Title

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

Authors

Rajendran, Pradeep
Hadaya, Joseph
Khalsa, Sahib
et al.

Publication Date

2024-03-15

DOI

10.1016/j.semcdb.2023.01.001

Peer reviewed

HHS Public Access

Author manuscript

Semin Cell Dev Biol. Author manuscript; available in PMC 2025 March 15.

Published in final edited form as:

Semin Cell Dev Biol. 2024 March 15; 156: 190–200. doi:10.1016/j.semcdb.2023.01.001.

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

Pradeep S. Rajendran[1,*], **Joseph Hadaya**[2,3], **Sahib S. Khalsa**[4,5], **Chuyue Yu**[6,7], **Rui Chang**[6,7], **Kalyanam Shivkumar**[2,3,*]

[1]Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA.

[2]University of California, Los Angeles (UCLA) Cardiac Arrhythmia Center and Neurocardiology Research Program of Excellence, David Geffen School of Medicine at UCLA, Los Angeles, California, USA.

[3]UCLA Molecular, Cellular, and Integrative Physiology Program, Los Angeles, California, USA.

[4]Laureate Institute for Brain Research, Tulsa, Oklahoma, USA.

[5]Oxley College of Health Sciences, University of Tulsa, Tulsa, Oklahoma, USA.

[6]Department of Neuroscience, Yale University School of Medicine, New Haven, Connecticut, USA.

[7]Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut, USA.

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. Since the seminal work of the Weber brothers' demonstrating that stimulation of the vagus nerve in a frog leads to slowing of heart rate, 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 parasympathetic nervous system in cardiovascular disease.

Address correspondence to: Pradeep Rajendran, MD, PhD, Massachusetts General Hospital, Department of Medicine, 55 Fruit Street Gray 730, Boston, Massachusetts 02142, USA, Phone: 630-306-1864, prajendran@partners.org; Kalyanam Shivkumar, MD, PhD, UCLA Cardiac Arrhythmia Center, UCLA Neurocardiology Research Program, David Geffen School of Medicine at UCLA, 100 Medical Plaza Suite 660, Los Angeles, California 90095, USA, Phone: 310-206-6433, kshivkumar@mednet.ucla.edu. *Co-corresponding authors.

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

Keywords

vagus nerve; cardiovascular physiology; cardiovascular disease; interoception; neuromodulation

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.

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 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 (ACV) and ambiguus cardiopulmonary (ACP) neurons. Utilizing Cre-dependent adeno-associated virus (AAV) labeling and optogenetic studies in transgenic mice, they showed that ACV and ACP neurons have distinct targets of innervation and physiologic functions. ACV neurons project to cardiac ganglia and are responsible for slowing heart rate and atrioventricular (AV) conduction velocity. ACP 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.

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-Botzinger 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 (HRV), which is widely considered to be an indirect measure of autonomic function.

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 AAV 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].

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 (BJR) 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 BJR 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 BJR 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.

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 HRV, 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 (HEP) [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 VNS (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 HRV 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.

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 (CNO), 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 (RTX) 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.

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 (Figure 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 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].

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 (EF < 35%) 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 (VT) or ventricular fibrillation (VF) during coronary artery occlusion and reperfusion, and increases VF threshold [140]. In rats, acute VNS applied 30 minutes prior to coronary artery ligation reduced the incidence of VT and stabilized gap junctions by preserving phosphorylated Connexin-43 [141]. In a canine model of chronic ischemia induced by LAD ligation, low-level VNS, initiated 2 hours after LAD 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 (Figure 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 (Figure 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.

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.

Sources of funding

The funding for this work was provided by the National Institutes of Health (NIH) through the Common Fund’s Stimulating Peripheral Activity to Relieve Conditions (SPARC) program Grants OT2 OD023848 and OT2 OD028201 and National Heart, Lung, and Blood Institute (NHLBI) Grant U01 EB025138 to Kalyanam Shivkumar.

References:

[1]. Weber E, Muskelbewegung, in: Wagner R (Ed.), Handworterbuch der Physiologie, Friedrich Vieweg, Braunschweig, 1846, pp. 1–122.

[2]. Standish A, Enquist LW, Schwaber JS, Innervation of the heart and its central medullary origin defined by viral tracing, Science 263(5144) (1994) 232–4. [PubMed: 8284675]

[3]. Standish A, Enquist LW, Escardo JA, Schwaber JS, Central neuronal circuit innervating the rat heart defined by transneuronal transport of pseudorabies virus, J Neurosci 15(3 Pt 1) (1995) 1998–2012. [PubMed: 7891147]

[4]. McAllen RM, Spyer KM, The location of cardiac vagal preganglionic motoneurones in the medulla of the cat, J Physiol 258(1) (1976) 187–204. [PubMed: 940054]

[5]. Geis GS, Wurster RD, Cardiac responses during stimulation of the dorsal motor nucleus and nucleus ambiguus in the cat, Circ Res 46(5) (1980) 606–11. [PubMed: 7363410]

[6]. Veerakumar A, Yung AR, Liu Y, Krasnow MA, Molecularly defined circuits for cardiovascular and cardiopulmonary control, Nature 606(7915) (2022) 739–746. [PubMed: 35650438]

[7]. Karemaker JM, The multibranched nerve: vagal function beyond heart rate variability, Biol Psychol 172 (2022) 108378. [PubMed: 35688294]

[8]. Higashiyama H, Hirasawa T, Oisi Y, Sugahara F, Hyodo S, Kanai Y, Kuratani S, On the vagal cardiac nerves, with special reference to the early evolution of the head-trunk interface, J Morphol 277(9) (2016) 1146–58. [PubMed: 27216138]

[9]. Taylor EW, Wang T, Leite CAC, An overview of the phylogeny of cardiorespiratory control in vertebrates with some reflections on the 'Polyvagal Theory', Biol Psychol 172 (2022) 108382. [PubMed: 35777519]

[10]. Prescott SL, Liberies SD, Internal senses of the vagus nerve, Neuron 110(4) (2022) 579–599. [PubMed: 35051375]

[11]. Monteiro DA, Taylor EW, Sartori MR, Cruz AL, Rantin FT, Leite CAC, Cardiorespiratory interactions previously identified as mammalian are present in the primitive lungfish, Sci Adv 4(2) (2018) eaaq0800. [PubMed: 29507882]

[12]. Taylor EW, Jordan D, Coote JH, Central control of the cardiovascular and respiratory systems and their interactions in vertebrates, Physiol Rev 79(3) (1999) 855–916. [PubMed: 10390519]

[13]. Spyer KM, Annual review prize lecture. Central nervous mechanisms contributing to cardiovascular control, J Physiol 474(1) (1994) 1–19. [PubMed: 8014887]

[14]. Panneton WM, Gan Q, The Mammalian Diving Response: Inroads to Its Neural Control, Frontiers in neuroscience 14 (2020) 524. [PubMed: 32581683]

[15]. Foley JO, Dubois FS, Quantitative studies of the vagus nerve in the cat, J Comp Neurol 67 (1937) 49–67.

[16]. Arora RC, Waldmann M, Hopkins DA, Armour JA, Porcine intrinsic cardiac ganglia, Anat Rec A Discov Mol Cell Evol Biol 271(1) (2003) 249–58. [PubMed: 12552641]

[17]. Yuan BX, Ardell JL, Hopkins DA, Losier AM, Armour JA, Gross and microscopic anatomy of the canine intrinsic cardiac nervous system, Anat Rec 239(1) (1994) 75–87. [PubMed: 8037379]

[18]. Armour JA, Potential clinical relevance of the 'little brain' on the mammalian heart, Exp Physiol 93(2) (2008) 165–76. [PubMed: 17981929]

[19]. Hoover DB, Shepherd AV, Southerland EM, Armour JA, Ardell JL, Neurochemical diversity of afferent neurons that transduce sensory signals from dog ventricular myocardium, Auton Neurosci 141(1-2) (2008) 38–45. [PubMed: 18558516]

[20]. Hoover DB, Isaacs ER, Jacques F, Hoard JL, Page P, Armour JA, Localization of multiple neurotransmitters in surgically derived specimens of human atrial ganglia, Neuroscience 164(3) (2009) 1170–9. [PubMed: 19747529]

[21]. Hoover DB, Ganote CE, Ferguson SM, Blakely RD, Parsons RL, Localization of cholinergic innervation in guinea pig heart by immunohistochemistry for high-affinity choline transporters, Cardiovasc Res 62(1) (2004) 112–21. [PubMed: 15023558]

[22]. Beaumont E, Salavatian S, Southerland EM, Vinet A, Jacquemet V, Armour JA, Ardell JL, Network interactions within the canine intrinsic cardiac nervous system: implications for reflex control of regional cardiac function, J Physiol 591(18) (2013) 4515–33. [PubMed: 23818689]

[23]. Rajendran PS, Nakamura K, Ajijola OA, Vaseghi M, Armour JA, Ardell JL, Shivkumar K, Myocardial infarction induces structural and functional remodelling of the intrinsic cardiac nervous system, J Physiol 594(2) (2016) 321–41. [PubMed: 26572244]

[24]. Rajendran PS, Challis RC, Fowlkes CC, Hanna P, Tompkins JD, Jordan MC, Hiyari S, Gabris-Weber BA, Greenbaum A, Chan KY, Deverman BE, Munzberg H, Ardell JL, Salama G, Gradinaru V, Shivkumar K, Identification of peripheral neural circuits that regulate heart rate using optogenetic and viral vector strategies, Nat Commun 10(1) (2019) 1944. [PubMed: 31028266]

[25]. Lazzara R, Scherlag BJ, Robinson MJ, Samet P, Selective in situ parasympathetic control of the canine sinoatrial and atrioventricular nodes, Circ Res 32(3) (1973) 393–401. [PubMed: 4691345]

[26]. Ardell JL, Randall WC, Selective vagal innervation of sinoatrial and atrioventricular nodes in canine heart, Am J Physiol 251(4 Pt 2) (1986) H764–73. [PubMed: 3021001]

[27]. Carlson MD, Geha AS, Hsu J, Martin PJ, Levy MN, Jacobs G, Waldo AL, Selective stimulation of parasympathetic nerve fibers to the human sinoatrial node, Circulation 85(4) (1992) 1311–7. [PubMed: 1555275]

[28]. Hanna P, Dacey MJ, Brennan J, Moss A, Robbins S, Achanta S, Biscola NP, Swid MA, Rajendran PS, Mori S, Hadaya JE, Smith EH, Peirce SG, Chen J, Havton LA, Cheng ZJ, Vadigepalli R, Schwaber J, Lux RL, Efimov I, Tompkins JD, Hoover DB, Ardell JL, Shivkumar K, Innervation and Neuronal Control of the Mammalian Sinoatrial Node a Comprehensive Atlas, Circ Res 128(9) (2021) 1279–1296. [PubMed: 33629877]

[29]. Cardinal R, Page P, Vermeulen M, Ardell JL, Armour JA, Spatially divergent cardiac responses to nicotinic stimulation of ganglionated plexus neurons in the canine heart, Auton Neurosci 145(1-2) (2009) 55–62. [PubMed: 19071069]

[30]. Ulphani JS, Cain JH, Inderyas F, Gordon D, Gikas PV, Shade G, Mayor D, Arora R, Kadish AH, Goldberger JJ, Quantitative analysis of parasympathetic innervation of the porcine heart, Heart Rhythm 7(8) (2010) 1113–9. [PubMed: 20381645]

[31]. Kawano H, Okada R, Yano K, Histological study on the distribution of autonomic nerves in the human heart, Heart Vessels 18(1) (2003) 32–9. [PubMed: 12644879]

[32]. Zipes DP, Mihalick MJ, Robbins GT, Effects of selective vagal and stellate ganglion stimulation of atrial refractoriness, Cardiovasc Res 8(5) (1974) 647–55. [PubMed: 4434368]

[33]. Schauerte P, Scherlag BJ, Pitha J, Scherlag MA, Reynolds D, Lazzara R, Jackman WM, Catheter ablation of cardiac autonomic nerves for prevention of vagal atrial fibrillation, Circulation 102(22) (2000) 2774–80. [PubMed: 11094046]

[34]. Yamakawa K, So EL, Rajendran PS, Hoang JD, Makkar N, Mahajan A, Shivkumar K, Vaseghi M, Electrophysiological effects of right and left vagal nerve stimulation on the ventricular myocardium, Am J Physiol Heart Circ Physiol 307(5) (2014) H722–31. [PubMed: 25015962]

[35]. Lewis ME, Al-Khalidi AH, Bonser RS, Clutton-Brock T, Morton D, Paterson D, Townend JN, Coote JH, Vagus nerve stimulation decreases left ventricular contractility in vivo in the human and pig heart, J Physiol 534(Pt. 2) (2001) 547–52. [PubMed: 11454971]

[36]. Coote JH, Myths and realities of the cardiac vagus, J Physiol 591(17) (2013) 4073–85. [PubMed: 23878363]

[37]. Neurocardiology: Structure-Based Function, Comprehensive Physiology, pp. 1635–1653.

[38]. Gatti PJ, Johnson TA, McKenzie J, Lauenstein JM, Gray A, Massari VJ, Vagal control of left ventricular contractility is selectively mediated by a cranioventricular intracardiac ganglion in the cat, J Auton Nerv Syst 66(3) (1997) 138–44. [PubMed: 9406117]

[39]. Levy MN, Sympathetic-parasympathetic interactions in the heart, Circ Res 29(5) (1971) 437–45. [PubMed: 4330524]

[40]. Levy MN, Cardiac sympathetic-parasympathetic interactions, Fed Proc 43(11) (1984) 2598–602. [PubMed: 6745448]

[41]. Herring N, Lokale MN, Danson EJ, Heaton DA, Paterson DJ, Neuropeptide Y reduces acetylcholine release and vagal bradycardia via a Y2 receptor-mediated, protein kinase C-dependent pathway, J Mol Cell Cardiol 44(3) (2008) 477–85. [PubMed: 17996892]

[42]. Herring N, Cranley J, Lokale MN, Li D, Shanks J, Alston EN, Girard BM, Carter E, Parsons RL, Habecker BA, Paterson DJ, The cardiac sympathetic co-transmitter galanin reduces acetylcholine release and vagal bradycardia: implications for neural control of cardiac excitability, J Mol Cell Cardiol 52(3) (2012) 667–76. [PubMed: 22172449]

[43]. Paintal AS, Vagal sensory receptors and their reflex effects, Physiol Rev 53(1) (1973) 159–227. [PubMed: 4568412]

[44]. Hainsworth R, Reflexes from the heart, Physiol Rev 71(3) (1991) 617–58. [PubMed: 2057525]

[45]. Crystal GJ, Salem MR, The Bainbridge and the "reverse" Bainbridge reflexes: history, physiology, and clinical relevance, Anesth Analg 114(3) (2012) 520–32. [PubMed: 21965361]

[46]. Aviado DM, Guevara Aviado D, The Bezold-Jarisch reflex. A historical perspective of cardiopulmonary reflexes, Ann N Y Acad Sci 940 (2001) 48–58. [PubMed: 11458703]

[47]. Campagna JA, Carter C, Clinical relevance of the Bezold-Jarisch reflex, Anesthesiology 98(5) (2003) 1250–60. [PubMed: 12717149]

[48]. Mancia G, Shepherd JT, Donald DE, Interplay among carotid sinus, cardiopulmonary, and carotid body reflexes in dogs, Am J Physiol 230(1) (1976) 19–24. [PubMed: 1251904]

[49]. Donald DE, Shepherd JT, Reflexes from the heart and lungs: physiological curiosities or important regulatory mechanisms, Cardiovasc Res 12(8) (1978) 446–69. [PubMed: 363264]

[50]. Oberg B, Thoren P, Circulatory responses to stimulation of medullated and non-medullated afferents in the cardiac nerve in the cat, Acta Physiol Scand 87(1) (1973) 121–32. [PubMed: 4687335]

[51]. Thames MD, Jarecki M, Donald DE, Neural control of renin secretion in anesthetized dogs. Interaction of cardiopulmonary and carotid baroreceptors, Circ Res 42(2) (1978) 237–45. [PubMed: 620444]

[52]. Jarecki M, Thoren PN, Donald DE, Release of renin by the carotid baroreflex in anesthetized dogs. Role of cardiopulmonary vagal afferents and renal arterial pressure, Circ Res 42(5) (1978) 614–9. [PubMed: 639184]

[53]. Recordati G, Schwartz PJ, Pagani M, Malliani A, Brown AM, Activation of cardiac vagal receptors during myocardial ischemia, Experientia 27(12) (1971) 1423–4. [PubMed: 5144849]

[54]. Thames MD, Klopfenstein HS, Abboud FM, Mark AL, Walker JL, Preferential distribution of inhibitory cardiac receptors with vagal afferents to the inferoposterior wall of the left ventricle activated during coronary occlusion in the dog, Circ Res 43(4) (1978) 512–9. [PubMed: 688555]

[55]. Robertson D, Hollister AS, Forman MB, Robertson RM, Reflexes unique to myocardial ischemia and infarction, J Am Coll Cardiol 5(6 Suppl) (1985) 99B–104B.

[56]. Ustinova EE, Schultz HD, Activation of cardiac vagal afferents in ischemia and reperfusion. Prostaglandins versus oxygen-derived free radicals, Circ Res 74(5) (1994) 904–11. [PubMed: 8156637]

[57]. Koren G, Weiss AT, Ben-David Y, Hasin Y, Luria MH, Gotsman MS, Bradycardia and hypotension following reperfusion with streptokinase (Bezold-Jarisch reflex): a sign of coronary thrombolysis and myocardial salvage, Am Heart J 112(3) (1986) 468–71. [PubMed: 3751860]

[58]. Cheng Z, Powley TL, Schwaber JS, Doyle FJ 3rd, A laser confocal microscopic study of vagal afferent innervation of rat aortic arch: chemoreceptors as well as baroreceptors, J Auton Nerv Syst 67(1 - 2) (1997) 1–14. [PubMed: 9470139]

[59]. Cheng Z, Powley TL, Schwaber JS, Doyle FJ 3rd, Vagal afferent innervation of the atria of the rat heart reconstructed with confocal microscopy, J Comp Neurol 381(1) (1997) 1–17. [PubMed: 9087415]

[60]. Lu HJ, Nguyen TL, Hong GS, Pak S, Kim H, Kim H, Kim DY, Kim SY, Shen Y, Ryu PD, Lee MO, Oh U, Tentonin 3/TMEM150C senses blood pressure changes in the aortic arch, J Clin Invest 130(7) (2020) 3671–3683. [PubMed: 32484458]

[61]. Lu Y, Ma X, Sabharwal R, Snitsarev V, Morgan D, Rahmouni K, Drummond HA, Whiteis CA, Costa V, Price M, Benson C, Welsh MJ, Chapleau MW, Abboud FM, The ion channel ASIC2 is

required for baroreceptor and autonomic control of the circulation, Neuron 64(6) (2009) 885–97. [PubMed: 20064394]

[62]. Lau OC, Shen B, Wong CO, Tjong YW, Lo CY, Wang HC, Huang Y, Yung WH, Chen YC, Fung ML, Rudd JA, Yao X, TRPC5 channels participate in pressure-sensing in aortic baroreceptors, Nat Commun 7 (2016) 11947. [PubMed: 27411851]

[63]. Zeng WZ, Marshall KL, Min S, Daou I, Chapleau MW, Abboud FM, Liberles SD, Patapoutian A, PIEZOs mediate neuronal sensing of blood pressure and the baroreceptor reflex, Science 362(6413) (2018) 464–467. [PubMed: 30361375]

[64]. Min S, Chang RB, Prescott SL, Beeler B, Joshi NR, Strochlic DE, Liberles SD, Arterial Baroreceptors Sense Blood Pressure through Decorated Aortic Claws, Cell Rep 29(8) (2019) 2192–2201 e3. [PubMed: 31747594]

[65]. Kupari J, Haring M, Agirre E, Castelo-Branco G, Ernfors P, An Atlas of Vagal Sensory Neurons and Their Molecular Specialization, Cell Rep 27(8) (2019) 2508–2523 e4. [PubMed: 31116992]

[66]. Bai L, Mesgarzadeh S, Ramesh KS, Huey EL, Liu Y, Gray LA, Aitken TJ, Chen Y, Beutler LR, Ahn JS, Madisen L, Zeng H, Krasnow MA, Knight ZA, Genetic Identification of Vagal Sensory Neurons That Control Feeding, Cell 179(5) (2019) 1129–1143 e23. [PubMed: 31730854]

[67]. Prescott SL, Umans BD, Williams EK, Brust RD, Liberles SD, An Airway Protection Program Revealed by Sweeping Genetic Control of Vagal Afferents, Cell 181(3) (2020) 574–589 e14. [PubMed: 32259485]

[68]. Zhao Q, Yu CD, Wang R, Xu QJ, Dai Pra R, Zhang L, Chang RB, A multidimensional coding architecture of the vagal interoceptive system, Nature 603(7903) (2022) 878–884. [PubMed: 35296859]

[69]. Kazci YE, Sahoglu Goktas S, Aydin MS, Karadogan B, Nebol A, Turhan MU, Ozturk G, Cagavi E, Anatomical characterization of vagal nodose afferent innervation and ending morphologies at the murine heart using a transgenic approach, Auton Neurosci 242 (2022) 103019. [PubMed: 35905544]

[70]. Khalsa SS, Adolphs R, Cameron OG, Critchley HD, Davenport PW, Feinstein JS, Feusner JD, Garfinkel SN, Lane RD, Mehling WE, Meuret AE, Nemeroff CB, Oppenheimer S, Petzschner FH, Pollatos O, Rhudy JL, Schramm LP, Simmons WK, Stein MB, Stephan KE, Van den Bergh O, Van Diest I, von Leupoldt A, Paulus MP, p. Interoception Summit, Interoception and Mental Health: A Roadmap, Biological psychiatry : cognitive neuroscience and neuroimaging 3(6) (2018) 501–513. [PubMed: 29884281]

[71]. Habecker BA, Anderson ME, Birren SJ, Fukuda K, Herring N, Hoover DB, Kanazawa H, Paterson DJ, Ripplinger CM, Molecular and cellular neurocardiology: development, and cellular and molecular adaptations to heart disease, J Physiol 594(14) (2016) 3853–75. [PubMed: 27060296]

[72]. Coste B, Mathur J, Schmidt M, Earley TJ, Ranade S, Petrus MJ, Dubin AE, Patapoutian A, Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels, Science 330(6000) (2010) 55–60. [PubMed: 20813920]

[73]. Dworkin BR, Interoception, Handbook of psychophysiology (3rd ed.). Cambridge University Press, New York, NY2007, pp. 898–506.

[74]. Chapleau MW, Baroreceptor reflexes, in: Robertson D, Biaggioni I, Burnstock G, Low PA, Paton JF, (Ed.), Primer on the autonomic nervous system, 3rd edition, Elsevier, London, 2012.

[75]. Marshall JM, Peripheral chemoreceptors and cardiovascular regulation, Physiol Rev 74(3) (1994) 543–94. [PubMed: 8036247]

[76]. Re RN, Mechanisms of disease: local renin-angiotensin-aldosterone systems and the pathogenesis and treatment of cardiovascular disease, Nature clinical practice. Cardiovascular medicine 1(1) (2004) 42–7. [PubMed: 16265259]

[77]. Janig W, Neurobiology of visceral afferent neurons: neuroanatomy, functions, organ regulations and sensations, Biol Psychol 42(1-2) (1996) 29–51. [PubMed: 8770369]

[78]. Armour JA, Murphy DA, Yuan BX, Macdonald S, Hopkins DA, Gross and microscopic anatomy of the human intrinsic cardiac nervous system, Anat Rec 247(2) (1997) 289–98. [PubMed: 9026008]

[79]. Ardell JL, Andresen MC, Armour JA, Billman GE, Chen PS, Foreman RD, Herring N, O'Leary DS, Sabbah HN, Schultz HD, Sunagawa K, Zucker IH, Translational neurocardiology: preclinical models and cardioneural integrative aspects, J Physiol 594(14) (2016) 3877–909. [PubMed: 27098459]

[80]. Shivkumar K, Ajijola OA, Anand I, Armour JA, Chen PS, Esler M, De Ferrari G, Fishbein MC, Goldberger JJ, Harper RM, Joyner MJ, Khalsa SS, Kumar R, Lane R, Mahajan A, Po S, Schwartz PJ, Somers VK, Valderrabano M, Vaseghi M, Zipes DP, Clinical neurocardiology-defining the value of neuroscience-based cardiovascular therapeutics, J Physiol 594(14) (2016) 3911–54. [PubMed: 27114333]

[81]. Berntson GG, Khalsa SS, Neural Circuits of Interoception, Trends in neurosciences 44(1) (2021) 17–28. [PubMed: 33378653]

[82]. Carvalho GB, Damasio A, Interoception and the origin of feelings: A new synthesis, Bioessays 43(6) (2021) e2000261. [PubMed: 33763881]

[83]. Barrett LF, Simmons WK, Interoceptive predictions in the brain, Nat Rev Neurosci 16(7) (2015) 419–29. [PubMed: 26016744]

[84]. Seth AK, Suzuki K, Critchley HD, An interoceptive predictive coding model of conscious presence, Frontiers in psychology 2 (2011) 395. [PubMed: 22291673]

[85]. Smith R, Thayer JF, Khalsa SS, Lane RD, The hierarchical basis of neurovisceral integration, Neurosci Biobehav Rev 75 (2017) 274–296. [PubMed: 28188890]

[86]. Ditting T, Hilgers KF, Scrogin KE, Stetter A, Linz P, Veelken R, Mechanosensitive cardiac C-fiber response to changes in left ventricular filling, coronary perfusion pressure, hemorrhage, and volume expansion in rats, American journal of physiology. Heart and circulatory physiology 288(2) (2005) H541–52. [PubMed: 15471986]

[87]. Hassanpour MS, Simmons WK, Feinstein JS, Luo Q, Lapidus RC, Bodurka J, Paulus MP, Khalsa SS, The Insular Cortex Dynamically Maps Changes in Cardiorespiratory Interoception, Neuropsychopharmacology 43(2) (2018) 426–434. [PubMed: 28726799]

[88]. Charbonneau JA, Maister L, Tsakiris M, Bliss-Moreau E, Rhesus monkeys have an interoceptive sense of their beating hearts, Proc Natl Acad Sci U S A 119(16) (2022) e2119868119. [PubMed: 35412910]

[89]. Maister L, Tang T, Tsakiris M, Neurobehavioral evidence of interoceptive sensitivity in early infancy, eLife 6 (2017).

[90]. Klein AS, Dolensek N, Weiand C, Gogolla N, Fear balance is maintained by bodily feedback to the insular cortex in mice, Science 374(6570) (2021) 1010–1015. [PubMed: 34793231]

[91]. Teed AR, Feinstein JS, Puhl M, Lapidus RC, Upshaw V, Kuplicki RT, Bodurka J, Ajijola OA, Kaye WH, Thompson WK, Paulus MP, Khalsa SS, Association of Generalized Anxiety Disorder With Autonomic Hypersensitivity and Blunted Ventromedial Prefrontal Cortex Activity During Peripheral Adrenergic Stimulation: A Randomized Clinical Trial, JAMA Psychiatry (2022).

[92]. De Ferrari GM, Schwartz PJ, Vagus nerve stimulation: from pre-clinical to clinical application: challenges and future directions, Heart Fail Rev 16(2) (2011) 195–203. [PubMed: 21165697]

[93]. Daban C, Martinez-Aran A, Cruz N, Vieta E, Safety and efficacy of Vagus Nerve Stimulation in treatment-resistant depression. A systematic review, J Affect Disord 110(1-2) (2008) 1–15. [PubMed: 18374988]

[94]. Villani V, Tsakiris M, Azevedo RT, Transcutaneous vagus nerve stimulation improves interoceptive accuracy, Neuropsychologia 134 (2019) 107201. [PubMed: 31562863]

[95]. Wolf V, Kuhnel A, Teckentrup V, Koenig J, Kroemer NB, Does transcutaneous auricular vagus nerve stimulation affect vagally mediated heart rate variability? A living and interactive Bayesian meta-analysis, Psychophysiology (2021) e13933. [PubMed: 34473846]

[96]. Poppa T, Benschop L, Horczak P, Vanderhasselt MA, Carrette E, Bechara A, Baeken C, Vonck K, Auricular transcutaneous vagus nerve stimulation modulates the heart-evoked potential, Brain Stimul 15(1) (2022) 260–269. [PubMed: 34933143]

[97]. Park HD, Blanke O, Heartbeat-evoked cortical responses: Underlying mechanisms, functional roles, and methodological considerations, Neuroimage 197 (2019) 502–511. [PubMed: 31051293]

[98]. Sclocco R, Garcia RG, Gabriel A, Kettner NW, Napadow V, Barbieri R, Respiratory-gated Auricular Vagal Afferent Nerve Stimulation (RAVANS) effects on autonomic outflow in hypertension, Annu Int Conf IEEE Eng Med Biol Soc 2017 (2017) 3130–3133. [PubMed: 29060561]

[99]. Staley R, Garcia RG, Stowell J, Sclocco R, Fisher H, Napadow V, Goldstein JM, Barbieri R, Modulatory Effects of Respiratory-Gated Auricular Vagal Nerve Stimulation on Cardiovagal Activity in Hypertension(.), Annu Int Conf IEEE Eng Med Biol Soc 2020 (2020) 2581–2584. [PubMed: 33018534]

[100]. Garcia RG, Cohen JE, Stanford AD, Gabriel A, Stowell J, Aizley H, Barbieri R, Gitlin D, Napadow V, Goldstein JM, Respiratory-gated auricular vagal afferent nerve stimulation (RAVANS) modulates brain response to stress in major depression, J Psychiatr Res 142 (2021) 188–197. [PubMed: 34365067]

[101]. Sevoz-Couche C, Laborde S, Heart rate variability and slow-paced breathing:when coherence meets resonance, Neurosci Biobehav Rev 135 (2022) 104576. [PubMed: 35167847]

[102]. Bates ME, Price JL, Leganes-Fonteneau M, Muzumdar N, Piersol K, Frazier I, Buckman JF, The Process of Heart Rate Variability, Resonance at 0.1 hz, and the Three Baroreflex Loops: A Tribute to Evgeny Vaschillo, Appl Psychophysiol Biofeedback (2022).

[103]. Kromenacker BW, Sanova AA, Marcus FI, Allen JJB, Lane RD, Vagal Mediation of Low-Frequency Heart Rate Variability During Slow Yogic Breathing, Psychosom Med 80(6) (2018) 581–587. [PubMed: 29771730]

[104]. Florea VG, Cohn JN, The autonomic nervous system and heart failure, Circ Res 114(11) (2014) 1815–26. [PubMed: 24855204]

[105]. Eckberg DL, Drabinsky M, Braunwald E, Defective cardiac parasympathetic control in patients with heart disease, N Engl J Med 285(16) (1971) 877–83. [PubMed: 4398792]

[106]. Jouven X, Zureik M, Desnos M, Guerot C, Ducimetiere P, Resting heart rate as a predictive risk factor for sudden death in middle-aged men, Cardiovasc Res 50(2) (2001) 373–8. [PubMed: 11334841]

[107]. Soliman EZ, Elsalam MA, Li Y, The relationship between high resting heart rate and ventricular arrhythmogenesis in patients referred to ambulatory 24 h electrocardiographic recording, Europace 12(2) (2010) 261–5. [PubMed: 19887457]

[108]. Zhang D, Shen X, Qi X, Resting heart rate and all-cause and cardiovascular mortality in the general population: a meta-analysis, CMAJ 188(3) (2016) E53–E63. [PubMed: 26598376]

[109]. Cauley E, Wang X, Dyavanapalli J, Sun K, Garrott K, Kuzmiak-Glancy S, Kay MW, Mendelowitz D, Neurotransmission to parasympathetic cardiac vagal neurons in the brain stem is altered with left ventricular hypertrophy-induced heart failure, Am J Physiol Heart Circ Physiol 309(8) (2015) H1281–7. [PubMed: 26371169]

[110]. Neff RA, Wang J, Baxi S, Evans C, Mendelowitz D, Respiratory sinus arrhythmia: endogenous activation of nicotinic receptors mediates respiratory modulation of brainstem cardioinhibitory parasympathetic neurons, Circ Res 93(6) (2003) 565–72. [PubMed: 12907666]

[111]. Neff RA, Mihalevich M, Mendelowitz D, Stimulation of NTS activates NMDA and non-NMDA receptors in rat cardiac vagal neurons in the nucleus ambiguus, Brain Res 792(2) (1998) 277–82. [PubMed: 9593939]

[112]. Lee HJ, Macbeth AH, Pagani JH, Young WS 3rd, Oxytocin: the great facilitator of life, Prog Neurobiol 88(2) (2009) 127–51. [PubMed: 19482229]

[113]. Garrott K, Dyavanapalli J, Cauley E, Dwyer MK, Kuzmiak-Glancy S, Wang X, Mendelowitz D, Kay MW, Chronic activation of hypothalamic oxytocin neurons improves cardiac function during left ventricular hypertrophy-induced heart failure, Cardiovasc Res 113(11) (2017) 1318–1328. [PubMed: 28472396]

[114]. Dyavanapalli J, Rodriguez J, Rocha Dos Santos C, Escobar JB, Dwyer MK, Schloen J, Lee KM, Wolaver W, Wang X, Dergacheva O, Michelini LC, Schunke KJ, Spurney CF, Kay MW, Mendelowitz D, Activation of Oxytocin Neurons Improves Cardiac Function in a Pressure-Overload Model of Heart Failure, JACC Basic Transl Sci 5(5) (2020) 484–497. [PubMed: 32478209]

[115]. Stramba-Badiale M, Vanoli E, De Ferrari GM, Cerati D, Foreman RD, Schwartz PJ, Sympathetic-parasympathetic interaction and accentuated antagonism in conscious dogs, Am J Physiol 260(2 Pt 2) (1991) H335–40. [PubMed: 1996679]

[116]. Bibevski S, Dunlap ME, Ganglionic mechanisms contribute to diminished vagal control in heart failure, Circulation 99(22) (1999) 2958–63. [PubMed: 10359742]

[117]. Vaseghi M, Salavatian S, Rajendran PS, Yagishita D, Woodward WR, Hamon D, Yamakawa K, Irie T, Habecker BA, Shivkumar K, Parasympathetic dysfunction and antiarrhythmic effect of vagal nerve stimulation following myocardial infarction, JCI Insight 2(16) (2017).

[118]. Dunlap ME, Bibevski S, Rosenberry TL, Ernsberger P, Mechanisms of altered vagal control in heart failure: influence of muscarinic receptors and acetylcholinesterase activity, Am J Physiol Heart Circ Physiol 285(4) (2003) H1632–40. [PubMed: 12829433]

[119]. Noda K, Sasaguri M, Ideishi M, Ikeda M, Arakawa K, Role of locally formed angiotensin II and bradykinin in the reduction of myocardial infarct size in dogs, Cardiovasc Res 27(2) (1993) 334–40. [PubMed: 8472285]

[120]. Dorheim TA, Wang T, Mentzer RM Jr., Van Wylen DG, Interstitial purine metabolites during regional myocardial ischemia, J Surg Res 48(5) (1990) 491–7. [PubMed: 2352425]

[121]. Wang HJ, Wang W, Cornish KG, Rozanski GJ, Zucker IH, Cardiac sympathetic afferent denervation attenuates cardiac remodeling and improves cardiovascular dysfunction in rats with heart failure, Hypertension 64(4) (2014) 745–55. [PubMed: 24980663]

[122]. Yoshie K, Rajendran PS, Massoud L, Mistry J, Swid MA, Wu X, Sallam T, Zhang R, Goldhaber JI, Salavatian S, Ajijola OA, Cardiac TRPV1-afferent signaling promotes arrhythmogenic ventricular remodeling after myocardial infarction, JCI Insight (2019).

[123]. Dibner-Dunlap ME, Thames MD, Control of sympathetic nerve activity by vagal mechanoreflexes is blunted in heart failure, Circulation 86(6) (1992) 1929–34. [PubMed: 1451264]

[124]. Schultz HD, Wang W, Ustinova EE, Zucker IH, Enhanced responsiveness of cardiac vagal chemosensitive endings to bradykinin in heart failure, Am J Physiol 273(2 Pt 2) (1997) R637–45. [PubMed: 9277549]

[125]. Beaumont E, Wright GL, Southerland EM, Li Y, Chui R, KenKnight BH, Armour JA, Ardell JL, Vagus nerve stimulation mitigates intrinsic cardiac neuronal remodeling and cardiac hypertrophy induced by chronic pressure overload in guinea pig, Am J Physiol Heart Circ Physiol 310(10) (2016) H1349–59. [PubMed: 26993230]

[126]. Zhang Y, Popovic ZB, Bibevski S, Fakhry I, Sica DA, Van Wagoner DR, Mazgalev TN, Chronic vagus nerve stimulation improves autonomic control and attenuates systemic inflammation and heart failure progression in a canine high-rate pacing model, Circ Heart Fail 2(6) (2009) 692–9. [PubMed: 19919995]

[127]. Li M, Zheng C, Sato T, Kawada T, Sugimachi M, Sunagawa K, Vagal nerve stimulation markedly improves long-term survival after chronic heart failure in rats, Circulation 109(1) (2004) 120–4. [PubMed: 14662714]

[128]. Beaumont E, Southerland EM, Hardwick JC, Wright GL, Ryan S, Li Y, KenKnight BH, Armour JA, Ardell JL, Vagus nerve stimulation mitigates intrinsic cardiac neuronal and adverse myocyte remodeling postmyocardial infarction, Am J Physiol Heart Circ Physiol 309(7) (2015) H1198–206. [PubMed: 26276818]

[129]. Furukawa Y, Hoyano Y, Chiba S, Parasympathetic inhibition of sympathetic effects on sinus rate in anesthetized dogs, Am J Physiol 271(1 Pt 2) (1996) H44–50. [PubMed: 8760156]

[130]. Schwartz PJ, De Ferrari GM, Sanzo A, Landolina M, Rordorf R, Raineri C, Campana C, Revera M, Ajmone-Marsan N, Tavazzi L, Odero A, Long term vagal stimulation in patients with advanced heart failure: first experience in man, European journal of heart failure 10(9) (2008) 884–91. [PubMed: 18760668]

[131]. De Ferrari GM, Crijns HJ, Borggrefe M, Milasinovic G, Smid J, Zabel M, Gavazzi A, Sanzo A, Dennert R, Kuschyk J, Raspopovic S, Klein H, Swedberg K, Schwartz PJ, I. CardioFit Multicenter Trial, Chronic vagus nerve stimulation: a new and promising therapeutic approach for chronic heart failure, Eur Heart J 32(7) (2011) 847–55. [PubMed: 21030409]

[132]. Zannad F, De Ferrari GM, Tuinenburg AE, Wright D, Brugada J, Butter C, Klein H, Stolen C, Meyer S, Stein KM, Ramuzat A, Schubert B, Daum D, Neuzil P, Botman C, Castel MA, D'Onofrio A, Solomon SD, Wold N, Ruble SB, Chronic vagal stimulation for the treatment of low ejection fraction heart failure: results of the NEural Cardiac TherApy foR Heart Failure (NECTAR-HF) randomized controlled trial, Eur Heart J 36(7) (2015) 425–33. [PubMed: 25176942]

[133]. De Ferrari GM, Tuinenburg AE, Ruble S, Brugada J, Klein H, Butter C, Wright DJ, Schubert B, Solomon S, Meyer S, Stein K, Ramuzat A, Zannad F, Rationale and study design of the NEuroCardiac TherApy foR Heart Failure Study: NECTAR-HF, European journal of heart failure 16(6) (2014) 692–9. [PubMed: 24846173]

[134]. De Ferrari GM, Stolen C, Tuinenburg AE, Wright DJ, Brugada J, Butter C, Klein H, Neuzil P, Botman C, Castel MA, D'Onofrio A, de Borst GJ, Solomon S, Stein KM, Schubert B, Stalsberg K, Wold N, Ruble S, Zannad F, Long-term vagal stimulation for heart failure: Eighteen month results from the NEural Cardiac TherApy foR Heart Failure (NECTAR-HF) trial, Int J Cardiol 244 (2017) 229–234. [PubMed: 28663046]

[135]. Gold MR, Van Veldhuisen DJ, Hauptman PJ, Borggrefe M, Kubo SH, Lieberman RA, Milasinovic G, Berman BJ, Djordjevic S, Neelagaru S, Schwartz PJ, Starling RC, Mann DL, Vagus Nerve Stimulation for the Treatment of Heart Failure: The INOVATE-HF Trial, J Am Coll Cardiol 68(2) (2016) 149–58. [PubMed: 27058909]

[136]. Hauptman PJ, Schwartz PJ, Gold MR, Borggrefe M, Van Veldhuisen DJ, Starling RC, Mann DL, Rationale and study design of the increase of vagal tone in heart failure study: INOVATE-HF, Am Heart J 163(6) (2012) 954–962 e1. [PubMed: 22709747]

[137]. Premchand RK, Sharma K, Mittal S, Monteiro R, Dixit S, Libbus I, DiCarlo LA, Ardell JL, Rector TS, Amurthur B, KenKnight BH, Anand IS, Autonomic Regulation Therapy via Left or Right Cervical Vagus Nerve Stimulation in Patients with Chronic Heart Failure: Results of the ANTHEM-HF Trial, Journal of cardiac failure 20 (2014) 808–816. [PubMed: 25187002]

[138]. Premchand RK, Sharma K, Mittal S, Monteiro R, Dixit S, Libbus I, DiCarlo LA, Ardell JL, Rector TS, Amurthur B, KenKnight BH, Anand IS, Extended Follow-Up of Patients With Heart Failure Receiving Autonomic Regulation Therapy in the ANTHEM-HF Study, J Card Fail 22(8) (2016) 639–42. [PubMed: 26576716]

[139]. Konstam MA, Udelson JE, Butler J, Klein HU, Parker JD, Teerlink JR, Wedge PM, Saville BR, Ardell JL, Libbus I, DiCarlo LA, Impact of Autonomic Regulation Therapy in Patients with Heart Failure: ANTHEM-HFrEF Pivotal Study Design, Circ Heart Fail 12(11) (2019) e005879. [PubMed: 31722536]

[140]. Vanoli E, De Ferrari GM, Stramba-Badiale M, Hull SS Jr., Foreman RD, Schwartz PJ, Vagal stimulation and prevention of sudden death in conscious dogs with a healed myocardial infarction, Circ Res 68(5) (1991) 1471–81. [PubMed: 2019002]

[141]. Ando M, Katare RG, Kakinuma Y, Zhang D, Yamasaki F, Muramoto K, Sato T, Efferent vagal nerve stimulation protects heart against ischemia-induced arrhythmias by preserving connexin43 protein, Circulation 112(2) (2005) 164–70. [PubMed: 15998674]

[142]. Zhao S, Dai Y, Ning X, Tang M, Zhao Y, Li Z, Zhang S, Vagus Nerve Stimulation in Early Stage of Acute Myocardial Infarction Prevent Ventricular Arrhythmias and Cardiac Remodeling, Frontiers in cardiovascular medicine 8 (2021) 648910. [PubMed: 33981734]

[143]. Wang Z, Yu L, Huang B, Wang S, Liao K, Saren G, Zhou X, Jiang H, Low-level transcutaneous electrical stimulation of the auricular branch of vagus nerve ameliorates left ventricular remodeling and dysfunction by downregulation of matrix metalloproteinase 9 and transforming growth factor beta1, Journal of cardiovascular pharmacology 65(4) (2015) 342–8. [PubMed: 25502306]

[144]. Yu L, Wang S, Zhou X, Wang Z, Huang B, Liao K, Saren G, Chen M, Po SS, Jiang H, Chronic Intermittent Low-Level Stimulation of Tragus Reduces Cardiac Autonomic Remodeling and Ventricular Arrhythmia Inducibility in a Post-Infarction Canine Model, JACC. Clinical electrophysiology 2(3) (2016) 330–339. [PubMed: 29766893]

[145]. Subramanian M, Edwards L, Melton A, Branen L, Herron A, Sivasubramanian MK, Monteiro R, Stansbury S, Balasubramanian P, Morris L, Elkholey K, Niewiadomska M, Stavrakis S, Non-invasive vagus nerve stimulation attenuates proinflammatory cytokines and augments antioxidant

levels in the brainstem and forebrain regions of Dahl salt sensitive rats, Sci Rep 10(1) (2020) 17576. [PubMed: 33067477]

[146]. Zhou L, Filiberti A, Humphrey MB, Fleming CD, Scherlag BJ, Po SS, Stavrakis S, Low-level transcutaneous vagus nerve stimulation attenuates cardiac remodelling in a rat model of heart failure with preserved ejection fraction, Experimental physiology 104(1) (2019) 28–38. [PubMed: 30398289]

[147]. Chen M, Zhou X, Liu Q, Sheng X, Yu L, Wang Z, Wang S, Zhou S, Left-sided Noninvasive Vagus Nerve Stimulation Suppresses Atrial Fibrillation by Upregulating Atrial Gap Junctions in Canines, Journal of cardiovascular pharmacology 66(6) (2015) 593–9. [PubMed: 26317165]

[148]. Yu L, Scherlag BJ, Li S, Fan Y, Dyer J, Male S, Varma V, Sha Y, Stavrakis S, Po SS, Low-level transcutaneous electrical stimulation of the auricular branch of the vagus nerve: a noninvasive approach to treat the initial phase of atrial fibrillation, Heart Rhythm 10(3) (2013) 428–35. [PubMed: 23183191]

[149]. Stavrakis S, Stoner JA, Humphrey MB, Morris L, Filiberti A, Reynolds JC, Elkholey K, Javed I, Twidale N, Riha P, Varahan S, Scherlag BJ, Jackman WM, Dasari TW, Po SS, TREAT AF (Transcutaneous Electrical Vagus Nerve Stimulation to Suppress Atrial Fibrillation): A Randomized Clinical Trial, JACC. Clinical electrophysiology 6(3) (2020) 282–291. [PubMed: 32192678]

[150]. Stavrakis S, Humphrey MB, Scherlag BJ, Hu Y, Jackman WM, Nakagawa H, Lockwood D, Lazzara R, Po SS, Low-level transcutaneous electrical vagus nerve stimulation suppresses atrial fibrillation, J Am Coll Cardiol 65(9) (2015) 867–75. [PubMed: 25744003]

[151]. Yu L, Huang B, Po SS, Tan T, Wang M, Zhou L, Meng G, Yuan S, Zhou X, Li X, Wang Z, Wang S, Jiang H, Low-Level Tragus Stimulation for the Treatment of Ischemia and Reperfusion Injury in Patients With ST-Segment Elevation Myocardial Infarction: A Proof-of-Concept Study, JACC Cardiovasc Interv 10(15) (2017) 1511–1520. [PubMed: 28797427]

[152]. Lin J, Scherlag BJ, Zhou J, Lu Z, Patterson E, Jackman WM, Lazzara R, Po SS, Autonomic mechanism to explain complex fractionated atrial electrograms (CFAE), J Cardiovasc Electrophysiol 18(11) (2007) 1197–205. [PubMed: 17916143]

[153]. Lellouche N, Buch E, Celigoj A, Siegerman C, Cesario D, De Diego C, Mahajan A, Boyle NG, Wiener I, Garfinkel A, Shivkumar K, Functional characterization of atrial electrograms in sinus rhythm delineates sites of parasympathetic innervation in patients with paroxysmal atrial fibrillation, J Am Coll Cardiol 50(14) (2007) 1324–31. [PubMed: 17903630]

[154]. Kurotobi T, Shimada Y, Kino N, Ito K, Tonomura D, Yano K, Tanaka C, Yoshida M, Tsuchida T, Fukumoto H, Features of intrinsic ganglionated plexi in both atria after extensive pulmonary isolation and their clinical significance after catheter ablation in patients with atrial fibrillation, Heart rhythm 12(3) (2015) 470–476. [PubMed: 25433142]

[155]. Katritsis DG, Pokushalov E, Romanov A, Giazitzoglou E, Siontis GC, Po SS, Camm AJ, Ioannidis JP, Autonomic denervation added to pulmonary vein isolation for paroxysmal atrial fibrillation: a randomized clinical trial, J Am Coll Cardiol 62(24) (2013) 2318–25. [PubMed: 23973694]

[156]. Katritsis DG, Giazitzoglou E, Zografos T, Pokushalov E, Po SS, Camm AJ, Rapid pulmonary vein isolation combined with autonomic ganglia modification: a randomized study, Heart rhythm 8(5) (2011) 672–8. [PubMed: 21199686]

[157]. Kim MY, Coyle C, Tomlinson DR, Sikkel MB, Sohaib A, Luther V, Leong KM, Malcolme-Lawes L, Low B, Sandler B, Lim E, Todd M, Fudge M, Wright IJ, Koa-Wing M, Ng FS, Qureshi NA, Whinnett ZI, Peters NS, Newcomb D, Wood C, Dhillon G, Hunter RJ, Lim PB, Linton NWF, Kanagaratnam P, Ectopy-triggering ganglionated plexuses ablation to prevent atrial fibrillation: GANGLIA-AF study, Heart rhythm 19(4) (2022) 516–524. [PubMed: 34915187]

[158]. Driessen AH, Berger WR, Krul SP, van den Berg NW, Neefs J, Piersma FR, Chan Pin Yin DR, de Jong JS, van Boven WP, de Groot JR, Ganglion Plexus Ablation in Advanced Atrial Fibrillation: The AFACT Study, J Am Coll Cardiol 68(11) (2016) 1155–65. [PubMed: 27609676]

[159]. Berger WR, Neefs J, van den Berg NWE, Krul SPJ, van Praag EM, Piersma FR, de Jong J, van Boven WP, Driessen AHG, de Groot JR, Additional Ganglion Plexus Ablation During Thoracoscopic Surgical Ablation of Advanced Atrial Fibrillation: Intermediate Follow-Up of the AFACT Study, JACC. Clinical electrophysiology 5(3) (2019) 343–353. [PubMed: 30898238]

[160]. Pachon JC, Pachon EI, Cunha Pachon MZ, Lobo TJ, Pachon JC, Santillana TG, Catheter ablation of severe neurally meditated reflex (neurocardiogenic or vasovagal) syncope: cardioneuroablation long-term results, Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology 13(9) (2011) 1231–42. [PubMed: 21712276]

[161]. Sun W, Zheng L, Qiao Y, Shi R, Hou B, Wu L, Guo J, Zhang S, Yao Y, Catheter Ablation as a Treatment for Vasovagal Syncope: Long-Term Outcome of Endocardial Autonomic Modification of the Left Atrium, J Am Heart Assoc 5(7) (2016).

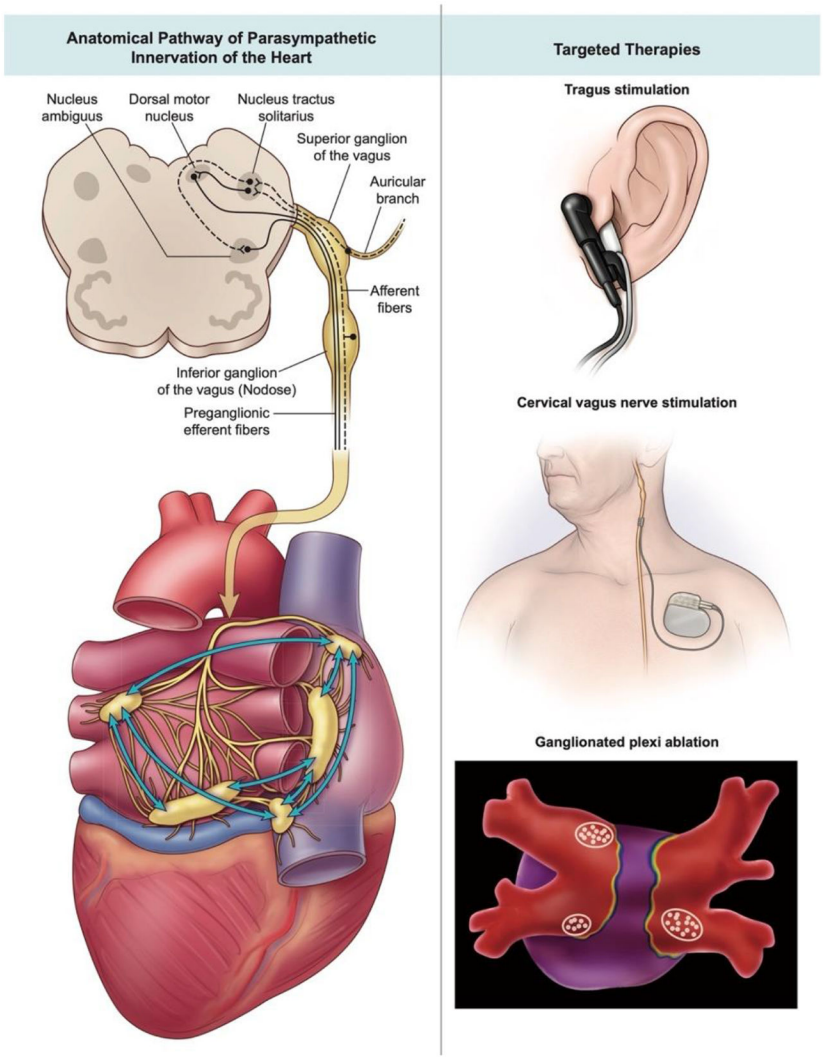

Figure 1. Parasympathetic innervation of the heart and therapies targeting the parasympathetic nervous system for treatment of cardiovascular diseases.

(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.