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Review

# The vagus nerve in cardiovascular physiology and pathophysiology: From evolutionary insights to clinical medicine

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ABSTRACT

The parasympathetic nervous system via the vagus nerve exerts profound influence over the heart. Together with the sympathetic nervous system, the parasympathetic nervous system is responsible for fine-tuned regulation of all aspects of cardiovascular function, including heart rate, rhythm, contractility, and blood pressure. In this review, we highlight vagal efferent and afferent innervation of the heart, with a focus on insights from comparative biology and advances in understanding the molecular and genetic diversity of vagal neurons, as well as interoception, parasympathetic dysfunction in heart disease, and the therapeutic potential of targeting the parasympathetic nervous system in cardiovascular disease.

The parasympathetic nervous system (PNS) via the vagus nerve exerts profound influence over the heart. Together with the sympathetic nervous system, the PNS is responsible for fine-tuned regulation of all aspects of cardiovascular function, including heart rate, rhythm, contractility, and blood pressure. Since the seminal work of the Weber brothers' demonstrating that stimulation of the vagus nerve in a frog leads to slowing of heart rate [1], numerous studies have been undertaken to characterize parasympathetic innervation of the heart. In this review, we highlight vagal efferent and afferent innervation of the heart, with a focus on insights from comparative biology and advances in understanding the molecular and genetic diversity of vagal neurons, as well as interoception, parasympathetic dysfunction in heart disease, and the therapeutic potential of targeting the PNS in cardiovascular disease.

## 1. Central origins of the vagus nerve in cardiovascular control

Parasympathetic preganglionic neurons originate in the medulla of the brainstem. The nuclei ambiguus (NA) are paired nuclei located in the medullary reticular formation, and the dorsal motor nuclei (DMN) are paired nuclei located near the floor of the fourth ventricle and running along the rostral to caudal medulla. Detailed anatomical and functional studies have demonstrated that these medullary regions project to the heart and mediate cardioinhibitory responses. Standish and colleagues performed retrograde neuronal tracing studies to identify the location of

*Abbreviations:* AF, atrial fibrillation; ANS, autonomic nervous system; AV, atrioventricular; ChAT, choline acetyltransferase; CNS, central nervous system; DMN, dorsal motor nuclei; DREADD, designer receptors exclusively activated by designer drugs; GP, ganglionated plexus; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; ICNS, intrinsic cardiac nervous system; MI, myocardial infarction; NA, nuclei ambiguus; NTS, nucleus tractus solitarius; NYHA, New York Heart Association; PNS, parasympathetic nervous system; RAVANS, respiratory-gated auricular vagus nerve stimulation; SA, sinoatrial; TRPV1, transient receptor potential vanilloid 1; VNS, vagus nerve stimulation.

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cardiac vagal motor neurons within the central nervous system (CNS) [2,3]. The investigators injected the trans-synaptic neuronal tracer pseudorabies virus into the sinoatrial (SA) node, epicardial fats pads, and the ventricular wall of rats. Cardiac vagal neurons were identified bilaterally in the NA, DMN, and in the tegmental field between these two nuclei. Interestingly, neurons in cardiac vagal motor neuron nuclei were labeled at early survival times after injection, whereas interneurons, neurons within the nucleus of the solitary tract (NTS), and various other central autonomic nuclei were labelled at later survival times [3], highlighting the extensive crosstalk that occurs between autonomic neural circuits centrally. Electrophysiologic data has provided a functional correlate to these anatomical studies. McAllen and Spyer showed that neurons within the NA were responsible for slowing heart rate in cats [4]. A cardiac branch of the vagus nerve was stimulated antidromically while recording from neurons in either the NA or DMN. They found that all the activated neurons resided in the NA and not the DMN. Consistent with these data, a subsequent study in cats showed that direct electrical stimulation of the NA slowed heart rate, and additionally, stimulation of the DMN reduced ventricular contractility [5].

Vagal nuclei innervate a variety of visceral organs in addition to the heart. While prior studies suggested that distinct subpopulations of neurons exist within these nuclei, the molecular, cellular, and functional diversity of these neurons has remained largely unknown. Recent work by Veerakumar et al. aimed to dissect central parasympathetic neurons involved in cardiovascular control [6]. The investigators first retrogradely-labeled and transcriptionally profiled cardiac-projecting neurons within the NA in mice, identifying two anatomically and molecularly distinct subtypes of neurons—termed ambiguus cardiovascular and ambiguus cardiopulmonary neurons. Utilizing Cre-dependent adeno-associated virus labeling and optogenetic studies in transgenic mice, they showed that ambiguus cardiovascular and ambiguus cardiopulmonary neurons have distinct targets of innervation and physiologic functions. Ambiguus cardiovascular neurons project to cardiac ganglia and are responsible for slowing heart rate and atrioventricular (AV) conduction velocity. Ambiguus cardiopulmonary neurons project to a different set of cardiac ganglia and are also able to slow heart rate and AV conduction velocity; however, these neurons also project to the lung and mediate the dive reflex, a simultaneous bradycardia and bronchoconstriction that occurs following water immersion. This work suggests that vagal neurons in the CNS are a heterogeneous population, and that molecularly and genetically distinct neuronal subtypes are involved in mediating the diverse physiological functions of the PNS.

## 2. Evolutionary characteristics of the vagus nerve

During mammalian embryological development the vagus nerve sprouts from the medulla and forms contacts with adjacent tissues that give rise to many internal organs that end up in distant locations in the chest and abdomen, including the heart, trachea/lungs, stomach/intestines, liver, and pancreas, thus explaining the diverse functions of this nerve [7]. Adequately understanding mammalian vagal control of the heart requires a broader appreciation for the fact that autonomic control of the cardiovascular system through the vagus nerve likely emerged early in the vertebrate evolutionary lineage. Parasympathetic innervation of the heart appears to have with the gnathostome head-trunk lineage [8]. For example, sharks exhibit phasic modulation of heart rate variability, as do non-mammalian species such as reptiles (e.g., snakes, lizards, turtles, etc.) [9]. From an autonomic standpoint, sharks represent a unique group because they lack sympathetic innervation of the heart; thus, in this species, the vagus nerve is the sole conduit responsible for extra-cardiac neural control [9]. Humans have a separate vagal and petrosal ganglia, whereas in mice these tend to be fused with the nodose and jugular ganglion prior to birth [10], although the functional significance of this anatomical arrangement is unclear. Phylogenetic differences in vagal innervation across species suggests that there may have been possible adaptive changes associated with the evolution of air breathing [11]. This is relevant to appreciating the highly interrelated cardiorespiratory control that is exhibited by mammalian species, such as the phenomenon of respiratory sinus arrythmia, in which changes in intrathoracic pressure during exhalation unload inhibition of vagal efferents leading to increased cholinergic outflow and reductions in heart rate. However, this cyclic respiratory-associated change is not purely mediated by pressure-related impacts on a peripheral nerve. Cardiac parasympathetic preganglionic neurons present in the NA and DMN form a central circuit sending projections via thoracic branches of the vagus nerve to the pacemaker cells of the heart and display an excitatory firing pattern during exhalation and an inhibitory pattern during inhalation [9]. Cardiac parasympathetic preganglionic neurons are also adjacent to neurons in the ventral respiratory groups including the pre-Bötzinger complex, the primary mammalian respiratory rhythm generator, and receive direct inhibitory input from them [12] as well as indirect inputs from pulmonary stretch receptors through the NTS [13]. Thus, in mammals, cyclic respiratory-associated changes in heart rate are associated with changes in breathing via engagement of brainstem cardiovagal and respiratory nuclei. However, these brainstem circuits themselves reflect intermediaries between the heart and brain and are responsive to centrally generated commands that may be voluntary in nature (e.g., cortical control over respiration), as well as to peripheral inputs that are usually automatic (e.g., baroreceptor or chemoreceptor-mediated, or the mammalian dive reflex, which involves trigeminal input [14]). The activity of these neural circuits collectively constitutes a major driver of heart rate variability, which is widely considered to be an indirect measure of autonomic function.

## 3. The cardiac vagus nerve and intrinsic cardiac nervous system

The vagus nerve is composed of co-fasciculating motor and sensory fibers from neurons in the medulla and the superior (jugular) and inferior (nodose) ganglia of the vagus nerve, respectively. Histological studies of the cervical vagus nerve in cats have shown that approximately 20% of the fibers are efferent and 80% are afferent, with most afferent fibers being unmyelinated [15]. Cardiac branches of the bilateral vagus nerves arise in the thorax near the hilum of the lungs. Efferent fibers from these branches synapse on the parasympathetic postganglionic neurons in the intrinsic cardiac nervous system (ICNS) and afferent fibers widely innervate the heart and vasculature. Thus, the vagus nerve allows for bidirectional communication between the heart and brain.

The mammalian ICNS is a distributed network of ganglia, termed ganglionated plexi (GP), located on the epicardial surface of the heart [16–18]. The ICNS is composed of a heterogeneous population of neurons that together with higher centers for the autonomic nervous system (ANS) regulate cardiac electrical and mechanical function. Immunohistochemical studies have shown that intrinsic cardiac neurons produce and respond to multiple neurotransmitters, suggesting that these neurons can transmit a diverse array of signals between the CNS and the heart [19,20]. Most neurons are immunoreactive for choline acetyltransferase (ChAT), consistent with a parasympathetic phenotype; however, a distinct subpopulation of neurons stains positive for antibodies against the sympathetic marker tyrosine hydroxylase [21]. These neurons are also encircled by varicosities from parasympathetic and sympathetic fibers as well as substance P and calcitonin gene related peptide-expressing sensory fibers. Consistent with these findings, in vivo neuronal recordings from GPs in pigs have demonstrated that intrinsic cardiac neurons receive inputs from parasympathetic preganglionic neurons in the brainstem and sympathetic postganglionic neurons in the stellate (cervicothoracic) ganglia [22,23]. Electrical stimulation of the vagus nerves and stellate ganglia modulate the firing rates of these neurons. These neurons also transduce the cardiac milieu and are responsive to a variety of chemical and mechanical stimuli. Thus, the ICNS is believed to receive diverse inputs from higher centers of the ANS including the cortical and brainstem regions via the vagus nerve and from the heart itself to coordinate cardiac function.

Parasympathetic postganglionic neurons within GPs of the ICNS project across the heart including to the SA node, AV node, and myocardial tissue to modulate cardiac electrical and mechanical function. Although each GP has a preferential sphere of influence, substantial overlap exists. In transgenic mice with Cre recombinase under the control of the ChAT promoter, Cre-dependent adeno-associated virus labeling and tracing experiments have identified that cholinergic neurons within a specific dorsal atrial GP project to the SA node [24]. Further, optogenetic stimulation of ChAT-positive neurons within this GP was shown to slow heart rate but not AV conduction velocity. Similarly, in the large mammals including canines, pigs, and humans, the right atrial GP, located in the intercaval region at the dorsal aspect of the right atrium, has been shown to control SA nodal function [25–28]. Electrical stimulation of the right atrial GP leads to bradycardia and ablation of the GP leads to complete loss of cervical vagus nerve stimulation (VNS)-mediated effects on heart rate, indicating that parasympathetic innervation of the SA node is relayed through this GP. Focal stimulation of all GPs by nicotine micro-injections results in changes in heart rate, suggesting both direct and indirect input from each GP to SA node [29]. Similarly, stimulation of most, but not all, GPs can result in AV block, and has varied effects on ventricular electrophysiology. Taken together, these findings suggest that there are interneurons connecting GPs and that these neurons are in constant communication to ensure that regional electromechanical activity is coordinated globally across the heart.

Both anatomical and functional data have shown that parasympathetic nerves richly innervate the heart in multiple species including pigs, canines, and humans. Immunohistochemical studies using acetylthiocholine, with precipitates with acetylcholinesterase and allows staining of cholinergic nerves, has shown the parasympathetic nerves are found throughout atrial and ventricular myocardial tissue [30,31]. Parasympathetic fibers innervating the ventricles project from GPs at the base of the heart and runs towards the apex, with large fiber trunks located on the epicardial surface and a fine meshwork of fibers on the endocardial surface. In the atria, VNS shortens the refractory period and increases susceptibility to atrial fibrillation (AF) [32]. Further, catheter-based ablation of fat pads has been shown to attenuate VNS-mediated shortening of the atrial refractory period and reduce AF [33]. Parasympathetic nerves have also been shown to modulate electrical and functional indices in the ventricles. VNS prolonged ventricular activation recovery interval, a surrogate for local action potential duration, and decreased contractility independent of its effect on heart rate in anesthetized pigs [34,35]. Furthermore, the effects of parasympathetic nerves on the myocardium can be seen independently of sympathetic nerve activity [36]. VNS lengthened the effective refractory period and duration of the monophasic action potential in the ventricle of isolated rabbit hearts where there was an absence of sympathetic tone [37]. In addition, VNS significantly reduced ventricular contractility in anaesthetized cats when the heart was paced and sympathetic tone was pharmacologically blocked [38]. While some effects of the PNS occur independent of the sympathetic nervous system, these two divisions of the ANS interact extensively at all levels of the cardiac neuroaxis including the heart [39,40]. For example, neuropeptide Y and galanin, co-transmitters released from sympathetic nerve endings, have been shown to reduce acetylcholine release and VNS-mediated bradycardia in isolated guinea pig atrial preparations [41,42].

## 4. Cardiac internal sensation via vagal afferents

The heart is under tight surveillance by the ANS. As a major conduit between the heart and brain, the vagus nerve forms specialized sensory endings on the heart and blood vessels to detect the cardiovascular milieu, including atrial and ventricular volume/pressure, cardiac contractility, and blood pressure as well as pathological states such as ischemic and inflammation [10,43,44]. Activation of vagal afferents triggers physiological reflexes to maintain cardiovascular homeostasis. Several cardiovascular reflexes mediated by vagal afferents have been described in humans and other species. Sensation of blood pressure changes and oxygen/$CO_2$ levels in the aorta via baroreceptor and chemoreceptor vagal neurons, respectively, regulate heart rate, blood pressure, and respiratory patterns in a similar manner to their carotid counterparts. The Bainbridge reflex, a tachycardia response to an increase of blood volume, is a physiological reflex initiated by stretch or distension detected by vagal afferent endings in the atria [45]. Like the baroreflex, the Bainbridge reflex is bi-directional such that a decrease in venous return (e.g., hemorrhage, hypotension) leads to heart rate slowing. The Bezold-Jarisch reflex is an inhibitory cardiac reflex attributed to activation of vagal afferent endings in the ventricles, resulting in severe bradycardia and hypotension [46,47]. Extensive evidence has shown that the Bezold-Jarisch reflex may play a role in blood pressure regulation [48–50], hypovolemia [51,52], and myocardial ischemia and reperfusion [53–57]. It is generally believed that the Bezold-Jarisch reflex may be triggered after myocardial infarction (MI) or by a sudden rise in cardiac vagal tone such as in vaso-vagal syncope to protect the heart from ischemic damage and exhaustion, yet the precise endogenous signals sensed by these ventricular afferents remain to be determined.

A variety of cardiovascular vagal afferents with distinct morphological and functional properties have been identified using conventional approaches. DiI-based anterograde tracing studies have shown flower-spray and end-net endings in the adventitia of the aorta [58]. Similar sensory ending structures have also been observed in the atria [59]. Electrophysiological properties of these afferents have been well summarized [43]. Multiple aortic afferent types have been reported with diverse sensory modalities, conduction velocities, activation thresholds, and response patterns. At least two specialized atrial vagal receptors have been described, both of which are fast-conducting A-fibers but may sense atrial filling and contractility differentially. Most ventricular vagal afferents are capsaicin-sensitive C-fibers that are also sensitive to alkaloids, bradykinin, and prostaglandins. These early studies clearly demonstrate the heterogeneity of cardiovascular vagal afferents; however, linking terminal morphologies, electrophysiological properties, molecular identities, and physiological roles has been challenging.

Recent advances in mouse genetics are rapidly improving our understanding of vascular vagal afferents. Many molecules including *Asic2*, *Trpc5*, and *Tmem150c* have been proposed to be critically involved in the baroreflex [60–62]. Among these candidates, the mechanosensitive ion channels Piezo1 and Piezo2 fulfill most criteria of being the primary baroreceptors that directly sense blood pressure changes [63]. Knocking out both Piezo1 and Piezo2 from vagal afferent neurons in Phox2b-Cre mice resulted in an almost complete loss of the baroreflex. The same phenotype was observed in mice in which Piezo2-positive vagal afferent neurons were ablated [64]. Meanwhile, optogenetic activation of Piezo2-positive vagal afferent neurons resulted in profound bradycardia, consistent with its role in the baroreflex [63]. Anatomically, Piezo2-positive vagal afferents form macroscopic claws around the aorta, which are decorated with end-net terminals [64]. Surprisingly, flower-spray endings are not involved in aortic blood pressure sensing as ablating these endings did not impact the baroreflex. While aortic blood pressure is sensed via end-net terminals, it is unclear whether vagal aortic afferents with different pressure sensitivities have similar or different genetic identities.

Much less in know about the genetic identities of cardiac vagal afferents. Recent single-cell studies have generated a molecular atlas for vagal sensory neurons and provided genetic roadmaps for the those innervating a number of visceral organs including the heart [65–68]. Several types of cardiac vagal afferents have been revealed, with their sensory ending structures characterized [68]. Similar ending structures were also observed using a transgenic approach [69]. Intriguingly, cardiac and aortic flower-spray endings seem to share similar molecular markers such as *Npy2r* and *Agtr1a*, suggesting that they may sense similar cues from different locations. However, the coding logic and the underlying sensory mechanisms for different cardiac signals remain to be elucidated.

## 5. Cardiac interoception and the vagus nerve

Interoception refers to the process by which the nervous system senses, interprets, and integrates signals originating from within the body, providing a moment-by-moment mapping of the body's internal landscape across conscious and unconscious levels [70]. Cardiac interoception thus relates to the process by which the nervous system senses, interprets, and integrates signals from the cardiovascular system. Mechanoreceptors and chemoreceptors are the primary sensors involved in cardiac interoception, which are responsive to stimulation by catecholamines (e.g., epinephrine, norepinephrine, dopamine), peptides (e.g., bradykinin, natriuretic peptides, neuropeptide Y), and by muscarinic, adenosinergic, or angtiotensinergic modulation (for a detailed review see [71]). Some of these act as sensory transducers (such as arterial baroreceptors), whereas others perform motoric functions as a part of regulatory responses (such as muscarinic and adrenergic receptors). The discovery of a new class of mechanically activated ion channels called PIEZOs [72] has resulted in the additional observation that these are key mechanosensors capable of mediating the baroreceptor reflex [63,64]. The signals relevant to cardiac interoception thus cover the full spectrum of cardiovascular function. They are predominantly transmitted throughout the nervous system via neural and humoral pathways via arterial baroreceptor reflex pathways [73,74], chemoreceptor pathways [75], the renin–angiotensin–aldosterone system [76], the ANS [77], and the ICNS [23,78]. These pathways ultimately provide afferent and efferent connections to the CNS at nearly every level of the neuraxis [79,80]. The consequence of this massive interconnectedness is that interoceptive brain regions play primary roles in continuously monitoring the autonomic, chemosensory, endocrine, and immune systems, which continuously relay information through peripheral nerves and direct neurochemical interfaces to the brainstem, hypothalamus, thalamus, and ultimately into cortical sectors including principally the insular and somatosensory cortices [81,82]. As a result, the neural circuits of interoception can process the current and future status of the body, at both conscious and unconscious levels, which allows for the development of inferential and predictive models of anticipated future body states, and the deployment of regulatory actions aimed at maintaining homeostasis [83,84].

With respect to human CNS control of cardiovagal input, we have integrated recent functional neuroanatomical findings and computational neuroscience models to propose that it is organized hierarchically within the nervous system [85]. According to this 'neurovisceral integration model', lower levels of networked regions (e.g., ICNS, NA, DMN, periaqueductal grey, thalamic and hypothalamic nuclei) primarily integrate afferent information from the body to regulate energy expenditure in response to current metabolic needs, whereas higher levels of networked regions (e.g. the amygdala, insular, anterior and posterior cingulate, parietal, ventromedial and dorsolateral prefrontal cortices) reciprocally process inputs from the lower networks and generate unconscious and conscious representations of cardiovascular states resulting in the perception of current somatic/visceral states and deployment of relevant regulatory actions focused on amplifying, maintaining, or suppressing representations. Information flows continuously and dynamically between these levels, with different weighting assigned to each level based on the relevance of the ongoing state of the organism. The consequence of this organization is such that some states will engage only lower level networks and not involve conscious processing (e.g., cardiocentric processing at the level of intra-thoracic or dorsal root ganglia in response to local changes in ventricular filling [86]) whereas others will engage higher level networks and involve conscious processing (e.g., cortical processing at the level of the insular cortices during an anxiety provoking adrenergic stressor [87]). While considerably broad in scope, the hierarchical neurovisceral model provides a comprehensive integrated perspective on cardiovagal function supporting delineations of peripheral versus central interoceptive dysfunction.

Recent animal and human studies are illuminating some of the relevant properties underlying cardiac interoception in states of health and disease. During a reinforced eye-tracking task, Rhesus monkeys gazed longer at audiovisual stimuli that were presented asynchronously versus synchronous to their heartbeat sensations, suggesting that they were able to make use of a rudimentary form of heartbeat sensation [88]. This approach parallels a similar study in human infants [89], raising the possibility that, in mammals, the capacity to integrate heartbeat signals with behavior emerges to language acquisition. A landmark study of fear processing in mice found that, optogenetic inhibition of the insular cortex extinguishes fear learning, consistent with a state-dependent regulation of fear [90]. Insular cortex responses to fear-evoking cues, which tracked the likelihood of harmful outcomes, were also reduced in concert with heart rate decelerations occurring during freezing responses, suggesting a possible role for vagal input in fear modulation. This was confirmed through left cervical VNS, which disrupted state-dependent fear processing in high fear versus low fear expressing mice in a manner similar to insular cortex inhibition. Women with generalized anxiety disorder showed hypersensitivity to peripheral adrenergic modulations of cardiovascular tone that were associated with heightened anxiety, cardiorespiratory sensations and increased insular cortex activity, but paradoxically, a blunting of ventromedial prefrontal cortex activity relative to healthy comparisons [91], providing evidence of both peripheral autonomic and CNS contributions to the pathophysiology of fear and anxiety in humans.

There is substantial interest in utilizing neuromodulation via VNS as a tool for generating improvements in a range of physical and mental health conditions including cardiac arrhythmias [92] and depression [93]. Preclinical studies have identified potential impacts of VNS on cardiac interoception, such as the observation that transcutaneous VNS increases the accuracy of heartbeat perception [94]. However, a major problem with transcutaneous VNS and intrathoracic VNS is the lack of specificity in terms of afferent and efferent signaling; this form of stimulation hits the entire branch of the vagus leading to downstream and upstream effects on multiple organ systems [7]. Auricular VNS is another form of stimulation which targets an accessory afferent branch innervating the external ear, and thus putatively results in a 'purely afferent' form of peripheral modulation. However, meta-analytic data suggest that auricular VNS does not appear to modulate the high-frequency component of heart rate variability, the primary indirect marker of cardiac activity that is commonly thought to be vagally mediated [95]. Transcutaneous stimulation of the auricular branch of the vagus nerve has been reported to modulate the heartbeat evoked potential [96], an electrophysiological signal that is presumed to be an afferent brain indicator representing the heartbeat sensation and associated mental processes [97], perhaps increasing the possibility that the effects of this form of stimulation are localized within afferent central autonomic networks. Respiratory-gated auricular vagal afferent nerve stimulation (RAVANS) is an innovative approach to VNS attempting to augment the respiratory-induced modulation of cardiac vagal activity by providing stimulation during the exhalation phase [98]. A preliminary study suggested that RAVANS was capable of modulating the high-frequency component of heart rate variability in patients with hypertension [99]. In depressed individuals, RAVANS was associated with engagement of brainstem and higher-level networks in the neurovisceral hierarchy [100]. However, the underlying mechanisms and optimal forms of stimulation are unclear. It is also worth mentioning that, beyond VNS, there is current interest in the application of non-invasive approaches intended to modulate cardiovagal signaling, primarily in the form of voluntary paced breathing at a reduced respiratory rate of 6 breaths per minute (0.1 Hertz). When repetitiously practiced, this form of 'resonance breathing' is thought to entrain cardiac vagal and baroreflex responses resulting in improvements in vagally-mediated heart rate variability and baroreflex sensitivity [101, 102]. Pharmacologic blockade studies in humans suggest that it appears to be predominantly mediated by cholinergic (presumably vagal) input [103]. Overall, these studies highlight the primacy of cardiac sensing across mammalian evolution, identify some key CNS checkpoints relevant to the regulation of fear, and point towards the role of the vagus nerve as a prominent conduit facilitating cardiac interoception.

## 6. Parasympathetic dysfunction in cardiovascular disease

Autonomic imbalance, characterized by hyperactivity of the sympathetic nervous system and diminished activity of the PNS, is a hallmark of many cardiovascular disease states such as MI, arrhythmias, and heart failure (HF) [104]. Eckberg and colleagues showed a profound abnormality of the PNS in patients with heart disease [105]. Atropine was used to block the PNS after blockade of the sympathetic nervous system with propranolol in normal patients and patients with heart disease. Atropine dramatically elevated heart rate in normal subjects compared to a more modest elevation in those with heart disease. In addition, the patients with HF had reduced heart rate slowing to elevations in arterial pressure produced by phenylephrine injection. Furthermore, resting heart rate is primarily governed by the PNS. Epidemiological data indicate that people with higher resting heart rate, or reduced parasympathetic tone, have worse cardiovascular outcomes including sudden cardiac death [106–108]. Taken together, these findings suggest a critical role for parasympathetic dysfunction in the pathophysiology of the disease state.

Multiple mechanisms, both central and peripheral, are responsible for altered parasympathetic regulation of cardiovascular function in the setting of heart disease. In the CNS, the activity of vagal motor neurons in the brainstem is attenuated following cardiac injury. *In vitro* patch clamp recordings from cardiac vagal motor neurons within the NA and DMN shows that they have blunted activity in rats with pressure overload-induced left ventricular hypertrophy [109]. Specifically, vagal motor neurons display diminished excitation due to an increase in GABAergic inhibitory transmission and a decrease in glutaminergic excitatory transmission. The principle sources of inhibitory inputs to vagal motor neurons is from the locus coeruleus and ventral respiratory group [110], and that of excitatory inputs is from the NTS and the paraventricular nucleus of the hypothalamus [111]. Moreover, increasing central parasympathetic outflow has been shown to have beneficial effects in HF. Oxytocin is a neuropeptide synthesized in the hypothalamus and released into the circulation from the posterior pituitary. While its classical effects relate to reproduction, childbirth, and social bonding [112], recent studies have identified a role for oxytocin in cardiovascular homeostasis via projections of oxytocin-producing neurons to cardiac vagal motor neurons [113,114]. Dyavanapalli et al. used a chemogenetic approach to study the effects of activation of oxytocin-producing neurons in the paraventricular nucleus of the hypothalamus in rats with left ventricular dysfunction [114]. Designer receptor exclusively activated by designer drugs (DREADD) is a chemogenetic tool that can be used to manipulate neuronal activity in a cell type-specific manner using synthetic ligands. In transgenic rats with Cre recombinase under the control of the OXT promoter, the investigators injected a Cre-dependent DREADD vector into the paraventricular nucleus. Left ventricular hypertrophy was then induced in the animals by transverse aortic constriction. Oxytocin-producing neurons in the paraventricular nucleus were selectively activated by intraperitoneally injecting the DEADD agonist clozapine-N-oxide, a DREADD agonist. Strikingly, they showed decreased inflammation and fibrosis, improved cardiac functional indices, and reduced mortality in rats with left ventricular hypertrophy compared to healthy animals.

Parasympathetic neurotransmission is altered in the peripheral nervous system and myocardium in cardiovascular disease. Under normal physiological conditions, complex interactions exist between the sympathetic and parasympathetic nervous system to regulate cardiovascular function. For instance, heart rate slowing to VNS is augmented by the presence of concomitant sympathetic nerve stimulation and heart rate rise to sympathetic nerve stimulation is blunted when on a background of VNS, a phenomenon termed as accentuated antagonism [39]. Further, VNS-induced heart rate response can be enhanced by exercise-induced sympathoexcitation in conscious dogs, indicating the physiological importance of this interaction [115]. Sympatho-vagal balance is mediated by pre- and post-junctional interactions in the myocardium [36]. In humans with HF, muscarinic receptor stimulation with intracoronary acetylcholine injection led to a reduction of norepinephrine release from sympathetic nerve endings, while there was no effect in patients with normal left ventricular function. Conversely, muscarinic blockade with intracoronary atropine injection had no effect in patients with HF but led to a significant increase in norepinephrine release in those with normal left ventricular function. These data indicate disrupted sympatho-vagal balance in heart disease. In a canine model of pacing-induced HF, there is diminished parasympathetic regulation of the SA nodal function [116]. Cervical VNS led to an attenuated heart rate slowing in animals with HF compared to healthy controls, whereas there was no difference in the heart rate response to stimulation of the right atrial GP, which exerts direct control over the SA node. These findings suggest interrupted neuronal transmission between parasympathetic preganglionic neurons in the brainstem and postganglionic neurons in the GP. In addition, in a porcine MI model, there was no difference in the electrophysiological response to VNS and acetylcholine levels in the infarct scar compared to a similar region compared to healthy animals [117]. Further, muscarinic receptors are upregulated, and acetylcholinesterase activity is reduced within the SA node of canines with pacing-induced HF [118]. Taken together, these data indicate diminished central parasympathetic signaling in heart disease and a potential for beneficial effects to restoring vagal tone.

Activation of cardiac afferent neurons plays an important role in pathogenesis of heart disease. During ischemia, numerous metabolites, including adenosine, bradykinin, and prostaglandins, are released locally in the myocardium, activate afferent neurons, and result in reflexive sympathetic activation [119,120]. Transient receptor potential vanilloid 1 (TRPV1)-expressing afferent neurons are responsible in part for detecting these ischemic metabolites and triggering acute and chronic sympathoexcitation, which contribute to adverse cardiac remodeling [121]. Resiniferatoxin is an ultrapotent agonist of the TRPV1 receptor and can cause degeneration of TRPV1-expressing nerve fibers or neuronal death. In both a rat and porcine model of MI, depletion of cardiac TRPV1-expressing nerve fibers by epicardial application of TRPV1 has been shown to reduce fibrosis, improve cardiac contractile function, and prevent hyperactivity of the sympathetic nervous system [121,122]. While the role of afferent neural signaling in mediating sympathoexcitation following cardiac injury is better characterized, its role in parasympathetic withdrawal is less well understood. Prostaglandin synthesis during myocardial ischemia and oxygen free radical formation during reperfusion have been shown to activate chemosensitive vagal afferent in rats [56]. In a canine model of pacing-induced HF, left atrial mechanoreceptors were activated by inflating a balloon in the left atrium, while simultaneously recording renal sympathetic nerve activity [123]. Animals with HF had an attenuated reduction in renal sympathetic nerve activity with activation of cardiac mechanoreceptors compared to healthy animals. There was no change in renal nerve activity following bilateral vagotomy. These findings suggest altered function of cardiac vagal mechanoreceptors in HF. Some vagally-mediated reflexes are enhanced in HF. Cardiac chemosensitive vagal afferent neurons sensitive to bradykinin had a greater response to exogenous bradykinin administration in dogs with pacing-induced HF compared to sham animals [124]. Afferent inputs to intrinsic cardiac neurons are also altered following MI. In a porcine MI model, in vivo recording of neuronal activity from a GP at the base of the left ventricle shows that intrinsic cardiac neurons have a differential response to activation of mechanoreceptors in the infarct scar versus border and remote regions of the heart. With recent advances in characterizing the molecular and genetic diversity of vagal afferents involved in cardiovascular homeostasis, future studies aimed at understanding how specific subtypes of vagal afferents are involved in the pathophysiology of heart disease will be crucial to understanding disease biology and developing novel therapeutics.

## 7. Targeting the parasympathetic nervous system for the treatment of cardiovascular disease

A growing body of pre-clinical and clinical evidence support a potential therapeutic role for VNS in cardiovascular disease, particularly HF (Fig. 1). In a guinea pig model of pressure overload, chronic VNS mitigated disease-associated reductions in ejection fraction and cardiac output and resulted in favorable remodeling of intrinsic cardiac neurons [125]. Similarly, in a canine model of pacing-induced cardiomyopathy, preemptive chronic VNS mitigated the development of HF [126]. Chronic VNS, initiated two weeks following left anterior descending coronary artery ligation in rats, improved left ventricular systolic function, reduced circulating catecholamine and brain natriuretic peptide levels, and improved survival, compared to rats receiving sham VNS [127]. Although the mechanisms by which VNS exerts its effects are not well defined, VNS has been shown to reduce myocardial inflammation following injury, reduce apoptosis, and favorably shift myocardial metabolism [125,128]. Moreover, VNS may exert anti-adrenergic effects on the heart itself both through peripheral processing within the ICNS as well as the nerve-myocyte interface [39,129]. VNS was first clinically evaluated in a series of 8 patients with heart failure with reduced fraction (HFrEF) and New York Heart Association (NYHA) class II-III symptoms in 2008 [130]. In this cohort, patients exhibited a significant increase in quality of life at 6 months and improvement in NYHA class. These findings were replicated by the same investigators in a larger, multicenter study of 32 patients, with improvement in NYHA class, quality of life, and left ventricular ejection fraction at 1 year [131].

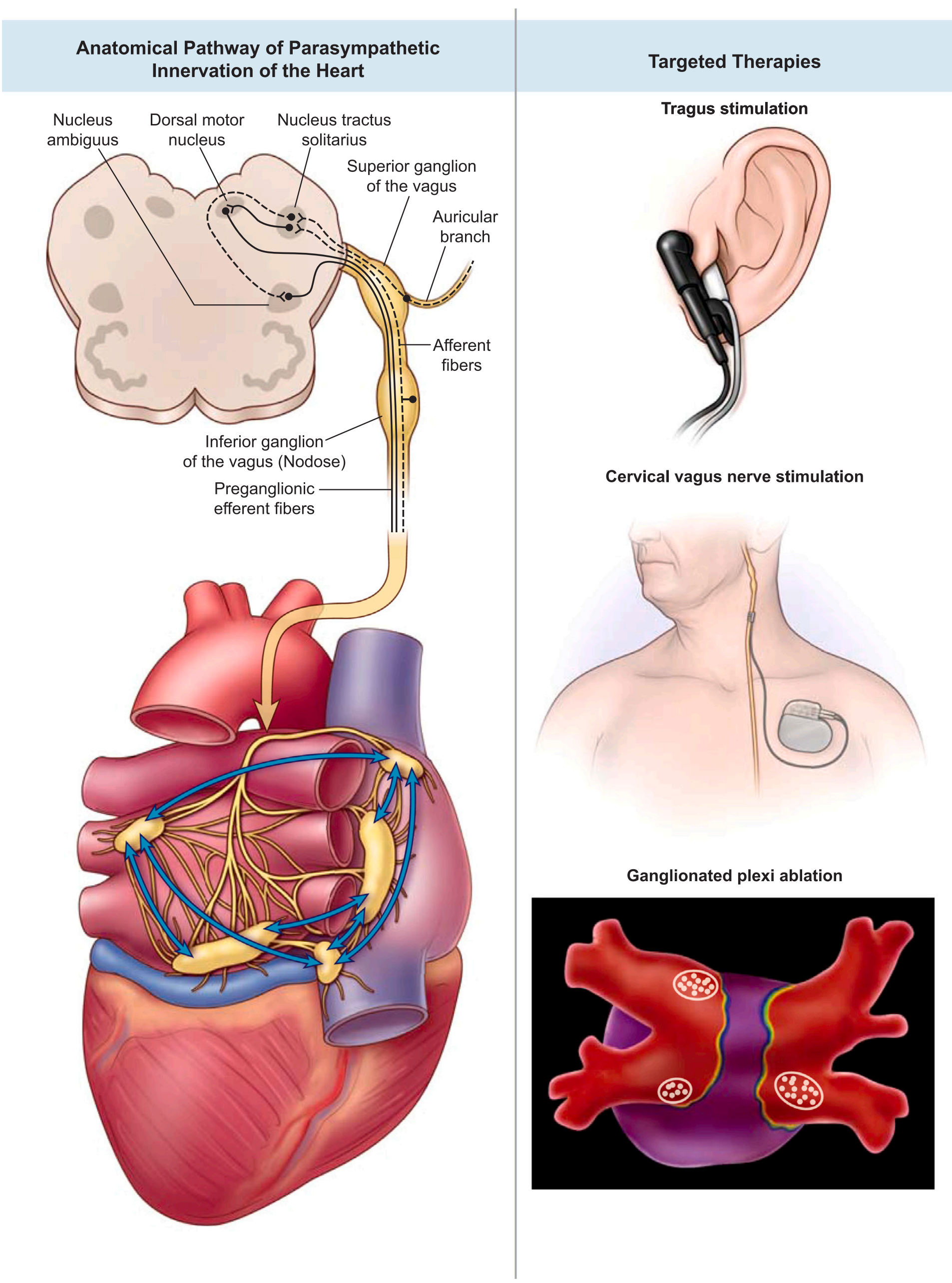


**Fig. 1.** Parasympathetic innervation of the heart and therapies targeting the parasympathetic nervous system for treatment of cardiovascular diseases. (Left) Parasympathetic preganglionic neurons innervating the heart originate in the medulla of the brainstem (top) and project via the vagus nerve to ganglionated plexi of the intrinsic cardiac nervous system (bottom). Postganglionic neurons in ganglionated plexi then project to atrial and ventricular myocardial tissue, the conduction system, and the vasculature. Vagal afferent neurons have cell bodies in the superior and inferior ganglia of the vagus and project to the medulla. (Right) The parasympathetic nervous system is being targeted at level of the auricular branch of the vagus nerve, which innervates the tragus of the external ear (top), the cervical vagus nerve (middle), and ganglionated plexi (bottom) for a variety of cardiovascular disease states including heart failure and arrhythmias.

Given promising pilot studies and robust pre-clinical data, three major trials have evaluated chronic VNS for HFrEF with mixed results, partly attributed to differences in study design and delivery of VNS therapy. Neural Cardiac Therapy for Heart Failure Study (NECTAR-HF) randomized 96 patients with HFrEF receiving guideline directed medical therapy to right cervical VNS or sham VNS [132,133]. At 6 months following randomization, no significant differences in left ventricular systolic function, left ventricular size, or pre-specified biomarkers were present between the two study groups, though improvements in quality of life and NYHA class were evident in those receiving VNS [132,134]. A retrospective analysis by the study investigators found that less than 15% of participants had evoked heart rate responses from VNS on Holter monitoring, suggesting ineffective activation of cardiac vagal efferent fibers [134]. The Increase of Vagal Tone in Heart Failure (INOVATE-HF) trial randomized approximately 700 patients with symptomatic (NYHA class III) HFrEF (EF $< 40\%$) to right VNS and guideline directed medical therapy versus medical therapy alone [135,136]. Although quality of life and NYHA class improved in those receiving VNS, there was no significant difference in all-cause mortality or unplanned HF rehospitalizations between the two groups [135]. In contrast to these two trials, the Autonomic Neural Therapy to Enhance Myocardial Function in Heart Failure (ANTHEM-HF) initial and extended pilot studies found an improvement in left ventricular ejection fraction and quality of life among patients with NYHA class II-III HFrEF at 6 and 12 month follow up [137,138]. Although this study was non-placebo controlled, VNS parameters were uniquely titrated for each patient based on heart rate dynamics, such that VNS evoked a 4–6 beat per minute bradycardia, supported by preclinical data. A follow-up randomized controlled trial by the ANTHEM group is underway, utilizing similar stimulation parameters and study cohort as the pilot study [139]. As such, chronic VNS remains an attractive therapeutic target for HF.

In addition to the putative cardioprotective effects of VNS on structural remodeling in the setting of HF, pre-clinical studies suggest acute VNS may exert an anti-arrhythmic effect for ventricular arrhythmias. In canine models, acute VNS reduces the probability of ventricular tachycardia or ventricular fibrillation during coronary artery occlusion and reperfusion, and increases ventricular fibrillation threshold [140]. In rats, acute VNS applied 30 min prior to coronary artery ligation reduced the incidence of ventricular tachycardia and stabilized gap junctions by preserving phosphorylated Connexin-43 [141]. In a canine model of chronic ischemia induced by left anterior descending coronary artery ligation, low-level VNS, initiated 2 h after ligation, reduced the incidence of ventricular arrythmias and reduced MI-associated sympathetic nerve sprouting [142]. Limited clinical data regarding direct electrical VNS for ventricular arrythmias exists, though this represents an area of intense study.

While VNS activates parasympathetic afferent and efferent fibers through direct electrical stimulation of the cervical vagus nerve, transcutaneous stimulation of the tragus has emerged as an alternative strategy (Fig. 1). The tragus is a small projection of the external ear and is innervated by the auricular branch of the vagus nerve, which is comprised of predominantly afferent fibers. It can be transcutaneously stimulated without the need for invasive surgery or device implantation. Chronic low-level intermittent tragus stimulation has been shown to reduce left ventricular remodeling and the inducibility of ventricular arrythmias in canine after MI [143,144]. Similarly, in Dahl salt sensitive rats, chronic intermittent low-level tragus stimulation reduces diastolic dysfunction, fibrosis, and inflammation induced by high salt diet [145, 146]. In healthy canine, tragus stimulation reduced the inducibility of AF by right atrial pacing [147,148]. Case series and a randomized clinical trial suggest that low-level tragus stimulation may reduce the burden of AF in selected patients with paroxysmal AF [149,150]. Limited clinical data exists regarding tragus stimulation in patients with MI or HF; however, one study reported that two hours of right low-level tragus stimulation reduced reperfusion-associated arrythmias and reduced regional left ventricular dyskinesia in those presenting with ST-segment elevation MI [151]. Given its safety profile and feasibility, further studies to evaluate tragus stimulation are necessary, particularly studies with larger, randomized cohorts and longitudinal follow up.

Modulation of the ICNS to treat AF has been an area of recent interest, as GPs regulate atrial electrical activation, conduction, and refractoriness (Fig. 1). For example, direct application of acetylcholine on the anterior right GP or inferior right GP in canines led to development of complex fractioned atrial electrograms and AF. Ablation of these GPs reduced fractioned electrograms and terminated episodes of AF [152]. Clinically, atrial GP can be localized by endocardial high frequency stimulation and the presence of an evoked vagal response, and subsequently ablated, although anatomic approaches are often used as well [153,154]. Among patients with paroxysmal AF, two randomized controlled trials have demonstrated that addition of left atrial GP ablation to conventional pulmonary vein isolation confers greater freedom from AF or atrial tachycardia [155,156]. A recent multi-center trial, GANGLIA-AF, randomized 102 patients with paroxysmal AF to pulmonary vein isolation or high-frequency stimulation guided left atrial GP ablation without pulmonary vein isolation, with a mean of 89 sites were tested high frequency ablation [157]. While there was no significant difference in atrial arrhythmias at 12-months, there was a greater reduction in antiarrhythmic drug dosages after GP ablation compared to pulmonary vein isolation. However, in the AF Ablation and Autonomic Modulation via Thorascopic Surgery (AFACT) randomized controlled trial, GP ablation did not reduce the recurrence of AF at 2 years and was associated with greater rates of pacemaker implantation [158,159]. These mixed results are likely attributed to selection criteria, ablation techniques, methods for localization of GPs (functional versus anatomic), underscoring the value of further study of the ICNS.

In addition to AF, GP ablation has recently been evaluated for neurally-mediated syncope and symptomatic bradycardia given the dense parasympathetic innervation of the atria and the role of enhanced vagal reflexes in these entities. Pachon and colleagues reported on 43 patients with neurally-mediated vasovagal syncope and found that GP ablation, guided by spectral mapping, markedly reduced episodes of spontaneous syncope, with syncope only recurring in 3 cases [160]. Consistent with this study, Sun et al. found excellent freedom from syncope in 57 patients with refractory vasovagal syncope undergoing either high-frequency stimulation guided left atrial GP ablation (100%) or with an anatomic approach (89.4%) at a mean follow up of 36 months [161]. While these early series are promising, further study of GP ablation in randomized cohorts and with comparison to standard of care therapy are necessary, as well as long term follow up to ensure lack of adverse effects from parasympathetic denervation.

## 8. Conclusions

The vagus nerve plays an essential role in communication between the heart and brain to maintain cardiovascular homeostasis. Recent studies have started to illuminate the molecular, cellular, and functional identify of vagal efferent and afferent neurons involved in cardiovascular physiology. Future advances in characterizing the diversity of vagal neurons will be crucial to not only unraveling mechanisms underlying pathophysiological states but also developing targeted neuromodulation therapies, which have already shown great promise.

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## Disclosures

Pradeep Rajendran and Kalyanam Shivkumar are co-founders of NeuCures, Inc. UCLA has patents developed by Kalyanam Shivkumar relating to cardiac neural diagnostics and therapeutics.

## Conflict of Interest

Pradeep Rajendran and Kalyanam Shivkumar are co-founders of NeuCures, Inc. University of California, Los Angeles has patents developed by Kalyanam Shivkumar relating to cardiac neural diagnostics and therapeutics.

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