The Vago-Coerulean Relay

The Anatomical and Neurochemical Mechanisms by Which the Vagus Nerve Modulates the Locus Coeruleus

Resolving the Single Arrow — How the Vagal Afferent Signal Reaches the Coeruleus Through a Dual, Predominantly Indirect Medullary Relay (Paragigantocellularis and Prepositus Hypoglossi), and the Experimental Methods That Establish Each Link

ONS Methodology — Mechanistic Monograph

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A Mechanistic Monograph Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Neuroscience. Companion to The Vagal Interface and The Coerulean Interface; the circuit-level reconstruction of the vagus-to-locus-coeruleus pathway on which those volumes depend.

“The vagus does not command the locus coeruleus. It petitions a medullary relay — one arm exciting, one arm inhibiting — and the relay decides what the coeruleus hears.”

— ONS Methodology Working Notes, 2026

For Gary Aston-Jones, whose restricted-afferent anatomy of the locus coeruleus gave the relay its first map, and for Liqun Luo, whose viral-genetic tracing redrew it — the two views between which this monograph locates the vagus.

THE COLLAPSE TRILOGY — MECHANISTIC MONOGRAPH

Companion volume — The Vagal Interface

Companion volume — The Coerulean Interface

Mechanistic Monograph — The Vago-Coerulean Relay □ this volume

The two companion volumes asserted that the afferent vagus modulates the locus coeruleus and built systems-level theory upon it, but each drew the connection as a single arrow — 'NTS □ LC'. This monograph resolves the arrow. It asks the narrower, more answerable question: by what concrete synapses, relay nuclei, transmitters, and receptors does vagal activity come to alter locus coeruleus firing, and by what experimental methods has each step been established? The answer is not a direct line but a dual, predominantly indirect medullary relay — excitatory through the paragigantocellularis, inhibitory through the prepositus hypoglossi — whose balance, not the nerve, decides the coeruleus's response.

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Abstract

The companion volumes *The Vagal Interface* and *The Coerulean Interface* asserted that the afferent vagus modulates the locus coeruleus and that this modulation matters for neurodegeneration, but they treated the vagus-to-coeruleus connection largely as a single arrow — "the nucleus tractus solitarius projects to the locus coeruleus" — without resolving the arrow into the specific synapses, relay nuclei, transmitters, and receptors that compose it. This monograph resolves the arrow. It asks a narrower and more answerable question than its companions: *by what concrete anatomical and neurochemical mechanisms does activity in the vagus nerve come to alter the firing of locus coeruleus neurons, and by what experimental methods has each step of that mechanism been established?* The answer is not a single projection but a small, well-characterised relay network, and the purpose of this monograph is to assemble that network, step by step, and to distinguish what is firmly established from what is inferred.

The mechanism is developed in five steps. First, the vagal afferent synapse: vagal sensory fibres, with cell bodies in the nodose ganglion, release glutamate onto second-order neurons of the nucleus tractus solitarius (NTS), the obligatory first central station of all vagal afferent traffic. Second, the polysynaptic relay from the NTS to the locus coeruleus, which this monograph argues is predominantly *indirect*: the dominant excitatory drive to the locus coeruleus arises from the nucleus paragigantocellularis (PGi) of the rostral ventrolateral medulla, which is glutamatergic and which the NTS innervates, while the dominant inhibitory drive arises from the GABAergic nucleus prepositus hypoglossi (PrH); a sparser direct NTS□locus coeruleus projection and a parabrachial route run in parallel. Third, the neurochemistry at the coeruleus itself, where glutamatergic excitation from the PGi acting at AMPA and NMDA receptors, GABAergic inhibition from the PrH, corticotropin-releasing factor, and enkephalin together set the balance between the locus coeruleus's tonic and phasic firing modes. Fourth, the humoral parallel: the area postrema, a circumventricular organ adjacent to the NTS and outside the blood–brain barrier, samples circulating cytokines directly and supplies a

blood-borne route to the same NTS relay, so that the vagal and humoral immune-to-brain channels converge before reaching the locus coeruleus. Fifth, the experimental methods — retrograde and monosynaptic viral tracing, antidromic electrophysiology, optogenetic and chemogenetic manipulation, immediate-early-gene mapping, cortical microdialysis, and, in humans, functional MRI, pupillometry, salivary α-amylase, and the P300 — by which each link has been demonstrated, together with a frank account of what each method can and cannot establish.

The monograph concludes with a wiring diagram of vagal control of the locus coeruleus and with the implications of the mechanism for the interpretation of vagus nerve stimulation: that the predominantly indirect, PGi-relayed, and dually excitatory-and-inhibitory architecture of the relay explains why vagal stimulation produces heterogeneous and sometimes null effects on noradrenergic markers, and why stimulation parameters, timing, and the balance of the excitatory and inhibitory relays are decisive. The vagus does not speak to the locus coeruleus directly; it speaks through a medullary relay whose properties determine what the locus coeruleus hears.

Keywords: vagus nerve, locus coeruleus, nucleus tractus solitarius, nucleus paragigantocellularis, nucleus prepositus hypoglossi, glutamate, GABA, corticotropin-releasing factor, area postrema, vagus nerve stimulation, pupillometry, retrograde tracing, noradrenaline

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1. Introduction

1.1 The Research Problem

The two companion volumes that precede this monograph — *The Vagal Interface* and *The Coerulean Interface* — both rest on a claim they do not themselves substantiate in mechanistic detail: that activity in the vagus nerve modulates the locus coeruleus. *The Vagal Interface* used the claim to argue that the afferent vagus delivers a chronic inflammatory load to the brainstem; *The Coerulean Interface* used it to argue that this load drives the locus coeruleus beyond its bioenergetic means. In both, the connection appears as a single arrow in a wiring diagram — "NTS □ LC" — and the load-bearing weight of two dissertations rests on that arrow. An arrow, however, is not a mechanism. To say that the nucleus tractus solitarius projects to the locus coeruleus is to assert the existence of a pathway without specifying whether it is monosynaptic or polysynaptic, excitatory or inhibitory, glutamatergic or peptidergic, dense or sparse, tonic or phasic in its influence — and these specifics determine whether the inferences the companion volumes draw from the connection are warranted.

This monograph is therefore deliberately narrower than its companions. It does not advance a systems-level theory of neurodegeneration; it resolves a single arrow into its constituent synapses. The question is: *by what concrete anatomical and neurochemical mechanisms does activity in the vagus nerve come to alter the firing of locus coeruleus neurons?* And, because the answer to a mechanistic question is only as good as the methods that established it, a second question runs alongside the first: *by what experimental methods has each step of that mechanism been*

demonstrated, and what does each method actually license us to conclude?

The central finding, developed across the chapters, is that the vagus does not modulate the locus coeruleus by a single direct projection. The dominant route is *indirect*, relayed through the nucleus paragigantocellularis of the rostral ventrolateral medulla; the relay is *dual*, comprising both a powerful glutamatergic excitatory arm and a powerful GABAergic inhibitory arm; and the net effect of vagal activity on the locus coeruleus is therefore not fixed but depends on the balance struck within the relay. This architecture is the mechanistic fact that the companion volumes' single arrow concealed, and its consequences — for the interpretation of vagal stimulation, for the heterogeneity of experimental results, and for the design of therapeutic protocols — are the subject of the final chapters.

1.2 Significance

The significance of resolving the arrow is threefold. First, it tests the load-bearing assumption of the companion volumes. If the vagus-to-coeruleus connection were weak, purely inhibitory, or non-existent, the inferences those volumes draw would collapse; establishing that the connection is real, substantial, and bidirectionally capable is a precondition for the larger theory, and this monograph supplies that establishment together with its qualifications. Second, the specific architecture of the relay — indirect, dual, and balance-dependent — has direct consequences for therapeutics. The heterogeneity and frequent null results of vagus nerve stimulation studies, particularly the inconsistent effects on pupillary and salivary noradrenergic markers in human transcutaneous stimulation, are predictable consequences of a relay that contains both an excitatory and an inhibitory arm and whose net output depends on stimulation parameters; a mechanism that includes the inhibitory arm explains the null results that a simple excitatory arrow cannot. Third, the methodological audit is itself a contribution: the vagus-to-coeruleus literature spans rodent tract-tracing, rodent and primate electrophysiology, and human neuroimaging and psychophysiology, and the inferential strength of these methods differs greatly, so that distinguishing what is demonstrated in rodent anatomy from what is inferred from a human pupillary correlate is necessary to weight the evidence correctly.

1.3 Scope and Limitations

This monograph concerns the afferent direction — how vagal activity reaches and modulates the locus coeruleus — and treats the efferent and propagative functions of the vagus, and the disease consequences of coeruleus modulation, only by reference to the companion volumes in which they are developed. It is concerned with mechanism, not with theory: it asks how the connection works, not what the connection means for neurodegeneration, which is the subject of *The Coerulean Interface*. The evidence base is heavily rodent, because the relevant tract-tracing and circuit-manipulation methods are feasible only in animals; the human evidence is correlative and indirect, depending on noradrenergic proxies whose validity is itself contested, and the monograph weights it accordingly. Finally, the monograph reflects an unresolved tension in the primary literature — between the classical "restricted afferent" view of locus coeruleus inputs, which holds that two medullary nuclei dominate, and the modern viral-tracing view, which finds the locus coeruleus to receive input from a far broader set of regions — and it does not pretend to resolve that tension, but states where the vagal relay sits within it.

2. Background: The Locus Coeruleus and Its Afferent Control

2.1 The Locus Coeruleus as a Controlled Nucleus

The locus coeruleus is a compact, bilateral nucleus of the dorsal pons, comprising on the order of tens of thousands of noradrenergic neurons per side in the human, that supplies noradrenaline to nearly the entire forebrain, cerebellum, and spinal cord. Its output is governed by two firing modes, characterised principally by Aston-Jones, Cohen, and colleagues: a *tonic* mode, in which the neurons fire at a slow, regular baseline rate that sets global arousal, and a *phasic* mode, in which a brief synchronous burst is evoked by salient stimuli and gates attention and behavioural responding. The balance between tonic and phasic firing — the "adaptive gain" of the locus coeruleus — is set by the nucleus's afferent control, and it is this afferent control that the vagal relay engages. The locus coeruleus is not an autonomous oscillator broadcasting a fixed signal; it is a controlled nucleus whose firing mode is continuously shaped by its inputs, and the question of this monograph is how the vagus participates in that shaping.

2.2 The Classical "Restricted Afferent" View

For two decades the dominant account of locus coeruleus afferent control was the "restricted afferent" view established by Aston-Jones, Ennis, and colleagues through combined retrograde tracing and electrophysiology in the rat. On this view, the locus coeruleus — despite its vast efferent reach — receives its major direct synaptic input from a surprisingly small number of sources, dominated by two medullary nuclei: the nucleus paragigantocellularis (PGi) of the rostral ventrolateral medulla, which supplies a powerful excitatory drive, and the nucleus prepositus hypoglossi (PrH), which supplies a powerful inhibitory drive. The restricted-afferent view is the framework within which the vagal relay was first situated, because the PGi is also a principal relay of visceral and autonomic information, including baroreceptor and chemoreceptor signals carried by the vagus and glossopharyngeal nerves. On this account, the route from the vagus to the locus coeruleus runs through the PGi, and the locus coeruleus's responses to visceral and cardiovascular stimuli are PGi-relayed.

2.3 The Modern Broad-Input View

The restricted-afferent view has been substantially revised by modern viral and genetic tracing. Schwarz, Luo, and colleagues, using monosynaptic rabies-virus tracing from genetically defined locus coeruleus neurons, reported in 2015 that the locus coeruleus receives input from a far broader set of regions than the restricted view held — over a hundred distinct sources — and that its input–output organisation is more modular than the picture of a few dominant medullary drivers implied. The reconciliation of the two views is partly methodological: retrograde tracing of the classical kind detects the strongest projections, while monosynaptic rabies tracing detects even sparse ones, so the "restricted" inputs are best read as the *dominant* inputs rather than the only ones. For the present monograph the reconciliation matters because it bears on the strength attributed to the vagal relay: the PGi and PrH remain among the heaviest direct inputs, so the PGi-relayed vagal route is a route through a major input, but the locus coeruleus also integrates the vagal signal with a wide field of other afferents, and the vagal influence is therefore one contribution among many rather than a controlling line.

2.4 Why the Distinction Governs Everything That Follows

The choice between these views is not academic; it determines how strong an effect of vagal activity on the locus coeruleus one should expect. On the restricted view, the vagus speaks to the locus coeruleus through one of its two dominant inputs, and vagal modulation should be substantial and reliable. On the broad-input view, the vagus is one of many voices, and its influence should be real but modest and easily masked. The empirical record — substantial locus coeruleus responses to strong vagal stimulation in animals, but heterogeneous and often weak effects of transcutaneous stimulation on noradrenergic markers in humans — is consistent with an intermediate reading: the vagal relay is through a major input (the PGi), but it is gated by a parallel inhibitory input (the PrH) and integrated with a broad afferent field, so its net effect is real, conditional, and parameter-dependent. The chapters that follow build the mechanism on this intermediate reading.

3. Methodology

This monograph employs the Organic Network Synthesis (ONS) methodology of the AdultCognitiveDisease.com corpus, here applied at a finer grain than in the companion volumes. Where the companion theses synthesised across systems, this monograph performs a *circuit reconstruction*: it assembles, from the primary neuroanatomical and neurophysiological literature, the specific chain of synapses connecting the vagus to the locus coeruleus, and it annotates each link with the method by which it was established and the inferential weight that method supports.

The method proceeds in three steps. First, *link enumeration*: the decomposition of the vagus-to-coeruleus connection into its candidate links — vagal afferent to NTS, NTS to PGi, NTS to PrH, direct NTS to locus coeruleus, PGi to locus coeruleus, PrH to locus coeruleus, parabrachial relay, and the humoral area-postrema parallel — so that the connection is treated as a network to be reconstructed rather than an arrow to be asserted. Second, *evidence grading*: the assignment to each link of the strongest available evidence and its method, with explicit attention to the hierarchy of inferential strength running from anterograde and monosynaptic tracing (which can establish a direct connection), through electrophysiology and circuit manipulation (which can establish function), to immediate-early-gene mapping (which establishes activation but not connectivity), to human neuroimaging and noradrenergic proxies (which establish correlation at low spatial resolution). Third, *limit statement*: for each link and for the whole, the explicit statement of what remains unestablished — in particular, the quantitative weight of the direct versus indirect routes in the human, which rodent anatomy cannot settle and human methods cannot resolve at the required resolution.

The methodology's central limitation is the species gap. The detailed circuit anatomy is rodent; the disease and much of the therapeutic interest is human; and the bridge between them — that the rodent vago-coerulean relay is conserved in humans — is plausible on grounds of brainstem conservation but is not directly demonstrated at the synaptic level. The monograph treats the rodent relay as the mechanism and the human data as consistent corroboration at lower resolution, and it does not claim that the quantitative balance of the relay's arms is known in humans.

4. Chapter I — The First Synapse: Vagal Afferents and the Nucleus Tractus Solitarius

4.1 The Vagal Afferent Neuron

The mechanism begins at the vagal afferent neuron, whose cell body lies in the inferior (nodose) vagal ganglion and whose peripheral process innervates the viscera — the gut wall, the hepatic portal region, the cardiovascular baro- and chemoreceptors, and the airways — while its central process enters the medulla and terminates in the nucleus tractus solitarius. The afferent neuron is, functionally, a transducer: it converts mechanical, chemical, metabolic, and immune states of the periphery into trains of action potentials. Its principal fast transmitter at the central terminal is glutamate, so that the first synapse of the entire vagus-to-coeruleus pathway is a glutamatergic excitatory synapse onto second-order NTS neurons. The afferent fibres are predominantly unmyelinated C-fibres and thinly myelinated A-delta fibres, consistent with the slow, tonic, modulatory character of visceral afference, and their conduction and recruitment properties are directly relevant to stimulation therapeutics, because the fibre populations recruited by a given stimulation intensity determine which afferent channels are engaged.

4.2 The Nucleus Tractus Solitarius as the Obligatory First Station

The NTS is the obligatory first central station of vagal afferent traffic: essentially all vagal sensory information passes through it before reaching any higher structure, including the locus coeruleus. It is viscerotopically organised, with gastrointestinal afferents terminating in its caudal and medial subnuclei and cardiorespiratory afferents in more rostral and lateral regions, and it performs the first integration of the vagal signal with local interneuronal processing and with the humoral signals sampled at the adjacent area postrema (Chapter IV). The functional significance of the NTS's obligatory position is that it is the single point at which the entire vagal signal can be modulated before it is distributed: any process that alters NTS excitability — local inflammation, the humoral milieu sampled at the area postrema, descending modulation — alters everything the locus coeruleus subsequently receives from the vagus. The NTS is the gate, and the locus coeruleus sees only what passes it.

4.3 The Branch Point: Where the Vagal Signal Goes Next

From the NTS, the integrated vagal signal is distributed along several ascending routes, of which only a subset reaches the locus coeruleus. The NTS projects heavily to the parabrachial nucleus (the principal ascending visceral relay to the forebrain), to the ventrolateral medulla including the PGi, to the hypothalamus, and to the dorsal motor nucleus and other autonomic premotor structures. The routes relevant to this monograph are those that converge on the locus coeruleus: the projection to the PGi (the principal indirect excitatory route), a direct but sparser NTS-to-locus-coeruleus projection, the projection to the PrH (an indirect inhibitory route), and the parabrachial route. The branch point at the NTS is therefore where the vagal signal is divided among destinations, and the locus coeruleus receives a processed, relayed fraction of it rather than the raw afferent volley. Chapter II reconstructs the routes by which that fraction arrives.

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5. Chapter II — The Polysynaptic Relay: From the Solitary Nucleus to the Coeruleus

5.1 The Indirect Excitatory Route Through the Paragigantocellularis

The dominant route from the NTS to the locus coeruleus is indirect, through the nucleus paragigantocellularis (PGi) of the rostral ventrolateral medulla. The PGi is the principal source of excitatory drive to the locus coeruleus on the classical restricted-afferent view, and it is simultaneously a major integrator of visceral and autonomic information, receiving NTS projections and relaying baroreceptor, chemoreceptor, and nociceptive signals to the locus coeruleus. The PGi-to-locus-coeruleus projection is functionally excitatory and is mediated substantially by glutamate acting at the locus coeruleus, producing the rapid activation of locus coeruleus neurons that follows salient or arousing visceral and somatic stimuli. The PGi also contains adrenergic (C1) neurons and releases other transmitters onto the locus coeruleus, so the projection is neurochemically mixed, but its dominant fast action is glutamatergic excitation. The significance of this route is that the principal way the vagus excites the locus coeruleus is *not* by a direct line but by driving the PGi, which then drives the locus coeruleus — a two-synapse minimum from NTS to coeruleus, three from the vagal afferent itself.

5.2 The Indirect Inhibitory Route Through the Prepositus Hypoglossi

Running in parallel with the excitatory PGi route is an inhibitory route through the nucleus prepositus hypoglossi (PrH), the principal source of GABAergic inhibitory input to the locus coeruleus on the restricted-afferent view. The PrH tonically inhibits the locus coeruleus, and it too receives visceral and autonomic information, so that vagal activity can engage the inhibitory PrH arm as well as the excitatory PGi arm. The existence of a powerful inhibitory relay is the single most consequential fact in this monograph's mechanism, because it means that the net effect of vagal activity on the locus coeruleus is not determined at the vagus or at the NTS but at the locus coeruleus, by the balance between the PGi excitation and the PrH inhibition that a given pattern of vagal activity evokes. A vagal volley that preferentially engages the PGi arm will excite the locus coeruleus; one that preferentially engages the PrH arm will inhibit it; and one that engages both may produce little net change. The dual relay is why "vagal stimulation activates the locus coeruleus" is true only conditionally.

5.3 The Direct NTS-to-Coeruleus Projection

A direct, monosynaptic projection from the NTS to the locus coeruleus also exists, demonstrated by anterograde and retrograde tracing, but it is sparser than the indirect PGi route and its functional weight is correspondingly smaller. Its existence matters because it provides a faster, less heavily processed route by which the vagal signal can reach the locus coeruleus, bypassing the PGi integration; but its sparseness means that the bulk of the vagal influence is relayed and processed rather than direct. The monograph's position is that the direct projection is real but minor, and that the dominant functional route is the indirect PGi one — a position consistent with the restricted-afferent electrophysiology, which found the PGi to be the dominant driver of locus coeruleus activation.

5.4 The Parabrachial and Other Parallel Routes

A further route runs through the parabrachial nucleus, the principal ascending visceral relay, which receives dense NTS input and projects to the locus coeruleus among many other targets. The parabrachial route carries integrated visceral and nociceptive information and contributes to the locus coeruleus's responses to interoceptive and aversive stimuli. Together with the broad afferent field identified by modern viral tracing (§2.3), these parallel routes mean that the vagal signal reaches the locus coeruleus along several convergent paths of differing speed, sign, and degree of processing, and that the locus coeruleus integrates them. The reconstruction is therefore not a single chain but a small convergent network, dominated by the excitatory PGi and inhibitory PrH arms, with the direct and parabrachial routes as parallel contributors.

5.5 The Reconstructed Relay

The reconstructed relay, in summary, runs: vagal afferent ☐ (glutamate) ☐ NTS ☐ {PGi (glutamatergic, excitatory) and PrH (GABAergic, inhibitory) and a sparse direct projection and a parabrachial route} ☐ locus coeruleus. The dominant functional arms are the excitatory PGi and the inhibitory PrH, and the net effect of vagal activity on locus coeruleus firing is the balance between them. This is the mechanistic content of the single arrow that the companion volumes drew, and it is both more substantial — a real, major-input relay — and more conditional — a dual, balance-dependent one — than the arrow implied.

6. Chapter III — The Neurochemistry at the Coeruleus: Setting the Firing Mode

6.1 Glutamatergic Excitation and the Phasic Mode

At the locus coeruleus, the glutamatergic input from the PGi acts at AMPA and NMDA receptors to depolarise and excite the noradrenergic neurons. The fast AMPA-mediated component drives the rapid, synchronous activation that underlies the phasic firing mode, so that a salient visceral or somatic stimulus relayed through the PGi evokes a phasic locus coeruleus burst and a corresponding pulse of forebrain noradrenaline. The NMDA component contributes a slower, integrative excitation. The glutamatergic PGi input is therefore the principal substrate by which vagal and visceral activity can drive the locus coeruleus into its phasic, attention-gating mode, and it is the mechanism behind the noradrenergic and arousal effects of strong vagal stimulation observed in animals.

6.2 GABAergic Inhibition and Tonic Restraint

The GABAergic input from the PrH acts at the locus coeruleus to provide tonic inhibitory restraint, setting a brake on baseline firing. The interplay between the PrH GABAergic brake and the PGi glutamatergic drive establishes the locus coeruleus's operating point — the tonic firing rate around which phasic bursts occur — and shifts in the balance between them move the locus coeruleus along the tonic–phasic continuum that determines adaptive gain. Vagal engagement of the inhibitory arm can therefore lower locus coeruleus tone, and the coexistence of the two arms means that the neurochemical outcome of vagal activity at the locus coeruleus is a subtraction, not an addition.

6.3 Corticotropin-Releasing Factor and the Stress Shift

Beyond the fast amino-acid transmitters, the locus coeruleus receives a corticotropin-releasing factor (CRF) input, principally from the central nucleus of the amygdala and Barrington's nucleus, characterised extensively by Valentino, Van Bockstaele, and colleagues. CRF acting at the locus coeruleus shifts its firing toward a high tonic mode — raising baseline rate and reducing phasic responsiveness — which is the locus coeruleus signature of stress and which biases the noradrenergic system toward scanning, high-arousal states. The CRF input is relevant to the vagal relay because visceral and inflammatory stress, sensed by the vagus and relayed centrally, engages the same stress circuitry, so that a chronically inflamed or stressed periphery can bias the locus coeruleus toward the high-tonic CRF-driven mode — a mechanism that connects this monograph's relay to the chronic locus coeruleus drive that *The Coerulean Interface* identified as bioenergetically costly.

6.4 Enkephalin, Opioid Tone, and the Push–Pull Balance

The PGi co-releases enkephalin alongside its excitatory transmission, and the locus coeruleus expresses opioid receptors whose activation inhibits firing; the locus coeruleus thus receives, from overlapping sources, both an excitatory and an opioidergic inhibitory influence, and the balance between CRF-driven excitation and opioid-driven inhibition is a further axis along which the nucleus is controlled. This push–pull arrangement, characterised in the locus coeruleus stress literature, means that the relay's effect on the locus coeruleus is set not by any single transmitter but by the summed balance of glutamate, GABA, CRF, and opioid actions, and that the same vagal input can produce different locus coeruleus outcomes depending on the prevailing peptidergic tone. The neurochemistry, like the anatomy, makes the vagal influence conditional rather than fixed.

6.5 The α2 Autoreceptor and Self-Regulation

Finally, the locus coeruleus regulates itself: noradrenaline released from its neurons and dendrites acts at α2-adrenergic autoreceptors to inhibit further firing, a negative-feedback loop that stabilises locus coeruleus output and limits the excursions that afferent drive can produce. The autoreceptor brake means that even a strong vagal-relayed excitation is self-limiting, and it is the mechanism exploited by α2 agonists such as clonidine to suppress locus coeruleus activity. For the vagal relay, the autoreceptor implies that the locus coeruleus's response to vagal drive is buffered and bounded, contributing further to the conditional, saturating character of the vagal influence.

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7. Chapter IV — The Humoral Parallel: Area Postrema and Cytokine Transduction

7.1 The Two Channels of Immune-to-Brain Signalling

The vagal relay reconstructed in the preceding chapters is the *neural* channel by which the peripheral state reaches the locus coeruleus, but it runs in parallel with a *humoral* channel that converges on the same NTS relay, and a complete mechanism must include both. The humoral channel is centred on the area postrema, a circumventricular organ lying in the floor of the fourth ventricle immediately adjacent to the NTS and, crucially, outside the blood–brain barrier. The area postrema's fenestrated vasculature allows it to sample circulating signals — including cytokines, hormones, and toxins — directly, and it projects to the NTS, so that blood-borne immune and metabolic signals gain access to the same relay that carries the neural vagal signal. The two channels converge at the NTS before either reaches the locus coeruleus.

7.2 Vagal Afferent Cytokine Sensing

The neural channel is itself immune-sensitive. Vagal afferents detect peripheral inflammation through several mechanisms characterised by Watkins, Maier, Goehler, and colleagues: receptors for interleukin-1 on or near the afferent terminals, the vagal paraganglia that lie along the nerve and respond to cytokines, and the dense innervation of immune-rich tissues. The functional demonstration is that subdiaphragmatic vagotomy attenuates the brain's response to intraperitoneal interleukin-1 and lipopolysaccharide — the fever, the sickness behaviour, and the central neural activation — establishing the afferent vagus as a necessary sensor for the brain's detection of inflammation arising below the diaphragm. The vagal relay therefore carries not only mechanical and metabolic visceral information but a genuine inflammatory signal, and it delivers that inflammatory signal, through the relay of Chapter II, toward the locus coeruleus.

7.3 Convergence and Redundancy

The convergence of the neural (vagal) and humoral (area postrema) channels at the NTS gives the brain two partly redundant routes for sensing peripheral inflammation, and the redundancy has consequences for both physiology and therapeutics. Physiologically, it means that the locus coeruleus is informed of peripheral inflammation by two independent paths, so that the noradrenergic and arousal responses to systemic inflammation are robust to the loss of either. Therapeutically, it means that interrupting the neural channel alone — by vagotomy or by failing to engage the afferent vagus in stimulation — does not silence the inflammatory signal to the locus coeruleus, because the humoral channel persists; and conversely, that anti-inflammatory interventions acting on the circulating cytokine load reduce the signal carried by both channels. The humoral parallel is why the vagal relay, though necessary for some inflammatory responses, is not the sole route, and why the companion volumes' emphasis on the vagus must be read alongside the humoral channel rather than as excluding it.

8. Chapter V — Demonstrating the Relay: Experimental Methods and Their Limits

8.1 Tract-Tracing: Establishing Connection

The anatomical backbone of the relay was established by tract-tracing. Retrograde tracers injected into the locus coeruleus and transported back to their cells of origin identified the PGi and PrH as dominant sources of input; anterograde tracers injected into the NTS and PGi and transported forward to their terminals confirmed the projections to the locus coeruleus; and modern monosynaptic rabies-virus tracing from genetically defined locus coeruleus neurons mapped the full afferent field and revealed its breadth (§2.3). Tract-tracing is the method that can establish that a connection *exists* and whether it is direct or relayed, and it is the strongest evidence in the reconstruction. Its limits are that it establishes anatomy, not function — a traced projection may be excitatory or inhibitory, strong or weak, tonically active or normally silent — and that the detailed tracing is rodent, leaving the human relay inferred from conservation rather than directly mapped.

8.2 Electrophysiology: Establishing Function and Sign

The functional properties of the relay — that the PGi excites and the PrH inhibits the locus coeruleus, and that the excitation is glutamatergic — were established by electrophysiology, principally the antidromic and orthodromic single-unit recording of Aston-Jones, Ennis, and colleagues, combined with local pharmacology. Stimulating the PGi while recording locus coeruleus units demonstrated excitation; local application of glutamate antagonists blocked it, establishing the transmitter; stimulating the PrH demonstrated inhibition blocked by GABA antagonists. Electrophysiology is the method that establishes the *sign and mechanism* of a connection, and it is what licenses the claim that the relay is dual. Its limits are spatial sampling — it records from the neurons one can reach — and, again, species: the definitive circuit electrophysiology is rodent.

8.3 Circuit Manipulation: Establishing Causation

Optogenetic and chemogenetic methods, which activate or silence genetically defined neuronal populations on command, allow the relay to be tested causally: silencing the PGi should reduce locus coeruleus responses to visceral drive, and activating defined NTS or PGi populations should drive the locus coeruleus. These methods, together with immediate-early-gene (c-Fos) mapping that reveals which neurons are activated by vagal stimulation, have been applied to the brainstem visceral circuitry and support the causal role of the relay nuclei. Circuit manipulation is the method that establishes *causation* rather than mere correlation or anatomy, and it is the modern standard. Its limits are that the genetic access required restricts it to animals, and that the artificial, synchronous activation it imposes may not reproduce the patterned, graded activity of the natural relay.

8.4 Neurochemical Readout: Microdialysis and Immediate-Early Genes

That vagal stimulation actually changes noradrenaline release was demonstrated by cortical microdialysis, which samples extracellular noradrenaline in the projection fields and has shown that vagus nerve stimulation increases cortical and hippocampal noradrenaline, and by c-Fos mapping, which shows locus coeruleus activation after stimulation. The animal electrophysiology of Dorr and Debonnel and of Manta and colleagues further showed that vagus nerve stimulation increases locus coeruleus firing rate, with a characteristic dependence on stimulation parameters and duration. These methods establish that the relay is not merely anatomically present but functionally engaged by stimulation, closing the loop from stimulus to noradrenergic output. Their limit is that they measure the output of the whole relay, not the contribution of any single arm, so they confirm the net effect without dissecting the excitatory–inhibitory balance.

8.5 Human Methods: Imaging and Noradrenergic Proxies

In humans, the relay cannot be traced or manipulated, and its engagement is inferred from indirect markers. Functional MRI of transcutaneous auricular vagus nerve stimulation has shown activation of the NTS and locus coeruleus and connected brainstem structures (Frangos and colleagues; Yakunina and colleagues), providing the principal direct human evidence that auricular stimulation reaches the relay, albeit at a spatial resolution that strains to resolve a nucleus as small as the locus coeruleus. Noradrenergic proxies are used as functional readouts: pupil diameter, which covaries with locus coeruleus activity, has been reported to increase with transcutaneous stimulation in some studies (Sharon and colleagues) but not others; salivary α-amylase, a peripheral noradrenergic marker; and the P300 event-related potential, which is locus-coeruleus-linked. The human evidence is the weakest link in the chain: the imaging is low-resolution, the proxies are indirect and their validity contested, and the results are heterogeneous. The heterogeneity is itself informative, and §10 argues that it is the predictable signature of the dual, balance-dependent relay rather than evidence of no effect.

8.6 The Inferential Hierarchy

Assembled, the methods form an inferential hierarchy: tract-tracing establishes that the relay exists and is largely indirect; electrophysiology establishes that it is dual, excitatory through the PGi and inhibitory through the PrH; circuit manipulation establishes that the relay nuclei causally control the locus coeruleus; microdialysis and immediate-early-gene mapping establish that stimulation engages the relay to change noradrenergic output; and human imaging and proxies establish, weakly and heterogeneously, that transcutaneous stimulation reaches the relay in people. The mechanism is therefore strongly established in rodent anatomy and physiology and weakly corroborated in human psychophysiology, and the honest summary is that the *existence and architecture* of the vago-coerulean relay are well demonstrated while its *quantitative net effect in the awake human* is not.

9. Chapter VI — Synthesis: A Wiring Diagram of Vagal Control of the Coeruleus

9.1 The Diagram

The synthesis of the monograph is a wiring diagram with the following elements. The *input* is vagal afferent activity, glutamatergic, arriving at the NTS. The *gate* is the NTS, which integrates the vagal signal with the humoral signal from the area postrema and distributes it. The *relay* is dual: an excitatory arm through the PGi (glutamatergic, driving the phasic mode) and an inhibitory arm through the PrH (GABAergic, restraining the tonic mode), with a sparse direct NTS projection and a parabrachial route in parallel. The *target* is the locus coeruleus, where the relayed signals are summed with CRF, opioid, and autoreceptor influences to set the tonic–phasic firing balance. The *output* is forebrain noradrenaline, whose phasic pulses gate attention and whose tonic level sets arousal and, as the companion volumes argue, restrains microglia. The diagram's defining feature is the dual relay: the vagus can both excite and inhibit the locus coeruleus, and what it does on any occasion is set within the medullary relay, not at the nerve.

9.2 What the Diagram Explains

The diagram explains several otherwise puzzling features of the literature. It explains why strong, direct vagal stimulation in animals reliably activates the locus coeruleus (it can be tuned to engage the excitatory PGi arm and to drive the relay hard) while transcutaneous stimulation in humans produces heterogeneous effects (it engages a mixed, lower-intensity afferent volley whose balance between the PGi and PrH arms is uncontrolled). It explains why locus coeruleus responses to visceral stimuli are graded and state-dependent rather than all-or-none (the dual relay and the peptidergic and autoreceptor modulation make the response conditional). And it explains why the companion volumes' single arrow, though correct in asserting a connection, was insufficient: the arrow omitted the inhibitory arm and the relay's conditionality, which are exactly the features that determine whether a given vagal intervention will raise or lower locus coeruleus output.

9.3 What the Diagram Leaves Open

The diagram leaves open the quantitative question that matters most for therapeutics: in the awake human, what is the net effect of a given transcutaneous stimulation protocol on locus coeruleus output, and how does it depend on stimulation parameters? The rodent anatomy and physiology fix the architecture but not the human quantitative balance; the human methods are too indirect to fix it; and the answer almost certainly depends on parameters — intensity, frequency, pulse width, duty cycle, and the timing relative to the locus coeruleus's state — that have not been systematically mapped. The diagram thus specifies the mechanism while identifying the parameter-mapping study that the field still requires, which §10 frames as a prediction.

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10. Chapter VII — Implications for Vagus Nerve Stimulation and Predictions

10.1 The Dual Relay Explains the Heterogeneous Stimulation Literature

The principal implication of the reconstructed mechanism is that the heterogeneous and frequently null effects of transcutaneous vagus nerve stimulation on noradrenergic markers are the *expected* consequence of a dual, balance-dependent relay, not evidence against a vagus-to-coeruleus connection. A relay that contains both a powerful excitatory arm and a powerful inhibitory arm will produce a net effect that depends on which arm a given stimulus preferentially engages, and a low-intensity, uncontrolled transcutaneous volley has no reason to engage them in a fixed ratio. The literature's heterogeneity is therefore the signature of the mechanism. This reframing matters because the null results have been read by some as showing that transcutaneous stimulation does not reach the locus coeruleus; the mechanism says instead that it reaches a relay whose net output is uncontrolled, and that the task is to control it.

10.2 Prediction: Parameter Dependence Is Lawful, Not Random

The framework predicts that the net effect of stimulation on locus coeruleus output is a lawful function of stimulation parameters, not random, and that systematically varying intensity, frequency, pulse width, and timing will reveal parameter regimes that reliably excite the locus coeruleus (by favouring the PGi arm) and regimes that do not. The prediction is testable with the existing human noradrenergic proxies, provided studies map parameter space rather than testing a single protocol, and its confirmation would convert transcutaneous stimulation from an intervention with unpredictable effects into a tunable one. The corollary prediction is that studies that fix a single arbitrary protocol will continue to produce heterogeneous results across laboratories, because they are sampling an uncontrolled point in a parameter-dependent function.

10.3 Prediction: The Inhibitory Arm Is a Therapeutic Target

The framework predicts that the inhibitory PrH arm is not merely a confound but a potential therapeutic target. If the goal in a given context is to *lower* locus coeruleus tone — for instance, to reduce the chronic high-tonic CRF-biased firing that *The Coerulean Interface* identifies as bioenergetically costly — then engaging the inhibitory arm, rather than the excitatory one, is the desired effect, and the dual relay provides the means. The prediction is that stimulation protocols can in principle be designed to bias the relay toward inhibition or excitation, and that the therapeutic objective should determine which is sought; a one-size-fits-all assumption that "more vagal stimulation is more locus coeruleus activation, which is good" is, on the mechanism, naive.

10.4 Prediction: Pupillometry Will Track the Relay Only When the Relay Is Engaged

The framework predicts that pupillometric and other noradrenergic proxies will track stimulation effects only under parameters that produce a net locus coeruleus change, and will appear null under parameters that balance the two arms, so that the validity of pupillometry as a locus coeruleus readout is itself parameter-conditional. This predicts that the contradictory pupillometry literature will resolve when studies report parameters and stratify by them, and it cautions against treating a null pupillary result as evidence that stimulation failed to reach the relay.

10.5 The Bridge Back to the Companion Volumes

The mechanism reconstructed here grounds the companion volumes' arrow. *The Coerulean Interface* argued that a chronically inflamed periphery drives the locus coeruleus beyond its bioenergetic means; the relay specifies how — through the glutamatergic PGi excitatory arm and the CRF stress shift, engaged by vagal and humoral inflammatory signalling converging at the NTS. *The Vagal Interface* argued that the afferent vagus delivers a chronic inflammatory load to the brainstem; the relay specifies the load's path and its dual, conditional character. The companion volumes' inferences survive the resolution of the arrow, but they acquire a qualification: the vagal influence on the locus coeruleus is real and substantial but conditional and balance-dependent, and the chronic drive they invoke is the net output of a relay whose architecture this monograph has reconstructed. The arrow was correct; the mechanism is richer than the arrow, and the richness is where the therapeutic leverage lies.

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11. Conclusion

This monograph set out to resolve a single arrow — the assertion, on which two companion dissertations rest, that the vagus nerve modulates the locus coeruleus — into the specific synapses, relay nuclei, transmitters, and receptors that compose it. The resolution yields a mechanism with five parts: a glutamatergic vagal afferent synapse onto the nucleus tractus solitarius, the obligatory first station; a predominantly indirect, dual relay from the solitary nucleus to the locus coeruleus, excitatory through the glutamatergic nucleus paragigantocellularis and inhibitory through the GABAergic nucleus prepositus hypoglossi, with a sparse direct projection and a parabrachial route in parallel; a neurochemistry at the coeruleus in which glutamate, GABA, corticotropin-releasing factor, enkephalin, and the α2 autoreceptor together set the tonic–phasic firing balance; a humoral parallel through the area postrema that converges on the same relay; and an evidentiary base that is strong in rodent anatomy and physiology and weak in human psychophysiology.

The central finding is that the vagus does not speak to the locus coeruleus directly or unconditionally. It speaks through a medullary relay that contains both an excitatory and an inhibitory arm, and the net effect of vagal activity on the locus coeruleus is set within that relay, by the balance the vagal signal strikes between its

arms. This single architectural fact — the dual, balance-dependent, predominantly indirect relay — is what the companion volumes' single arrow concealed, and it is the fact that explains the heterogeneous and frequently null effects of vagus nerve stimulation on noradrenergic markers, that identifies stimulation parameters as the decisive and under-mapped variable, and that opens the inhibitory arm as a therapeutic target in its own right. The companion volumes' inferences survive, but they are now grounded in, and qualified by, a mechanism: the vagal load on the locus coeruleus is real, substantial, and conditional, and the conditionality is where the therapy will be found.

The vagus does not command the locus coeruleus. It petitions a medullary relay, and the relay decides what the coeruleus hears.

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