
The Gate and the Ransom

*The cholinergic nucleus basalis in Alzheimer's disease — what the cell is paid to do,
what it must pay to live, and why the receptor is surrendered before the phenotype and
the phenotype before the cell*

Ch4 · TrkA and p75 · proNGF · MMP-9 · Retrograde transport · Attention · Slow-wave sleep

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In a cognitively normal brain, roughly 197,000 neurons of this nucleus display the receptor for the signal they cannot live without, and roughly 210,000 display the enzyme that makes their transmitter. By the stage of mild cognitive impairment the first number has halved and the second has not moved. The cell is still alive, still cholinergic, and has stopped collecting what it is owed.

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Abstract

The nucleus basalis of Meynert is the most consequential single nucleus in the history of Alzheimer research. It supplied the field with its first mechanism, its first drug class and its first serious error. Davies and Maloney reported a selective loss of central cholinergic markers in 1976; Perry and colleagues correlated the loss with plaque counts and mental test scores in 1978; Whitehouse, Price and colleagues located the lesion in the basal forebrain in 1981 and 1982; Arendt and colleagues put the depletion at seventy per cent in 1983, and within a decade the finding had produced the cholinesterase inhibitors. The error in that sequence was not the anatomy but the arithmetic of time: a figure obtained at autopsy in advanced disease was read as the natural history of the lesion, and a nucleus was described as dying when what it was mostly doing was going quiet.

This paper reconstructs the chronology from the quantitative human record and finds three failures, not one, separated in time and in kind. Mufson and colleagues, counting nucleus basalis neurons that display the high-affinity nerve growth factor receptor TrkA, found 196,632 in the cognitively normal, 106,110 in mild cognitive impairment and 86,978 in Alzheimer's disease — a forty-six per cent loss already complete before dementia, not significantly worse after it, and uncorrelated with Braak stage. Gilmor and colleagues, counting the same nucleus for choline acetyltransferase in a cohort from the same programme, found approximately 210,000 cholinergic neurons per hemisphere in the cognitively normal and no significant loss in mild cognitive impairment or early disease. Read together — and we are explicit throughout about the hazards of reading two series together — these numbers describe a cell that is still present, still cholinergic, and has stopped displaying the receptor for the signal it cannot live without. The receptor is surrendered first; the transmitter phenotype next, and only in established disease, cortical choline acetyltransferase activity being unchanged or transiently *elevated* in mild cognitive impairment; the cell dies last.

The second half asks why. The nucleus basalis holds its survival on loan: of the six cholinergic sectors of the human brain, it is the one that carries nerve growth factor receptor protein, and the factor it depends on is not delivered ready to use. It is released as a precursor and matured in the extracellular space by a plasmin-dependent protease cascade, with matrix metalloproteinase 9 degrading the mature product, as Bruno and Cuellar established. Four independent human measurements show that pathway failing, and failing before dementia: precursor nerve growth factor rises in cortex; matrix metalloproteinase 9 activity rises; TrkA falls while the pro-apoptotic co-receptor p75 is held constant; and nerve growth factor protein is *increased* throughout the Alzheimer brain while falling in the nucleus basalis itself — a gradient that can only be read as a delivery failure. The road is independently compromised: rab4, rab5, rab7 and rab27 are upregulated in these neurons in mild cognitive impairment, and in trisomic mice an extra dose of the amyloid precursor protein gene alone enlarges the signalling endosome, blocks transport and degenerates the cells. The ransom is not scarce. It is uncut, over-degraded, unrecepted and

undelivered.

This resolves the field's most awkward disagreement about the nucleus. Two independent ADNI samples show that basal forebrain volume predicts subsequent entorhinal degeneration while the reverse model receives negligible support, and that it classifies amyloid status better than hippocampal volume does; against this, a 779-participant tau-PET study published in 2025 finds Ch4 volume coupled to mesial-temporal tau specifically in preclinical disease. Granted the trophic lesion, these are not in conflict: a locally generated failure of trophic maturation injures the hostage before the hostage-taker arrives, and the tangle in the adjacent limbic cortex supplies the second hit. We decline the stronger claim the imaging is often made to carry. Upstream is not origin, and a nucleus that shrinks is not a nucleus that dies.

On function the paper is deflationary in a way that matters for therapy. The nucleus basalis is not a memory store: lesions of it in monkeys do not reliably impair memory, they impair attention. Cortical acetylcholine rises in sub-second transients when a cue is *detected*, and — the finding with the sharpest clinical edge — a *decrease* in pre-cue cholinergic activity predicts detection while an increase predicts a miss. Cholinergic neurons burst with theta in waking and REM sleep and virtually cease firing in slow-wave sleep; raising cholinergic tone during slow-wave sleep with physostigmine abolishes declarative consolidation in healthy humans while sparing non-declarative learning. The signal is a difference, not a level. The disease flattens a rhythm; a cholinesterase inhibitor raises a mean. The drugs are efficacious and the effect is small — 2.7 points on a 70-point scale — and we propose that this is the predictable consequence of treating a modulation-depth lesion with a tonic agent.

We close with the nucleus's second job, which the cholinergic hypothesis never assigned it. Loss of basal forebrain volume in cognitively normal adults with abnormal amyloid and tau tracks rising soluble TREM2 and complement C3, most steeply in APOE ε4 carriers, and an open-label 2026 stimulation series reports a shift toward anti-inflammatory cytokines. The nucleus may be a brake on microglial reactivity as well as a gate on cortical plasticity — which would make its early failure a permission rather than merely a deficit. The paper ends with a graded ledger, six conditions of refutation named in advance, and nine experiments, beginning with the one measurement that would settle the ordering argument: TrkA, choline acetyltransferase and tau scored in the same neurons, in the same sections, across the same clinical continuum.

Part I — The Cell and Its Office



1. The Nucleus That Founded a Hypothesis

The cholinergic hypothesis of Alzheimer's disease was assembled in seven years from four observations, and it is worth setting them down in order, because the order is where the trouble entered.

In December 1976 Davies and Maloney published a one-page letter in *The Lancet* reporting a selective loss of central cholinergic neurons in Alzheimer's disease, inferred from reduced choline acetyltransferase and acetylcholinesterase activity in necropsy brain (Davies & Maloney, 1976). In 1978 Perry, Tomlinson, Blessed and colleagues tied the biochemistry to both the pathology and the patient: both enzyme activities fell significantly as mean plaque count rose, and the choline acetyltransferase reduction correlated with intellectual impairment on a memory and information test (Perry et al., 1978). This was the first demonstration in the field that a specific transmitter deficit tracked both a specific lesion and a specific clinical measure, and it remains one of the cleanest ever produced.

What neither study could do was locate the cells. That came from Whitehouse, Price, Struble, Clark, Coyle and DeLong, first in *Annals of Neurology* in 1981 — a single case compared with one age- and sex-matched control — and then in the paper everyone cites, a short report in *Science* in 1982: in Alzheimer's disease and senile dementia there is a profound and apparently selective loss of neurons in the basal forebrain, centred on the nucleus basalis of Meynert in the substantia innominata (Whitehouse et al., 1981, 1982). Classical morphometry the following year placed the depletion at approximately seventy per cent, in a series that also found seventy-seven per cent loss in paralysis agitans and forty-seven per cent in Korsakoff's disease, and that reported the loss to be restricted to the large neurons of the nucleus basalis, with the immediately adjacent neurons of the globus pallidus externus unaffected (Arendt et al., 1983).

Four observations, one conclusion, and a decade later a drug class. The inference was reasonable on the evidence available and it was wrong in two specific ways, both of which this paper is about.

The first error was about identity. If this disease destroys cholinergic neurons, and the cholinergic neurons counted are the ones that die, then being cholinergic looks like what places a neuron at risk. The inference was rarely stated so plainly, but it organised a generation of pharmacology — and it is false. Woolf, Jacobs and Butcher examined the mesopontine cholinergic cell groups in 1989 — Ch5 and Ch6, as large, as isodendritic and as acetylcholinesterase-rich as the

nucleus basalis — in the same disease, and found no loss of neuronal somata in any diagnostic category: cholinergic phenotype, they concluded, is not a sufficient condition for vulnerability. What distinguishes the nucleus basalis from its mesopontine counterparts is not its transmitter but its trophic dependency, and Part III is an account of that dependency and of the four ways it fails.

The second error was about time, and it is the one the field has still not absorbed. The seventy per cent figure comes from advanced disease. It was treated as the natural history of the lesion, and the whole therapeutic programme was addressed to it: replace the transmitter the dead cells are no longer making. But the quantitative work that followed, overwhelmingly from Mufson's group working with the Rush cohorts, established that at the stage when a patient is merely impaired the cells are largely still there. Gilmor and colleagues found approximately 210,000 choline-acetyltransferase-immunopositive neurons per hemisphere in the cognitively unimpaired, no decline at all in mild cognitive impairment, and a non-significant reduction of roughly fifteen per cent in early disease (Gilmor et al., 1999). DeKosky and colleagues found cortical choline acetyltransferase activity not reduced in mild cognitive impairment or mild disease at all — in hippocampus and superior frontal cortex it was significantly *elevated* — falling below normal only at end stage (DeKosky et al., 2002).

So the founding figure was real and the inference from it was wrong. For the years in which the patient is declining and might be treated, the nucleus basalis is not a field of dead cells. It is a nucleus of living cells that have stopped doing something. The question this paper exists to answer is what, exactly, they stop doing, and in what order.

2. What Ch4 Is

The nucleus basalis is not a nucleus in the compact sense. It is a sheet of large, hyperchromic, magnocellular neurons dispersed through the substantia innominata beneath the globus pallidus and anterior commissure, intercalated among fibre bundles, with no sharp cytoarchitectonic border on any side. This geometry is not a detail. It determines what can be counted, what can be imaged, and how much of the literature's disagreement is real.

The functional taxonomy that organises the system is Mesulam's. Beginning in 1983, Mesulam, Mufson, Levey and Wainer partitioned the cholinergic neurons of the primate and human basal forebrain into sectors Ch1 through Ch4 (Mesulam et al., 1983; Mesulam & Geula, 1988). Ch1 occupies the medial septal nucleus and Ch2 the vertical limb of the diagonal band, together the principal cholinergic supply of the hippocampus; Ch3, in the horizontal limb, supplies the olfactory bulb; Ch4 is the nucleus basalis of Meynert itself, containing the large majority of the forebrain system's cells and broadcasting acetylcholine across the entire cortical mantle and the amygdala. Two further sectors, Ch5 and Ch6, lie outside the forebrain in the mesopontine tegmentum and supply the thalamus; they are not this paper's subject, but their existence is the control condition that made the first founding error visible.

Ch4 is internally differentiated, and the differentiation matters for lesion anatomy. Mesulam and Geula's enzyme mapping of the human brain divided it into anteromedial, anterolateral, intermediate and posterior subsectors — Ch4am, Ch4al, Ch4i and Ch4p — each with a preferential cortical territory: the anteromedial weighting toward medial cortex and cingulate, the anterolateral toward amygdala and frontoparietal cortex, the posterior toward superior temporal gyrus and temporal pole (Mesulam & Geula, 1988). The human brain additionally contains a cell group with no clear rodent homologue, the nucleus subputaminalis.

Two features of Ch4's connectivity are load-bearing later, and Mesulam's review states them most compactly (Mesulam, 2013). First, Ch4 sits at the confluence of the limbic and reticular activating systems: it receives dopaminergic input from the ventral tegmental area and substantia nigra, serotonergic input from the raphe and noradrenergic input from the locus coeruleus, and its heaviest cortical reciprocal relationships are with limbic and paralimbic cortex. That limbic affiliation is the most plausible structural explanation for the nucleus's early involvement by neurofibrillary pathology: it is anatomically continuous with the basomedial limbic structures where that pathology begins. Second, the projection is not purely volumetric — more than half of the presynaptic varicosities along Ch4's cortical axons make conventional synaptic contacts with cortical neurons. The nucleus addresses the cortex; it does not merely perfuse it.

The counting problem deserves its own statement, because the numbers in this paper come from it. Design-based stereology on immunostained human tissue gives the most trustworthy figures, and Gilmor's series puts the cognitively normal nucleus at approximately 210,000 choline-acetyltransferase-positive and 174,000 vesicular-acetylcholine-transporter-positive neurons per hemisphere, with biological variation calculated at seventeen per cent (Gilmor et al., 1999). For imaging, the reference standard is a probabilistic atlas rather than a visible structure: Zaborszky and colleagues traced the magnocellular cell groups in ten human postmortem brains and warped them into a standard stereotaxic space, producing the open-source maps on which essentially all subsequent basal forebrain MRI volumetry is based (Zaborszky et al., 2008). Every in vivo "basal forebrain volume" discussed in Chapters 7 and 8 is a measurement made inside a mask derived from those ten brains — not a criticism of a method that was a considerable achievement and remains the only one available, but a statement of what the measurement is.

Table 1 — The human cholinergic basal forebrain: what is counted and by what means.

Quantity	Value	Method	Source
ChAT-positive neurons, nucleus basalis, per hemisphere (no cognitive impairment)	~210,000	Design-based stereology, immunocytochemistry	Gilmor et al. (1999)
VAcHT-positive neurons, same nucleus and cases	~174,000	Same	Gilmor et al. (1999)
Biological variation across normal cases	17%	Same	Gilmor et al. (1999)
TrkA-immunoreactive neurons, nucleus basalis (no cognitive impairment)	196,632 ± 12,093	Unbiased stereology, Religious Orders Study	Mufson et al. (2000)
Stereotaxic atlas of Ch1–Ch4 for MRI	10 postmortem brains, silver-stained serial sections	Cytoarchitectonic 3-D reconstruction into MNI space	Zaborszky et al. (2008)
Cortical cholinergic varicosities forming conventional synapses	>50%	Ultrastructural survey	Mesulam (2013)

3. The Most Expensive Axon in the Brain

Before asking why this neuron fails, it is worth establishing what it is being asked to maintain, because the single most arresting fact about the basal forebrain cholinergic neuron is a measurement of its axon.

The measurement was not possible until genetically directed sparse labelling made it possible to recover an entire arbor from a single cell. Wu, Williams and Nathans did this in the mouse and reported individual basal forebrain cholinergic arbors spanning multiple cortical columns, carrying more than a thousand branch points, with total axon lengths up to fifty centimetres. From published human morphometric data they then calculated the figure that should be better known than it is: the mean axon length of a human basal forebrain cholinergic neuron is on the order of **one hundred metres** (Wu, Williams & Nathans, 2014).

Three consequences follow, and the first two are arithmetic.

The first is that the nucleus is a bottleneck of extraordinary leverage: approximately two hundred thousand neurons per hemisphere supply the entire cortical mantle. Mesulam's formulation is exact — because neurofibrillary degeneration in a small number of these neurons can perturb neurotransmission in every cortical area, early involvement of Ch4 has a magnifying effect on Alzheimer pathology (Mesulam, 2013). Nothing else in the forebrain converts so little cell loss into so much cortical consequence.

The second is that this is a maintenance problem of a different order from the one a cortical pyramidal neuron faces. A hundred metres of thinly myelinated axon with a thousand branch points must be supplied with membrane, mitochondria, vesicle machinery and acetylcholine-synthesising enzyme from a soma of ordinary size, continuously, for eight or nine decades — and the same cell's survival signal must travel that road in the opposite direction. We are not aware of any serious estimate of the fraction of this neuron's energy budget consumed by axonal maintenance and decline to invent one; but that the trophic signal and the maintenance supply share a single long, thin, highly branched conduit is the structural fact that makes the rest of this paper's mechanism plausible rather than merely possible.

The third is empirical and comes from the same paper: in an Alzheimer model, cholinergic axons were lost slowly, with an accumulation of axon-derived material in discrete puncta. The axon degenerates by a process that leaves debris rather than by clean retraction — which is what one expects if the failure is of transport and clearance rather than of transcription.

4. The Gate, Not the Store

The cholinergic hypothesis was, in its popular form, a hypothesis about memory. Drachman and Leavitt's 1974 demonstration that scopolamine in healthy young subjects produces a pattern of impairment resembling normal ageing gave the idea its most memorable experimental anchor (Drachman & Leavitt, 1974), and the correlation between cortical choline acetyltransferase activity and memory scores supplied the clinical one (Perry et al., 1978). The inference drawn was that acetylcholine is a memory transmitter and the nucleus basalis therefore, loosely, a memory structure. Half a century of work has established something different and more useful: Ch4 does not store anything. It issues permissions.

The lesion evidence is the first reason to think so, and the primate data are cleaner than the rodent data. Voytko, reviewing selective basal forebrain lesions in monkeys, drew a distinction the field had not made: the medial septal and diagonal band sectors may well matter for memory, but lesions of the nucleus basalis itself *do not consistently disrupt mnemonic function*. What they disrupt is attention — impairments of which, she noted, are an early and significant feature of Alzheimer's disease (Voytko, 1996). A structure whose destruction fails to produce amnesia is not a memory store.

The second reason is the timescale and sign of the signal itself. Parikh, Kozak, Martinez and Sarter used choline-sensitive microelectrodes to measure cortical acetylcholine release at sub-second resolution in rats performing a cue-detection task, and the result is the most important single finding in this chapter. Cues that were behaviourally detected evoked transient increases in cholinergic activity in medial prefrontal cortex, on a timescale of seconds; cues that were missed evoked no transient. Cholinergic deafferentation of that cortex impaired detection. And — the detail with the sharpest clinical edge — *decreases* in pre-cue cholinergic activity predicted subsequent detection, while *increases* in pre-cue activity predicted misses. These second-scale transients were superimposed on slower changes at the scale of minutes (Parikh et al., 2007).

Read that result carefully and it says that cortical acetylcholine carries information in its excursions, not in its concentration, and that the detectability of a signal depends on the quiet that precedes it. A high tonic level is not a strong version of the signal. It is the condition under which the signal cannot be read.

The third reason is that acetylcholine's role in plasticity is explicitly permissive. Kilgard and Merzenich paired an auditory stimulus with episodic electrical stimulation of the nucleus basalis in the adult rat and produced massive progressive reorganisation of primary auditory cortex, with receptive fields narrowing, broadening or unchanged depending on the properties of the paired

stimulus, in a pattern paralleling the remodelling produced by behavioural training; the basal forebrain, they concluded, plays an active instructional role in representational plasticity (Kilgard & Merzenich, 1998). The nucleus does not carry the content. It marks which content is worth keeping, and the mark must be *paired* to have that effect.

The fourth reason is the sleep-wake dependence, and it completes the argument. Lee, Hassani, Alonso and Jones recorded identified basal forebrain units across the sleep-wake cycle with immunohistochemical confirmation of cell type, and found that cholinergic neurons discharge in bursts at maximal rates during active waking and paradoxical sleep, synchronised with theta, when cortical gamma and theta are maximal — and **virtually cease firing during slow-wave sleep** (Lee et al., 2005). The system does not idle at night. It switches off.

That the off-state is functional and not merely a rest was demonstrated in humans. Gais and Born infused physostigmine during slow-wave-sleep-rich sleep in healthy subjects and *completely blocked* the sleep-related consolidation of declarative memory for word pairs, without interfering with a non-declarative mirror-tracing task and without altering consolidation during waking, when endogenous tone is already maximal (Gais & Born, 2004). Raising acetylcholine at the wrong point in the cycle destroys a function that depends on acetylcholine being low.

Hasselmo and Sarter's synthesis draws the general conclusion: cholinergic neuromodulation operates in distinct modes on distinct timescales, with second-scale changes triggered by sensory cues facilitating detection, and the distribution of nicotinic and muscarinic receptors in target circuits determining what a transient does when it arrives (Hasselmo & Sarter, 2011).

The office of Ch4, then, is to open and close a gate: to raise the gain on cortical representations that are worth encoding, at the moment they occur, against a background that must be quiet for the signal to mean anything, and to withdraw entirely during the state in which the cortex consolidates what it has taken in. The clinical consequence of losing such a system is not primarily that memories are erased. It is that the cortex loses the ability to mark which of its current activity deserves to become a memory, and loses the nightly silence in which the marking is cashed. We return to the therapeutic implication in Chapter 13, and we flag here that the implication is an inference of ours, not a demonstrated fact.

What else the basal forebrain is

One complication must be admitted before the chronology, because it limits what every imaging result in this paper can mean.

The basal forebrain is not a cholinergic structure with impurities. It is a mixed structure in which the cholinergic population is a minority by number, intermingled with GABAergic, glutamatergic and peptidergic neurons, several of which project to cortex in parallel. The functional consequence was demonstrated decisively by Kim and colleagues: cortically projecting GABAergic

basal forebrain neurons containing parvalbumin control cortical gamma-band oscillations — optogenetic stimulation increased cortical gamma power by entraining a cortical oscillator resonant near forty hertz, bilateral inhibition reduced the forty-hertz auditory steady-state response, and, the crucial control, selective saporin lesioning of the basal forebrain *cholinergic* neurons did not alter the gamma enhancement (Kim et al., 2015).

Two things follow. An attractive and much-cited story — basal forebrain degeneration explains the gamma abnormalities of Alzheimer's disease, therefore gamma entrainment addresses the cholinergic lesion — is not licensed by this evidence, because the pathway generating cortical gamma from the basal forebrain is demonstrably not the cholinergic one. And, more consequentially here, a basal forebrain *volume* measured in an MRI mask is not a cholinergic neuron count: it is the volume of a region containing cholinergic neurons, non-cholinergic projection neurons, dendrites, fibre bundles and glia, measured inside a boundary interpolated from ten postmortem brains (Zaborszky et al., 2008). When basal forebrain volume predicts something, what has been shown is that the region predicts it; attributing the effect to the cholinergic population is a further inference, and the parvalbumin result makes it non-trivial.

Part II — The Chronology



5. Three Clocks: The Receptor, The Phenotype, The Cell

This chapter contains the paper's principal empirical claim, and it is assembled by reading two stereological series against each other. We state the hazard of doing so at the outset and return to it at the end, because the claim is strong and the method of reaching it is indirect.

The receptor clock. Mufson and colleagues applied unbiased stereology to the nucleus basalis in thirty people from the Religious Orders Study, all cognitively tested within twelve months of death, counting neurons immunoreactive for TrkA, the high-affinity nerve growth factor receptor. In the nine with no cognitive impairment the mean count was $196,632 \pm 12,093$; in the twelve with mild cognitive impairment, $106,110 \pm 14,565$; in the nine with probable Alzheimer's disease, $86,978 \pm 12,141$. Both impaired groups differed significantly from the unimpaired — forty-six and fifty-six per cent reductions — and, critically, *they did not differ significantly from each other*. The count correlated with the Boston Naming Test and a global cognitive measure, and not with the Mini-Mental State Examination, age at death, education, apolipoprotein E genotype, sex, or **Braak score** (Mufson et al., 2000).

Two features of that result are usually passed over. Nearly the entire loss of the receptor-bearing population is complete *before dementia* and does not accelerate across the transition to it — the authors say so in their closing sentence. And the absence of correlation with Braak stage means this failure is not simply a readout of how far cortical neurofibrillary pathology has advanced.

The phenotype clock. Gilmor and colleagues counted the same nucleus in a cohort from the same research programme with the same class of method, but stained for choline acetyltransferase and the vesicular acetylcholine transporter rather than the receptor. In the unimpaired the counts were approximately 210,000 and 174,000 per hemisphere. In mild cognitive impairment there was *no decline*; in early Alzheimer's disease, a non-significant reduction of approximately fifteen per cent. The counts correlated with dementia severity but not with apolipoprotein E status, age, sex, education or postmortem interval. Cholinergic neurons, they concluded, are relatively preserved in early disease (Gilmor et al., 1999).

The cortical half of the phenotype clock runs the same way and, for a period, runs *backwards*. DeKosky and colleagues assayed choline acetyltransferase activity in hippocampus and four cortical regions across fifty-eight individuals. Only the end-stage group had activity reduced below normal; in mild cognitive impairment and mild disease it was unchanged in inferior parietal, superior temporal and anterior cingulate cortex, significantly *elevated* in superior frontal cortex, and

significantly *higher* in hippocampus than in either the unimpaired or the demented. Their interpretation — that the cholinergic system is capable of compensatory responses during early dementia, and that the earliest cognitive deficits therefore involve changes other than simply cholinergic loss — has been widely cited and insufficiently integrated (DeKosky et al., 2002).

The cell clock. This is the one the field has had since 1983, and it is the slowest. Seventy per cent depletion of nucleus basalis neurons in Alzheimer's disease by morphometry, with losses exceeding seventy-five per cent described in advanced cases (Arendt et al., 1983; Whitehouse et al., 1982) — against a fifteen per cent non-significant reduction in early disease (Gilmor et al., 1999). The large cell loss is real and it is late.

Putting the three together. In the cognitively normal nucleus basalis there are approximately 210,000 cholinergic neurons and approximately 197,000 TrkA-bearing neurons — close enough, given seventeen per cent biological variation, to indicate that in health these are substantially the same population: the cholinergic neuron of this nucleus is by default a receptor-bearing neuron. In mild cognitive impairment the cholinergic count is unchanged at roughly 210,000 while the TrkA count has fallen to 106,110; in early-to-moderate disease the cholinergic count has fallen by a non-significant fifteen per cent and the TrkA count stands at 86,978. Only later does the nucleus lose seventy per cent of its cells.

If these series are commensurable, the ordering is unambiguous. The neuron surrenders the receptor for the signal it depends on while retaining its transmitter identity, retains its transmitter identity while cortical activity of the synthesising enzyme is compensatorily raised, and dies last, long after both.

The hazards, stated. These are different cohorts, different antibodies and different years, and a difference between two counts in two studies is not the evidential object that a difference between two counts in the same sections would be. An antibody can fail to detect a receptor for reasons short of the protein's absence — epitope masking, expression falling below a detection threshold, redistribution from the membrane to an endosomal compartment — so a count of immunoreactive perikarya is a count of cells displaying detectable antigen, not a census of cells possessing the protein. Both series come from the same research programme and autopsy cohorts, which materially strengthens the comparison, and the direction of the effect is consistent with the independent literature on receptor expression reviewed in Part III. But the ordering claim is an inference across studies, graded as such in Chapter 15. Chapter 16 specifies the single experiment that would convert it into an observation: TrkA, choline acetyltransferase and tau scored in the same neurons, in the same sections, across the same clinical continuum. It has not been done, and there is no methodological obstacle to doing it.

Table 2 — Three clocks in one nucleus.

Stage	TrkA-immunoreactive neurons (Mufson 2000)	ChAT-immunopositive neurons (Gilmor 1999)	Cortical/hippocampal ChAT activity (DeKosky 2002)	Large-neuron count (Arendt 1983)
No cognitive impairment	196,632 ± 12,093	~210,000 per hemisphere	reference	reference
Mild cognitive impairment	106,110 ± 14,565 (−46%, p < 0.001)	no decline	unchanged in 3 regions; elevated in superior frontal; elevated in hippocampus	—
Mild / moderate AD	86,978 ± 12,141 (−56%; not different from MCI)	−15%, non-significant	unchanged except at end stage	—
Advanced / end-stage AD	—	—	reduced below normal	~70% loss

6. The Tangle Arrives Early, and in the Cholinergic Cell

The chronology of Chapter 5 is a chronology of molecules and cells. It needs a pathological chronology beside it, and the question the pathology has to answer is a precise one: does neurofibrillary pathology reach the nucleus basalis early, and does it reach the cholinergic neurons specifically?

Mesulam, Shaw, Mash and Weintraub answered both. Using thioflavin-S histofluorescence for tangles, the tau antibodies AT8 and Alz-50 for pre-tangle cytopathology, and — the methodological point that makes the study decisive — concurrent visualisation of tauopathy and choline acetyltransferase in the same tissue, they examined five index cases who had died at the stage of mild cognitive impairment or early Alzheimer's disease, identified by longitudinal neuropsychological assessment, against seven age-matched cognitively normal subjects. Tangles and tau immunostaining were present within cholinergic nucleus basalis neurons *even in the cognitively normal subjects*. The percentage of tauopathy-containing neurons was greater in the cognitively impaired and correlated significantly with memory scores obtained one to eighteen months before death. Cytopathology in cortical cholinergic pathways, they concluded, is a very early event in the continuum from advanced age through mild cognitive impairment to disease (Mesulam et al., 2004).

Three things are worth separating. The pathology is early, present before any cognitive impairment at all; it is *in the cholinergic cell*, not merely in the territory; and its extent tracks a clinical measure taken close to death. What it is not is specific — tangles in the nucleus basalis of cognitively normal elderly subjects are a feature of ageing, so their presence cannot by itself distinguish the disease. The quantity, not the fact, carries the information.

Mesulam's anatomical explanation for the early involvement is the one given in Chapter 2 and the most parsimonious available: the nucleus is anatomically continuous with the basomedial limbic structures in which neurofibrillary degeneration begins, and its densest reciprocal cortical relationships are with limbic and paralimbic cortex. On this account it is not singled out by a special intrinsic vulnerability to tau; it is caught early because of where it sits and what it is wired to (Mesulam, 2013). That is compatible with, and does not require, the trophic mechanism of Part III, and we will argue that both operate.

The largest quantitative study of the nucleus in a pathologically subtyped cohort adds a dimension the founding studies could not see. Hanna Al-Shaikh and colleagues, working with the Florida Autopsied Multi-Ethnic cohort, quantified thioflavin-S-positive tangles and neuronal density

in the anterior nucleus basalis in 1,361 neuropathologically diagnosed Alzheimer cases and 103 non-demented controls, blinded to grouping, with the cohort stratified by corticolimbic tangle distribution into hippocampal-sparing, typical and limbic-predominant subtypes. Tangle counts and neuronal densities ran in opposite directions across the three (Table 3). Higher tangle burden in the nucleus was associated with younger age at onset in both hippocampal-sparing and typical disease, and in typical disease also with female sex, the apolipoprotein E $\epsilon 4$ allele, and lower Mini-Mental State Examination scores (Hanna Al-Shaikh et al., 2020).

The subtype gradient bears directly on the therapeutic question. The lesion is heaviest in the subtype with the youngest onset and the most neocortical, least limbic-weighted tangle distribution, and lightest in the oldest, most limbic-confined subtype. Whatever else that means, it means "the cholinergic deficit of Alzheimer's disease" is not one quantity: it varies by a factor approaching two across pathological subtypes that are routinely pooled in trials.

Table 3 — The nucleus basalis across neuropathological subtypes of Alzheimer's disease.

Subtype	Median age at death	Median nbM tangle count per 0.125 mm ²	Median nbM neuronal density per mm ²	n
Hippocampal-sparing AD	72 (66–80)	14 (9–20)	22 (17–28)	163 / 148
Typical AD	81 (76–86)	10 (5–16)	25 (19–30)	937 / 727
Limbic-predominant AD	86 (82–90)	8 (5–11)	26 (19–32)	163 / 127

Interquartile ranges in parentheses. Counts and densities from Hanna Al-Shaikh et al. (2020); n given for tangle count / neuronal density.

7. The Imaging Claim That Inverts the Staging Model

The prevailing model of Alzheimer progression in human neuroimaging is cortical-first: degeneration begins in entorhinal cortex and spreads through the temporoparietal cortex. That model has organised a great deal of research, and for the basal forebrain it has a specific implication — the nucleus basalis, dependent on its cortical targets, should degenerate *after* them.

Two studies claim the opposite, and they are the reason this nucleus is now contested territory rather than settled history.

Schmitz and Spreng examined whether longitudinal decreases in basal forebrain and entorhinal grey matter volume were interdependent and sequential, in a large cohort spanning cognitively normal to Alzheimer's disease. Basal forebrain volume predicted longitudinal entorhinal degeneration; models of parallel degeneration, or of entorhinal origin, received what the authors describe as negligible support. Comparing cognitively matched normal subgroups split by an amyloid biomarker, they found abnormal degeneration in the basal forebrain but *not* the entorhinal cortex, with both appearing only among prodromal, mildly amnesic individuals (Schmitz & Spreng, 2016).

Fernández-Cabello and colleagues then tested whether the comparative evidence generalised — the question that matters and that the field too rarely asks. Using two independent ADNI samples of 284 and 553 participants, with harmonised cerebrospinal fluid assays for amyloid- β and phosphorylated tau and two years of longitudinal structural MRI, they applied a predictive modelling strategy in which baseline volume in a seed region must account for longitudinal change in a target region. Predictive spread favoured the nucleus-basalis-to-entorhinal model over its reverse, and generalised across both samples. The cerebrospinal fluid ratio of phosphorylated tau to amyloid- β moderated the relationship — which the authors read as consistent with trans-synaptic spread — and the moderation survived adjustment for clinical diagnosis. An exploratory voxel-wise analysis found smaller baseline nucleus basalis volume predicting degeneration specifically in localised entorhinal and perirhinal regions, while smaller baseline entorhinal volume predicted degeneration in medial temporal cortex, recapitulating the prior staging model from the other seed (Fernández-Cabello et al., 2020).

Two further results give the structural claim purchase. Teipel and colleagues compared basal forebrain and hippocampal volume as predictors of cortical amyloid burden on florbetapir PET across 670 participants in four diagnostic groups: basal forebrain volume classified amyloid status

significantly more accurately, while hippocampal volume identified the clinical categories better; in logistic regression both discriminated impaired from normal, but *only* basal forebrain volume predicted amyloid status (Teipel et al., 2014). In a multicentre study of 79 patients with mild cognitive impairment, 35 of whom converted over a mean of twenty-one months, reduced basal forebrain grey matter volume was among the predictors of conversion, and increased mean diffusivity in the right basal forebrain predicted increased risk (Brueggen et al., 2015).

Two observations about normal ageing frame all of this. Zaborszky's atlas paper reported both right and left Ch4 significantly smaller with age as a covariate (Zaborszky et al., 2008); and Wolf and colleagues found, in 43 healthy elderly subjects, that basal forebrain volumes were associated with general intelligence, most strongly in the regions corresponding to Ch4p and the nucleus subputaminalis, whose volumes were in turn related to widespread temporal, frontal and parietal grey and white matter (Wolf et al., 2014). Ch4 shrinks with age in everyone, and how much of it a person has is associated with how well they perform in the absence of disease.

8. The Counterweight: What Volume Means, and What the Order Licenses

A paper that has found a result it likes should go looking for the study that complicates it. Here is that study, and it is recent and large.

Xia and colleagues analysed 779 older individuals from the Australian Imaging, Biomarker and Lifestyle study, all of whom had undergone amyloid PET with NAV4694, tau PET with the early-stage-sensitive tracer MK-6240, and structural MRI. Co-occurrence of amyloid and mesial-temporal tau was associated with reduced Ch4 volume in cognitively unimpaired individuals, and mesial-temporal tau burden was associated with Ch4 volume *exclusively* in cognitively unimpaired individuals with established amyloid pathology — an association not observed for the hippocampus. It persisted into mild cognitive impairment and was not apparent in Alzheimer's dementia (Xia et al., 2025).

This is not a refutation of the preceding chapter and should not be presented as one. Both literatures agree that Ch4 is affected in the preclinical period, that the effect is specific to Ch4 rather than a general atrophy, and that amyloid status conditions it. Where they pull apart is direction of dependence: Fernández-Cabello's predictive modelling makes the nucleus the upstream term with respect to subsequent entorhinal volume change, while Xia's cross-sectional association makes mesial-temporal tau the term that accounts for Ch4 volume in exactly the population — cognitively unimpaired, amyloid-positive — in which the upstream claim is made. A longitudinal predictive model and a cross-sectional association are not the same evidential object, and both can be correct if the relationship is bidirectional: tau in the adjacent limbic cortex loads the nucleus, and the compromised nucleus accelerates degeneration in the cortex that loaded it. That reading is a proposal, not a finding, and we grade it accordingly.

What should not be waved past is the question of what a basal forebrain volume measures, because the chronological argument of both chapters rests on it.

Two problems are technical. The mask contains cholinergic neurons, parvalbumin-positive GABAergic projection neurons whose function is demonstrably independent of them (Kim et al., 2015), other non-cholinergic cells, dendrites, afferent and efferent fibre bundles, vasculature and glia; a reduction in its volume is as consistent with lost afferents or dendritic retraction as with cholinergic cell loss. And the structure is small, has no visible boundary on standard structural MRI, and is localised by warping an atlas built from ten postmortem brains into each subject's space (Zaborszky et al., 2008) — registration error and partial-volume effects in a thin, fibre-interdigitated

sheet adjacent to cerebrospinal fluid and to the globus pallidus are not small, and are not independent of atrophy elsewhere.

The third problem is the one the post-mortem literature makes unavoidable, and it is the most interesting. In the clinical period during which the imaging detects volume change, the nucleus is not losing many cells: Gilmor's count is flat in mild cognitive impairment. So whatever the MRI tracks in the preclinical and prodromal period is largely *not* neuronal death but somatic atrophy, dendritic simplification, loss of afferents or shrinkage of the neuropil — exactly what a cell undergoing trophic withdrawal and phenotypic downregulation would undergo while remaining alive. Arendt's morphometry, which counted *large* neurons, is subject to the same reading: a size-thresholded count in a nucleus whose cells are shrinking records as missing the cells that have fallen below the threshold.

This is not a reason to discard the imaging. It is a reason to read it as what it most likely is: an *in vivo* measure of the atrophy of a nucleus under trophic stress, available years before the cells are lost. Read that way, the imaging and the stereology stop disagreeing. The nucleus shrinks early, which the MRI sees; it dies late, which the cell counts see; and the gap between the two is the interval this paper is about.

What the order actually licenses

The chronological literature on this nucleus has acquired a rhetorical register that outruns its evidence, and the deflation is worth performing explicitly, because the argument of Part III does not need the overstated version. The strongest claim in circulation is that Alzheimer's disease originates in the basal forebrain: Schmitz and Spreng's title says degeneration there "precedes and predicts" cortical spread, and their abstract says the finding challenges the belief that the disease has a cortical origin. Fernández-Cabello's conclusion is more carefully worded — that this is a robust and reliable *upstream* event — and the careful wording is the correct one. Four limits apply.

Upstream is not origin. A predictive model establishes that baseline volume in one region accounts for subsequent change in another. It does not establish that the first region is where the disease begins, because both regions may be downstream of something neither model contains. In this disease the candidates are not hypothetical: Braak, Thal, Ghebremedhin and Del Tredici, staging 2,332 unselected brains, found 58 cases with abnormal tau confined to subcortical sites, predominantly the locus coeruleus, with no abnormal cortical tau at all, and reported that the first neocortical plaques appeared only after the onset of brainstem tauopathy (Braak et al., 2011) — and the nucleus basalis receives a noradrenergic projection from that nucleus (Mesulam, 2013). A model with two regions in it can only order those two regions.

Volume is not death. Established above. The nucleus that the imaging sees shrinking is a nucleus whose cell count is, at that stage, preserved.

The region is not the cell type. Established in Chapter 4.

Early is not specific. Tauopathy in cholinergic nucleus basalis neurons is present in cognitively normal elderly subjects (Mesulam et al., 2004); Ch4 shrinks with age in people who never develop dementia (Zaborszky et al., 2008); and basal forebrain volume in healthy elderly adults is associated with general intelligence rather than with disease (Wolf et al., 2014). An early change that is also a normal change requires a quantitative threshold argument, not a categorical one, and the threshold has not been established.

What the order does license is narrower and more useful. By the time a patient reaches mild cognitive impairment, this nucleus has already lost roughly half of its receptor-bearing neurons (Mufson et al., 2000) without losing its cells (Gilmor et al., 1999), while its cortical enzyme activity is in places compensatorily raised (DeKosky et al., 2002); and it is measurably smaller in vivo, years earlier, in people who are cognitively normal and amyloid-positive (Schmitz & Spreng, 2016; Teipel et al., 2014). Something is happening to this neuron, early, that is neither cortical cell death nor its own. The next part identifies what, and the identification is not speculative: every element of the mechanism has been measured in human tissue.

Part III — The Ransom



9. The Contract, and the Signal Made Outside the Cell

The basal forebrain cholinergic neuron does not manufacture its own licence to live. It depends for trophic support on nerve growth factor supplied by the cortical and hippocampal territories it innervates, taken up at its distal terminals, and conveyed to the soma by retrograde axonal transport in a signalling endosome, with the two nerve growth factor receptors — the high-affinity tyrosine kinase TrkA and the low-affinity p75 neurotrophin receptor — determining what the arriving signal does. This is the organising principle of basal forebrain neurobiology and it is among the best-supported facts in the field (Mufson et al., 2008).

Two properties of this arrangement make it a liability of a very particular shape.

The first is that it is *conditional on a target*. The survival of the neuron is contracted out to tissue that the disease dismantles. If cortical synapses are lost, the terminals from which the factor is taken up are lost with them, and the supply falls for reasons that have nothing to do with the health of the nucleus.

The second is that it is *conditional on a road*. Not only must the factor be present; it must be captured, internalised, and carried a hundred metres against the direction of normal cargo flow, through a thinly branched axon, to a soma that will only respond when the endosome arrives. A trophic dependency implemented through retrograde transport is a dependency on the integrity of the transport apparatus, and the transport apparatus is not a passive conduit — it is endosomal machinery with its own failure modes.

The contract is also, importantly, specific to this sector. The mesopontine cholinergic groups, which share the nucleus basalis's transmitter, its size, its isodendritic geometry and its position in the reticular core, do not carry nerve growth factor receptor protein, and they do not degenerate appreciably in this disease. The asymmetry has been visible in the literature since 1989 (Mesulam, Geula, Bothwell & Hersh, 1989; Woolf, Jacobs & Butcher, 1989) and it is the cleanest available demonstration that what the disease destroys in the basal forebrain is not the cholinergic cell but the *dependent* cell.

What follows is an account of a contract that fails in four places at once, in a pattern that is measurable in human tissue, and that begins before dementia.

The signal that has to be made outside the cell

The usual mental model of a neurotrophin — a finished protein released by a target and captured by a terminal — is wrong, and the way in which it is wrong is the hinge of this paper.

Bruno and Cuello demonstrated that nerve growth factor is released into the extracellular space in an activity-dependent manner *in its precursor form*, proNGF, and that its maturation and its degradation both take place in that extracellular compartment, through the coordinated release and action of proenzymes and enzyme regulators. The converting cascade and its endogenous regulators — tissue plasminogen activator, plasminogen, neuroserpin, precursor matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1 — are colocalised in cortical neurons and released on neuronal stimulation. Plasmin, generated from plasminogen by tissue plasminogen activator, cleaves proNGF to mature nerve growth factor; matrix metalloproteinase 9 degrades the mature product. The authors showed the mechanism operates *in vivo*: applying inhibitors of the converting and degrading enzymes to the central nervous system produced dramatic alterations in tissue levels of precursor or mature nerve growth factor respectively. They named the pathological corollary explicitly — that alterations of this cascade might cause or contribute to a lack of proper neuronal trophic support in conditions including Alzheimer's disease (Bruno & Cuello, 2006).

Three consequences follow, and each creates a failure mode a simple ligand-scarcity model cannot see.

First, the quantity that matters is not how much nerve growth factor the cortex contains but the ratio of two enzymatic rates — conversion of the precursor against destruction of the product. A cortex rich in nerve growth factor immunoreactivity can be trophically impoverished if most of that immunoreactivity is uncut precursor, or if the mature product is degraded faster than it is captured.

Second, because the cascade is released on neuronal stimulation, trophic supply is *activity-dependent*: a target that has become less active supplies less conversion, independently of how much precursor it still makes.

Third, the precursor is not an inert reservoir. proNGF binds p75 in concert with sortilin and, in that configuration, can signal toward apoptosis rather than survival, so a shift from mature to precursor is potentially the substitution of a negative signal rather than merely the loss of a positive one. That consequence has been established principally in cellular systems, and we grade it accordingly.

This is the architecture. The next chapter is the evidence that every component of it is deranged in human Alzheimer's disease, and deranged early.

10. Four Independent Failures in One Pathway

The four measurements below were made by different groups, in different tissues, with different methods. They are not four readings of one phenomenon. They are four separate derangements of a single pathway, and they point the same way.

One: the precursor accumulates, and it accumulates before dementia. Peng, Wu, Mufson and Fahnstock measured proNGF by Western blot in parietal cortex from twenty individuals with no cognitive impairment, twenty with mild cognitive impairment and nineteen with mild-to-moderate Alzheimer's disease. proNGF was 1.4-fold greater in mild cognitive impairment and 1.6-fold greater in mild-to-moderate disease than in the unimpaired, consistent with the group's earlier two-fold increase at end stage, and correlated negatively with Mini-Mental State Examination score (Peng et al., 2004).

Two: the enzyme that destroys the mature product is more active, and it is more active before dementia. Bruno, Mufson, Wu and Cuello assayed matrix metalloproteinase 9 activity in human frontal and parietal cortex across the same three categories and found it significantly greater in both Alzheimer's disease and mild cognitive impairment than in the unimpaired ($p < 0.01$), with inverse correlations against both Global Cognitive Score and Mini-Mental State Examination score — a metabolic failure in the maturation and degradation pathway driving exacerbated degradation of mature nerve growth factor in early disease (Bruno et al., 2009).

Taken together, these two measurements describe the cascade failing from both ends in the same clinical stage: less conversion of the precursor, more destruction of the product.

Three: the receiving apparatus is dismantled asymmetrically, and the asymmetry favours the pro-apoptotic arm. In the nucleus basalis, the number of TrkA-immunoreactive perikarya falls by forty-six per cent before dementia (Mufson et al., 2000). In the cortex, the picture is more specific than is usually reported and we state it exactly: Counts, Nadeem, Wu, Ginsberg, Saragovi and Mufson measured p75 and TrkA protein by quantitative immunoblotting in five cortical regions across four clinical groups, and found cortical p75 levels *stable* across all groups, while TrkA was reduced by approximately fifty per cent in mild-to-moderate and severe Alzheimer's disease compared with both the unimpaired and mild cognitive impairment, in every region examined except visual cortex, with TrkA levels correlating with Mini-Mental State Examination score in anterior cingulate, superior frontal and superior temporal cortex (Counts et

al., 2004). The cortical TrkA loss, then, arrives *later* than the perikaryal TrkA loss — it is absent in mild cognitive impairment — which is informative: the receptor disappears from the cell body before it disappears from the projection field.

In both compartments the direction of the asymmetry is the same. The survival-signalling receptor is lost; the receptor capable of signalling toward death is retained. Mufson and colleagues' formulation of the therapeutic implication — that drugs maintaining a homeostatic balance between TrkA and p75 might slow the onset of disease — follows directly (Mufson et al., 2008).

Four: the factor is present in the cortex and missing in the nucleus, which can only be read as a delivery failure. This is the most underused result in the literature. Scott, Mufson, Weingartner, Skau and Crutcher measured nerve growth factor protein by two-site ELISA across ten brain regions in Alzheimer's disease and aged normal brain, with tissue from three independent brain banks. Immunoreactivity was at least moderately *increased* in virtually every region examined — hippocampus, superior temporal and frontal gyri, inferior parietal lobule, frontal and occipital poles, cerebellum, amygdala, putamen — with one exception: in the nucleus basalis of Meynert it was significantly *decreased*. The authors drew the only available inference, that the pattern is consistent with impaired retrograde transport of nerve growth factor to the nucleus (Scott et al., 1995).

A ligand that is abundant at the source and deficient at the destination is not a scarce ligand. It is an undelivered one.

Table 4 — Four failures in the nerve growth factor pathway, by clinical stage.

Failure	Measurement	Earliest stage detected	Source
Precursor not converted	proNGF protein in parietal cortex 1.4-fold (MCI), 1.6-fold (mild–moderate AD); negative correlation with MMSE	Mild cognitive impairment	Peng et al. (2004)
Mature product over-degraded	MMP-9 activity in frontal and parietal cortex ($p < 0.01$); inverse correlation with global cognition and MMSE	Mild cognitive impairment	Bruno et al. (2009)
Survival receptor lost, death receptor kept	TrkA-immunoreactive nbM perikarya –46%; cortical TrkA –50% in AD but not MCI; cortical p75 unchanged throughout	MCI (perikarya); AD (cortex)	Mufson et al. (2000); Counts et al. (2004)
Product not delivered	NGF protein in nearly every cortical and subcortical region, significantly in nucleus basalis	Established AD	Scott et al. (1995)

II. The Road Home: Endosomes, Transport, and an Unbuffered Cell

The delivery failure Scott's gradient implies has since been given a mechanism, and the mechanism was found in a disease that is not Alzheimer's disease but becomes it.

Salehi, Delcroix, Belichenko and colleagues, with Nixon, Goldstein and Mobley, used the Ts65Dn and Ts1Cje mouse models of Down syndrome to ask what, in trisomy, degenerates the basal forebrain cholinergic neuron. The answer was specific: increased dose of the amyloid precursor protein gene, *App*, markedly decreases retrograde nerve growth factor transport and causes these neurons to degenerate. Transport was also decreased in mice expressing wild-type human APP or a familial-Alzheimer mutant APP — significantly, but less markedly and without evident degeneration. Because the evidence suggested an intra-axonal defect, they examined early endosomes within cholinergic axons: nerve growth factor-containing early endosomes were *enlarged* in Ts65Dn mice, with increased App content (Salehi et al., 2006).

That is a gene-dose experiment identifying a single gene, central to Alzheimer's disease, as sufficient to break the retrograde delivery of the signal on which this particular cell type depends — and identifying the lesion as an enlargement of the signalling endosome itself.

The human basal forebrain shows the corresponding derangement, and shows it before dementia. Ginsberg, Mufson, Alldred, Counts, Wu, Nixon and Che microdissected cholinergic basal forebrain neurons from postmortem brains across the same three clinical groups and found selective upregulation of the genes regulating early and late endosomes: *rab4*, *rab5*, *rab7* and *rab27* were upregulated in both impaired groups, the upregulation correlated with antemortem cognitive decline, the result was validated by quantitative PCR, and *rab5* protein itself was elevated on immunoblot. Endosomal pathology, they proposed, accelerates endocytosis and endosome recycling and may underlie the selective vulnerability of these neurons (Ginsberg et al., 2011).

Put the two results beside each other. In the mouse, extra App dose enlarges the signalling endosome and blocks the retrograde transport of nerve growth factor, and the cells die. In the human nucleus basalis, the regulators of exactly those endosomal compartments are upregulated at the stage of mild cognitive impairment, in proportion to cognitive decline. And in the human brain, nerve growth factor protein accumulates in the cortex while falling in the nucleus (Scott et al., 1995). Three independent observations, one road.

The Down syndrome literature supplies the natural experiment in people. Iulita, Ower, Barone and colleagues, with Cuello, measured plasma markers in thirty-one individuals with Down syndrome, with and without dementia, against thirty-one healthy controls, with cognitive follow-up over two years. proNGF, matrix metalloproteinases 1 and 3, metalloproteinase 9 activity and the inflammatory cytokines tumour necrosis factor α , interleukin 6 and interleukin 10 were all higher in Down syndrome plasma *even at Alzheimer-asymptomatic stages*; declining plasma amyloid- β 42 and rising proNGF correlated with decline across the two years; and a combined measure of amyloid and inflammatory molecules strongly predicted prospective deterioration (Iulita et al., 2016).

In a population genetically guaranteed to develop Alzheimer pathology, the derangement of the nerve growth factor maturation pathway is detectable in blood before the dementia, and it tracks the decline. That is the strongest available human evidence that the trophic failure is not a late consequence of cortical destruction.

Calcium, calpain, and the unbuffered cell

The trophic account is not the only mechanism of selective vulnerability operating in this nucleus, and a paper that argued it was would be overselling. The most substantial independent candidate concerns calcium handling, and it has the virtue of being measured in the same human cells.

Geula's group has pursued the observation that basal forebrain cholinergic neurons lose the calcium-binding protein calbindin-D28K with age, and that the loss is associated with tangle formation and degeneration in Alzheimer's disease. Riascos and colleagues extended this to a panel of calcium-responsive proteins, combining laser capture of identified cholinergic neurons with analysis of basal forebrain homogenates. Calbindin and calcium/calmodulin-dependent protein kinase I messenger RNA were progressively downregulated in captured cholinergic neurons across the normal-aged-Alzheimer continuum; calbindin, CaMKI δ and GAP43 proteins were progressively lost in homogenates in ageing and disease; and activated μ -calpain — a calcium-sensitive protease that degrades both CaMKI and GAP43 — was significantly increased in the normal aged basal forebrain and *ten times higher* in Alzheimer basal forebrain, the overactivation confirmed by proteolytic fragments of its substrate spectrin (Riascos et al., 2014).

Three features deserve emphasis. The calbindin loss begins in normal ageing, making it a candidate substrate for why this nucleus is vulnerable in the first place rather than a consequence of the disease. The calpain activation is present in aged controls and amplified tenfold in disease — a quantitative rather than categorical difference, the same structure the tangle data have. And the substrates being degraded are proteins of signalling and growth: a cell losing its calcium buffer and gaining a calcium-activated protease is a cell whose capacity to *respond* to a trophic signal is being destroyed from inside, at the same time as the signal itself fails to arrive.

That last point is why this chapter sits inside Part III rather than beside it. The two mechanisms are not competitors. A neuron that has lost half its TrkA, receives uncut precursor rather than mature factor, cannot carry what it does capture back to the soma, and is cleaving its own response machinery with an overactive calpain, is a neuron in which every stage of a trophic response has been independently compromised. We do not know their relative contributions and decline to assign them.

12. Why Giving Nerve Growth Factor Did Not Work

If the lesion were a shortage of nerve growth factor, supplying nerve growth factor would help. It has been tried, in the most serious way such a thing has been tried in any adult neurodegenerative disease, and the result is one of the most informative negative findings in the field — informative because of what it rules out.

Tuszynski's group initiated nerve growth factor gene therapy for Alzheimer's disease in 2001, the first gene delivery programme in an adult neurodegenerative disorder, and in 2015 reported postmortem findings in ten patients surviving one to ten years after treatment. The results establish something important and positive: degenerating neurons in the Alzheimer brain *responded*. All ten showed axonal sprouting toward the nerve growth factor source; in the three who had received unilateral gene transfer, allowing a within-subject comparison, cholinergic neuronal hypertrophy occurred on the treated side ($p < 0.05$); cellular signalling and functional markers were activated in the two given adeno-associated viral delivery; neurons bearing tau pathology as well as neurons free of it expressed the transgene, meaning a cell already carrying the disease's cytoskeletal lesion can still be infected and still activate signalling. Sprouting persisted for ten years, with no adverse pathological effects attributable to the factor (Tuszynski et al., 2015).

The phase 2 trial did not translate this into benefit. Rafii and colleagues randomised forty-nine participants with mild-to-moderate disease one-to-one to stereotactically guided intracerebral injection of adeno-associated viral vector serotype 2 carrying nerve growth factor into the nucleus basalis of each hemisphere, or to sham surgery, across ten US academic centres. Delivery was safe and well tolerated through twenty-four months, with no significant difference from placebo on the primary outcome, the cognitive subscale of the Alzheimer's Disease Assessment Scale ($p = .17$), and no effect on selected biomarkers. The authors' closing sentence names the gap: pathological confirmation of accurate gene targeting is needed (Rafii et al., 2018).

Two readings are available and they are not mutually exclusive.

The first is a delivery failure. A vector injected into a diffuse, border-free sheet of neurons dispersed among fibre bundles may or may not be where the cells are, and the trial's authors say as much; nothing in the phase 2 result distinguishes "the mechanism is wrong" from "the protein did not reach enough of the right cells".

The second is the one this paper's mechanism predicts. Even perfectly delivered, mature nerve growth factor addresses one of the four failures in Table 4 and leaves three standing: it does not restore TrkA to the forty-six per cent of perikarya that have stopped displaying it, does not slow the metalloproteinase degrading the mature product, and does not repair the retrograde transport that Scott's gradient and Ginsberg's rab upregulation indicate is failing. Supplying more of a factor to a cell that has lost its receptor, cannot carry what it binds, and is cleaving its own downstream signalling machinery with an overactive calpain is an intervention addressed to the one element that was arguably never limiting. Scott's data say so directly: in the Alzheimer brain, nerve growth factor is already *increased* nearly everywhere except in the nucleus that needs it.

We grade this reading as inference. It is, however, an inference with a clean experimental test, specified in Chapter 16: a trial of trophic support in this nucleus should be stratified or combined by receptor and transport status, not conducted as though ligand concentration were the variable.

Part IV — The Consequence and the Clinic

13. What the Cortex Loses, and Why Raising the Mean Cannot Restore a Rhythm

The nucleus basalis fails in the basal forebrain and the deficit is suffered in the cortex. Three classes of measurement describe what arrives there, and they agree.

The terminals. The most direct in vivo measure of the cholinergic projection is a ligand for the vesicular acetylcholine transporter, which is expressed in presynaptic cholinergic terminals. Aghourian, Legault-Denis, Soucy, Rosa-Neto, Gauthier, Kostikov, Gravel and Bédard imaged twelve participants — six with Alzheimer's disease, six healthy — with three consecutive PET sessions each: fluoroethoxybenzovesamicol for cholinergic terminals, NAV4694 for amyloid, and fluorodeoxyglucose for metabolism. Reductions in fluoroethoxybenzovesamicol uptake were major, were evident in *every* individual Alzheimer subject, and were present across multiple cortical areas. The cholinergic tracer distinguished the groups with greater sensitivity than either the metabolic or the amyloid tracer; its uptake correlated positively with metabolism and negatively, in restricted areas, with amyloid; and both the Mini-Mental State Examination and the Montreal Cognitive Assessment correlated significantly with the cholinergic and metabolic tracers but not with the amyloid tracer (Aghourian et al., 2017).

The cohort is six against six, and no conclusion about relative biomarker performance should be drawn from twelve people. What the study establishes is narrower and still valuable: cortical cholinergic terminal loss in established disease is large enough to be visible in every single patient.

The receptors. Kendziorra and colleagues imaged the $\alpha 4 \beta 2$ nicotinic acetylcholine receptor in nine patients with moderate Alzheimer's disease, eight with amnesic mild cognitive impairment and seven age-matched controls. Both patient groups showed significantly reduced binding potential in typically affected regions, binding correlated with severity of impairment, and — the prognostically interesting detail — among the mild cognitive impairment patients only the five who subsequently converted showed a reduction (Kendziorra et al., 2011).

The enzyme. Cortical choline acetyltransferase activity, the measurement that founded the field, falls below normal only at end stage (DeKosky et al., 2002), having been unchanged or elevated throughout the clinical period in which patients are treated.

Set the three beside one another and a specific picture emerges. During mild cognitive impairment the synthesising enzyme is intact or compensatorily raised, the postsynaptic nicotinic receptor is already reduced in those who will convert, and the nucleus of origin has lost half its trophic receptors without losing its cells. The system is not, in that window, short of the capacity to make acetylcholine. Something about its regulation, its delivery or its reception fails first.

Why raising the mean cannot restore a rhythm

The cholinesterase inhibitors work. The Cochrane synthesis of thirteen randomised, double-blind, placebo-controlled trials of donepezil, galantamine and rivastigmine at recommended doses over six months to a year found improvements in cognitive function averaging 2.7 points (95% CI -3.0 to -2.3) in the midrange of the 70-point ADAS-Cog scale, with benefits also on global clinical state, activities of daily living and behaviour, and with nothing to suggest the effects are smaller in severe or in mild dementia. It also found that more patients leave treatment than placebo on account of adverse events — approximately twenty-nine per cent against eighteen per cent — with nausea, vomiting and diarrhoea significantly more frequent. The reviewer's conclusion was that the three drugs are efficacious for mild to moderate Alzheimer's disease and that none of the treatment effects are large (Birks, 2006).

The converse evidence that the system is load-bearing is, if anything, stronger. Coupland and colleagues examined 58,769 patients with a dementia diagnosis against 225,574 matched controls aged fifty-five or older in English general practice, calculating cumulative exposure to fifty-six strongly anticholinergic drugs. The adjusted odds ratio for dementia rose from 1.06 (95% CI 1.03–1.09) in the lowest exposure band to 1.49 (95% CI 1.44–1.54) in the highest, with significant elevations for anticholinergic antidepressants, antiparkinson drugs, antipsychotics, bladder antimuscarinics and antiepileptics; the associations persisted when exposure windows were restricted to three-to-thirteen and five-to-twenty years before the index date, were stronger in cases diagnosed before eighty, and yielded a population-attributable fraction of 10.3 per cent (Coupland et al., 2019). This is observational and subject to the usual confounding by indication and prodromal prescribing. But blocking the system is associated with harm at a magnitude that dwarfs the benefit of enhancing it.

Why should pharmacological enhancement of a load-bearing, demonstrably depleted system produce only 2.7 points?

The standard explanations are correct as far as they go: the drugs do not address the degeneration, and by diagnosis the terminals from which acetylcholine would be released are already substantially lost (Aghourian et al., 2017). We propose a third, complementary explanation, which follows from Part I and is an inference of ours rather than a result.

The cholinergic signal is a difference, not a level, and three independent lines establish it. Cortical acetylcholine carries information in sub-second transients at cue detection, with a *decrease* in pre-cue activity predicting detection and an *increase* predicting a miss (Parikh et al., 2007). The source neurons burst with theta in waking and REM and virtually cease firing in slow-wave sleep (Lee et al., 2005). And raising cholinergic tone during slow-wave sleep in healthy humans abolishes declarative consolidation while sparing non-declarative learning and having no effect during waking (Gais & Born, 2004).

A cholinesterase inhibitor raises the ambient concentration of acetylcholine wherever it is released, continuously, for as long as the drug is in the body. On the evidence above that intervention has two opposing consequences: it increases the transmitter available at each phasic release event, which should help, and it raises the floor against which phasic events must be detected while elevating tone during the state in which the physiological signal is near zero and elevation is experimentally harmful. The net effect of a benefit with a structurally opposed cost is a small net benefit, which is what forty years of trials have found.

The prediction that follows is testable and is specified in Chapter 16: an intervention that restores modulation *depth* — a phasic, state-gated or circadian-timed delivery, or stimulation of the source timed to behaviour — should outperform an equivalent average increase in tone. The nearest existing test of that idea is not pharmacological, and it is the subject of the next section.

Two honest caveats. First, nothing in the human cholinesterase-inhibitor literature was designed to test timing, so the argument above is an interpretation of trial results rather than a result. Second, the drugs' pharmacokinetics are not flat, and the degree to which they actually abolish the diurnal cholinergic excursion in human cortex has not, to our knowledge, been measured; it should be, and that measurement is cheaper than any trial.

14. The Brake on Microglial Reactivity, and Stimulating the Source

The cholinergic hypothesis assigned this nucleus one job: supplying the cortex with a transmitter for cognition. There is reason to think it has a second, and that the second may be the one that matters for the disease's progression.

The starting point is outside the brain. In the peripheral nervous system, cholinergic input restrains the inflammatory responses of macrophages, promoting clearance while preventing runaway inflammation; microglia are the brain's resident macrophages. Schmitz, Soreq, Poirier and Spreng asked whether basal forebrain cholinergic input performs the analogous function on microglial responses to amyloid and tau, dividing cognitively normal older ADNI adults by the relative concentrations of phosphorylated tau and amyloid in cerebrospinal fluid into neurotypical and preclinical subgroups and following them longitudinally with structural MRI, cerebrospinal fluid soluble TREM2 and complement expression in the blood transcriptome.

Longitudinal loss of basal forebrain volume was greater in the preclinical subgroup, and across preclinical adults it was associated with greater accumulation of soluble TREM2 and higher peripheral blood complement C3 expression — none of it attributable to whole-brain grey matter degeneration. Preclinical APOE $\epsilon 4$ carriers showed the largest volume loss and the highest C3 expression. The authors' reading is that an age-related loss of cholinergic neuromodulation may remove a key check on microglial reactivity to accumulating amyloid and tau (Schmitz et al., 2020).

This is an association in observational data and the causal direction is not established by it: microglial reactivity could as easily be damaging the basal forebrain as being released by its loss. What makes the inflammatory reading more than decorative is a piece of interventional evidence, small and open-label, reported in the next chapter.

If the cholinergic brake is real, the consequence for how this nucleus is understood is substantial. A nucleus that merely supplies a cognitive transmitter fails and produces a deficit. A nucleus that also restrains the brain's immune effectors fails and produces a *permission* — a removal of restraint at the moment, early in the preclinical period, when amyloid and tau begin to accumulate and microglial reactivity is the mechanism by which that accumulation is either cleared or converted into damage. On that reading the early failure of Ch4 is not only the first cognitive lesion; it is a licence granted to everything downstream of it. We hold this as a proposal, and name what it needs: a demonstration that cholinergic input restrains microglial responses to amyloid and tau in brain tissue, with the direction of causation established, rather than an association between a

volume and a biomarker.

Stimulating the source

If the function of Ch4 is phasic and the drug is tonic, the obvious experiment is to drive the nucleus directly. It has been done three times, all small, all open-label or near-open-label, and the results are consistent enough to be worth stating carefully and weak enough that overstating them would be indefensible.

Kuhn, Hardenacke, Lenartz and colleagues at Cologne implanted six patients with mild to moderate disease for bilateral **low-frequency** stimulation of the nucleus basalis, with a four-week double-blind sham-controlled phase followed by eleven months open label; the primary outcome was the ADAS-Cog. On the basis of stable or improved primary outcome at twelve months, four of six were considered responders, and no severe or non-translational stimulation-related side effects occurred. The authors' conclusion, which we endorse, is that taking into account all the limitations of a pilot study, stimulation of this nucleus is technically feasible and well tolerated (Kuhn et al., 2015). A companion report found nutritional status mainly stable over the year, with five of six maintaining or improving body composition — relevant because progressive weight loss is a feature of advancing disease (Noreik et al., 2015).

A single severe case improved from a Mini-Mental State Examination score of 5 to 9 and an ADAS-Cog total of 43 to 33 over ten weeks, with a postoperative subdural effusion that resolved and an episode of abnormal thermoregulation on parameter changes (Zhang et al., 2021). A case report establishes nothing about efficacy; the thermoregulatory event is worth recording because the substantia innominata is adjacent to hypothalamic territory.

The most recent series is the most informative, and it connects this section to the last. Chen and colleagues stimulated nine individuals with moderate-to-severe disease bilaterally in an open-label trial, six completing the full twelve-month protocol with serial neuropsychiatric assessment and serum cytokine profiling. Stratified by baseline severity the trajectories diverged: the four with moderate disease maintained preoperative performance on the Montreal Cognitive Assessment and the Boston Naming Test over twelve months, while the two with severe disease declined significantly on both. Serum analysis showed a significant immunomodulatory effect — elevated anti-inflammatory interleukin 10 and interleukin 27, reduced pro-inflammatory CXCL10 and RANTES at twelve months (Chen et al., 2026).

What can and cannot be concluded should be stated bluntly. Six completers, no sham arm, no blinding, a severity stratification performed on four and two patients — and cognitive stability in an open-label dementia trial is the least reliable observation in clinical neurology. Nothing here demonstrates efficacy.

What the series does supply is a mechanistic signal predicted by a different literature. Schmitz's association linked loss of basal forebrain volume to rising soluble TREM2 and complement C3 in preclinical disease and proposed the nucleus as a brake on microglial reactivity (Schmitz et al., 2020). An independent group, stimulating that nucleus, reports a shift of circulating cytokines toward the anti-inflammatory. The convergence of a human observational association and a human interventional cytokine shift, from groups not addressing each other's hypotheses, is the kind of coincidence worth following, and it is the basis of experiment four in Chapter 16.

The second reason to take source stimulation seriously is conceptual rather than empirical, and it is the argument of the preceding section stated positively. Stimulation of the nucleus can be patterned; a drug in the bloodstream cannot. Kilgard and Merzenich's demonstration that *episodic* nucleus basalis stimulation paired with a stimulus reorganises adult cortex — with the direction of the reorganisation set by the properties of the paired stimulus (Kilgard & Merzenich, 1998) — is a demonstration that the therapeutic variable in this system is pairing, not dose. No trial in Alzheimer's disease has yet delivered stimulation of this nucleus paired with behaviour. That is an omission, not a negative result.

Part V — Ledger, Tests and Conclusion



15. A Graded Ledger

The argument of this paper mixes settled human anatomy, two large stereological series, a contested *in vivo* imaging literature, animal physiology that is strong but not human, a handful of very small interventional studies, and three mechanistic inferences of our own. Presenting these at a uniform level of confidence would misrepresent all of them. The grades below are deliberately coarse.

Established — supported by replicated human data or direct measurement, and unlikely to be overturned.

- Cortical and hippocampal cholinergic markers are reduced in advanced disease, the reduction correlating with plaque burden and cognitive impairment, and the nucleus basalis loses a large majority of its large neurons (Davies & Maloney, 1976; Perry et al., 1978; Whitehouse et al., 1982; Arendt et al., 1983).
- Ch4 is the principal cholinergic source for the cortical mantle and amygdala, is internally subdivided with preferential cortical territories, and is most densely interconnected with limbic and paralimbic cortex (Mesulam et al., 1983; Mesulam & Geula, 1988; Mesulam, 2013).
- Ch4 neurons carry high levels of nerve growth factor receptor protein and the mesopontine cholinergic neurons of Ch5–Ch6 do not; the mesopontine system accumulates neurofibrillary change without losing somata, so cholinergic phenotype alone cannot be a sufficient condition for vulnerability (Mesulam, Geula, Bothwell & Hersh, 1989; Woolf, Jacobs & Butcher, 1989).
- Nucleus basalis cholinergic neuron number is essentially preserved in mild cognitive impairment and reduced only non-significantly (~15%) in early disease (Gilmor et al., 1999).
- The number of nucleus basalis neurons displaying TrkA immunoreactivity falls ~46% in mild cognitive impairment, is not significantly lower in early disease, and does not correlate with Braak stage (Mufson et al., 2000).
- Cortical choline acetyltransferase activity is unchanged in mild cognitive impairment and mild disease, is significantly elevated in superior frontal cortex and hippocampus in mild cognitive impairment, and falls below normal only at end stage (DeKosky et al., 2002).
- Tau pathology is present within cholinergic nucleus basalis neurons in cognitively normal elderly subjects, is greater in the cognitively impaired, and correlates with antemortem memory scores (Mesulam et al., 2004).

- Nerve growth factor is released as a precursor and matured and degraded extracellularly by a plasmin- and MMP-9-dependent protease cascade with identified endogenous regulators (Bruno & Cuello, 2006).
- proNGF and MMP-9 activity are both increased in cortex in mild cognitive impairment, each correlating inversely with cognition (Peng et al., 2004; Bruno et al., 2009).
- Cortical TrkA protein is reduced ~50% in Alzheimer's disease but not in mild cognitive impairment, while cortical p75 is unchanged across all stages (Counts et al., 2004).
- Nerve growth factor protein is increased throughout the Alzheimer brain and significantly decreased in the nucleus basalis itself (Scott et al., 1995).
- rab4, rab5, rab7 and rab27 are upregulated in cholinergic basal forebrain neurons in mild cognitive impairment and Alzheimer's disease, in proportion to cognitive decline (Ginsberg et al., 2011); and increased *App* gene dose alone enlarges nerve growth factor-containing early endosomes, impairs retrograde transport and degenerates these neurons in trisomic mice (Salehi et al., 2006).
- Calbindin-D28K is progressively lost from human basal forebrain cholinergic neurons in ageing and disease, and activated μ -calpain is ~10-fold higher in Alzheimer basal forebrain (Riascos et al., 2014).
- A single basal forebrain cholinergic neuron maintains an axon arbor of up to 50 μ m in mouse with >1,000 branch points; the calculated human mean is on the order of 100 μ m (Wu, Williams & Nathans, 2014).
- These neurons fire maximally in theta-synchronised bursts during waking and REM sleep and virtually cease firing in slow-wave sleep (Lee et al., 2005); cortical acetylcholine rises in sub-second transients on cue detection, with pre-cue decreases predicting detection and increases predicting misses (Parikh et al., 2007); and raising cholinergic tone during slow-wave sleep in healthy humans abolishes declarative consolidation while sparing non-declarative learning (Gais & Born, 2004).
- Episodic nucleus basalis stimulation paired with a sensory stimulus reorganises adult primary sensory cortex, with the direction set by the paired stimulus (Kilgard & Merzenich, 1998).
- Cortically projecting parvalbumin-positive GABAergic basal forebrain neurons control cortical gamma oscillations independently of the cholinergic population (Kim et al., 2015).
- Cholinesterase inhibitors improve cognition by approximately 2.7 points on the 70-point ADAS-Cog, with higher withdrawal for adverse events than placebo (Birks, 2006).

- Cortical vesicular acetylcholine transporter binding is reduced in every individual with established disease examined by FEOBV PET (Aghourian et al., 2017), and $\alpha 4\beta 2$ nicotinic receptor availability is reduced in Alzheimer's disease and in converting mild cognitive impairment (Kendziorra et al., 2011).
- Nerve growth factor gene delivery induces axonal sprouting and cholinergic hypertrophy in the Alzheimer brain persisting up to ten years, including in tau-bearing neurons (Tuszynski et al., 2015), and produced no clinical or biomarker benefit in a sham-controlled phase 2 trial (Raffi et al., 2018).

Probable — supported by the best available evidence, with acknowledged limits of power, design or replication.

- Nucleus basalis lesions in primates impair attention rather than reliably impairing memory (Voytko, 1996 — a review of a small primate literature).
- Basal forebrain volume predicts subsequent entorhinal degeneration more strongly than the reverse, and the result generalises across two independent ADNI samples (Schmitz & Spreng, 2016; Fernández-Cabello et al., 2020 — large samples, but a single analytic strategy applied by one group).
- Basal forebrain volume classifies cortical amyloid status more accurately than hippocampal volume (Teipel et al., 2014).
- Nucleus basalis tangle burden is highest in hippocampal-sparing and lowest in limbic-predominant Alzheimer's disease, with neuronal density running in the opposite direction (Hanna Al-Shaikh et al., 2020 — large, single-centre, cross-sectional).
- Derangement of the nerve growth factor maturation pathway is detectable in plasma in Down syndrome before dementia and tracks subsequent cognitive decline (Iulita et al., 2016 — 31 cases, 2-year follow-up).
- Mesial-temporal tau burden is associated with Ch4 volume specifically in cognitively unimpaired amyloid-positive individuals, and not with hippocampal volume (Xia et al., 2025 — large, cross-sectional).
- Cumulative anticholinergic drug exposure is associated with incident dementia in a dose-dependent manner (Coupland et al., 2019 — large, observational, vulnerable to confounding by indication and prodromal prescribing).
- Loss of basal forebrain volume in preclinical Alzheimer's disease tracks rising soluble TREM2 and complement C3, most steeply in APOE $\epsilon 4$ carriers (Schmitz et al., 2020 — observational; direction of causation not established).

Weak — reported, and not yet load-bearing.

- Deep brain stimulation of the nucleus basalis is associated with cognitive stability in some patients with moderate disease (Kuhn et al., 2015, n = 6, four-week sham phase then open label; Chen et al., 2026, n = 6 completers, open label; Zhang et al., 2021, single case). No sham-controlled trial of adequate duration or size exists.
- Nucleus basalis stimulation shifts circulating cytokines toward an anti-inflammatory profile (Chen et al., 2026 — six completers, open label, no control group).

Inference — derived from established premises, not directly tested.

- *The ordering claim.* The nucleus basalis neuron surrenders detectable TrkA before it surrenders its cholinergic phenotype, and surrenders its phenotype before it dies. The premises are established and come from the same research programme and cohorts; the comparison is nonetheless made across two studies with different antibodies and cases, and a count of immunoreactive perikarya is not a census of cells possessing the protein. Experiment one in Chapter 16 converts this into an observation or refutes it.
- *The modulation-depth argument.* Because the cholinergic signal carries information in phasic excursions against a low background, and because elevating cholinergic tone at the wrong point in the sleep-wake cycle is experimentally harmful, a tonic cholinesterase inhibitor has a benefit and a structurally opposed cost, and the small net effect observed in trials is the predictable result. The premises are established; the application to the drug class is ours.
- *The delivery reading of the gene-therapy result.* Mature nerve growth factor addresses one of four demonstrated failures in the trophic pathway and leaves receptor loss, accelerated degradation and transport failure standing; a ligand already increased throughout the Alzheimer brain was unlikely to be the limiting term.

Proposal — advanced for testing, with the test specified in Chapter 16.

- The chronological disagreement between the predictive-spread imaging literature and the tau-PET association is resolved by bidirectionality: a locally generated failure of trophic maturation injures the nucleus before its targets are lost, and tau in the adjacent limbic cortex supplies a second, later insult to the same cells.
- The early failure of Ch4 constitutes a *permission* as well as a deficit, through the withdrawal of cholinergic restraint on microglial reactivity at the stage when amyloid and tau begin to accumulate.
- An intervention that restores modulation depth — phasic, state-gated or behaviourally paired — will outperform an equivalent average increase in cholinergic tone.

Two entries deserve comment, because they are where a critical reader should press. The ordering claim is the paper's load-bearing contribution and it is an inference across studies; we would rather carry it at that grade, visibly, than present it as the observation it is not. The second is the foundational literature itself, and the point is historical hygiene: the 1981 report that located the lesion in this nucleus was a *single case* compared with one age- and sex-matched control (Whitehouse et al., 1981), the series followed in 1982 and the morphometry in 1983. A field-defining anatomical claim entered the literature on an n of one, was corroborated, and was then treated as describing a natural history it had never measured. That sequence is why this paper spends five chapters on chronology.

16. What Would Refute This, and What Should Be Done

Conditions of refutation. The following results would falsify specific claims of this paper, and we name them in advance.

1. **Co-labelling showing that TrkA loss and phenotype loss are coincident rather than sequential.** If TrkA, choline acetyltransferase and tau are scored in the same neurons across the same continuum and the receptor-negative cells prove to be the cells that have already lost choline acetyltransferase, the ordering claim of Chapter 5 fails and the paper's central contribution goes with it.
2. **Demonstration that the TrkA deficit is an epitope or threshold artefact.** If TrkA messenger RNA, or TrkA protein by a method not dependent on the same epitope, is preserved in mild cognitive impairment, the receptor is present and not displayed — which changes the mechanism from receptor loss to receptor trafficking and requires Part III to be rewritten rather than refined.
3. **Perfectly targeted trophic delivery producing clinical benefit.** If a trial delivers mature nerve growth factor to a verified target, with pathological confirmation of transduction in a substantial fraction of the cells, and achieves benefit, then ligand supply was the limiting term and the four-failure account of Chapter 10 is overcomplicated.
4. **A phasic or paired cholinergic intervention performing no better than a tonic one at matched average exposure.** This refutes the modulation-depth inference of Chapter 13.
5. **Evidence that preclinical basal forebrain volume change does reflect cell loss.** If stereology in a cohort matched to the imaging studies' preclinical stage shows substantial neuronal loss, the reconciliation offered in Chapter 8 is wrong.
6. **Demonstration that cholinergic input does not restrain microglial responses to amyloid or tau in brain tissue.** This reduces the proposal of Chapter 14 to an epiphenomenal association.

Experiments, in order of what they would settle per unit of effort.

1. **Triple labelling for TrkA, choline acetyltransferase and phosphorylated tau in the same nucleus basalis sections, across no cognitive impairment, mild**

cognitive impairment and early disease. This is the experiment the paper exists to prompt. Both stereological series it relies on used tissue from the same autopsy programme, the sections and antibodies exist, and concurrent visualisation of tau and choline acetyltransferase has already been done in this nucleus (Mesulam et al., 2004). The outcome settles the ordering claim, settles whether the receptor-negative population is tau-bearing, and costs a fraction of any imaging study.

2. **TrkA messenger RNA and non-epitope-dependent TrkA protein in laser-captured cholinergic basal forebrain neurons across the same continuum.** Distinguishes receptor loss from receptor non-display, which determines whether the therapeutic target is expression or trafficking. The method has already been applied to these neurons (Ginsberg et al., 2011; Riascos et al., 2014).
3. **A matched post-mortem and in vivo cohort to calibrate what basal forebrain volume measures.** Antemortem MRI in the Zaborszky mask, then stereology of the cholinergic population, somatic volumes and non-cholinergic populations in the same brains. Nothing in the imaging literature can be interpreted confidently until someone has done this.
4. **A sham-controlled trial of nucleus basalis stimulation with pre-specified cytokine endpoints.** The convergence between a human observational association (Schmitz et al., 2020) and a human interventional cytokine shift (Chen et al., 2026) currently rests on six open-label patients. A trial powered for the inflammatory endpoint rather than for cognition would be smaller, faster and more informative than another underpowered cognitive trial.
5. **Behaviourally paired stimulation of the nucleus basalis.** Kilgard and Merzenich's result says the therapeutic variable is pairing, and no Alzheimer trial has delivered stimulation paired with a cognitive task. To be tested in a primate model before patients.
6. **Measurement of the diurnal cholinergic excursion in human cortex under cholinesterase inhibition.** Chapter 13 predicts the drugs compress it. Whether they do is, to our knowledge, unmeasured; FEOBV and $\alpha 4\beta 2$ ligands plus a timed design could establish it.
7. **Stratification of any future trophic trial by receptor and transport status.** If half of these neurons have stopped displaying TrkA before dementia, a trial that does not stratify is averaging over two populations with different mechanisms. Cerebrospinal fluid proNGF, MMP-9 activity and plasminogen-cascade markers are measurable (Bruno et al., 2009; Iulita et al., 2016) and could serve as the stratifier.
8. **Prospective testing of the nerve growth factor maturation profile as a preclinical biomarker in Down syndrome.** Iulita's plasma panel predicted

deterioration in thirty-one people; trisomy 21 is the population in which the trophic hypothesis is most directly testable in life, at an attainable sample size.

9. Sub-sector resolution in vivo. The post-mortem data say the lesion varies nearly two-fold across pathological subtypes (Hanna Al-Shaikh et al., 2020) and the normal-ageing data say the posterior sector carries the strongest cognitive associations (Wolf et al., 2014). Imaging that separates Ch4am, Ch4al, Ch4i, Ch4p and the nucleus subputaminalis would convert a single volume into an anatomically interpretable measure.

17. Conclusion — The Gate and the Ransom

The nucleus basalis of Meynert does two things that nothing else in the brain does, and the disease takes both.

The first is that it holds the gate on cortical encoding. It is not a memory store — destroying it in a primate does not reliably produce amnesia — and the signal it sends is not a level but a difference: sub-second transients at the moment a cue is detected, against a background that must be quiet for the transient to be legible, from neurons that burst with theta in waking and in dreaming and fall silent in the deep sleep during which the cortex consolidates what those transients marked. Raise that background pharmacologically at the wrong hour and healthy subjects lose the memories they formed that day. The office of the nucleus is to say *this, now, is worth keeping*, and then to get out of the way.

The second is that it pays a ransom for its own life. Of the six cholinergic sectors of the human brain, Ch4 is the one that carries nerve growth factor receptor protein, and the mesopontine sectors that do not carry it do not die in this disease. The factor it depends on is not delivered finished: it is released as a precursor, cut to maturity in the extracellular space by a plasmin-dependent cascade, destroyed there by a metalloproteinase, captured at a terminal up to a hundred metres from the cell body, and carried home inside an endosome. Four measurements in human tissue show that pathway failing before dementia — precursor accumulating, metalloproteinase activity rising, the survival receptor disappearing while the death receptor is retained, and the factor itself piling up in a cortex that cannot get it to the nucleus. One extra copy of one Alzheimer gene is enough, in a mouse, to swell the endosome and sever the road.

Set the two together and the chronology falls into an order the field has held the pieces for since 2000 without assembling. Approximately 197,000 of this nucleus's neurons display TrkA in a cognitively normal brain and approximately 210,000 display choline acetyltransferase; in health they are substantially the same neurons. By the stage of mild cognitive impairment the TrkA count has halved, the cholinergic count has not moved, and in the cortex the enzyme that makes acetylcholine has in places been turned up. Only much later do seventy per cent of the cells go. The receptor is surrendered first, the phenotype second, the cell last — and the first of those surrenders does not correlate with Braak stage, which means it is not simply a shadow cast by the cortex.

That ordering, if the single experiment in Chapter 16 confirms it, dissolves the field's awkwardest disagreement about this nucleus. The imaging that finds basal forebrain degeneration

upstream of entorhinal degeneration and the tau-PET study that finds Ch4 volume coupled to mesial-temporal tau are both describing a hostage harmed before the hostage-taker arrives, and then harmed again when it does. And it explains the therapeutic record without needing to call any of it a failure of execution. We gave the nucleus more transmitter and raised a mean where the physiology encodes in excursions, and got 2.7 points on a 70-point scale. We gave it more of a growth factor that was already increased everywhere except where it was needed, and reached, as the trialists themselves said, a target we could not confirm we had hit. Neither intervention was addressed to the thing that had actually gone wrong, because the thing that had actually gone wrong was not a shortage. It was a contract that stopped being honoured — unripened, over-degraded, unreceipted, undelivered — in a cell that was still alive, still cholinergic, and still, for years, waiting to be paid.

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