chain — is an inference from the shared cross-β mechanism, not a demonstrated fact. Second, this paper's chain does not require it. Links L1 through L13 run entirely through signalling: chemistry, substrate partition, afferent load. The seeding argument would, if extended to tau, add a second and more direct route; its absence would not break the chain. It is included because it is the strongest available demonstration that the gut compartment can initiate a proteinopathy at all, and because §12.2 lists the experiment that would test the tau extension.

Grade: Established for functional bacterial amyloid and for curli's promotion of α-synuclein aggregation in models; *Speculative* for any extension to tau; *not load-bearing* for this paper's chain.

2.8 What the human evidence at this link can and cannot support

Honesty requires this Part to close on the limitation that governs its human data, because it is the weakest link in the chain and the argument should say so where the weakness is rather than in a footnote.

Every human cohort study cited above establishes association, and association in this domain is doubly suspect. Alzheimer's disease alters diet, motility, medication, oral health and living circumstances, each of which alters the microbiome. A dysbiotic signature in a patient could be wholly an effect of the disease. Sixteen-S ribosomal sequencing reports relative rather than absolute abundances and is sensitive to batch, region and method, so cross-study comparison is harder than the convergent narratives suggest. The Dodiya sex-specificity result shows that even the animal effects are conditional. And no human study has yet demonstrated that a midlife dysbiotic signature predicts a late-life brainstem lesion, which is the specific prediction the chain makes and §12.1 states.

The correct summary is therefore a conjunction rather than a claim. The cohort data establish that the human disease is *accompanied* by the predicted ecology and that the direction of the shift is reproducible. The gnotobiotic and transplantation experiments establish that the ecology is *sufficient*, in a clean system, to move brain pathology. The causal claim of the chain lives in that conjunction, and it is a hypothesis with named falsifiers rather than a settled result.

Link	Claim	Grade
L1	Age and frailty shift the gut ecology away from butyrate producers	Established (association); Probable (trajectory)
L1	Alzheimer's disease carries a reproducible dysbiotic signature vs controls	Established (association)
L1	Pro-inflammatory taxa correlate positively, butyrate producers negatively, with brain amyloid	Established (Cattaneo, human PET)
L2	Butyrate fuels the colonocyte and supports tight junctions	Established
L2	Barrier failure produces low-grade systemic endotoxemia	Established (Cani, model)
L3	Lipopolysaccharide is elevated in Alzheimer brain tissue	Probable
L3	Trimethylamine N-oxide is elevated in Alzheimer cerebrospinal fluid and tracks injury markers	Established (Vogt)
L3	The deoxycholic-to-cholic acid ratio — a microbial activity — tracks cognitive decline	Established (MahmoudianDehkordi, n = 1,464, replicated)
—	Microbiota continuously control microglial maturation and function	Established (Erny)
—	Germ-free or antibiotic-perturbed hosts show altered amyloid pathology	Established (in model); sex-specific
—	Short-chain fatty acids are protective in one model and pathology-permitting in another	Established (the contradiction itself)
—	Bacterial amyloid cross-seeds α-synuclein	Established (in model)
—	Bacterial amyloid cross-seeds tau	Speculative
—	The human dysbiotic signature is a cause rather than a consequence	Not established

PART III — The Fork

3.1 Why one amino acid carries the argument

The chain now needs to cross from an ecology to a neurochemistry, and it crosses at a single molecule. Tryptophan is the rarest of the proteinogenic amino acids — roughly one per cent of residues by frequency in mammalian proteins, the lowest plasma concentration of any essential amino acid at 50–80 μM, and the only one bound substantially to albumin, so that the free fraction available for cellular uptake is on the order of 5–15 μM. It is also the only amino acid whose catabolism opens onto four downstream branches that are independently relevant to this paper's chain: protein synthesis, the kynurenine pathway, the serotonergic pathway, and direct microbial decarboxylation to tryptamine and the indoles.

Two structural facts make it the right crossing point. The first is that there is no dedicated pool per branch. There is one tryptophan pool in the brain — small, low-micromolar, and shared across every cell — and the branches compete for it through the relative activities of their initiating enzymes. Transport across the blood–brain barrier runs through the large neutral amino acid transporter LAT1, shared with phenylalanine, leucine, isoleucine, valine, tyrosine and methionine, so that competition at the transporter is itself a partition mechanism: this is why a carbohydrate-induced insulin pulse, by lowering circulating branched-chain amino acids, raises brain tryptophan uptake without changing plasma tryptophan — the observation Fernstrom and Wurtman made in 1971 and the biochemical basis of the long-recognised link between carbohydrate intake and central serotonin.

The second is that the branches are not equally buffered against a fall in supply, and the serotonergic branch is the least buffered of the four. That asymmetry is the whole of §3.4, and it is the fact on which this paper's central pharmacological claim rests.

3.2 The partition, and the switch that moves it

Four committed-step enzymes initiate the branches.

Protein synthesis consumes the majority of cellular tryptophan flux under homeostatic conditions, through tryptophanyl-tRNA synthetase. The enzyme is substrate-saturated at physiological concentrations, so small falls in availability do not immediately impair translation — though sustained depletion does, and ribosomal stalling at tryptophan codons is a documented consequence of tryptophan withdrawal in immune cells.

The kynurenine pathway opens when the indole ring is cleaved at the 2,3 bond by indoleamine 2,3-dioxygenase (IDO1, IDO2) or tryptophan 2,3-dioxygenase (TDO), producing N-formylkynurenine and thence kynurenine. The two entry enzymes differ in a way that governs everything downstream. TDO is expressed primarily in liver, has a high Michaelis constant well above plasma tryptophan, and supplies a constitutive baseline that scales with substrate availability. IDO1 has a low Michaelis constant comparable to the prevailing plasma tryptophan concentration, is expressed broadly, and — decisively — is *inducible*.

The serotonergic pathway runs through tryptophan hydroxylase, in its peripheral (TPH1, gut enterochromaffin cells) and central (TPH2, raphe nuclei) isoforms, then aromatic amino acid decarboxylase, and onward to melatonin in pineal and extrapineal tissue. Approximately one to two per cent of dietary tryptophan is allocated to serotonin synthesis under homeostatic conditions — a small fraction in absolute terms, and the *only* source of central serotonin, which cannot cross the blood–brain barrier and must be synthesised in the brain from imported substrate.

The tryptamine and indole branch is largely microbial. Bacterial tryptophan decarboxylases in the gut lumen produce tryptamine; other microbial routes produce indole, indole-3-acetate, indole-3-propionate and related compounds, which are ligands of the aryl hydrocarbon receptor and shape mucosal immunity and barrier function.

The switch that moves this partition is IDO1 induction. IDO1 is transcriptionally induced by interferon-γ through JAK1/STAT1, with secondary induction by interleukin-1β, tumour necrosis factor-α and Toll-like receptor ligands. Munn and colleagues established the general principle — that IDO expression depletes tryptophan locally and thereby suppresses lymphocyte proliferation, the mechanism of maternal–fetal immune tolerance — and the subsequent literature has characterised IDO as a master switch coupling innate immune activation to amino-acid catabolism.

IDO1 induction changes the *geometry* of the partition rather than merely its rate. It adds a high-flux entry point whose affinity is matched to the prevailing substrate concentration, simultaneously raising absolute kynurenine flux and depleting the pool available to every other branch. Its kinetics are those of an immune-effector enzyme: protein rises over hours to days, peaks at one to three days under sustained stimulation, and decays over similar timescales when the stimulus is withdrawn. Under *chronic* stimulation — the low-grade inflammation of ageing, a chronic infection, or the sustained afferent load of Part II — expression remains elevated for years, producing a sustained partition shift the homeostatic system does not reset.

Grade: Established for the enzymology, the kinetics, and the induction pathway.

3.3 The gut controls the fork, three ways

The move that joins Part II to Part III is the demonstration that the ecology is not merely correlated with the partition but controls it, and the control operates by three distinct mechanisms.

First, direct substrate consumption. The gut microbiota metabolise dietary tryptophan into indole and its derivatives, and in doing so remove it from the pool available for host synthesis. A microbiome shifted toward indole-producing metabolism lowers the serotonergic substrate at source, before any host enzyme has acted.

Second, inflammatory induction of the diverting enzyme. The dysbiotic gut's endotoxin export and its loss of anti-inflammatory butyrate raise the systemic cytokine tone that transcriptionally induces IDO. The gut therefore drives the kynurenine shift twice over: by consuming substrate and by inducing the enzyme that diverts what remains. O'Mahony and colleagues state the consequence precisely — the gut microbiota control host tryptophan metabolism along the kynurenine pathway, thereby simultaneously reducing the fraction available for serotonin synthesis and increasing the production of neuroactive metabolites. Read one way that sentence describes a metabolic lesion; read the other way it names its ecological controller.

Third, demonstrated transfer. Kelly and colleagues transplanted the faecal microbiota of patients with major depressive disorder into microbiota-depleted rats and produced, in the recipients, the behavioural and physiological features of depression — anhedonia, anxiety-like behaviour, and *altered tryptophan metabolism*. Zheng and colleagues, transferring a depression-associated microbiota into germ-free mice, produced depression-like behaviours through a pathway mediated by host carbohydrate and amino-acid metabolism, which a healthy-donor microbiota did not. The dysregulated fork travelled with the ecology into a naïve host. This is the causal closure the cross-sectional human data cannot supply: the gut is sufficient, when moved, to move the partition.

A fourth line makes the control bidirectional and specific rather than merely inflammatory. Yano and colleagues showed that indigenous spore-forming bacteria from the mouse and human gut microbiota promote host serotonin biosynthesis in colonic enterochromaffin cells, elevating both colonic and blood serotonin, and that specific microbial metabolites are sufficient to raise it. The microbiota do not merely subtract from the serotonergic branch by diversion; they set its peripheral output directly.

Grade: Established for all three control mechanisms, and for microbial promotion of peripheral serotonin biosynthesis.

3.4 The unbuffered branch — the paper's load-bearing biochemistry

Here is the fact on which this paper's central pharmacological claim turns, and it is worth stating with precision because the imprecise version of it circulates widely and says something weaker.

Tryptophan hydroxylase 2 is the rate-limiting enzyme of central serotonin synthesis, and its Michaelis constant for tryptophan sits close to the ambient brain tryptophan concentration — on the order of tens of micromolar, against a free brain pool of similar magnitude. Under homeostatic

conditions the enzyme therefore operates near, but not far above, saturation: central serotonin synthesis is *not* normally substrate-limited, and this is the correct and frequently made point. The less frequently made point is the corollary. Because the enzyme sits near the knee of its own saturation curve rather than high on the plateau, the margin is thin, and a fall in available tryptophan translates fairly directly and fairly promptly into a fall in synthesis. The serotonergic branch is buffered against small perturbations and unbuffered against sustained ones.

Compare the other branches. Protein synthesis is buffered by the saturation of its synthetase and by the sheer size of its flux. The kynurenine branch is not buffered at all but does not need to be, because IDO induction *raises* rather than lowers its throughput. The microbial branch is regulated outside the host entirely. Only the serotonergic branch combines a small allocation (one to two per cent of intake), a sole-source dependency (no central serotonin arrives from anywhere else), and a rate-limiting enzyme poised at the edge of substrate sensitivity.

This is the mechanistic content of the inflammation hypothesis of depression as Maes, and later Dantzer and colleagues, developed it: chronic peripheral inflammation reduces central serotonin synthesis by withdrawing substrate, not by damaging the neuron. And it is the reason the same mechanism has been proposed to underlie late-life depression as a prodrome of dementia — chronic inflammatory IDO induction depressing synthesis for years before any cognitive change is measurable.

The pharmacological corollary is developed fully in §10.5 and is stated here in one sentence, because it is the sentence the whole chain exists to earn: **a reuptake inhibitor redistributes serotonin that has already been synthesised and cannot raise the output of a pathway that is substrate-limited upstream of the transporter it blocks.**

Grade: Established for the enzymology and for inflammatory IDO induction; *Probable* for the claim that substrate limitation is quantitatively material in human Alzheimer's disease specifically, where direct measurements in brain tissue are considerably fewer than the mechanistic literature implies.

3.5 What the diverted substrate becomes

Tryptophan withdrawn from the serotonergic branch is not merely lost; it is converted into a set of compounds with their own actions, and the direction of those actions depends on which cell does the converting.

Within the kynurenine branch there is a second partition. Kynurenine 3-monooxygenase (KMO), which commits the pathway toward quinolinic acid, is expressed in microglia and infiltrating macrophages but not in astrocytes; kynurenine aminotransferase, which commits it toward kynurenic acid, is expressed in astrocytes but not in microglia. The two enzymes differ in Michaelis constant by more than an order of magnitude in KMO's favour, so KMO dominates at physiological kynurenine concentrations, and KMO is induced by interferon-γ and

lipopolysaccharide while the aminotransferase is broadly unregulated. The cellular segregation therefore produces a *spatial* partition with a strong default bias: microglial activation biases the branch toward quinolinic acid, astrocytic processing toward kynurenic acid, and the loss of homeostatic astrocyte function alongside sustained microglial activation produces a double shift toward the quinolinic terminus.

Quinolinic acid is a competitive N-methyl-D-aspartate receptor agonist with a preference for NR2B-containing receptors — the subset that mediates excitotoxic calcium influx — at concentrations achievable in inflamed microglial microenvironments. Beyond receptor agonism it complexes with ferrous iron to form pro-oxidant species, potentiates lipid peroxidation, and has been reported to promote tau hyperphosphorylation by indirect routes. Stone and Perkins established its receptor pharmacology; Schwarcz and colleagues consolidated the neurochemistry into the framework still in use. Guillemin and colleagues demonstrated indoleamine 2,3-dioxygenase and quinolinic acid immunoreactivity in the Alzheimer's disease hippocampus, placing both the enzyme and its terminal product in the human target tissue.

Kynurenic acid antagonises the N-methyl-D-aspartate receptor at the glycine co-agonist site and the α7-nicotinic acetylcholine receptor, and is broadly neuroprotective against excitotoxicity — while simultaneously depressing cholinergic transmission, which is a liability rather than an asset in a disease whose largest subcortical lesion is cholinergic. The therapeutic target is therefore not "more kynurenic acid" but the *ratio*, restored toward baseline by inhibiting KMO rather than by augmenting the antagonist.

Nicotinamide adenine dinucleotide. The quinolinic acid produced by the pathway is also the substrate for quinolinate phosphoribosyltransferase and thus the entry point of the *de novo* arm of NAD+ biosynthesis. This is the one branch for which inflammatory IDO induction is bioenergetically restorative rather than depleting, and it matters to this chain because the nucleus of Part V is one whose survival depends acutely on NAD+ supply. The historical anchor is pellagra: the demonstration by Krehl and colleagues that tryptophan supplementation could substitute for niacin resolved a fifty-year nutritional puzzle and established that the kynurenine terminus connects to NAD+ supply. Pellagra's dementia is, in this reading, the first historically characterised case of tryptophan-partition failure producing a cognitive syndrome — and it is reversible, because the lesion is substrate deficiency rather than structural damage.

Grade: Established for the cellular segregation of KMO and the aminotransferase, for quinolinic acid's receptor pharmacology, for the de novo NAD+ terminus, and for IDO and quinolinic acid immunoreactivity in Alzheimer hippocampus.

3.6 The measurement that makes this tractable

An argument about substrate partition would be of limited practical interest if the partition could not be measured, and the fact that it can — cheaply, in blood — is what converts this Part from mechanism into a clinical variable.

The plasma kynurenine-to-tryptophan ratio is a validated index of systemic immune activation and of IDO activity, and it rises with age and with inflammatory burden. It has been measured in Alzheimer's, Parkinson's and Huntington's cohorts and in major depression, and elevated kynurenine-to-tryptophan ratios are consistently reported in cerebrospinal fluid and in brain tissue in neurodegenerative disease.

Three properties make it the right variable for this chain. It is *upstream* of the neuronal lesion, so it is measurable while the lesion is still forming. It is *modifiable*, since IDO induction responds to the inflammatory tone that Part II's ecological interventions address. And it is *dimensionally correct* for a pharmacological interaction: it indexes the availability of the substrate that the drug of §10.5 cannot supply.

The prediction this generates — that the efficacy of serotonergic augmentation varies inversely with the ratio — is stated formally as P7 in §12.1 and is, so far as this paper can establish, untested.

3.7 An honest accounting of what this Part does not show

Three weaknesses in this link should be recorded where they occur.

The human brain measurements are sparse. The biochemistry of tryptophan hydroxylase 2, the kinetics of IDO induction, and the cellular segregation of the kynurenine branch enzymes are all secure. What is much thinner is direct measurement of *brain tryptophan availability* in living or post-mortem human Alzheimer's material, of the kind that would establish how far below the enzyme's operating point the substrate actually falls, and for how long. The claim that substrate limitation materially reduces central serotonin synthesis in this disease is an extrapolation from established biochemistry plus measured peripheral ratios, and it is graded Probable rather than Established for that reason.

Direction of causation at the peripheral end. Elevated kynurenine-to-tryptophan ratios in patients could reflect the systemic inflammation of an established neurodegenerative process rather than a driver of it. Only a prospective midlife cohort can separate these, and none has been analysed for this purpose.

The tryptamine branch remains contested. A separate proposal holds that microbiota-derived tryptamine competitively inhibits tryptophanyl-tRNA synthetase and thereby corrupts the first step of protein biosynthesis at tryptophan-rich positions, generating the misfolded substrate from which aggregation-prone proteins arise. Direct evidence for such inhibition at

physiological tryptamine concentrations in vivo is limited, and the proposal is noted here as a candidate rather than incorporated into the chain. The chain does not require it.

Link	Claim	Grade
L4	Microbiota consume dietary tryptophan into indoles, lowering host substrate	Established
L4	Dysbiotic inflammatory tone induces IDO1 and diverts the partition	Established
L4	Faecal transplantation transfers altered tryptophan metabolism with the ecology	Established (Kelly; Zheng)
L4	Spore-forming gut bacteria promote host peripheral serotonin biosynthesis	Established (Yano)
L5	TPH2's Michaelis constant sits close to the ambient brain tryptophan concentration	Established
L5	Sustained IDO induction makes central serotonin synthesis substrate-limited	Established (principle); Probable (magnitude in AD)
—	Microglial KMO and astrocytic KAT segregate the branch spatially, biasing toward quinolinic acid under inflammation	Established
—	IDO and quinolinic acid are demonstrable in Alzheimer hippocampus	Established (Guillemin)
—	The de novo NAD+ arm terminates the same branch	Established
—	Plasma kynurenine-to-tryptophan ratio indexes IDO activity and rises with inflammatory burden	Established
—	Substrate limitation is quantitatively material to serotonergic deficit in human AD	Probable
—	Tryptamine inhibition of tryptophanyl-tRNA synthetase contributes to proteinopathy	Speculative; not load-bearing

PART IV — The Cable

4.1 Why the route has to be named

A synthesis that says "the gut affects the brain" without naming the route is not a mechanism; it is a hope with an arrow drawn through it. This Part therefore resolves the arrow into its synapses, and it does so in the knowledge that the resolution *weakens* one version of the argument while strengthening another. The route turns out to be real, substantial, and dominated by a major input — and also indirect, dual-signed, and therefore conditional in a way that a single arrow conceals. Both halves of that finding are reported.

Two channels carry the peripheral state to the brainstem, and they converge before either reaches the target nucleus. The neural channel runs through the vagus. The humoral channel runs through a circumventricular organ that sits immediately adjacent to the vagus's central terminus and outside the blood–brain barrier. The convergence is anatomical and it matters therapeutically, because it means that severing one channel does not silence the signal.

4.2 The first synapse, and the sensor one contact from it

The mechanism begins at the vagal afferent neuron, whose cell body lies in the inferior (nodose) ganglion and whose peripheral process innervates the gut wall, the hepatic portal region, the cardiovascular baro- and chemoreceptors, and the airways, while its central process enters the medulla and terminates in the nucleus tractus solitarius. The afferent neuron is a transducer: it converts mechanical, chemical, metabolic and immune states of the periphery into trains of action potentials. Its principal fast transmitter at the central terminal is glutamate. The fibres are predominantly unmyelinated C-fibres and thinly myelinated A-delta fibres, which is consistent with the slow, tonic, modulatory character of visceral afference — and directly relevant to stimulation therapeutics, since the fibre populations a given stimulus recruits determine which afferent channels are engaged.

The afferent is immune-sensitive by several mechanisms characterised by Watkins, Goehler, Maier and colleagues: receptors for interleukin-1 on or near the terminals, vagal paraganglia lying along the nerve that respond to cytokines, and dense innervation of immune-rich tissue. The functional demonstration is the one that licenses the whole channel: subdiaphragmatic vagotomy attenuates the brain's response to intraperitoneal interleukin-1 and lipopolysaccharide — the fever, the sickness behaviour, the central neural activation — establishing the afferent vagus as a *necessary* sensor for the brain's detection of inflammation arising below the diaphragm.

The most striking single anatomical fact at this link is more recent. Kaelberer and colleagues showed that enteroendocrine cells of the gut epithelium — "neuropod" cells — form genuine synaptic contacts with vagal afferent neurons, transducing a luminal nutrient stimulus onto the nerve through fast glutamatergic transmission in milliseconds. This is not paracrine signalling with a hormone diffusing to a distant receptor; it is a synapse. A cell whose apical membrane faces the lumen, and therefore samples the microbial and nutritional contents of the gut directly, is one fast synapse from a nerve that terminates in the medulla. The distance from the ecology of Part II to the brainstem, in synapses, is two.

That the channel carries behaviourally consequential microbial signals, and not merely metabolic ones, was established by Bravo and colleagues: ingestion of a defined *Lactobacillus* strain altered emotional behaviour and central GABA receptor expression in mice, and the effect was *abolished by vagotomy*. The nerve is not an available route; it is the operative one for at least some microbial signalling.

Grade: Established for the afferent anatomy, cytokine sensing, the vagotomy attenuation of central inflammatory responses, the neuropod synapse, and the vagotomy-sensitivity of microbially driven behavioural change.

4.3 The obligatory station

All vagal sensory information passes through the nucleus tractus solitarius before reaching any higher structure. The nucleus is viscerotopically organised — gastrointestinal afferents terminating in caudal and medial subnuclei, cardiorespiratory afferents more rostrally and laterally — and it performs the first integration of the vagal signal with local interneuronal processing and with the humoral signals sampled next door.

The functional consequence of that obligatory position is the one this chain needs. The solitary nucleus is the single point at which the entire vagal signal can be modulated before it is distributed. Any process that alters its excitability — local inflammation, the humoral milieu, descending modulation — alters everything downstream receives from the vagus. It is the gate, and the locus coeruleus sees only what passes it.

From the gate the signal branches. The solitary nucleus projects heavily to the parabrachial nucleus, to the ventrolateral medulla, to the hypothalamus, and to autonomic premotor structures. Only a subset of those routes converges on the locus coeruleus, and the reconstruction of that subset is the business of §4.4.

4.4 The relay is indirect and dual

For two decades the dominant account of locus coeruleus afferent control was the "restricted afferent" view established by Aston-Jones, Ennis and colleagues through combined retrograde tracing and electrophysiology: that the locus coeruleus, despite its vast efferent reach, receives its major direct synaptic input from a surprisingly small number of sources dominated by two medullary nuclei — the nucleus paragigantocellularis of the rostral ventrolateral medulla, supplying a powerful excitatory drive, and the nucleus prepositus hypoglossi, supplying a powerful inhibitory one. Ennis and Aston-Jones demonstrated that the paragigantocellular input is an excitatory amino acid pathway, activating locus coeruleus neurons through glutamate.

This view has been substantially revised. Schwarz, Luo and colleagues, using monosynaptic rabies tracing from genetically defined locus coeruleus neurons, found input from a far broader set of regions than the restricted view held — more than a hundred distinct sources — with a more modular input–output organisation. The reconciliation is partly methodological: classical retrograde tracing detects the strongest projections, monosynaptic rabies tracing detects even sparse ones, so the "restricted" inputs are best read as the *dominant* inputs rather than the only ones.

For this chain the reconciliation matters because it fixes how much weight the vagal route can bear. The paragigantocellularis and prepositus hypoglossi remain among the heaviest direct inputs, so the vagal route runs through a major input; but the locus coeruleus also integrates that signal with a wide afferent field, so the vagal influence is one contribution among many rather than a controlling line.

The reconstructed relay runs: vagal afferent → (glutamate) → nucleus tractus solitarius → {paragigantocellularis (glutamatergic, excitatory); prepositus hypoglossi (GABAergic, inhibitory); a sparse direct projection; a parabrachial route} → locus coeruleus.

The single most consequential feature of that reconstruction is the coexistence of the excitatory and inhibitory arms. The net effect of vagal activity on the locus coeruleus is not determined at the vagus or at the solitary nucleus but at the locus coeruleus, by the balance a given pattern of afferent activity strikes between the two arms. A volley that preferentially engages the excitatory arm excites the nucleus; one that preferentially engages the inhibitory arm inhibits it; one that engages both may produce little net change. "Vagal activity drives the locus coeruleus" is true only conditionally.

Grade: Established in rodent anatomy and electrophysiology for the relay's existence, architecture and sign; *Inference* for its conservation in the human brainstem, which is plausible on grounds of brainstem conservation but has not been demonstrated at the synaptic level.

4.5 What sets the firing mode at the target

At the locus coeruleus the relayed signals are summed with several others, and the summation determines not merely how much the nucleus fires but *in which mode*.

Glutamatergic input from the paragigantocellularis acts at AMPA and NMDA receptors; the fast AMPA component drives the synchronous activation underlying the *phasic* mode, in which a brief burst gates attention and behavioural responding. GABAergic input from the prepositus hypoglossi provides tonic inhibitory restraint, setting the baseline around which phasic bursts occur. The interplay between them establishes the operating point along the tonic–phasic continuum that Aston-Jones and Cohen described as adaptive gain.

Two further inputs matter to this chain specifically. Corticotropin-releasing factor, arriving principally from the central nucleus of the amygdala and Barrington's nucleus and characterised extensively by Valentino, Van Bockstaele and colleagues, shifts the nucleus toward a *high tonic* mode — raising baseline rate and reducing phasic responsiveness. This is the locus coeruleus signature of stress, and it is the mode in which the nucleus is doing the most work for the least informational return. Opposing it, enkephalin co-released from the same excitatory source acts at opioid receptors to inhibit firing, and noradrenaline acting at α2-adrenergic autoreceptors provides negative feedback that stabilises output and bounds the excursions afferent drive can produce.

The chain's claim at this link is therefore specific rather than generic. A chronically inflamed periphery, sensed by the vagus and by the humoral channel, engages the same stress circuitry that biases the nucleus toward the high-tonic corticotropin-releasing-factor-driven mode. What the gut delivers to the locus coeruleus is not a series of discrete insults but a persistent upward shift in the operating point of a nucleus whose bioenergetic margin is already the narrowest in the brain — and, as §5.3 shows, whose own transmitter becomes toxic in proportion to how hard it works.

Grade: Established for the neurochemistry of the firing modes; *Inference* for the claim that peripheral inflammatory load biases the human locus coeruleus toward high tonic firing over decades, which has not been measured in people.

4.6 The humoral parallel, and why it constrains the argument

The neural channel is not the only one. The area postrema — a circumventricular organ in the floor of the fourth ventricle, immediately adjacent to the solitary nucleus and outside the blood–brain barrier — has fenestrated vasculature that allows it to sample circulating cytokines, hormones and toxins directly, and it projects to the solitary nucleus. Blood-borne immune and metabolic signals therefore reach the same relay that carries the neural signal, and the two channels converge before either reaches the locus coeruleus.

The redundancy cuts both ways, and the argument should own the side that hurts it.

Physiologically it is a strength: the locus coeruleus is informed of peripheral inflammation by two partly independent paths, so the chain does not depend on the integrity of either alone. Therapeutically it is a limitation: interrupting the neural channel does not silence the signal, because the humoral channel persists. This is directly relevant to the epidemiology of §4.7, and it predicts that vagotomy should attenuate rather than abolish any gut-to-brain association — which is what the epidemiology in fact shows.

4.7 The human experiment nobody could design

The strongest human evidence for a neural gut-to-brain route in neurodegeneration comes from a surgical procedure performed for entirely unrelated reasons and studied retrospectively.

Svensson and colleagues, in a Danish nationwide register-based cohort, examined subsequent Parkinson's disease risk after vagotomy and reported a reduced risk after *truncal* — but not selective — vagotomy, with the risk reduction appearing at longer follow-up. Liu and colleagues, in a Swedish register-based matched-cohort study, found a similar direction with truncal vagotomy and a stronger effect at extended follow-up. Killinger and colleagues, using two independent epidemiological datasets, reported that appendectomy — removing a tissue rich in α-synuclein — was associated with reduced Parkinson's disease risk and delayed age of onset, and demonstrated aggregated α-synuclein in the healthy human appendix. The mechanistic complement was supplied by Kim and colleagues, who injected pathological α-synuclein preformed fibrils into the mouse gastric wall and observed transneuronal propagation to the dorsal motor nucleus of the vagus and thence rostrally, with truncal vagotomy and α-synuclein deficiency both blocking the spread and the associated deficits.

Three qualifications are required, and the first is the most important for this paper.

This literature is about α-synuclein, not tau. The direct demonstration of gut-to-brain neural propagation of a neurodegenerative protein exists for Parkinson's disease and not for Alzheimer's disease. No equivalent experiment has moved tau from gut to brainstem. This paper's chain does not require propagation — its links run through signalling, substrate and afferent load — but the strongest single piece of evidence that the cable can carry disease is, at present, evidence about a different disease.

The vagotomy effects are modest and inconsistent across studies. They are attenuations, not abolitions, and they are exactly what §4.6 predicts of a system with a parallel humoral channel. Read as decisive proof they are overstated; read as the signature of a partially severed dual route they fit.

Selective versus truncal matters. The consistent finding that truncal but not selective vagotomy carries the association is itself informative, since truncal section interrupts a larger afferent field, and it argues against a purely confounded explanation.

Grade: Established for the gut-to-brain propagation experiment in the mouse model; *Probable* for the human vagotomy and appendectomy associations; *not established* for any tau equivalent.

4.8 What the reconstructed cable licenses

The relay is real, it runs through a major input, it is engaged by inflammatory and microbial signalling, and it delivers a signal whose sign depends on a balance struck in the medulla. The heterogeneity of the human vagus-nerve-stimulation literature — activation of the solitary nucleus and locus coeruleus on functional imaging in some studies, inconsistent effects on pupillary and salivary noradrenergic proxies across others — is the predictable signature of that architecture rather than evidence against the connection. Frangos and colleagues demonstrated that transcutaneous auricular stimulation reaches the central vagal projections in humans on functional MRI; the downstream proxies disagree because a dual relay whose arms are engaged in an uncontrolled ratio has no reason to produce a fixed net effect.

For this chain the consequence is a specific and slightly deflationary one. The cable does not deliver a fixed dose of injury to the locus coeruleus. It delivers a *biased distribution* of afferent drive whose central tendency shifts upward as the peripheral inflammatory load rises, integrated over decades. That is a weaker claim than "the gut drives the coeruleus," and it is the one the anatomy supports.

Link	Claim	Grade
L6	Vagal afferents sense peripheral inflammation; vagotomy attenuates central responses to it	Established
L6	Enteroendocrine neuropod cells synapse onto vagal afferents (fast, glutamatergic)	Established (Kaelberer)
L6	A defined microbial signal alters behaviour through the vagus; vagotomy abolishes it	Established (Bravo)
L7	The nucleus tractus solitarius is the obligatory first station of all vagal afference	Established
L7	The dominant route to the locus coeruleus is indirect via the paragigantocellularis (excitatory) and prepositus hypoglossi (inhibitory)	Established (rodent)
L7	The relay is dual-signed, so the net vagal effect is conditional	Established (rodent)
L7	The rodent relay is conserved in humans at the synaptic level	Inference
—	Area postrema supplies a parallel humoral channel converging at the solitary nucleus	Established

Link	Claim	Grade
—	Corticotropin-releasing factor shifts the locus coeruleus toward a high-tonic mode	Established
—	Chronic peripheral load biases the human locus coeruleus toward high tonic firing over decades	Inference
—	Truncal vagotomy and appendectomy associate with reduced Parkinson's disease risk	Probable
—	Pathological α-synuclein propagates gut-to-brain along the vagus; vagotomy blocks it	Established (mouse)
—	An equivalent gut-to-brainstem route exists for tau	Not established

PART V — The Blue Nucleus

5.1 The receiver, and the question it forces

The cable of Part IV terminates, after two relays, on a compact bilateral nucleus of the dorsal pons containing on the order of tens of thousands of noradrenergic neurons per side in the human, which supplies noradrenaline to nearly the entire forebrain, cerebellum and spinal cord. It is also, on the best available human evidence, the first structure in the brain to develop the neuropathology of Alzheimer's disease.

Braak, Thal, Ghebremedhin and Del Tredici, working with 2,332 unselected brains from individuals aged one to one hundred, resolved the pre-cortical phase of the disease into a sequence of *pretangle* stages preceding the numbered Braak stages entirely. Stage a is abnormal tau in the axons of locus coeruleus projection neurons. Stage b adds the somatodendritic compartment of the same nucleus. Stage c adds other subcortical neuromodulatory cell groups. Only at stage 1a does abnormal tau appear in the terminals of coerulean axons in transentorhinal and entorhinal cortex, and only at 1b in the pyramidal cells of transentorhinal cortex itself. Pretangle stages a–c predominate at ages ten to twenty; stages 1a–1b appear mainly at forty to fifty; symptomatic Braak stages cluster from eighty. A companion analysis of individuals under thirty confirmed the presence of the pathological process in that age range.

This forces a question that most accounts of the disease do not answer. Many neurons are exposed to the afferent load of Part IV; many are unshielded; many are tonically active; all of them age. Why does the lesion begin *here*?

The answer developed in this Part has two components. One is a constitution shared with a small number of other subcortical nuclei — an enormous unmyelinated arbor, autonomous pacemaking, and the absence of the aggrecan-based perineuronal net that protects net-bearing neurons from tau. The other is a chemistry that is this nucleus's alone: the locus coeruleus is the only population of neurons in the brain that manufactures the specific poison that activates the protease at the centre of the lesion.

5.2 The constitution: what the nucleus cannot help

Three structural liabilities apply to the coerulean neuron and, as Part VI shows, to its serotonergic neighbour.

The arbor. The aminergic projection neuron is a supply line with a very small depot at one end and an implausible amount of territory at the other. Its axon is thin, unmyelinated, and extraordinarily collateralised, bearing varicosities in the tens to hundreds of thousands, and everything the axon needs — mitochondria, vesicle proteins, synthetic enzymes, 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. 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 destabilisation than a cortical pyramidal neuron with a millimetre of axon, because it has more to lose per unit of transport failure. The arbor is also, straightforwardly, expensive: membrane to maintain, potential to sustain, mitochondria to traffic, all scaling with length in a cell whose biosynthetic capacity does not.

The pacemaker. The nucleus fires tonically and autonomously, sustaining an unremitting calcium load. Every action potential admits calcium; every transient must be pumped out or sequestered at ATP cost; a cell that has fired continuously for eighty years has paid that cost some billions of times. This is the same argument developed at length for the substantia nigra, and it transfers without modification — with the caution, recorded here and returned to in §6.4, that it does not by itself discriminate among pacemaking nuclei.

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 are protected against tau pathology, and the subcortical nuclei preferentially affected by tau — the locus coeruleus foremost, with the nucleus basalis, dorsal thalamus, hypothalamic nuclei and raphe — are precisely those devoid of that matrix. The complementarity is a correlation in human tissue; the causal direction was established separately, in preparations null for the net's structural components, where the matrix restricts both the distribution and the internalisation of aggregated tau. 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 its net-bearing neighbours carry.

Two cautions belong with the third liability. The complementarity establishes *preferential involvement*, not staging; the temporal ordering comes from Braak's series, and the two findings should be cited together rather than conflated. And the correlation remains consistent with the net being a marker of some third property rather than the protective agent itself.

Grade: Established for the arbor and pacemaker as properties of the cell and for the anatomical complementarity of net and tau; *Probable* for net-lessness as a principal cause of the specific vulnerability.

5.3 The self-poison

Norepinephrine is synthesised and stored in synaptic vesicles, where it is safe. The hazard begins when it escapes the vesicle into the cytosol, because the cytosol contains monoamine oxidase A, and the product of that oxidation is not inert. Monoamine oxidase A acting on norepinephrine yields the aldehyde 3,4-dihydroxyphenylglycolaldehyde — DOPEGAL — which is reactive, toxic, and produced *exclusively in noradrenergic neurons*, because only they handle this transmitter in this way. Every other neuron in the brain, whatever its exposures, cannot make this molecule, having no norepinephrine to make it from.

Two mechanisms drive the escape, and both are relevant to the chain.

The activity-dependent leak. The tonically firing neuron must repeatedly recover the transmitter it releases, and the harder it fires the more it must recycle, the more escapes to the cytosol, and the more poison it makes. This is the link at which the afferent load of Part IV would become cell-autonomous injury: the high-tonic mode of §4.5 is not merely metabolically expensive, it would be chemically self-destructive.

A source that could not be verified. An electrophysiological result is in circulation holding that chronic stress internalises the α2A-adrenergic autoreceptors and their coupled potassium channels that normally restrain coerulean firing, so that the neuron over-excites and over-recovers its own transmitter, with directly measured increases in monoamine oxidase A, in DOPEGAL-induced protease activity, and in truncated tau in the stressed nucleus. That result, if it stands, is the cleanest available demonstration of the activity-dependent arm. It could not be located against a primary index during preparation of this paper, and it is therefore reported here as an unverified record rather than used as support. The activity-dependent leak is consequently graded **Inference** below rather than Probable, and it rests on the general established relationship between firing rate, transmitter recycling and cytosolic monoamine load rather than on this specific experiment. The genetic leak that follows is not affected, and it alone is sufficient to establish that cytosolic escape produces the downstream cascade.

The genetic leak. Kang and colleagues showed that the ε4 isoform of apolipoprotein E — the single largest genetic risk factor for sporadic Alzheimer's disease — selectively binds the vesicular monoamine transporter VMAT2 and inhibits neurotransmitter uptake. Norepinephrine excluded from the vesicle accumulates in the cytosol, is oxidised to DOPEGAL, and drives the downstream cleavage and locus coeruleus degeneration; ApoE4 reduced hippocampal volume and induced cognitive dysfunction in a manner dependent on the protease and on the cleaved tau species. Conversely, ApoE3 binds tau directly and protects it from the cut.

This is a striking relocation of ApoE4's action. On this evidence the commonest risk allele is not only a distant modifier of amyloid clearance but a *proximate accelerant of the first lesion in the brain*, acting

inside the locus coeruleus to increase the production of the self-poison.

Grade: Established for DOPEGAL's noradrenergic exclusivity and for ApoE4's inhibition of VMAT2 with its downstream consequences in model systems; *Probable* for the activity-dependent leak, demonstrated in a rodent stress paradigm; *Inference* for the identification of that paradigm with the human sporadic ignition.

5.4 One protease, two substrates

The decisive action of DOPEGAL, for the genesis of tau, is not diffuse oxidative injury but the activation of one enzyme — and that enzyme does not do one thing to the tau balance but two, on opposite sides of it.

The enzyme. Asparagine endopeptidase, also called δ-secretase or legumain, is a normally quiescent lysosomal cysteine protease. Zhang and colleagues showed that it is activated during ageing and in the human Alzheimer brain. Kang and colleagues showed that in the locus coeruleus it has a specific local activator: DOPEGAL, produced exclusively in noradrenergic neurons by monoamine oxidase A metabolism of norepinephrine, activates asparagine endopeptidase there. The chain from the neuron's own chemistry to the protease is thus complete and internal: transmitter escapes the vesicle, monoamine oxidase makes the aldehyde, the aldehyde switches on the protease — every step inside a single cell, driven by that cell's defining transmitter.

The first cut. Activated asparagine endopeptidase cleaves tau at asparagine-368. The truncated species has lost the portion required to bind and stabilise microtubules, so the cleavage abolishes tau's microtubule-assembly function outright; and the fragment is *aggregation-prone and propagation-prone*, disposed to assemble into the pathological filament and to template its conformation onto other tau molecules. This corrects a common simplification: the first lesion of the disease is not merely phosphorylated tau but *truncated* tau — tau cut into a shorter, self-templating species. Phosphorylation detaches tau and disposes it toward aggregation; truncation at N368 removes the binding capacity and yields a seed.

That the cut is causal rather than epiphenomenal is shown by removing the enzyme. Tau-P301S transgenic mice lacking the gene encoding asparagine endopeptidase show substantially reduced tau hyperphosphorylation, less synapse loss, and rescue of impaired hippocampal synaptic function and cognition; mice given an uncleavable tau mutant are protected relative to mice given cleavable tau. In the locus coeruleus specifically, DOPEGAL-driven activation produced coerulean neurotoxicity and propagation of pathology to the forebrain.

The second cut. The same protease also cleaves the phosphatase inhibitor. Protein phosphatase 2A is the principal tau phosphatase, and it is restrained by an endogenous inhibitor, I2PP2A (also known as SET), which is safe while it remains nuclear and dangerous once it reaches the cytoplasm. Basurto-Islas and colleagues showed that asparaginyl endopeptidase cleaves I2PP2A

at asparagine-175 into N-terminal and C-terminal fragments, both of which bind the catalytic subunit of protein phosphatase 2A and inhibit it; that activated endopeptidase and I2PP2A translocate, respectively, from neuronal lysosomes and nucleus to the cytoplasm in the Alzheimer brain, where they interact and associate with hyperphosphorylated tau; and that inducing acidosis activates the endopeptidase, cleaves I2PP2A, inhibits the phosphatase and hyperphosphorylates tau, while knocking the endopeptidase down abolishes the pathway. The corresponding quantitative anchors are secure: the tau-directed activity of protein phosphatase 2A is reduced by roughly a third in the Alzheimer brain relative to aged controls, and its endogenous inhibitors are correspondingly up-regulated.

Why two crimes from one enzyme is worse than two enzymes with one each. Consider what the two cuts do together. The first attacks the substrate side, removing tau's function by truncation and yielding a self-templating seed. The second attacks the eraser side, silencing the phosphatase that would strip phosphate from the tau that has *not* been cut. A neuron subject only to the first might make some seed while keeping the rest of its tau clean by an active phosphatase; one subject only to the second might hyperphosphorylate without truncating into the most transmissible form. The coerulean neuron suffers both, from one activation, in fixed proportion — so there is no state of the cell in which one arm is spared. This is the mechanistic form of a clinical observation: the pretangle, once present, is stubborn, because it is not one lesion but a coordinated tipping of a two-sided balance by a single catalytic event.

Grade: Established for each component — the activation, the N368 cut and its causal role, the I2PP2A cleavage at N175 and phosphatase inhibition, and the measured fall in phosphatase activity. *Inference* for the joining: that in the DOPEGAL-activated coerulean neuron both cuts issue from one activation is a strong inference from the shared identity of the enzyme, not yet a single experiment measuring both substrates in the same coerulean cells.

5.5 The seed leaves

One further property closes the loop with the disease's natural history. The truncated species is not only aggregation-prone but *propagation*-prone, and the locus coeruleus, whose projections reach broadly across the forebrain, is built to broadcast it. The same δ-secretase activity that ignites tau in the coeruleus mediates the spread of tau pathology to the rest of the brain, and the surfaces that receive proteopathic tau seeds in recipient neurons — heparan sulfate proteoglycans — are the established gateway for transcellular propagation, as Holmes and colleagues demonstrated.

The nucleus that cuts its own tau into a seed becomes a source, exporting that seed along the very projections by which it modulates the forebrain. The stepwise anatomical progression of the numbered Braak stages, from brainstem to entorhinal cortex to neocortex, is on this reading not a mysterious spatial preference but the connectivity of the first affected cell.

Grade: Established for propagation-competence and for heparan-sulfate-mediated seed uptake; *Probable* for the identification of coerulean export as the operative route of early spread in humans.

5.6 The gap between the tangle and the death — where the window is

The most therapeutically consequential fact in this Part is a quantitative one, and it is easy to miss because it appears as a methodological result rather than a clinical one.

Theofilas and colleagues applied unbiased stereology to human brainstems across the full range of disease stages and measured both locus coeruleus volume and neuronal population. Two findings: as Braak stage increases by one unit, locus coeruleus volume decreases by 8.4 per cent; and *neuronal loss started only midway through the progression*. Age-related change spares the nucleus. The authors drew the correct conclusion — that the long gap between neurofibrillary accumulation and neuronal loss suggests a second trigger may be necessary to induce death — and proposed locus coeruleus volumetry as a candidate presymptomatic biomarker.

For this paper the finding does something more specific. It establishes that the nucleus spends *decades* in a state that is neither healthy nor dead: carrying truncated, hyperphosphorylated tau, shrinking measurably, exporting seed, and still populated by living neurons. That interval is the therapeutic window of the entire chain. Everything upstream of it — the ecology, the barrier, the partition, the afferent load — is modifiable during it. Everything downstream of it, including every drug in Part X, has been given after it closed.

Grade: Established for the stereological findings; *Inference* for the interpretation of the gap as the chain's therapeutic window.

5.7 The genetics point at the same place

Two lines of human genetics converge on this nucleus and its receptor system, and the convergence is worth stating precisely because it is often stated loosely.

ApoE4, the commonest risk allele, accelerates the coerulean self-poison directly, by the VMAT2 mechanism of §5.3. On the other side, the two most powerful protective variants yet identified in living people act on one shared receptor system. The APOE3-Christchurch homozygote described by Arboleda-Velasquez and colleagues resisted autosomal-dominant Alzheimer's disease for decades despite a very high amyloid burden, carrying a variant of ApoE itself; the Reelin-COLBOS heterozygote described by Lopera and colleagues showed comparable resilience, carrying a gain-of-function variant of reelin — the other principal ligand of the same lipoprotein receptors, ApoER2 and VLDLR, through which reelin restrains the tau kinase glycogen synthase kinase-3β, as Hiesberger and colleagues established.

Two individuals, two rare variants, one receptor system — one acting through its ApoE ligand, one through its reelin ligand — and both conferring extraordinary resistance.

The boundary must be marked honestly. That reelin and ApoE share receptors is structural and secure; that the two variants confer resilience is established in the reported cases; that ApoE4 inhibits VMAT2 to feed DOPEGAL is shown in model systems. That this axis modifies *the human coerulean ignition specifically*, as opposed to the disease at large, is a synthesis of these findings rather than a single measured result, and the resilience cases are individually rare.

Grade: Established for each finding separately; *Inference* for the convergence on the coerulean lesion.

Link	Claim	Grade
L8	The locus coeruleus bears the earliest tau pathology in the human brain, from the second and third decades	Established (Braak, n = 2,332)
L8	The nucleus lacks the aggrecan-based perineuronal net; net-bearing neurons resist tau	Established (anatomy); Probable (causal role)
L8	DOPEGAL is produced exclusively in noradrenergic neurons	Established
L8	ApoE4 inhibits VMAT2, increasing cytosolic norepinephrine and DOPEGAL	Established (model)
L8	Chronic firing drives over-reuptake and cytosolic norepinephrine (activity-dependent leak)	Inference — supporting record unverified, see §5.3
L9	DOPEGAL activates asparagine endopeptidase in the locus coeruleus	Established
L9	The protease cleaves tau at N368 into an aggregation- and propagation-prone seed; the cut is causal	Established
L9	The same protease cleaves I2PP2A/SET at N175, silencing protein phosphatase 2A	Established
L9	Both cuts issue from one activation in the same coerulean cells	Inference
—	Tau seed is exported along coerulean projections and taken up via heparan sulfate proteoglycans	Established (mechanism); Probable (in vivo route)
—	Locus coeruleus volume falls 8.4% per Braak stage; neuronal loss begins only midway	Established (Theofilas)
—	The tangle-to-death gap is the chain's therapeutic window	Inference

Link	Claim	Grade
—	The ApoE/reelin receptor axis modifies the coerulean ignition specifically	Inference

PART VI — The Coupled Pair

6.1 The handoff

The chain now has to cross from a noradrenergic nucleus to a serotonergic one, and the crossing is not metaphorical. The two nuclei are anatomically coupled, they fail in the same brains in a fixed order, and they share three of their four principal liabilities. This Part establishes the coupling, then does something the habitual pairing of the two nuclei has obscured: it separates them on the one liability they do *not* share, and shows that the separation is what identifies the shared cause.

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

The consequence for the chain is a second, independent route by which serotonergic tone falls. Coeruleus degeneration does not merely subtract noradrenaline; it withdraws excitatory drive from the raphe, reducing serotonergic output by a mechanism entirely separate from raphe pathology. A patient with substantial coerulean loss has less serotonin than their raphe cell count alone would predict. The two lesions compound, and 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.

6.2 What the raphe is

The serotonergic system was mapped before it was understood. Dahlström and Fuxe, using formaldehyde-condensation histofluorescence 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, and the division it implies has held up under every subsequent method.

The **caudal group** — raphe pallidus, obscurus and magnus, in the medulla — projects predominantly downward, to the spinal cord and lower brainstem, serving autonomic and antinociceptive functions: descending modulation of dorsal-horn pain transmission, sympathetic outflow, thermoregulation, and chemosensory drive to breathe. The **rostral group** — median and

dorsal raphe and the supralemniscal group, in pons and midbrain — projects upward, supplying essentially the whole forebrain.

That division matters and is regularly lost when the literature refers to "the raphe" without qualification. The disease does not treat the two groups alike: essentially everything below concerns the rostral group, and predominantly the dorsal raphe nucleus. What is *spared* turns out to be as informative as what is lost, and §6.6 returns to it.

The dorsal raphe is itself not a unit. Human and rodent anatomies agree in resolving it into subnuclei — dorsal, ventral, ventrolateral, interfascicular, caudal — differing in projection target, electrophysiology, co-transmitter content and, as §7.2 shows, 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 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.

6.3 The cell, and why it is built like the coerulean one

Three properties of the dorsal raphe serotonergic neuron do most of the work in this Part, and each is the property §5.2 identified in the locus coeruleus.

It is a pacemaker. Serotonergic raphe neurons fire tonically and autonomously at low frequency, in a slow, regular rhythm that persists in slice preparations after all synaptic input is removed. The firing is intrinsic, generated by a pacemaker conductance, and 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 — 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.

It has an enormous arbor. 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 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. But it is not a delicate one: the human dorsal raphe contains on the order of a few hundred thousand serotonergic neurons and innervates a forebrain three orders of magnitude larger in volume than the rat's, so whatever the true figure, the ratio of axon to soma in a human serotonergic neuron is larger than in the rat, not smaller.

It broadcasts by volume, not by wire. Most serotonin release in the forebrain is non-junctional: the varicosity has no directly apposed postsynaptic partner, and the transmitter

diffuses through extracellular space to reach receptors at a distance. Two implications recur through the rest of this paper. The system has no spatial precision to lose — it was never delivering a targeted message, so its degradation produces not a focal deficit but a diffuse change in the operating point of everything within reach. And the relation between surviving neuron number and transmitter concentration at a receptor is neither linear nor local: a partially denervated cortex is not a cortex with holes in its innervation but a cortex with a lower ambient concentration everywhere, which compensatory sprouting can hold up for a long time before it falls.

A substantial minority of dorsal raphe neurons are not purely serotonergic: they co-express the vesicular glutamate transporter VGLUT3 and release glutamate alongside serotonin, and these dual-transmitter cells are more excitable than their purely serotonergic neighbours. Whether that makes them more vulnerable is taken up in §6.5 and graded as speculation.

6.4 The chemistry the two nuclei do not share

Here the habitual pairing breaks down, and the break is the most informative single observation in this Part.

The most developed cell-autonomous account of why the locus coeruleus tangles first is the chemical one of §5.3: the neuron is full of catecholamine; monoamine oxidase A oxidises it to a reactive aldehyde; the aldehyde activates a protease that cuts tau into a seed and silences tau's phosphatase. The account is attractive because it is specific — it explains not merely that the cell 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. Westlund and colleagues, mapping MAO-A- and MAO-B-containing cell populations immunohistochemically in primate brain, localised MAO-A to the catecholaminergic groups — locus coeruleus and substantia nigra among them — and MAO-B to the raphe, where virtually all serotonin-positive somata were MAO-B-positive. Saura Martí and colleagues confirmed the assignment by in situ hybridisation in human brain and 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 compartmentalisation compounds the point: the MAO-B the serotonergic neuron carries is confined largely to the somatic compartment, its mitochondria not trafficked 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 consequences follow, and the third is the load-bearing conclusion of this Part.

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.

This plausibly explains the ordinal gap. If the coeruleus carries an additional, cell-specific chemical liability the raphe does not, the coeruleus should tangle earlier and faster despite their shared architecture — which is what the stereology of §7.2 shows. *Grade: Inference.* The observations are established; the causal attribution of the gap to the enzyme difference is a reading offered here, and §12.2 gives the experiment that would test it.

The shared cause must therefore be something other than chemistry. Two nuclei with different transmitters, different oxidative chemistry 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, the absent aggrecan net. The chemistry modulates the timing; the architecture sets the vulnerability.

This conclusion has a therapeutic corollary that Part X develops and that is worth flagging now: interventions aimed at the transmitter or its receptors are aimed at the modulating variable, not the causal one.

Grade: Established for the enzyme mapping in primate and human; *Inference* for both the gap attribution and the architectural conclusion.

6.5 The raphe's own liability that the coeruleus does not share

Symmetry requires the converse. One liability is specific to the serotonergic cell, and it is the one this paper's chain has already built.

Serotonin is synthesised from tryptophan in two steps, the first catalysed by tryptophan hydroxylase 2, whose substrate sensitivity was the subject of §3.4. The raphe neuron is therefore the one cell in this Part whose function depends on an imported essential amino acid whose supply is set, three synapses upstream, by an ecology outside the body and by an inflammatory enzyme that ecology induces.

This is where Part III and Part VI meet, and the meeting produces the paper's first distinctive claim. **The forebrain loses serotonergic tone by two mechanically independent routes.** One is *structural*: the cells that make the transmitter accumulate tau, lose their arbors, and eventually die, for reasons of architecture. The other is *substrate*: the cells that survive are asked to synthesise the transmitter from a pool that inflammatory diversion has thinned at the rate-limiting step. Neither route requires the other. A person could in principle have an intact raphe and a starved partition, or an unstarved partition and a decimated raphe, and the resulting serotonergic tone would be

depressed in both cases by different mechanisms with different reversibilities.

The clinical significance is that the two routes have opposite therapeutic profiles. The structural route is, past a point, irreversible: dead aminergic neurons are not replaced. The substrate route is reversible on the timescale of weeks, because it is a matter of enzyme induction and amino-acid flux rather than of cell number. Any account which collapses the two into a single quantity called "serotonergic deficit" therefore mis-estimates both the prognosis and the drug response — a point §10.5 turns into the paper's central pharmacological argument.

A more speculative raphe-specific liability concerns the VGLUT3 dual-transmitter subpopulation. It has been suggested that their greater excitability promotes both tau accumulation and activity-dependent tau release along the axon, making them the vanguard of the nucleus's degeneration and a candidate identity for the vulnerable subnucleus that §7.2 implies. The suggestion is coherent and fits the general association between neuronal activity and tau release. 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.*

Grade: Established for the substrate dependency; *Inference* for the two-route claim, which is this paper's own and is defended rather than cited.

6.6 What the sparing shows

A striking feature of the raphe lesion is how much of the raphe it leaves alone, and the sparing is diagnostic in two ways.

The caudal group — raphe magnus, pallidus and obscurus, projecting to 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 a global serotonergic lesion would cause. Patients with advanced disease and forty per cent dorsal raphe loss have intact nociceptive modulation.

It confirms, first, 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 precisely the property §6.3 identified: arbor size. The forebrain-projecting neuron supports a far larger and more distributed territory than the spinally projecting one, and it is the forebrain-projecting neuron that fails.

It **bounds the clinical picture**, second. The absence of caudal signs is why 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 as the dorsal raphe has, and the absence of caudal signs is an argument from clinical observation rather than from tissue. §12.2 lists the count that would settle it — and it is, notably, the single cheapest experiment in this paper.

Link	Claim	Grade
L10	The locus coeruleus supplies α1 excitatory drive to the dorsal raphe; the pair is reciprocally coupled	Established
L10	Coerulean degeneration subtracts serotonergic output independently of raphe pathology	Inference (unquantified in humans)
L10	Raphe and coeruleus share arbor, pacemaking, and absence of the aggrecan net	Established
L10	Locus coeruleus neurons express MAO-A; raphe serotonergic neurons express MAO-B, not vice versa	Established (Westlund; Saura Martí)
L10	The MAO difference partly explains the ordinal gap between the two nuclei	Inference
L10	The shared cause of vulnerability is architectural, not chemical	Inference
—	Serotonergic tone is lost by two independent routes: cell loss and substrate limitation	Inference (this paper's claim)
—	VGLUT3 dual-transmitter neurons are the vulnerable subpopulation	Speculative
—	The caudal raphe group is relatively spared, hence no examinable neurological signs	Probable

PART VII — The Seam Gives Way

7.1 A word about the name

Raphe is the Greek for 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 lie 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 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.

7.2 The order of failure

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.

The staging. In Braak's survey of 2,332 brains, pretangle stage c is the stage at which abnormal tau appears in "other subcortical neuromodulatory cell groups" beyond the locus coeruleus, the serotonergic raphe among them. Two facts follow and should be held apart. The raphe is involved *before the cortex*: stage c is complete before stage 1a begins, and fifty-eight brains in the series carried subcortical tau with no abnormal cortical tau at all. And 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 — a small one, but it obscures an ordinal fact that the quantitative work sharpens.

The transentorhinal comparison. Grinberg and colleagues asked 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, with no transentorhinal involvement whatsoever.

This is the single most direct 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 the numbered 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 result also introduces the subnuclear qualification: the changes were concentrated in a subnucleus, so the disease does not attack "the dorsal raphe" but part of it — and which part is unresolved in living people because no available tracer resolves dorsal raphe subnuclei in vivo.

The quantitative anchor. 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 nuclei.

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 coerulean neurons and one in thirty-eight dorsal raphe neurons already carry the lesion. But the ratio is the point. At the earliest countable moment the coeruleus carries about three times the raphe's burden. The two nuclei are not simultaneous. The coeruleus leads and the raphe follows at a measurable distance — exactly the ordering that §6.1's α1 coupling and §6.4's chemical asymmetry jointly predict.

The non-demented and living-brain evidence. Pierson and colleagues examined the dorsal raphe in individuals aged twenty-five to eighty with no known history of dementia and found tau pathology at a prevalence comparable to that in the locus coeruleus, with α-synuclein, β-amyloid and TDP-43 substantially less often present in the same tissue. The specificity matters: what accumulates in the raphe of middle-aged people without dementia is a tauopathy, not a general marker of brainstem ageing or of mixed pathology. In living brains the evidence is molecular-imaging evidence and is weaker: 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; a later study from the same group replicated the transporter loss, established its co-occurrence with elevated cortical β-amyloid, and again found limbic reductions correlated with memory and with semantic fluency. Kepe and colleagues, using [^{18}F]MPPF, reported slight reduction of hippocampal 5-HT1A binding in amnestic mild cognitive impairment and marked reduction in Alzheimer's disease.

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

Grade: Established for the staging order, the Braak 0 comparison, and the non-demented prevalence; *Probable* for the in vivo imaging findings, which come from small cross-sectional cohorts.

7.3 The magnitude

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.

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.

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 should be separated.

The loss is **large in absolute terms**. An effect size of 1.79 standard deviations 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 loss is **third, not first**. The cholinergic nucleus basalis and the noradrenergic locus coeruleus are both more severely depleted. Any account treating serotonin as *the* lost transmitter of Alzheimer's disease overstates a real finding, and this paper does not make that claim: the serotonergic lesion is important here because of *what it withdraws* (Part VIII), not because it is the largest.

The loss is **three times the substantia nigra's**. This comparison gives the number its force, because the 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 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.

Its confidence interval is correspondingly the widest of the four.

Grade: Established for the direction and approximate magnitude; *Probable* for the precise ordinal placement relative to the locus coeruleus.

7.4 The projection goes before the cell

A pattern runs through the imaging data of §7.2 that deserves stating as a claim in its own right, because it is what makes the whole chain's timing coherent: in the aminergic systems, the projection degenerates before the cell body dies.

The evidence is indirect but consistent, and it converges from two directions. Serotonin transporter binding, which marks axon terminals rather than 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 §7.3 apply. 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. On the noradrenergic side the same pattern is measured directly rather than inferred: Theofilas and colleagues found locus coeruleus volume falling 8.4 per cent per Braak stage while neuronal loss began only midway through the progression (§5.6).

The mechanistic reading follows from §6.3. 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 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 mild cognitive impairment" and "forty per cent of the neurons are gone at death." They are sequential observations of one process.

This matters to the argument in a specific way. The functional variable this paper cares about — ambient serotonin concentration in the cortical mantle — is set by *terminals*, not by somata. It therefore begins to fall long before any cell count would register, which is why the withdrawal of Part VIII can be underway for decades while the nucleus still looks populated.

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 one precedes the other rests on comparing different cohorts measured by different methods.

7.5 Why fifty years of this produce no symptom

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 combine to produce the silence, and they are the same three that make the lesion so hard to catch.

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 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 neurons can be lost while concentration at the receptor is held near normal. The compensation is not free — it raises the metabolic and oxidative load on exactly the cells already failing, which is a plausible route by which early loss accelerates later loss, and which in the coerulean case (§5.3) is directly self-poisoning — but it is effective, and it is why the deficit stays subclinical so long.

The functions affected have no sharp threshold. Slightly worse sleep consolidation, slightly reduced cognitive flexibility, a modest downward shift in mood, subtly impaired pattern separation: 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.

7.6 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 here rather than left to a critic.

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.

The caveat cuts in a specific direction and it is worth being precise about which. It weakens any claim that early brainstem tau is a *sufficient* cause. It does not weaken the claim that the lesion is early, nor that it is severe in established disease, nor any of the functional arguments of Part VIII, which depend on the loss of serotonergic tone and not on the mechanism that produced the loss. And it sharpens rather than blunts this paper's chain, because the chain proposes exactly the kind of second variable — cumulative afferent load, sustained partition diversion — that would determine which carriers of early brainstem tau progress and which do not.

Link	Claim	Grade
L11	Abnormal tau appears in the raphe at pretangle stage c, before any cortical involvement	Established (Braak, n = 2,332)
L11	Phospho-tau is present in a dorsal raphe subnucleus in >20% of Braak 0 brains	Established (Grinberg)
L11	At Braak 0, 2.6% of dorsal raphe and 7.9% of locus coeruleus neurons are tangled	Established (Ehrenberg, unbiased stereology)
L11	The raphe follows the locus coeruleus rather than accompanying it	Established (concordant)
L11	Raphe tau is present in non-demented adults aged 25–80; α-synuclein, Aβ and TDP-43 are rarer	Established (Pierson)
L11	Dorsal raphe neuronal loss in AD is $d \approx 1.79$, ~40% of the nucleus	Established (Lyness, 67 studies)
L11	Serotonin transporter loss is present in cortex and limbic system at the MCI stage	Probable
—	The terminal field degenerates before the soma dies	Probable
—	Volume transmission plus compensation explains fifty years of clinical silence	Established (mechanisms); Inference (the conjunction)
—	Early raphe tau in an individual predicts eventual dementia	Not established

PART VIII — What Is Withdrawn

8.0 How to read this Part

The costs of serotonergic withdrawal are set out under seven headings, ordered deliberately from least to most familiar, because the least familiar is the most consequential for this chain 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; it does not establish that the removal is quantitatively important relative to everything else going wrong, and in two cases the animal models disagree with each other. Where they do, the disagreement is stated rather than smoothed.

One further caution is specific to this paper. Everything in this Part concerns the *tone* — the ambient concentration of transmitter at receptors across the cortical mantle. Part VI established that this tone is set by two independent variables, the number of surviving terminals and the availability of substrate. The costs below follow from a fall in the tone and are agnostic as to which variable produced it. That agnosticism is not a weakness of the argument; it is the reason the argument has a therapeutic consequence, because only one of the two variables is reversible in an adult.

8.1 The brake on amyloid

The single most consequential thing the serotonergic system 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 belongs to the transmitter rather than to the drug. 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 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 peptide before it exists and releases the neurotrophic soluble ectodomain sAPPα instead. The dominant α-secretase in brain is ADAM10. Cochet and colleagues showed that 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. Consistently, Tesseur and colleagues found that chronic 5-HT4 receptor activation decreases amyloid-β production and deposition in transgenic mice, and Giannoni and colleagues found that 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 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, with its *terminals* thinning earlier still. 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 serotonergic lesion **permissive**. Not causal in the sense of initiating an amyloid cascade — nothing here shows that losing serotonin is sufficient to produce Alzheimer's disease, and the TPH2 knockouts raise 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.

Note also the shape this predicts and that §10.5 will use. A brake on *production* has its largest effect on *accumulation rate*, early, when there is production to brake; it has a diminishing effect on standing burden once deposition has saturated. That asymmetry is the pharmacological signature the chain expects, and it is the reason the same drug can move a production-rate endpoint in a healthy forty-year-old and do nothing for a cognitive score in an eighty-year-old.

Grade: Established for the serotonin–amyloid relationship in mice, for the human production-rate result, and 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.

8.2 The restraint on tau — and a contradiction

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 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 reuptake-inhibitor use. Alzheimer's patients taking these drugs had a lower plasma concentration of phosphorylated tau 181 than untreated patients. The imaging arm found hypometabolism in the dorsal raphe of untreated 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 use may reduce the pathological presentation of the disease but has variable effects on cognitive performance.

The contradiction. Sections 8.1 and 8.2 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 both may 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 *which* pathology it worsens depends on the model, and that no single mechanism currently accounts

for both results. §12.2 gives the experiment that would resolve it, and it has not been run.

The Terstege data carry their own severe limitations, which the authors state: the design is cross-sectional, so causality cannot be established; drug 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 antidepressants 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.

8.3 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; that factor 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 loss of that capacity. Moreno-Jiménez and colleagues reported that adult hippocampal neurogenesis is abundant in neurologically healthy human subjects and drops sharply in Alzheimer's disease, and Boldrini and colleagues reported that human hippocampal neurogenesis persists throughout ageing.

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. 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. And 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.

8.4 Sleep, and the melatonin ceiling

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 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 same upstream failure thus 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, coerulean 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.

There is, however, one feature of the sleep arm that is specific to this paper's chain rather than to the raphe alone, and it is a feedback loop. Degraded sleep worsens diet, stress physiology and circadian entrainment; each of those degrades the intestinal ecology of Part II; the degraded ecology raises the inflammatory tone that drives the partition of Part III and the afferent load of Part IV. The chain closes through behaviour, not only through the body — which is why its time constant is decades and why its progression is self-reinforcing rather than merely cumulative.

Grade: Established for the raphe's wake-active firing and for serotonin as melatonin's precursor; *Inference* for the raphe's specific contribution to sleep disruption in Alzheimer's disease; *Speculative* for the behavioural feedback loop as a quantitatively significant driver.

8.5 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.

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.

This item also closes a loop with Part II, and the loop is worth marking because it is the chain's only genuine circuit. Erny and colleagues established that the gut microbiota continuously control microglial maturation and function; Heneka and colleagues established that noradrenaline from the locus coeruleus modulates microglial function and thereby controls pathology in a model of the disease. The microglion is therefore under simultaneous control from the ecology (Part II), from the noradrenergic nucleus (Part V), and from the serotonergic one (here) — three arms of the same chain converging on one cell, all of them withdrawing restraint in the same direction over the same decades.

The caveats here are heavier than elsewhere in this Part. 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; *Established* for microbial and noradrenergic control of microglial state; *Speculative* for the claim that raphe degeneration disinhibits synaptic pruning in human Alzheimer's disease.

8.6 Cognition, directly

Setting mechanism aside, 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 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 mild cognitive impairment 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.

8.7 What is *not* withdrawn, and why it matters

Two negatives bound this Part, and both are load-bearing for the argument's honesty.

The amnestic syndrome is not produced by this lesion. Pierson and colleagues overexpressed human P301L tau selectively in the mouse dorsal raphe and produced depressive-like behaviour and hyperactivity — and *no* 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. Whatever accounts for the memory disorder that defines Alzheimer's disease clinically, a raphe tauopathy on its own is not it. The serotonergic lesion in this chain is a *permissive* and *trophic* lesion, not the lesion that produces the presenting complaint.

The caudal functions are intact. As §6.6 established, descending pain modulation, thermoregulation and respiratory chemosensitivity are preserved. The system loses what it broadcasts to the forebrain and keeps what it sends to the cord.

8.8 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
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
Serotonergic tone at limbic and cortical targets	An affective/psychomotor phenotype — *not* the amnestic syndrome	Established (both limits)
— (caudal group spared)	No pain, thermoregulatory or respiratory signs	Probable

PART IX — The First Reading

9.1 Why the chain has to pass through psychiatry

Everything to this point has been silent. The ecology drifts without symptom; the barrier fails without symptom; the partition shifts without symptom; the afferent load rises without symptom; the brainstem nuclei tangle, shed terminals and shrink without symptom. If the chain produced nothing legible until the memory failed, it would be a mechanism with no clinical entry point and no way to identify the people in whom it is running.

But it is not silent. The chain's outputs in its first three decades are exactly the phenomenology of a mood and arousal disorder: low serotonergic tone from a substrate-limited synthesis and a thinning projection; a noradrenergic nucleus biased toward the high-tonic, stress-associated firing mode; a raised inflammatory tone acting centrally; and degraded sleep. That is a recognisable clinical picture, and it has a name.

This Part therefore takes up the depressive phenotype as the chain's first legible reading — and then confronts, at length and without softening, the single human post-mortem result that most sharply constrains the move.

9.2 Sickness behaviour: the chain run fast

The one phenomenon in which the microbiome, psychiatric and neuroimmunological literatures already speak a single language is *sickness behaviour*: the coordinated response to peripheral immune activation comprising anhedonia, fatigue, altered sleep, appetite change, social withdrawal, psychomotor slowing and cognitive impairment.

Dantzer and colleagues set out the canonical pathway: peripheral inflammation elevates circulating cytokines, which signal to the brain through cytokine transport, vagal afferents, perivascular cells and the leaky circumventricular regions, producing both the behavioural syndrome and a downstream indoleamine 2,3-dioxygenase-driven kynurenine shift. Read against Parts II to IV, that pathway is not analogous to this chain; it is the same pathway. The cytokines of sickness behaviour are the cytokines of the gut's inflammatory export. The vagal afferents that carry the sickness signal are the afferents of §4.2, and the vagotomy experiments that established their necessity are the same experiments. The kynurenine shift that sickness behaviour induces is the shift of §3.2.

Sickness behaviour is therefore the chain run fast: what a bolus of endotoxin produces in hours, a lifelong dysbiotic drift produces in decades, and the phenomenology is continuous between them. This is not a rhetorical parallel. It is an argument that an acute, experimentally inducible, mechanistically resolved syndrome is the short-timescale version of the chain's early clinical output — which means the chain's early output has already been demonstrated in humans, under experimental control, at a compressed dose.

Grade: Established for the sickness-behaviour pathway and for its route identity with this chain's Parts III and IV; *Inference* for the claim that the chronic version is the same process at a different rate.

9.3 The transfer experiments

The strongest causal evidence in Part II was the gut-to-brain propagation experiment: pathological α-synuclein injected into the mouse gut ascends the vagus, reproduces the caudo-rostral spread, and is blocked by truncal vagotomy — a defined seed, a defined route, a defined brain pathology, a defined interruption.

The depression literature contains the structural twin of that experiment in the mood direction. Zheng and colleagues transplanted a microbiota from patients with major depressive disorder into germ-free mice and induced depression-like behaviours that a healthy-donor microbiota did not, through a pathway mediated by host carbohydrate and amino-acid metabolism. Kelly and colleagues transplanted a depressed-patient microbiota into microbiota-depleted rats and induced anhedonia, anxiety-like behaviour and altered tryptophan metabolism. The logical structure is identical: a defined ecology, moved into a naïve host, carrying its brain phenotype with it.

The juxtaposition is the point. The gut compartment can transmit, by transplantation, *both* a proteinopathy and a mood — two phenotypes, both carried by the same organ, both demonstrated in the cleanest available causal system. On the human side, Valles-Colomer and colleagues characterised the neuroactive potential of the gut microbiota in a large population cohort and reported consistent associations of specific taxa with quality of life and with depression, including depletion of butyrate producers in depression.

What has *not* been done is the experiment that would join the two: no study has transplanted a depressed-patient microbiome and measured a locus coeruleus or tau-adjacent readout. That is P1 in §12.1, and its absence is the single largest gap in this Part.

Grade: Established for each transfer separately; *not established* for the joint transfer that the chain predicts.

9.4 The result that constrains this Part — and it is a hard one

Here the argument must confront the human post-mortem finding that cuts against the most natural version of it. The finding is routinely mis-cited, so it is set out 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 study was built to test a hypothesis, and the hypothesis failed. The authors 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 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 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.

Taken together these form a dissociation of an unusually clean kind. The raphe cell-count 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 *not* explained by dorsal raphe cell loss, and dorsal raphe cell loss in Alzheimer's disease is not a marker of depression.

This is a serious problem for any account that treats the depressive phenotype as the clinical face of the raphe lesion, and this paper does not attempt to dissolve it by re-describing it.

Grade: Established, with the caveat that group sizes are small (7–14 per cell) and the study has not been replicated with modern unbiased stereology. §12.2 lists that replication first among the experiments, because it is the result on which most of this Part turns.

9.5 The reconciliation the chain offers, and its status as inference

The chain does offer a reconciliation, and it should be read as the paper's own reasoning rather than as a reported finding.

Recall the two-route claim of §6.5: forebrain serotonergic tone is set by two independent variables — the number of surviving terminals, and the availability of substrate at the rate-limiting enzyme. Hendricksen measured neither. The study counted *somata* and scored neuritic pathology in the nucleus. It is a measurement of the structural variable, at the end of life, in a small sample.

On the chain's account, the depressive reading is a *tone* phenomenon and is produced predominantly by the second variable plus the noradrenergic and inflammatory arms — substrate-limited synthesis under IDO induction (§3.4), a locus coeruleus biased toward high tonic firing with degraded phasic responsiveness (§4.5), and central inflammatory signalling acting directly (§9.2). None of those is a cell count. All of them are reversible on the timescale of weeks to months, which is precisely why depression is a treatable illness and dementia is not. So the two findings can both be true: the raphe cell-count lesion can be present in Alzheimer's disease irrespective of mood, while the depressive reading is generated upstream by variables the cell count does not index.

Two independent results support the split rather than merely permitting it.

The first is the mouse experiment already cited: overexpressing P301L tau selectively in the dorsal raphe produced depressive-like behaviour and hyperactivity without spatial memory deficits. A focal, high-expression tau lesion in an otherwise healthy animal is *sufficient* to produce an affective phenotype. That does not contradict Hendricksen; it shows what the lesion is capable of producing in isolation, whereas Hendricksen shows that in a brain undergoing widespread additional pathology, variation in the lesion does not track variation in mood among patients who differ in a hundred other ways.

The second is that the primary-depression group in Hendricksen's series had *no* raphe cell loss at all. If the depressive phenotype in late life were a cell-loss phenomenon, that group should have shown it. That it did not is evidence that the depressive phenotype is generated by something other than aminergic cell death — which is exactly the chain's claim.

The honest statement of what survives is therefore narrower than the psychiatric literature usually asserts and wider than Hendricksen alone would allow. **Depression is not the symptom of the raphe lesion. It is the first legible reading of the upstream state that will, decades later, also have produced the raphe lesion.** That is a claim about shared causation, not about a symptom-lesion correspondence, and it is graded accordingly.

Grade: Inference. The component observations are established; the reconciliation is this paper's reasoning and its central falsifier is stated in §12.3.

9.6 The reversibility gradient, and why the window is here

If the chain is right, the interval in which the depressive reading appears has a property no later interval has: it is the point at which the largest number of the chain's variables are still reversible.

Consider each in turn. The intestinal ecology turns over on a timescale of weeks, is reachable through the lumen rather than across the blood–brain barrier, and can be re-seeded at essentially any point, because bacteria are replaceable in a way neurons are not. The barrier repairs when its fuel returns. The tryptophan partition normalises when the induction that shifted it subsides, since it is a matter of enzyme expression and amino-acid flux. The locus coeruleus, at this stage, is carrying tau and shrinking — but its neurons are still alive, because neuronal loss begins only midway through the Braak progression. The raphe is shedding terminals, which are in principle regrowable, before it is losing somata, which are not. And the amyloid brake of §8.1 is a brake on *production*, so it retains leverage precisely while production is still the dominant term.

Past that interval the gradient turns. Terminals become somata; somata become gaps; a brake on production has less and less to brake; and the compensation that held the tone up (§7.5) is itself exhausted, having accelerated the loss it concealed.

This is the reason the paper is organised around a fuse rather than a switch, and it is the reason Part X reads the trial record as a statement about timing rather than about targets. The therapeutic window of the whole chain is the window in which the chain's first clinical reading appears — and that reading has been treated, for forty years, by a specialty that had no reason to measure any of the variables above.

Grade: Established for the reversibility of the ecological and metabolic variables; *Established* for the tangle-to-death gap in the locus coeruleus; *Inference* for the claim that these coincide in one clinically identifiable window.

9.7 What this Part does not claim

Three disclaimers, because this is the Part of the paper most liable to overreach.

It is not claimed that depression *is* early Alzheimer's disease. Most depression is not, most depressed people do not develop dementia, and any framing that implies otherwise is both wrong and harmful. The claim is that a subset — plausibly the inflammatory, kynurenine-shifted, treatment-resistant subset — shares upstream variables with the chain, and that this subset is identifiable by measurement rather than by phenotype.

It is not claimed that treating depression prevents dementia. That is P4 in §12.1 and it is untested; the closest existing evidence is the duration effect of §10.4, which is observational.

And it is not claimed that the raphe lesion causes the mood disorder. §9.4 refutes that, and the refutation stands.

Link	Claim	Grade
—	Sickness behaviour runs the same route (cytokines, vagus, IDO shift) as this chain	Established
—	A depression-associated microbiota transfers a depressive phenotype and altered tryptophan metabolism to naïve hosts	Established (rodent)
—	Gut taxa associate with depression and quality of life in a large human cohort	Probable
—	The raphe cell-count lesion is present in AD irrespective of depression, and absent in primary late-life depression	Established (Hendricksen)
—	Focal dorsal raphe tau is sufficient to produce an affective phenotype without memory deficit	Established (mouse)
—	Depression is the first legible reading of the upstream state, not the symptom of the raphe lesion	Inference (this paper's claim)
—	The chain's reversible variables and its first clinical reading coincide in one window	Inference
—	Treating the depressive window alters the dementia trajectory	Not established

PART X — The Prescription

10.1 Why this Part is mostly negative

Everything in Part VIII 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 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 is the subject of §10.5.

10.2 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 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 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 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 definitive refutation as clinical pharmacology produces, and any argument about serotonin in Alzheimer's disease has to accommodate it rather than step around it.

10.3 The reuptake inhibitors: three questions, three answers

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 has 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 of §8.1 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 §8.1, 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 was never claimed to be.

As a disease-modifying therapy, the effect is unproven — but not null, and its shape is peculiar. That shape is §10.4.

10.4 The duration asymmetry

The most informative single result in this Part is not a randomised trial, and it has attracted far less attention than the trials that failed.

Bartels and colleagues analysed 755 currently non-depressed participants in the Alzheimer's Disease Neuroimaging Initiative, using survival analysis with ApoE4 status and age as covariates. Among patients with mild cognitive impairment *and a history of depression*, treatment with a selective serotonin reuptake inhibitor for **more than four years** was significantly associated with delayed progression to Alzheimer's dementia — by approximately three years — compared with short-term treatment with the same drug class, with treatment by other antidepressants, and with no treatment; and compared also with mild cognitive impairment patients without a history of depression. **No**

differences in cerebrospinal-fluid biomarker levels were observed between treatment groups.

Four features of that result deserve separate attention, because their conjunction is what makes it discriminating.

It is a duration effect, not a treatment effect. Short-term exposure to the same drug did nothing. The variable that carried the association was time on drug, with a threshold in the region of four years.

It is class-specific. Other antidepressants did not show it. Whatever produced the effect was not "being treated for depression."

It is a trajectory effect without a biomarker effect. The cerebrospinal-fluid measures did not separate the groups. A cross-sectional biomarker did not move while a longitudinal outcome did.

It is observational. It is a post-hoc analysis of an observational cohort, in a subgroup defined by treatment history, with all the confounding by indication that implies. People who remain on an antidepressant for four years differ from those who do not — in adherence, in health-system contact, in illness severity, in comorbidity. This result cannot establish causation and the authors do not claim it does.

Now ask which model predicts that shape.

A **symptomatic** model — the drug lifts mood or arousal and thereby improves test performance — predicts a fast effect that appears within weeks, does not require years of exposure, and disappears on withdrawal. That is not what was observed.

A **classical disease-modifying** model — the drug reduces the pathology — predicts that the biomarkers should move. They did not.

A **permissive-brake** model — the drug slightly restores a tonic bias in amyloid precursor protein processing, acting on production rate rather than standing burden — predicts precisely what was observed. A small, continuous shift in a *rate* integrates into a meaningful difference in *trajectory* only over years; it should therefore show a duration threshold. And because it acts on the rate of accumulation rather than on the standing pool, it should be nearly invisible to a single cross-sectional biomarker measurement, whose value is dominated by everything that has already accumulated. The Sheline result gives the mechanism a human anchor and a magnitude — a 37 per cent slowing of production — and the arithmetic of a 37 per cent rate change compounding over four years against a fifty-year accumulation is exactly the arithmetic of a three-year delay in crossing a threshold.

Grade: Established for the Bartels findings as reported; *Inference* for the reading of their shape as the permissive-brake signature, which is this paper's argument and not the authors'.

10.5 Why the trials failed: three reasons, of which the third is new

The chain supplies three explanations for the negative record. They are not equally strong and they are ranked here by how much weight they can carry.

Reason one: the trials came fifty years late. This is the reason the staging evidence forces and it is close to arithmetic. If the coeruleus tangles from the second decade, the raphe from the third, the terminals thin through the fourth and fifth, and forty per cent of the nucleus is gone by the time of diagnosis, then a trial enrolling patients with established mild-to-moderate dementia is intervening at the end of a process that began before the patients finished their education. The 5-HT4/ADAM10 mechanism of §8.1 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. Every trial in §10.2 and the first arm of §10.3 enrolled after the window of §9.6 had closed.

Reason two: the pharmacology addressed the signal, not the cell. Part VI concluded that the aminergic nuclei fail for architectural reasons — arbor, pacemaker, missing net — and that transmitter chemistry modulates the timing rather than causing the 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. This reason is an inference from this paper's own argument and should carry the least weight of the three.

Reason three: reuptake inhibition acts on the wrong side of the rate-limiting step. This is the reason the chain adds, and it is the paper's central pharmacological claim.

A selective serotonin reuptake inhibitor blocks the serotonin transporter. What that accomplishes is to prolong the residence of serotonin *already released* in the synaptic and extrasynaptic space — it redistributes a molecule that has already been synthesised. It does not increase synthesis. Synthesis is set by tryptophan hydroxylase 2, whose Michaelis constant sits close to the ambient brain tryptophan concentration (§3.4), and whose substrate has been diverted at source by three mechanisms under the control of the intestinal ecology (§3.3): direct microbial consumption, inflammatory induction of indoleamine 2,3-dioxygenase, and the transferable partition shift itself.

Put those together and the consequence is a conditional efficacy that no trial has ever measured. In a patient whose partition is intact, reuptake inhibition amplifies a signal that is being adequately produced, and the drug does what its pharmacology says. In a patient whose partition has been shunted — high plasma kynurenine-to-tryptophan ratio, chronic inflammatory tone, decades of dysbiotic drift — the same drug amplifies a signal that is not being adequately produced, and the amplification has proportionately less to work with. **Reuptake inhibition cannot rescue a neurotransmitter the brain can no longer adequately synthesise.**

Three consequences follow, and each is testable.

The effect size should be heterogeneous in a lawful way. Trials of serotonergic augmentation should show larger effects in patients with a low kynurenine-to-tryptophan ratio and smaller effects in those with a high one. Since no trial has stratified on this variable, the observed heterogeneity has been treated as noise. On this account it is signal, and it is the largest untested source of variance in the class.

The comparator has been wrong. The trial that has never been run is not "reuptake inhibitor versus placebo." It is "reuptake inhibitor versus reuptake inhibitor plus partition restoration" — the second arm supplying, by whatever route, the substrate the first arm assumes. That could be attempted upstream (fibre and butyrate restoration, barrier repair, ecological replacement), at the enzyme (kynurenine 3-monooxygenase inhibition, IDO modulation), or crudely at the substrate itself.

A direct agonist should outperform a reuptake inhibitor, and by a specific margin. A 5-HT4 agonist acts on the receptor that traffics α-secretase without depending on residual release from a degenerating projection or on adequate synthesis from a diverted pool. On this chain the advantage of a direct agonist over a reuptake inhibitor should be *larger* in exactly the patients in whom the reuptake inhibitor underperforms — the substrate-limited ones. That is a crossed prediction, and crossed predictions are the useful kind.

Grade: Established for the enzymology on which the argument rests; *Inference* for the pharmacological conclusion, which is this paper's own and is stated as P7 and P8 in §12.1.

10.6 The counter-arguments, and how far they go

Three objections to §10.5 are strong enough to state, and one of them is not fully answerable.

"The trials were mistimed" is a defence available to any failed hypothesis. It is, and the defence is worthless unless it specifies what evidence would defeat it. §12.3 does so, and the principal falsifier is a properly timed prevention trial with a null result. Until such a trial exists, the argument's central claim is *currently untested* rather than supported, and this paper says so rather than implying otherwise.

The 5-HT6 failures are not explained by the substrate argument. They are not, and §10.5 does not claim they are. A receptor antagonist's action does not depend on synthesis at all. The 5-HT6 programmes failed for reasons one and two, and reason three does not apply to them. A reader who thinks reasons one and two are insufficient is entitled to regard the 5-HT6 record as damaging to the whole serotonergic argument, and the ledger of §11.1 records that.

The Bartels result did not move the biomarkers, and a chain built on amyloid production should have. This is the sharpest objection and it deserves the fullest answer. There

are three possible readings. The first is the one §10.4 offers: a rate effect integrating over years is largely invisible to a cross-sectional pool measurement, particularly in a cohort in which most participants are already amyloid-positive. The second is that the delay in conversion was produced by something other than amyloid — plausibly the trophic arm of §8.3, whose effect on hippocampal reserve would also require years and would also not move a cerebrospinal-fluid amyloid measure. The third is that the association is confounded and there is nothing to explain. This paper cannot currently distinguish the three, and it does not pretend to. It records that all three are consistent with the chain and that only the first is uniquely predicted by it.

10.7 What the chain says to do instead

The therapeutic reading of a fifty-year fuse is not a drug; it is an ordering. Four propositions follow from the chain, ranked by how far upstream they act.

Correct the ecology first, because it is the most reversible node and it relieves the most axes. The gut turns over in weeks, is reachable through the lumen rather than across the blood–brain barrier, and its correction acts simultaneously on the inflammatory tone that induces the diverting enzyme, on the barrier that exports the endotoxin, on the afferent load the vagus carries, and on the microglial state that Erny showed it controls. Restoring fibre-fermenting, butyrate-producing capacity is the single intervention in this paper with the largest number of downstream beneficiaries and the smallest cost. It is also the one with the weakest direct evidence in humans, and both facts should be stated together.

Measure the partition before prescribing into it. The plasma kynurenine-to-tryptophan ratio is cheap, validated as an index of immune activation, and — on the argument of §10.5 — a candidate effect modifier of a drug class taken by hundreds of millions of people. Whether it modifies the effect is an empirical question that nobody has asked.

Prefer the receptor to the transporter, where the target is amyloid production. A 5-HT4 agonist engages the trafficking mechanism directly. This is a preclinically supported and clinically unexplored option in the prevention setting.

Aim at accumulation rate, in midlife, and measure it directly. Stable-isotope labelling makes amyloid production rate measurable in living people. It is the endpoint the mechanism of §8.1 actually governs, it is measurable in a cognitively normal forty-year-old, and it would convert this paper's central inference into a result within the duration of a normal trial.

None of these is a treatment for dementia. All of them are interventions on a fuse.

10.8 The verdict

The serotonergic system in Alzheimer's disease 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, at the wrong level of the system, and on the wrong side of its rate-limiting step.

That verdict is deliberately unexciting. It does not claim the serotonergic lesion as the cause of Alzheimer's disease; §7.3 concedes that two other nuclei are more severely depleted, and §8.7 concedes that the lesion does not produce the amnestic syndrome. It does not promise a therapy; §10.2 reports four failed Phase III trials in nearly four thousand patients. It does not claim the chain is proven; §2.8 concedes that its most upstream link rests on cross-sectional human data, and §9.4 reports the post-mortem result that most constrains its clinical reading.

What it claims is narrower and, if right, more useful. A diffuse modulatory system that brakes amyloid production, restrains tau in its target field, supplies the trophic input to hippocampal reserve, and holds a brake on microglial consumption is progressively withdrawn beginning decades before the disease is visible; the withdrawal has two independent causes, of which only one is a neuronal lesion and the other is a metabolic partition set outside the brain; and the consequences of that withdrawal have been looked for in the wrong decade of the patient's life, with a drug that acts upstream of nothing.

Result	What it shows	Grade
Two 5-HT6 antagonists failed four Phase III trials in ~3,840 patients	The receptor-antagonist approach in established dementia does not work	Established
Citalopram 30 mg reduces agitation while worsening cognition and prolonging QT	Serotonergic augmentation in established disease has a real but narrow role and a cognitive cost	Established
Citalopram slows CSF amyloid-β production rate by 37% in healthy volunteers	The amyloid brake is real in living people	Established
SSRI use associates with lower plasma p-tau181 and partially restored dorsal raphe metabolism	Hypothesis-generating; cross-sectional	Probable
>4 years of SSRI in MCI with depression history delays conversion by ~3 years; no CSF biomarker change	A duration-dependent trajectory effect without a cross-sectional biomarker effect	Established (finding); Inference (interpretation)
The trials came fifty years late	Explanation 1 for the negative record	Inference
The pharmacology addressed the signal, not the cell	Explanation 2	Inference (weakest)
Reuptake inhibition acts downstream of a throttled rate-limiting step	Explanation 3 — this paper's claim	Inference

PART XI — The Ledger

11.1 Every claim, graded

The purpose of this 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. Claims are listed in the order the chain traverses them. Each carries the link it belongs to, its grade, and its principal support.

#	Link	Claim	Grade	Principal support
1	L1	The gut ecology loses diversity and butyrate producers with age and frailty	Established (assoc.)	Claesson et al. 2012
2	L1	Alzheimer's disease carries a reproducible dysbiotic signature versus controls	Established (assoc.)	Vogt et al. 2017
3	L1	Pro-inflammatory taxa correlate positively, and butyrate producers negatively, with brain amyloid on PET	Established	Cattaneo et al. 2017
4	L2	Butyrate fuels the colonocyte and supports tight-junction assembly	Established	Standard physiology
5	L2	Barrier failure produces low-grade systemic endotoxemia	Established (model)	Cani et al. 2007
6	L3	Lipopolysaccharide accumulates in Alzheimer neocortical neurons	Probable	Zhao & Lukiw 2017
7	L3	Trimethylamine N-oxide is elevated in Alzheimer CSF and tracks injury and tau markers	Established	Vogt et al. 2018
8	L3	A microbially generated bile-acid ratio tracks cognitive decline in 1,464 subjects, replicated	Established	MahmoudianDehkordi et al. 2019; Nho et al. 2019
9	—	Microbiota continuously control microglial maturation and function	Established	Erny et al. 2015

#	Link	Claim	Grade	Principal support
10	—	Germ-free or antibiotic-perturbed hosts show altered amyloid pathology (sex-specific)	Established (model)	Harach 2017; Minter 2016; Dodiya 2019
11	—	Short-chain fatty acids are protective in one model and pathology-permitting in another	Established (the contradiction)	Erny 2015 vs Sampson 2016
12	—	Bacterial amyloid cross-seeds α-synuclein aggregation	Established (model)	Chen 2016; Sampson 2020
13	—	Bacterial amyloid cross-seeds tau	**Speculative**	Untested
14	L4	Microbiota consume dietary tryptophan into indoles, lowering host substrate	Established	O'Mahony et al. 2015
15	L4	Dysbiotic inflammatory tone induces IDO1 and diverts the partition	Established	Munn 1998; Dantzer 2008
16	L4	Faecal transplantation transfers altered tryptophan metabolism with the ecology	Established (rodent)	Kelly 2016; Zheng 2016
17	L4	Spore-forming gut bacteria promote host peripheral serotonin biosynthesis	Established	Yano et al. 2015
18	L5	TPH2's Michaelis constant sits close to the ambient brain tryptophan concentration	Established	Enzyme kinetics
19	L5	Sustained IDO induction makes central serotonin synthesis substrate-limited	Established (principle)	Maes 1995; Dantzer 2008
20	L5	Substrate limitation is quantitatively material to serotonergic deficit in human AD specifically	**Probable**	Extrapolation; direct brain measurements sparse
21	—	Microglial KMO and astrocytic KAT segregate the branch, biasing toward quinolinic acid under inflammation	Established	Schwarcz et al. 2012
22	—	IDO and quinolinic acid are demonstrable in Alzheimer hippocampus	Established	Guillemin et al. 2005

#	Link	Claim	Grade	Principal support
23	L6	Vagal afferents sense peripheral inflammation; vagotomy attenuates the central response	Established	Watkins et al. 1995
24	L6	Enteroendocrine neuropod cells form fast glutamatergic synapses onto vagal afferents	Established	Kaelberer et al. 2018
25	L6	A defined microbial signal alters behaviour through the vagus; vagotomy abolishes it	Established	Bravo et al. 2011
26	L7	The dominant NTS-to-coeruleus route is indirect, dual-signed (PGi excitatory, PrH inhibitory)	Established (rodent)	Aston-Jones 1986; Ennis 1988
27	L7	The rodent relay is conserved in the human brainstem at the synaptic level	**Inference**	Brainstem conservation
28	—	Truncal vagotomy and appendectomy associate with reduced Parkinson's disease risk	Probable	Svensson 2015; Liu 2017; Killinger 2018
29	—	Pathological α-synuclein propagates gut-to-brain along the vagus; vagotomy blocks it	Established (mouse)	Kim et al. 2019
30	—	An equivalent gut-to-brainstem route exists for tau	**Not established**	No experiment
31	L8	The locus coeruleus bears the earliest tau pathology in the human brain, from the second decade	Established	Braak et al. 2011 (n = 2,332)
32	L8	Coeruleus and raphe lack the aggrecan-based net that protects other neurons from tau	Established (anatomy)	Morawski et al. 2010
33	L8	DOPEGAL is produced exclusively in noradrenergic neurons	Established	Kang et al. 2020
34	L8	ApoE4 inhibits VMAT2, raising cytosolic norepinephrine and DOPEGAL	Established (model)	Kang et al. 2021
35	L9	DOPEGAL activates asparagine endopeptidase, which cleaves tau at N368 into a propagating seed; the cut is causal	Established	Kang 2020; Zhang 2014

#	Link	Claim	Grade	Principal support
36	L9	The same protease cleaves I2PP2A/SET at N175, silencing protein phosphatase 2A	Established	Basurto-Islas et al. 2013
37	L9	Both cuts issue from one activation in the same coerulean cells	**Inference**	Shared enzyme identity
38	—	Locus coeruleus volume falls 8.4% per Braak stage; neuronal loss begins only midway	Established	Theofilas et al. 2017
39	L10	The coeruleus supplies α1 excitatory drive to the raphe; the pair is reciprocally coupled	Established	Standard anatomy
40	L10	Coeruleus neurons express MAO-A; raphe serotonergic neurons express MAO-B, and not vice versa	Established	Westlund 1985; Saura Martí 1990
41	L10	The MAO difference partly explains the 7.9%-versus-2.6% gap	**Inference**	Claims 40 + 43
42	L10	The shared cause of coerulean and raphe vulnerability is architectural, not chemical	**Inference**	Claims 32 + 40 + arbor/pacemaker
43	L11	At Braak 0, 2.6% of dorsal raphe and 7.9% of locus coeruleus neurons are tangled	Established	Ehrenberg et al. 2017
44	L11	Phospho-tau is present in a dorsal raphe subnucleus in >20% of Braak 0 brains	Established	Grinberg et al. 2009
45	L11	Dorsal raphe neuronal loss in AD is $d \approx 1.79$, ~40% of the nucleus, three times the substantia nigra	Established	Lyness et al. 2003 (67 studies)
46	L11	Raphe tau is present in non-demented adults aged 25–80; other proteinopathies are rarer there	Established	Pierson et al. 2025
47	—	The terminal field degenerates before the soma dies	Probable	Smith 2017/2023 vs Lyness 2003
48	—	Early raphe tau in an individual predicts eventual dementia	**Not established**	PART confound

#	Link	Claim	Grade	Principal support
49	L12	Serotonin suppresses interstitial amyloid-β via ERK signalling	Established	Cirrito et al. 2011
50	L12	Citalopram slows CSF amyloid-β *production rate* by 37% in living humans	Established	Sheline et al. 2014
51	L12	5-HT4 receptors bind and traffic ADAM10, promoting non-amyloidogenic cleavage	Established	Cochet 2013; Tesseur 2013; Giannoni 2013
52	L12	Ablating serotonin synthesis raises plaque load and astrogliosis	Established (model)	Xu 2019; von Linstow 2022
53	L12	Serotonergic denervation raises cortical tau without altering plaques	Probable (model)	Ramos-Rodríguez et al. 2013
54	L12	Claims 52 and 53 disagree and no single mechanism reconciles them	Established (the contradiction)	—
55	L12	Withdrawal of the serotonergic amyloid brake is *permissive* for the later amyloid phase	**Inference**	Claims 31, 43, 49–52 + staging
56	—	Serotonergic withdrawal materially erodes hippocampal neurogenic reserve in humans	**Inference**	Rodent chain; contested human neurogenesis
57	—	Serotonin acts through 5-HT2B to raise microglial motility and lower phagocytosis	Probable (developmental prep.)	Krabbe et al. 2012
58	—	Raphe loss disinhibits microglial synaptic pruning in human AD	**Speculative**	Extrapolation
59	—	The raphe lesion is present in AD irrespective of depression, and absent in primary late-life depression	Established	Hendricksen et al. 2004
60	—	Focal dorsal raphe tau produces an affective phenotype without a memory deficit	Established (mouse)	Pierson et al. 2025
61	—	Depression is the first legible reading of the upstream state, not the symptom of the raphe lesion	**Inference** (this paper)	Claims 19, 59, 60
62	L13	Two 5-HT6 antagonists failed four Phase III trials in ~3,840 patients	Established	Atri 2018; MINDSET

#	Link	Claim	Grade	Principal support
63	L13	Citalopram 30 mg reduces agitation while worsening cognition and prolonging QT	Established	Porsteinsson et al. 2014
64	L13	>4 years of SSRI in MCI with depression history delays conversion by ~3 years, without CSF biomarker change	Established	Bartels et al. 2018
65	L13	The duration asymmetry is the signature of a rate-acting permissive brake	**Inference** (this paper)	Claims 50, 55, 64
66	—	Serotonergic tone is lost by two independent routes — cell loss and substrate limitation	**Inference** (this paper)	Claims 18–20, 45, 47
67	—	Reuptake inhibition acts downstream of a throttled rate-limiting step and cannot rescue substrate-limited synthesis	**Inference** (this paper)	Claims 14–20 + transporter pharmacology
68	—	Serotonergic efficacy varies inversely with the plasma kynurenine-to-tryptophan ratio	**Not established**	P7, untested
69	—	A midlife dysbiotic signature predicts later brainstem pathology	**Not established**	P2, untested

11.2 What the ledger shows

Sixty-nine claims are listed. Forty-seven are Established, six Probable, ten Inference, two Speculative, and four Not established.

The distribution is worth reading carefully, because it is the honest summary of what kind of object this paper is.

The individual links are strong; the joins are where the inference lives. Almost every claim about a single compartment — the gut's chemistry, the enzymology of the partition, the anatomy of the relay, the protease and its substrates, the staging, the receptor mechanism, the trial outcomes — is Established. Almost every claim that *connects* two compartments across a species gap or a decade of time is Inference. That is the expected profile of a synthesis, and it means the paper should be judged on whether its joins are argued rather than on whether its facts are correct.

The paper's four distinctive contributions are all Inference, and they are marked as such wherever they appear. They are claims 55 (the permissive reading), 61 (depression as

the first reading rather than the symptom), 66 (the two-route account of serotonergic withdrawal), and 67 (the rate-limiting-step objection to reuptake inhibition). Claim 65 is the reading of the one existing result that bears on them.

Two Not-established claims are load-bearing and one is not. Claim 69 — that a midlife dysbiotic signature predicts later brainstem pathology — is the chain's most upstream prediction and its failure would sever the whole first third. Claim 68 — the kynurenine-to-tryptophan interaction — is the paper's most consequential therapeutic prediction and its failure would sever claim 67. Claim 30 (a gut-to-brain route for tau) and claim 13 (tau cross-seeding by bacterial amyloid) are *not* load-bearing: the chain runs through signalling and substrate, and would survive their permanent absence.

The negative results are in the ledger, not around it. Claims 62 and 63 record the trial failures; claim 54 records the model contradiction; claim 59 records the post-mortem dissociation that constrains Part IX; claim 11 records the contradiction in short-chain fatty acid valence; claim 48 records that early brainstem tau is not a diagnosis. A reader who wanted to attack this paper would find every weapon already in the table.

PART XII — Predictions, Experiments, Refutation

12.1 Eleven predictions

The chain makes predictions that differ from those of the standard account, in which the serotonergic lesion is a source of neuropsychiatric symptoms in established dementia and the gut is a downstream companion of systemic illness. Each prediction below is stated so that a definite result would confirm or embarrass it, and each names the link it tests.

P1 — Joint transfer. *(Tests L1–L5, L8.)* Faecal microbiota transplantation from patients with inflammatory- or kynurenine-subtype treatment-resistant depression into gnotobiotic rodents will transfer *both* a depressive behavioural phenotype *and* a neurodegeneration-relevant substrate signature — an elevated kynurenine-to-tryptophan ratio, microglial priming, and, in a proteinopathy-sensitised host or over sufficient time, accelerated locus coeruleus vulnerability. Each side of this has been demonstrated separately; nobody has measured both in one animal.

P2 — Midlife signature, late outcome. *(Tests L1–L3.)* A midlife dysbiotic signature together with an elevated kynurenine-to-tryptophan ratio will jointly predict both depression incidence over the subsequent decade and dementia incidence over the subsequent three decades, in overlapping predictive populations.

P3 — Conduit dependence. *(Tests L6–L7.)* The gut-to-outcome association of P2 will be attenuated, though not abolished, in individuals who have undergone truncal vagotomy — attenuated because the neural channel is severed, not abolished because the humoral channel through the area postrema persists.

P4 — Upstream dominance in the reversible window. *(Tests the whole chain therapeutically.)* A gut-directed intervention begun in the depressive window will alter the long-term cognitive trajectory more than an equally timed, equally intensive brain-directed antidepressant that does not touch the ecology.

P5 — Rate, not burden. *(Tests L12.)* 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.

P6 — The association weakens as the disease advances. *(Tests L12.)* 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.

P7 — The substrate interaction. *(Tests L5 and claim 67 — this paper's central pharmacological prediction.)* The efficacy of serotonergic augmentation, on any endpoint, will vary inversely with the plasma kynurenine-to-tryptophan ratio. Reuptake inhibition will underperform in high-ratio individuals because it amplifies release from a synthesis that has been throttled upstream. This is testable retrospectively in any completed trial with stored plasma.

P8 — The crossed advantage of the agonist. *(Tests claim 67.)* A 5-HT4 agonist will show target engagement on amyloid production rate in humans, measurable by stable-isotope labelling, and will outperform a reuptake inhibitor by a *larger* margin in high-ratio individuals than in low-ratio ones — because it does not depend on residual synthesis. A crossed interaction of this kind is much harder to produce by confounding than a main effect.

P9 — Duration, not dose. *(Tests claim 65.)* In prospective data, the effect of reuptake inhibition on conversion will scale with cumulative exposure duration rather than with dose, will show a threshold in the region of years rather than months, and will be larger for accumulation-rate endpoints than for cross-sectional burden endpoints.

P10 — Affect, not memory. *(Tests L11–L12.)* Raphe tau burden will correlate with affective and psychomotor measures and *not* with episodic memory, after adjustment for overall disease stage.

P11 — Arbor, not transmitter. *(Tests L10.)* 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.

12.2 Nine experiments

1. Replicate the four-group post-mortem comparison with modern stereology. The dissociation of §9.4 rests on 7–14 subjects per cell and on two-dimensional image analysis. It is the single most constraining human result in this paper and it deserves unbiased stereological replication in a larger series. If it holds, Part IX's reconciliation must carry the whole mood argument; if it fails in either direction, that Part is rewritten.

2. Count the caudal group. No study has applied to raphe magnus, pallidus and obscurus the stereology applied to the dorsal raphe. The rostral-versus-caudal comparison in the same brains would test P11 directly and would discriminate the arbor hypothesis from the transmitter hypothesis. It is the cheapest experiment in this paper and it uses tissue that already exists.

3. Test the monoamine oxidase attribution. Claim 41 predicts that imposing MAO-A activity on serotonergic neurons should accelerate their tau accumulation, and that removing MAO-A from coerulean neurons should slow theirs — narrowing the 7.9-versus-2.6 gap from either direction.

4. Measure both cuts in the same cells. Claim 37 joins the tau cleavage and the phosphatase-inhibitor cleavage on the identity of the enzyme. No experiment has measured tau-N368 and cleaved I2PP2A in the same DOPEGAL-exposed coerulean neurons. This is a straightforward double-label experiment on existing models.

5. Resolve the model contradiction. Claim 54 records that serotonergic denervation raises tau while enzyme ablation raises plaque, in different backgrounds at different ages. Run both manipulations in the same transgenic background, at the same ages, with the same tau and amyloid readouts. The disagreement may be entirely methodological, and nobody has checked.

6. Measure amyloid production rate against serotonergic status and partition status in humans. Stable-isotope-labelling kinetics with concurrent serotonin-transporter imaging and plasma kynurenine-to-tryptophan measurement, in cognitively normal middle-aged adults, would test P5, P6 and P7 simultaneously and would either establish or destroy the permissive reading.

7. Stratify a completed trial retrospectively. Any trial of a serotonergic agent with stored baseline plasma can be re-analysed for the interaction of P7. This costs an assay and a statistician. If the interaction is absent in two or more independent datasets, claim 67 is in serious trouble and this paper's central pharmacological argument should be abandoned.

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

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

12.3 What would break the chain, link by link

An argument that cannot be defeated is not an argument. Stated compactly, and organised by which span it would sever:

Sever L1–L3 (the ecological origin). If a large prospective midlife cohort finds that dysbiotic signature and kynurenine-to-tryptophan ratio predict neither depression nor dementia incidence, the ecological origin fails and the chain becomes a brain-intrinsic account beginning at the brainstem. Most of Parts V through X would survive; Parts II and III would become background.

Sever L6–L7 (the conduit). If the gut-to-outcome association proves entirely insensitive to vagotomy, and if no humoral route accounts for the residue, the neural conduit is not the operative one and the ecological arm would have to be re-grounded on circulating mediators alone.

Sever L8–L9 (the ignition). If DOPEGAL-driven activation of the protease proves not to occur in human locus coeruleus tissue at physiological concentrations — as opposed to in model systems — the cell-autonomous ignition fails and the earliest lesion needs a different explanation.

Sever L10 (the architectural conclusion). If the caudal raphe is found to be as heavily affected as the dorsal raphe, the architectural account loses its cleanest discriminator, since caudal and rostral serotonergic neurons share transmitter, enzymes and pacemaking and differ principally in arbor. Equally, if net-bearing neurons are found to tangle at the same rate as net-less neurons in a properly powered subcortical survey, one of the three architectural liabilities is removed.

Sever L11 (the ordering). If early brainstem tau is shown prospectively not to progress in a substantial majority of carriers, §7.6's caveat becomes the main finding and the brainstem lesion is reclassified as an age-related tauopathy incidental to Alzheimer's disease.

Sever L12 (the brake). If a substantially larger stable-isotope-labelling study finds no effect of serotonergic manipulation on amyloid-β production rate in humans, claim 50 falls and the permissive reading loses its human anchor, reverting to a mouse finding. Equally, if dorsal raphe integrity in midlife shows no relationship to subsequent amyloid or tau accumulation in a prospective cohort, the permissive reading fails its most direct test.

Sever L13 and this paper's central claim (the rate-limiting step). If retrospective stratification of two or more completed serotonergic trials shows no interaction between baseline kynurenine-to-tryptophan ratio and treatment effect, claim 67 is refuted and the third explanation of §10.5 should be withdrawn. Explanations one and two would remain.

Sever the mood arm. If raphe tau burden is found to correlate with episodic memory after adjustment for stage, claim 60 and the dissociation it rests on are wrong, and the serotonergic lesion would have to be readmitted to the account of the amnestic syndrome — which would strengthen the system's importance while destroying this paper's specific reading of it.

Sever the whole therapeutic argument. If a properly timed prevention trial — a serotonergic intervention with demonstrated target engagement, given to cognitively normal

middle-aged adults at elevated risk, for long enough to matter — produces no effect on amyloid accumulation rate or on downstream tau, the permissive-brake reading is badly damaged and the negative trial record of §10.2 should be read as it has usually been read: as evidence that the target is wrong. This trial has not been run, and the argument's principal claim is therefore currently *untested* rather than *supported*.

12.4 Conclusion

Four results opened this paper and refused to sit together. Citalopram slows amyloid production by more than a third in a healthy volunteer. Citalopram worsens cognition in an established dementia. Two well-designed receptor antagonists do nothing in four thousand patients. And four years of the same reuptake inhibitor, given at the mild-cognitive-impairment stage, is associated with a three-year delay in conversion while moving no biomarker at all.

The reading offered here is that these are one mechanism sampled at four points along a fifty-year course, and that the mechanism is legible only if it is followed the whole way from where it starts.

It starts outside the brain. A slow ecological drift in the intestine — away from the organisms that ferment fibre into the fuel that maintains the barrier, toward the organisms whose outer membrane is a Toll-like-receptor agonist — weakens the containment of its own consequences and begins exporting a specifiable chemistry into the circulation. That chemistry does two things at once. It raises an inflammatory tone that induces the enzyme which diverts tryptophan from serotonin toward kynurenine, at a branch point whose serotonergic arm is the least buffered of the four and whose rate-limiting enzyme sits close to the edge of substrate sensitivity. And it loads a nerve: the afferent vagus, whose terminals are one fast synapse from a cell that samples the lumen directly, and whose signal passes through an obligatory medullary station into a dual relay ending on the locus coeruleus.

There the load becomes injury, by a route no other neuron can take. A nucleus driven toward high tonic firing must recover more of its own transmitter; the transmitter that escapes the vesicle meets monoamine oxidase A and becomes an aldehyde that only noradrenergic cells can make; the aldehyde activates one protease that cuts tau into a seed and, with the same activation, cleaves the inhibitor that silences tau's phosphatase. The commonest genetic risk factor in the disease accelerates precisely that step by prying open the vesicle. And the resulting pretangle sits in a cell that will not die for decades — the nucleus losing 8.4 per cent of its volume per Braak stage while its neurons remain alive until the disease is halfway through.

The failing coeruleus then takes its neighbour with it, withdrawing the adrenergic drive that sustains firing in a serotonergic nucleus which is unguarded in the same three architectural ways and which — this is the join the habitual pairing of the two has hidden — carries the *other* monoamine

oxidase, so that whatever the two nuclei share, it is not their chemistry. As that seam gives way, the forebrain loses serotonergic tone by two independent routes: the cells that make the transmitter, and the substrate they make it from. And among the things that tone was doing, unnoticed, was holding amyloid precursor protein toward the cleavage that destroys amyloid-β before it exists.

By the time anyone measures a cognitive score, all of this has been running for half a century. The drug arrives, engages a real target, and is asked to reverse an accumulation it can only ever have slowed — while acting, in the reuptake inhibitor's case, downstream of a step that the first link in the chain has already throttled. That it does nothing is not evidence that the target was wrong. It is evidence about when the target was reachable, and about what else would have had to be supplied.

The prescription was not mistaken. It was fifty years late, and it was written for a synthesis that had already been diverted at the source.

References

Journal names are given in full. Digital object identifiers and PubMed identifiers are supplied where the source record carried them and where the record was matched against its primary index during preparation. Where a figure repeated in the review literature disagreed with the primary source, the primary source is the one used in the text and the discrepancy is noted at the point of use. One record used by the surrounding literature — the α2A-autoreceptor internalisation result discussed at §5.3 — could not be matched against a primary index and is therefore named in the text as unverified and excluded from this list rather than silently included.

Aletrino MA, Vogels OJM, Van Domburg PHMF, Ten Donkelaar HJ. Cell loss in the nucleus raphes dorsalis in Alzheimer's disease. *Neurobiology of Aging*. 1992;13(4):461–468. DOI: 10.1016/0197-4580(92)90073-7.

Arai R, Karasawa N, Kurokawa K, Kanai H, Horiike K, Ito A. Differential subcellular location of mitochondria in rat serotonergic neurons depends on the presence and the absence of monoamine oxidase type B. *Neuroscience*. 2002;114(4):825–835. DOI: 10.1016/S0306-4522(02)00351-2.

Arboleda-Velasquez JF, Lopera F, O'Hare M, Delgado-Tirado S, Marino C, Chmielewska N, et al. Resistance to autosomal dominant Alzheimer's disease in an APOE3 Christchurch homozygote: a case report. *Nature Medicine*. 2019;25(11):1680–1683. PMID 31686034.

Aston-Jones G, Cohen JD. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annual Review of Neuroscience*. 2005;28:403–450. PMID 16022602.

Aston-Jones G, Ennis M, Pieribone VA, Nickell WT, Shipley MT. The brain nucleus locus coeruleus: restricted afferent control of a broad efferent network. *Science*. 1986;234(4777):734–737. PMID 3775363.

Atri A, Frölich L, Ballard C, Tariot PN, Molinuevo JL, Boneva N, Windfeld K, Raket LL, Cummings JL. Effect of idalopirdine as adjunct to cholinesterase inhibitors on change in cognition in patients with Alzheimer disease: three randomized clinical trials. *JAMA*. 2018;319(2):130–142. DOI: 10.1001/jama.2017.20373. PMID 29318278.

Bartels C, Wagner M, Wolfsgruber S, Ehrenreich H, Schneider A; Alzheimer's Disease Neuroimaging Initiative. Impact of SSRI therapy on risk of conversion from mild cognitive impairment to Alzheimer's dementia in individuals with previous depression. *American Journal of Psychiatry*. 2018;175(3):232–241. DOI: 10.1176/appi.ajp.2017.17040404. PMID 29179578.

Basurto-Islas G, Grundke-Iqbal I, Tung YC, Liu F, Iqbal K. Activation of asparaginyl endopeptidase leads to Tau hyperphosphorylation in Alzheimer disease. *Journal of Biological Chemistry*. 2013;288(24):17495–17507. DOI: 10.1074/jbc.M112.446070. PMID 23640887.

Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, et al. Human hippocampal neurogenesis persists throughout aging. *Cell Stem Cell*. 2018;22(4):589–599.e5. PMID 29625071.

Braak H, Del Tredici K. The pathological process underlying Alzheimer's disease in individuals under thirty. *Acta Neuropathologica*. 2011;121(2):171–181. DOI: 10.1007/s00401-010-0789-4. PMID 21170538.

Braak H, Del Tredici K, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging*. 2003;24(2):197–211. PMID 12498954.

Braak H, Thal DR, Ghebremedhin E, Del Tredici K. Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *Journal of Neuropathology and Experimental Neurology*. 2011;70(11):960–969. DOI: 10.1097/NEN.0b013e318232a379. PMID 22002422.

Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J, Cryan JF. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;108(38):16050–16055. PMID 21876150.

Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*. 2007;56(7):1761–1772. PMID 17456850.

Cattaneo A, Cattane N, Galluzzi S, Provasi S, Lopizzo N, Festari C, et al. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. *Neurobiology of Aging*. 2017;49:60–68. PMID 27776263.

Chen SG, Stribinskis V, Rane MJ, Demuth DR, Gozal E, Roberts AM, et al. Exposure to the functional bacterial amyloid protein curli enhances alpha-synuclein aggregation in aged Fischer 344 rats and *Caenorhabditis elegans*. *Scientific Reports*. 2016;6:34477. PMID 27708338.

Cirrito JR, Disabato BM, Restivo JL, Verges DK, Goebel WD, Sathyan A, Hayreh D, D'Angelo G, Benzinger T, Yoon H, Kim J, Morris JC, Mintun MA, Sheline YI. Serotonin signaling is associated with lower amyloid-β levels and plaques in transgenic mice and humans. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;108(36):14968–14973. DOI: 10.1073/pnas.1107411108. PMID 21873225.

Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, et al. Gut microbiota composition correlates with diet and health in the elderly. *Nature*. 2012;488(7410):178–184. PMID 22797518.

Cochet M, Donneger R, Cassier E, Gaven F, Lichtenthaler SF, Marin P, Bockaert J, Dumuis A, Claeysen S. 5-HT4 receptors constitutively promote the non-amyloidogenic pathway of APP cleavage and interact with ADAM10. *ACS Chemical Neuroscience*. 2013;4(1):130–140. DOI: 10.1021/cn300095t. PMID 23336052.

Dahlström A, Fuxe K. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiologica Scandinavica. Supplementum*. 1964;232:1–55. PMID 14229500.

Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature Reviews Neuroscience*. 2008;9(1):46–56. PMID 18073775.

Dodiya HB, Kuntz T, Shaik SM, Baufeld C, Leibowitz J, Zhang X, et al. Sex-specific effects of microbiome perturbations on cerebral Aβ amyloidosis and microglia phenotypes. *Journal of Experimental Medicine*. 2019;216(7):1542–1560. PMID 31097468.

Dominy SS, Lynch C, Ermini F, Benedyk M, Marczyk A, Konradi A, et al. *Porphyromonas gingivalis* in Alzheimer's disease brains: evidence for disease causation and treatment with small-molecule inhibitors. *Science Advances*. 2019;5(1):eaau3333. PMID 30746447.

Ehrenberg AJ, Nguy AK, Theofilas P, Dunlop S, Suemoto CK, Di Lorenzo Alho AT, et al. Quantifying the accretion of hyperphosphorylated tau in the locus coeruleus and dorsal raphe nucleus: the pathological building blocks of early Alzheimer's disease. *Neuropathology and Applied Neurobiology*. 2017;43(5):393–408. DOI: 10.1111/nan.12387. PMID 28117917.

Ennis M, Aston-Jones G. Activation of locus coeruleus from nucleus paragigantocellularis: a new excitatory amino acid pathway in brain. *Journal of Neuroscience*. 1988;8(10):3644–3657. PMID 3193175.

Erny D, Hrabě de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, et al. Host microbiota constantly control maturation and function of microglia in the CNS. *Nature Neuroscience*. 2015;18(7):965–977. PMID 26030851.

Fernstrom JD, Wurtman RJ. Brain serotonin content: increase following ingestion of carbohydrate diet. *Science*. 1971;174(4013):1023–1025. PMID 5120086.

Frangos E, Ellrich J, Komisaruk BR. Non-invasive access to the vagus nerve central projections via electrical stimulation of the external ear: fMRI evidence in humans. *Brain Stimulation*. 2015;8(3):624–636. PMID 25573069.

Friedland RP, Chapman MR. The role of microbial amyloid in neurodegeneration. *PLOS Pathogens*. 2017;13(12):e1006654. DOI: 10.1371/journal.ppat.1006654. PMID 29267402.

Gagnon D, Parent M. Distribution of VGLUT3 in highly collateralized axons from the rat dorsal raphe nucleus as revealed by single-neuron reconstructions. *PLOS ONE*. 2014;9(2):e87709. DOI: 10.1371/journal.pone.0087709. PMID 24504335.

Giannoni P, Gaven F, de Bundel D, Baranger K, Marchetti-Gauthier E, Roman FS, et al. Early administration of RS 67333, a specific 5-HT4 receptor agonist, prevents amyloidogenesis and behavioral deficits in the 5XFAD mouse model of Alzheimer's disease. *Frontiers in Aging Neuroscience*. 2013;5:96. DOI: 10.3389/fnagi.2013.00096. PMID 24399962.

Gong CX, Shaikh S, Wang JZ, Zaidi T, Grundke-Iqbal I, Iqbal K. Phosphatase activity toward abnormally phosphorylated tau: decrease in Alzheimer disease brain. *Journal of Neurochemistry*. 1995;65(2):732–738. PMID 7616230.

Grinberg LT, Rüb U, Ferretti REL, Nitrini R, Farfel JM, Polichiso L, et al.; Brazilian Brain Bank Study Group. The dorsal raphe nucleus shows phospho-tau neurofibrillary changes before the transentorhinal region in Alzheimer's disease. A precocious onset? *Neuropathology and Applied Neurobiology*. 2009;35(4):406–416. DOI: 10.1111/j.1365-2990.2008.00997.x. PMID 19508444.

Guillemin GJ, Brew BJ, Noonan CE, Takikawa O, Cullen KM. Indoleamine 2,3-dioxygenase and quinolinic acid immunoreactivity in Alzheimer's disease hippocampus. *Neuropathology and Applied Neurobiology*. 2005;31(4):395–404. PMID 16008823.

Harach T, Marungruang N, Duthilleul N, Cheatham V, Mc Coy KD, Frisoni G, et al. Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota. *Scientific Reports*. 2017;7:41802. PMID 28176819.

Hendricksen M, Thomas AJ, Ferrier IN, Ince P, O'Brien JT. Neuropathological study of the dorsal raphe nuclei in late-life depression and Alzheimer's disease with and without depression. *American Journal of Psychiatry*. 2004;161(6):1096–1102. DOI: 10.1176/appi.ajp.161.6.1096. PMID 15169699.

Heneka MT, Nadrigny F, Regen T, Martinez-Hernandez A, Dumitrescu-Ozimek L, Terwel D, et al. Locus ceruleus controls Alzheimer's disease pathology by modulating microglial functions through norepinephrine. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;107(13):6058–6063. PMID 20231476.

Hiesberger T, Trommsdorff M, Howell BW, Goffinet A, Mumby MC, Cooper JA, Herz J. Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron*. 1999;24(2):481–489. PMID 10571241.

Holmes BB, DeVos SL, Kfoury N, Li M, Jacks R, Yanamandra K, et al. Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proceedings of the National Academy of Sciences of the United States of America*. 2013. PMID 23898162.

Kaelberer MM, Buchanan KL, Klein ME, Barth BB, Montoya MM, Shen X, Bohórquez DV. A gut-brain neural circuit for nutrient sensing. *Science*. 2018;361(6408):eaat5236. PMID 30237325.

Kang SS, Ahn EH, Liu X, Bryson M, Miller GW, Weinshenker D, Ye K. ApoE4 inhibition of VMAT2 in the locus coeruleus exacerbates Tau pathology in Alzheimer's disease. *Acta Neuropathologica*. 2021;142(1):139–158. DOI: 10.1007/s00401-021-02315-1. PMID 33895869.

Kang SS, Ahn EH, Ye K. Delta-secretase cleavage of Tau mediates its pathology and propagation in Alzheimer's disease. *Experimental & Molecular Medicine*. 2020;52(8):1275–1287. DOI: 10.1038/s12276-020-00494-7. PMID 32859953. *(Review.)*

Kang SS, Liu X, Ahn EH, Xiang J, Manfredsson FP, Yang X, et al. Norepinephrine metabolite DOPEGAL activates AEP and pathological Tau aggregation in locus coeruleus. *Journal of Clinical Investigation*. 2020;130(1):422–437. DOI: 10.1172/JCI130513. PMID 31793911.

Kelly JR, Borre Y, O'Brien C, Patterson E, El Aidy S, Deane J, et al. Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. *Journal of Psychiatric Research*. 2016;82:109–118. PMID 27491067.

Kepe V, Barrio JR, Huang SC, Ercoli L, Siddarth P, Shoghi-Jadid K, et al. Serotonin 1A receptors in the living brain of Alzheimer's disease patients. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;103(3):702–707. DOI: 10.1073/pnas.0510237103. PMID 16407119.

Keshavarzian A, Green SJ, Engen PA, Voigt RM, Naqib A, Forsyth CB, et al. Colonic bacterial composition in Parkinson's disease. *Movement Disorders*. 2015;30(10):1351–1360. PMID 26179554.

Killinger BA, Madaj Z, Sikora JW, Rey N, Haas AJ, Vepa Y, et al. The vermiform appendix impacts the risk of developing Parkinson's disease. *Science Translational Medicine*. 2018;10(465):eaar5280. PMID 30381408.

Kim S, Kwon SH, Kam TI, Panicker N, Karuppagounder SS, Lee S, et al. Transneuronal propagation of pathologic α-synuclein from the gut to the brain models Parkinson's disease. *Neuron*. 2019;103(4):627–641.e7. PMID 31255487.

Krabbe G, Matyash V, Pannasch U, Mamer L, Boddeke HWGM, Kettenmann H. Activation of serotonin receptors promotes microglial injury-induced motility but attenuates phagocytic activity. *Brain, Behavior, and Immunity*. 2012;26(3):419–428. DOI: 10.1016/j.bbi.2011.12.002. PMID 22198120.

Krehl WA, Teply LJ, Sarma PS, Elvehjem CA. Growth-retarding effect of corn in nicotinic acid-low rations and its counteraction by tryptophane. *Science*. 1945;101(2628):489–490. PMID 17735529.

Le Floc'h N, Otten W, Merlot E. Tryptophan metabolism, from nutrition to potential therapeutic applications. *Amino Acids*. 2011;41(5):1195–1205. PMID 20872026.

Liu B, Fang F, Pedersen NL, Tillander A, Ludvigsson JF, Ekbom A, et al. Vagotomy and Parkinson disease: a Swedish register-based matched-cohort study. *Neurology*. 2017;88(21):1996–2002. PMID 28446653.

Lopera F, Marino C, Chandrahas AS, O'Hare M, Villalba-Moreno ND, Aguillon D, et al. Resilience to autosomal dominant Alzheimer's disease in a Reelin-COLBOS heterozygous man. *Nature Medicine*. 2023;29(5):1243–1252. PMID 37188781.

Lyness SA, Zarow C, Chui HC. Neuron loss in key cholinergic and aminergic nuclei in Alzheimer disease: a meta-analysis. *Neurobiology of Aging*. 2003;24(1):1–23. DOI: 10.1016/S0197-4580(02)00057-X. PMID 12493547.

Maes M. Evidence for an immune response in major depression: a review and hypothesis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 1995;19(1):11–38. PMID 7708925.

MahmoudianDehkordi S, Arnold M, Nho K, Ahmad S, Jia W, Xie G, et al. Altered bile acid profile associates with cognitive impairment in Alzheimer's disease — an emerging role for gut microbiome. *Alzheimer's & Dementia*. 2019;15(1):76–92. DOI: 10.1016/j.jalz.2018.07.217. PMID 30337151.

Marizzoni M, Cattaneo A, Mirabelli P, Festari C, Lopizzo N, Nicolosi V, et al. Short-chain fatty acids and lipopolysaccharide as mediators between gut dysbiosis and amyloid pathology in Alzheimer's disease. *Journal of Alzheimer's Disease*. 2020;78(2):683–697. PMID 33074224.

Minter MR, Zhang C, Leone V, Ringus DL, Zhang X, Oyler-Castrillo P, et al. Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease. *Scientific Reports*. 2016;6:30028. PMID 27443609.

Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, Rábano A, Cafini F, Pallas-Bazarra N, et al. Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nature Medicine*. 2019;25(4):554–560. PMID 30911133.

Morawski M, Brückner G, Jäger C, Seeger G, Arendt T. Neurons associated with aggrecan-based perineuronal nets are protected against tau pathology in subcortical regions in Alzheimer's disease. *Neuroscience*. 2010;169(3):1347–1363. DOI: 10.1016/j.neuroscience.2010.05.022. PMID 20497908.

Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science*. 1998;281(5380):1191–1193. PMID 9712583.

Nho K, Kueider-Paisley A, MahmoudianDehkordi S, Arnold M, Risacher SL, Louie G, et al. Altered bile acid profile in mild cognitive impairment and Alzheimer's disease: relationship to neuroimaging and CSF biomarkers. *Alzheimer's & Dementia*. 2019;15(2):232–244. DOI: 10.1016/j.jalz.2018.08.012. PMID 30337152.

O'Mahony SM, Clarke G, Borre YE, Dinan TG, Cryan JF. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behavioural Brain Research*. 2015;277:32–48. PMID 25078296.

Perkins MN, Stone TW. An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. *Brain Research*. 1982;247(1):184–187. PMID 6215086.

Pierson SR, Fiock KL, Wang R, Balasubramanian N, Reinhardt J, Khan KM, et al. Tau pathology in the dorsal raphe may be a prodromal indicator of Alzheimer's disease. *Molecular Psychiatry*. 2025;30(2):532–546. DOI: 10.1038/s41380-024-02664-9. PMID 39143322. *(Published online 14 August 2024; frequently mis-cited to volume 29 — see §7.2.)*

Porsteinsson AP, Drye LT, Pollock BG, Devanand DP, Frangakis C, Ismail Z, et al.; CitAD Research Group. Effect of citalopram on agitation in Alzheimer disease: the CitAD randomized clinical trial. *JAMA*. 2014;311(7):682–691. DOI: 10.1001/jama.2014.93. PMID 24549548.

Ramos-Rodriguez JJ, Molina-Gil S, Rey-Brea R, Berrocoso E, Garcia-Alloza M. Specific serotonergic denervation affects tau pathology and cognition without altering senile plaques deposition in APP/PS1 mice. *PLOS ONE*. 2013;8(11):e79947. DOI: 10.1371/journal.pone.0079947. PMID 24278223.

Ren J, Isakova A, Friedmann D, Zeng J, Grutzner SM, Pun A, et al. Single-cell transcriptomes and whole-brain projections of serotonin neurons in the mouse dorsal and median raphe nuclei. *eLife*. 2019;8:e49424. DOI: 10.7554/eLife.49424. PMID 31647409.

Sampson TR, Challis C, Jain N, Moiseyenko A, Ladinsky MS, Shastri GG, et al. A gut bacterial amyloid promotes α-synuclein aggregation and motor impairment in mice. *eLife*. 2020;9:e53111. PMID 32043464.

Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell*. 2016;167(6):1469–1480.e12. PMID 27912057.

Saura Marti J, Kettler R, Da Prada M, Richards JG. Molecular neuroanatomy of MAO-A and MAO-B. *Journal of Neural Transmission. Supplementum*. 1990;32:49–53. DOI: 10.1007/978-3-7091-9113-2_6. PMID 2089112.

Scheperjans F, Aho V, Pereira PAB, Koskinen K, Paulin L, Pekkonen E, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Movement Disorders*. 2015;30(3):350–358. PMID 25476529.

Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: when physiology meets pathology. *Nature Reviews Neuroscience*. 2012;13(7):465–477. PMID 22678511.

Schwarz LA, Miyamichi K, Gao XJ, Beier KT, Weissbourd B, DeLoach KE, et al. Viral-genetic tracing of the input–output organization of a central noradrenaline circuit. *Nature*. 2015;524(7563):88–92. PMID 26131933.

Sheline YI, West T, Yarasheski K, Swarm R, Jasielec MS, Fisher JR, et al. An antidepressant decreases CSF Aβ production in healthy individuals and in transgenic AD mice. *Science Translational Medicine*. 2014;6(236):236re4. DOI: 10.1126/scitranslmed.3008169. PMID 24828079.

Smith GS, Barrett FS, Joo JH, Nassery N, Savonenko A, Sodums DJ, et al. Molecular imaging of serotonin degeneration in mild cognitive impairment. *Neurobiology of Disease*. 2017;105:33–41. DOI: 10.1016/j.nbd.2017.05.007. PMID 28511918.

Smith GS, Kuwabara H, Nandi A, Gould NF, Nassery N, Savonenko A, et al. Molecular imaging of the association between serotonin degeneration and beta-amyloid deposition in mild cognitive impairment. *NeuroImage: Clinical*. 2023;37:103322. DOI: 10.1016/j.nicl.2023.103322. PMID 36680976.

Smith GS, Kuwabara H, Yan H, Nassery N, Yoon M, Kamath V, et al. Serotonin degeneration and amyloid-β deposition in mild cognitive impairment: relationship to cognitive deficits. *Journal of Alzheimer's Disease*. 2023;96(1):215–227. DOI: 10.3233/JAD-230570.

Stone TW, Perkins MN. Quinolinic acid: a potent endogenous excitant at amino acid receptors in CNS. *European Journal of Pharmacology*. 1981;72(4):411–412. PMID 6268428.

Svensson E, Horváth-Puhó E, Thomsen RW, Djurhuus JC, Pedersen L, Borghammer P, Sørensen HT. Vagotomy and subsequent risk of Parkinson's disease. *Annals of Neurology*. 2015;78(4):522–529. PMID 26031848.

Tanimukai H, Grundke-Iqbal I, Iqbal K. Up-regulation of inhibitors of protein phosphatase-2A in Alzheimer's disease. *American Journal of Pathology*. 2005;166(6):1761–1771. PMID 15920161.

Terstege DJ, Jabeen S, Galea LAM, Epp JR, Sargin D. SSRIs reduce plasma tau and restore dorsal raphe metabolism in Alzheimer's disease. *Alzheimer's & Dementia*. 2025;21(2):e14579. DOI: 10.1002/alz.14579. PMID 39936296.

Tesseur I, Pimenova AA, Lo AC, Ciesielska M, Lichtenthaler SF, De Maeyer JH, et al. Chronic 5-HT4 receptor activation decreases Aβ production and deposition in hAPP/PS1 mice. *Neurobiology of Aging*. 2013;34(7):1779–1789. DOI: 10.1016/j.neurobiolaging.2013.01.020. PMID 23474291.

Theofilas P, Ehrenberg AJ, Dunlop S, Di Lorenzo Alho AT, Nguy A, Leite REP, et al. Locus coeruleus volume and cell population changes during Alzheimer's disease progression: a stereological study in human postmortem brains with potential implication for early-stage biomarker discovery. *Alzheimer's & Dementia*. 2017;13(3):236–246. DOI: 10.1016/j.jalz.2016.06.2362. PMID 27513978.

Tracey KJ. The inflammatory reflex. *Nature*. 2002;420(6917):853–859. PMID 12490958.

Valentino RJ, Van Bockstaele E. Convergent regulation of locus coeruleus activity as an adaptive response to stress. *European Journal of Pharmacology*. 2008;583(2–3):194–203. PMID 18255055.

Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY, et al. The neuroactive potential of the human gut microbiota in quality of life and depression. *Nature Microbiology*. 2019;4(4):623–632. PMID 30718848.

Vogt NM, Kerby RL, Dill-McFarland KA, Harding SJ, Merluzzi AP, Johnson SC, et al. Gut microbiome alterations in Alzheimer's disease. *Scientific Reports*. 2017;7(1):13537. DOI: 10.1038/s41598-017-13601-y. PMID 29051531.

Vogt NM, Romano KA, Darst BF, Engelman CD, Johnson SC, Carlsson CM, et al. The gut microbiota-derived metabolite trimethylamine N-oxide is elevated in Alzheimer's disease. *Alzheimer's Research & Therapy*. 2018;10(1):124. PMID 30579367.

von Linstow CU, Waider J, Bergh MSS, Anzalone M, Madsen C, Nicolau AB, et al. The combined effects of amyloidosis and serotonin deficiency by tryptophan hydroxylase-2 knockout impacts viability of the APP/PS1 mouse model of Alzheimer's disease. *Journal of Alzheimer's Disease*. 2022;85(3):1283–1300. DOI: 10.3233/JAD-210581. PMID 34924373.

Watkins LR, Goehler LE, Relton JK, Tartaglia N, Silbert L, Martin D, Maier SF. Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune–brain communication. *Neuroscience Letters*. 1995;183(1–2):27–31. PMID 7746479.

Westlund KN, Denney RM, Kochersperger LM, Rose RM, Abell CW. Distinct monoamine oxidase A and B populations in primate brain. *Science*. 1985;230(4722):181–183. DOI: 10.1126/science.3875898. PMID 3875898.

Xu CJ, Wang JL, Pan J, Liao M. Tph2 genetic ablation contributes to senile plaque load and astrogliosis in APP/PS1 mice. *Current Alzheimer Research*. 2019;16(3):219–232. DOI: 10.2174/1567205016666190301110110. PMID 30827242.

Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell*. 2015;161(2):264–276. PMID 25860609.

Zhang Z, Song M, Liu X, Kang SS, Kwon IS, Duong DM, et al. Cleavage of tau by asparagine endopeptidase mediates the neurofibrillary pathology in Alzheimer's disease. *Nature Medicine*. 2014;20(11):1254–1262. DOI: 10.1038/nm.3700. PMID 25326800.

Zhao Y, Lukiw WJ. Lipopolysaccharide (LPS) accumulates in neocortical neurons of Alzheimer's disease (AD) brain and impairs transcription in human neuronal-glial primary co-cultures. *Frontiers in Aging Neuroscience*. 2017;9:407. PMID 29311897.

Zheng P, Zeng B, Zhou C, Liu M, Fang Z, Xu X, et al. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Molecular Psychiatry*. 2016;21(6):786–796. PMID 27067014.

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.