Autonomic and Vagal Regulation: Physiology, Measurement, and Evidentiary Boundaries

Scientific White Paper SeaKing Solace Science Review 03

Document Status

This scientific white paper was prepared by SeaKing Solace as an evidence-based review of the published scientific literature. It has not been peer reviewed or published by an academic journal. Its purpose is to establish the scientific and evidentiary framework used to inform the SeaKing Solace research program. The underlying studies cited throughout the paper include peer-reviewed publications; their inclusion should not be interpreted as independent validation of SeaKing Solace's hypotheses or proposed applications.

1. Abstract

Autonomic regulation is often described through simplified constructs such as sympathetic versus parasympathetic activity, vagal tone, autonomic balance, or changes in individual biomarkers. Human physiology supports a more complex model. Autonomic control is distributed, state-dependent, regionally differentiated, and effector-specific, integrating visceral afferent information with central processing and organ-specific sympathetic and parasympathetic outputs. The vagus nerve is an important component of this system, but its sensory and motor pathways are anatomically and functionally heterogeneous; cardiac vagal influence therefore cannot be assumed to represent whole-vagus or whole-body parasympathetic activity.

This review examines the physiological basis of autonomic and vagal regulation, the hierarchy of evidence available for measuring these processes, and the limits of commonly used autonomic biomarkers. Direct neural recording provides the closest available measurement of activity in a specified peripheral pathway, while selective pharmacological blockade, controlled physiological perturbation, regional neurotransmitter kinetics, and validated downstream effector measures provide progressively more indirect but complementary evidence. No method is universally superior; evidentiary strength depends on whether the measurement directly addresses the pathway, organ, or mechanism being claimed.

Heart rate variability, including RMSSD and respiratory-linked HRV, can provide useful information about cardiac parasympathetic modulation under appropriately characterized conditions, but it is not a direct measure of vagal nerve activity or global autonomic health. Respiration, mean heart rate, posture, age, recording duration, behavioral state, and analytical method constrain interpretation. Other practical measures, including electrodermal activity, pre-ejection period, pupillometry,

catecholamines, blood pressure, skin blood flow, and heart rate, similarly reflect specific downstream physiological processes rather than interchangeable measurements of a unitary autonomic state. Multimodal measurement can strengthen inference through convergent and divergent patterns across non-equivalent channels, but statistical integration does not by itself create a valid measure of “autonomic balance,” “autonomic coherence,” or global regulation.

Controlled vagus nerve stimulation further illustrates the distinction between pathway recruitment and endogenous physiology. Demonstrating electrically evoked vagal recruitment establishes that particular neural elements can be engaged under specified stimulation conditions; it does not establish a scalar vagal tone, whole-vagus activation, or the mechanism responsible for a clinical effect. Likewise, commonly used concepts including sympathovagal balance, parasympathetic dominance, unqualified vagal tone, and Polyvagal-derived ventral/dorsal state mappings exceed the evidence when presented as established global physiological states.

Finally, physiological change and physiological improvement are not equivalent. Regulatory quality must be evaluated dynamically across baseline state, challenge, response, within-exposure behavior, recovery or reconfiguration, and repeated exposure. Both exaggerated and blunted responses can be maladaptive depending on context, and neither higher HRV, lower sympathetic-effector activity, smaller reactivity, faster recovery, habituation, nor greater cross-signal concordance is intrinsically beneficial. A claim of improved autonomic regulation requires evidence that the response is appropriate to the physiological demand, reproducible under comparable conditions, tolerable, and associated with meaningful functional or clinical benefit.

For SeaKing Solace, these findings establish a deliberately bounded Phase 1 interpretation. Synchronized physiological measurements collected during controlled natural-motion exposure may identify reproducible multidimensional baseline-exposure-recovery and repeated-session response phenotypes. Such findings would constitute physiological discovery rather than proof of a specific vagal, sympathetic, or central mechanism and would not, by themselves, establish improved autonomic regulation. Mechanistic attribution and therapeutic benefit require subsequent controlled validation. This framework provides the evidentiary foundation for investigating motion-associated autonomic physiology while preserving the distinction between measured response, inferred mechanism, and demonstrated benefit.

2. Purpose and Scope

This scientific white paper examines autonomic and vagal regulation as the physiological framework necessary for interpreting measurable responses to motion. Its purpose is not to establish that motion or vestibular stimulation can influence autonomic physiology. That question is addressed separately within the preceding SeaKing Solace scientific reviews. Instead, the present paper asks a downstream question: when autonomic physiology changes, what has actually changed, how can it be measured, and what evidence is required before that change can be assigned mechanistic or functional meaning?

This distinction defines the paper's place within the broader SeaKing Solace scientific framework. Scientific Review 01 addresses the physical-input problem: how natural motion produces structured, multidimensional, head-centered vestibular stimulation. Scientific Review 02 addresses vestibular-autonomic interaction: the evidence that vestibular input can influence autonomic physiology, the dependence of those responses on stimulus characteristics and physiological state, and the boundaries of what such observations can establish. Review 03 begins from that interface rather than re-establishing it. Its subject is the autonomic system itself: its organization, the role and limits of vagal influence, the dynamics of regulation, the validity of available measurements, and the evidentiary distinction between physiological response, causal mechanism, and demonstrated benefit.

The foundation carried forward from Review 02 is mechanistic rather than merely anatomical: direct human microneurography demonstrates that vestibular perturbation can modify both muscle

sympathetic nerve activity (MSNA) and skin sympathetic nerve activity (SSNA). Those responses are not uniform; they vary with the stimulus, the effector being measured, and physiological state. Review 03 therefore begins from an established vestibulo-autonomic interaction and asks how autonomic and vagal physiology should be understood and measured once that interaction is granted.

A central concern is the tendency to describe autonomic physiology using global constructs that exceed what was actually measured. Terms such as vagal tone, sympathetic tone, autonomic balance, and parasympathetic dominance can imply unitary physiological quantities even though autonomic control is distributed across anatomically and functionally differentiated pathways and effectors. Likewise, heart rate variability, electrodermal activity, pupil dynamics, blood pressure, pre-ejection period, catecholamines, respiration, and related measures each arise from particular physiological processes and cannot be assumed to provide interchangeable estimates of a single autonomic state. This paper therefore evaluates autonomic claims at the level supported by the measurement and experimental method used to generate them. [1–6]

The review follows an explicit hierarchy of mechanistic proximity. Direct neural recording provides the closest evidence of activity within a specified neural pathway. Selective pharmacological blockade, ganglionic or controlled physiological perturbation, regional neurotransmitter kinetics, and validated downstream organ or effector measurements provide complementary but progressively more indirect evidence. This hierarchy does not imply that the most invasive study is universally the best study. Rather, the strongest evidence for a particular autonomic claim is the most direct valid measurement of the pathway or effector being claimed, ideally supported by convergent evidence from non-equivalent methods. [7–9]

Regulation is also treated as a dynamic process rather than a preferred resting value. The relevant sequence includes baseline state, challenge or exposure, response magnitude and pattern, within-exposure dynamics, recovery or reconfiguration, and change across repeated exposures. Neither greater nor smaller reactivity is presumed beneficial in isolation. Similarly, higher HRV, lower heart rate, reduced sympathetic-effector activity, faster recovery, habituation, or greater agreement among biomarkers does not independently establish improved autonomic regulation. Such an interpretation requires evidence that the physiological response is appropriate to the demand and participant state, reproducible under comparable conditions, tolerable, and ultimately related to meaningful function or clinical outcome. [2, 10–14]

The scope of this paper is evidentiary rather than therapeutic. It does not establish that a particular motion exposure treats autism, POTS, anxiety, or any other clinical condition. It does not assume that a physiological response to motion is vagally mediated, nor that a response resembling one produced by vagus nerve stimulation shares the same mechanism. It does not treat biomarker movement as proof of therapeutic efficacy. Disease-specific efficacy, dose optimization, mechanism validation, and clinical endpoints require separate empirical study.

For SeaKing Solace, the immediate relevance is methodological. Phase 1 is designed as a discovery program in which synchronized physiological measurements are collected during controlled natural-motion exposure. Within the boundaries developed in this paper, those measurements may identify reproducible multidimensional response phenotypes across baseline, exposure, recovery, and repeated sessions. They can reveal candidate relationships among motion characteristics and physiological responses and can generate hypotheses for later controlled testing. They cannot, without additional validation, establish a specific vagal, sympathetic, or central mechanism or demonstrate that an observed physiological pattern represents improved autonomic regulation.

The objective of this white paper is specific: to establish the physiological vocabulary, measurement hierarchy, and evidentiary rules necessary to describe what Phase 1 observes without claiming more than the data can support. The sections that follow move from autonomic architecture and vagal physiology through dynamic regulation and measurement, then examine common interpretive

shortcuts and conclude with the evidence that would be required to progress from observed physiological change to a defensible claim of improved regulation.

3. What Is Autonomic Regulation?

3.1 Beyond the Sympathetic-Parasympathetic Seesaw

Autonomic regulation is not the maintenance of a single preferred physiological state. It is the context-dependent adjustment of physiological function as internal conditions, environmental demands, behavior, and anticipated needs change. Effective regulation may therefore require substantial changes in cardiovascular, respiratory, thermoregulatory, metabolic, or other autonomically influenced functions rather than preservation of a resting value. [15–17]

Homeostasis provides one foundation for this model. Rather than implying literal constancy, homeostatic regulation uses interacting feedback processes to preserve physiologically viable conditions despite disturbance. Allostatic frameworks emphasize a complementary feature: coordinated physiological and behavioral change, including anticipatory adjustment in predictive formulations, in relation to expected demand. These concepts need not be treated as competing explanations. Both support a view in which regulation depends on the relationship between physiological response and physiological need, rather than movement toward one universally preferred autonomic state. [15–17]

The familiar representation of sympathetic and parasympathetic activity as opposite ends of a single balance is physiologically incomplete. At dually innervated cardiac targets, reciprocal control is important, but it is not obligatory. Experimental evidence supports partially independent sympathetic and parasympathetic dimensions capable of reciprocal change, coactivation, coinhibition, or change in one branch without a corresponding change in the other. [2, 18–21]

For descriptive purposes, cardiac autonomic control can therefore be represented by six broad modes: reciprocal sympathetic activation, in which sympathetic influence increases while parasympathetic influence decreases; reciprocal parasympathetic activation, with the opposite pattern; coactivation, in which both increase; coinhibition, in which both decrease; uncoupled sympathetic change; and uncoupled parasympathetic change. These are physiological configurations, not rankings of regulatory quality. The evidence does not establish coactivation as inherently healthier than reciprocal control, nor coinhibition as inherently pathological. [2, 18, 19]

This distinction also limits what can be inferred from an end-organ response. Heart rate and heart period reflect the net behavior of the cardiac effector, not direct measurements of the neural inputs producing that behavior. Similar heart-rate changes can arise from different underlying combinations of sympathetic and parasympathetic influence, and branch interactions can be nonlinear. Tachycardia, bradycardia, or an unchanged heart rate therefore cannot by themselves identify which autonomic configuration produced the observed response. [21–23]

Human evidence adds another caution. Group averages can produce an apparent reciprocal pattern even when sympathetic and parasympathetic responses are not correlated in the same way within individuals. Population-level association and within-person autonomic coordination are therefore not interchangeable forms of evidence. [24]

The six-mode framework is intentionally cardiac and illustrative. It demonstrates why obligatory reciprocity and a one-dimensional “balance” model are inadequate, but it should not be expanded into a two-variable model of the entire autonomic nervous system. Autonomic output is regionally and effector-specific, and cardiac branch dynamics cannot establish global sympathetic, parasympathetic, or vagal activity elsewhere in the body. [1, 22, 23, 25] The anatomical basis for that distributed organization is addressed in Section 4, while cardiac vagal and regional sympathetic physiology are treated separately in Sections 6 and 8.

3.2 Regulation Is a Trajectory

A resting measurement captures only one interval in an ongoing regulatory process. A more informative description asks what the physiological state was before a demand, what challenge or exposure occurred, how the system responded, what happened while the demand continued, how physiology reconfigured afterward, and what changed when comparable exposures were repeated. The relevant sequence is therefore: baseline → challenge/exposure → reactivity → within-exposure dynamics → recovery/reconfiguration → repeated exposure. [26–29]

Reactivity is the change in a specified physiological variable from a defined baseline during a defined challenge. Its direction and magnitude depend on the variable being measured, the nature and intensity of the challenge, posture, participant state, and the analytical window. Greater task demand can itself produce greater autonomic response, demonstrating why response magnitude cannot be interpreted independently of the demand that elicited it. [29]

Nor does the evidence support the assumption that smaller reactivity is universally preferable. Prospective studies have associated exaggerated physiological stress responses with adverse outcomes, but blunted responses have also predicted distinct adverse outcomes. A context-appropriate response may therefore involve substantial activation, withdrawal, coactivation, or relatively little change depending on the physiological system and demand. [10, 30–32]

Recovery is similarly a trajectory rather than simply “the value after the task.” Its interpretation depends on when measurement begins, whether the challenge has actually ended, what the participant is doing during recovery, and which physiological system is being observed. Different effectors can recover over different timescales, and return toward the preceding baseline is not necessarily equivalent to restoration of function when the organism or environment has entered a new state. [11, 12, 26–28]

Repeated exposure adds another dimension. Responses may reproduce, diminish, increase, drift, or reorganize across comparable exposures. Habituation describes reduced responding with repeated sufficiently similar stimulation, but the observation of a smaller response does not itself establish beneficial adaptation. Changes across sessions can also reflect altered baseline state, anticipation, learning, fatigue, exposure differences, or measurement effects. Repeated-exposure trajectories should therefore be described before they are assigned functional meaning. [13, 33–35]

Within this framework, autonomic flexibility is best used descriptively for the capacity to alter physiological configuration as demands change and to reconfigure again when those demands change. It is not synonymous with high resting HRV, greater reactivity, faster recovery, or frequent switching among physiological states. No single practical biomarker has been established as a general measure of this capacity. [26–28]

The same regulatory systems that are adaptive during an acute demand can become biologically costly when activation is frequent or prolonged, adaptation fails, recovery is delayed, or an inadequate response requires compensatory activity elsewhere. The concept of allostatic load was developed to describe this cumulative burden across interacting physiological systems. [36]

Empirical allostatic-load indices have shown prospective relationships with later health and functional outcomes, supporting the importance of cumulative multisystem physiology. However, studies vary substantially in the biomarkers selected, thresholds applied, and algorithms used to construct these indices. Allostatic load is therefore useful here as a framework for cumulative physiological burden, not as evidence that autonomic regulation itself can be represented by a single universal composite score. [37–39]

Taken together, autonomic regulation is best understood as distributed, context-dependent, dynamic control rather than movement toward one preferred autonomic state. Characterizing it requires identifying the demand, the physiological systems involved, their response trajectories, their recovery or reconfiguration, and their behavior across repeated exposure. Whether a particular

trajectory represents improved regulation is a separate evidentiary question. That judgment requires functional meaning and outcome evidence and is reserved for Section 17. [10, 11, 14, 40]

4. Functional Architecture of the Autonomic Nervous System

The autonomic nervous system is often introduced through its sympathetic and parasympathetic efferent divisions. That organization is useful, but incomplete. Autonomic regulation begins with information about the internal environment, integrates that information with central and local physiological state, and produces differentiated output through peripheral pathways and organ-specific effectors. A more complete functional architecture therefore includes visceral sensory input, central integration, sympathetic and parasympathetic efferent pathways, peripheral ganglia and local circuits, target-organ effectors, and enteric circuitry. [41–44]

This architecture is better understood as a set of interacting control loops than as a one-way command system. Visceral signals continuously ascend toward the central nervous system, central and peripheral circuits integrate those signals with other information, and autonomic outputs alter organ function. Those changes in turn modify subsequent sensory input. Local reflexes and enteric circuits can also regulate function without requiring every adjustment to originate from a higher central command. [25, 43]

4.1 Visceral Sensory Input

Regulation requires information about the physiological state being regulated. Visceral afferent pathways convey information from cardiovascular, respiratory, gastrointestinal, and other internal systems toward the central nervous system. Vagal visceral afferents project prominently to the nucleus tractus solitarius in the brainstem, while spinal visceral afferents enter through dorsal-root pathways and project into the spinal dorsal horn. [43]

These afferent systems make the autonomic nervous system fundamentally sensory as well as motor. Internal mechanical and chemical conditions can therefore participate in ongoing regulatory loops rather than autonomic function consisting simply of motor commands sent outward from the brain. The relative contribution of vagal and spinal afferent pathways varies by organ, stimulus, and physiological function; the detailed organization of the vagus itself is addressed in Section 5. [25, 43]

4.2 Distributed Central Integration

Visceral information does not converge on a single autonomic command center. Autonomic control is distributed across interconnected regions of the brainstem, hypothalamus, limbic system, and cerebral cortex. The central autonomic network framework was developed to describe this distributed organization and its reciprocal, parallel, and state-dependent interactions. [42, 45] Human neuroimaging provides additional evidence for distributed central autonomic processing. Meta-analytic findings identify overlapping but task- and division-dependent associations among cortical and subcortical regions during autonomically relevant conditions rather than one monolithic cortical autonomic network. [46]

The anatomical boundaries and functional membership of a “central autonomic network,” however, are not fixed. Human functional imaging measures neural correlates rather than peripheral autonomic nerve traffic, and many detailed circuit assignments derive from animal neuroanatomical and physiological studies. The CAN is therefore useful as an organizing framework for distributed central integration, not as a single anatomically closed structure whose activation can be inferred from a peripheral biomarker. [42, 45, 46] Its detailed state-dependent organization is considered in Section 11.

4.3 Peripheral Efferent Organization

The conventional sympathetic and parasympathetic divisions describe major peripheral efferent pathways through which central and reflex control reaches target tissues. Both use preganglionic neurons whose axons leave the central nervous system and communicate through peripheral

autonomic ganglia before postganglionic signaling reaches many target organs. Their anatomical origins, ganglionic organization, neurotransmitters, and target distributions differ, and parasympathetic pathways include important cranial as well as sacral outflow. [41, 44] Figure 1 summarizes this conventional peripheral efferent architecture; it should be read as an organizational schematic rather than as a model of two unitary whole-body autonomic states.

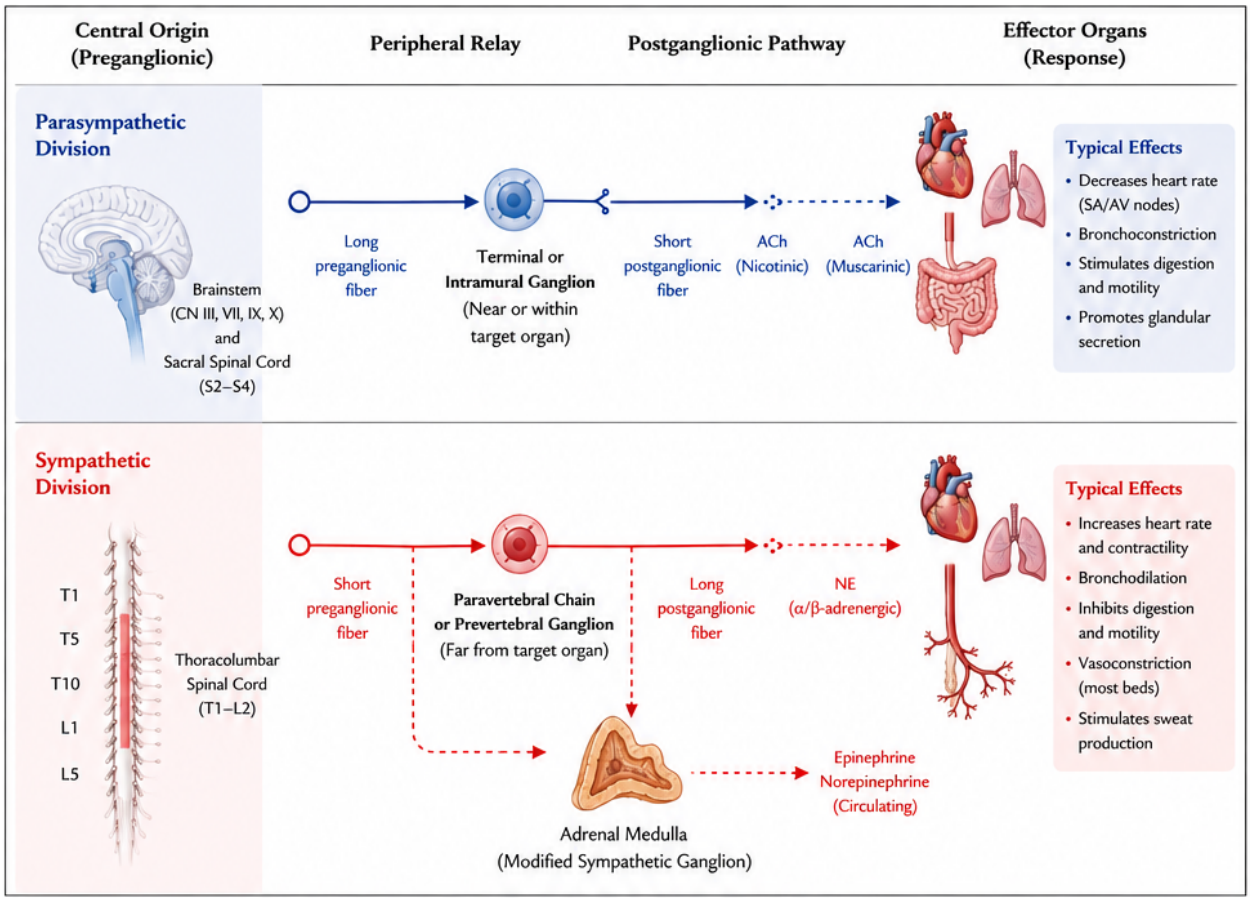


Figure 1. Conventional peripheral efferent organization of the autonomic nervous system. Simplified schematic of major sympathetic and parasympathetic pathways, ganglionic organization, principal neurotransmitters, and representative target effects. The diagram is organizational and should not be interpreted as evidence that sympathetic or parasympathetic outflow behaves as a single whole-body state. [41,44]

This classification should not be mistaken for two unitary whole-body output channels. Peripheral autonomic pathways are organized according to target and function. Experimental evidence supports organ- and tissue-specific reflex patterns, particularly within sympathetic outflow, rather than obligatory parallel activation of every sympathetic effector. [1] The regional organization of sympathetic physiology is examined in Section 8.

The same caution applies to parasympathetic physiology. The vagus is a major parasympathetic pathway to thoracic and abdominal viscera, but “parasympathetic” and “vagal” are not interchangeable descriptions of the entire division. Nor does activity directed toward one vagally innervated organ establish activity at another. The anatomical and functional heterogeneity of the vagus is therefore treated separately in Sections 5–7 rather than collapsed into the present macro-architecture. [25, 41, 44]

4.4 Ganglia, Local Circuits, and the Enteric Nervous System

Peripheral autonomic ganglia are not merely anatomical relay points in a diagram from brain to organ. They participate in the organization and transmission of autonomic output, while additional local neural circuits can shape organ function closer to the target. This becomes particularly apparent in the gastrointestinal tract, where extensive enteric circuitry supports local sensory processing, interneuronal integration, and motor control while remaining functionally connected with extrinsic sympathetic and parasympathetic pathways. [25, 41, 44]

For this reason, the enteric nervous system is commonly discussed alongside the sympathetic and parasympathetic divisions, but the three-part taxonomy should not be interpreted as evidence that the body occupies discrete global “sympathetic,” “parasympathetic,” or “enteric” states. The labels describe useful anatomical and functional organization; they do not define mutually exclusive whole-organism modes. [41, 44, 47]

Taken together, autonomic architecture can be represented schematically as:

visceral state → afferent sensing → distributed integration → differentiated efferent output → ganglionic/local processing → organ-specific effectors → altered visceral state

The arrows should not be interpreted as a strictly linear hierarchy. Autonomic organization includes recurrent feedback, local processing, reflex circuitry, and interactions between central state and peripheral physiology. The literature does not support reducing that organization to exclusively “top-down” or “bottom-up” control. [25, 43]

This architecture explains why the physiological specificity established in Section 3 is necessary. A change measured at one effector occupies only one location within a larger distributed system. It may provide valid information about that effector or a validated pathway contributing to it, but it cannot automatically identify the state of the entire autonomic nervous system. Likewise, observing a peripheral response does not by itself establish which central circuit generated it. [1, 25, 46]

The conventional autonomic vocabulary remains useful when applied at the appropriate anatomical scale. Recent scholarship has challenged aspects of the historical “autonomic,” “sympathetic,” and “parasympathetic” taxonomy and proposed alternative visceral-neural formulations. [47] Those critiques reinforce the importance of anatomical and functional specificity, but they do not require abandoning conventional terminology for this paper. Here, sympathetic, parasympathetic, vagal, enteric, afferent, and efferent labels are retained where they identify defined pathways or functions rather than presumed global physiological states.

The resulting model is therefore neither a two-branch seesaw nor a single central controller. It is a distributed, recurrent sensor-integrator-effector system whose outputs are differentiated by pathway, organ, and physiological demand. That architecture provides the necessary context for the next question: what exactly is meant by the vagus nerve, and which parts of autonomic physiology can legitimately be attributed to it?

5. The Vagus Nerve: Anatomy, Afferent-Efferent Organization, and Functional Scope

The vagus nerve is frequently described as the principal parasympathetic nerve, but that shorthand can obscure its actual anatomy. The vagus is a mixed cranial nerve containing sensory afferent fibers, branchiomotor fibers, and visceral efferent fibers. Only the visceral efferent component is appropriately described as parasympathetic. The physical nerve therefore carries information in both directions and serves multiple functions rather than constituting a single parasympathetic output channel. [48, 49]

This distinction is foundational for interpreting vagal physiology. A change associated with one vagal pathway does not establish that all components of the nerve changed similarly. “Vagal activity” is

consequently incomplete as a mechanistic description unless the direction of signaling, relevant pathway or target, and physiological domain are specified.

5.1 Vagal Afferents: Multiple Sensory Channels

Vagal sensory neuron cell bodies are located primarily in the superior, or jugular, and inferior, or nodose, sensory ganglia. These populations have different embryological origins and, particularly in experimental models, exhibit substantial molecular and functional specialization. [49–51]

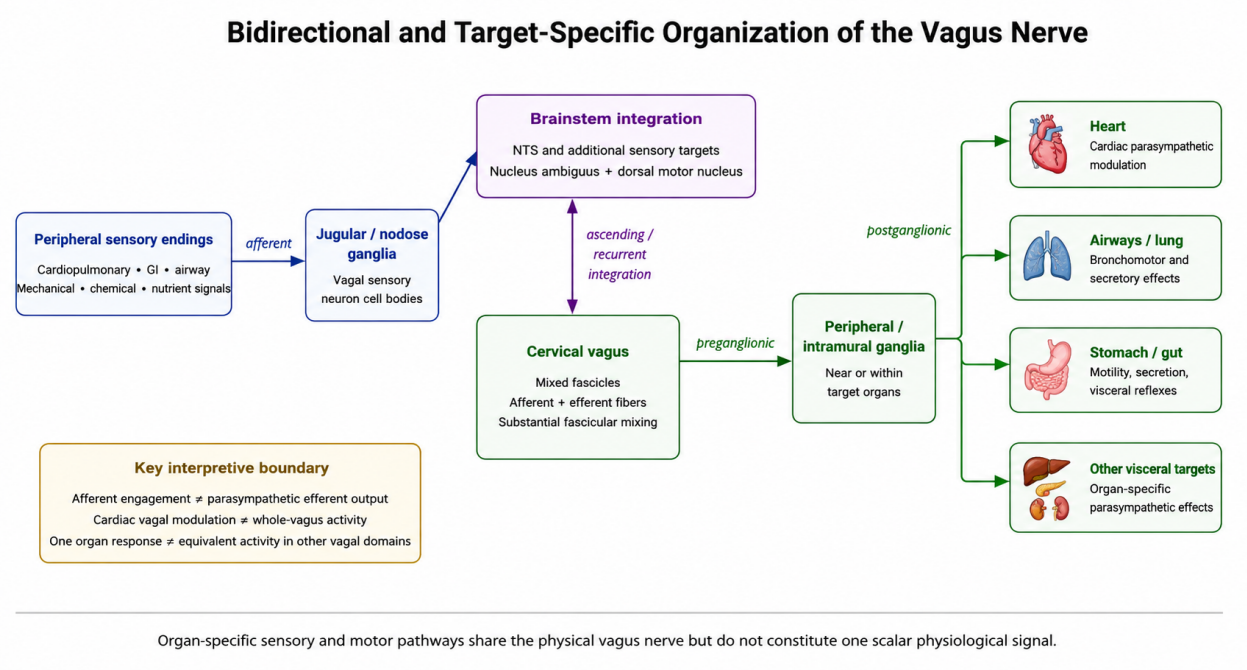
Their peripheral endings sample a wide range of internal conditions. Cardiopulmonary and gastrointestinal vagal afferents can respond to mechanical deformation, pressure, distension, nutrients, chemical signals, and other organ-specific events. These fibers should therefore not be conceptualized as one homogeneous stream carrying a generic measure of internal state. Different sensory populations encode different physiological variables and project through partially differentiated pathways. [50, 52, 53]

The nucleus tractus solitarius (NTS) is the major medullary recipient of visceral vagal afferent traffic, but it is not the exclusive termination site. Selected vagal sensory populations also project to paratrigeminal and spinal trigeminal regions and other medullary targets. [49, 54] The common diagram of "vagus → NTS" is therefore useful as a first approximation, but it should not be mistaken for a complete map of vagal sensory processing.

At the level needed for this paper, the afferent pathway can be summarized as:

organ-specific physiological event → specialized vagal sensory ending → jugular/nodose sensory neuron → NTS and/or additional medullary sensory-processing pathways

This sensory architecture is important because an intervention capable of influencing vagal afferent traffic would not necessarily be equivalent to one directly changing parasympathetic efferent output. Afferent engagement and efferent parasympathetic action are anatomically different propositions. Figure 2 places this afferent pathway alongside the distinct parasympathetic efferent pathway to show why neither can be treated as a scalar measure of the whole vagus.



Organ-specific sensory and motor pathways share the physical vagus nerve but do not constitute one scalar physiological signal.

Figure 2. Bidirectional and target-specific organization of the vagus nerve. Vagal afferents arise from organ-specific sensory endings with cell bodies in jugular and nodose ganglia and project principally to the NTS and additional medullary sensory targets. Parasympathetic efferents arise from defined brainstem motor populations, travel through vagal pathways, and relay in peripheral or intramural ganglia before reaching target organs. The schematic emphasizes that afferent engagement, cardiac parasympathetic modulation, and whole-vagus activity are not interchangeable constructs. [48–56]

5.2 Vagal Efferents: Parasympathetic Output Is Target Specific

Preganglionic vagal motor neurons arise principally from the nucleus ambiguus and the dorsal motor nucleus of the vagus. Efferent vagal control is therefore heterogeneous even at its central origin rather than being generated by one generic "vagal center." [48, 55, 56] These neurons send preganglionic axons through vagal pathways toward peripheral or intramural ganglia located near or within target organs. Postganglionic neurons then provide the final neural connection to many visceral effectors. Vagal parasympathetic control is consequently not a simple one-neuron projection from brainstem to organ. [48, 49]

Different vagal motor populations contribute to different physiological functions. Cardiac vagal neurons are represented in both nucleus ambiguus and dorsal motor nucleus populations, although their relative contributions and subtype organization remain areas of active investigation. [55, 56] Gastrointestinal and other visceral vagal outputs have their own patterns of preganglionic organization and peripheral integration.

The appropriate anatomical hierarchy is therefore:

central integrative circuitry → defined vagal preganglionic population → vagal trunk/branch → peripheral or intramural ganglion → target-organ effector

This hierarchy matters because a demonstrated change at one target does not establish equivalent efferent activity at another. Cardiac vagal control is developed in Section 6, while representative noncardiac vagal functions are examined in Section 7.

5.3 The Human Vagus Is Not a Uniform Cable

The physical organization of the human vagus further argues against treating it as a unitary signal. Human cervical and abdominal vagus nerves contain multiple fascicles composed of mixtures of myelinated and unmyelinated axons, with substantial variation across anatomical level and between individuals. [57, 58] Nor do human cervical fascicles appear to behave as fixed parallel cables running unchanged from one end of the neck to the other. Microstructural studies show repeated splitting and merging over relatively short distances. Recent human microCT tracing supports partial organ-related organization near some branch points while also demonstrating substantial mixing elsewhere along the nerve. [59, 60]

This has two important consequences. First, a simple fixed fascicular map cannot presently be assumed to apply throughout the human cervical vagus or across individuals. Second, stimulation or recording at one cervical location cannot be presumed anatomically selective for one organ or function merely because some degree of organ-related fascicular organization exists elsewhere.

The cervical vagus is also not necessarily a chemically pure parasympathetic bundle. Human anatomical studies have identified tyrosine-hydroxylase-positive fibers within cervical vagal fascicles, with evidence that at least some originate from sympathetic structures. [61] Although this does not provide a complete functional map of sympathetic signaling within the vagus, it establishes an important anatomical boundary: the physical cervical nerve trunk should not be equated with a pure collection of parasympathetic fibers.

The statement that most vagal fibers are afferent is widely used as a teaching approximation and captures an important feature of vagal organization: sensory traffic constitutes a major component of the nerve. However, a fixed numerical ratio should not be treated as a universal human constant.

Human vagal morphology varies by cervical, thoracic, and abdominal level, and available structural methods do not assign every axon unambiguously by direction, target, and function. Fiber composition can also change as branches leave or join the nerve. [48, 57, 58] For this reason, statements such as “the vagus is 80% afferent and 20% efferent” are better understood as broad teaching shorthand than as a precise anatomical property applicable to every human vagal segment.

The scientifically important conclusion does not depend on an exact percentage: the vagus carries substantial sensory as well as motor traffic, and those components must be distinguished when interpreting physiological measurements or stimulation effects.

5.4 From Anatomy to Evidentiary Language

The anatomy establishes several limits on what can legitimately be called “vagal engagement.” A change in heart rate or heart-rate variability cannot establish total vagal activity. Stimulation of the cervical vagus cannot be assumed to selectively recruit one direction of signaling, one fiber class, one organ pathway, or one brainstem target without additional evidence. Likewise, activity in one vagally mediated organ system cannot establish equivalent activity elsewhere. [48, 57, 59, 60]

Accordingly, mechanistic language should identify the anatomical level actually supported by the evidence. Depending on the experiment, defensible descriptions might include vagal afferent signaling, cardiac parasympathetic modulation, vagal preganglionic output to a specified organ, or electrically evoked cervical vagal recruitment. The unqualified phrase “increased vagal activity” collapses anatomically distinct processes and should be avoided when the measurement does not distinguish among them.

This distinction becomes particularly important for biomarkers. A cardiac measurement may provide information about cardiac parasympathetic modulation under validated conditions, but cardiovagal physiology represents only one subset of vagal function. It cannot be promoted into a measure of the entire vagus. [48, 49] The validity and limitations of cardiac autonomic biomarkers are examined later in this paper.

The vagus should therefore be understood as a shared anatomical conduit containing multiple directions of information flow, fiber classes, central origins and destinations, peripheral branches, and organ-specific functions. Its anatomy does not support a single scalar quantity called “vagal activity” that rises or falls uniformly throughout the organism. Instead, every vagal claim should answer three questions:

Which direction? Which target? Which physiological function?

Those questions provide the anatomical boundary for the sections that follow. Section 6 examines the specific domain of cardiac vagal control, while Section 7 demonstrates why physiology at other vagally influenced organs cannot be inferred from the cardiac domain.

6. Cardiac Vagal Control and Parasympathetic Modulation

Cardiac vagal control is one of the best-characterized parasympathetic efferent domains, but it is also one of the most common sources of overly broad vagal inference. The relevant physiology is specific: defined vagal efferent pathways influence cardiac targets and thereby alter cardiac timing and conduction. This is cardiac parasympathetic control, not a measurement of activity throughout the vagus nerve. [55, 56]

At a useful mechanistic level, the pathway can be represented as:

central/reflex input → cardiac vagal preganglionic neurons → vagal cardiac pathways → peripheral cardiac ganglia → postganglionic acetylcholine release → muscarinic action at cardiac targets → altered nodal timing and conduction

The strongest established effects occur at the sinoatrial and atrioventricular nodes. Parasympathetic postganglionic signaling is cholinergic, with acetylcholine acting through muscarinic mechanisms to slow sinoatrial pacemaker rate and atrioventricular conduction. [48] More detailed claims about ventricular vagal effects or individual receptor and ion-channel mechanisms require narrower evidence and are not necessary for the present framework.

6.1 Tonic Cardiac Parasympathetic Influence

The resting heart is not simply operating at an intrinsic pacemaker rate until autonomic input is recruited by a challenge. Heart rate at any moment reflects intrinsic sinoatrial activity together with ongoing autonomic influences. Human pharmacological blockade provides particularly important evidence for this point. Removal of cardiac parasympathetic influence produces substantial changes in resting heart rate, demonstrating meaningful tonic parasympathetic restraint of cardiac chronotropy under resting conditions. [62]

This provides a legitimate, target-specific meaning for tonic cardiac parasympathetic influence. It does not establish a corresponding amount of activity elsewhere in the vagus, nor does resting heart rate alone quantify that influence. The observed heart rate remains the net result of intrinsic pacemaker activity, sympathetic influence, parasympathetic influence, and their interactions. For that reason, a lower resting heart rate cannot by itself be interpreted as greater cardiac vagal activity. The same endpoint could result from reduced sympathetic drive, altered intrinsic pacemaker behavior, mixed branch changes, or increased parasympathetic influence. [22, 23]

A defining feature of cardiac parasympathetic control is its rapid temporal action. Changes in vagal influence can alter cardiac timing substantially faster than changes in cardiac sympathetic influence, whose effector kinetics generally develop more slowly. [63] This difference matters because the two branches can contribute to the same cardiac endpoint while operating on different timescales. Rapid vagal modulation can shape individual cardiac intervals and beat-to-beat heart-period variability, whereas slower sympathetic effects may be less visible in very short temporal fluctuations. [63]

The temporal distinction also creates a measurement problem. Averaging physiology over long intervals can obscure brief cardiovagal changes, while very short measurements can become vulnerable to respiratory timing, transient state, ectopy, movement, and other sources of variability. Measurement windows therefore cannot be interpreted independently of the kinetics of the physiological process being studied. Fast vagal action should not, however, be equated with better regulation. A rapid parasympathetic response is first a physiological observation. Whether its magnitude, timing, persistence, or recovery is appropriate depends on the challenge and functional context established in Sections 3 and 17.

6.2 Respiratory Modulation of Cardiac Vagal Output

Cardiac vagal influence is not constant across the respiratory cycle. Respiration phasically modulates cardiac parasympathetic outflow, contributing substantially to respiratory-linked oscillation in heart period. This phenomenon underlies respiratory sinus arrhythmia and much of the respiratory-frequency variability observable in cardiac timing. [64–66]

The presence of respiratory-linked cardiac variability therefore provides physiologically meaningful information about cardiac parasympathetic modulation under appropriate conditions. But respiration is not merely a nuisance variable superimposed on an otherwise pure vagal signal. Breathing rate, depth, mechanical effects, and physiological state participate in generating the observed pattern. [64–66]

This distinction becomes especially important in experimental work in which the exposure itself may alter respiration. If breathing changes during motion, the resulting change in respiratory-linked heart-period variability cannot be interpreted without measuring the respiratory change as well. Respiration should therefore be treated as part of the physiology rather than simply corrected away statistically.

Section 10 examines respiratory-autonomic coupling in detail, and Section 14 addresses the validity and limitations of RSA, HF-HRV, RMSSD, and related measures. The purpose here is narrower: to establish why rapid respiratory-linked variability can contain real information about cardiac parasympathetic modulation without being equivalent to a direct recording of vagal nerve activity.

6.3 Cardiac Vagal and Sympathetic Influences Interact

As established in Section 3, cardiac sympathetic and parasympathetic influences are not required to behave as opposite ends of a seesaw. The cardiac vagal literature adds an additional layer: their effects can interact nonlinearly at the heart. [20, 21] One well-described example is accentuated antagonism, in which the cardiac effect of vagal activity can depend on the concurrent level of sympathetic drive rather than representing a fixed amount that can simply be subtracted from sympathetic influence. [21] This is another reason why heart rate cannot be decomposed reliably into a simple numerical quantity of 'sympathetic minus parasympathetic' activity.

Reflex context can also produce different relationships between the branches. Reciprocal responses are physiologically important, but coactivation and uncoupled branch changes are also possible. [20, 21] Section 3 established those multiple modes conceptually; they need not be reclassified here. The practical implication is that cardiac vagal influence must be interpreted in the context of concurrent cardiac physiology. The same apparent parasympathetic change can have different cardiac consequences depending on sympathetic state, intrinsic cardiac properties, respiration, and the timing of observation.

This physiology creates a critical distinction between the underlying neural pathway and the measurable cardiac result.

The causal sequence contains several steps:

vagal neural traffic → cardiac ganglionic transmission → acetylcholine release → muscarinic action → sinoatrial/atrioventricular response → measurable cardiac timing

Heart rate, heart period, RSA, RMSSD, and HF-HRV therefore sit downstream of the neural activity that contributes to them. They may contain valid information about cardiac parasympathetic modulation, but they are not interchangeable with the underlying neural signal.

Pharmacological blockade is valuable precisely because it helps establish how much a downstream cardiac measure depends on parasympathetic contribution. Even with such validation, however, the relationship is not one-to-one, because respiration, intrinsic cardiac behavior, branch interaction, and measurement conditions also shape the observed signal. [62, 64–67] Accordingly, cardiac parasympathetic modulation is generally more precise than the unqualified phrase vagal activity when the evidence comes from cardiac timing. Higher RSA, RMSSD, or HF-HRV does not demonstrate globally increased activity throughout cranial nerve X, and slower heart rate does not establish increased vagal firing.

The distinction is anatomical as well as methodological. Cardiac vagal physiology is one specific subset of the heterogeneous system described in Section 5. Evidence of increased cardiac parasympathetic influence therefore cannot establish increased vagal activity in gastrointestinal, pulmonary, inflammatory, or other vagally influenced pathways. [48, 49] Cardiac vagal control is consequently best understood as a rapid, target-specific parasympathetic efferent process that modulates cardiac timing and conduction through cholinergic muscarinic mechanisms while interacting dynamically with intrinsic cardiac and sympathetic influences.

That precision is important because cardiovagal physiology gives us a particularly measurable autonomic domain without giving us a scalar measure of the entire vagus. The next section makes that distinction concrete by examining vagal physiology outside the heart and asking whether different vagal organ systems necessarily change together.

7. Non-Cardiac Vagal Physiology

The cardiac domain provides unusually accessible evidence of parasympathetic modulation, but it represents only one subset of vagal physiology. Outside the heart, vagal pathways participate in sensory and efferent functions involving the airways, gastrointestinal tract, pancreas, metabolic regulation, and neuroimmune signaling. These functions are organized through distinct organ-specific circuits rather than one shared output that rises and falls uniformly throughout the body. [48, 49, 68] Figure 3 summarizes these organ-specific vagal domains.

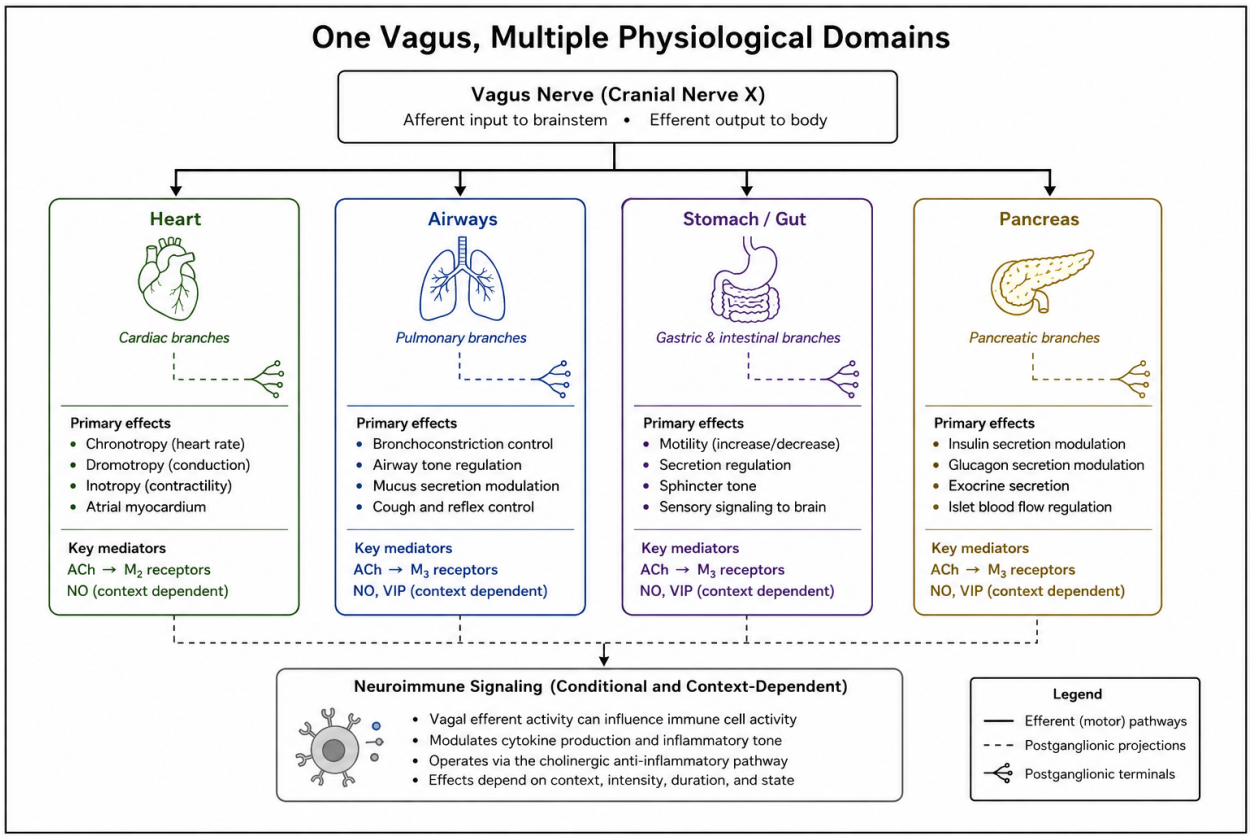


Figure 3. One vagus, multiple physiological domains. The vagus contains afferent and efferent pathways serving distinct organ systems, including cardiac, pulmonary, gastrointestinal, and pancreatic domains. Organ-specific pathways and effects should not be collapsed into a single scalar measure of whole-vagus activity; neuroimmune signaling is shown as conditional and context-dependent.

This distinction is empirically important. If the vagus behaved as one global physiological variable, measures of vagal control at different organs should covary consistently. Human experiments demonstrate that they need not. Similarly, a single stimulus associated with vagal activation can alter one noncardiac output while leaving another unchanged. The relevant physiological unit is therefore the specified pathway and target, not an assumed whole-vagus state.

7.1 Airway Vagal Physiology

The respiratory system illustrates both sides of vagal organization: specialized sensory afference and organ-specific parasympathetic efferent control. Airway vagal afferents comprise heterogeneous sensory populations responsive to mechanical and chemical events within the airways and lungs. These pathways participate in respiratory regulation and protective reflexes such as cough. [69] Their activity reflects airway-specific information and cannot be inferred from cardiac vagal output.

On the efferent side, parasympathetic pathways are a major autonomic influence on human airway smooth muscle and secretory function. [70] Bronchial cholinergic control is therefore a genuine vagally mediated physiological domain, but it is mechanistically distinct from vagal chronotropic control at the sinoatrial node.

Direct human evidence makes this distinction unusually clear. In healthy participants studied with cholinergic blockade, both resting bronchial cholinergic control and cardiac vagal control varied substantially between individuals, yet the two measures were not significantly correlated. [71] A person with greater cardiac parasympathetic influence therefore did not necessarily have proportionally greater bronchial vagal influence.

That experiment provides direct evidence against treating vagal control as one organism-wide scalar. It also establishes a practical boundary for cardiac biomarkers: respiratory sinus arrhythmia or other cardiovagal measures cannot be assumed to report vagal control of airway caliber or secretion. [71, 72]

7.2 Gastrointestinal Vagal Control and Sensory Signaling

The gastrointestinal system provides another example of highly differentiated vagal function. Upper gastrointestinal vagal circuits participate in gastric motility and accommodation through vagovagal loops that combine visceral afferent information, brainstem integration, efferent output, enteric circuitry, and local organ mechanisms. [73, 74] Gut vagal afferents also encode multiple categories of meal-related information, including mechanical stretch, nutrient content, and metabolic state. [75] These sensory channels reinforce the anatomical point established in Section 5: visceral vagal afference is not one generic stream reporting a single degree of bodily calm or parasympathetic activity.

Human cephalic-phase experiments provide functional evidence of vagal involvement in gastrointestinal secretion. Sham feeding can increase gastric acid and bicarbonate secretion, and atropine substantially inhibits those responses, supporting cholinergically mediated vagal control. [76] Yet even within the gastrointestinal domain, “vagal activation” is not sufficient as a complete mechanistic description. Gastric motility, accommodation, secretion, sensory signaling, and downstream metabolic responses involve different pathways and local circuits. Evidence that one gastric function changes therefore should not be assumed to establish a parallel change in every gastrointestinal vagal output.

7.3 Pancreatic and Metabolic Responses

Pancreatic physiology provides an especially useful demonstration of why a single vagal stimulus need not produce uniform downstream responses. Vagal regulation of pancreatic function operates through dedicated vagovagal circuits interacting with local neural, endocrine, and metabolic mechanisms. [77, 78] More broadly, autonomic metabolic regulation involves organ-specific afferent and efferent circuits linking gut, brain, liver, pancreas, and other tissues rather than one common parasympathetic command. [79]

Pancreatic polypeptide has often been used experimentally as an indirect marker of vagal-cholinergic activation. Human sham-feeding studies support a genuine cholinergic contribution: modified sham feeding increases pancreatic-polypeptide release, and atropine can abolish that response. Yet adrenergic blockade can also modify part of the response, demonstrating that even this commonly used organ-specific vagal-associated marker can reflect mixed autonomic physiology. [80]

More importantly, the same cephalic-vagal stimulus does not necessarily move different endocrine outputs together. In healthy humans, sham feeding increased pancreatic polypeptide but did not significantly increase insulin or GIP in the same experiment. [77] Vagal influence on insulin secretion under other physiological conditions remains possible; the narrower point for this review is that one vagally associated stimulus did not generate one uniform pancreatic or endocrine response.

Pancreatic polypeptide therefore cannot serve as a measure of whole-vagus activity any more than HRV can. Each is an organ- and mechanism-constrained downstream signal.

7.4 Neuroimmune Signaling Requires Particular Caution

Vagal involvement in inflammatory regulation has generated substantial experimental and therapeutic interest, but this domain also illustrates why functional effect and anatomical mechanism must be separated carefully. Experimental vagus nerve stimulation can alter inflammatory responses, and vagally associated pathways can participate in neuroimmune regulation. However, those observations do not establish a single simple efferent pathway running directly from the brain through the vagus to every immune target. [81–83]

The spleen is the clearest boundary case. Direct vagal innervation of splenic parenchyma should not be presented as established. Contemporary anatomical evidence attributes labeled intrasplenic fibers to sympathetic rather than direct vagal origin in the studied model, while leaving open indirect multisynaptic, immune-cell, enteric, sympathetic-relay, and other routes through which vagal stimulation may influence inflammatory physiology. [84, 85]

This distinction matters methodologically. Demonstrating that VNS suppresses an inflammatory response establishes a functional effect of the intervention. It does not by itself identify whether the relevant pathway was afferent, efferent, sympathetic-relay mediated, endocrine, immune-cell mediated, enteric, or some combination of these. The endogenous inflammatory reflex and the mechanism of electrically evoked VNS effects are related scientific questions, but they are not automatically the same question.

For this reason, statements such as “greater vagal tone reduces inflammation” compress multiple disputed anatomical and physiological propositions into one phrase. A defensible neuroimmune claim should instead identify the stimulus, measured inflammatory endpoint, demonstrated neural pathway, and degree to which that pathway was causally established.

Across these representative systems, the common result is not that vagal functions are unrelated. They are integrated within broader autonomic and central regulatory networks. The important result is that integration does not imply uniformity.

Cardiac and bronchial vagal control can dissociate within the same individuals. [71] A single cephalic-vagal challenge can change pancreatic polypeptide without changing insulin or GIP. [77] Airway afferents encode respiratory mechanical and chemical events, while gastrointestinal afferents encode stretch, nutrients, and metabolic state through different sensory populations. [69, 75] Neuroimmune effects can involve pathways whose anatomical organization differs still further.

There is consequently no single practical measurement that simultaneously quantifies vagal outflow to the heart, airways, stomach, pancreas, intestinal circuits, and immune interfaces. Cardiac timing measures such as RSA or HRV cannot validate noncardiac vagal engagement, just as pancreatic polypeptide cannot validate cardiac or whole-vagus engagement.

This provides the physiological counterpart to the anatomical boundary established in Section 5. The phrase vagal activity should be replaced, wherever the evidence permits, by a statement naming the relevant direction and target: cardiac parasympathetic modulation, airway vagal afference, bronchial cholinergic control, gastric vagovagal regulation, pancreatic vagal-associated response, or another defined domain.

Noncardiac vagal physiology therefore confirms that the vagus is not a unitary whole-body regulatory variable. Airway, gastrointestinal, pancreatic, metabolic, and neuroimmune functions are carried through distinct sensory, motor, and reflex circuits whose outputs can dissociate. Cardiovagal measures characterize the cardiac vagal domain; they cannot be promoted into evidence of global vagal engagement or regulation.

8. Sympathetic Regulation Is Regional, Not Unitary

The phrase sympathetic activation is often used as though the sympathetic nervous system were a single output channel whose activity rises or falls throughout the body. Human physiology does not support that model. Sympathetic efferent traffic is organized into differentiated pathways serving specific tissues and organs, and those pathways can be recruited in different magnitudes or even different directions during the same physiological state or challenge. [86–88]

Shared central drives and reflexes can produce parallel sympathetic activation across multiple regions; the evidentiary requirement is that parallel recruitment must be demonstrated rather than presumed. [89]

Accordingly, the scientifically meaningful question is generally not “Did sympathetic activity increase?” but rather:

Which sympathetic outflow changed, toward which effector, under what physiological condition?

8.1 Muscle Sympathetic Nerve Activity

Microneurography permits direct recording of postganglionic sympathetic neural traffic from accessible peripheral nerves in awake humans. One of its most established applications is muscle sympathetic nerve activity (MSNA), recorded from sympathetic fibers directed primarily toward skeletal-muscle vascular beds. [86, 90] MSNA is strongly linked to vascular resistance and arterial-pressure regulation and exhibits prominent cardiac rhythmicity and baroreflex modulation. It is therefore an unusually direct and valuable human measure of sympathetic vasoconstrictor traffic to skeletal muscle. [86, 90]

Its directness does not make it global. An electrode recording MSNA from an accessible peripheral nerve measures activity within that local neural population. It does not directly record sympathetic traffic to the skin, heart, kidney, gastrointestinal organs, adrenal medulla, or other sympathetic targets. [86, 90] Even MSNA itself is not completely homogeneous. Analysis of action potentials within human muscle sympathetic bursts demonstrates neuronal subpopulations with different recruitment thresholds and degrees of baroreflex control. [91] Regional specificity therefore persists even within a commonly treated sympathetic domain.

8.2 Skin Sympathetic Nerve Activity

Skin sympathetic nerve activity (SSNA) is a different postganglionic signal. It contains neural activity related to cutaneous vasomotor and sudomotor control and is influenced strongly by thermoregulation, emotional arousal, and other nonbaroreflex inputs. [92] This functional organization differs materially from MSNA. Cutaneous sympathetic pathways can contribute to vasoconstriction during cold exposure, sweating during heat or arousal, and specialized active vasodilator responses during heat stress. These are all sympathetic functions, but they are not physiologically interchangeable.

Human exercise studies demonstrate the distinction directly. Central command and muscle-afferent feedback can contribute differently to skin and muscle sympathetic responses during the same exercise challenge. [93, 94] SSNA therefore cannot be used as a surrogate for MSNA, and MSNA cannot be used as a surrogate for SSNA. A change in sweating or electrodermal activity likewise does not establish a corresponding change in muscle vasoconstrictor or visceral sympathetic traffic. [92, 95, 96]

This becomes particularly important for practical experiments because electrodermal activity is relatively easy to measure. Its accessibility does not expand its physiological domain. Section 15 addresses electrodermal measurement specifically; here the relevant point is simply that sudomotor sympathetic activity is one sympathetic effector pathway, not a meter of the entire sympathetic nervous system.

8.3 Visceral Sympathetic Outflows

Sympathetic regionalization extends beyond accessible peripheral nerves. The heart, kidneys, and hepatosplanchnic organs receive functionally differentiated sympathetic inputs that cannot routinely be measured through standard peripheral microneurography. Regional norepinephrine-spillover methods provide one important window into these internal organs. By combining tracer kinetics with regional sampling, spillover approaches can estimate sympathetic neurotransmitter release from particular vascular beds and reveal preferential organ activation that may be obscured in peripheral venous catecholamine concentrations. [87, 88]

These measurements have demonstrated that cardiac, renal, muscle, and other sympathetic responses do not necessarily move together. Cardiac sympathetic activity and MSNA may show some association at rest yet exhibit different relative changes during handgrip and mental stress. [89] During mental arithmetic, cardiac norepinephrine spillover can increase substantially even when antecubital venous norepinephrine changes little. [97] Conversely, after a large meal, renal norepinephrine spillover and MSNA can increase while cardiac norepinephrine spillover remains essentially unchanged. [98]

These examples are difficult to reconcile with a single scalar quantity called “sympathetic tone.” They are readily explained by differentiated recruitment of organ-specific sympathetic pathways. Regional spillover nevertheless remains downstream of neural firing. It reflects neurotransmitter release into plasma together with reuptake, blood flow, clearance, and tracer assumptions. It is therefore mechanistically closer to an organ-specific sympathetic process than peripheral venous norepinephrine concentration, but it is not equivalent to a direct nerve recording. [88]

8.4 Human Dissociation Is Observable Across States

Regional differentiation is not merely a theoretical possibility inferred from anatomy. It appears in human experimental physiology, aging, and disease. Across age groups, MSNA can increase while SSNA decreases, with selective impairment of thermoregulatory skin sympathetic responses. [95] In chronic renal failure, markedly elevated MSNA has been observed alongside SSNA and arousal-related SSNA responses that did not differ comparably from controls. [96] These findings do not mean that aging or renal disease produces a universal pattern that should be expected in healthy individuals. Their importance is narrower: they demonstrate directly that sympathetic outflows within the same person or population can dissociate substantially. Figure 4 summarizes this differentiated-output architecture.

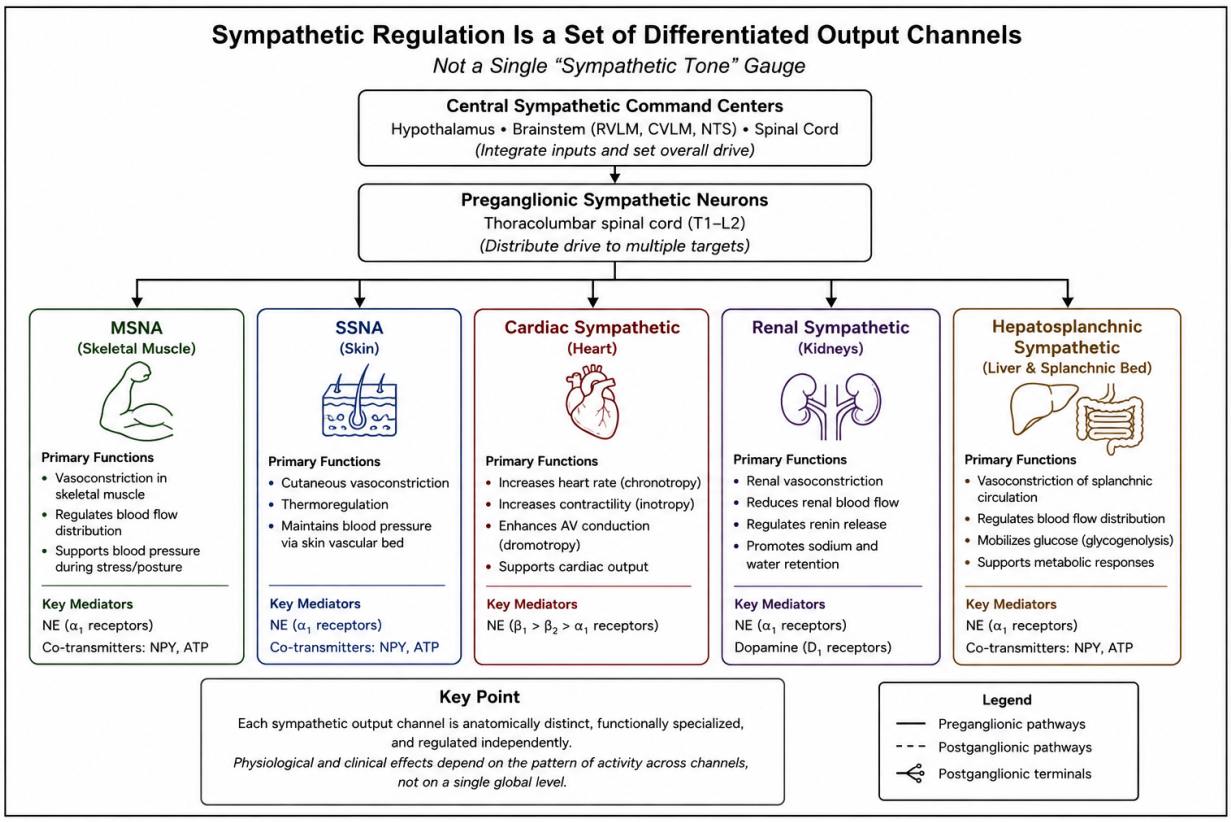


Figure 4. Sympathetic regulation is a set of differentiated output channels. Sympathetic neural traffic is organized into target-specific outflows serving skeletal muscle, skin, heart, kidney, and hepatosplanchnic organs. These channels may covary under some conditions but can also dissociate; activity in one regional effector should therefore not be treated as a single body-wide measure of sympathetic tone.

The same principle appears during acute challenges. Mental stress may preferentially recruit cardiac sympathetic activity. [97] Feeding may recruit renal and muscle sympathetic pathways without comparable cardiac activation. [98] Exercise can engage skin and muscle sympathetic pathways through different combinations of central command and peripheral reflex input. [93] Thus, both chronic physiological state and acute context can alter the pattern of sympathetic recruitment rather than merely changing the amplitude of one global sympathetic signal.

8.5 What a Sympathetic Measurement Can and Cannot Establish

The measurement hierarchy introduced later in Section 13 becomes especially important for sympathetic physiology because measures differ substantially in mechanistic proximity.

Direct MSNA provides strong evidence about postganglionic sympathetic neural traffic to skeletal-muscle vasculature. Direct SSNA provides information about cutaneous sympathetic neural traffic, although its mixed vasomotor and sudomotor composition can make quantitative interpretation more context dependent. Regional norepinephrine spillover provides organ-specific information about sympathetic neurotransmission where direct neural recording is usually unavailable. Peripheral plasma catecholamines, heart rate, arterial pressure, skin conductance, and other practical signals are progressively more integrated downstream observations.

None of those measurements becomes more global simply because it is convenient. A rise in MSNA does not establish increased SSNA, cardiac sympathetic activity, renal sympathetic activity, or adrenal catecholamine secretion. [86, 95, 96] A rise in SSNA or electrodermal activity does not

establish increased muscle or visceral sympathetic traffic. [92, 95, 96] Peripheral venous norepinephrine concentration cannot identify reliably which organ-specific sympathetic outflow changed and may miss selective regional activation. [88, 97]

Heart rate and arterial pressure are even more integrated endpoints. Both can change through combinations of sympathetic, parasympathetic, vascular, mechanical, endocrine, metabolic, and intrinsic cardiac mechanisms. Their direction alone cannot specify which sympathetic efferent pathway changed. The correct terminology should therefore follow the measurement. Where the evidence supports it, terms such as muscle sympathetic nerve activity, cutaneous sympathetic activity, sudomotor sympathetic response, cardiac sympathetic activation, or renal sympathetic activation are preferable to the unqualified phrase sympathetic tone.

Regional sympathetic physiology also prevents a second common error: assuming that less sympathetic activity necessarily means better regulation. Sympathetic activation performs essential physiological functions. Vasoconstrictor traffic contributes to maintenance of arterial pressure; cardiac sympathetic activation can support increased circulatory demand; renal sympathetic pathways participate in renal and volume regulation; cutaneous pathways support thermoregulation; and sudomotor activity supports heat dissipation.

The existence of differentiated sympathetic patterns therefore does not establish one preferred pattern for all contexts. An increase in a particular outflow can be appropriate or maladaptive depending on the demand, magnitude, duration, recovery, and functional consequence. Conversely, an absent or blunted response can itself be inappropriate when the physiological situation requires activation. Section 17 owns the evidentiary question of when a physiological trajectory can be called improved regulation. For the present section, the conclusion is deliberately narrower.

Human sympathetic physiology is organized as differentiated neural outflows to specific effectors and organs, not as a single body-wide sympathetic state. MSNA, SSNA, cardiac sympathetic activity, renal sympathetic activity, and other visceral outflows can covary under some conditions and dissociate markedly under others. Even within an individual sympathetic outflow, neuronal recruitment can be heterogeneous. [89, 91, 95, 96]

Sympathetic interpretation should therefore begin by naming the effector or organ actually measured. A change in one sympathetic domain cannot automatically be generalized to the sympathetic nervous system as a whole.

9. Baroreflex Physiology and Cardiovascular Regulation

The arterial baroreflex is a rapid negative-feedback system that helps stabilize arterial pressure during ongoing physiological change. Stretch-sensitive receptors in the carotid sinus and aortic arch respond to pressure-related deformation of the arterial wall and transmit afferent information through glossopharyngeal and vagal pathways to brainstem integrative circuits. [45, 49, 99] Figure 5 summarizes the baroreflex as a closed-loop, multi-effector feedback system with nonlinear, state-dependent operating characteristics.

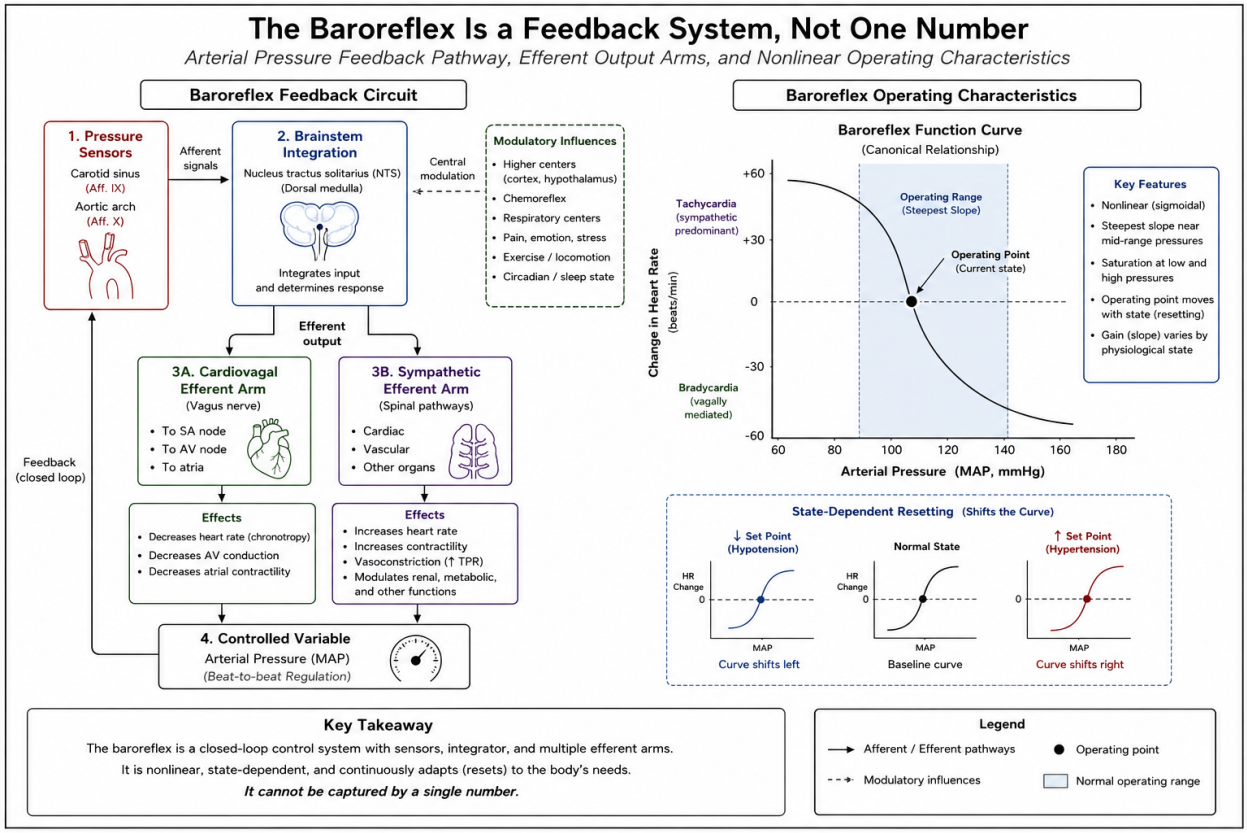


Figure 5. The baroreflex is a feedback system, not one number. Arterial pressure is sensed at carotid and aortic baroreceptors and integrated centrally before influencing distinct cardiovagal and sympathetic efferent arms. Baroreflex behavior is nonlinear and depends on operating point and physiological state; resetting can shift the pressure-response relationship without reducing the system to a single scalar measure.

A rise in arterial pressure generally increases baroreceptor afferent signaling and favors greater cardiovagal influence together with reduced sympathetic vasoconstrictor outflow. A fall in pressure produces the opposite compensatory tendency. The resulting changes in cardiac timing, cardiac sympathetic influence, vascular resistance, and arterial pressure alter the stimulus presented to the baroreceptors, completing the feedback loop.

At a functional level, the system can be represented as:

arterial pressure → carotid/aortic wall deformation → baroreceptor afference → brainstem integration → cardiac-vagal and sympathetic efferent responses → cardiac/vascular effects → arterial pressure

No single measurement within that sequence represents the entire reflex.

9.1 One Reflex, Multiple Efferent Arms

The baroreflex is sometimes discussed primarily as a heart-rate reflex, but pressure regulation requires more than cardiovagal control. The cardiovagal arm alters sinoatrial and atrioventricular timing through the cardiac parasympathetic pathways described in Section 6. Changes in this arm can be observed as changes in R-R interval or heart rate. A sympathetic cardiac arm can alter cardiac sympathetic drive and thereby influence chronotropy, conduction, and contractile performance. A sympathetic vasomotor arm alters regional vascular resistance, including through baroreflex control of muscle sympathetic nerve activity. [86, 90]

These branches need not possess identical sensitivity. Human studies demonstrate that cardiovagal baroreflex sensitivity and MSNA-based sympathetic baroreflex sensitivity can dissociate substantially. In a large healthy young cohort, cardiac BRS did not predict sympathetic MSNA BRS. [100] Spontaneous cardiac and sympathetic BRS likewise show only modest correspondence overall, with some relationships differing by sex. [101]

Thus, the phrase baroreflex sensitivity is incomplete unless the reflex output being measured is identified. Cardiovagal BRS and sympathetic BRS are related physiological quantities, but they are not interchangeable measures of one underlying number.

9.2 Baroreflex Sensitivity Is a Gain, Not the Reflex Itself

Baroreflex sensitivity, or BRS, describes the responsiveness of a specified reflex output to a specified pressure input under defined conditions. In general terms, it is a gain: how much the selected output changes for a given change in arterial-pressure stimulus. The conventional phenylephrine or Oxford cardiovagal approach relates changes in systolic pressure to changes in R-R interval. It therefore measures a cardiac branch of the baroreflex rather than all baroreflex function. [102] Sympathetic BRS can instead relate changes in diastolic pressure to MSNA burst probability or activity, quantifying a muscle-vasoconstrictor sympathetic component. [100, 101]

A BRS value is therefore physiologically interpretable only when several things are known:

pressure input → selected output → participant state → perturbation or analytic method → operating region

Two values both called “BRS” may represent different parts of the feedback system.

This is not merely a semantic concern. Different baroreflex-testing techniques applied to the same individuals show only modest intercorrelation, demonstrating that method-specific physiology can be concealed by referring to all results as “the BRS.” [103]

9.3 The Baroreflex Is Nonlinear

The full relationship between arterial-pressure stimulus and reflex output is not adequately described by one linear slope across all pressures. Baroreflex stimulus-response curves are generally conceptualized as nonlinear, with regions of low response at the extremes and a steeper region of higher gain between them. This creates several distinct concepts: threshold, below which further pressure change may produce little additional response; saturation, beyond which additional pressure change likewise produces little additional response; maximal gain, representing the steepest portion of the stimulus-response relationship; and the operating point, the location on that curve around which the system is functioning under the current physiological state.

The distinction matters because a locally measured slope depends partly on where the person is operating on the curve. A different local slope does not necessarily mean that the reflex's maximum possible responsiveness has changed. [104] This is one reason a single BRS number should not be treated as an intrinsic permanent property of an individual.

9.4 State Dependence and Baroreflex Resetting

The baroreflex does not operate around one immutable pressure set point. Its operating point changes with posture, exercise, central command, muscle-afferent input, cardiopulmonary loading, and other physiological states. [105, 106] Exercise provides a particularly clear demonstration. As arterial pressure rises to support increased metabolic demand, the baroreflex does not simply switch off. Instead, the stimulus-response relationship can reset upward and rightward, allowing the system to regulate pressure around a higher operating range while retaining substantial reflex sensitivity. [105, 106]

This distinction prevents an important interpretive error. A higher arterial pressure during a challenge does not necessarily indicate loss of baroreflex control, just as a lower local BRS value in a new

state does not necessarily indicate global reflex impairment. The operating point may simply have moved to a different portion of a reset response curve. [104] Cardiovagal response latency is state dependent as well. Human carotid-stimulation experiments show that vagally mediated response delay changes with posture and autonomic state. [107] In synchronized physiological recordings, temporal relationships should therefore be estimated rather than assumed to remain fixed across baseline and challenge.

Resetting is a physiological property, not automatically evidence of adaptation or benefit. Whether a state-dependent change represents appropriate regulation is a separate question reserved for Sections 12 and 17.

9.5 Perturbation-Based Baroreflex Measurement

Different experimental methods interrogate different portions of the baroreflex. The classic phenylephrine/Oxford method pharmacologically raises arterial pressure and relates the pressure change to reflex R-R interval prolongation. It has historically provided a strong perturbational measure of cardiovagal BRS. [102] The modified Oxford method uses sequential vasodilator and vasoconstrictor perturbations to create a wider pressure range. This allows broader characterization of cardiac and sympathetic pressure-response relationships than a narrow spontaneous fluctuation may provide. [100, 104]

Neck pressure and suction manipulate carotid sinus transmural pressure more selectively and can map carotid-cardiac and carotid-vasomotor stimulus-response curves, including threshold, saturation, operating point, and maximal gain. [103, 105, 108] These methods, however, primarily manipulate carotid baroreceptor loading and do not reproduce aortic stimulation in the same way as systemic blood-pressure perturbation.

The Valsalva maneuver creates endogenous phase-dependent pressure changes and is clinically useful, but it simultaneously alters respiration, intrathoracic pressure, venous return, and multiple autonomic inputs. BRS estimates derived from it are consequently not physiologically identical to those produced by pharmacological or neck-chamber methods. [103] These methods are not competing attempts to measure one perfectly method-independent number. They probe overlapping but non-identical parts of a dynamic feedback system.

9.6 Spontaneous Baroreflex Sensitivity

Natural beat-to-beat fluctuations in arterial pressure and cardiac timing also permit estimation of baroreflex-related coupling without deliberately perturbing the participant. Sequence methods identify spontaneous runs in which systolic pressure rises or falls and is followed by concordant changes in R-R interval. The pressure-R-R slope across those sequences provides an estimate of local cardiovagal coupling. Transfer-function, spectral, and alpha-index approaches quantify frequency-domain relationships between spontaneous arterial-pressure and heart-period oscillations.

Comparative studies show that several common spontaneous methods can correlate under ordinary conditions, although their estimates depend on algorithmic choices and may become unreliable in recordings with very low apparent BRS. [109] The physiological interpretation should remain narrow. Spontaneous cardiovagal BRS primarily samples local gain near the person's current operating point. During upright tilt, for example, spontaneous estimates can reflect a lower local slope even while the maximal gain of the reset full baroreflex curve remains substantially higher. [104]

Spontaneous methods are therefore valuable for repeated and naturalistic measurement, but they do not reconstruct the complete baroreflex stimulus-response curve and do not directly record baroreceptor afferent firing or all efferent branches. [104, 110] Natural pressure-heart-period coupling can also contain contributions from respiration and common central drives. Temporal sequence and coherence strengthen the interpretation that the signals are coupled, but they do not transform the measurement into a direct recording of one-way baroreceptor causality. [109, 110]

9.7 BRS, HRV, and Blood-Pressure Variability Are Not Equivalent

The baroreflex influences cardiac timing, and cardiac parasympathetic activity contributes to both HRV and cardiovagal BRS. The measures are therefore related, but they are not the same physiological quantity. A change in HRV does not prove that baroreflex sensitivity changed. Resting cardiac vagal influence also contains baroreflex-independent components. [111] Similarly, BRS contributes to short-term blood-pressure buffering, but it does not uniquely determine spontaneous blood-pressure or heart-rate variability because numerous other physiological processes contribute to those signals. [112]

This distinction will become important when HRV is treated directly in Section 14. Section 9 requires only the conceptual boundary: HRV describes variation in cardiac timing, whereas cardiovagal BRS describes the relationship between a pressure input and a cardiac timing response under specified conditions.

The arterial baroreflex is best understood as a rapid, multi-effector, state-dependent cardiovascular feedback system, not a single cardiovagal reflex or scalar autonomic variable. Cardiac and sympathetic baroreflex gains can dissociate, and the full stimulus-response relationship is nonlinear and capable of resetting around different operating pressures. This places several boundaries on interpretation.

A cardiovagal BRS value cannot establish sympathetic baroreflex function. An MSNA-based sympathetic BRS value cannot establish baroreflex control of every sympathetic organ. A spontaneous pressure-R-R slope cannot be treated as a direct measure of baroreceptor afference or the complete reflex. And neither higher nor lower BRS is intrinsically evidence of better or worse autonomic regulation without consideration of physiological state, operating point, method, repeated measurement, and functional meaning.

For a naturalistic study such as SeaKing Phase 1, synchronized beat-to-beat arterial pressure and ECG could support (see Phase 1 Protocol, Section X) analyses of pressure-heart-period coupling and its trajectory across baseline, exposure, and recovery. The defensible claim would remain method-specific: an observed change could indicate altered local cardiovagal baroreflex coupling under the measured condition. It could not by itself establish global baroreflex improvement, a specific vestibulo-baroreflex mechanism, or improved autonomic regulation.

That is the level at which BRS becomes useful: not as another global autonomic score, but as a defined property of a specific pressure-response relationship.

10. Respiration as an Autonomic Modulator and Measurement Confound

Respiration is often treated in autonomic research as a variable that must be controlled so that the “real” autonomic signal can be observed. That framing is incomplete. Breathing is itself an active component of cardiovascular and autonomic physiology. Across each respiratory cycle, neural respiratory activity, pulmonary afferent feedback, intrathoracic-pressure changes, cardiac filling, arterial pressure, baroreflex input, cardiac parasympathetic modulation, and sympathetic neural traffic interact dynamically. [113, 114] Figure 6 summarizes respiration as both an input to and an output of integrated autonomic and cardiovascular regulation.

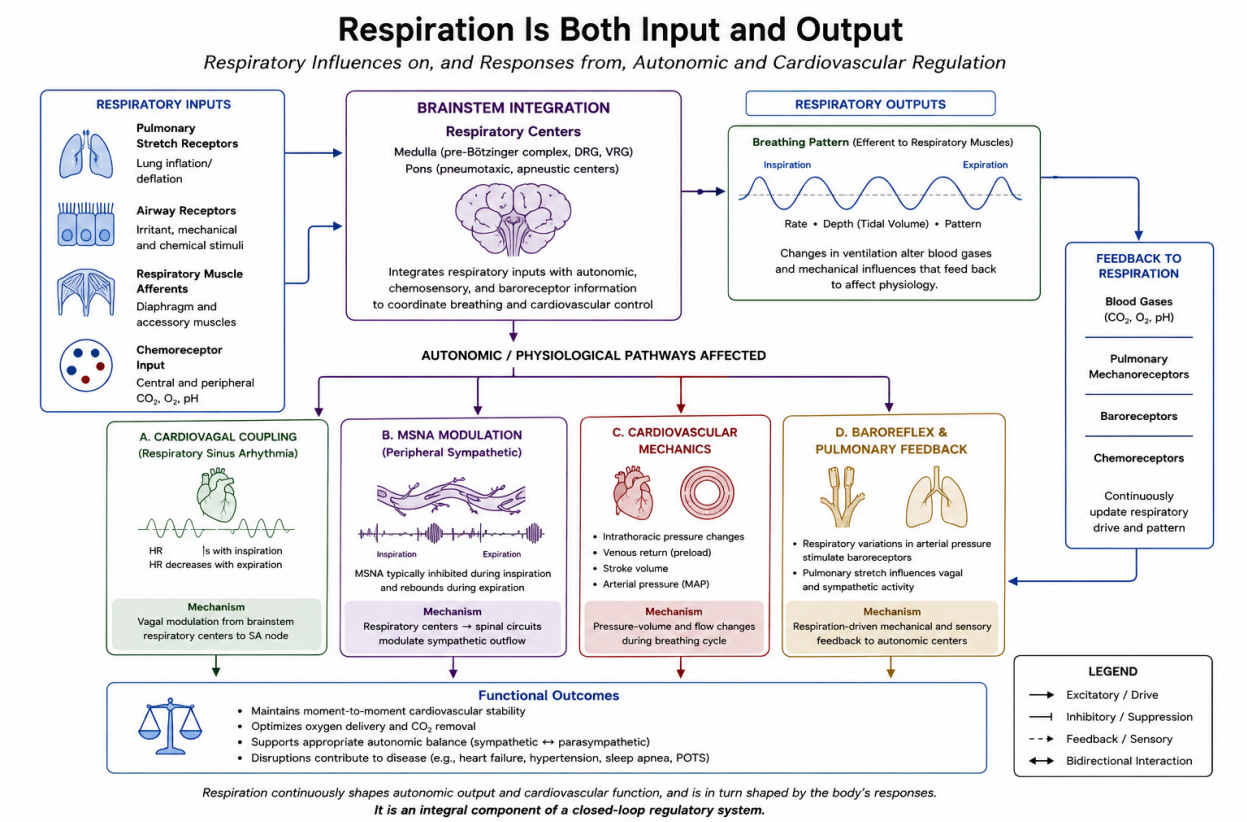


Figure 6. Respiration is both input and output. Respiratory sensory inputs and central respiratory pattern generation interact with cardiovagal timing, regional sympathetic outflow, cardiovascular mechanics, baroreflex function, and pulmonary feedback. Because breathing both shapes and responds to these processes, respiratory state must be retained when interpreting synchronized autonomic and cardiovascular measurements.

Respiration should therefore be understood as both input and output within the regulatory system. Autonomic and behavioral state can change breathing, while the resulting respiratory pattern can in turn alter downstream cardiovascular and autonomic measurements. This distinction is especially important in an experimental exposure capable of changing comfort, arousal, posture, speech, nausea, effort, or breathing itself. A physiological change observed during that exposure may be partly mediated through respiration rather than occurring independently of it.

10.1 Respiratory Heart Rate Variability

Heart rate normally varies across the respiratory cycle. This phenomenon has historically been termed respiratory sinus arrhythmia (RSA). Following recent international expert recommendations, this paper uses the more descriptive term respiratory heart rate variability (RespHRV), retaining RSA when discussing historical literature or established metric names. [115] RespHRV reflects respiratory-linked variation in sinoatrial timing. In intact humans, cardiac parasympathetic modulation contributes strongly to this oscillation, which is why respiratory-frequency HRV can provide useful information about cardiovagal physiology. [64, 65, 116]

The oscillation, however, is not determined by cardiac parasympathetic influence alone. Its amplitude depends materially on the respiratory stimulus itself. Breathing frequency and tidal volume alter the magnitude of RespHRV, and the respiration-to-heart-rate relationship changes in both magnitude and phase as breathing frequency changes. [116, 117]

Accordingly:

larger RespHRV ≠ proportionally greater vagal neural firing

RespHRV is better understood as the cardiac result of respiratory modulation acting through interacting neural, reflex, afferent, and mechanical pathways.

Several mechanisms contribute to respiratory-linked cardiovascular oscillation, and their relative importance can change with breathing pattern and physiological state. Central respiratory-autonomic coupling provides one route. Respiratory rhythm-generating networks interact with neural circuits influencing cardiac vagal and sympathetic output, allowing respiratory timing to shape autonomic discharge.

Pulmonary and cardiopulmonary afferent feedback provides another. Lung inflation and associated mechanical events generate sensory information that can modify respiratory-linked autonomic responses. Human lung-denervation evidence supports a meaningful contribution from pulmonary vagal afference: after lung transplantation, the normal relationships among tidal volume, respiratory effort, breathing frequency, and RespHRV are substantially altered. [118]

Mechanical and hemodynamic coupling adds another pathway. Inspiration changes intrathoracic pressure, venous return, cardiac filling, stroke volume, and arterial pressure. These mechanical changes can influence subsequent cardiac timing through cardiovascular reflexes, including the baroreflex. The contribution of neural autonomic pathways is nevertheless substantial. Human total cardiac autonomic blockade causes respiration-to-heart-rate and pressure-to-heart-rate impulse responses to nearly disappear, demonstrating major autonomic mediation of these relationships. [119]

Yet autonomic mediation is not exclusive. Respiratory-frequency heart-rate oscillations can persist at reduced magnitude in denervated transplanted hearts. [120] The mere presence of a respiratory cardiac oscillation therefore does not prove intact cardiac vagal innervation or identify a single neural mechanism.

The appropriate model is not “RSA equals vagal activity.” It is:

respiratory pattern → central + afferent + mechanical interactions → cardiac-autonomic modulation → measurable respiratory variation in cardiac timing

10.2 Respiration Also Modulates Sympathetic Outflow

Respiratory coupling is not confined to the parasympathetic cardiac domain. Direct human microneurographic recordings demonstrate pronounced within-breath modulation of muscle sympathetic nerve activity. MSNA is generally inhibited around inspiration and postinspiration and becomes more prominent toward expiration, although the exact pattern depends on physiological conditions. [121, 122] Respiratory phase can also modify reflex responsiveness. Carotid-baroreceptor perturbation produces different sympathetic responses depending on the phase of breathing in which the perturbation occurs. [121]

These findings prevent another overly simple inference. Greater cardiorespiratory coupling cannot automatically be interpreted as “more parasympathetic.” Breathing can simultaneously organize cardiac parasympathetic timing, sympathetic neural traffic, mechanical cardiovascular oscillations, and baroreflex expression. As established in Section 8, the sympathetic effect must also retain its effector identity. Respiratory modulation of MSNA demonstrates respiratory-sympathetic coupling in the skeletal-muscle vascular domain; it does not establish identical respiratory modulation of every sympathetic organ.

10.3 Rate Alone Is Not Enough

Breathing frequency is important, but it is not an adequate description of respiratory state.

At minimum, several respiratory dimensions can influence autonomic interpretation: frequency determines how rapidly the respiratory cycle repeats and influences where respiratory-linked cardiac variability appears in the frequency spectrum; depth or tidal volume alters pulmonary stretch, mechanical cardiovascular effects, and RespHRV amplitude; inspiratory-to-expiratory timing changes respiratory-autonomic coupling even when nominal breathing frequency is held constant; respiratory phase influences the instantaneous pattern of cardiac and sympathetic neural activity; effort and mode differ between spontaneous, voluntarily paced, resisted, assisted, and passive ventilation; and ventilation and carbon dioxide can change when depth and rate change, potentially recruiting chemoreflex and vascular mechanisms. [116, 117, 123]

Human experiments demonstrate that changing inspiratory and expiratory timing can alter both HRV and spontaneous baroreflex estimates despite an unchanged nominal breathing frequency. [123] Recording only breaths per minute can therefore miss physiologically meaningful changes in respiratory waveform. Posture and behavioral state add further context because they change the autonomic background against which respiration operates.

10.4 Slow Breathing and Cardiovascular Resonance

Slow breathing provides one of the clearest demonstrations that respiratory manipulation can produce large cardiovascular oscillations without corresponding to a simple increase in one autonomic branch. At breathing frequencies near approximately 0.1 Hz, respiratory forcing can interact strongly with cardiovascular and baroreflex dynamics. Heart-rate and arterial-pressure oscillations can become unusually large as the systems approach a resonant relationship. [104, 105, 124] Slow breathing frequently increases cardiac HRV measures commonly interpreted as cardiovagally sensitive, and meta-analysis supports increases during slow breathing, immediately afterward, and following multisession interventions. [125]

Those findings are physiologically meaningful. They should not, however, be described as evidence that slow breathing simply delivers a larger “vagal dose.” Resonance, altered respiratory mechanics, cardiac parasympathetic modulation, and baroreflex interaction all contribute to the observed response. [124–126] Nor is approximately six breaths per minute a universal optimum. Individual resonance characteristics and cardiorespiratory phase relationships vary with person and state. Most importantly for interpretation, a larger oscillation created by respiratory resonance is not automatically evidence of greater baseline autonomic capacity, improved regulation, or therapeutic benefit.

10.5 Respiration Can Move Vagal HRV Into the LF Band

Slow breathing exposes a fundamental problem with assigning fixed autonomic branches to fixed HRV frequency bands. Under ordinary adult resting breathing rates, much respiratory-linked cardiac variability commonly appears within the conventional high-frequency range. When breathing slows, however, the respiratory oscillation itself can move below approximately 0.15 Hz and into the conventional low-frequency band. Human pharmacological-blockade evidence demonstrates that LF heart-rate variability generated during slow breathing can remain predominantly vagally mediated. [126]

Therefore:

LF power ≠ sympathetic activity

and

HF power ≠ the only location of respiratory cardiovagal modulation

The physiological location of the respiratory peak must be determined from the person's actual breathing rather than assumed from a fixed spectral band. Current expert recommendations explicitly recognize that RespHRV can occupy conventional LF or HF ranges depending on

respiratory frequency. [115] The detailed validity of HF-HRV, RMSSD, RespHRV, LF power, and related HRV metrics belongs to Section 14. The narrower conclusion here is that respiration itself can move autonomically mediated cardiac variability across conventional frequency boundaries.

Respiratory and cardiovascular signals can display coherence, phase locking, and other forms of synchronization. Such relationships can provide useful information about physiological coupling, but they should not be promoted automatically into proof of a specific neural pathway. Cardiorespiratory phase synchronization is intermittent, and apparent synchronization has also been demonstrated in surrogate data and in heart-transplant physiology. [127]

The evidentiary ceiling is nevertheless clear: correlation, coherence, spectral overlap, or phase locking demonstrate statistical relationships between signals. They do not independently identify whether the coupling arose from central respiratory drive, vagal afference, cardiac vagal efference, baroreflex interaction, mechanical coupling, or a shared upstream influence.

The same principle applies to motion-associated physiological synchronization. Temporal alignment between motion, respiration, heart rate, HRV, or pressure may generate mechanistic hypotheses, but temporal correspondence alone cannot identify the causal pathway.

10.6 Respiration Must Be Measured, Not Assumed

Because respiration changes both autonomic physiology and the signals used to infer it, rigorous interpretation requires respiratory measurement alongside cardiac and pressure measurements. Respiratory rate alone may be insufficient when depth, inspiratory-to-expiratory ratio, waveform, effort, or ventilation changes. Chest or abdominal effort bands can provide useful information about respiratory phase, rate, and relative amplitude, although they do not provide absolute tidal volume without appropriate calibration.

Continuous respiratory measurement becomes particularly important when the experimental environment can alter breathing spontaneously. Speech, sighs, breath holds, coughing, swallowing, exertion, nausea, discomfort, and motion sickness can all modify respiratory pattern and thereby alter downstream HRV and blood-pressure measurements. Measurement duration matters as well. Long averaging windows can obscure within-breath and short-latency interactions, whereas very short windows may provide unstable spectral estimates. If an analysis requires distinguishing changes in ventilation or chemoreflex state rather than simply tracking respiratory timing, end-tidal carbon dioxide may also become relevant.

The general rule is straightforward:

If respiratory-linked cardiac variability or spontaneous pressure-heart-period coupling is being interpreted, respiration should be measured continuously (see Phase 1 Protocol, Section X).

For SeaKing Phase 1, respiration has a particularly important interpretive status. Natural motion may coincide with changes in breathing. Those changes could occur through numerous pathways, including altered arousal, vestibular or visceral experience, posture, relaxation, nausea, behavioral state, speech, or other responses to the environment. The present evidence does not allow those initiating pathways to be identified from respiratory or cardiac signals alone.

If breathing changes and HRV changes at the same time, at least several interpretations remain possible:

motion → cardiac-autonomic pathway → HRV change

motion → respiratory change → cardiac-autonomic/mechanical coupling → HRV change

motion → shared upstream state change → respiratory + cardiac changes

or combinations of these pathways.

Respiration is therefore not simply a confound to be statistically removed. It may be part of the physiological response itself. The same logic applies to baroreflex-related measurements. Respiratory mechanics alter arterial pressure and respiratory phase alters reflex responsiveness, so a motion-associated change in spontaneous BRS cannot be interpreted independently of the respiratory context in which it occurred.

For discovery-phase research, the appropriate objective is therefore to preserve the coupled trajectories (see Phase 1 Protocol, Section X):

motion exposure → respiration → cardiac timing → arterial pressure → other autonomic effectors

while avoiding premature assignment of one causal pathway.

Respiration is best understood as a rhythmic physiological input and output that modifies cardiac parasympathetic timing, sympathetic neural traffic, cardiovascular mechanics, and baroreflex expression while itself changing with behavior and experimental state. RespHRV is consequently a coupled cardiac phenomenon whose amplitude depends on both autonomic modulation and the breathing pattern that helps generate it. For SeaKing, that means an HRV change cannot be assigned to vagal regulation independently of respiration. Breathing must be measured and modeled as a potential response, mediator, measurement context, and competing mechanistic pathway.

11. Central Autonomic Network and State Integration

Peripheral autonomic pathways do not operate independently of the brain state in which they are embedded. Visceral sensory information ascends into central circuits, cardiovascular and respiratory reflexes are integrated within the brainstem, and autonomic output is continuously influenced by hypothalamic, midbrain, limbic, insular, cingulate, and prefrontal systems. The resulting organization is better understood as a distributed and recurrent network than as a single autonomic control center. [42, 45, 46, 128]

The term central autonomic network (CAN) is useful for describing this interacting organization, but it should not be interpreted as a sharply bounded anatomical organ. Different formulations include somewhat different regions depending on the physiological function, task, and method being studied. [128] The common principle is more important than any one fixed map: central autonomic regulation emerges through coordinated interactions among systems that receive bodily information, evaluate internal and external demands, organize behavior, and influence peripheral autonomic effectors.

11.1 Brainstem Infrastructure for Autonomic Integration

The brainstem contains essential circuitry for rapid autonomic reflexes and patterned visceral control. The nucleus tractus solitarius receives major visceral afferent input, while ventrolateral medullary regions, the nucleus ambiguus, dorsal motor nucleus of the vagus, and interconnected brainstem systems contribute to cardiovascular and visceral efferent organization. These structures should not be understood simply as a linear chain from sensory input to motor output. They interact reciprocally with one another and with higher central systems, allowing reflex responses to be modified by physiological and behavioral state.

Human imaging combined with controlled physiological perturbation provides direct support for this organization. During pharmacologically manipulated baroreflex conditions, brainstem fMRI responses have been identified in regions corresponding to classical baroreflex circuitry, including the NTS, ventrolateral medulla, nucleus ambiguus, and dorsal motor nucleus. [129]

This type of evidence is particularly useful because the physiological input is experimentally manipulated while central responses are measured. Even then, however, a BOLD response is not a direct recording of neural firing or autonomic efferent traffic. It establishes involvement of a region within the manipulated physiological process, not exclusive ownership of the process by that region.

The nervous system continuously receives information about internal bodily conditions. Cardiovascular pressure, cardiac timing, respiratory state, gastrointestinal distension and nutrients, metabolic signals, and other visceral events provide afferent input that can alter central processing.

This creates a recurrent relationship:

visceral physiology → afferent signaling → central representation/integration → autonomic and behavioral response → altered visceral physiology

Visceral information therefore does more than support unconscious homeostatic reflexes. Human and experimental evidence indicates that bodily signals can influence perception, emotion, cognition, motivation, and behavior. [130–132] This relationship is often discussed under the broader concept of interoception, the sensing and representation of internal bodily state. Human heartbeat-detection experiments, for example, associate interoceptive awareness with activity in insular and cingulate regions. [131]

Interoception should nevertheless be distinguished from autonomic regulation itself. Greater awareness of a bodily signal does not establish that the physiological state being sensed is better regulated, healthier, or more adaptive. A person may perceive an exaggerated, blunted, or otherwise maladaptive physiological response accurately. [131, 132]

Thus:

awareness of physiological state ≠ quality of physiological regulation

That distinction becomes important whenever subjective experience and autonomic measurements change together.

11.2 Context Can Modify Autonomic Output

The recurrent architecture also operates in the opposite direction. Central state can modify how peripheral autonomic systems respond to the same or similar sensory input. Hypothalamic, midbrain, amygdalar, insular, cingulate, and prefrontal systems participate in integrating visceral information with environmental context, salience, effort, motivation, cognition, and behavioral demands. [128]

This helps explain why autonomic responses cannot be interpreted solely from the physical stimulus. The physiological significance of standing, exercise, threat, anticipation, cognitive effort, pain, social interaction, nausea, or a familiar repeated exposure differs even when some peripheral sensory inputs overlap. Central integration contributes to organizing the response appropriate to that broader state. The relationship should not be reduced to a simple “top-down” model, however. Central state changes peripheral physiology, but altered peripheral physiology also changes subsequent central input. The system is recurrent rather than hierarchical in only one direction.

For experimental interpretation, central state can therefore function as a mediator or modifier between an exposure and the observed peripheral response.

A simplified model is:

stimulus/exposure → central-state change → peripheral autonomic response

but an equally relevant pathway is:

stimulus/exposure → peripheral/visceral change → central-state change → further peripheral and behavioral change

and in normal physiology, both can operate simultaneously.

11.3 Human Evidence for Causal Cortical Access

Neuroimaging associations alone cannot establish that a cortical region causes an autonomic response. Human lesion and intracranial-stimulation evidence therefore provides an important additional level of inference. Studies involving cingulate and insular lesions demonstrate that damage to particular cortical regions can alter autonomic responses to behavioral or cognitive challenges. Intracranial electrical stimulation likewise shows that stimulation within insular and cingulate regions can produce changes in heart rate, blood pressure, and related cardiovascular outputs. [133–137]

These findings establish an important principle: higher cortical regions do not merely correlate with autonomic activity. They have causal access to cardiovascular autonomic output. The sign and magnitude of those effects, however, depend on stimulation location, cortical subregion, participant state, and other conditions. A given cortical structure therefore should not be assigned one fixed autonomic function. Human cortical causality supports distributed integration, not a simple map in which one cortical area is the sympathetic center and another is the parasympathetic center.

Early interpretations of insular-autonomic physiology sometimes proposed a relatively simple lateralization, with the right insula characterized as predominantly sympathetic and the left as predominantly parasympathetic. The broader human evidence does not support that formulation as an absolute rule. Later stimulation and lesion studies demonstrate bilateral involvement and substantial subregional complexity. [134, 135, 138]

Lateralization may exist for particular functions, tasks, or anatomical subregions. What is not justified is converting those findings into a global rule such as:

right hemisphere = sympathetic

left hemisphere = parasympathetic

Central autonomic organization is more topographically and functionally differentiated than that. This mirrors the pattern already established in the peripheral nervous system. Just as “the sympathetic system” is not one homogeneous peripheral channel and “the vagus” is not one homogeneous parasympathetic signal, the brain does not appear to contain two global cortical controllers corresponding neatly to those labels.

11.4 HRV-Brain Associations Are Associations

A substantial literature has related HRV to activity within cortical and subcortical regions associated with central autonomic regulation. Meta-analytic and systematic-review evidence supports meaningful brain-heart covariation across distributed regions. [139–141] Those findings are useful. They provide evidence that cardiac timing varies in relation to central neural state and are consistent with recurrent brain-body integration. They do not establish that HRV is a direct measurement of CAN activity.

Several causal arrangements remain possible when HRV and brain activity covary:

central activity → cardiac autonomic change → HRV

peripheral physiological change → afferent feedback → central activity

shared upstream state → both brain and cardiac change

or recurrent combinations of all three.

BOLD imaging and HRV are also measurements at different physiological levels. BOLD reflects regional hemodynamic correlates of neural activity, while HRV reflects beat-to-beat timing at the sinoatrial effector. Correlation between them does not collapse those levels into one measure.

Modern systematic review also shows substantial heterogeneity across HRV-neuroimaging studies, reinforcing the need to avoid treating one HRV value as a direct readout of a fixed central network. [139–141]

Section 14 owns the detailed validity of HRV. The boundary needed here is simply:

HRV can participate in evidence of brain-heart coupling; it cannot identify the CNS mechanism that produced it.

11.5 “CAN Activation” Is Too Broad Without Specification

The phrase CAN activation creates many of the same problems as “vagal activation” or “sympathetic activation.” A distributed network does not have to increase or decrease uniformly. Different regions may change in different directions, at different times, and for different reasons. A baroreflex perturbation, cognitive challenge, emotional stimulus, exercise condition, visceral event, or respiratory manipulation may recruit overlapping but non-identical central patterns. The CAN concept therefore identifies an organizational framework, not a scalar variable.

A scientifically precise central-autonomic claim should specify, where evidence permits, the measured region or network, experimental condition, measurement modality, peripheral physiological relationship, and whether the evidence is correlational, perturbational, lesion-based, or stimulation-based. Even direct evidence that a region contributes causally to cardiovascular output does not establish that its activation is beneficial. Central-state change is first a physiological observation.

The central-autonomic literature reinforces the dynamic model established earlier in this paper. Autonomic output is conditioned by what the organism is doing and what the situation requires. The same peripheral value can therefore have different meanings in different central and behavioral states. A heart rate appropriate during exertion may be inappropriate at rest. A sympathetic vascular response necessary during standing may look very different from one occurring during psychological threat. Respiratory and cardiac coupling may change during voluntary slow breathing for reasons different from those producing similar numerical changes during spontaneous rest.

Central integration is one reason physiological context cannot be stripped away when interpreting biomarkers. Accordingly, no central configuration can be labeled intrinsically calm, safe, adaptive, regulated, or therapeutic from state-integration evidence alone. The CAN-STATE evidence does not establish one optimal brain-autonomic pattern. The question of regulatory quality remains dependent on demand, trajectory, recovery, reproducibility, tolerability, and functional meaning, which are treated in Sections 12 and 17.

For Phase 1, peripheral physiological measurements can establish response patterns but cannot localize those patterns to a specific central-autonomic region or network state. Central mechanisms remain hypotheses for later targeted validation. [129, 131]

12. Dynamic Regulation: Baseline, Reactivity, Recovery, and Repeated Exposure

Autonomic regulation cannot be determined adequately from a single physiological value measured at one moment. Regulation is expressed through how physiological systems maintain viable conditions, respond when demands change, reorganize during the demand, and reconfigure afterward. This requires a temporal framework: baseline → challenge → response → within-exposure dynamics → recovery → repeated exposure → functional meaning

Each stage contains information that can be lost when physiology is reduced to a resting value, a simple pre-versus-post difference, or a single average across an exposure. The central question is therefore not merely whether a physiological variable increased or decreased. It is whether the

observed trajectory was appropriate to the physiological demand and what happened when that demand changed.

12.1 Homeostasis and Allostasis

Homeostasis is sometimes described too narrowly as maintenance of physiological constancy. Biological homeostasis is better understood as the use of interacting feedback processes to preserve viable internal conditions despite ongoing disturbance. [15] That objective does not require every physiological variable to remain fixed. Standing requires cardiovascular changes that would be unnecessary while supine. Exercise requires substantial alterations in heart rate, cardiac output, ventilation, vascular distribution, and metabolic activity. A challenge that appropriately changes physiology is therefore not evidence that homeostasis has failed. Change can be part of regulation.

Allostasis emphasizes this point from another direction. In allostatic and predictive formulations, physiological and behavioral systems can adjust in anticipation of expected demand rather than waiting for every regulated variable to depart from a desired range before responding. [15–17] Homeostasis and allostasis therefore need not be treated as competing theories. They emphasize complementary aspects of regulation: feedback processes that preserve physiological viability and coordinated adjustment that allows the organism to meet changing or anticipated demands.

Neither term identifies one directly measurable autonomic variable. There is no singular “homeostasis level” or “allostasis level” that can be read from HRV, heart rate, blood pressure, electrodermal activity, pupil size, or another peripheral signal.

12.2 Baseline Is a State, Not a Universal Zero

Every response measurement begins somewhere. That starting condition matters. A baseline should characterize the physiological and behavioral state immediately relevant to the subsequent challenge. It is not necessarily a universal representation of the individual's normal physiology. Posture, respiration, recent activity, anticipation, circadian timing, hydration, temperature, medications, food intake, emotional state, illness, sleep, and prior exposures can all influence the state from which a response begins. This creates an important measurement problem. Reactivity is usually calculated relative to baseline, so an altered baseline can change the apparent size of a response even when the challenge-period value itself is similar.

Anticipatory activation can be particularly important. If physiology begins changing before the formally defined exposure starts, the measured “baseline” may already contain part of the response. Regression to the mean can create additional distortion when participants are selected or classified partly because of an unusually high or low initial value. Baseline should therefore be treated as the first measured state in a trajectory, not as a physiological zero against which all subsequent values automatically acquire meaning.

12.3 Reactivity Is Context Dependent

Reactivity describes the change in a specified physiological variable from a defined baseline to a defined challenge period. That definition is intentionally neutral. A larger response is not inherently worse, and a smaller response is not inherently better. Response magnitude depends partly on what the organism is being asked to do. Greater task demand can produce greater cardiovagal HRV reactivity, demonstrating that the size of a response can reflect properties of the challenge rather than a stable person-level quality. [29]

The same principle applies across autonomic domains. A substantial sympathetic vasoconstrictor response may be appropriate during orthostatic challenge. Increased heart rate may be necessary during exercise. Respiratory and cardiovascular oscillations may become larger during slow breathing near cardiovascular resonance. The fact that physiology moved farther from its resting value does not establish dysregulation. Conversely, failure to change can be abnormal when the demand requires a response.

Prospective stress literature provides an important boundary. Both exaggerated and blunted stress responses have been associated with adverse later outcomes. [31] Greater cardiovascular reactivity has been associated with cardiovascular risk, but favorable psychological characteristics do not uniformly predict smaller responses; optimism, for example, has been associated with greater cardiac reactivity during cognitive stress. [30, 142]

There is therefore no defensible universal rule that: smaller response = better regulation or that: larger response = worse regulation The relevant question is whether the magnitude, direction, latency, and duration of the response are appropriate for the specific physiological system and demand.

A response rarely consists of one instantaneous change that remains constant until an exposure ends. Physiology can rise rapidly and stabilize, continue drifting, oscillate, partially recover while the exposure continues, escalate over time, or show different temporal patterns across different effectors. Two participants can therefore have the same average value during an exposure while having very different physiological trajectories. For example, one signal might change sharply during the first minutes and then return toward its initial state despite continued exposure. Another might change gradually and reach its largest deviation near the end. A third might oscillate around its starting value.

Collapsing each of those responses into one exposure mean would obscure potentially important regulatory information. Different physiological systems also operate on different timescales. Heart rate, arterial pressure, RespHRV, electrodermal activity, pupil dynamics, endocrine responses, and other measures should not be expected to peak simultaneously. Multimodal dissociation can therefore be compatible with coordinated regulation. Different effectors can change in different directions and at different times while participating in the same organism-level response. This is one reason synchronization across measurement channels is informative but not mandatory evidence of regulation.

12.4 Recovery Is a Trajectory

Recovery is not simply “the value after the task.” It is the trajectory that follows a defined challenge under defined postchallenge conditions. That trajectory can include rapid return, gradual return, overshoot, persistent displacement, oscillation, or different recovery rates across physiological systems. Recovery also contains information that peak reactivity does not. Delayed cardiovascular recovery has been associated with cardiovascular risk factors and prospectively with adverse cardiovascular outcomes, supporting recovery as a physiologically separable dimension. [27, 30]

Cardiac vagal recovery research similarly supports the importance of postchallenge dynamics, while also demonstrating substantial methodological heterogeneity in recovery windows, posture, and experimental conditions. [28] Consequently, the statement that someone “recovered faster” is incomplete unless the physiological variable, beginning of the recovery interval, observation duration, posture, activity, and persistence or termination of the challenge are defined. Nor must successful recovery always mean returning precisely to the prestimulus value.

If the physiological or environmental context has changed, the appropriate postchallenge operating state may differ from the original baseline. Baroreflex resetting during exercise, discussed in Section 9, provides one example of why regulation can remain functional around a changed operating point. Recovery should therefore be described before it is judged.

12.5 Repeated Exposure: Reproducibility, Habituation, and Sensitization

A single exposure reveals an acute response. Repeated exposures reveal whether that response is stable or changes with experience. Several longitudinal patterns are possible: reproducibility, in which a broadly similar response recurs; habituation, in which response magnitude decreases across sufficiently comparable repeated exposures; sensitization, in which response magnitude increases; drift, in which baseline or response characteristics change without a clear stimulus-

specific pattern; or more complex adaptation in which timing, recovery, or cross-system organization changes even when peak magnitude does not.

Habituation has a specific meaning. It describes decreased responsiveness to a sufficiently comparable repeated stimulus and is dependent on stimulus identity and exposure history. [33] Human cardiovascular responses can habituate across repeated acute-stress exposures, and individuals differ in those longitudinal patterns. [34] A smaller response on a later exposure, however, is not automatically evidence of beneficial habituation. Altered baseline state, anticipation, fatigue, learning, sickness adaptation, different received exposure, measurement drift, or loss of physiological responsiveness can all produce apparent response decrement. [33]

Even genuine habituation is not inherently beneficial. Reducing an unnecessary response to a familiar, harmless stimulus may reduce physiological cost. Losing a response that remains necessary to meet physiological demand may instead be maladaptive. Repeated exposure therefore adds information, but it does not eliminate the need for context and functional validation.

The term autonomic flexibility can be useful if it is defined precisely. For this paper, autonomic flexibility means: the capacity to alter physiological configuration in relation to changing demand and subsequently reconfigure when the demand changes again. This is a systems-level description, not a biomarker. Flexibility is therefore not synonymous with high resting HRV. Recent human evidence found that resting RMSSD predicted RMSSD reactivity and recovery but did not predict heart-rate reactivity or recovery, subjective stress, psychological distress, or emotion-regulation strategies. [35]

Nor is flexibility synonymous with large reactivity. As already established, both exaggerated and blunted responses can be associated with adverse outcomes depending on context. [31] A meaningful claim about autonomic flexibility therefore requires, at minimum, specification of the stimulus, physiological domain, direction and magnitude of response, timing, recovery, and reproducibility. This definition also permits different physiological channels to behave differently. Flexibility does not require every measured variable to move together, nor does it require sympathetic and parasympathetic outputs to behave reciprocally. It requires context-appropriate reconfiguration, not movement toward one preferred scalar state.

12.6 Allostatic Load Is Cumulative, Not Momentary

The concept of allostatic load addresses a different temporal scale. Physiological mediators that are useful during an acute demand can become costly when activation is too frequent, prolonged, inadequately terminated, poorly adapted, or compensated for repeatedly by other systems. [36] Allostatic load provides a conceptual framework for this cumulative biological cost. Multisystem allostatic-load scores have predicted later mortality and functional decline in longitudinal cohorts, supporting the broader construct's relevance to long-term health. [37]

The operationalization of allostatic load, however, remains heterogeneous. Contemporary reviews document substantial differences in biomarker selection, cutoffs, scoring methods, and algorithms, with no universally accepted individual-level allostatic-load meter. [38, 39] Review 03 therefore uses allostatic load as a conceptual model of cumulative dysregulation, not as permission to create a new SeaKing score by combining whatever biomarkers happen to be available. HRV, blood pressure, electrodermal activity, pupil dynamics, respiration, or other signals cannot simply be normalized, summed, and labeled “allostatic load” or “autonomic regulation.” Acute dynamic regulation and cumulative allostatic load answer different questions.

12.7 Regulation Is a Trajectory, Not a Direction

The preceding evidence supports a useful way to organize dynamic autonomic responses. The seven-stage trajectory used here is a literature-informed organizing framework for preserving temporally distinct features of response; it is not a validated biological decomposition of autonomic regulation. A physiological response can be characterized across the following stages:

1. Baseline state: What was the physiological and behavioral condition before the challenge?

2. Challenge or exposure: What stimulus was actually received, at what magnitude and duration, and under what participant state?

3. Reactivity: Which physiological domains changed, in what direction, by how much, and with what latency?

4. Within-exposure dynamics: Did those responses persist, stabilize, oscillate, escalate, or adapt while the exposure continued?

5. Recovery: What happened after the challenge, over what time course, and under what postchallenge conditions?

6. Repeated exposure: Did the trajectory reproduce, habituate, sensitize, drift, or otherwise change with exposure history?

7. Functional meaning: Only after the preceding trajectory is established should the response be related to tolerability, symptoms, function, or later clinical benefit. This sequence is the core dynamic-regulation framework for the remainder of this paper. It also makes clear why a single arrow is insufficient. An increase can be appropriate or inappropriate. A decrease can be appropriate or inappropriate. No change can be appropriate or inappropriate. Even rapid recovery can be appropriate or inappropriate depending on whether the physiological demand has actually ended. Direction alone is not a value judgment. Figure 7 summarizes this dynamic-regulation framework.

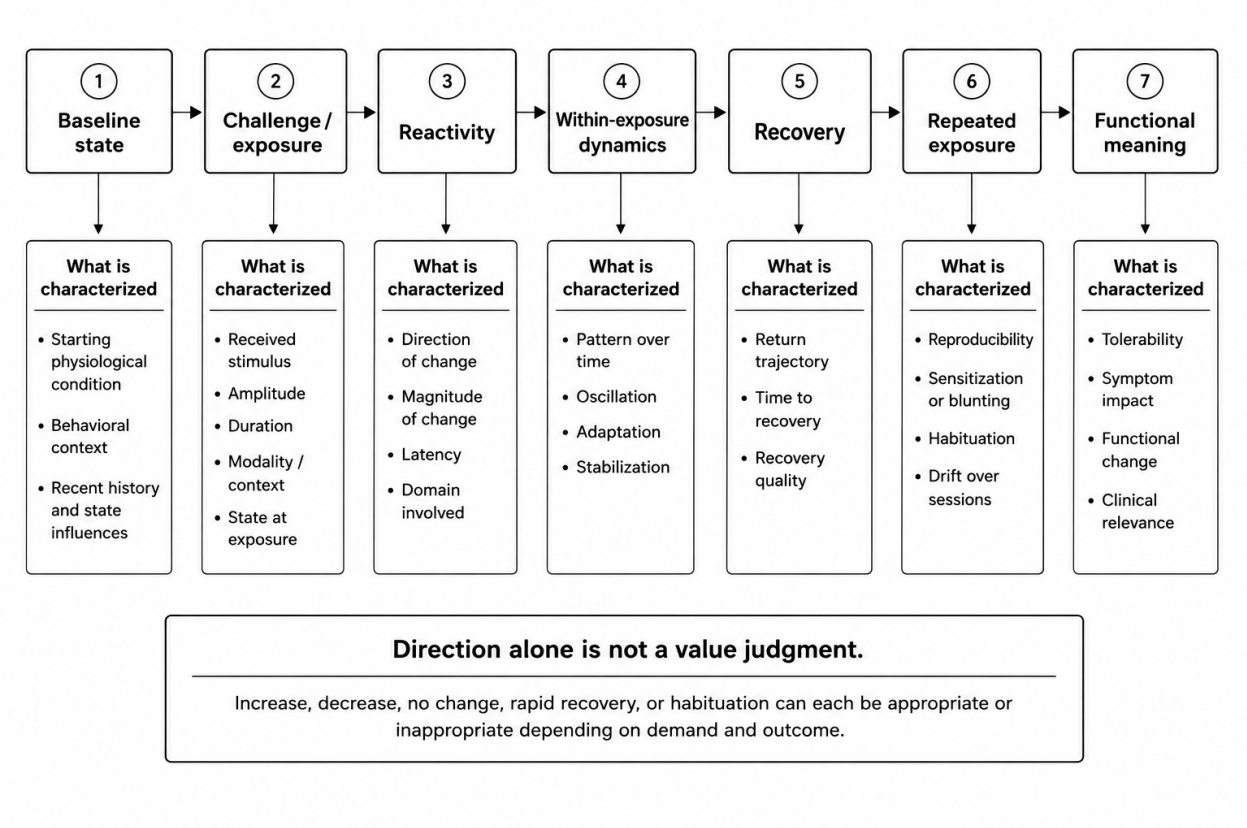


Figure 7. Dynamic regulation is a trajectory, not a direction. Physiological interpretation proceeds from baseline state through the received challenge, reactivity, within-exposure dynamics, recovery, repeated exposure, and functional meaning. Direction or magnitude alone does not determine whether a response is adaptive or maladaptive; that judgment depends on the physiological demand, trajectory, reproducibility, tolerability, and outcome. [31, 33, 35]

Phase 1 should preserve continuous synchronized trajectories across baseline, motion onset, sustained exposure, motion cessation, recovery, and repeated sessions while retaining the received motion dose (see Phase 1 Protocol, Section X). The seven-stage framework can organize interpretation, but the underlying time series should remain continuous and findings should be tested for robustness to prospectively specified alternative window definitions rather than assuming that the proposed stages are fixed physiological states. Different channels may show different latencies and directions. The appropriate discovery output is a set of reproducible response trajectories, not an unvalidated "autonomic regulation score." Whether any trajectory represents improvement is reserved for Section 17. [31, 33, 35]

13. Evidence Hierarchy for Autonomic Mechanistic Claims

Autonomic physiology can be measured at several levels between neural activity and the observable behavior of an organ. Those levels are not interchangeable. A heart-rate change, sweat response, neurotransmitter concentration, pharmacological-blockade effect, and direct neural recording may all provide legitimate autonomic information, but they answer different questions. For this paper, mechanistic evidence is organized by proximity to the specific pathway or effector being claimed:

1. Direct neural recording
2. Selective pharmacological blockade
3. Ganglionic or controlled physiological perturbation
4. Regional neurotransmitter kinetics
5. Validated downstream organ or effector measures

This five-tier hierarchy is an evidentiary framework used in Review 03 to organize methods by mechanistic proximity; it is not an empirically validated taxonomy of autonomic measurement or universal study quality. A highly controlled downstream measurement may be better suited to a naturalistic longitudinal question than an invasive neural recording that samples the wrong pathway or materially alters the experimental environment. [7–9] There is therefore no single universal autonomic "gold standard." The strongest evidence for a claim is the most direct valid measurement of the specific neural pathway, organ, or effector being claimed, ideally triangulated with non-equivalent methods.

13.1 Tier 1: Direct Neural Recording

Direct neural recording provides the closest practical observation of autonomic neural traffic in humans. Microneurography allows action potentials or multiunit activity to be recorded from identified peripheral autonomic pathways in awake participants. Human sympathetic microneurography is well established, including direct recordings of muscle sympathetic nerve activity and skin sympathetic nerve activity. [7, 86, 92]

Its directness remains local. An MSNA recording provides strong evidence that sympathetic neural traffic directed toward skeletal-muscle vascular beds changed. It does not establish simultaneous sympathetic activity at the heart, kidney, skin, adrenal medulla, or another organ. Likewise, SSNA directly samples cutaneous sympathetic traffic rather than total-body sympathetic activity. [86, 92] Even within a named sympathetic outflow, single-unit recordings demonstrate heterogeneous neuronal recruitment. [90] Direct recording therefore solves one inferential problem while preserving another: direct measurement of a pathway ≠ measurement of the entire autonomic nervous system.

Direct human cervical-vagus recording has recently become technically feasible. Ultrasound-guided microneurography has recorded axonal activity directly from the cervical vagus in awake humans. [143] Subsequent multiunit work demonstrated cardiac and respiratory modulation across vagal recording sites, while single-unit analysis has isolated myelinated and unmyelinated axons with cardiac-rhythmic firing characteristics, including a subset compatible with cardioinhibitory efferent function. [144, 145] These studies materially advance human vagal physiology because they move measurement upstream from organ-level cardiac timing toward the nerve itself. They do not create a global vagal meter.

As Section 5 established, the cervical vagus is a mixed nerve. Recording an action potential within it does not automatically identify whether that axon is afferent or efferent, cardiac or pulmonary, gastrointestinal or laryngeal, or what physiological effect its activity produces. Functional identity still requires anatomical, temporal, physiological, or other convergent evidence. [143–145]

Human cervical-vagus microneurography should therefore be regarded as an emerging high-directness method, not a routine measurement of whole-body “vagal tone.”

13.2 Tier 2: Selective Pharmacological Blockade

Direct recording asks what a neural pathway is doing. Pharmacological blockade asks a different causal question: What part of an observed response remains when a defined autonomic contribution is removed? In cardiac physiology, muscarinic blockade with atropine can remove major parasympathetic chronotropic influence, while beta-adrenergic blockade can remove major sympathetic cardiac influence. Combined blockade can reveal cardiac behavior after both major autonomic chronotropic inputs have been substantially suppressed. [8, 62, 146, 147]

These experiments are powerful because they can validate downstream measures mechanistically. If a cardiac signal changes substantially when muscarinic influence is removed, that provides causal evidence that parasympathetic signaling contributes to the intact signal. It does not make the downstream signal identical to neural firing. For example, respiratory-linked HRV is strongly attenuated by parasympathetic blockade, supporting its sensitivity to cardiac parasympathetic modulation. Yet it does not proportionally rank cardiac vagal influence across individuals without accounting for respiratory and other determinants. [146]

Blockade also has its own inferential limitations. Drugs differ in specificity and completeness, branches interact, and removing one component can alter reflex and compensatory activity elsewhere. [8] Combined blockade creates an experimentally altered physiological state rather than directly revealing normal autonomic traffic. [147] The defensible inference is therefore: blockade can establish causal contribution without converting a downstream biomarker into a direct neural measurement.

13.3 Tier 3: Ganglionic and Controlled Physiological Perturbation

Ganglionic blockade provides a broader intervention. By interrupting transmission through autonomic ganglia, agents such as trimethaphan can markedly suppress autonomically mediated cardiovascular reflexes and HRV. [148] This provides strong evidence that autonomic neural transmission contributes to an observed process. Its breadth is also its limitation. Sympathetic and parasympathetic pathways are interrupted together, so ganglionic blockade cannot by itself identify which branch produced a particular response. [148] Controlled physiological perturbations provide another form of causal evidence. Rather than removing a pathway, they manipulate a defined input and measure the resulting output.

The baroreflex methods discussed in Section 9 illustrate this approach. Pharmacological pressure perturbation or carotid neck pressure/suction alters a specified cardiovascular input while cardiac or sympathetic responses are measured. [100, 102, 108] Such experiments provide stronger evidence of input-output structure, timing, and gain than spontaneous correlation alone. Their conclusions remain bounded by the reflex arm measured, stimulus range, operating point, and experimental method.

13.4 Tier 4: Regional Neurotransmitter Kinetics

Some important autonomic organs cannot be accessed routinely through direct neural recording. Regional norepinephrine-spillover methods partially bridge that gap by estimating sympathetic neurotransmitter release in defined organ territories, including cardiac, renal, and hepatosplanchnic circulations. [88] This makes regional spillover substantially more informative about organ-specific sympathetic physiology than a peripheral venous norepinephrine concentration. It nevertheless remains downstream of neural firing.

Measured norepinephrine spillover reflects not only neural release but also neuronal reuptake, local blood flow, clearance, and tracer kinetics. [88] Simultaneous MSNA and cardiac-spillover studies show why maintaining that distinction matters: different regional sympathetic domains can exhibit both shared and divergent recruitment during the same challenge. [89] Regional neurotransmitter kinetics therefore provide organ-specific evidence of sympathetic neurotransmission, not a count of sympathetic action potentials and not a biochemical measure of global sympathetic activity.

13.5 Tier 5: Validated Downstream Effector Measures

Most practical human autonomic research occurs at the downstream end of the hierarchy. Heart-period variability, pre-ejection period, electrodermal activity, sweating, arterial pressure, pupil dynamics, respiration, heart rate, and related signals are attractive because they can be measured repeatedly, noninvasively, and under naturalistic conditions. Their scientific validity does not require pretending they are direct neural recordings. A downstream measure becomes mechanistically useful when experimental validation establishes a reproducible relationship between that signal and a defined autonomic pathway or effector under specified conditions.

Human sudomotor research provides a particularly clear example. Direct SSNA bursts precede measurable sweat expulsions by seconds, and neural burst magnitude relates to subsequent sweat output. [9] That evidence validates sweating and electrodermal physiology as measures of a cutaneous sympathetic-sudomotor effector domain. It simultaneously establishes the limitation: the measurable sweat response occurs downstream of neural activity, has a neural-to-effector latency, and does not represent global sympathetic activity.

Cardiac blockade studies provide an analogous framework for selected cardiac measures. Parasympathetic- and sympathetic-sensitive cardiac signals can be validated against selective blockade without becoming direct recordings of those nerves. [62] The inferential chain should therefore remain explicit: measured signal → validated organ/effector process → supported autonomic contribution under defined conditions It should stop there unless additional evidence justifies another mechanistic step.

A methodological hierarchy can be misused if “more direct” is interpreted as “better for every research question.” That is not the purpose of this framework. Microneurography may provide exceptionally direct information about one neural pathway while being unsuitable for long-duration repeated naturalistic exposure. A wearable downstream measure may be mechanistically less direct while providing thousands of synchronized observations across baseline, exposure, recovery, and repeated sessions. A biomarker can also be highly reproducible or clinically useful without being a direct measure of mechanism. Conversely, an invasive neural measurement can be extremely direct but narrow in anatomical scope.

The relevant methodological question is therefore: Is this measurement valid for the claim being made? rather than: Is this the most invasive measurement available?

13.6 Triangulation Across Non-Equivalent Methods

The strongest mechanistic inference often arises when different methods converge. Direct recording can establish that target-specific neural traffic changed. Selective blockade can test whether a particular receptor or branch contribution is necessary for a downstream response. Controlled perturbation can establish input-output timing and gain. Regional transmitter kinetics can extend organ-specific inference to sites inaccessible to direct neural recording. Validated noninvasive measures can then characterize how the relevant effector behaves repeatedly under realistic conditions. When these non-equivalent methods point toward the same physiological model, mechanistic confidence increases. Disagreement is equally important.

If a downstream biomarker changes while the relevant direct neural measure does not, or if pharmacological blockade fails to alter a presumed branch-specific signal, that discrepancy should refine the model rather than be averaged away. [7–9] Triangulation therefore means convergence

among measurements with different inferential weaknesses, not repetition of several versions of the same downstream signal.

13.7 The Evidence Ladder

For Review 03, the hierarchy can be summarized as:

1. Direct neural recording; 2. Selective pharmacological blockade; 3. Ganglionic or controlled physiological perturbation; 4. Regional neurotransmitter kinetics; 5. Validated downstream organ/effector measures.

The downward progression represents increasing distance between the measured variable and neural activity. It does not represent decreasing scientific value. Every level remains anatomically and functionally bounded. A direct MSNA recording is still muscle-vascular sympathetic activity, not whole-body sympathetic activity. A cervical-vagus recording remains local neural traffic whose functional identity may require additional evidence. Atropine-sensitive HRV remains downstream cardiac physiology rather than vagal firing. Regional norepinephrine spillover remains transmitter kinetics rather than nerve traffic. Electrodermal activity remains a cutaneous sudomotor effector measure rather than “sympathetic tone.”

The hierarchy therefore prevents a common inferential error: measurement precision does not authorize expansion beyond the physiological domain actually measured.

Phase 1 appropriately emphasizes scalable downstream physiological measurements during natural motion. These can characterize reproducible response phenotypes and generate mechanistic hypotheses, but they cannot by themselves establish direct vagal activation, global sympathetic suppression, or a specific central mechanism. Later studies can move upward in mechanistic proximity using methods matched to the hypothesis. [7–9, 148]

14. Heart Rate Variability as a Cardiac Autonomic Measure

Heart rate variability (HRV) describes variation in the timing between successive heartbeats. When derived appropriately from normal sinus beats, short-term HRV can provide useful information about modulation of sinoatrial timing, including an important contribution from cardiac parasympathetic influence. It is therefore a valuable autonomic measurement. It is not a direct recording of the vagus nerve.

As established by the evidence hierarchy in Section 13, HRV is a downstream cardiac-effector signal. Neural traffic, ganglionic transmission, neurotransmitter action, sinoatrial-node physiology, respiration, hemodynamics, and intrinsic cardiac properties all intervene between autonomic neural activity and the R-R intervals from which HRV is calculated. [3, 8, 146] The appropriate question is not whether HRV is “vagal” or “not vagal.” The more useful question is: Under what conditions does a particular HRV measure provide valid information about cardiac parasympathetic modulation, and where must that inference stop?

14.1 Pharmacological Validation

Selective pharmacological blockade provides the strongest causal validation for cardiovagal interpretations of short-term HRV. Muscarinic blockade markedly reduces respiratory-linked and short-term beat-to-beat heart-period variability, demonstrating that cardiac parasympathetic influence contributes substantially to those signals under appropriate conditions. [3, 8, 62, 146] This supports a legitimate inference: selected short-term HRV measures are sensitive to cardiac parasympathetic modulation. It does not support a stronger identity statement: HRV = vagal nerve activity.

Blockade establishes contribution rather than equivalence. The measured HRV signal remains the downstream behavior of the sinoatrial effector after several physiological transformations. This distinction also separates tonic cardiac parasympathetic influence from temporal cardiac parasympathetic modulation. Pharmacological removal of muscarinic influence can estimate the

tonic parasympathetic contribution to resting cardiac rate, whereas HRV primarily describes temporal variation in cardiac timing. Those quantities overlap physiologically but are not interchangeable. [64, 146] Accordingly, a person with greater resting HRV does not necessarily have proportionally greater atropine-defined tonic cardiac parasympathetic control.

The root mean square of successive differences, or RMSSD, quantifies short-term variation between successive normal R-R intervals. Because cardiac parasympathetic effects operate rapidly, RMSSD is commonly sensitive to rapid cardiovagal modulation and is one of the more useful short-term time-domain HRV measures. [63, 67, 149] RMSSD also avoids one problem inherent in fixed frequency bands: it does not require respiratory-linked variability to fall inside a predetermined spectral range. That does not make RMSSD respiration independent.

Changes in breathing can alter successive beat-to-beat timing, and RMSSD remains influenced by mean heart rate, rhythm, ectopy, artifact, measurement duration, posture, and physiological state. [150, 151] The most defensible wording is therefore: RMSSD provides a downstream index sensitive to short-term cardiac parasympathetic modulation under defined conditions. It should not be described as a direct quantitative measure of vagal firing or total parasympathetic activity.

14.2 HF-HRV and RespHRV

High-frequency HRV is often interpreted as a cardiovagal measure because, during ordinary resting breathing, respiratory-linked cardiac variability commonly occupies the conventional HF frequency range. That interpretation is strongest when three conditions are satisfied: the cardiac rhythm is suitable for sinus-node HRV analysis; respiratory-linked variability is actually present within the analyzed band; and respiration is measured sufficiently to establish where the respiratory oscillation lies. [3, 115, 126] When those conditions are met, respiratory-frequency HF-HRV can provide meaningful information about cardiac parasympathetic modulation. The respiratory dependence is not incidental.

As established in Section 10, respiratory frequency and tidal volume can change the magnitude of respiratory cardiac variability substantially. Blockade studies show that adding information about respiratory cycle length and tidal volume can materially improve the relationship between RSA and pharmacologically defined cardiac parasympathetic control. [64, 146] Respiratory heart rate variability, or RespHRV, is therefore best interpreted as respiratory modulation of cardiac timing with a substantial cardiovagal contribution, rather than as a direct measure of tonic vagal output.

A larger respiratory oscillation does not necessarily mean a proportionally larger amount of tonic cardiac parasympathetic activity. Increasing respiratory interval can increase HF-HRV substantially even when mean R-R interval changes little. [152] Similarly, changing respiratory rate or tidal volume can substantially alter spectral HRV while average heart period remains comparatively stable. [153] The respiration-linked signal is real. Its amplitude simply cannot be assigned to neural drive alone.

14.3 LF Power and LF/HF

The low-frequency band has historically been assigned a sympathetic interpretation in some HRV frameworks. The evidence does not support that simplification. Human pharmacological and postural experiments demonstrate that LF cardiac variability can contain substantial parasympathetic contribution. Its branch composition changes with physiological state, and during slow breathing respiratory-linked, predominantly vagally mediated cardiac oscillation can move directly into the conventional LF band. [3, 126] Therefore: LF power is not a pure measure of sympathetic activity.

For the same reason, dividing LF power by HF power does not transform two mixed, context-dependent cardiac frequency measures into a quantitative measure of reciprocal sympathetic-versus-parasympathetic control. LF/HF does not measure sympathovagal balance. [154] Review 03 should therefore not use LF/HF as a mechanistic or regulatory endpoint. This does not make LF power physiologically meaningless. It means its interpretation must remain tied to the actual oscillatory processes, respiratory frequency, posture, baroreflex conditions, and measurement context rather than being assigned to one autonomic branch by definition.

HRV magnitude is strongly related to mean heart rate and heart period. Part of this relationship is mathematical: the same absolute timing change has different proportional consequences at different baseline R-R intervals. Part is physiological. Experimental evidence, including isolated cardiac preparations and sinoatrial-node studies, shows that intrinsic pacemaker properties contribute to variability even when normal autonomic innervation is absent. [150, 151] HRV amplitude therefore cannot be treated as though it were determined solely by the amount of autonomic neural traffic reaching the heart. This has practical implications for comparison.

If one condition produces both a slower mean heart rate and a higher RMSSD, some of the HRV change may reflect the altered cardiac operating rate itself rather than a proportional increase in parasympathetic neural modulation. Mathematical correction for mean heart rate can reduce some of this dependence, but correction also changes the physiological quantity being analyzed. [150, 151] Mean heart rate should therefore be reported and modeled, not automatically normalized away.

14.4 Posture, Age, and Between-Person Comparison

HRV is also state dependent. Posture changes cardiac loading, sympathetic and parasympathetic influence, baroreflex conditions, and the branch composition of spectral power. Supine, seated, standing, and moving recordings are therefore not interchangeable reference states. [3, 62] Age produces substantial shifts in population HRV distributions, and sex can influence some metrics. [155] This limits universal cut points and simple between-person ranking. A raw RMSSD of one value cannot be interpreted identically across individuals of different ages, heart rates, respiratory patterns, postures, rhythms, medications, and intrinsic cardiac characteristics.

The problem is especially clear in pharmacological validation studies. Across individuals, resting RSA only moderately tracks atropine-defined cardiac parasympathetic control, and RSA alone does not reliably rank individuals according to tonic cardiac vagal influence. [64, 146] For discovery research, within-person change under comparable conditions is therefore a narrower and generally more defensible inference than assigning physiological meaning to one person's HRV relative to another person's value. This does not eliminate between-person analysis. It means such analysis requires substantially more contextual adjustment and should not be interpreted as though HRV were a direct person-level quantity of “vagal capacity.”

14.5 Recording Duration and Dynamic Measurement

HRV estimates depend on the duration and temporal characteristics of the recording. Conventional short-term HRV is often characterized using approximately five-minute stationary segments. Some studies demonstrate that substantially shorter recordings can provide acceptable agreement for RMSSD in static resting conditions, while other metrics, particularly frequency-domain measures, require longer or metric-specific windows. [156, 157] Evidence validating ultra-short resting RMSSD does not automatically validate ultra-short spectral analysis during a dynamic challenge. This distinction matters for SeaKing because the scientifically interesting signal may not be stationary.

Motion onset, physiological adaptation during exposure, motion cessation, and recovery can all produce changing cardiovascular states. Conventional spectral methods assume sufficient stationarity within the analyzed interval; that assumption can fail when the underlying physiology is changing rapidly. Dynamic HRV analysis should therefore be understood as a sequence of local temporal estimates, not as though an entire exposure represented one homogeneous autonomic state. Window length should be selected according to the metric and physiological question before examining the result rather than being optimized retrospectively for the largest apparent effect.

14.6 Rhythm, Artifact, and Motion

HRV depends fundamentally on identifying the timing of normal sinus beats accurately. Ectopic beats, missed detections, false detections, pacing, atrial arrhythmias, and motion artifact can produce large artificial changes in successive-difference and spectral measures. ECG-derived normal-to-normal intervals are therefore preferred when HRV is being used for mechanistic interpretation. This issue is particularly important during vessel motion. Movement can introduce

signal artifact precisely during the period in which the physiological response is of greatest interest. Artifact rejection cannot therefore be treated simply as generic data cleaning. Excessive or state-dependent artifact can create systematic missingness or apparent physiological change.

Any pulse-derived variability measure used as a backup should be identified as pulse-rate variability rather than assumed to be interchangeable with ECG-derived HRV unless it has been validated under the relevant motion conditions. Protocol-specific artifact thresholds and exclusion rules belong in the Phase 1 methods plan rather than this physiology review, but the epistemic requirement is clear: the validity of an HRV inference begins with the validity of the beat series from which it was calculated.

Nonlinear HRV measures can characterize aspects of temporal organization that conventional time- and frequency-domain metrics do not capture. That mathematical richness can be useful. It does not confer additional autonomic specificity by itself. Entropy measures, Poincare-derived metrics, fractal measures, proprietary composites, or machine-derived HRV features remain downstream descriptions unless the specific metric has been independently validated against the mechanism being claimed. [149] A more complex HRV algorithm does not move upward on the evidence hierarchy simply because it extracts additional structure from the same R-R series.

Accordingly, nonlinear HRV should be treated as additional cardiac time-series information, not automatically as a superior measure of vagal activity, autonomic flexibility, or regulation.

14.7 What HRV Can Legitimately Tell Us

The HRV evidence therefore supports a narrow but valuable conclusion. Under appropriate conditions, RMSSD, respiratory-frequency HF-HRV, and RespHRV can provide validated downstream information sensitive to cardiac parasympathetic modulation at the sinoatrial effector. Pharmacological blockade provides causal support for that interpretation. The inference becomes stronger when sinus rhythm and beat detection are valid; respiration is recorded and incorporated into interpretation; mean heart rate is reported; posture and activity are defined; measurement duration matches the metric; within-person conditions are comparable; and the HRV result is considered alongside non-equivalent physiological measures.

The evidence does not support using HRV as a direct recording of vagal nerve activity; a quantitative measure of the entire vagus or parasympathetic nervous system; a measure of global autonomic balance; a stand-alone measure of autonomic flexibility; or evidence that regulation improved. Higher HRV is therefore not intrinsically better, calmer, healthier, or more parasympathetically “dominant.”

For Phase 1, HRV is most useful as a within-person cardiac response measure embedded in a synchronized trajectory. ECG-derived normal-to-normal intervals, respiration, mean heart rate, posture/activity, exposure timing, recovery, and repeated-session context should be retained (see Phase 1 Protocol, Section X). Reproducible changes in RMSSD or respiration-informed HRV can support altered cardiac parasympathetic modulation under defined conditions; they do not establish whole-vagus activation, improved regulation, or therapeutic benefit. [3, 146, 150, 156]

15. Multimodal Autonomic Measurement and Triangulation

No practical noninvasive biomarker provides a direct measurement of the autonomic nervous system as a whole. Instead, commonly used autonomic measures sample different downstream physiological domains. Electrodermal activity reflects cutaneous sudomotor physiology. Pre-ejection period reflects cardiac electromechanical behavior with conditional sensitivity to beta-adrenergic influence. Pupil dynamics reflect interacting ocular sympathetic and parasympathetic control. Blood pressure reflects the integrated hemodynamic result of cardiac, vascular, neural, mechanical, and local influences. Respiration is both an autonomic context and physiological response. Heart rate is a final cardiac output influenced by multiple pathways. [4, 5, 158, 159]

Their scientific value lies precisely in this non-equivalence. Multiple measurements can reveal whether an exposure produces a reproducible physiological response across several domains. They should not be treated as interchangeable estimates of one hidden quantity called "autonomic activity." The frozen biomarker review therefore concludes that practical autonomic measures are useful because they measure different downstream effectors, not because they provide multiple versions of the same signal. [4, 158–160]

15.1 Electrodermal Activity

Electrodermal activity (EDA) measures changes in the electrical properties of skin associated primarily with eccrine sweat-gland activity. As established in Section 13, direct sympathetic recordings provide unusually strong validation for this relationship. Cutaneous sympathetic sudomotor bursts precede measurable sweat responses, linking the downstream skin signal to a defined sympathetic effector pathway. EDA can support a specific inference: change in cutaneous sympathetic-sudomotor effector activity under defined recording conditions. It should not be promoted into a measure of whole-body sympathetic activity. [4, 9]

Sweat-gland distribution also matters. Palmar and plantar surfaces contain dense eccrine innervation and are not necessarily interchangeable with wrist or other wearable recording sites. [4] Temperature, humidity, electrode contact, skin properties, movement, and thermoregulatory sweating can further alter the measured signal. Increased or decreased EDA should therefore be interpreted within the cutaneous sympathetic-sudomotor domain rather than as evidence of global sympathetic activation or withdrawal. The signal is physiologically meaningful because its effector is specific.

15.2 Pre-Ejection Period

Pre-ejection period (PEP) is the interval between ventricular electrical activation and opening of the aortic valve. It is commonly estimated using ECG and impedance cardiography. Experimental validation supports sensitivity of PEP to cardiac beta-adrenergic influence, making it useful as a conditional cardiac sympathetic-sensitive measure. [158, 161] The word conditional is important. PEP is an electromechanical interval rather than a neural recording. Ventricular preload, afterload, contractile state, posture, respiration, ventricular function, and identification of impedance landmarks can change the measured interval independently of a simple change in sympathetic nerve firing. [158, 161]

A shortening of PEP under controlled conditions can support increased cardiac beta-adrenergic influence. It cannot establish global sympathetic activation or even necessarily increased sympathetic nerve firing to the heart without additional evidence. This distinction is particularly important during motion, where posture, thoracic mechanics, electrode movement, and impedance morphology may change simultaneously.

15.3 Catecholamines

Catecholamine measurements occupy a different level of the evidence hierarchy. As Section 13 established, regional norepinephrine spillover can provide meaningful information about sympathetic neurotransmitter release within defined organ territories. Static plasma norepinephrine concentration is considerably less specific. The measured concentration reflects not simply release but the balance among release, neuronal reuptake, vascular distribution, metabolism, blood flow, and clearance. Sampling location also matters. [88] Epinephrine requires an additional distinction because circulating epinephrine has a major adrenal source and therefore should not be treated as a direct measure of peripheral sympathetic nerve traffic.

Plasma norepinephrine and epinephrine provide useful biochemical information, but neither is a direct measure of sympathetic nerve firing or a body-wide readout of peripheral sympathetic neural activity. Their mechanistic meaning depends strongly on sampling and kinetic method.

15.4 Pupil Dynamics

The pupil is controlled through interacting sympathetic and parasympathetic ocular pathways. Parasympathetic activity contributes strongly to pupillary constriction, while sympathetic pathways contribute to dilation. This makes pupil diameter and pupillary reflex dynamics potentially useful autonomic-effector measures. The measured pupil, however, is not a branch-specific autonomic meter. Luminance, accommodation and near response, gaze angle, baseline pupil diameter, age, ocular disease, medications, cognitive effort, attention, arousal, and other state variables can materially influence pupil behavior. [159, 162, 163]

The relative contribution of sympathetic and parasympathetic pathways can also vary across experimental conditions. Pupil dilation cannot be assigned uniquely to sympathetic activation, nor constriction to parasympathetic dominance. Pupillometry can support a claim that an ocular autonomic-effector response occurred when visual conditions and relevant context are controlled, but it cannot by itself identify whole-body sympathetic or parasympathetic state. Nor should pupil behavior be converted into a direct measurement of the central autonomic network or locus coeruleus. Section 11 established why central inference requires separate evidence.

15.5 Blood Pressure, Heart Rate, Respiration, and Skin Blood Flow

Several additional measures are highly informative even though they have relatively low branch specificity when interpreted alone. Blood pressure is a regulated cardiovascular output produced through interactions among cardiac output, vascular resistance, arterial mechanics, autonomic reflexes, local vascular control, respiration, posture, and other determinants. Baroreflex sensitivity can provide more specific information about defined reflex relationships, but as Section 9 established, different BRS methods characterize different components and should not be collapsed into one autonomic score. [99, 108]

Heart rate is similarly valuable as an outcome while remaining mechanistically nonspecific. Sympathetic and parasympathetic cardiac influences, intrinsic sinoatrial behavior, respiration, temperature, activity, circulating mediators, and other factors can all change heart rate. Respiration is especially important because, as Section 10 established, it can simultaneously function as physiological response, mediator, mechanical input, and autonomic measurement context. Skin blood flow provides information about a peripheral vascular effector, but local temperature, endothelial and metabolic regulation, probe location and pressure, and thermoregulatory state can substantially modify the signal. [160]

These measurements should be preserved because each describes a different part of the physiological response. Their limited branch specificity is not a reason to discard them. It is a reason to name them correctly.

If every autonomic biomarker measured the same underlying quantity, strong correlation among them should be expected. That is not what human physiology shows. Different autonomic effectors can respond differently to the same challenge. Regional sympathetic outflows can dissociate. Cardiac parasympathetic modulation can change without an equivalent change in cutaneous sudomotor activity. Pupil behavior can change under cognitive or visual conditions that produce different cardiovascular effects. Different channels also have different latencies and recovery kinetics. This phenomenon is sometimes described as response fractionation. It is not necessarily measurement failure. [5, 164, 165]

Divergence may reflect different autonomic branches; different organs or effectors; regional sympathetic specialization; different central control relationships; different response latencies; or different nonautonomic influences on the measured signal. The frozen multimodal review specifically finds partial coherence, output-specific central associations, individual and situational patterning, and time-varying relationships among cardiac, electrodermal, respiratory, pupillary, and other physiological signals. [5, 164–166] A useful multimodal framework must preserve that disagreement rather than force all channels to agree.

15.6 Triangulation Across Non-Equivalent Channels

Multimodal measurement becomes most powerful when the channels retain their physiological identities. Suppose a defined exposure is followed reproducibly by changes in respiratory pattern, RMSSD, EDA, pupil dynamics, and blood pressure. The correct interpretation is not: five biomarkers show autonomic activation. Instead, each signal contributes a separate observation: cardiac parasympathetic-sensitive timing changed; respiration changed; cutaneous sudomotor output changed; ocular autonomic-effector behavior changed; hemodynamic state changed. If those changes repeatedly occur with a defined temporal relationship to the same exposure, confidence increases that the exposure is associated with a real multidomain physiological response.

That is triangulation. Its strength comes from convergence among non-equivalent measurements with different physiological generators and different inferential weaknesses. This is the same principle established in Section 13, now applied to practical discovery measurement.

Multimodal inference should not require every signal to change in the same direction or at the same time. A physiologically coherent response may contain increased cardiac parasympathetic-sensitive HRV; transiently increased EDA; little change in heart rate; a delayed pupil response; and a respiratory change that precedes the cardiac response. Whether that pattern is plausible depends on context and reproducibility, not on whether every signal points toward one imagined autonomic axis. Likewise, two signals may initially move together and later separate during sustained exposure or recovery. That divergence can itself reveal structure.

Section 12 established that regulation unfolds across time. Multimodal analysis extends that principle by asking whether different physiological channels have distinct but reproducible trajectories across the same exposure. Therefore: cross-signal agreement strengthens evidence of convergence; cross-signal disagreement can contain mechanistic information. Neither alone establishes whether the response is beneficial.

15.7 Multivariate Pattern Discovery

Multimodal datasets also permit analyses that cannot be performed with one biomarker at a time. Principal components, clustering, latent-variable models, classifiers, machine-learning embeddings, and other multivariate approaches can identify recurring structure across many physiological channels. Such methods may be particularly useful in discovery research when the physiological response is multidimensional and not known in advance. But statistical structure does not automatically acquire physiological identity. A principal component that explains substantial variance across HRV, EDA, respiration, pupil, and blood pressure is still a statistical component. A classifier that distinguishes motion from baseline with high accuracy is a predictive model. [167–169]

A cluster separating participants into different response phenotypes is a data-derived grouping. None can automatically be renamed autonomic state, vagal state, sympathetic state, autonomic balance, or regulatory quality without independent physiological validation against that construct. [167–170] Prediction and mechanism are different scientific questions.

15.8 Why a Global Autonomic Score Is Not Justified

The temptation to combine multiple measurements into one number is understandable. A single score is easier to display, compare, optimize, and eventually feed into a closed-loop controller. Physiological validity still requires justification. Combining HRV, EDA, pupil diameter, heart rate, respiration, blood pressure, or PEP requires decisions about scaling, weighting, direction, temporal alignment, and the meaning assigned to each component. Those decisions implicitly assume that the measures contribute to one common construct. The evidence reviewed here does not establish such a construct.

For example, why should increased RMSSD and decreased EDA both receive a positive value? Under what physiological demand should that combination be considered preferable? How should a necessary increase in sympathetic vascular support be scored? What happens when pupil and cardiac signals diverge appropriately? Without external validation, the resulting number would

primarily encode the designer's assumptions. SeaKing Phase 1 should not create an ad hoc autonomic score, regulation score, balance score, or coherence score by mathematically combining available biomarkers. A future composite could become valid if it were prospectively defined and independently validated against a specific physiological or clinically meaningful endpoint.

Phase 1 should preserve each channel as a named physiological measure with its own signal-quality rules and align all channels to the same motion timeline (see Phase 1 Protocol, Section X). Analyses can examine channel-specific trajectories, within-person reproducibility, time-lagged relationships, and exploratory multivariate phenotypes. Data-derived components, clusters, or classifiers should remain statistical constructs until independently validated; the study should not manufacture an ad hoc global autonomic score. [4, 5, 160, 171]

16. Terminology and Epistemic Boundaries

Scientific precision depends not only on what is measured, but on what the measurement is called. Autonomic terminology frequently compresses complex, pathway-specific physiology into convenient scalar labels: “vagal tone,” “sympathetic tone,” “autonomic balance,” “parasympathetic dominance,” “CAN activation,” or “improved autonomic regulation.” Such language can be useful shorthand when a construct has been narrowly defined and validated. Without that definition, however, the terminology can imply substantially more than the underlying evidence establishes. [6, 172, 173]

The governing rule for this paper is simple:

Name the measured physiological domain first. Expand the interpretation only as far as independent evidence permits.

A biomarker should not acquire the identity of the nerve, autonomic branch, central network, mechanism, or regulatory quality that may contribute to it. This boundary is carried forward across the entire Review 03 research record.

16.1 “Vagal Tone”

The phrase vagal tone is particularly vulnerable to overextension because the vagus is not one homogeneous efferent signal. As Sections 5–7 established, it contains sensory afferents, branchiomotor fibers, and parasympathetic efferents serving different organs. Cardiac parasympathetic modulation is consequently specific to the cardiac domain unless independent evidence supports extension to pulmonary, gastrointestinal, inflammatory, or broader vagal activity. [48, 49, 71]

For that reason, this paper preferentially uses cardiac parasympathetic influence or cardiac vagal modulation when the evidence concerns sinoatrial cardiac control. The phrase cardiac vagal tone may be defensible when it is explicitly defined as tonic parasympathetic influence on the heart and supported by an appropriate method, such as selective pharmacological blockade. [62, 146]

HRV alone does not establish that quantity. Higher RMSSD or greater RespHRV can support changes in cardiac parasympathetic modulation under appropriate conditions, but neither quantifies whole-body vagal tone or total vagal nerve activity. Cardiac vagal modulation should likewise remain a cardiac-domain inference unless separate evidence supports broader vagal recruitment. The narrower terminology is not semantic caution for its own sake. It identifies the physiology the measurement can actually support. [3, 48]

16.2 “Sympathetic Tone”

The same problem applies to sympathetic tone. Section 8 established that sympathetic outflow is regionally differentiated. Muscle sympathetic nerve activity, cutaneous sympathetic activity, cardiac sympathetic influence, renal sympathetic activity, adrenal output, and other sympathetic effectors can change differently during the same physiological challenge. [86, 89, 95] A measure from one domain should retain that domain in its name. Examples include muscle sympathetic nerve activity,

cutaneous sympathetic-sudomotor activity, cardiac beta-adrenergic influence, or regional norepinephrine spillover rather than simply sympathetic tone.

The phrase “sympathetic activation” is acceptable only when the activated pathway or effector is specified or when unusually broad evidence genuinely supports a system-level inference. EDA, PEP, plasma catecholamines, blood pressure, or pupil diameter alone cannot establish global sympathetic tone. [4, 88, 158, 159]

16.3 “Autonomic Balance”

The sympathetic and parasympathetic systems are often presented as opposite ends of one physiological axis. Section 3 established why that model is inadequate. Sympathetic and parasympathetic cardiac influences can change reciprocally, independently, or together. Regional sympathetic outputs can also dissociate from one another. [2, 18, 19, 89] There is no universal scalar quantity corresponding to sympathetic ← autonomic balance → parasympathetic. This is also why LF/HF should not be interpreted as a quantitative measure of “sympathovagal balance.” Section 14 established that LF and HF power do not map uniquely onto sympathetic and parasympathetic branches. [126, 154]

Review 03 does not use autonomic balance, sympathovagal balance, parasympathetic dominance, or sympathetic dominance as global physiological states unless a specific validated construct is explicitly defined. When multiple autonomic outputs change, those changes should be described rather than compressed into an assumed balance axis. [172, 173]

The term autonomic coherence creates a related problem. Different physiological channels can become correlated or synchronized, but Section 15 established that cross-channel concordance is evidence of convergence, not evidence that the channels have become one physiological process. Divergence can also be physiologically appropriate. [5, 174] Synchronized changes in HRV, respiration, pupil dynamics, EDA, blood pressure, or other signals are better described literally as multimodal convergence, cross-channel coupling, or synchronized physiological change. They should not automatically be labeled “autonomic coherence.”

A proprietary composite or latent variable derived from multiple channels likewise does not become a physiological mechanism merely because it receives a physiological-sounding name. [171]

16.4 “CAN Activation”

The central autonomic network is a distributed and recurrent organization, not a scalar central autonomic variable. Section 11 established that different central regions contribute differently across tasks, physiological states, and experimental conditions. [128, 139, 140] Peripheral changes in HRV, pupil dynamics, EDA, blood pressure, or respiration may be compatible with altered central-autonomic processing, but they do not identify the participating regions, the direction of their activity, or the pathway involved.

Even neuroimaging requires region-, task-, and method-specific interpretation. For SeaKing Phase 1, a peripheral physiological response may be compatible with central-autonomic involvement but does not demonstrate CAN activation. Localization and causal attribution require appropriate central measurement or perturbation.

16.5 “Autonomic Flexibility”

Section 12 defined autonomic flexibility narrowly as the capacity to alter physiological configuration in relation to changing demand and subsequently reconfigure when that demand changes again. That is a dynamic systems property, not a synonym for one biomarker. [35]

No single feature establishes autonomic flexibility. High resting HRV, large reactivity, rapid recovery, and multimodal variability each describe only part of a dynamic response. Flexibility requires evidence that physiological configuration changes appropriately with demand and reconfigures as that demand changes.

Any claim of flexibility must identify the demand, physiological domain, response trajectory, recovery or reconfiguration, and relevant repeated-exposure context. The term is useful only when the dynamic behavior it describes is visible.

The phrase autonomic dysregulation is legitimate when the abnormality is specified and supported. Used alone, however, it can become an explanation rather than a description. A scientifically useful statement should identify the feature at issue, for example: exaggerated orthostatic tachycardia; blunted cardiovagal baroreflex response; delayed cardiovascular recovery after challenge; abnormal regional sympathetic recruitment; or another defined physiological abnormality.

Only after that abnormal feature has been established should the broader label “autonomic dysregulation” be applied. This prevents a circular inference in which an unusual biomarker is called dysregulation and then “dysregulation” is used to explain the unusual biomarker. [6, 30, 31]

16.6 “Improved Autonomic Regulation”

The strongest value judgment is improved autonomic regulation, and no single direction of physiological change is sufficient to establish it. Higher HRV, lower EDA, slower heart rate, smaller reactivity, faster recovery, or greater cross-channel convergence may each be meaningful in context, but none is intrinsically evidence of improvement. [5, 30, 31] Sections 12 and 15 established why: exaggerated and blunted responses can both be maladaptive, different effectors can legitimately diverge, and regulation unfolds across baseline, challenge, response, within-exposure dynamics, recovery, and repeated exposure.

Whether a physiological trajectory can legitimately be called improved requires another evidentiary step. Section 17 owns that threshold. Until then, the correct terminology is descriptive: increased, decreased, attenuated, augmented, faster, slower, more reproducible, habituated, sensitized, convergent, divergent, or changed, followed by the specific physiological variable and experimental context. Description should precede judgment.

Terminological discipline also applies to stimulation studies. Vagus nerve stimulation is a controlled perturbation, not a direct measurement of endogenous vagal physiology. Electrode placement near a nerve does not by itself prove which fibers were recruited. Demonstrated evoked recruitment does not establish that the same fibers participate in an endogenous physiological state. A clinical effect following stimulation does not independently establish the pathway through which that effect occurred. [175–177]

The frozen project guardrails therefore keep three distinctions explicit: electrode location does not by itself establish target engagement; evoked recruitment does not establish that the same fibers participate in endogenous vagal physiology; and clinical efficacy does not by itself identify the mechanism responsible for benefit.

This is important for SeaKing because stimulation literature can provide evidence that perturbing a pathway is capable of producing an effect. It cannot automatically establish that natural motion recruits the same pathway. That mechanistic bridge must be demonstrated rather than assumed.

The same principle applies to computational analysis. A machine-learning classifier may distinguish two exposure conditions accurately. A clustering algorithm may identify reproducible participant groups. A latent-variable model may identify a component shared across several physiological signals. A composite score may predict an outcome.

Those results can be scientifically valuable without automatically acquiring mechanistic meaning. Prediction does not identify mechanism; a cluster does not by itself identify a physiological pathway; and latent factors or composite scores remain statistical constructs unless independently validated against the physiological or functional meaning assigned to them. [164, 171]

Naming is not validation. This boundary becomes especially important in high-dimensional Phase 1 data, where sophisticated analyses may discover patterns that are statistically robust before their

biological meaning is known. The appropriate initial description is a data-derived response phenotype or pattern, followed by subsequent validation.

16.7 A Claim Ladder for SeaKing

The terminology rules can be reduced to a practical claim ladder.

Level 1: Observation

A measured signal changed.

Example: RMSSD increased during a defined motion exposure.

Level 2: Validated physiological inference

External validation supports interpretation of that signal within a defined physiological domain.

Example: The change is consistent with altered cardiac parasympathetic modulation under the measured respiratory and cardiac conditions.

Level 3: Multimodal physiological inference

Non-equivalent measurements converge on a reproducible physiological response.

Example: The exposure was associated with a reproducible multidomain physiological response involving cardiac, respiratory, sudomotor, and pupillary effectors.

Level 4: Mechanistic inference

Controlled validation demonstrates that a specified pathway causally contributes to the response.

Example: A defined neural or reflex pathway contributes causally to the observed physiological response.

Level 5: Regulatory or functional inference

The physiological trajectory is shown to correspond to context-appropriate regulation, meaningful function, symptom improvement, or another validated outcome.

Each level requires additional evidence. A finding at Level 1 cannot be promoted directly to Level 4 or Level 5 because the interpretation is attractive. This is the epistemic structure that separates observation, physiological inference, mechanism, and benefit.

SeaKing should use the most specific language its measurements permit: cardiac, respiratory, sudomotor, pupillary, hemodynamic, multimodal, and within-person response descriptions first; broader autonomic or mechanistic labels only after separate validation. Phase 1 should not claim increased “vagal tone,” reduced “sympathetic tone,” restored “autonomic balance,” “CAN activation,” “autonomic coherence,” or “improved autonomic regulation” from discovery biomarkers alone. [6, 154, 171, 172]

17. When Can Autonomic Regulation Be Called Improved?

A physiological change is not automatically a physiological improvement. This distinction is fundamental to autonomic research because many variables commonly assigned positive or negative meaning are inherently context dependent. Higher HRV may be appropriate in one state and uninformative in another. Reduced cardiovascular reactivity may represent efficient regulation or an inadequate response to demand. Faster recovery may be favorable after one challenge while premature withdrawal of support may be inappropriate during another. Habituation can reflect useful

adaptation, but reduced response across repeated exposures can arise through several mechanisms. Therefore, improved autonomic regulation is not a direction on a biomarker scale. [10, 11, 32]

It is a higher-order inference about whether physiological systems respond appropriately to a defined demand, reconfigure appropriately as that demand changes or ends, reproduce that behavior under comparable conditions, remain tolerable, and ultimately support meaningful function or clinical outcome. [10, 11, 32]

17.1 Measurement Validity and Benefit Are Different Questions

A biomarker can be valid without being beneficial. RMSSD can validly provide information sensitive to cardiac parasympathetic modulation without higher RMSSD necessarily representing better health. EDA can validly reflect cutaneous sympathetic-sudomotor activity without lower EDA necessarily representing improved regulation. PEP can contain valid information about cardiac beta-adrenergic influence without a longer or shorter PEP necessarily being preferable.

The first question is: What physiological process does this measurement validly reflect? The second is: What happened to that process under the defined condition? Only then comes the separate question: Was that response functionally appropriate or beneficial? Collapsing these questions creates a common inferential error: validated biomarker → observed direction → assumed benefit. The middle observation does not supply the final value judgment.

17.2 Direction Alone Cannot Define Improvement

Section 12 established that both exaggerated and blunted physiological responses can be maladaptive depending on the demand. Prospective human evidence reinforces this boundary. Greater stress reactivity has been associated with adverse outcomes in some contexts, while unusually small or blunted responses have also predicted adverse outcomes in others. [10, 32] There is therefore no universal rule such as: less sympathetic response = better; more parasympathetic response = better; lower heart rate = better; higher HRV = better; lower EDA = better; or smaller reactivity = better.

The appropriate response depends on what the organism is being asked to do. Standing requires cardiovascular compensation against gravity. Exercise requires increased cardiac output. Thermal stress may require sympathetic sudomotor and vascular responses. Cognitive or emotional challenge may appropriately produce transient physiological mobilization. Suppressing a necessary response would not constitute improved regulation merely because the resulting biomarker appears “calmer.” Conversely, a large response is not inherently pathological if the demand requires it and the system reconfigures appropriately afterward. Improvement must be judged relative to demand, not relative to a preferred direction on a graph.

17.3 Baseline Is a Reference, Not Necessarily a Target

Baseline measurements are essential because they establish the physiological state from which a response begins. They should not automatically be treated as the state to which the system must return as rapidly as possible. The post-challenge physiological environment may differ from the pre-challenge environment. Metabolic demand, circulating mediators, temperature, fluid distribution, emotional state, respiratory pattern, and other variables may remain altered after the nominal stimulus ends. Recovery is better understood as appropriate reconfiguration after demand, rather than compulsory restoration of every variable to its original value. This also prevents another common assumption: closer to a population mean = healthier. [27, 28, 30]

Population reference distributions can identify unusual physiology. They do not establish the optimal state for every individual under every condition. For within-person discovery research, an individual's own comparable baseline is often the more informative reference for characterizing change. Whether movement toward or away from that baseline represents improvement requires additional functional evidence.

17.4 Recovery Adds Information Beyond Peak Response

Peak reactivity captures only one part of a physiological trajectory. Two individuals can exhibit similar peak responses while differing substantially in how long the response persists, whether different effectors recover together, and whether the system reconfigures appropriately after the challenge. Prospective evidence indicates that delayed cardiovascular recovery can carry information beyond peak reactivity itself. [11] This makes recovery scientifically important. But the same interpretive rule applies: faster recovery is not universally better simply because it is faster.

The relevant question is whether recovery timing is appropriate for the preceding demand and associated with favorable functional consequences. A response that disappears before physiological support is no longer required may be inadequate. A response that remains elevated long after the demand has ended may represent inefficient or prolonged activation. What matters is the shape and context of the recovery trajectory, not simply its slope.

17.5 Reproducibility Is Necessary Before Value Judgment

One favorable-looking response during one exposure is weak evidence of improved regulation. Physiology varies naturally from day to day and moment to moment. Sleep, hydration, meals, medications, posture, prior activity, circadian timing, respiration, emotional state, illness, environmental conditions, and measurement noise can all influence autonomic signals. Before a response phenotype is assigned functional meaning, it should therefore demonstrate reasonable within-person reproducibility under comparable conditions. That does not require identical waveforms. Biological regulation is not mechanical repetition. [33, 34]

It means that the important characteristics of the response recur sufficiently to distinguish a participant-specific pattern from transient noise or uncontrolled context. Repeated measurement is not merely a way to increase sample size. It is part of the evidence required to determine whether the observed response represents a stable physiological characteristic.

17.6 Repeated Exposure: Adaptation Is Not Automatically Improvement

Repeated exposure adds another level of inference. A physiological response may become smaller, larger, faster, slower, or differently coordinated across sessions. Those changes can represent habituation, sensitization, learning, altered expectation, physiological adaptation, changing tolerance, or other processes. A smaller repeated response should not automatically be labeled beneficial habituation, nor a larger repeated response harmful sensitization, without context. [33, 34]

A reduced response may indicate efficient adaptation to a familiar benign stimulus. It may also reflect fatigue, reduced engagement, altered measurement conditions, or inadequate physiological recruitment. Similarly, persistence of a response across sessions is not necessarily failure to adapt if the response remains appropriate to the recurring demand. Repeated-exposure change becomes more informative when it is stimulus specific, reproducible, compatible with maintained physiological function, and associated with stable or improved tolerance and meaningful outcomes. The direction of the adaptation alone does not establish its value.

17.7 Tolerability Is Part of the Evidence

A physiological trajectory should not be labeled improved while ignoring whether the exposure producing it is tolerable. This is particularly important for motion. A motion profile might produce a highly reproducible cardiovascular or respiratory response while simultaneously producing nausea, dizziness, distress, disequilibrium, fatigue, or another adverse experience. The physiological pattern would remain scientifically interesting. It would not thereby become beneficial. Tolerability provides a necessary boundary condition between measurable physiological response and candidate beneficial response. This does not mean subjective comfort alone establishes improved regulation. Pleasantness and physiological benefit are also not identical.

Rather, an intervention that consistently produces unacceptable adverse effects cannot be considered favorably regulated simply because selected biomarkers move in a theoretically desirable direction.

17.8 Multimodal Convergence Strengthens the Response Claim, Not the Benefit Claim

Section 15 established that convergence among non-equivalent physiological channels can strengthen confidence that a real multidomain response occurred. It does not establish that the response is beneficial. Suppose motion produces a reproducible pattern involving respiration, cardiac variability, EDA, pupil dynamics, and blood pressure. That convergence can substantially strengthen the conclusion that the exposure produced a coordinated physiological response. It still does not answer whether that response improved regulation, reduced symptoms, improved function, or produced therapeutic benefit. Likewise, divergence among channels does not establish dysregulation.

Multimodal measurement improves description and physiological inference. Functional meaning remains a separate evidentiary step.

17.9 Association With Meaningful Function

The claim of improvement becomes substantially stronger when a reproducible physiological trajectory is associated with a meaningful functional outcome. Depending on the population and research question, such outcomes might include validated measures of symptoms, task performance, tolerance, daily function, behavior, quality of life, or another prespecified clinically meaningful endpoint. The specific endpoint must be appropriate to the condition being studied. This is where the word improved begins to acquire empirical meaning. If a physiological pattern repeatedly occurs alongside better function, greater tolerance, reduced symptom burden, or another validated outcome, the pattern becomes a candidate marker of beneficial regulation. [12, 40]

Even then, association does not establish causation. The physiological response may contribute to the functional improvement, accompany it, or arise from another process that produces both. Mechanistic validation remains a separate question.

17.10 Biomarkers Are Not Automatically Surrogate Endpoints

A biomarker can correlate with clinical outcome without becoming a validated substitute for that outcome. Suppose a particular Phase 1 physiological phenotype later predicts symptom improvement in a clinical population. That would make the phenotype potentially useful as a predictive or prognostic biomarker. It would not automatically establish that changing the biomarker causes the clinical improvement or that the biomarker can replace the clinical endpoint in an intervention trial. A surrogate endpoint requires stronger evidence: changes in the surrogate must reliably capture the relevant effect of intervention on the meaningful outcome across appropriate validation settings. [12, 40]

Biomarker association, surrogate validation, and mechanism are separate inferential steps. Review 03 need not resolve future surrogate-endpoint methodology. The important boundary is that a physiologically attractive marker should not be promoted into a therapeutic endpoint merely because it predicts outcome once.

17.11 An Evidence Ladder for Improvement

The evidence required to call a physiological response beneficial can therefore be represented as a progression:

1. Observed physiological change: A valid measurement changes during or after a defined exposure.

2. Reproducible context-specific response: The important response characteristics recur within the individual under comparable conditions.

3. Appropriate recovery or reconfiguration: The response evolves after the demand in a manner consistent with the physiological context.

4. Repeated-session stability or adaptation: The trajectory remains interpretable across repeated exposures, including any habituation, sensitization, or other adaptation.

5. Tolerability: The exposure and resulting physiological response occur without unacceptable adverse effects.

6. Association with meaningful function or clinical outcome: The physiological phenotype corresponds to prespecified functional, symptomatic, behavioral, or clinical benefit.

7. Validated intervention benefit: Controlled evidence demonstrates that the intervention produces meaningful benefit and establishes the role, if any, of the physiological phenotype as mediator, predictor, or validated surrogate.

Each step answers a different question. Skipping steps does not make the conclusion more ambitious. It makes it less defensible.

17.12 What Phase 1 Can Establish

Phase 1 can establish measurable physiological change, within-person trajectories, recovery patterns, multimodal convergence or divergence, reproducibility, repeated-session adaptation, and tolerability. Those findings can define candidate response phenotypes. Discovery measurements alone cannot establish improved autonomic regulation, therapeutic efficacy, a validated surrogate endpoint, or a specific vagal, sympathetic, or central causal mechanism. [10–12]

17.13 What Later Phases Must Add

Later controlled studies must test causal mechanisms, while clinical studies determine whether a discovered phenotype is associated with meaningful improvement and whether deliberately producing it changes functional or clinical outcomes. The sequence is: discover the response, test its mechanism, establish whether it matters, and only then evaluate it as an intervention target or surrogate. [12, 40]

17.14 The Standard for “Improved Regulation”

Review 03 therefore uses a demanding standard: improved autonomic regulation requires a response appropriate to a defined demand, appropriate recovery or reconfiguration, reproducibility under comparable conditions, tolerability, and association with meaningful functional or clinical benefit. Stronger causal language requires evidence that the intervention produced the benefit and that the physiological response contributes to it. No single biomarker direction, global score, or multimodal concordance satisfies this standard by itself. [10, 11, 32, 40]

18. Implications for SeaKing Solace

The evidence reviewed in this paper supports a clear scientific opportunity, but not yet a therapeutic conclusion. Human autonomic physiology is dynamic, regional, multimodal, and strongly dependent on context. Cardiac parasympathetic activity, regional sympathetic outflows, respiration, baroreflex function, central integration, pupil dynamics, sudomotor activity, hemodynamics, and other physiological processes interact without collapsing into one universal autonomic state. [2, 5, 26, 86] Natural motion introduces a similarly multidimensional exposure.

The first scientific question is not:

Does natural motion improve the autonomic nervous system?

It is:

Does defined natural motion produce reproducible, dose-related physiological response phenotypes in humans, and if so, what are their temporal and multimodal characteristics?

That is the appropriate question for SeaKing Phase 1. [13]

18.1 Natural Motion as an Experimental Exposure

Natural vessel motion is a multidimensional, time-varying exposure involving combinations of heave, pitch, roll, sway, surge, yaw, acceleration, frequency, amplitude, direction, and duration. Received exposure also varies with vessel location, posture, participant movement, visual environment, and sea state. Phase 1 should analyze time-resolved motion rather than reduce the exposure to “boat versus no boat,” aligning motion with baseline, response, within-exposure dynamics, and recovery.

18.2 Phase 1 Is a Discovery Study

The principal Phase 1 objective is phenotype discovery: identifying reproducible patterns linking measured motion to cardiac, respiratory, pupillary, sudomotor, hemodynamic, and other physiological trajectories. A phenotype is a description, not a diagnosis, mechanism, or treatment effect. Its value lies in defining what repeatedly occurs, when it occurs, and how it changes across sessions. To prevent a rich multichannel dataset from becoming a post hoc search for whichever signal appears favorable, the Phase 1 protocol and statistical analysis plan should prospectively distinguish the primary endpoint or primary endpoint family from secondary and exploratory channels, with multiplicity handled accordingly (see Phase 1 Protocol/SAP, Section X). [5, 10]

18.3 Motion Must Be Measured With the Physiology

Physiological differences between sessions are uninterpretable without the received motion dose. Synchronized motion and physiology allow testing of exposure-response relationships, thresholds or nonlinearities, latency, reproducibility, and whether recovery begins when motion ends or while exposure continues. These are discovery questions and do not presume that any motion profile is beneficial.

18.4 Within-Person Structure Is Central

Because autonomic measurements vary with age, posture, respiration, heart rate, medication, sleep, hydration, prior activity, and baseline state, Phase 1 should emphasize within-person comparisons across comparable exposures. Between-person analyses remain useful for heterogeneity and candidate responder phenotypes, but universal biomarker thresholds should not be invented before population-specific validation. [29, 150, 155]

18.5 Multimodal Measurement Should Remain Multimodal

Each physiological channel should retain its identity. HRV, EDA, pupil dynamics, respiration, heart rate, blood pressure, and other measures provide non-equivalent information; convergence strengthens evidence of a multidomain response, while divergence can itself be informative. Phase 1 should seek reproducible configurations rather than average the channels into an unvalidated global autonomic score. [4, 5, 159, 171]

18.6 Phase 1 Can Generate Mechanistic Hypotheses

Discovery can generate targeted mechanistic hypotheses without presuming them. A reproducible cardiac-respiratory phenotype might justify selective cardiovagal testing; vascular or sudomotor phenotypes might motivate sympathetic or reflex studies; other patterns may justify central investigation. The sequence is discover → characterize → hypothesize → perturb → validate, using the evidence hierarchy from Section 13.

18.7 Phase 1 Does Not Need to Prove a Vagal Mechanism

The vagus remains relevant to the SeaKing hypothesis, but Phase 1 does not need to demonstrate direct vagal activation to succeed. Respiration-informed cardiac parasympathetic modulation would be meaningful without establishing whole-vagus activation; likewise, sympathetic-sensitive or central-compatible patterns remain bounded to the domains actually measured. [64, 129, 146]

18.8 Phase 1 Does Not Need to Prove Benefit

A successful Phase 1 result may be a reproducible, dose-related, temporally structured, well-tolerated physiological phenotype whose mechanism and functional significance remain unknown. Section 17 establishes why that result cannot yet be called improved autonomic regulation or therapeutic efficacy. [10, 40] Whether the primary Phase 1 premise is supported should be determined prospectively rather than by selecting the most favorable channel or analysis after data collection. The protocol and statistical analysis plan should therefore define, before enrollment or unblinded analysis, the primary endpoint structure, reproducibility criterion, and channel-specific smallest effect size of scientific interest (SESOI) where an independently defensible threshold can be derived from external reliability, test-retest, measurement-error, reliable-change, or other relevant literature. Where no defensible SESOI exists, the endpoint should remain exploratory rather than receive an arbitrary threshold (see Phase 1 Protocol/SAP, Section X).

18.9 Prospective Outcomes: Supportive, Inconclusive, and Non-Supportive

Falsifiability requires all three outcomes to be reachable in practice. For the prospectively defined primary endpoint or endpoint family, a supportive result should require an effect that meets the prespecified scientific threshold and reproducibility criteria with adequate precision. An inconclusive result should be reserved for estimates whose uncertainty still spans both scientifically meaningful and negligible effects, or for prespecified failures of data quality or exposure adequacy that prevent a valid decision. A non-supportive result should be reachable when the study achieves the prespecified measurement and exposure conditions and the resulting precision excludes effects at or above the independently justified SESOI. Failure to reject a conventional null hypothesis is not, by itself, evidence of no meaningful effect.

Because Phase 1 contains repeated sessions, time-varying motion dose, multiple physiological channels, and potentially position-dependent exposure, these decision rules require a mixed-effects or hierarchical analysis that respects the nested data structure. Power and precision should be justified by simulation using the actual participant × session × exposure × channel design rather than by treating repeated observations as independent. The statistical analysis plan should also prespecify primary analysis windows, alternative sensitivity windows, and a concordance rule defining what constitutes robustness across reasonable segmentation choices (see Phase 1 Protocol/SAP, Section X). These specifications belong in the protocol/SAP; Review 03 establishes the evidentiary requirement rather than choosing numerical thresholds in advance of that statistical work.

18.10 From Natural Motion to Controlled Stimulation

If reproducible motion-response relationships emerge, later phases can reproduce candidate motion characteristics under controlled conditions to determine which axes, frequencies, amplitudes, durations, or temporal patterns are necessary. This progression from ecological discovery to experimental control can separate motion effects from the broader vessel experience.

18.11 From Controlled Response to Closed-Loop Investigation

Closed-loop control requires a validated target. Candidate phenotypes must first be reproducible under controlled conditions, linked to meaningful function or clinical outcome, and tested to determine whether deliberately producing them improves that outcome. Only then should they be considered closed-loop targets. [12, 40]

18.12 The Scientific Opportunity

The literature establishes a testable physiological question rather than an autonomic therapy: motion can be quantified, autonomic effectors can be measured synchronously, within-person trajectories can be followed, mechanistic hypotheses can later be tested with more direct methods, and clinical significance can be evaluated in subsequent populations.

18.13 Current Evidentiary Position

Natural motion is a plausible multidimensional physiological exposure whose autonomic effects in humans warrant synchronized, dose-resolved study. The unresolved questions are substantive: the optimal motion profile, whether any response phenotype is beneficial, whether particular effects are vagally or sympathetically mediated, which central mechanisms participate, and whether the findings ultimately have therapeutic relevance for autism, POTS, or another condition. [10, 40, 172]

18.14 Conclusion

Autonomic physiology is dynamic, distributed, and context dependent. SeaKing Phase 1 can pair quantified natural motion with synchronized, non-equivalent physiological measurements to determine whether reproducible exposure-response phenotypes exist. If they do, later studies can test mechanism and functional significance. The scientific progression is: measure the motion → measure the physiology → discover the response → test the mechanism → establish functional meaning → determine whether deliberate control can produce benefit. The first step is discovery. If the prospectively defined primary analysis can, with adequate precision, exclude a scientifically meaningful motion-linked response under the tested conditions, the premise required for progression is not supported and subsequent development should be redirected rather than advanced on the present hypothesis.

References

1. Jänig W, Häbler HJ. Specificity in the organization of the autonomic nervous system: a basis for precise neural regulation of homeostatic and protective body functions. Prog Brain Res. 2000;122:351-367. doi:10.1016/S0079-6123(08)62150-0. PMID: 10737070.
2. Berntson GG, Cacioppo JT, Quigley KS. Autonomic determinism: the modes of autonomic control, the doctrine of autonomic space, and the laws of autonomic constraint. Psychol Rev. 1991;98(4):459-487. doi:10.1037/0033-295X.98.4.459. PMID: 1660159.
3. Pomeranz B, Macaulay RJ, Caudill MA, Kutz I, Adam D, Gordon D, Kilborn KM, Barger AC, Shannon DC, Cohen RJ, et al. Assessment of autonomic function in humans by heart rate spectral analysis. Am J Physiol. 1985;248(1 Pt 2):H151-H153. doi:10.1152/ajpheart.1985.248.1.H151. PMID: 3970172.
4. Boucsein W, Fowles DC, Grimnes S, Ben-Shakhar G, Roth WT, Dawson ME, Filion DL; Society for Psychophysiological Research Ad Hoc Committee on Electrodermal Measures. Publication recommendations for electrodermal measurements. Psychophysiology. 2012;49(8):1017-1034. doi:10.1111/j.1469-8986.2012.01384.x. PMID: 22680988.
5. Kreibig SD. Autonomic nervous system activity in emotion: a review. Biol Psychol. 2010;84(3):394-421. doi:10.1016/j.biopsycho.2010.03.010. PMID: 20371374.
6. Eckberg DL. Sympathovagal balance: a critical appraisal. Circulation. 1997;96(9):3224-3232. doi:10.1161/01.CIR.96.9.3224. PMID: 9386196.
7. Mano T, Iwase S, Toma S. Microneurography as a tool in clinical neurophysiology to investigate peripheral neural traffic in humans. Clin Neurophysiol. 2006;117(11):2357-2384. doi:10.1016/j.clinph.2006.06.002. PMID: 16904937.
8. Berntson GG, Cacioppo JT, Quigley KS. Autonomic cardiac control. I. Estimation and validation from pharmacological blockades. Psychophysiology. 1994;31(6):572-585. doi:10.1111/j.1469-8986.1994.tb02350.x. PMID: 7846218.
9. Sugenoya J, Iwase S, Mano T, Ogawa T. Identification of sudomotor activity in cutaneous sympathetic nerves using sweat expulsion as the effector response. Eur J Appl Physiol Occup Physiol. 1990;61(3-4):302-308. doi:10.1007/BF00357617. PMID: 2282916.
10. O'Riordan A, Howard S, Gallagher S. Blunted cardiovascular reactivity to psychological stress and prospective health: a systematic review. Health Psychol Rev. 2023;17(1):121-147. doi:10.1080/17437199.2022.2068639. PMID: 35445639.
11. Steptoe A, Marmot M. Impaired cardiovascular recovery following stress predicts 3-year increases in blood pressure. J Hypertens. 2005;23(3):529-536. doi:10.1097/01.hjh.0000160208.66405.a8. PMID: 15716693.
12. Fleming TR, Powers JH. Biomarkers and surrogate endpoints in clinical trials. Stat Med. 2012;31(25):2973-2984. doi:10.1002/sim.5403. PMID: 22711298; PMCID: PMC3551627.
13. Minjoz S, Jeanne R, Pellissier S, Hot P. Psychophysiological effects of heart rate variability biofeedback versus sham biofeedback: A randomized controlled trial. Biol Psychol. 2026;206:109254. doi:10.1016/j.biopsycho.2026.109254. PMID: 41905438.
14. Krugers HJ, Joels M. The Stress Response Is Adaptive in a Context- and State-Dependent Manner. Cells. 2025;14(24):1957. doi:10.3390/cells14241957. PMID: 41439976; PMCID: PMC12731636.
15. Ramsay DS, Woods SC. Clarifying the roles of homeostasis and allostasis in physiological regulation. Psychol Rev. 2014;121(2):225-247. doi:10.1037/a0035942. PMID: 24730599; PMCID: PMC4166604.
16. Sterling P. Allostasis: a model of predictive regulation. Physiol Behav. 2012;106(1):5-15. doi:10.1016/j.physbeh.2011.06.004. PMID: 21684297.
17. Schulkin J, Sterling P. Allostasis: A Brain-Centered, Predictive Mode of Physiological Regulation. Trends Neurosci. 2019;42(10):740-752. doi:10.1016/j.tins.2019.07.010. PMID: 31488322.
18. Berntson GG, Cacioppo JT, Quigley KS. Cardiac psychophysiology and autonomic space in humans: empirical perspectives and conceptual implications. Psychol Bull. 1993;114(2):296-322. doi:10.1037/0033-2909.114.2.296. PMID: 8416034.
19. Berntson GG, Cacioppo JT, Quigley KS, Fabro VT. Autonomic space and psychophysiological response. Psychophysiology. 1994;31(1):44-61. doi:10.1111/j.1469-8986.1994.tb01024.x. PMID: 8146254.
20. Kollai M, Koizumi K. Reciprocal and non-reciprocal action of the vagal and sympathetic nerves innervating the heart. J Auton Nerv Syst. 1979;1(1):33-52. doi:10.1016/0165-1838(79)90004-3. PMID: 553085.
21. Paton JFR, Boscan P, Pickering AE, Nalivaiko E. The yin and yang of cardiac autonomic control: vago-sympathetic interactions revisited. Brain Res Brain Res Rev. 2005;49(3):555-565. doi:10.1016/j.brainresrev.2005.02.005. PMID: 16269319.
22. Cacioppo JT, Uchino BN, Berntson GG. Individual differences in the autonomic origins of heart rate reactivity: the psychometrics of respiratory sinus arrhythmia and preejection period. Psychophysiology. 1994;31(4):412-419. doi:10.1111/j.1469-8986.1994.tb02449.x. PMID: 10690921.
23. Gianaros PJ, Quigley KS. Autonomic origins of a nonsignal stimulus-elicited bradycardia and its habituation in humans. Psychophysiology. 2001;38(3):540-547. doi:10.1017/S004857720100004X. PMID: 11352143; PMCID: PMC2898889.

24. Berntson GG, Cacioppo JT, Binkley PF, Uchino BN, Quigley KS, Fieldstone A. Autonomic cardiac control. III. Psychological stress and cardiac response in autonomic space as revealed by pharmacological blockades. Psychophysiology. 1994;31(6):599-608. doi:10.1111/j.1469-8986.1994.tb02352.x. PMID: 7846220.

25. Karemaker JM. An introduction into autonomic nervous function. Physiol Meas. 2017;38(5):R89-R118. doi:10.1088/1361-6579/aa6782. PMID: 28304283.

26. Chida Y, Hamer M. Chronic psychosocial factors and acute physiological responses to laboratory-induced stress in healthy populations: a quantitative review of 30 years of investigations. Psychol Bull. 2008;134(6):829-885. doi:10.1037/a0013342. PMID: 18954159.

27. Schuler JL, O'Brien WH. Cardiovascular recovery from stress and hypertension risk factors: a meta-analytic review. Psychophysiology. 1997;34(6):649-659. doi:10.1111/j.1469-8986.1997.tb02141.x. PMID: 9401420.

28. Roddick CM, Seo YS, Barkovich SL, Forrester L, Chen FS. Cardiac vagal recovery following acute psychological stress in human adults: A scoping review. Neurosci Biobehav Rev. 2025;176:106268. doi:10.1016/j.neubiorev.2025.106268. PMID: 40571137.

29. Manser P, Thalmann M, Adcock M, Knols RH, de Bruin ED. Can Reactivity of Heart Rate Variability Be a Potential Biomarker and Monitoring Tool to Promote Healthy Aging? A Systematic Review With Meta-Analyses. Front Physiol. 2021;12:686129. doi:10.3389/fphys.2021.686129. PMID: 34393813; PMCID: PMC8359814.

30. Chida Y, Steptoe A. Greater cardiovascular responses to laboratory mental stress are associated with poor subsequent cardiovascular risk status: a meta-analysis of prospective evidence. Hypertension. 2010;55(4):1026-1032. doi:10.1161/HYPERTENSIONAHA.109.146621. PMID: 20194301.

31. Turner AI, Smyth N, Hall SJ, Torres SJ, Hussein M, Jayasinghe SU, Ball K, Clow AJ. Psychological stress reactivity and future health and disease outcomes: A systematic review of prospective evidence. Psychoneuroendocrinology. 2020;114:104599. doi:10.1016/j.psyneuen.2020.104599. PMID: 32045797.

32. Cole CR, Blackstone EH, Pashkow FJ, Snader CE, Lauer MS. Heart-rate recovery immediately after exercise as a predictor of mortality. N Engl J Med. 1999;341(18):1351-1357. doi:10.1056/NEJM199910283411804. PMID: 10536127.

33. Grissom N, Bhatnagar S. Habituation to repeated stress: get used to it. Neurobiol Learn Mem. 2009;92(2):215-224. doi:10.1016/j.nlm.2008.07.001. PMID: 18667167; PMCID: PMC2773683.

34. Tyra AT, Brindle RC, Hughes BM, Ginty AT. Cynical hostility relates to a lack of habituation of the cardiovascular response to repeated acute stress. Psychophysiology. 2020;57(12):e13681. doi:10.1111/psyp.13681. PMID: 32920855.

35. Csigai P, Sparrow L, Maquet L, Pique A, Kozieja P, De Zorzi L, Sequeira H. Resting HRV predicts cardiac vagal control during stress, not psychological distress. Sci Rep. 2026 May 23. doi:10.1038/s41598-026-52956-z. PMID: 42177247.

36. McEwen BS. Stress, adaptation, and disease. Allostasis and allostatic load. Ann N Y Acad Sci. 1998;840:33-44. doi:10.1111/j.1749-6632.1998.tb09546.x. PMID: 9629234.

37. Seeman TE, McEwen BS, Rowe JW, Singer BH. Allostatic load as a marker of cumulative biological risk: MacArthur studies of successful aging. Proc Natl Acad Sci U S A. 2001;98(8):4770-4775. doi:10.1073/pnas.081072698. PMID: 11287659; PMCID: PMC31909.

38. McCrory C, McLoughlin S, Layte R, NiCheallaigh C, O'Halloran AM, Barros H, et al. Towards a consensus definition of allostatic load: a multi-cohort, multi-system, multi-biomarker individual participant data (IPD) meta-analysis. Psychoneuroendocrinology. 2023;153:106117. doi:10.1016/j.psyneuen.2023.106117. PMID: 37100008; PMCID: PMC10620736.

39. Carbone JT, Clift J, Alexander N. Measuring allostatic load: Approaches and limitations to algorithm creation. J Psychosom Res. 2022;163:111050. doi:10.1016/j.jpsychores.2022.111050. PMID: 36228435.

40. Christensen R, Ciani O, Manyara AM, Taylor RS. Surrogate endpoints: a key concept in clinical epidemiology. J Clin Epidemiol. 2024;167:111242. doi:10.1016/j.jclinepi.2023.111242. PMID: 38142762.

41. Wehrwein EA, Orer HS, Barman SM. Overview of the Anatomy, Physiology, and Pharmacology of the Autonomic Nervous System. Compr Physiol. 2016;6(3):1239-1278. doi:10.1002/cphy.c150037. PMID: 27347892.

42. Benarroch EE. Physiology and Pathophysiology of the Autonomic Nervous System. Continuum (Minneap Minn). 2020;26(1):12-24. doi:10.1212/CON.0000000000000817. PMID: 31996619.

43. Jänig W. Neurobiology of visceral afferent neurons: neuroanatomy, functions, organ regulations and sensations. Biol Psychol. 1996;42(1-2):29-51. doi:10.1016/0301-0511(95)05145-7. PMID: 8770369.

44. Gibbons CH. Basics of autonomic nervous system function. Handb Clin Neurol. 2019;160:407-418. doi:10.1016/B978-0-444-64032-1.00027-8. PMID: 31277865.

45. Benarroch EE. The central autonomic network: functional organization, dysfunction, and perspective. Mayo Clin Proc. 1993;68(10):988-1001. doi:10.1016/S0025-6196(12)62272-1. PMID: 8412366.

46. Beissner F, Meissner K, Bär KJ, Napadow V. The autonomic brain: an activation likelihood estimation meta-analysis for central processing of autonomic function. J Neurosci. 2013;33(25):10503-10511. doi:10.1523/JNEUROSCI.1103-13.2013. PMID: 23785162; PMCID: PMC3685840.

47. Barboi AC, Macefield VG. The autonomic nervous system: Time for a conceptual reframing? J Physiol. 2025;603(22):6719-6731. doi:10.1113/JP288973. PMID: 40956984; PMCID: PMC12645539.

48. Ottaviani MM, Macefield VG. Structure and Functions of the Vagus Nerve in Mammals. Compr Physiol. 2022;12(4):3989-4037. doi:10.1002/cphy.c210042. PMID: 35950655.

49. Neuhuber WL, Berthoud HR. Functional anatomy of the vagus system - emphasis on the somato-visceral interface. Auton Neurosci. 2021;236:102887. doi:10.1016/j.autneu.2021.102887. PMID: 34634680; PMCID: PMC8627476.

50. Kupari J, Haring M, Agirre E, Castelo-Branco G, Ernfors P. An Atlas of Vagal Sensory Neurons and Their Molecular Specialization. Cell Rep. 2019;27(8):2508-2523.e4. doi:10.1016/j.celrep.2019.04.096. PMID: 31116992; PMCID: PMC6533201.

51. Atsumi K, Yajima T, Tachiya D, Kokubun S, Shoji N, Sasano T, Ichikawa H, Sato T. Sensory neurons in the human jugular ganglion. Tissue Cell. 2020;64:101344. doi:10.1016/j.tice.2020.101344. PMID: 32473709.

52. Berthoud HR, Neuhuber WL. Functional and chemical anatomy of the afferent vagal system. Auton Neurosci. 2000;85(1-3):1-17. doi:10.1016/S1566-0702(00)00215-0. PMID: 11189015.

53. Berthoud HR, Munzberg H, Morrison CD, Neuhuber WL. Gut-brain communication: Functional anatomy of vagal afferents. Curr Opin Neurobiol. 2025;93:103058. doi:10.1016/j.conb.2025.103058. PMID: 40451136; PMCID: PMC12353194.

54. Driessen AK. Vagal Afferent Processing by the Paratrigeminal Nucleus. Front Physiol. 2019;10:1110. doi:10.3389/fphys.2019.01110. PMID: 31555145; PMCID: PMC6722180.

55. Gourine AV, Machhada A, Trapp S, Spyer KM. Cardiac vagal preganglionic neurones: An update. Auton Neurosci. 2016;199:24-28. doi:10.1016/j.autneu.2016.06.003. PMID: 27396874.

56. Wang YB, Saunders SE, Campbell JN, Boychuk CR. Cardiac vagal motor neurons. Curr Opin Neurobiol. 2025;93:103068. doi:10.1016/j.conb.2025.103068. PMID: 40570503; PMCID: PMC12353181.

57. Havton LA, Biscola NP, Stern E, et al. Human organ donor-derived vagus nerve biopsies allow for well-preserved ultrastructure and high-resolution mapping of myelinated and unmyelinated fibers. Sci Rep. 2021;11:23831. doi:10.1038/s41598-021-03248-1. PMID: 34903749; PMCID: PMC8668909.

58. Pelot NA, Goldhagen GB, Cariello JE, Musselman ED, Clissold KA, Ezzell JA, Grill WM. Quantified Morphology of the Cervical and Subdiaphragmatic Vagus Nerves of Human, Pig, and Rat. Front Neurosci. 2020;14:601479. doi:10.3389/fnins.2020.601479. PMID: 33250710; PMCID: PMC7672126.

59. Upadhye AR, Kolluru C, Druschel L, et al. Fascicles split or merge every approximately 560 microns within the human cervical vagus nerve. J Neural Eng. 2022;19(5):054001. doi:10.1088/1741-2552/ac9643. PMID: 36174538; PMCID: PMC10353574.

60. Thompson N, Mastitskaya S, Iacoviello F, Turhani F, Shearing PR, Aristovich K, Holder D. Human vagus nerve fascicular anatomy and its implications for targeted cardiac stimulation: a microCT segmentation and histological pilot anatomical study. Front Neurosci. 2026;20:1731234. doi:10.3389/fnins.2026.1731234. PMID: 41788551; PMCID: PMC12956647.

61. Ruigrok TJH, Mantel SA, Orlandini L, de Knegt C, Vincent AJPE, Spoor JKH. Sympathetic components in left and right human cervical vagus nerve: implications for vagus nerve stimulation. Front Neuroanat. 2023;17:1205660. doi:10.3389/fnana.2023.1205660. PMID: 37492698; PMCID: PMC10364449.

62. Cacioppo JT, Berntson GG, Binkley PF, Quigley KS, Uchino BN, Fieldstone A. Autonomic cardiac control. II. Noninvasive indices and basal response as revealed by autonomic blockades. Psychophysiology. 1994;31(6):586-598. doi:10.1111/j.1469-8986.1994.tb02351.x. PMID: 7846219.

63. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation. 1996;93(5):1043-1065. doi:10.1161/01.CIR.93.5.1043. PMID: 8598068.

64. Grossman P, Kollai M. Respiratory sinus arrhythmia, cardiac vagal tone, and respiration: within- and between-individual relations. Psychophysiology. 1993;30(5):486-495. doi:10.1111/j.1469-8986.1993.tb02072.x. PMID: 8416075.

65. Grossman P, Taylor EW. Toward understanding respiratory sinus arrhythmia: relations to cardiac vagal tone, evolution and biobehavioral functions. Biol Psychol. 2007;74(2):263-285. doi:10.1016/j.biopsycho.2005.11.014. PMID: 17081672.

66. Ritz T. Putting back respiration into respiratory sinus arrhythmia or high-frequency heart rate variability: implications for interpretation, respiratory rhythmicity, and health. Biol Psychol. 2024;185:108728. doi:10.1016/j.biopsycho.2023.108728. PMID: 38092221.

67. Laborde S, Mosley E, Thayer JF. Heart rate variability and cardiac vagal tone in psychophysiological research: recommendations for experiment planning, data analysis, and data reporting. Front Psychol. 2017;8:213. doi:10.3389/fpsyg.2017.00213. PMID: 28265249.

68. Karemaker JM. The multibranched nerve: vagal function beyond heart rate variability. Biol Psychol. 2022;172:108378. doi:10.1016/j.biopsycho.2022.108378. PMID: 35688294.

69. Mazzone SB, Undem BJ. Vagal Afferent Innervation of the Airways in Health and Disease. Physiol Rev. 2016;96(3):975-1024. doi:10.1152/physrev.00039.2015. PMID: 27279650; PMCID: PMC4982036.

70. Undem BJ, Potenzieri C. Autonomic neural control of intrathoracic airways. Compr Physiol. 2012;2(2):1241-1267. doi:10.1002/cphy.c110032. PMID: 23798300.

71. Horváth I, Argay K, Herjavecz I, Kollai M. Relation between bronchial and cardiac vagal tone in healthy humans. Chest. 1995;108(3):701-705. doi:10.1378/chest.108.3.701. PMID: 7656619.

72. Ritz T. Studying noninvasive indices of vagal control: the need for respiratory control and the problem of target specificity. Biol Psychol. 2009;80(2):158-168. doi:10.1016/j.biopsycho.2008.08.003. PMID: 18775468.

73. Browning KN, Travagli RA. Central control of gastrointestinal motility. Curr Opin Endocrinol Diabetes Obes. 2019;26(1):11-16. doi:10.1097/MED.0000000000000449. PMID: 30418187; PMCID: PMC6512320.

74. Febo-Rodriguez L, Chumpitazi BP, Sher AC, Shulman RJ. Gastric accommodation: Physiology, diagnostic modalities, clinical relevance, and therapies. Neurogastroenterol Motil. 2021;33(12):e14213. doi:10.1111/nmo.14213. PMID: 34337824.

75. Mendez-Hernandez R, Braga I, Bali A, Yang M, de Lartigue G. Vagal Sensory Gut-Brain Pathways That Control Eating-Satiety and Beyond. Compr Physiol. 2025;15(2):e70010. doi:10.1002/cph4.70010. PMID: 40229922; PMCID: PMC12090708.

76. Feldman M. Gastric H+ and HCO3- secretion in response to sham feeding in humans. Am J Physiol. 1985;248(2 Pt 1):G188-G191. doi:10.1152/ajpgi.1985.248.2.G188. PMID: 3970199.

77. Taylor IL, Feldman M. Effect of cephalic-vagal stimulation on insulin, gastric inhibitory polypeptide, and pancreatic polypeptide release in humans. J Clin Endocrinol Metab. 1982;55(6):1114-1117. doi:10.1210/jcem-55-6-1114. PMID: 6752165.

78. Mussa BM, Verberne AJM. The dorsal motor nucleus of the vagus and regulation of pancreatic secretory function. Exp Physiol. 2013;98(1):25-37. doi:10.1113/expphysiol.2012.066472. PMID: 22660814.

79. Imai J, Katagiri H. Regulation of systemic metabolism by the autonomic nervous system consisting of afferent and efferent innervation. Int Immunol. 2022;34(2):67-79. doi:10.1093/intimm/dxab023. PMID: 33982088.

80. Koop H, Arnold R, Creutzfeldt W. Pancreatic polypeptide and gastrin release during sham feeding in man. Digestion. 1984;30(1):47-52. doi:10.1159/000199090. PMID: 6489644.

81. Martelli D, McKinley MJ, McAllen RM. The cholinergic anti-inflammatory pathway: a critical review. Auton Neurosci. 2014;182:65-69. doi:10.1016/j.autneu.2013.12.007. PMID: 24411268.

82. McAllen RM, McKinley MJ, Martelli D. Reflex regulation of systemic inflammation by the autonomic nervous system. Auton Neurosci. 2022;237:102926. doi:10.1016/j.autneu.2021.102926. PMID: 34906897.

83. Matteoli G, Boeckxstaens GE. The vagal innervation of the gut and immune homeostasis. Gut. 2013;62(8):1214-1222. doi:10.1136/gutjnl-2012-302550. PMID: 23023166; PMCID: PMC3711371.

84. Khan A, Tinajero A, Moresco JJ, Yang J, Gautron L. Anatomical reappraisal of the relationship between vagal preganglionic motor neurons and the spleen in the male mouse. Auton Neurosci. 2026;264:103394. doi:10.1016/j.autneu.2026.103394. PMID: 41724013.

85. Gautron L. The parasympathetic innervation of the spleen: are we chasing a ghost? J Anat. 2022;240(4):772-774. doi:10.1111/joa.13586. PMID: 34729780; PMCID: PMC8930805.

86. Macefield VG. Sympathetic microneurography. Handb Clin Neurol. 2013;117:353-364. doi:10.1016/B978-0-444-53491-0.00028-6. PMID: 24095138.

87. Esler M. The 2009 Carl Ludwig Lecture: Pathophysiology of the human sympathetic nervous system in cardiovascular diseases: the transition from mechanisms to medical management. J Appl Physiol (1985). 2010;108(2):227-237. doi:10.1152/japplphysiol.00832.2009. PMID: 19940096.

88. Esler M. Clinical application of noradrenaline spillover methodology: delineation of regional human sympathetic nervous responses. Pharmacol Toxicol. 1993;73(5):243-253. doi:10.1111/j.1600-0773.1993.tb00579.x. PMID: 8115306.

89. Wallin BG, Esler M, Dorward P, Eisenhofer G, Ferrier C, Westerman R, Jennings G. Simultaneous measurements of cardiac noradrenaline spillover and sympathetic outflow to skeletal muscle in humans. J Physiol. 1992;453:45-58. doi:10.1113/jphysiol.1992.sp019217. PMID: 1464839; PMCID: PMC1175546.

90. Macefield VG, Wallin BG. Physiological and pathophysiological firing properties of single postganglionic sympathetic neurons in humans. J Neurophysiol. 2018;119(3):944-956. doi:10.1152/jn.00004.2017. PMID: 29142091.

91. Klassen SA, Shoemaker JK. Action potential subpopulations within human muscle sympathetic nerve activity: Discharge properties and governing mechanisms. Auton Neurosci. 2021;230:102743. doi:10.1016/j.autneu.2020.102743. PMID: 33202287.

92. Greaney JL, Kenney WL. Measuring and quantifying skin sympathetic nervous system activity in humans. J Neurophysiol. 2017;118(4):2181-2193. doi:10.1152/jn.00283.2017. PMID: 28701539; PMCID: PMC5626908.

93. Vissing SF. Differential activation of sympathetic discharge to skin and skeletal muscle in humans. Acta Physiol Scand Suppl. 1997;639:1-32. PMID: 9421582. PMID: 9421582.

94. McCarthy B, Datta S, Sesa-Ashton G, et al. Differential control of sympathetic outflow to muscle and skin during physical and cognitive stressors. Clin Auton Res. 2024;34:177-189. doi:10.1007/s10286-024-01015-6. PMID: 38308178.

95. Grassi G, Seravalle G, Turri C, Bertinieri G, Dell'Oro R, Mancia G. Impairment of thermoregulatory control of skin sympathetic nerve traffic in the elderly. Circulation. 2003;108(6):729-735. doi:10.1161/01.CIR.0000081769.02847.A1. PMID: 12885757.

96. Grassi G, Seravalle G, Arenare F, Buccianti G, Furiani S, Ilardo V, Bolla G, Mancia G. Behaviour of regional adrenergic outflow in mild-to-moderate renal failure. J Hypertens. 2009;27(3):562-566. doi:10.1097/HJH.0b013e32831fb371. PMID: 19330916.

97. Esler M, Jennings G, Lambert G. Measurement of overall and cardiac norepinephrine release into plasma during cognitive challenge. Psychoneuroendocrinology. 1989;14(6):477-481. doi:10.1016/0306-4530(89)90047-4. PMID: 2623135.

98. Cox HS, Kaye DM, Thompson JM, Turner AG, Jennings GL, Itsiopoulos C, Esler MD. Regional sympathetic nervous activation after a large meal in humans. Clin Sci (Lond). 1995;89(2):145-154. doi:10.1042/cs0890145. PMID: 7554755.

99. Swenne CA. Baroreflex sensitivity: mechanisms and measurement. Neth Heart J. 2013;21(2):58-60. doi:10.1007/s12471-012-0346-y. PMID: 23179611; PMCID: PMC3547418.

100. Dutoit AP, Hart EC, Charkoudian N, Wallin BG, Curry TB, Joyner MJ. Cardiac baroreflex sensitivity is not correlated to sympathetic baroreflex sensitivity within healthy, young humans. Hypertension. 2010;56(6):1118-1123. doi:10.1161/HYPERTENSIONAHA.110.158329. PMID: 21060001; PMCID: PMC3879599.

101. Taylor CE, Witter T, El Sayed K, Hissen SL, Johnson AW, Macefield VG. Relationship between spontaneous sympathetic baroreflex sensitivity and cardiac baroreflex sensitivity in healthy young individuals. Physiol Rep. 2015;3(11):e12536. doi:10.14814/phy2.12536. PMID: 26564059; PMCID: PMC4673619.

102. Smyth HS, Sleight P, Pickering GW. Reflex regulation of arterial pressure during sleep in man. A quantitative method of assessing baroreflex sensitivity. Circ Res. 1969;24(1):109-121. doi:10.1161/01.RES.24.1.109. PMID: 4303309.

103. Goldstein DS, Horwitz D, Keiser HR. Comparison of techniques for measuring baroreflex sensitivity in man. Circulation. 1982;66(2):432-439. doi:10.1161/01.CIR.66.2.432. PMID: 7094250.

104. Schwartz CE, Medow MS, Messer Z, Stewart JM. Spontaneous fluctuation indices of the cardiovagal baroreflex accurately measure the baroreflex sensitivity at the operating point during upright tilt. Am J Physiol Regul Integr Comp Physiol. 2013;304(12):R1107-R1113. doi:10.1152/ajpregu.00559.2012. PMID: 23576616; PMCID: PMC3680794.

105. Fadel PJ, Raven PB. Human investigations into the arterial and cardiopulmonary baroreflexes during exercise. Exp Physiol. 2012;97(1):39-50. doi:10.1113/expphysiol.2011.057554. PMID: 22002871; PMCID: PMC3253263.

106. Raven PB, Young BE, Fadel PJ. Arterial Baroreflex Resetting During Exercise in Humans: Underlying Signaling Mechanisms. Exerc Sport Sci Rev. 2019;47(3):129-141. doi:10.1249/JES.0000000000000190. PMID: 30921029.

107. Keyl C, Schneider A, Dambacher M, Bernardi L. Time delay of vagally mediated cardiac baroreflex response varies with autonomic cardiovascular control. J Appl Physiol (1985). 2001;91(1):283-289. doi:10.1152/jappl.2001.91.1.283. PMID: 11408442.

108. Fritsch JM, Smith ML, Simmons DT, Eckberg DL. Differential baroreflex modulation of human vagal and sympathetic activity. Am J Physiol. 1991;260(3 Pt 2):R635-R641. doi:10.1152/ajpregu.1991.260.3.R635. PMID: 2001013.

109. Laude D, Elghozi JL, Girard A, et al. Comparison of various techniques used to estimate spontaneous baroreflex sensitivity (the EuroBaVar study). Am J Physiol Regul Integr Comp Physiol. 2004;286(1):R226-R231. doi:10.1152/ajpregu.00709.2002. PMID: 14500269.

110. Pinna GD, Maestri R, La Rovere MT. Assessment of baroreflex sensitivity from spontaneous oscillations of blood pressure and heart rate: proven clinical value? Physiol Meas. 2015;36(4):741-753. doi:10.1088/0967-3334/36/4/741. PMID: 25798657.

111. Kollai M, Jokkel G, Bonyhay I, Tomcsanyi J, Naszlady A. Relation between baroreflex sensitivity and cardiac vagal tone in humans. Am J Physiol. 1994;266(1 Pt 2):H21-H27. doi:10.1152/ajpheart.1994.266.1.H21. PMID: 8304501.

112. Mancia G, Parati G, Pomidossi G, Casadei R, Di Rienzo M, Zanchetti A. Arterial baroreflexes and blood pressure and heart rate variabilities in humans. Hypertension. 1986;8(2):147-153. doi:10.1161/01.HYP.8.2.147. PMID: 3080371.

113. Elstad M, O'Callaghan EL, Smith AJ, Ben-Tal A, Ramchandra R. Cardiorespiratory interactions in humans and animals: rhythms for life. Am J Physiol Heart Circ Physiol. 2018;315(1):H6-H17. doi:10.1152/ajpheart.00701.2017. PMID: 29522373.

114. Fisher JP, Zera T, Paton JFR. Respiratory-cardiovascular interactions. Handb Clin Neurol. 2022;188:279-308. doi:10.1016/B978-0-323-91534-2.00006-0. PMID: 35965029.

115. Menuet C, Ben-Tal A, Linossier A, et al. Redefining respiratory sinus arrhythmia as respiratory heart rate variability: an international Expert Recommendation for terminological clarity. Nat Rev Cardiol. 2025;22(12):978-984. doi:10.1038/s41569-025-01160-z. PMID: 40328963.

116. Hirsch JA, Bishop B. Respiratory sinus arrhythmia in humans: how breathing pattern modulates heart rate. Am J Physiol Heart Circ Physiol. 1981;241(4):H620-H629. doi:10.1152/ajpheart.1981.241.4.H620. PMID: 7315987.

117. Saul JP, Berger RD, Chen MH, Cohen RJ. Transfer function analysis of autonomic regulation. II. Respiratory sinus arrhythmia. Am J Physiol Heart Circ Physiol. 1989;256(1):H153-H161. doi:10.1152/ajpheart.1989.256.1.H153. PMID: 2912177.

118. Taha BH, Simon PM, Dempsey JA, Skatrud JB, Iber C. Respiratory sinus arrhythmia in humans: an obligatory role for vagal feedback from the lungs. J Appl Physiol (1985). 1995;78(2):638-645. doi:10.1152/jappl.1995.78.2.638. PMID: 7759434.

119. Triedman JK, Perrott MH, Cohen RJ, Saul JP. Respiratory sinus arrhythmia: time domain characterization using autoregressive moving average analysis. Am J Physiol Heart Circ Physiol. 1995;268(6 Pt 2):H2232-H2238. doi:10.1152/ajpheart.1995.268.6.H2232. PMID: 7611472.

120. Bernardi L, Keller F, Sanders M, Reddy PS, Griffith B, Meno F, Pinsky MR. Respiratory sinus arrhythmia in the denervated human heart. J Appl Physiol (1985). 1989;67(4):1447-1455. doi:10.1152/jappl.1989.67.4.1447. PMID: 2793748.

121. Eckberg DL, Nerhed C, Wallin BG. Respiratory modulation of muscle sympathetic and vagal cardiac outflow in man. J Physiol. 1985;365:181-196. doi:10.1113/jphysiol.1985.sp015766. PMID: 4032310; PMCID: PMC1192996.

122. St Croix CM, Satoh M, Morgan BJ, Skatrud JB, Dempsey JA. Role of respiratory motor output in within-breath modulation of muscle sympathetic nerve activity in humans. Circ Res. 1999;85(5):457-469. doi:10.1161/01.RES.85.5.457. PMID: 10473675.

123. Wang YP, Kuo TBJ, Lai CT, Chu JW, Yang CCH. Effects of respiratory time ratio on heart rate variability and spontaneous baroreflex sensitivity. J Appl Physiol (1985). 2013;115(11):1648-1655. doi:10.1152/japplphysiol.00163.2013. PMID: 24092689.

124. Radaelli A, Raco R, Perfetti P, Viola A, Azzellino A, Signorini MG, Ferrari AU. Effects of slow, controlled breathing on baroreceptor control of heart rate and blood pressure in healthy men. J Hypertens. 2004;22(7):1361-1370. doi:10.1097/01.hjh.0000125446.28861.51. PMID: 15201553.

125. Laborde S, Allen MS, Borges U, Dosseville F, Hosang TJ, Iskra M, et al. Effects of voluntary slow breathing on heart rate and heart rate variability: A systematic review and a meta-analysis. Neurosci Biobehav Rev. 2022;138:104711. doi:10.1016/j.neubiorev.2022.104711. PMID: 35623448.

126. Kromenacker BW, Sanova AA, Marcus FI, Allen JJB, Lane RD. Vagal Mediation of Low-Frequency Heart Rate Variability During Slow Yogic Breathing. Psychosom Med. 2018;80(6):581-587. doi:10.1097/PSY.0000000000000603. PMID: 29771730.

127. Toledo E, Akselrod S, Pinhas I, Aravot D. Does synchronization reflect a true interaction in the cardiorespiratory system? Med Eng Phys. 2002;24(1):45-52. doi:10.1016/S1350-4533(01)00114-X. PMID: 11891139.

128. Saper CB. The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. Annu Rev Neurosci. 2002;25:433-469. doi:10.1146/annurev.neuro.25.032502.111311. PMID: 12052916.

129. Gerlach DA, Manuel J, Hoff A, Kronsbein H, Hoffmann F, Heusser K, et al. Novel Approach to Elucidate Human Baroreflex Regulation at the Brainstem Level: Pharmacological Testing During fMRI. Front Neurosci. 2019;13:193. doi:10.3389/fnins.2019.00193. PMID: 30890917; PMCID: PMC6411827.

130. Critchley HD, Mathias CJ, Dolan RJ. Neuroanatomical basis for first- and second-order representations of bodily states. Nat Neurosci. 2001;4(2):207-212. doi:10.1038/84048. PMID: 11175883.

131. Critchley HD, Wiens S, Rotshtein P, Öhman A, Dolan RJ. Neural systems supporting interoceptive awareness. Nat Neurosci. 2004;7(2):189-195. doi:10.1038/nn1176. PMID: 14730305.

132. Critchley HD, Harrison NA. Visceral influences on brain and behavior. Neuron. 2013;77(4):624-638. doi:10.1016/j.neuron.2013.02.008. PMID: 23439117.

133. Critchley HD, Mathias CJ, Josephs O, O'Doherty J, Zanini S, Dewar BK, Cipolotti L, Shallice T, Dolan RJ. Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. Brain. 2003;126(Pt 10):2139-2152. doi:10.1093/brain/awg216. PMID: 12821513.

134. Chouchou F, Mauguière F, Vallayer O, Catenoix H, Isnard J, Montavont A, et al. How the insula speaks to the heart: Cardiac responses to insular stimulation in humans. Hum Brain Mapp. 2019;40(9):2611-2622. doi:10.1002/hbm.24548. PMID: 30815964; PMCID: PMC6865697.

135. Tian X, Wang J, Wang X, Yang X, Li T, Deng J, Tang C, Wang M, Luan G. Topography of the insular cortex in heart rate control: high-precision mapping reveals critical role of the middle short gyrus. Front Neurosci. 2025;19:1665378. doi:10.3389/fnins.2025.1665378. PMID: 41040956; PMCID: PMC12484038.

136. Sanchez-Larsen A, Principe A, Ley M, Navarro-Cuartero J, Rocamora R. Characterization of the Insular Role in Cardiac Function through Intracranial Electrical Stimulation of the Human Insula. Ann Neurol. 2021;89(6):1172-1180. doi:10.1002/ana.26074. PMID: 33783847.

137. Caruana F, Gerbella M, Avanzini P, Gozzo F, Pelliccia V, Mai R, et al. Motor and emotional behaviours elicited by electrical stimulation of the human cingulate cortex. Brain. 2018;141(10):3035-3051. doi:10.1093/brain/awy219. PMID: 30107501.

138. Oppenheimer SM, Gelb A, Girvin JP, Hachinski VC. Cardiovascular effects of human insular cortex stimulation. Neurology. 1992;42(9):1727-1732. doi:10.1212/WNL.42.9.1727. PMID: 1513461.

139. Thayer JF, Åhs F, Fredrikson M, Sollers JJ 3rd, Wager TD. A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. Neurosci Biobehav Rev. 2012;36(2):747-756. doi:10.1016/j.neubiorev.2011.11.009. PMID: 22178086.

140. Matusik PS, Zhong C, Matusik PT, Alomar O, Stein PK. Neuroimaging Studies of the Neural Correlates of Heart Rate Variability: A Systematic Review. J Clin Med. 2023;12(3):1016. doi:10.3390/jcm12031016. PMID: 36769662; PMCID: PMC9917610.

141. Napadow V, Dhond R, Conti G, Makris N, Brown EN, Barbieri R. Brain correlates of autonomic modulation: Combining heart rate variability with fMRI. Neuroimage. 2008;42(1):169-177. doi:10.1016/j.neuroimage.2008.04.238. PMID: 18524629; PMCID: PMC2603289.

142. DuPont CM, Weis TM, Manuck SB, Marsland AL, Matthews KA, Gianaros PJ. Does well-being associate with stress physiology? A systematic review and meta-analysis. Health Psychol. 2020;39(10):879-890. doi:10.1037/hea0000979. PMID: 32686951.

143. Ottaviani MM, Wright L, Dawood T, Macefield VG. In vivo recordings from the human vagus nerve using ultrasound-guided microneurography. J Physiol. 2020;598(17):3569-3576. doi:10.1113/JP280077. PMID: 32538473.

144. Patros M, Ottaviani MM, Wright L, Dawood T, Macefield VG. Quantification of cardiac and respiratory modulation of axonal activity in the human vagus nerve. J Physiol. 2022;600(13):3113-3126. doi:10.1113/JP282994. PMID: 35524982.

145. Farmer DGS, Patros M, Ottaviani MM, Dawood T, Kumric M, Bozic J, et al. Firing properties of single axons with cardiac rhythmicity in the human cervical vagus nerve. J Physiol. 2025;603(7):1941-1958. doi:10.1113/JP286423. PMID: 39320231; PMCID: PMC11955867.

146. Kollai M, Mizsei G. Respiratory sinus arrhythmia is a limited measure of cardiac parasympathetic control in man. J Physiol. 1990;424:329-342. doi:10.1113/jphysiol.1990.sp018070. PMID: 2391653; PMCID: PMC1189816.

147. Jose AD, Taylor RR. Autonomic blockade by propranolol and atropine to study intrinsic myocardial function in man. J Clin Invest. 1969;48(11):2019-2031. doi:10.1172/JCI106167. PMID: 5398888; PMCID: PMC297454.

148. Diedrich A, Jordan J, Shannon JR, Robertson D, Biaggioni I. Modulation of QT interval during autonomic nervous system blockade in humans. Circulation. 2002;106(17):2238-2243. doi:10.1161/01.CIR.0000035241.76918.6C. PMID: 12390954.

149. Berntson GG, Bigger JT Jr, Eckberg DL, Grossman P, Kaufmann PG, Malik M, et al. Heart rate variability: origins, methods, and interpretive caveats. Psychophysiology. 1997;34(6):623-648. doi:10.1111/j.1469-8986.1997.tb02140.x. PMID: 9401419.

150. Sacha J. Interaction between heart rate and heart rate variability. Ann Noninvasive Electrocardiol. 2014;19(3):207-216. doi:10.1111/anec.12148. PMID: 24602150; PMCID: PMC6932101.

151. Monfredi O, Lyashkov AE, Johnsen AB, Inada S, Schneider H, Wang R, Nirmalan M, Wisloff U, Maltsev VA, Lakatta EG, Zhang H, Boyett MR. Biophysical characterization of the underappreciated and important relationship between heart rate variability and heart rate. Hypertension. 2014;64(6):1334-1343. doi:10.1161/HYPERTENSIONAHA.114.03782. PMID: 25225208.

152. Hayano J, Mukai S, Sakakibara M, Okada A, Takata K, Fujinami T. Effects of respiratory interval on vagal modulation of heart rate. Am J Physiol. 1994;267(1 Pt 2):H33-H40. doi:10.1152/ajpheart.1994.267.1.H33. PMID: 7914066.

153. Brown TE, Beightol LA, Koh J, Eckberg DL. Important influence of respiration on human R-R interval power spectra is largely ignored. J Appl Physiol (1985). 1993;75(5):2310-2317. doi:10.1152/jappl.1993.75.5.2310. PMID: 8307890.

154. Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. Front Physiol. 2013;4:26. doi:10.3389/fphys.2013.00026. PMID: 23431279; PMCID: PMC3576706.

155. Zhang J. Effect of age and sex on heart rate variability in healthy subjects. J Manipulative Physiol Ther. 2007;30(5):374-379. doi:10.1016/j.jmpt.2007.04.001. PMID: 17574955.

156. Munoz ML, van Roon A, Riese H, Thio C, Oostenbroek E, Westrik I, de Geus EJC, Gansevoort R, Lefrandt J, Nolte IM, Snieder H. Validity of (Ultra-)Short Recordings for Heart Rate Variability Measurements. PLoS One. 2015;10(9):e0138921. doi:10.1371/journal.pone.0138921. PMID: 26414314; PMCID: PMC4586373.

157. Burma JS, Graver S, Miutz LN, Macaulay A, Copeland PV, Smirl JD. The validity and reliability of ultra-short-term heart rate variability parameters and the influence of physiological covariates. J Appl Physiol (1985). 2021;130(6):1848-1867. doi:10.1152/japplphysiol.00955.2020. PMID: 33856258.

158. Sherwood A, Allen MT, Fahrenberg J, Kelsey RM, Lovallo WR, van Doornen LJP. Methodological guidelines for impedance cardiography. Psychophysiology. 1990;27(1):1-23. doi:10.1111/j.1469-8986.1990.tb02171.x. PMID: 2187214.

159. McDougal DH, Gamlin PD. Autonomic control of the eye. Compr Physiol. 2015;5(1):439-473. doi:10.1002/j.2040-4603.2015.tb00603.x. PMID: 25589275; PMCID: PMC4919817.

160. Bernardi L, Hayoz D, Wenzel R, Passino C, Calciati A, Weber R, Noll G. Synchronous and baroceptor-sensitive oscillations in skin microcirculation: evidence for central autonomic control. Am J Physiol. 1997;273(4):H1867-H1878. doi:10.1152/ajpheart.1997.273.4.H1867. PMID: 9362255.

161. Newlin DB, Levenson RW. Pre-ejection period: measuring beta-adrenergic influences upon the heart. Psychophysiology. 1979;16(6):546-552. doi:10.1111/j.1469-8986.1979.tb01519.x. PMID: 229507.

162. Steinhauer SR, Condray R, Pless ML. Pharmacological isolation of cognitive components influencing the pupillary light reflex. J Ophthalmol. 2015;2015:179542. doi:10.1155/2015/179542. PMID: 26090217; PMCID: PMC4458281.

163. Larson MD, Tayefeh F, Sessler DI, Daniel M, Noorani M. Sympathetic nervous system does not mediate reflex pupillary dilation during desflurane anesthesia. Anesthesiology. 1996;85(4):748-754. doi:10.1097/00000542-199610000-00009. PMID: 8873544.

164. Quigley KS, Barrett LF. Is there consistency and specificity of autonomic changes during emotional episodes? Guidance from the Conceptual Act Theory and psychophysiology. Biol Psychol. 2014;98:82-94. doi:10.1016/j.biopsycho.2013.12.013. PMID: 24388802; PMCID: PMC4041070.

165. Eisenbarth H, Chang LJ, Wager TD. Multivariate Brain Prediction of Heart Rate and Skin Conductance Responses to Social Threat. J Neurosci. 2016;36(47):11987-11998. doi:10.1523/JNEUROSCI.3672-15.2016. PMID: 27881783; PMCID: PMC5125248.

166. Constantinou E, Vlemincx E, Panayiotou G. Testing emotional response coherence assumptions: Comparing emotional versus non-emotional states. Psychophysiology. 2023;60(11):e14359. doi:10.1111/psyp.14359. PMID: 37282750.

167. Kragel PA, LaBar KS. Multivariate pattern classification reveals autonomic and experiential representations of discrete emotions. Emotion. 2013;13(4):681-690. doi:10.1037/a0031820. PMID: 23527508; PMCID: PMC3745776.

168. Simon L, Rab SL, Goldstein P, Magal N, Admon R. Multi-trajectory analysis uncovers latent associations between psychological and physiological acute stress response patterns. Psychoneuroendocrinology. 2022;145:105925. doi:10.1016/j.psyneuen.2022.105925. PMID: 36115320.

169. Vos G, Trinh K, Sarnyai Z, Rahimi Azghadi M. Generalizable machine learning for stress monitoring from wearable devices: A systematic literature review. Int J Med Inform. 2023;173:105026. doi:10.1016/j.ijmedinf.2023.105026. PMID: 36893657.

170. Cuve HCJ, Harper J, Catmur C, Bird G. Coherence and divergence in autonomic-subjective affective space. Psychophysiology. 2023;60(6):e14262. doi:10.1111/psyp.14262. PMID: 36740720; PMCID: PMC10909527.

171. Kettunen J, Ravaja N, Näätänen P, Keskivaara P, Keltikangas-Järvinen L. The synchronization of electrodermal activity and heart rate and its relationship to energetic arousal: a time series approach. Biol Psychol. 1998;48(3):209-225. doi:10.1016/S0301-0511(98)00017-9. PMID: 9788761.

172. Berntson GG, Cacioppo JT, Grossman P. Whither vagal tone. Biol Psychol. 2007;74(2):295-300. doi:10.1016/j.biopsycho.2006.08.006. PMID: 17046142.

173. Grossman P. Respiratory sinus arrhythmia (RSA), vagal tone and biobehavioral integration: Beyond parasympathetic function. Biol Psychol. 2024;186:108739. doi:10.1016/j.biopsycho.2023.108739. PMID: 38151156.

174. Marwitz M, Stemmler G. On the status of individual response specificity. Psychophysiology. 1998;35(1):1-15. doi:10.1111/1469-8986.3510001. PMID: 9499701.

175. Farmer AD, Strzelczyk A, Finisguerra A, et al. International Consensus Based Review and Recommendations for Minimum Reporting Standards in Research on Transcutaneous Vagus Nerve Stimulation (Version 2020). Front Hum Neurosci. 2021;14:568051. doi:10.3389/fnhum.2020.568051. PMID: 33854421; PMCID: PMC8040977.

176. Butt MF, Albusoda A, Farmer AD, Aziz Q. The anatomical basis for transcutaneous auricular vagus nerve stimulation. J Anat. 2020;236(4):588-611. doi:10.1111/joa.13122. PMID: 31742681; PMCID: PMC7083568.

177. Frangos E, Ellrich J, Komisaruk BR. Non-invasive Access to the Vagus Nerve Central Projections via Electrical Stimulation of the External Ear: fMRI Evidence in Humans. Brain Stimul. 2015;8(3):624-636. doi:10.1016/j.brs.2014.11.018. PMID: 25573069; PMCID: PMC4458242.