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Cardiac neuroanatomy - Imaging nerves to define functional control

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Abstract

The autonomic nervous system regulates normal cardiovascular function and plays a critical role in the pathophysiology of cardiovascular disease. Further understanding of the interplay between the autonomic nervous and cardiovascular systems holds promise for the development of neuroscience-based cardiovascular therapeutics. To this end, techniques to image myocardial innervation will help provide a basis for understanding the fundamental underpinnings of cardiac neural control. In this review, we detail the evolution of gross and microscopic anatomical studies for functional mapping of cardiac neuroanatomy.

Keywords

neurocardiology; intracardic nervous system; myocardial innervation; autonomic nervous system

INTRODUCTION

The autonomic nervous system (ANS) enables integrative control of the viscera to ensure survival of the organism.[1] Specifically, the cardiac ANS plays a crucial role to maintain normal rhythm and sustain the circulation of blood. The cardiac ANS intricately regulates all the critical physiological functions of the heart (chronotropy, dromotropy, inotropy, and lusitropy). The interplay of the ANS and the heart is readily apparent in the pathophysiology of most cardiovascular diseases including hypertension, heart failure, myocardial infarction and arrhythmias.[2–6] The study of such interactions has led to the development of neuromodulatory therapies that treat cardiac disease, ranging from vagus nerve stimulation to bilateral cardiac sympathetic decentralization for heart failure and ventricular arrhythmias.[7–9] A more thorough characterization of cardiac innervation will promote

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further study of neural control of cardiac function and allow for the development of targeted cardiovascular therapeutics.[10] In this review, we describe available and burgeoning techniques in mapping the structure and function of cardiac neuroanatomy.

Functional organization of the cardiac nervous system

Anatomically, cardiac nerves are complex, and detailed atlases of cardiac innervation are few in number (Figures 1–3). Macroscopic images of cardiac nerves depicted in anatomical texts since the late 1500s are reproduced in books and anatomical studies without histological confirmation of whether structures labeled as 'nerves' are actually neural. Indeed, when neural structures are surgically targeted for therapeutics in clinical care (e.g. bilateral cardiac sympathetic decentralization), intra-operative histological confirmation is sought to confirm that neural tissue was indeed removed.[11] The problem at the level of the heart is compounded by the lack of technology for high-resolution microscopic studies until very recently. Furthermore, methods to handle the very large image datasets and the bioinformatics tools for neuronal tracing in images and three-dimensional reconstruction have only recently been developed. Placing these images in their functional context, using state-of-the-art physiological approaches from micro-stimulation to neural recordings, are required for interpretation of normal physiology. Finally, integrating anatomical and functional data would provide the framework needed to precisely define the changes in innervation due to disease (neural remodeling) and assure correct utilization and monitoring of new or pre-existing methods for neuromodulation.

Gross anatomy of neurotransmission to and from the heart

The gross anatomy of cardiac innervation has been the subject of investigation for a number of years. Dissections of mammals and human cadavers have illustrated that myocardial innervation is conserved to a significant degree among species.[12–18] The ANS innervating the heart has been categorized into: i) central; ii) intrathoracic extra-cardiac; and iii) intrinsic cardiac components (Figures 1–3). The intrathoracic extrinsic cardiac nervous system connects the intrinsic cardiac nervous system within the heart to the central nervous system. It is composed of parasympathetic and sympathetic motor components that exert opposing effects on cardiac electrical and mechanical indices.

Parasympathetic motor neurons—Preganglionic neurons of the parasympathetic nervous system are located in the nucleus ambiguus and dorsal motor nucleus of the medulla oblongata as well as scattered regions in between these two structures.[19,20] These project axons via the vagus nerve and its multiple intrathoracic cardiopulmonary branches to efferent postganglionic parasympathetic neurons in the numerous intrinsic cardiac ganglia.[21] Postganglionic efferent parasympathetic neurons located in individual cardiac ganglia receive preganglionic inputs from both the right and left vagal trunks.[22] Anatomic dissections in canines have demonstrated that postganglionic vagal neurons to the sinoatrial (SA) node are found predominantly in a ganglionated plexus next to the right pulmonary vein-atrial junction, while the postganglionic vagal neurons that influence the atrioventricular (AV) node are located predominantly in the region adjacent to the inferior vena cava-inferior left atrium junction.[23]

Sympathetic motor neurons—Sympathetic efferent preganglionic neurons originate in the intermediolateral cell column of the spinal cord and project via C7-T6 rami to postganglionic sympathetic neurons in the superior cervical, middle cervical, cervicothoracic (stellate) ganglia (sympathetic chain), and mediastinal ganglia.[16,18] These postganglionic neurons project axons via multiple cardiopulmonary nerves to atrial and ventricular myocardium and limited populations of intrinsic cardiac adrenergic neurons. Kawashima published a detailed study in 2005 that analyzed the origin and course of the autonomic nerves in human cadavers using microscopy, highlighting the conserved nature of the superior and middle cervical, mediastinal, and stellate ganglia across species.[18]

Sensory neurons—Cardiac afferents provide beat-to-beat sensory information of cardiac function and microenvironment to the neuraxis, and additional information is conveyed by arterial mechano-and chemoreceptors (Figure 2). Afferent nerves travel within the vagal trunk and ‘sympathetic’ fibers to the nodose and dorsal root ganglia, respectively. The processing of afferent information at multiple levels, including the intrinsic cardiac nervous system, extracardiac intrathoracic ganglia, spinal cord, brain stem, and higher centers, provides an elegant mechanism of interacting feedback loops that modulate efferent cardiomotor (sympathetic and parasympathetic) signals for maintaining normal rhythm and life-sustaining circulation.

The intrinsic cardiac nervous system—In contrast to the extrinsic components of the ANS, intrinsic cardiac neurons are found in intramural ganglia and in epicardial fat pads that obscure direct visualization (Figure 4).[24,25] While these neuronal somata were identified early in the last century, the location and connections of these neurons have remained poorly understood.[26] Subsequent study using light and electron microscopy confirmed these structures in the human heart (Figures 4C–E).[25] It is now known that the intrinsic cardiac ganglionated plexuses contain complex networks of neurons and interconnecting nerves that include the following subtypes: i) afferent neurons; ii) motor (parasympathetic and sympathetic) neurons; and iii) interconnecting local circuit neurons.[27] Taken together, this neural network at the level of the heart is thought to play an important role in modulating cardio-cardiac reflexes.

Histochemical and immunohistochemical techniques

Histochemical techniques have characterized the richness of myocardial innervation. Cresyl violet staining has been used to identify the cytoplasmic Nissl substance in autonomic neurons.[17,24,28] The retrograde transport of horseradish peroxidase and vital dyes applied to various cardiac tissues had been used to identify the locations of cardiac vagal preganglionic neurons in the medulla.[29,30] This technique has helped characterize the locations of sympathetic postganglionic neuronal somata and vagal preganglionic efferent neurons innervating feline and canine hearts.[16] This marker has also proved helpful in delineating locations of afferent neurons in nodose and dorsal root ganglia that transduce the mechanical and/or chemical milieu of various regions of the heart or great vessels to the nervous system.[31–33] Xrhodamine-5-(and 6)-isothiocyanate (XRITC) is another marker that serves as a retrograde fluorescent tracer for preganglionic vagal neurons.[34,35] As mentioned above, visualization of the intrinsic cardiac nervous system has proved challenging, and fluorescent

dyes, Fast Blue and bisbenzimide, have been used to demonstrate retrograde transport that confirm the presence of interneuronal connections.[36–38] Antibodies against cholera toxin subunit B have also been used in immunohistochemical studies of the intracardiac ganglia.[39]

Specific identification of neuron types in the two limbs of the ANS is made possible by targeting select neurotransmitters associated with sympathetic or parasympathetic neuronal somata located in the peripheral cardiac ANS. Postganglionic parasympathetic neurons utilize the neurotransmitter acetylcholine, degraded by the enzyme acetylcholinesterase selectively associated with parasympathetic motor neurons. As such, many studies use acetylcholine to mimic the effects of vagal stimulation.[40,41] Karnovsky and Roots published a histochemical method in 1964 that utilized a thiocholine ester that is hydrolyzed by cholinesterase to form copper thiocholine, which appears brown when treated with ammonium sulfide.[42] This method and refinements of this method including, but not limited to, the use of acetylthiocholine and the use of a marker of the peroxidase activity of the reaction product have been used to study the distribution of cholinergic neurons in the heart (Figure 5).[43–49] For example, Pauza et al. showed that the number of intrinsic cholinergic neurons in the heart was directly proportional to ganglia area in guinea pig, rat, dog, and human hearts using such methods.[48,50,51]

While this histologic method identifies cholinesterase activity associated with select populations of neuronal somata, it is not specific for acetylcholinesterase activity in vagal neurons. As such, immunohistochemcial methods have been used to identify parasympathetic neurons using antibodies against choline acetyltransferase (ChAT, Figure 5A–C). This methodology was initially utilized in the characterization of guinea pig myenteric plexus neurons associated with the gastrointestinal tract.[52] It was thereafter applied to study of guinea pig cardiac ganglia and is now in widespread use.[53,54] Additional markers such as choline transporter and vesicular acetylcholine transporter, which are more specific for the identification cholinergic neurons, have since been developed.[55,56]

Similarly, sympathetic neurons can be identified using immunohistochemcial markers that target an enzyme specific to these neurons. With respect to adrenergic neurons, tyrosine hydroxylase (TH) is the enzyme involved in the rate-limiting step in norepinephrine synthesis and, as such, is specific for norepinephrine-producing neurons (Figure 5D–F). Pickel et al. developed antibodies against TH that performed well for localization of neurons involved in biosynthesis of catecholamines.[28] Immunohistochemistry targeting of TH has been used regularly to characterize the sympathetic innervation of the heart across different animal models.[45,49,53,57,58] Pan-neuronal markers are used to identify cardiac nerves, and examples include the neurofilament markers β-tubulin III and protein gene product 9.5 that are found almost exclusively in neurons (Figure 6).[45,50,56,57,59–62] Presynaptic nerves are targeted using antibodies against synapsin I, which is a member of a family of neuron-specific proteins that interact with synaptic vesicles.[61,63]

While the majority of these intracardiac neurons are either parasympathetic or sympathetic in nature, intracardiac nerves are not exclusively parasympathetic or sympathetic. Thus, the use of additional markers has helped elucidate the mixed cholinergic and adrenergic phenotype of intrinsic cardiac neurons and nerves in the heart. For example, Hoard et al.

demonstrated the presence of cholinergic neurons in intrinsic cardiac ganglia that are associated with tyrosine hydroxylase, dopamine β-hydroxylase, and norepinephrine transporter, yet do not contain vesicular monoamine transporter type 2 (VMAT2) and thereby precludes the ability to store norepinephrine.[62] Rysevaite et al. showed that 83% of intrinsic cardiac neurons were ChAT immunoreactive, 4% were TH immunoreactive, and 14% were immunoreactive for both ChAT and TH.[64] This study also demonstrated immunoreactivity to calcitonin gene-related peptide and substance P, neuropeptides found in nociceptive neurons, to identify a population of afferent neurons in the intrinsic cardiac nervous system.[64]

Antibody-based approaches have inherent limitations with respect to fidelity of results. In that regard, the use of transgenic mice has allowed for a more detailed structural analysis, as in the morphogenesis of the cardiac nervous system. In mouse studies, wingless type integration site family, member 1 (Wnt1)-Cre recombinase (Cre) mice have been used to identify tissues derived from neural crest cells, as *Wnt1* is only expressed during embryonic development of the central nervous system.[65] White et al. demonstrated that crossing these mice with mice expressing the tdTomato reporter results in neural crest-derived cells that are labeled with red fluorescence and imaged using epifluorescent stereomicroscopy to image the subepicardial neural network in exquisite detail.[61]

Mapping the fine neural network across large distances in organs has typically been performed using histological sections that are then reconstructed, a time-consuming process that also suffers from loss in fidelity due to tissue distortion. Light scatter due to tissue inhomogeneity has hindered imaging larger volumes of tissue. Tissue clearing approaches, initially developed to better visualize neural networks in the central nervous system, attempt to minimize light scatter by decreasing tissue inhomogeneity and allowing for deeper imaging in thick sections or intact tissues while preserving the three-dimensional molecular and cellular architecture. Two main subgroups of techniques include solvent-based methods, such as BABB,[66] tetrahydrofuran and dibenzylether,[67] 3DISCO[68] and iDISCO, or aqueous-based methods, including Sca*l*e, SeeDB, CUBIC, *Clear*^{T}, *Clear*^{T2}, CLARITY, and PACT, are used to remove the lipid membranes of cells, which are the major source of tissue heterogeneity and, hence, light diffraction, to generate optically transparent tissue.[67–75] While various challenges face these differing techniques including time intensity, degree of tissue clearing, preservation of fluorescence of reporters, and tissue expansion, the rapidly evolving field holds the promise of improved three-dimensional imaging at depth. In conjunction with these developments, advances in microscopy in the form of laser-scanning confocal, two-photon, and light-sheet microscopy permit volumetric imaging of cleared tissues. The premise of these techniques is to image discrete planes within a volume and minimize out-of-focus light to yield improved imaging at depth.

Functional mapping

Given the complex structure of parasympathetic, sympathetic motor and other intrinsic cardiac neurons within the heart, mapping studies have been performed to elucidate the functional architecture of the cardiac nervous system. Studies have evaluated how autonomic nerve stimulation affects mechanical and electrocardiographic parameters of the heart

(Figure 7).[76–79] Norris et al. placed strain gauges on the epicardium and electrically stimulated sympathetic nerves in canines to measure regional inotropy to determine gross functional innervation patterns.[80,81] The positive and negative chronotropic effect of sympathetic and parasympathetic simulation on SA node activity, respectively, were originally described in studies employing nerve stimulation while monitoring heart rate. These studies determine that the right vagus nerve primarily affecting the SA node while the left vagus nerve exerted predominant influence on the AV node in the canine heart.[82,83] Randall et al. demonstrated that parasympathetic stimulation resulted in decreased chronotropy and decreased AV conduction, while Loeb and DeTarnowsky showed how sympathetic nerve stimulation resulted in increased AV conduction through the use of intracardiac electrograms in *in vivo* experiments in dog.[84,85] More recently, a model of isolated Langendorff rabbit heart with intact dual autonomic innervation has allowed for *in vitro* preparations that are devoid of confounding neurohormonal influences of *in vivo* models.[86]

Optogenetic Techniques

In contrast to optical mapping, which is helpful at the organ level, optogenetics provide functional information on myocardial innervation at the cellular level. Optogenetic methods utilize light-activated ion channels called opsins to activate or inhibit specific cell types such as neural populations *in vivo*, providing spatiotemporal resolution in unprecedented detail and allowing the study of structure and function.

The application of optogenetics in neurons was developed over a decade ago and has since been translated to the study in neurocardiology.[87–89] Channelrhodopsin 2 (ChR2) is an opsin, or light-gated ion channel, identified in unicellular green alga *Chlamydomonas reinhardtii*.[88] When ChR2 is expressed in a neuron, inward currents are evoked within 50ms of a flash of blue light, and this technology has be used for fast neuronal photostimulation in mammals.[89] Improvements of this tool include ChR2 variants that increase the magnitude of photocurrents induced.[90–92] In the brain, multidiode probe arrays have been used in studies of local brain tissues to allow for multisite neuronal light-activation.[93] Alternatively, various ways of patterned illumination have been developed for differential light activation in a sample.[94]

Gene delivery systems, such as viruses are currently been used to deliver opsins to select neuronal populations. Examples of such vectors include herpes, rabies and adeno- and lenti-associated viruses, with the latter two being the most commonly used.[95] For example, Boyden et al. transfected cultured neurons with lentiviruses bearing the ChR2 protein tagged with a yellow fluorescent protein.[89] Adeno-associated viruses are small, nonpathogenic and replication-defective parvoviruses with single-stranded DNA genome. Multiple subtypes exist; for instance, AAV2 in particular has been shown to have high transduction efficiency in neurons while AAV9 has been used to deliver the *cop4* gene, which encodes the ChR2 protein, into rat ventricular cardiomyocytes *in vivo*.[94–96]. Additional delivery systems include the use of transgenes and the Cre/*loxP* recombination systems, as mentioned above, to target photoactivation of specific neural populations. In this system, *loxP* cassettes flank the *cop4* gene with a reporter gene encoding fluorescent protein and the Cre driver

expressing Cre recombinase under the control of a cell-specific promoter such as tyrosine hydroxylase or choline acetyltransferase.[35,97,98]

The initial application of optogenetics to cardiovascular research was in 2010 to locate and control cardiac pacemaker cells in zebrafish and in embryonic stem cell-derived cardiomyocytes in mice, and several studies have since been published regarding optogenetic control of cardiomyocytes.[99–105] Wang et al. have been able to demonstrate that optogenetic stimulation of noradrenergic neurons in the locus cereleus of the brain inhibits parasympathetic cardiac vagal neuron outflow that results in tachycardia.[35] Their group have further evaluated the neural axis in the murine heart by identifying and stimulating a subpopulation of cardiomyocytes that bear the *phenylethanolamine n-methyltransferase* gene, which encodes the enzyme that converts norepinephrine to epinephrine, to exert control on heart rhythm through the use of optogenetics.[106] Wengrowski et al. have shown photoactivation of cardiac sympathetic nerves affects cardiac electrical properties to increase heart rate along with cardiac contractile force (Figure 8).[97] To study the effects of vagal tone on exercise capacity, Machhada et al. utilized a viral vector system to target neurons in the rat brainstem dorsal ventral motor nucleus, the main vagal nucleus in the central nervous system, with channelrhodopsin ChIEF.[107] Activation of the dorsal ventral motor nucleus neurons resulted in increased cardiac contractility and exercise capacity. In sum, modern viral-based as well as Cre transgenic approaches, have been used to map the structural and functional properties of the autonomic nervous system controlling the heart.

Clinical Neuroimaging

Imaging techniques of myocardial innervation, although limited, do allow for the study of innervation in animal studies and living humans. [^{14}C]2-deoxyglucose has been used to radiolabel neurons in culture and *in vivo* in sympathetic ganglia in canine with neural uptake induced by stimulation and imaged using autoradiography.[108,109] C-11 hydroxyephedrine or radioiodinated metaiodobenzylguanidine (*m*IBG), which are catecholamine analogues that are taken up by sympathetic nerve terminals, can be used to visualize sympathetic innervation to the heart *in vivo* in dogs and humans using a gamma camera and positron emission tomography, respectively.[110–115] Bravo et al. used three different C-11 labeled catecholamines to identify neurotransmitter metabolites involved in vesicular storage, transport and degradation in the sympathetic nerve terminal and outlined the dynamic regeneration process of sympathetic nerve function in the transplanted heart.[116] In studies of *m*IBG, the radiotracer is injected and the mean counts per pixel in the heart are compared to those of the mediastinum to generate a heart/mediastinal (H/M) uptake ratio. Quantification of the H/M ratio has resulted in the identification of cutoffs that have been associated with disease progression and response to therapy.[117–124] In short, cardiac imaging employing radiotracers of sympathetic output to the heart has diagnostic and prognostic implications in heart failure and arrhythmic and ischemic heart disease that warrants further study.

Summary and Future Directions

The functional anatomy of the cardiac nervous system is complex. Early studies focused on describing the anatomy of the cardiac nervous system to help elucidate the role of the ANS in regional cardiac control. Concepts regarding cardiac neural control have been revised in

recent years with new physiological data and have created a new and exciting framework for understanding regulatory control of the mammalian heart. Direct single neural and network recordings from intrinsic cardiac and extra-cardiac ganglia provide the methods to study organ level physiology and a proper framework for interpretation.[27,125,126] The field is now poised from a clinical and instrumentation front to take full advantage of the cellular and molecular work that has been done in the past to probe autonomic circuits.

More recently, technological advances in optical mapping and optogenetics hold the promise of functionally mapping the specificities of cardiac neural innervation to understand the role that the autonomic nervous system plays in the pathophysiology of cardiovascular disease, with particular regard to therapeutically targeting select neural elements therein. As such, further discoveries in this arena hold the promise of developing neuroscience-based cardiovascular therapeutics in the future.

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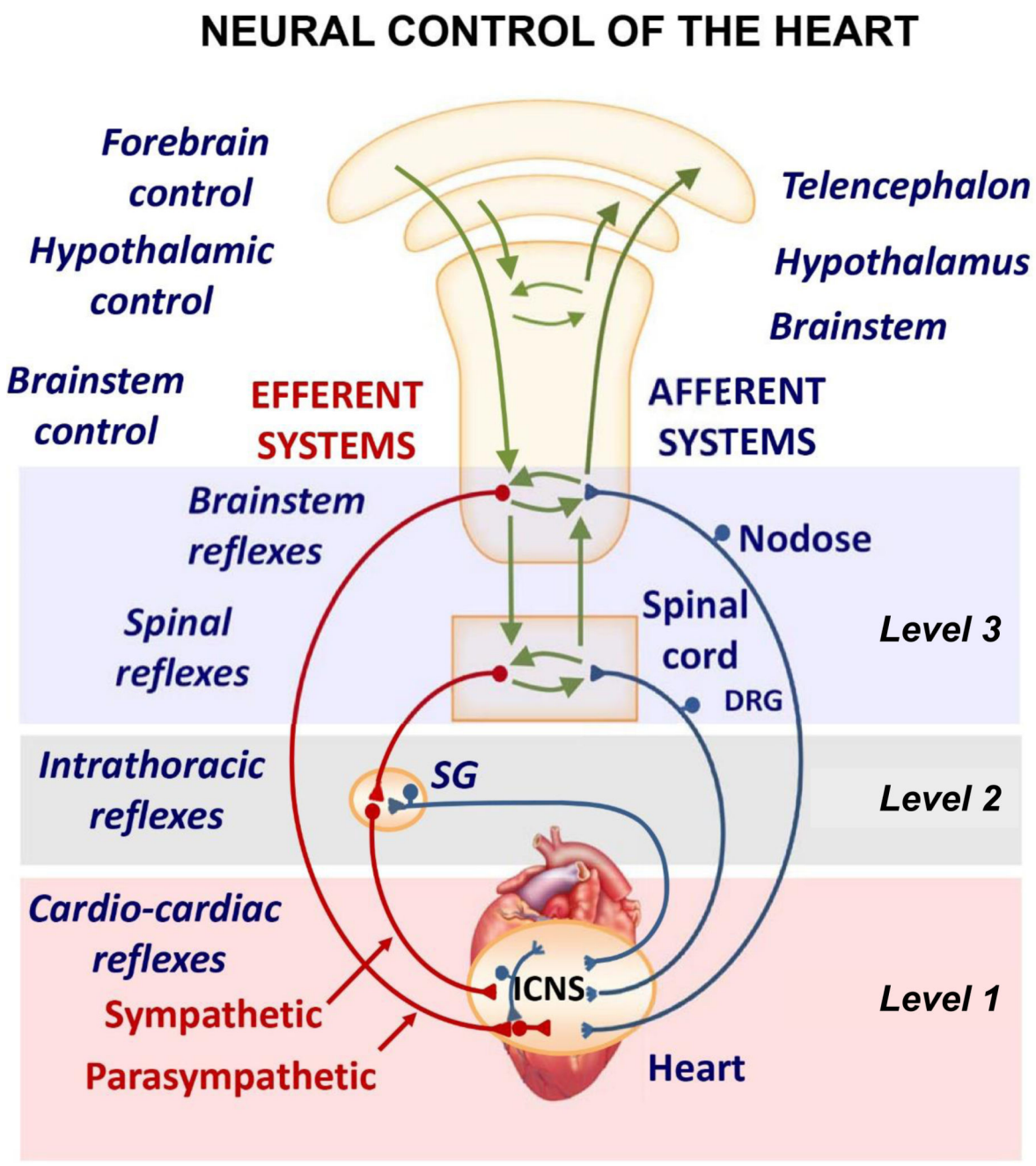


Figure 1.

Neural control of the heart. Autonomic control of the heart is comprised of a series of nested feedback loops, from the intracardiac neurons (level 1) to the intrathoracic extra-cardiac neurons (levels 2) and the central nervous system (level 3). SG, sympathetic ganglion; DRG, dorsal root ganglion; ICNS, intracardiac nervous system. Adapted from *Janig*.[127]

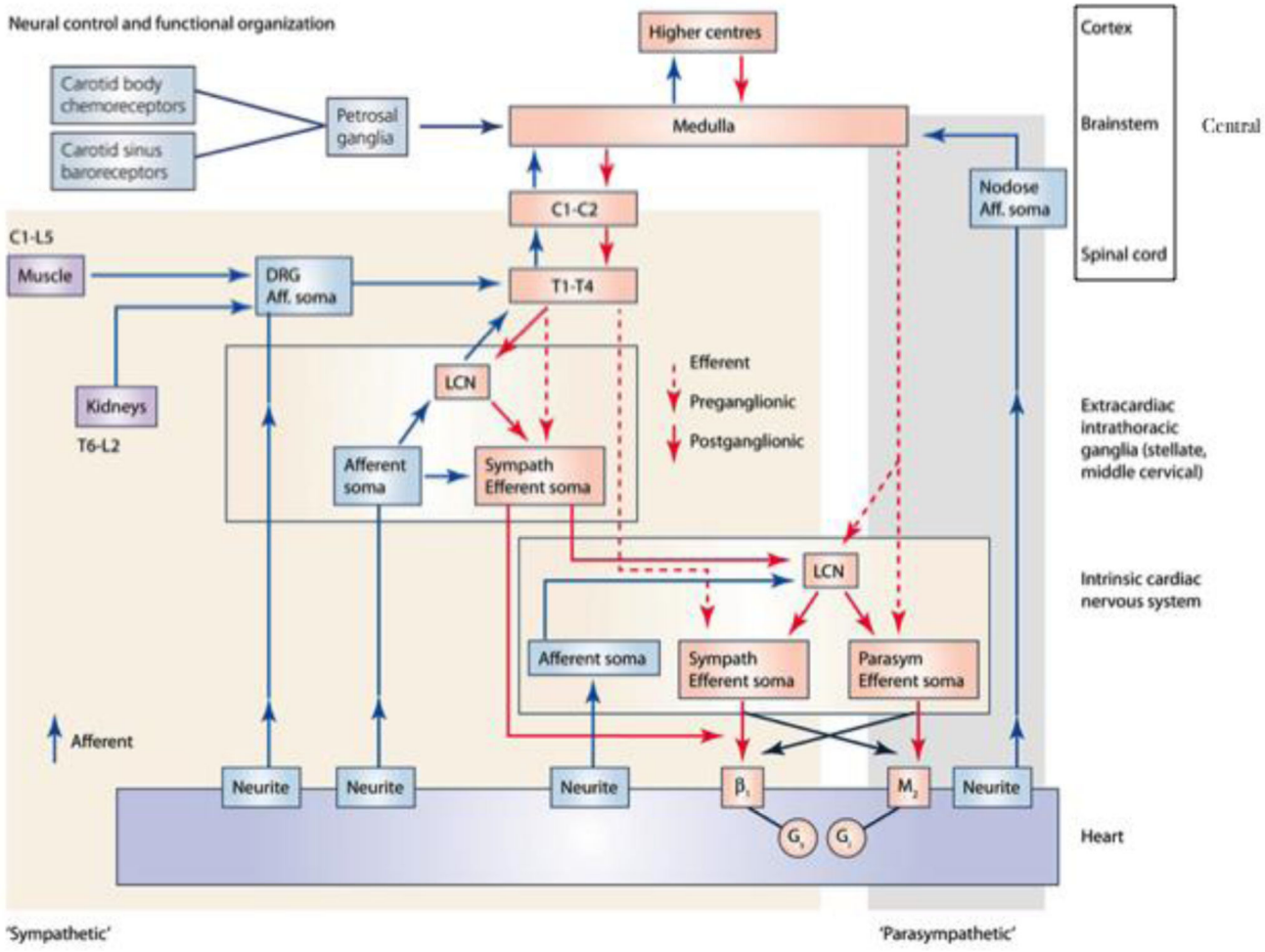
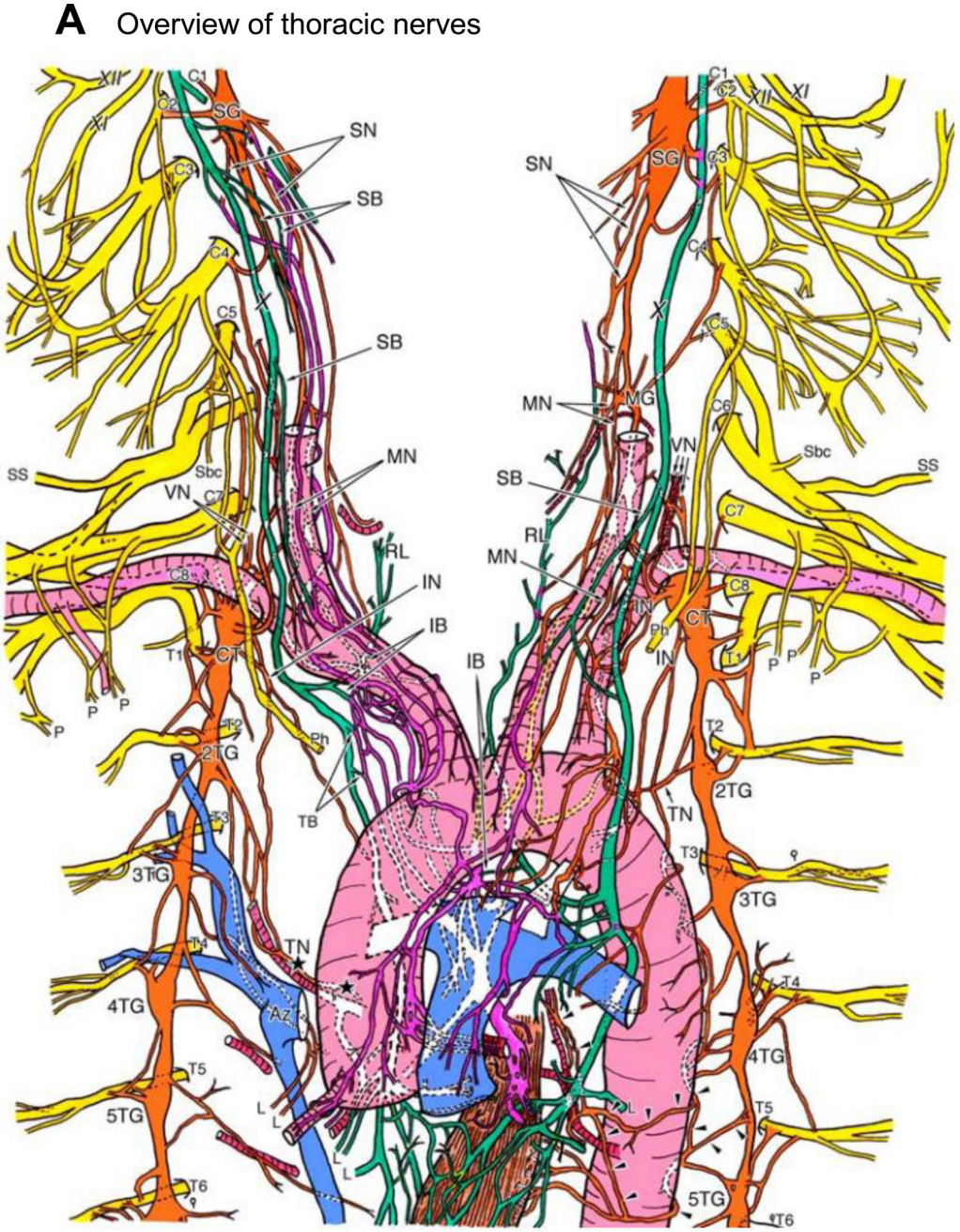


Figure 2.

Neurohumoral control and functional organization of cardiac innervation. Aff, afferent; β_1, β-adrenergic receptor; C, cervical; DRG, dorsal root ganglion; G_i, inhibitory G-protein; G_s, stimulatory G-protein; L, lumbar; LCN, local circuit neuron; M_2, muscarinic receptor; T, thoracic. Adapted from *Shivkumar et al.*[10]



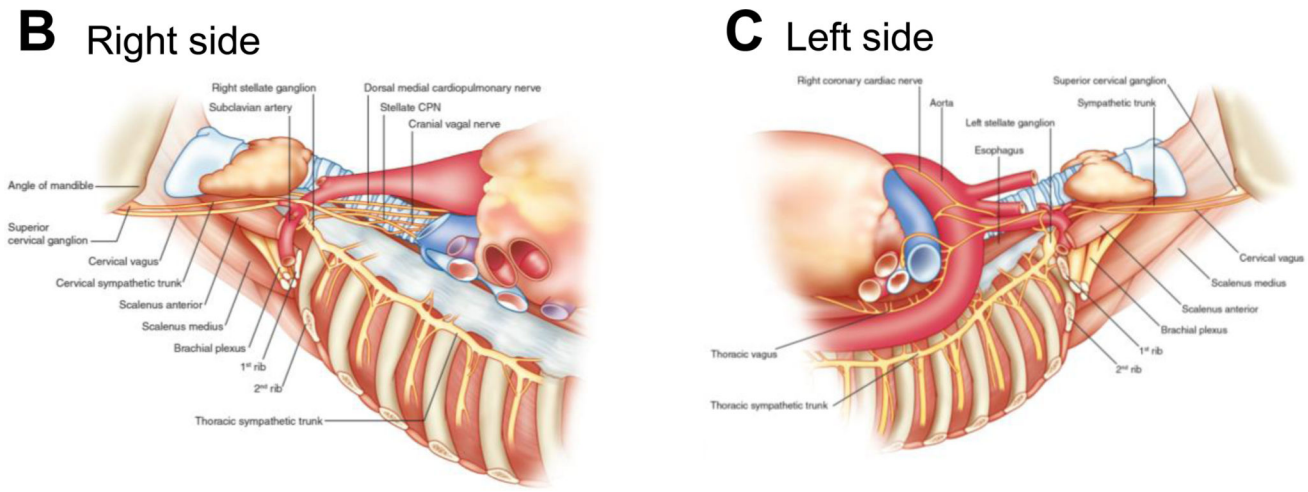


Figure 3.

A, Detailed illustration of the course of the autonomic cardiac nerves. The innervation of the heart via the right thoracic cardiac nerve and the inferior cardiac nerve is shown by the black stars. The arrowheads highlight the course of the left thoracic cardiac nerve. The sympathetic cardiac nerves, vagal cardiac branches, and the cardiac plexuses are colored in orange, green and purple, respectively. AI, anterior interventricular branch; Ao, aorta; Az, azygos vein; CB, circumflex branch; CC, common carotid artery; CT, cervicothoracic (stellate) ganglion; GV, great cardiac vein; IB, inferior (vagal) cardiac branch; IG, inferior cervical ganglion; IN, inferior cervical cardiac nerve; L, lung; LA, left atrium; LCA, left coronary artery; MG, middle cervical ganglion; MN, middle cardiac nerve; P, pectoral nerve; Ph, phrenic nerve; PT, pulmonary trunk; RA, right atrium; RCA, right coronary artery; RL, recurrent laryngeal nerve of vagus nerve; SB, superior (vagal) cardiac branch; Sbc, nerve to subclavian muscle; SG, superior cervical ganglion; SN, superior cardiac nerve; SS, suprascapular nerve; SVC, superior vena cava; TB, thoracic (vagal) cardiac branch; TG, thoracic ganglia; TN, thoracic cardiac nerve; VG, vertebral ganglion; VN, vertebral nerve; X, vagus nerve; XI, accessory nerve; XII, hypoglossal nerve. Adapted from *Kawashima*.[18] Course of the right (B) and left (C) sympathetic trunks, vagus nerve and their major branches in an embalmed cadaver. Adapted from *Janes et al*. and *Dilsizian and Narula*.[17,128]

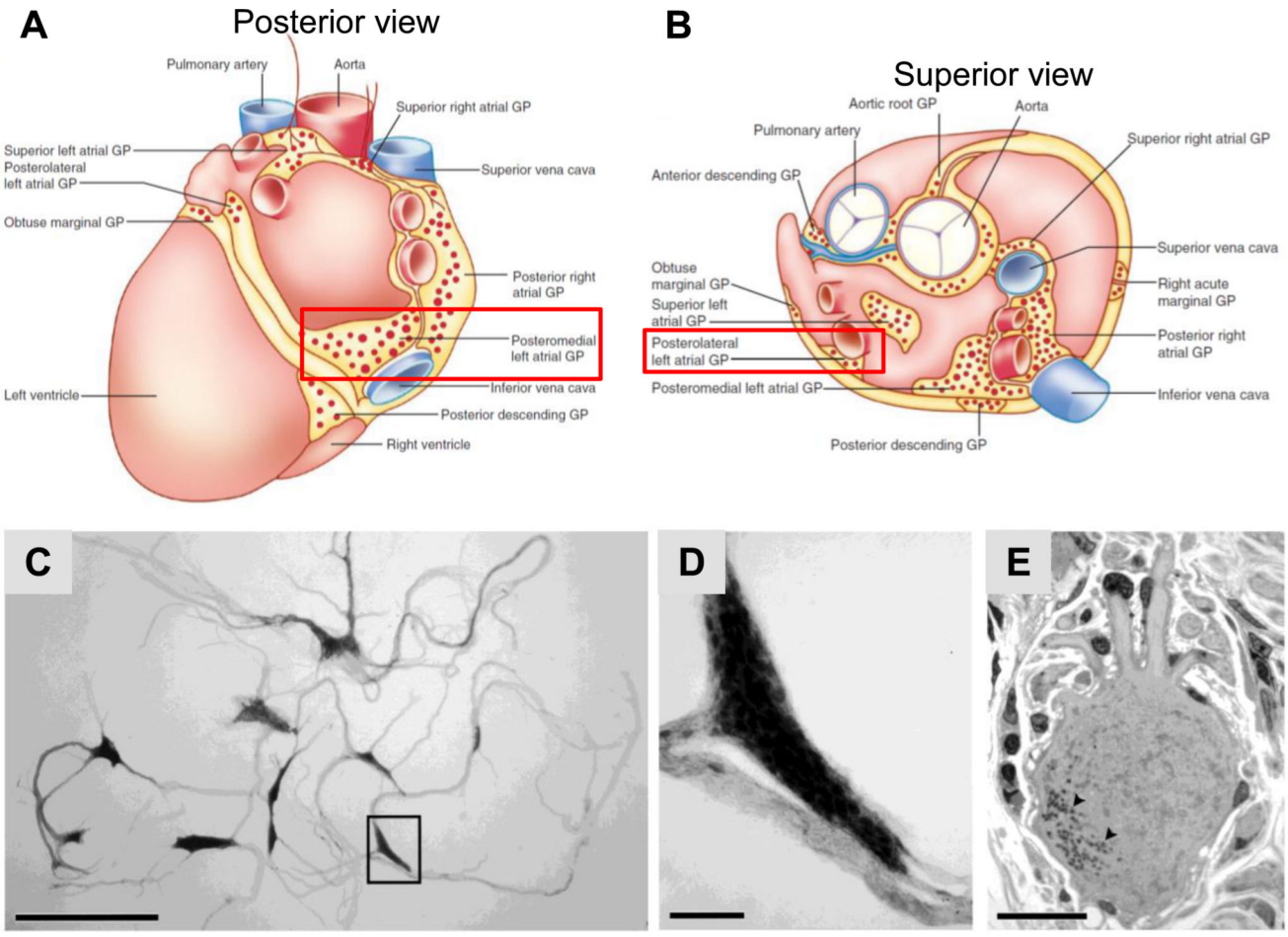


Figure 4.

A–B, Distribution of the intrinsic cardiac ganglionated plexuses (GP) in the heart. A, posterior; B, superior. Adapted from *Dilsizian and Narula*.[128] C–E, Light photomicrographs of human intrinsic cardiac nerves, ganglia and neurons. C, Network of ganglia and nerves stained with methylene blue and dissected from the posteromedial left atrial ganglionated plexus. The ganglia appear as expansions along a nerve (box). D, Enlargement of boxed area in A illustrating a ganglion composed of approximately 150–200 nerve cell bodies. E, High-magnification micrograph of a multipolar neuron. Note the accumulation of lipofuscin granules (arrowheads). Scale bars 52.5mm in C, 250μm in D, 25μm in E. Adapted from *Armour et al*.[25]

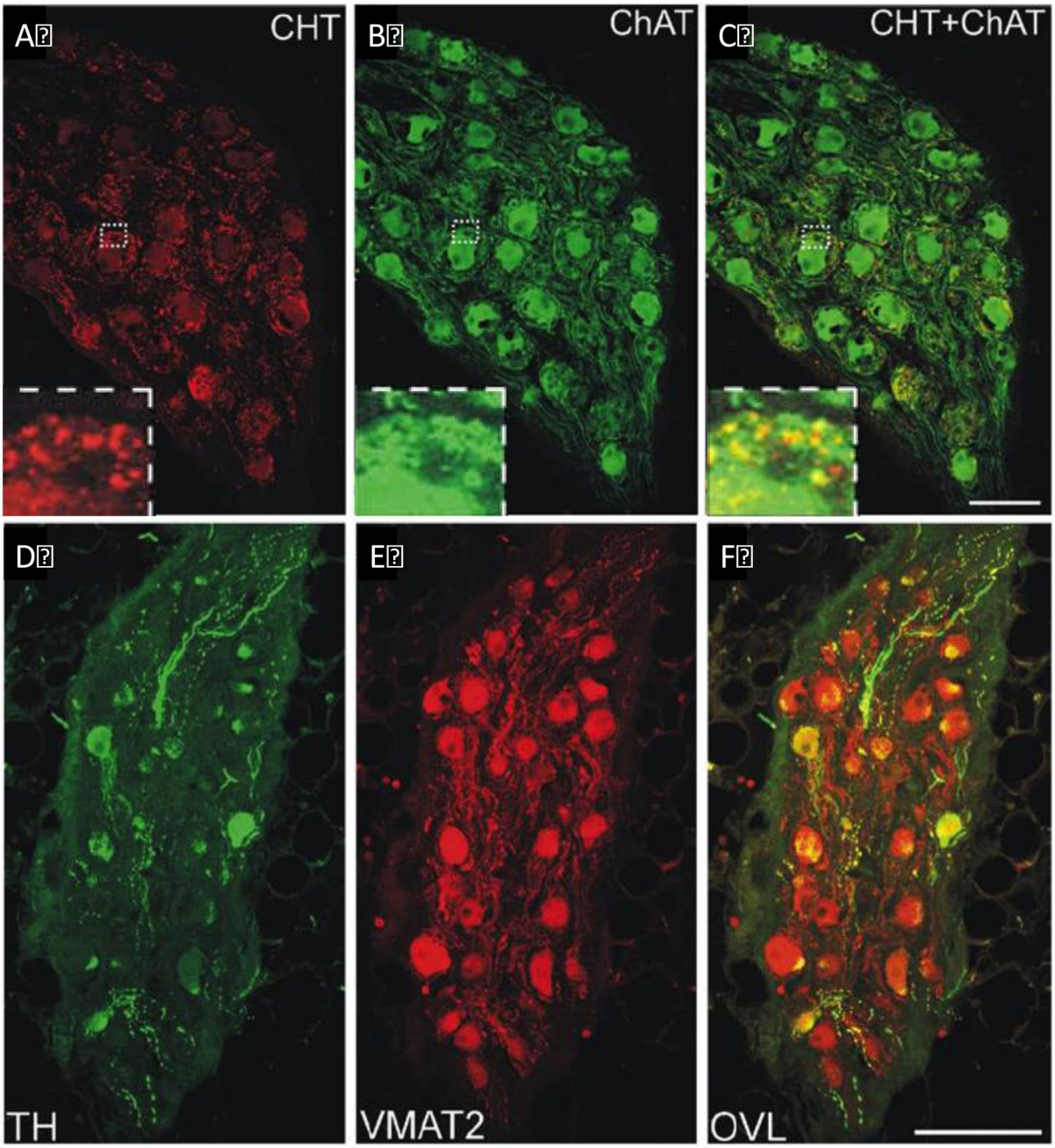


Figure 5.

Confocal images of human intrinsic cardiac neurons expressing the cholinergic and adrenergic phenotypes. A–C, Confocal images of a ganglion that was double labeled to show CHT (A) and ChAT (B). CHT immunoreactivity (A, red) was prominent in varicose nerve fibers around intrinsic cardiac neurons and faint or absent in the neuronal cell bodies. In contrast, staining for ChAT (B, green) was notable in neuronal cell bodies and generally less intense in surrounding nerve processes. (C) Colocalization of CHT and ChAT (yellow) was noted in some cell bodies and nerve processes in the overlay image (CHT+ChAT). Inserts at lower left show boxed areas at higher magnification. All panels contain maximum projection

images compiled from confocal scans that spanned 8μm. Scale bar = 100μm in A–C. D–F Confocal images of a section that was double labeled to show TH (D, green) and VMAT2 (E, red). (D) Few neurons and nerve fibers stained for TH. (B) Prominent staining for VMAT2 occurred in most neurons and many nerves fibers. (C) Overlap (OVL) of TH and VMAT2 images shows that a significant amount of the TH colocalizes (yellow) with VMAT2. All panels contain maximum projection images compiled from confocal scans that spanned 10μm. Scale bar = 150μm. Adapted from *Hoover et al.*[129]

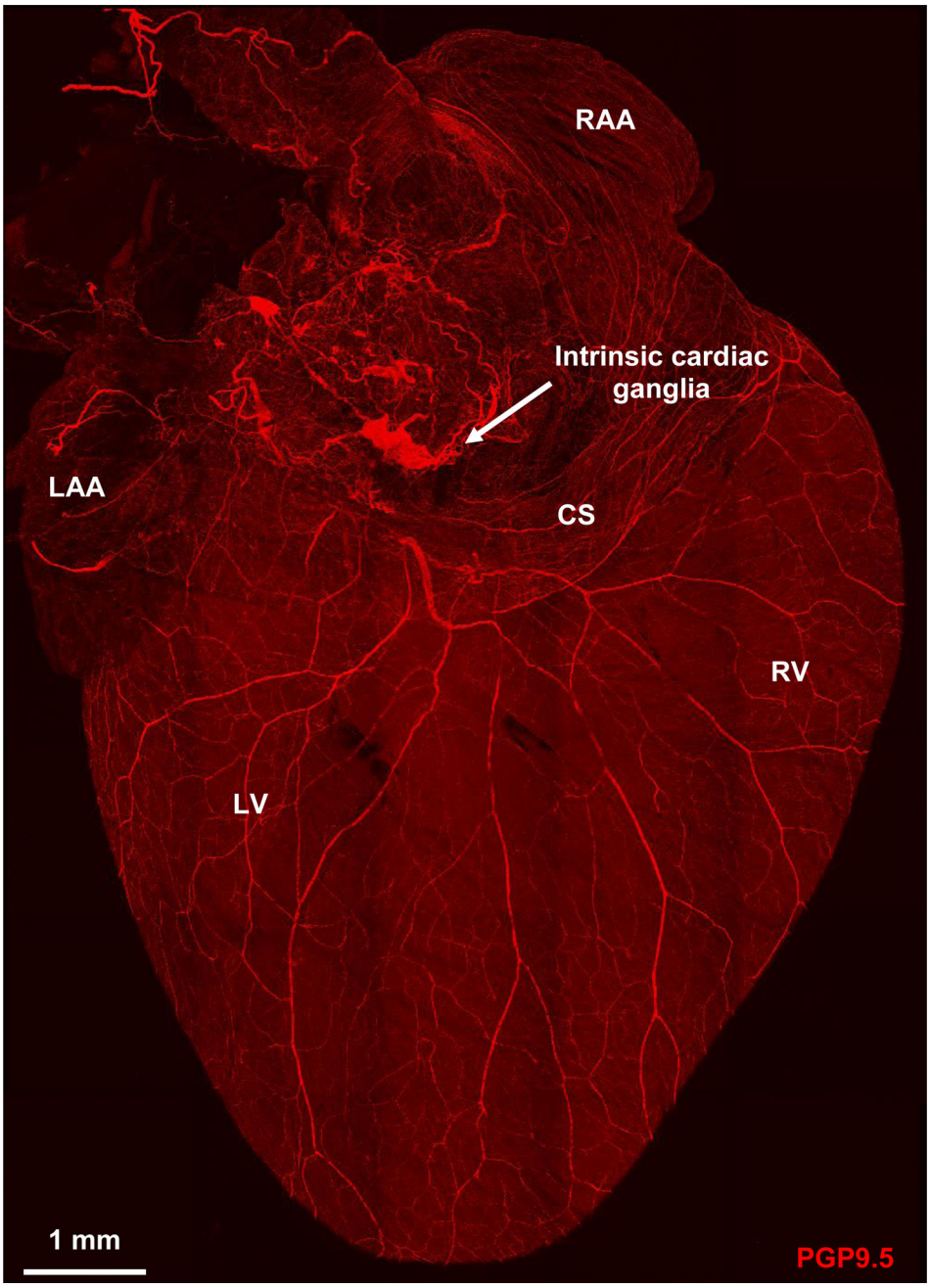


Figure 6.

Confocal image of dorsal surface of mouse heart stained with pan-neuronal marker protein gene product 9.5 (PGP9.5) and demonstrating the rich innervation of the heart. CS, coronary sinus; LAA, left atrial appendage; LV, left ventricle; RAA, right atrial appendage; RV, right ventricle.

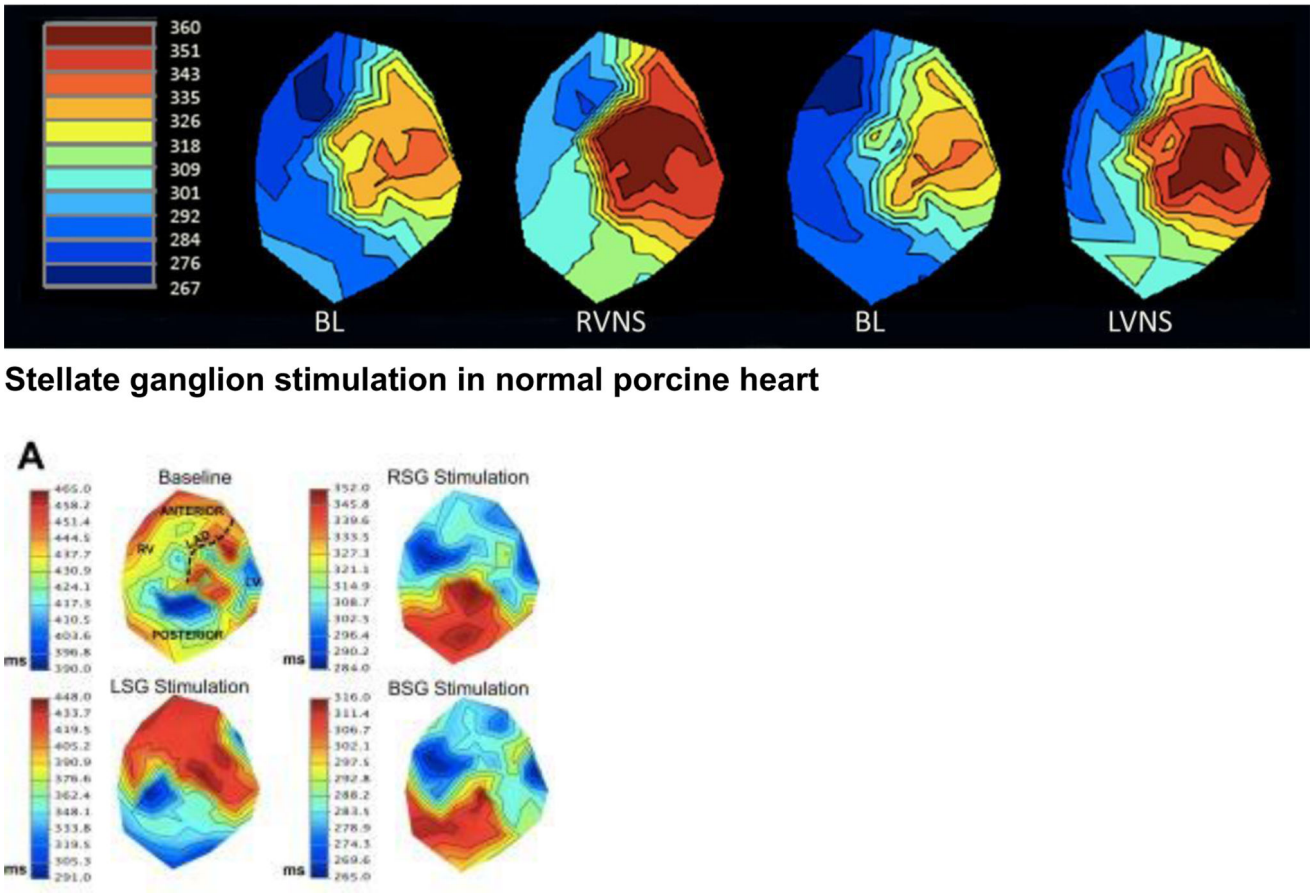
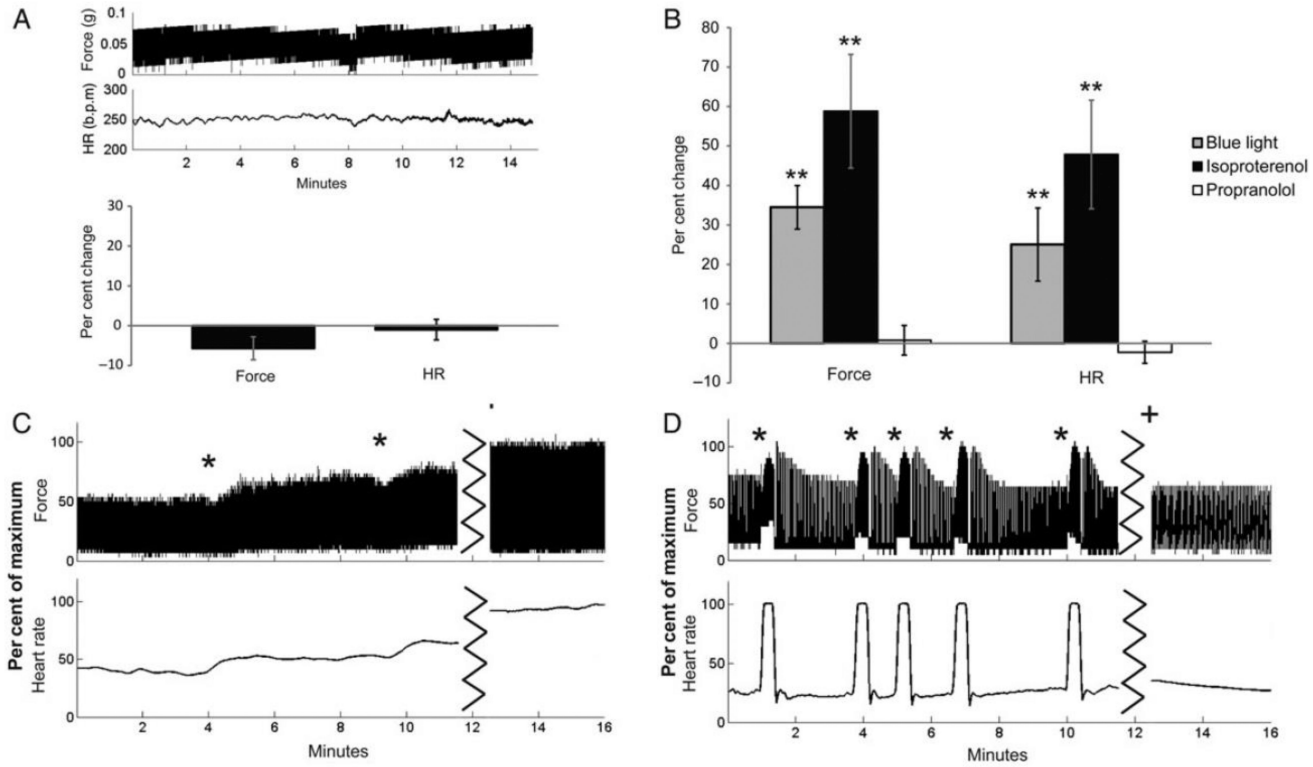


Figure 7.

A, Activation recovery interval (ARI) maps at baseline and during right (RVNS) and left (LVNS) vagal nerve stimulation in a normal porcine heart. No significant regional differences in responses were found. Adapted from *Yamakawa et al.*[79] B, ARI maps in control porcine hearts at baseline (BL) and during right (RSG), left (LSG) and bilateral (BSG) stellate ganglion stimulation. Myocardial regions are displayed in the BL control map. Adapted from *Ajijola et al.*[130]



Effects of blue light photostimulation on heart rate and contractile force in Langendorff-perfused mouse hearts. A, Stability of force and heart rate shown over 15 minutes (top) and expressed as percent change (bottom, n = 8). B, Percent change from control phase after stimulating with blue light (grey, n = 10), administering isoproterenol (black, n = 5), or stimulating with blue light after administering propranolol (white, n = 5). C, Changes in force and heart rate after two rounds of blue light photostimulation (*) and addition of isoproterenol (+). D, Changes in force and heart rate after several rounds of blue light photostimulation (*) and response to photostimulation after administration of propranolol (+). **P<0.05 statistically different form baseline. Adapted from *Wengrowski et al.*[97]