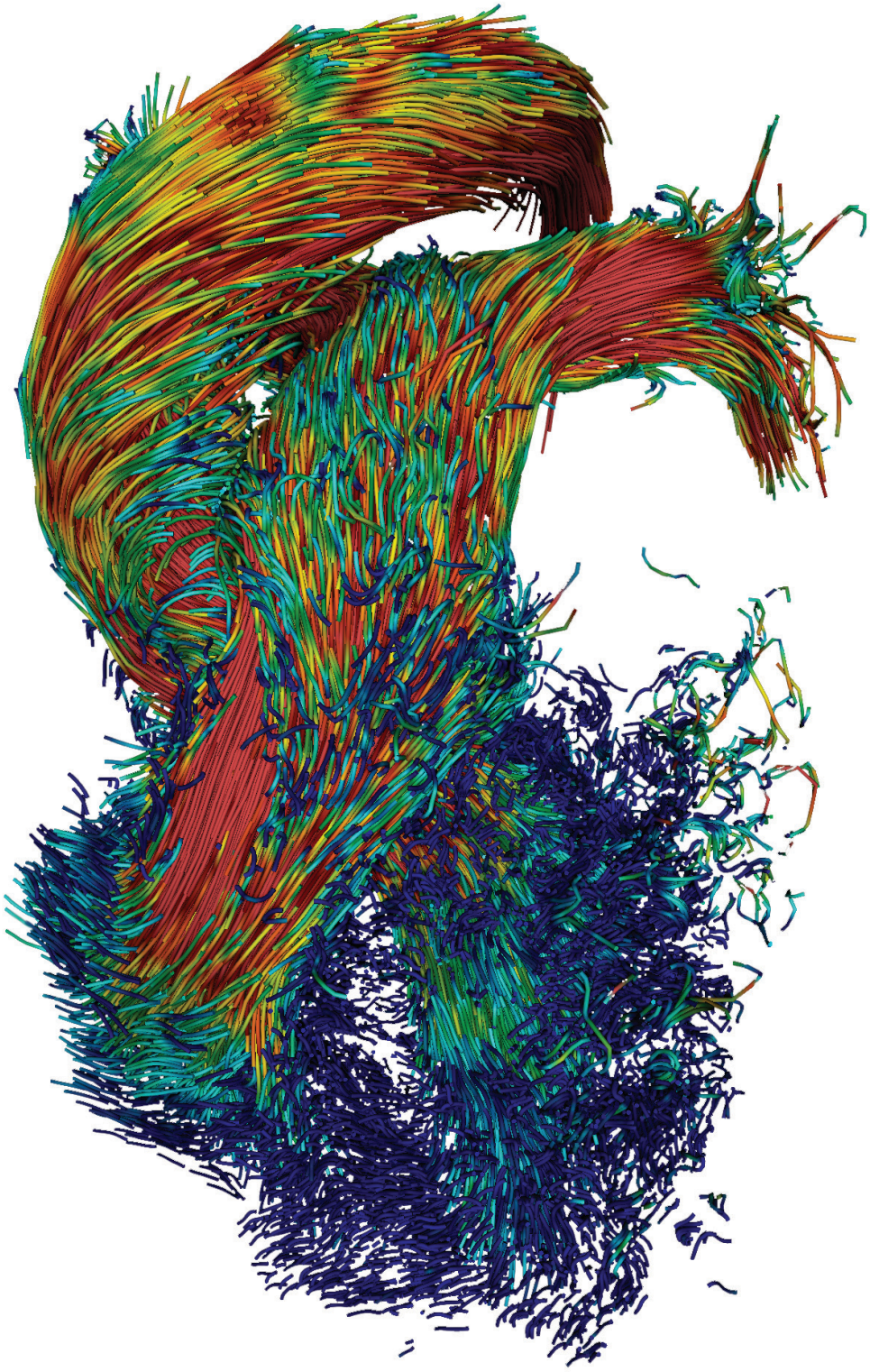
Cardiovascular effects of thoracic epidural anaesthesia

Wink, J.



Section II

Anatomy and physiology

Chapter 3

Human Cardiac Sympathetic Innervation: Controversies in anatomy and relevance for cardiac neuronal modulation

Jeroen Wink, Rogier van Delft, Robbert GE Notenboom,
Patrick F Wouters, Marco C DeRuiter, Monique RM Jongbloed

Submitted

Introduction

A balanced function of the cardiac autonomic nervous system (cANS) is essential to maintain cardiovascular homeostasis. The sympathetic nervous system has been attributed an important role in the perioperative stress response induced by surgery and anesthesia but is also implicated in the genesis and maintenance of atrial and ventricular arrhythmias[1-3] as well as in the pathogenesis of heart failure[4, 5]. Thoracic epidural anesthesia (TEA) and paravertebral blockade are regularly employed as analgesic techniques in cardiothoracic anesthesia. Besides sensory and motor blockade, TEA (T1-T5) and paravertebral blockade also induce blockade of sympathetic outflow to the heart **(Figure 1)**.

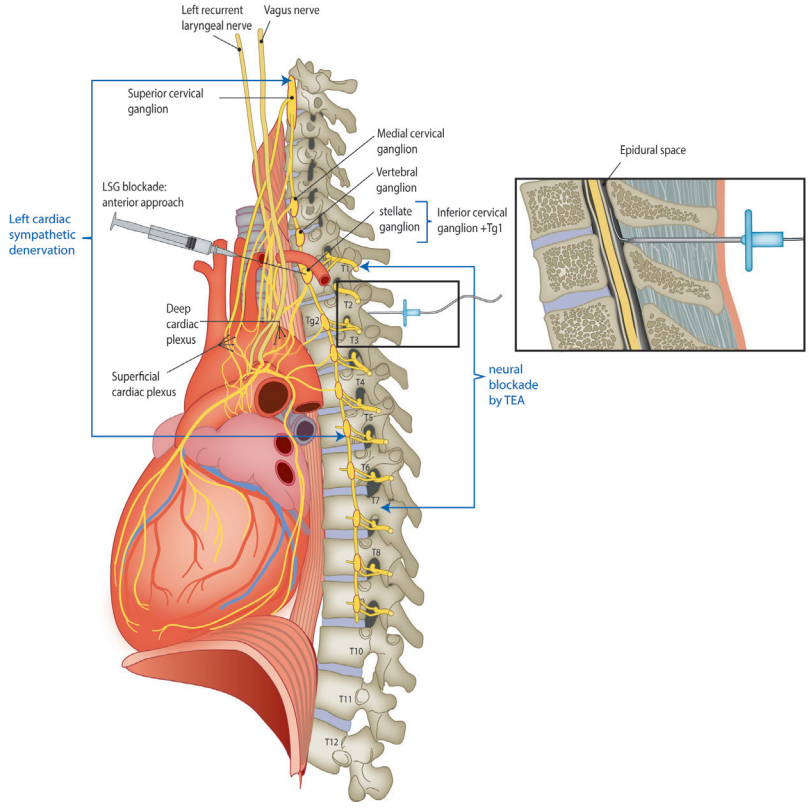


Figure 1. Relationship between level of neuronal modulation and sympathetic input to the heart

Preganglionic cardiac sympathetic axons synapse with postganglionic sympathetic neurons in the cervical or upper thoracic ganglia (Tg); postganglionic fibers from these ganglia form the sympathetic cardiac nerves that innervate the heart via the deep and superficial cardiac plexus. Neuronal modulation of cardiac sympathetic innervation may be achieved by TEA at the spinal level, by left stellate ganglion (LSG) blockade, by blockade of the upper thoracic ganglia, or by paravertebral blockade (i.e. blockade of sympathetic chain ganglia). TEA with upper border of analgesia above spinal segment T7, but certainly above T5, includes blockade of the sympathetic cardiac nerves. The amount of spinal levels blocked depends on the dose of local anesthetic drugs administered epidurally.

This reduces the chronotropic and inotropic state of the heart, the occurrence and magnitude of which seem to vary between individuals and conditions[6]. In heart failure, an increased sympathetic tone is considered to underlie a chain of detrimental effects with detrimental impact on prognosis[5, 7, 8]. Several arrhythmias have been related to an imbalance of autonomic innervation. Blockade of cardiac sympathetic innervation has been shown to improve myocardial blood flow and myocardial oxygen balance during stress[9], but is also a novel therapeutic approach for arrhythmias and heart failure[9-14].

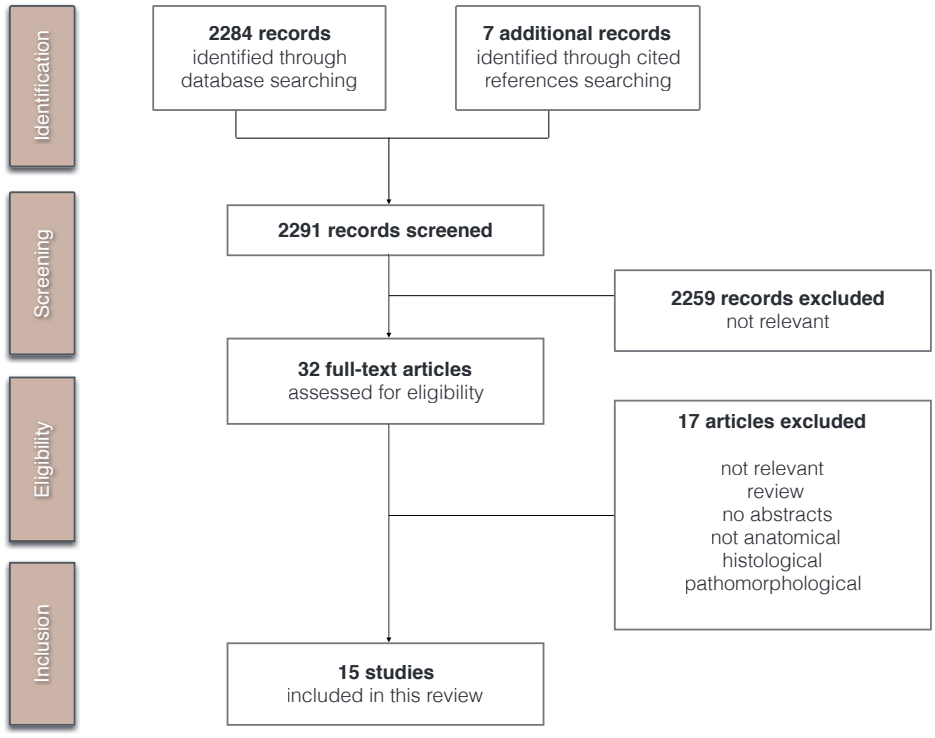
Although these techniques are aimed at targeting cardiac autonomic innervation, controversies regarding the anatomy of the human cardiac autonomic nerve system still exist. State of the art information on unresolved details and controversies regarding the anatomy of cardiac sympathetic innervation is relevant for understanding the cardiovascular effects elicited by cardiac sympathetic denervation. In addition, anatomical variability in cardiac sympathetic innervation between human subjects may contribute to inter-individual diversity in physiological effects and cardiovascular side effects of targeted cardiac sympathetic blockade.

This review aims to provide an update on current knowledge on anatomy and function of the human cardiac sympathetic nervous system and focuses on controversies as well as gaps in knowledge on this subject with reference to the clinical practice of neuraxial modulation of the cardiac sympathetic nervous system. The sympathetic innervation of the heart is known to vary by species[15]. In the current review we will discuss primarily the human anatomical arrangement of sympathetic outflow from the spinal cord and sympathetic ganglia to the heart, its heterogeneity and inter-individual variability. In addition, a brief overview of embryogenesis of cardiac innervation, including the cervicothoracic ganglia, is provided.

Methods

To summarize the current knowledge on human anatomy of cardiac autonomic innervation, below we present a narrative review of the extant literature on this topic. Papers regarding macroscopic human anatomy of the cANS were systemically reviewed. In addition an overview of major morphogenetic processes as well physiological background information is provided, which were not part of the systemic literature search. The database Pubmed was searched to identify anatomical studies of human cardiac sympathetic innervation.

The search strategy consisted of the following thesaurus terms and text words: ("innervation" [Subheading] OR innervat*[ti] OR re-innervat*[ti] OR "nerve"[ti] OR "nerves"[ti] OR "nervous"[ti] OR "neural"[ti]) AND ("sympathetic"[tw] OR "sympathic"[tw] OR "autonomic"[tw]) AND ("Heart"[majr] OR "Heart"[ti] OR "cardiac"[ti] OR "epicardial"[ti] OR "epicardiac"[ti] OR "Pericardium"[majr] OR "epicardium"[ti] OR "intracardiac"[ti] OR "extracardiac"[ti]) AND ("anatomy and histology"[Subheading] OR "anatomy"[tw] OR "anatomy"[MeSH] OR "anatomic"[tw] OR "anatomical"[tw]) NOT ("Animals"[Mesh] NOT "Humans"[Mesh]) NOT (Clinical Study[ptyp] OR Clinical Trial[ptyp] OR Controlled Clinical Trial[ptyp] OR "trial"[tw] OR "trials"[tw]) AND (Dutch[lang] OR English[lang] OR German[lang]). English papers were included and additional filters on species (human) and language (English or German) were employed. Physiological studies, pathomorphological studies, studies based on microscopy/histological findings only, studies without abstracts reviews, research concerning embryology, animals or organs other than the cardiovascular system were excluded. The search yielded a total of 2284 references an 7 additional references identified through cited references. After screening only 15 studies were included in the review (**Flowchart 1**). The anatomy of the human cardiac autonomic nerve system from the central level towards the end organ (i.e. the heart) was described using these literature sources (**Table 1**) and special attention was given to discrepancies and variations in anatomy as described from these reports.



Flowchart 1. Flow diagram of literature search

Overview of cardiac neural hierarchy: from brain to heart

Several forebrain areas, including the insular cortex, anterior cingulate cortex, central nucleus of the amygdala, and several hypothalamic nuclei project to medullary and spinal nuclei controlling cardiac function **(Figure 2)**; these projections are either direct or via a relay in the periaqueductal gray[16].

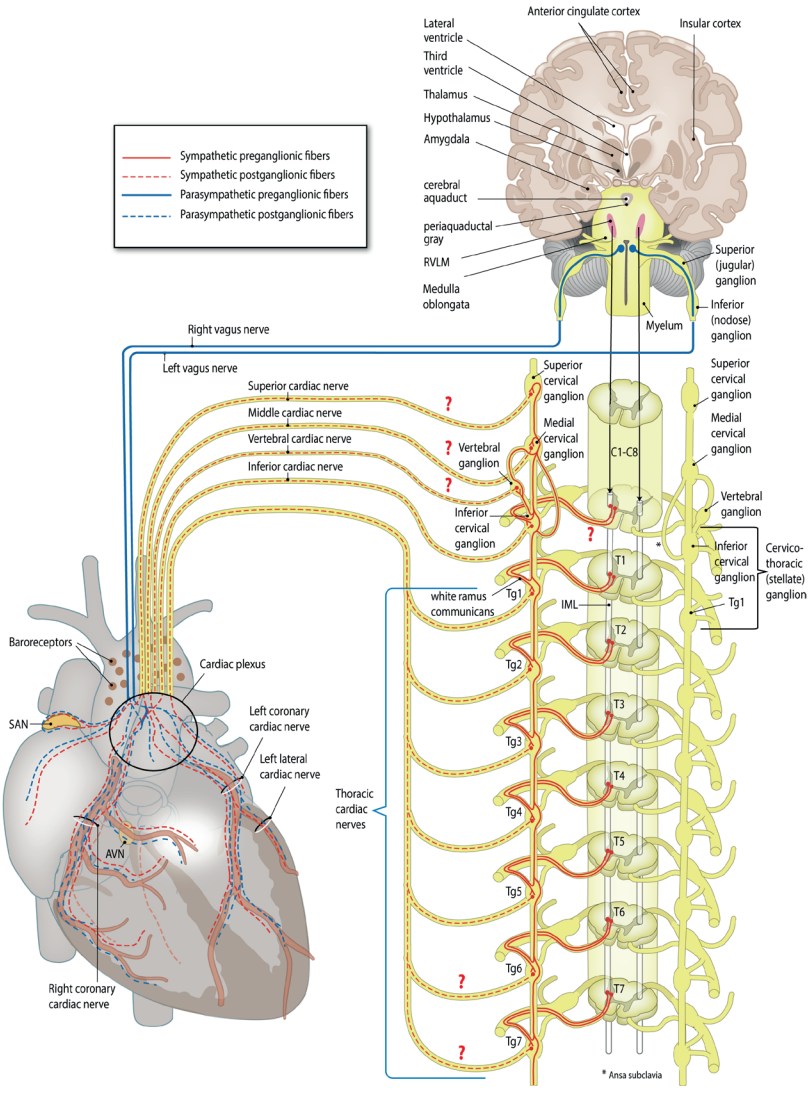


Figure 2. Overview of cardiac innervation

Schematic drawing of the cardiac autonomic nervous system. Preganglionic cardiac parasympathetic axons arise from neurons in either the nucleus ambiguus or dorsal vagal nucleus; they run in vagal cardiac branches vagus nerve (blue, solid lines) to synapse in cardiac plexuses and ganglia from where postganglionic fibers (blue, dotted lines) innervate the sino-atrial node (SAN), atrioventricular node (AVN), coronary arteries and

ventricular myocytes. Preganglionic cardiac sympathetic axons (red, solid lines) arise from neurons in the IMLs of the upper four or five (possibly six or seven) thoracic spinal segments, that receive modulating input from several forebrain centers (e.g. the insular cortex, anterior cingulate cortex, central nuclei of the amygdala, and several hypothalamic nuclei interneurons) via the intermediolateral cell column of the spinal cord (IML); they leave the spinal cord through anterior (ventral) roots, enter the anterior (ventral) rami of spinal nerves and pass to the sympathetic chains through white rami communicantes to synapse in the upper thoracic (Tg) or cervical ganglia; postganglionic fibers (red, dotted lines) from these ganglia form the sympathetic cardiac nerves. At the heart parasympathetic and sympathetic nerves converge to form the cardiac plexus from which atrial and ventricular autonomic innervation is arranged.

The red question marks (?) indicate anatomical structures of which existence and/or involvement in cardiac sympathetic innervation are debated.

The cANS can be divided into extrinsic and intrinsic components. The extrinsic cardiac nervous system (i.e. the part of the cANS outside the heart) comprises sympathetic and parasympathetic nerves that control the intrinsic cardiac nervous system (i.e. the cANS situated at the cardiac surface and within the cardiac chambers) in an opposing fashion. The interaction between sympathetic and parasympathetic activity is complex and is modulated by input from chemoreceptors and baroreceptors via the visceral sensory fibers[17]. The intrinsic cardiac nervous system is formed by a complex network of ganglionated plexuses located in the myocardial wall and pericardial fat[18, 19]. Within this network functional communications between neurons of the ganglionated plexuses exists, supplying the myocardial tissue including the cardiac conduction system[20, 21]. In general, sympathetic activation is triggered by neurons of the rostral ventrolateral medulla (RVLM, situated in the medulla oblongata, **Figure 2**), hich sends excitatory projections to preganglionic sympathetic neurons of the intermediolateral column (IML) of the spinal cord, that extends from spinal levels C8/T1 to T2/T3[22]. After synapsing in paravertebral ganglia, postganglionic sympathetic fibers will eventually innervate heart, via cardiac nerves that sprout directly from the sympathetic chain ganglia. These cardiac nerves (superior, middle, vertebral and inferior nerves) can either innervate the heart directly or may combine with other postganglionic (sympathetic and parasympathetic) nerves to form plexuses consisting of combined nerves innervating the heart (intrinsic cardiac nervous system)[23] (**Figure 2**). From the cardiac plexuses, mixed cardiac nerves arise that largely run along the course of coronary vessels to innervate the coronary vasculature and myocardium (i.e the right coronary, left coronary and left lateral cardiac nerves). Parasympathetic output to the heart is mediated by preganglionic neurons located in either the dorsal motor nucleus of the vagus nerve or near the nucleus ambiguus[24]. In contrast to sympathetic preganglionic neurons that synapse on postganglionic neurons in the sympathetic chain, the parasympathetic preganglionic neurons send long axons that synapse on cholinergic and non-cholinergic postganglionic neurons located in the cardiac ganglia, situated in proximity to the heart (intrinsic cardiac nervous system).

Embryogenesis of cardiac autonomic innervation- overview of major morphogenic processes

Literature on human development of the autonomic nerve system consists of several historical papers describing human embryos based on observations using light and electron microscopy, some dating back as far as 1893[25]. More current molecular studies providing insight into embryological background and genetic pathways involved, are largely conducted in animal models.

Spinal level and intermediolateral column (IML).

The central parts of the nervous system, i.e. the brain and spinal cord (myelum) derive from the embryonic neural tube, which starts to form in humans at approximately 3 week of gestation (reviewed in[26]). In the neural tube, the first sign of preganglionic sympathetic motor neurons has been described in rat in the ventrolateral zone of the spinal cord, where also the somatic motor neurons are situated. During further development, these autonomic motor neurons will separate from the somatic motor neurons and are then found more dorsally where they will form the IML[27] **(Figure 2)**. The autonomic motor neurons in the IML will send their extensions out via the anterior aspect of the neural tube, i.e. the future anterior (ventral) root. These extensions will synapse with clusters of postganglionic motor neurons that develop outside the central nervous system, the sympathetic chain ganglia, that will give rise to the sympathetic innervation of the heart.

Development of sympathetic chain ganglia and output towards the heart.

In chicken embryos, the first clusters of catecholamine positive cells have been observed at the thoracolumbar level and bilateral to the aorta[28]. These cellular clusters will expand from the thoracic to the cervical region. During further development the clusters of cells on both sides of the aorta will form continuous cords, regarded as a primitive sympathetic chain, that will further differentiate and give rise to secondary (permanent) paravertebral chains[28], directed by several ligands and their receptors[29]. The cell type most well established in development of the ganglia of the paravertebral sympathetic chains is the neural crest cell. Neural crest cells are a population of multipotent cells that migrate from the region of the neural tube (Figure 3) and have multiple functions during development[30]. Innervation of heart occurs via the arterial and venous poles, corresponding to different subpopulations of neural crest cells[31] the arterial pole seems to be the major source of input of sympathetic nerves to the heart, whereas parasympathetic nerves arrive at the heart mainly via the venous pole[31].

Not all cardiac autonomic nerves tissues are neural crest cell derived, and other cell types, such as those derived from the neurogenic placode, may also contribute.

The sympathetic chains will give rise to the rami communicanti, connecting them to the anterior (ventral) rami of spinal nerves. In addition, autonomic cardiac nerves will leave the ganglia forming cardiac sympathetic nerves, which is first observed in chick in the lower cervical/upper thoracic region[32]. During further development, sympathetic cardiac nerves will also connect to

vagus nerves and other cardiac sympathetic nerves towards the heart will form[33]. Development of parasympathetic peripheral nerves (derived from the cranially situated cardiac neural crest), precedes the development of sympathetic peripheral nerves[34].

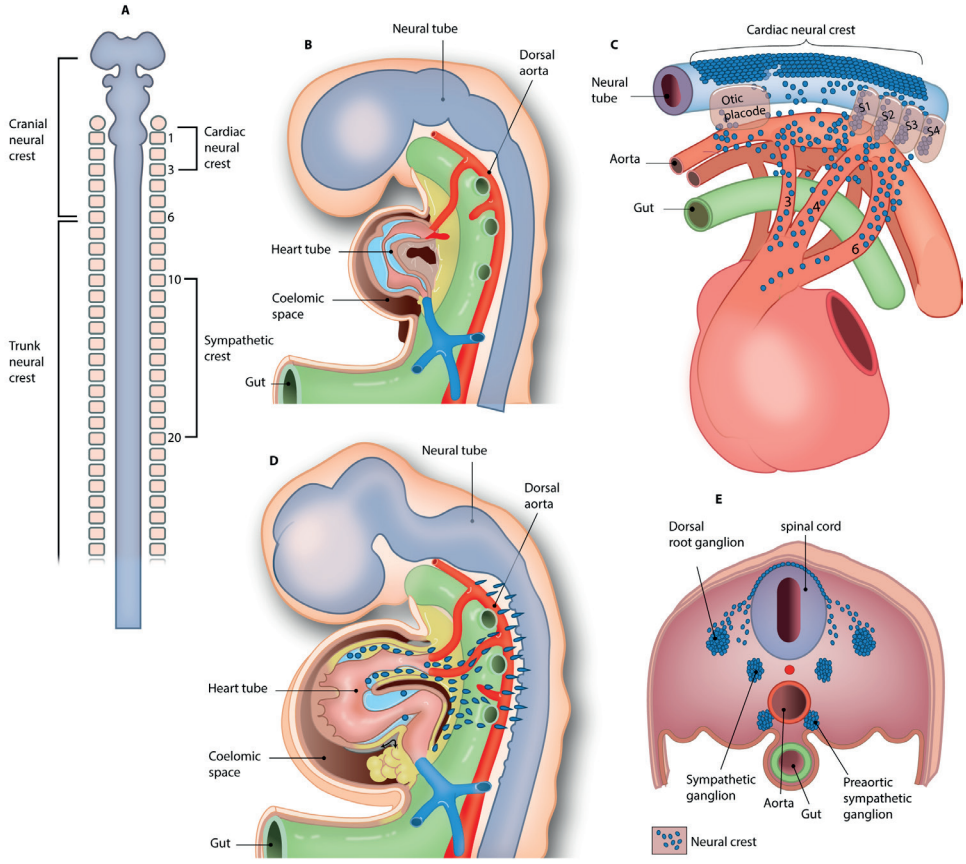


Figure 3. Embryology of cardiac autonomic innervation

a. The central parts of the nervous system, i.e. the brain and spinal cord derive from the embryonic neural tube (light blue), that develops in human at approximately 4 weeks of gestation. **b-d.** During development, neural crest cells (indicated in dark blue) will migrate from the region of the neural tube and will migrate towards multiple locations in the body, including the heart. They can differentiate in multiple cell types Neural crest cells contributing to the sympathetic chain originate from the chain neural crest. The first clusters of cathecholamine positive cells have been observed at the thoracolumbar level and bilateral to the aorta. These cellular clusters will expand from the thoracic to the cervical region. During further development the clusters of cells on both sides of the aorta will form primitive sympathetic chains that will further differentiate and give rise to paravertebral sympathetic chains. Neural crest cells also contribute to other ganglia, including the dorsal root and pre-vertebral (pre-aortic) ganglia. Panel a is modified after Kirby[33]. Panels b and c modified after Vegh et al[38].

Development of cardiac ganglia, plexuses and nerves.

In human embryo's, cardiac ganglia and nerves could be identified at the 20 mm stage, corresponding to approx. 7-8 weeks of gestation[25, 35]. Saburkina et al. describe prenatal development of epicardiac ganglia in 20 hearts of human fetuses of gestational age 15-40 weeks[36] showing the presence of epicardiac neural plexuses in all stages examined. The size of the ganglia, as well as the number of inter-ganglionic nerves, increased with gestational age, although interindividual variations were observed[36].

Navaratnam report development of the deep cardiac plexus to result from fusion of nerves at the venous pole with innervation at the arterial pole at later developmental stages (40 mm stage, > 8 gestational weeks), due to definitive positioning of the heart with the venous and arterial pole becoming more closely situated. The superficial cardiac plexus was recognized at the site of the arterial duct[25]. In human, the first presence of nerve fibers into the heart has been described at approximately 6 weeks intra-uterine life[25]. During development the amount of cardiac innervation is orchestrated by neural chemo-attractants and chemo-repellents, the balance of which determines the extent of cardiac innervation[37, 38].

Innervation of conduction tissues.

In early histological studies in human embryos, the putative sites of the sino-atrial node (SAN) and atrioventricular node (AVN), i.e. the embryonic right sinus horn and the dorsal AV canal, were shown to be heavily innervated at 5-6 weeks post-ovulation, even prior to development of the nodes[25]. Early studies in human report contributions of the so called right sinus nerve to innervation of the sino-atrial node, of the left sinus nerve to the AVN and contributions from both sides of the body to the single pulmonary vein. More recent studies based on lineage tracing in mouse, however, showed innervation of the nodes only at embryonic day (E) 13.5 and E14.5 (corresponding to >7 weeks in human). Of interest, these nerve fibers were not derived from neural crest cells but likely from another source[31]. In postnatal humans, density of innervation was found to be highest in the sino-atrial node, with decreasing density towards AV node and more distal parts of the cardiac conduction system. Moreover, there was an initial sympathetic dominance in nerve supply of the CCS in childhood, with gradual transition into a sympathetic and parasympathetic co-dominance in adulthood[39].

Maturation of the ANS is reflected by an increase in heart rate variability with an overt increment of sympathetic activity[40]. Functional studies based on human fetal cardiotocography indicate that the period from 21 to 31 gestational weeks seem critical to ANS development[41], although many of these functional studies not include embryonic/early fetal stages.

Ventricular innervation.

During development of ventricular innervation, cardiac nerves extend parallel to coronary vessels. Vascular smooth muscle cells of the coronary vessels have been shown to secrete nerve growth factor, a neurotrophic factor, thus guiding the patterning of autonomic (sympathetic)

ventricular innervation[42]. The autonomic nerve system maintains some plasticity after birth and in disease states[43]. This, along with the fact that neural crest cells are multipotent and can currently be derived from human pluripotent stem cells stem cells (hiPSCs), opens avenues for potential future applications in patients with autonomic nerve damage[44].

Controversial issues.

Cervical ganglia have been shown to contribute to cardiac innervation both in animal models as well as in human[45, 46], although reports in literature differ (discussed in paragraph 6.1). The origin of the cervical sympathetic chain ganglia is still debated. Based on the observation that cellular clusters will expand from the thoracic to the cervical region as describe above[26], it has been speculated that cervical ganglia are generated from the thoracic sympathetic chain[45, 47]. As there are only 3-4 cervical ganglia in the cervical region whereas at the thoracic level each spinal level has a corresponding ganglion, alternatively, it has been suggested that the development of sympathetic ganglia is associated initially with the intersegmental vessels[48]. The limited number of cervical ganglia could thus be attributed to regression of most of the cervical intersegmental arteries, and remodeling and fusion of the corresponding ganglia. The upper 4 cervical ganglia would thus form the superior cervical sympathetic ganglion, anatomically related or induced by the developing external carotid artery[48]. The number of ganglionated plexuses reported in fetal hearts differs somewhat between studies: Saburkina et al report the presence of 7 ganglionated plexuses[36], whereas in an earlier study of Smith 4 groups of ganglia were identified[49]. The same study reported "darkly staining cells lying between the aorta and pulmonary artery "to be visible at the 15 mm stage, indicative of a deep cardiac plexus[49], whereas a distinction between deep and superficial plexuses could not be made by Than et al[50].

With regard to innervation of the myocardium, there is controversy on the density of autonomic innervation in atrial versus ventricular tissues. Parasympathetic (vagal) nerves appear to be more densely distributed in the atria at both neonatal and adult stages[51, 52]. Some authors describe equal densities of sympathetic nerve fibers in atria and ventricles, at least in the neonatal stage[51], whereas other authors describe more sympathetic nerves in the ventricles in the adult heart[52]. This might indicate that differentiation of the cANS still occurs after birth, supported by observation of dynamic changes in nerve supply throughout life[39] .

Sympathetic output from the spinal cord towards the sympathetic chain

In general, exit of sympathetic outflow from the spinal cord only occurs in designated levels, primarily from the first thoracic to the second or third lumbar spinal level[22].

Bonica and colleagues documented that the human preganglionic cardiac sympathetic output from the spinal cord originates from the first to the fourth, and sometimes even fifth thoracic spinal cord segment[53]. This study is often cited in thoracic epidural anesthesia studies as an anatomical reference of cardiac sympathetic innervation. To our knowledge the study of Bonica and colleagues is to date the only human anatomical document describing cardiac sympathetic

innervation from the level of the spinal column in humans. The IML fibers contributing to sympathetic autonomic innervation of the heart leave the spinal cord in anterior roots, After leaving the spinal cord in anterior (motor) roots, preganglionic sympathetic fibers enter the spinal nerves, pass through the anterior rami and travel via white (i.e. myelinated) rami communicantes towards the paravertebral ganglia of the sympathetic chain **(Figure 4)**, where they synapse with postganglionic neurons **(Figures 2, 4).** Preganglionic neurons may synapse with as many postganglionic neurons[54]. The synapse may occur at the same level, or the preganglionic fiber may ascend and, possibly, descend before synapsing **(Figure 4)**.

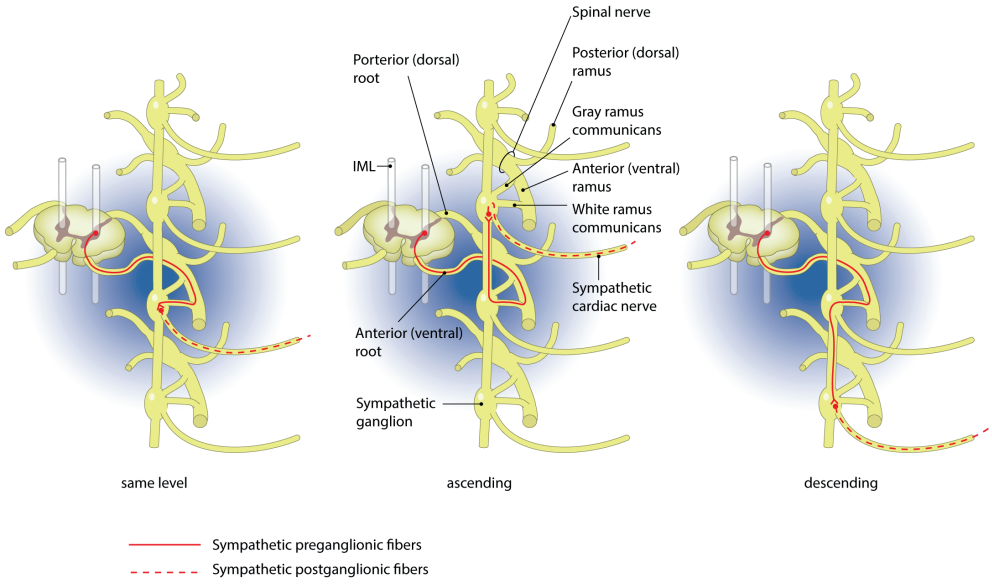


Figure 4. Courses taken by preganglionic sympathetic fibers

After leaving the spinal cord via the anterior (ventral) root, the axons of preganglionic sympathetic neurons enter the anterior (ventral) ramus of the spinal nerve and pass to the sympathetic chain through a white ramus communicans. Within the sympathetic chain preganglionic fibers may 1) synapse immediately with a postganglionic neuron of the paravertebral ganglion at that level, 2) ascend in the sympathetic chain to synapse with a postganglionic neuron of a higher paravertebral ganglion, or 3) descend to synapse with a postganglionic neuron of a lower paravertebral ganglion.

IML, intermediolateral column of the spinal cord.

Controversial issues regarding levels of sympathetic output form the spinal cord towards sympathetic ganglia.

Some authors suggest that sympathetic nerves may also emerge from the cervical myelum[55, 56]. Although there is a clear transition from C8 to T1 in the amount of sympathetic fibers present, Sheehan and colleagues observed sympathetic fibers in the anterior roots of C8 of humans (see question marks in **Figure 2**). It is however important to emphasize that the amount of fibers

observed in the cervical roots was scarce, especially compared to the amount of fibers in the anterior roots of T1-T2[55]. More importantly, presence of sympathetic fibers in the anterior root of C8 does not necessarily imply that these fibers are cardiac destined fibers.Controversy also exists on the level where preganglionic cardiac sympathetic fibers synapse within the sympathetic chain. The sympathetic preganglionic neurons of spinal cord T1-T5 have been described to either synapse in the first sympathetic ganglion they reach or to ascend within the sympathetic chain to synapse in a ganglion at a higher level, particularly to the three or four cervical ganglia[54]. In addition, sympathetic preganglionic neurons of spinal level T6 to as low as T10 have been described to either terminate in the first sympathetic ganglion they reach, ascent or even descent to a higher or lower thoracic ganglion[54] **(Figure 2, Figure 4)**. This raises the question from which thoracic spinal cord levels the preganglionic sympathetic fibers originate that eventually end up as thoracic cardiac nerves innervating the heart. This is relevant since TEA blocks sympathetic outflow at the spinal level. Several animal studies using transneural retrograde labelling support the observation of Bonica that preganglionic sympathetic neurons can ascent or descent to a higher or lower ganglion from where postganglionic fibers are projected towards the heart. Markers injected in the stellate (cervicothoracic) ganglion of rat were found in spinal segments C8 to T8 with a peak at T2[57]. However, whether these spinal segments are involved in sympathetic innervation of the heart or that other regions are targeted remains to be elucidated. In addition, the latter study suggests that the axons of sympathetic preganglionic neurons from one spinal cord segment may branch and have axonal projections to multiple cervical and thoracic ganglia. In another study, rat hearts were injected with retrograde tracers, which were subsequently found in the preganglionic sympathetic neurons of spinal cord levels T1-T7 and in some rats even in spinal levels T8-T11[58].

A problem in interpreting results and extrapolating them to the human situation, is that many studies have been performed on animals and sympathetic innervation of the heart varies by species[15]. Another limitation is that a substantial number of reference studies are dated several decades ago, when limited techniques e.g. for neuronal tracing and specific immunohistochemical labelling were available, or altogether unavailable. Therefore it seems difficult if not impossible to state with certainty that the small fibers described as representing the sympathetic outflow really are of sympathetic origin. Bonica did not describe the method that was used to detail the preganglionic origin of cardiac sympathetic innervation[54]. Moreover, neural tracing techniques cannot be performed in humans. Interpretation of results of immunohistochemical stainings in many cases relies on (alleged) specificity of immunohistochemical stainings to discriminate the different divisions of the autonomic nervous system. If preganglionic sympathetic fibers from spinal level T5-T10 indeed ascent to higher paravertebral ganglia, this would imply that thoracic ganglia Tg1-Tg5 could be innervated from spinal levels below T5. From a theoretical point of view, it is not unlikely that preganglionic sympathetic neurons from spinal levels below T5 might be involved in cardiac sympathetic innervation.

In conclusion, the cranial border of spinal sympathetic outflow to the heart is most likely confined to T1 in most cases. However, sympathetic outflow from the cervical myelum has been described for C8 and to date it remains unclear whether this is a common variation and whether spinal cervical sympathetic outflow is involved in cardiac sympathetic innervation (see question marks in, Figure 2). Therefore the upper border of preganglionic sympathetic neurons originating from the spinal cord to the paravertebral ganglia providing the heart with postganglionic sympathetic fibers, might involve cervical spinal segment C8 as well. Similarly, the lower border of preganglionic cardiac sympathetic neurons may involve spinal levels below T5. This information might be relevant in case where for instance TEA is targeted to block all spinal cardiac sympathetic segments.

Sympathetic ganglia giving rise to postganglionic output to the heart

The sympathetic chain, including the stellate ganglion and other cervical ganglia, is a chain of paravertebral ganglia that exists on both anterolateral sides of the vertebral column. It is within the sympathetic chain that the preganglionic sympathetic neurons synapse to the cell bodies of postganglionic sympathetic neurons that extent via cardiac nerves to the heart (**Figure 2**).
The cervical ganglia, receiving preganglionic sympathetic input from the thoracic spinal cord via ascending fibers within the thoracic ganglia, are a well described extension of the sympathetic chain[45, 54, 59, 60]. Although nomenclature differs in literature over the past 50 years, the most accepted names that are therefore used in this review are: the superior cervical ganglion, the middle cervical ganglion, the vertebral ganglion and the inferior cervical ganglion **(Figure 2)**. Cervical ganglia that are generally accepted to provide postganglionic cardiac nerves are the inferior cervical ganglion, that is fused with the first thoracic ganglion in about 80% of humans to form the cervicothoracic or stellate ganglion[45, 61] **(Figure 2)**.

Controversies regarding involvement of cervicothoracic ganglia in cardiac sympathetic innervation.

As stated earlier, Bonica and colleagues reported preganglionic cardiac sympathetic outflow to emerge from cervical and the upper four to five thoracic spinal cord segments and postganglionic cardiac sympathetic outflow from the upper five thoracic paravertebral ganglia[53, 54]. However, there is controversy on the exact origin of (postganglionic) sympathetic cardiac innervation. Several anatomical studies on human cadavers show a wide variation in the lower limit of origin of cardiac sympathetic innervation. Where most human studies report that the thoracic cardiac nerves emerge from the first to fourth thoracic ganglia, others reported contributions from the fifth thoracic[54, 62], sixth thoracic[63] and even from the seventh thoracic ganglia[64] to the thoracic cardiac nerves (**Figure 2**). By contrast, Janes and colleagues reported no cardiopulmonary nerves arising from the superior cervical ganglia and sympathetic chain inferior of the stellate ganglion[59]. In conclusion the origin of cardiac sympathetic innervation, i.e. the level of the sympathetic ganglia giving rise to postganglionic cardiac nerves, has been shown to differ between anatomical studies and inter-individual variations may occur. Whether cervical ganglia

besides the stellate ganglion play a role in transmission of cardiac sympathetic signals is unclear. Similarly, there is debate on the origin of cardiac nerves from different thoracic ganglia. In some patients, thoracic ganglia Tg6 and Tg7 (see question marks in, **Figure 2**) might be involved in cardiac sympathetic innervation. If so, preganglionic sympathetic neurons from spinal segments T6 and T7 or, if ascending, even from spinal segments below T7 are likely to be involved in cardiac sympathetic innervation. In addition, these anatomical studies demonstrated inter-individual and intra-individual variety (asymmetry in left to right sympathetic innervation) in the anatomy of cardiac autonomic innervation. These variations may be relevant in procedures targeting the cardiac output to the heart, e.g. during left cardiac sympathetic denervation targeting the lower cervical/upper thoracic nerves.

Postganglionic output to the heart: Sympathetic cardiac nerves

The sympathetic chain gives off gray (i.e. unmyelinated) rami communicantes carrying sympathetic fibers to the spinal nerves which serve as motor nerves to the effector organs such as the skin and glands. However, postganglionic nerves to the heart from the cervical and thoracic sympathetic chain do not travel via gray rami with the spinal nerves but originate as separate (unmyelinated) cardiac nerves from the paravertebral ganglia to the heart (**Figure 2**). Thus, after passing the thoracic and cervical ganglia, sympathetic signals reach the heart via different nerves. The cardiac sympathetic nerves enter the heart through the vascular (arterial and venous) pole of the heart. At the arterial pole, the cardiac nerves extend along the common carotid, subclavian and brachiocephalic arteries towards the aorta and branches also extend along the pulmonary chain. At the venous pole, cardiac nerves run along the superior vena cava. Thoracic cardiac nerves are described to descend obliquely along the thoracic vertebrae or the intercostal vessels, sometimes following complex courses through the mediastinum, before heading towards the heart[45].

Cardiac nerves: Variations and controversies

Despite extensive anatomical research, the route and even number of these postganglionic nerves remains unclear. Kawashima described an important connection between the superior cervical ganglion and the heart, by the existence of the so called superior cardiac nerve[45, 60]. He further described the middle cardiac nerve originating from the middle cervical and the vertebral ganglion, and the inferior cardiac nerve originating from the inferior cervical/ cervicothoracic (stellate) ganglion. Besides these cervical ganglia, he reported that each of the upper 4 to 5 thoracic paravertebral ganglia has a sympathetic connection running towards the heart, mostly sharing the combined name of 'thoracic cardiac nerves' (**Figure 2**). This study by Kawashima confirms the previously reported cervical and thoracic sympathetic contributions to the cardiac plexuses by Pather et al[63]. In addition, de Gama and colleagues described in 41% of individuals a separate cardiopulmonary nerve from the vertebral ganglion, the vertebral cardiac nerve[65] (**Figure 2**). As mentioned above, Janes and colleagues, however, state that cardiopulmonary nerves only arise from the stellate (cervicothoracic) ganglia and the caudal halves of the cervical

sympathetic chains[59], leaving no role for transmission of sympathetic input to the heart for the superior cervical ganglion nor the thoracic paravertebral ganglia (see question marks in, **Figure 2**). In conclusion, it is still matter of debate which cervical ganglia play a role in the transmission of cardiac sympathetic signals. Regardless, there is evidence that besides the stellate ganglion other cervical and thoracic ganglia are involved in the transmission of sympathetic signals to the heart with potential relevance for cardiac neuronal modulation.

Combination of cardiac nerves to form mixed cardiac plexuses

Upon entering the heart, both postganglionic sympathetic and preganglionic parasympathetic (branches of vagus and recurrent laryngeal) nerves converge at the cardiac surface to form plexuses[20, 45, 60] (**Figure 1**). The superficial (ventral) cardiac plexus is located near the aortic arch and the left pulmonary artery on the left side and near the ascending aorta and brachiocephalic chain on the right side. The deep (dorsal) cardiac plexus is located between the aortic arch and the tracheal bifurcation[65]. Next to sympathetic nerves, the plexuses also receive parasympathetic contributions from the vagal nerve. Of interest, the vagal nerves have been shown to carry sympathetic nerve fibers[66]. Upon entering the pericardial sac, mixed autonomic nerves project to cardiac ganglia that are interconnected by neurons, thus forming ganglionated plexuses or epicardial neural plexuses at the vascular (arterial and venous) pole of the heart. These plexuses are embedded in the epicardial fat (**Figure 5**). The largest amount of ganglia is located at multiple sides near the atria. Ventricular ganglia are mostly distributed in the epicardial fat near the aortic root and adjacent to major branches of the coronary arteries[18, 67, 68]. With a total amount of cardiac ganglia observed between 706 and 1,506 and an estimated amount of neurons in the epicardial neural plexus between 14,000 and 43,000 the human intrinsic cardiac nervous system is very extensive[67, 69]. The highly interconnected and integrated cardiac ganglia have intrinsic activity that is modulated by sympathetic or parasympathetic (vagal) inputs[16]. These plexuses thus contain mixed cardiac nerves, i.e. nerves originating from different cardiac sympathetic nerves but also from parasympathetic nerves. The use of markers such as tyrosine hydroxylase (TH) and choline acetyltransferase (ChAT) has helped elucidate the composition of cholinergic and adrenergic intrinsic cardiac neurons and nerves in the heart. Petraitiene and colleagues[70] obtained tissue samples of intrinsic nerves from seven ganglionated plexuses from human hearts as described by Pauza et al: the left and right coronary subplexuses, the ventral right atrial and ventral left atrial subplexuses, the left dorsal subplexus, the middle dorsal subplexus, the dorsal right atrial subplexus[67]. They demonstrated that autonomic fibers to the ganglionated plexuses innervating the right atrium predominantly contain cholinergic fibers. In contrast, plexuses innervating the left atrium and left and right ventricle are predominantly innervated by adrenergic fibers[70].

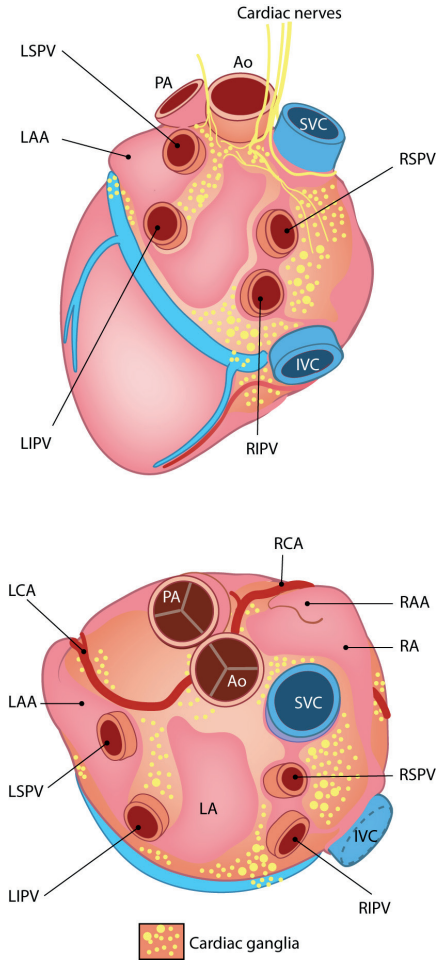


Figure 5. Cardiac plexuses

Drawing of a postero-inferior and a superior view of the human heart illustrating the distribution of ganglionated plexuses on the surface of the atria and ventricles. Modified after Armour et al[18].

Ao, aorta; IVC, inferior vena cava; LA, left atrium; LAA, left atrial appendage; LCA, left coronary artery; LIPV, left inferior pulmonary vein; LSPV, left superior pulmonary vein; Pa, pulmonary artery; RA, right atrium; RAA, right atrial appendage; RCA, right coronary artery; RIPV, right inferior pulmonary vein; RSPV, right superior pulmonary vein; SVC, superior vena cava

Three main large (mixed sympathetic and parasympathetic) nerves that follow the coronary arteries or their major branches can be recognized that contribute to innervation of the atria and ventricles: the left coronary cardiac nerve (which runs along the anterior interventricular (descending) branch of the left coronary artery), the left lateral cardiac nerve (which runs along the circumflex artery) and the right coronary cardiac nerve (which runs along the right coronary artery)[59]. Additional cardiopulmonary nerves connect to these (coronary) cardiac nerves distal from the plexuses (**Figure 2**), innervating coronary vessels and myocardial cells. Both cholinergic and adrenergic nerves run from the epicardium into the myocardium[52]. However, there are more cholinergic nerves at the subendocardial than at the subepicardial area of the myocardium. Corresponding to Petraitiene and colleagues[70], Kawano et al.[52] report a general distribution pattern of atria being more densely innervated by cholinergic nerves whereas the ventricles are predominantly innervated by adrenergic fibers. The AVN and SAN are more densely innervated than the His bundle and bundle branches, although the latter components of the cardiac conduction system still receive more innervation than the adjacent ventricular myocardium[71].

Controversies in distribution of cholinergic and adrenergic nerve fibers and location of ganglionated plexuses.

The cardiac topography of the intracardiac ganglionated plexuses, consisting of numerous ganglia on atria and ventricles, seems to be according to a pattern. The epicardial neural plexus has been described as a system of six to 10 subplexuses localized at discrete cardiac regions[18, 67]

(**Figure 5**). Armour and colleagues consistently identified five atrial and five ventricular locations where ganglionated plexuses could be observed. The group of Pauza described a system of seven subplexuses consistently observed at five atrial and two ventricular locations[67]. The ganglionated plexuses are interconnected suggesting that a plexus might have interaction with several topographic regions of the heart[18]. Although ganglionated plexuses were observed to be located at specific cardiac regions, variability seems to exist in the exact location of ganglia. The results of the studies of Kawano[52] and Petraitiene[70] are partly conflicting since Petraitiene reported that the left atrium is predominantly innervated by adrenergic nerve fibers where according to Kawano it is more densely innervated by cholinergic fibers.

Clinical correlation: Physiology of cardiac innervation

Anatomically it seems that the myelum at T1-T3, which incorporates 1/3 of approximately 90,000 preganglionic sympathetic neurons of the thoracic myelum[72] (not specifically cardiac sympathetic neurons), would be an important contributor of sympathetic outflow to the heart. Interestingly, the thoracic cardiac nerves reported to deliver the most substantial contribution to the cardiac plexus are the third and fourth[62] or the fourth and fifth thoracic segments[64]. However, the amount of neurons does not necessarily correlate to the strength of the physiological effects elicited after stimulation of these nerves. Physiological studies examining the effects of electrical stimulation of sympathetic nerves from different spinal cord levels can provide information on the contribution of different spinal segments to the innervation of the heart. Electrophysiological studies in animals reveal that each cervical or thoracic paravertebral ganglion is innervated by a subset of spinal segments. Ganglia are almost always most strongly innervated by one particular spinal segment with contributions from adjacent spinal segments that diminish as a function of distance from the dominant segment[73, 74]. In cats, maximal evoked responses in several cardiac nerves were demonstrated after stimulation of T2 and responses gradually decreased after stimulation of T1, T3, T4 and T5 white rami[73]. Another study in guinea pigs demonstrated that the majority of superior cervical ganglion cells receives input from spinal segments T1-T4, the majority of stellate ganglion cells from spinal segments T2-T6 and the majority of fifth thoracic ganglion cells from T4-T8 with a main supply from spinal level T5. Although the majority of segments involved in innervation of the fifth thoracic ganglion arose from the T4-T8 spinal segments, even the spinal segments of T9 and T10 were occasionally involved[74].

To our knowledge there are no human electrophysiological studies assessing the projections of sympathetic preganglionic neurons to the cervical and thoracic ganglia. However, there is a human study by Randall and colleagues that assessed elevations in blood pressure and cardiac acceleration after stimulation of separate ganglia of the upper thoracic sympathetic chain during surgery. Elevations in heart rate and blood pressure were reported after stimulation of thoracic ganglia Tg1-Tg5, but not after stimulation below Tg5 (cervical levels were not included in this study). Considerable variation was found in thoracic levels of sympathetic innervation of the hearts of different patients. Similarly, there was variability in which thoracic ganglion stimulation elicited the strongest response[75].

Besides variation in which ganglion evokes the strongest cardiac response, differential effects from left and right sided cardiac sympathetic structures have been described. Left stellate ganglion (LSG) and right stellate ganglion (RSG) stimulation have differential effects on heart rate reflected by a more substantial increase in heart rate (73-78 %) after RSG stimulation compared to heart rate effects after LSG stimulation (0-49 %)[76, 76-79]. Stimulation of both ganglia elicits increases of contractile forces in the basal and apical parts of the left ventricle as well as the right ventricle[79]. There remains controversy on which region of the atria and/or ventricles is being innervated by either LSG or RSG. A previous animal study indicates that the LSG primarily innervates the posterior aspect of the right and left ventricle and RSG predominantly innervates the anterior aspect of both ventricles[77]. Other animal studies suggests the same heterogeneous innervation pattern of the left ventricle leading to increased left ventricle asynchrony after unilateral stellate ganglion stimulation[80, 76]. Selective innervation of the anterior and posterior parts of the left ventricle by respectively the RSG and LSG is disputed by other animal studies demonstrating that both the RSG and the LSG innervate the anterior wall of the left ventricle[76, 79, 81] and both innervate the right ventricle[79].

In summary, from electrophysiological studies in animals it can be concluded that each cervical and thoracic paravertebral ganglion is innervated by sympathetic preganglionic neurons from multiple spinal levels. However, there always seems to be one spinal level with the strongest input with input diminishing when adjacent spinal levels are more distant from the main contributing spinal segment. Therefore it seems likely that besides its main supply from spinal level T5, Tg5 in humans is innervated by preganglionic sympathetic neurons from spinal levels below T5, and that inter-individual variations in ganglionic dominancy may occur . Electrophysiological studies also support the aforementioned inter-individual variations in anatomy. Although there remains controversy about exact innervation of the ventricles by the LSG and RSG, both sympathetic chains probably innervate the atria and the ventricles heterogeneously.

Conclusions and clinical implications

The exact origin of preganglionic sympathetic neurons innervating the human heart is controversial and remains a matter of debate. Although human cardiac sympathetic innervation is regularly described to emerge from spinal cord segments T1-T4 or T5, several human anatomical studies report involvement of the sixth and even seventh thoracic ganglia in cardiac sympathetic innervation. Consequently preganglionic sympathetic neurons from spinal levels T6 and T7 (or even more caudal segments) may be involved in cardiac sympathetic innervation. Therefore, complete blockade of cardiac sympathetic innervation may require blockade of spinal segments below T5 or thoracic ganglia below Tg5. Another consequence would be that involvement of sympathetic cardiac segments in neural blockade may, besides high TEA, more likely apply to mid-thoracic epidural analgesia, often used in abdominal surgical procedures, with cranial spread of anesthetic blockade. Human anatomical studies also demonstrate controversial results regarding involvement of the cervical paravertebral ganglia in cardiac sympathetic innervation. Along with the stellate ganglion other cervical paravertebral ganglia

may be involved in the transduction of cardiac sympathetic signals. Besides ambiguous cranial and caudal borders of cardiac sympathetic innervation there is considerable inter-individual and intra-individual anatomical variation. The anatomy of the cardiac sympathetic output to the heart is extremely variable, which likely accounts for part of the anatomical controversies encountered in literature. This variability renders the outcome of procedures targeting neuronal modulation of cardiac sympathetic innervation, such as stellate ganglion and paravertebral blockade, unpredictable.

Reference list

1. Schwartz PJ, Motolese M, Pollavini G, Lotto A, Ruberti U, Trazzi R, Bartorelli C, Zanchetti A. Prevention of sudden cardiac death after a first myocardial infarction by pharmacologic or surgical antiadrenergic interventions. J Cardiovasc Electrophysiol 1992;3:2-16.
2. Shen MJ, Zipes DP. Role of the autonomic nervous system in modulating cardiac arrhythmias. Circ Res 2014;114:1004-1021.
3. Looi KL, Tang A, Agarwal S. Ventricular arrhythmia storm in the era of implantable cardioverter-defibrillator. Postgrad Med J 2015;91:519-526.
4. Shuang W, Shiying F, Fengqi L, Renhai Q, Lanfeng W, Zhuqin L, Xu W . Use of a high thoracic epidural analgesia for treatment of end-stage congestive heart failure secondary to coronary artery disease: effect of HTEA on CHF. Int J Cardiol 2008;125:283-285.
5. Florea VG, Cohn JN. The autonomic nervous system and heart failure. Circ Res 2014;114:1815-1826.
6. Wink J, Veering BT, Aarts LP, Wouters PF. Effect of increasing age on the haemodynamic response to thoracic epidural anaesthesia: an observational study. Eur J Anaesthesiol 2014;31:597-605.
7. Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, Francis GS, Simon AB, Rector T. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N Engl J Med 1984;311:819-823.
8. Anand IS, Fisher LD, Chiang YT, Latini R, Masson S, Maggioni AP, Glazer RD, Tognoni G, Cohn JN. Changes in brain natriuretic peptide and norepinephrine over time and mortality and morbidity in the Valsartan Heart Failure Trial (Val-HeFT). Circulation 2003;107:1278-1283.
9. Nygard E, Kofoed KF, Freiberg J, Holm S, Aldershvile J, Eliasen K, Kelbaek H. Effects of high thoracic epidural analgesia on myocardial blood flow in patients with ischemic heart disease. Circulation 2005;111:2165-2170.
10. Ogawa M, Zhou S, Tan AY, Song J, Gholmieh G, Fishbein MC, Luo H, Siegel RJ, Karagueuzian HS, Chen LS, Lin SF, Chen PS. Left stellate ganglion and vagal nerve activity and cardiac arrhythmias in ambulatory dogs with pacing-induced congestive heart failure. J Am Coll Cardiol 2007;50:335-343.
11. Olausson K, Magnusdottir H, Lurje L, Wennerblom B, Emanuelsson H, Ricksten SE. Anti-ischemic and anti-anginal effects of thoracic epidural anesthesia versus those of conventional medical therapy in the treatment of severe refractory unstable angina pectoris. Circulation 1997;96:2178-2182.
12. Do DH, Bradfield J, Ajijola OA, Vaseghi M, Le J, Rahman S, Mahajan A, Nogami A, Boyle NG, Shivkumar K. Thoracic Epidural Anesthesia Can Be Effective for the Short-Term Management of Ventricular Tachycardia Storm. J Am Heart Assoc 2017;6:1-10.
13. Ma D, Liu L, Zhao H, Zhang R, Yun F, Li L, Wang Y, Qu R, Liu P, Liu F. Thoracic Epidural Anesthesia Reversed Myocardial Fibrosis in Patients With Heart Failure Caused by Dilated Cardiomyopathy. J Cardiothorac Vasc Anesth 2017;31:1672-1675.

14. Ajijola OA, Lellouche N, Bourke T, Tung R, Ahn S, Mahajan A, Shivkumar K. Bilateral cardiac sympathetic denervation for the management of electrical storm. J Am Coll Cardiol 2012;59:91-92.
15. Wallis D, Watson AH, Mo N. Cardiac neurones of autonomic ganglia. Microsc Res Tech 1996;35:69-79.
16. Palma JA, Benarroch EE. Neural control of the heart: recent concepts and clinical correlations. Neurology 2014;83:261-271.
17. Vaseghi M, Shivkumar K. The role of the autonomic nervous system in sudden cardiac death. Prog Cardiovasc Dis 2008;50:404-419.
18. Armour JA, Murphy DA, Yuan BX, Macdonald S, Hopkins DA. Gross and microscopic anatomy of the human intrinsic cardiac nervous system. Anat Rec 1997;247:289-298.
19. Hou Y, Scherlag BJ, Lin J, Zhang Y, Lu Z, Truong K, Patterson E, Lazzara R, Jackman WM, Po SS. Ganglionated plexi modulate extrinsic cardiac autonomic nerve input: effects on sinus rate, atrioventricular conduction, refractoriness, and inducibility of atrial fibrillation. J Am Coll Cardiol 2007;50:61-68.
20. Jamali HK, Waqar F, Gerson MC. Cardiac autonomic innervation. J Nucl Cardiol 2016;24:1558-1570.
21. Randall DC, Brown DR, McGuirt AS, Thompson GW, Armour JA, Ardell JL. Interactions within the intrinsic cardiac nervous system contribute to chronotropic regulation. Am J Physiol Regul Integr Comp Physiol 2003;285:R1066-R1075.
22. Spinal cord: internal organization. In: Susan Standring, editor. Gray's Anatomy: The Anatomical Basis of Clinical Practice. 40th ed. New York: Elsevier limited; 2008. p. 257-273.
23. Kapa S, Venkatachalam KL, Asirvatham SJ. The autonomic nervous system in cardiac electrophysiology: an elegant interaction and emerging concepts. Cardiol Rev 2010;18:275-284.
24. Heart and great vessels. In: Susan Standring, editor. Gray's Anatomy: The Anatomical Basis of Clinical Practice. 40th ed. New York: Elsevier limited; 2008. p. 959-987.
25. Navaratnam V. Development of the nerve supply to the human heart. Br Heart J 1965;27:640-650.
26. Sadler TW. Embryology of neural tube development. Am J Med Genet C Semin Med Genet 2005;135C:2-8.
27. Phelps PE, Barber RP, Vaughn JE. Embryonic development of choline acetyltransferase in thoracic spinal motor neurons: somatic and autonomic neurons may be derived from a common cellular group. J Comp Neurol 1991;307:77-86.
28. Kirby ML, Gilmore SA. A correlative histofluorescence and light microscopic study of the formation of the sympathetic trunks in chick embryos. Anat Rec 1976;186:437-449.
29. Britsch S, Li L, Kirchhoff S, Theuring F, Brinkmann V, Birchmeier C, Riethmacher D. The ErbB2 and ErbB3 receptors and their ligand, neuregulin-1, are essential for development of the sympathetic nervous system. Genes Dev 1998;12:1825-1836.
30. Le Douarin NM, Dupin E. The "beginnings" of the neural crest. Dev Biol 2018.

31. Hildreth V, Webb S, Bradshaw L, Brown NA, Anderson RH, Henderson DJ. Cells migrating from the neural crest contribute to the innervation of the venous pole of the heart. J Anat 2008;212:1-11.
32. Kirby ML, McKenzie JW, Weidman TA. Developing innervation of the chick heart: a histofluorescence and light microscopic study of sympthetic innervation. Anat Rec 1980;196:333-340.
33. Kirby M. Innervation of the developing heart. In: Kirby M, editor. Cardiac Development. 1 ed. New York: Oxford University Press; 2007. p. 179-197.
34. Baptista CA, Kirby ML. The cardiac ganglia: cellular and molecular aspects. Kaohsiung J Med Sci 1997;13:42-54.
35. Gordon L, Polak JM, Moscoso GJ, Smith A, Kuhn DM, Wharton J. Development of the peptidergic innervation of human heart. J Anat 1993;183:131-140.
36. Saburkina I, Pauziene N, Pauza DH. Prenatal development of the human epicardiac Ganglia. Anat Histol Embryol 2009;38:194-199.
37. Kimura K, Ieda M, Fukuda K. Development, maturation, and transdifferentiation of cardiac sympathetic nerves. Circ Res 2012;110:325-336.
38. Vegh AMD, Duim SN, Smits AM, Poelmann RE, Ten Harkel ADJ, DeRuiter MC, Goumans MJ, Jongbloed MRM. Part and Parcel of the Cardiac Autonomic Nerve System: Unravelling Its Cellular Building Blocks during Development. J Cardiovasc Dev Dis 2016;3.
39. Chow LT, Chow SS, Anderson RH, Gosling JA. Autonomic innervation of the human cardiac conduction system: changes from infancy to senility--an immunohistochemical and histochemical analysis. Anat Rec 2001;264:169-182.
40. Gerstner T, Sprenger J, Schaible T, Weiss C, Koenig S. Maturation of the autonomic nervous system: differences in heart rate variability at different gestational weeks. Z Geburtshilfe Neonatol 2010;214:11-14.
41. Wallwitz U, Schneider U, Nowack S, Feuker J, Bauer S, Rudolph A, Hoyer D. Development of integrative autonomic nervous system function: an investigation based on time correlation in fetal heart rate patterns. J Perinat Med 2012;40:659-667.
42. Nam J, Onitsuka I, Hatch J, Uchida Y, Ray S, Huang S, Li W, Zang H, Ruiz-Lozano P, Mukouyama YS. Coronary veins determine the pattern of sympathetic innervation in the developing heart. Development 2013;140:1475-1485.
43. Hasan W. Autonomic cardiac innervation: development and adult plasticity. Organogenesis 2013;9:176-193.
44. Zhu Q, Lu Q, Gao R, Cao T. Prospect of Human Pluripotent Stem Cell-Derived Neural Crest Stem Cells in Clinical Application. Stem Cells Int 2016;2016:7695836.
45. Kawashima T. The autonomic nervous system of the human heart with special reference to its origin, course, and peripheral distribution. Anat Embryol (Berl) 2005;209:425-438.
46. Verberne ME, Gittenberger-De Groot AC, Van IL, Poelmann RE. Contribution of the cervical sympathetic ganglia to the innervation of the pharyngeal arch arteries and the heart in the chick embryo. Anat Rec 1999;255:407-419.

47. Rubin E. Development of the rat superior cervical ganglion: ganglion cell maturation. J Neurosci 1985;5:673-684.
48. Kanagasuntheram R, Dharshini P. The cervical sympathetic trunk--a new hypothesis. Ann Acad Med Singapore 1994;23:923-928.
49. Smith RB. The development of the intrinsic innervation of the human heart between the 10 and 70 mm stages. J Anat 1970;107:271-279.
50. Than M, Dharap AS. Variations in the formation of the cardiac plexus--a study in human foetuses. Z Morphol Anthropol 1996;81:179-188.
51. Chow LT, Chow SS, Anderson RH, Gosling JA. The innervation of the human myocardium at birth. J Anat 1995;187:107-114.
52. Kawano H, Okada R, Yano K. Histological study on the distribution of autonomic nerves in the human heart. Heart Vessels 2003;18:32-39.
53. Bonica JJ. The Management of Pain.Philedelphia: Philedelphia, Lea & Febiger; 1953.
54. Bonica JJ. Autonomic innervation of the viscera in relation to nerve block. Anesthesiology 1968;29:793-813.
55. Sheehan D. Spinal autonomic outflows in man and monkey. J Comp Neurol 1941;75:341-370.
56. Neuwirth E. Current concepts of the cervical portion of the sympathetic nervous system. J Lancet 1960;80:337-338.
57. Pyner S, Coote JH. Evidence that sympathetic preganglionic neurones are arranged in target-specific columns in the thoracic spinal cord of the rat. J Comp Neurol 1994;342:15-22.
58. Ter Horst GJ, Hautvast RW, De Jongste MJ, Korf J. Neuroanatomy of cardiac activity-regulating circuitry: a transneuronal retrograde viral labelling study in the rat. Eur J Neurosci 1996;8:2029-2041.
59. Janes RD, Brandys JC, Hopkins DA, Johnstone DE, Murphy DA, Armour JA. Anatomy of human extrinsic cardiac nerves and ganglia. Am J Cardiol 1986;57:299-309.
60. Kawashima T, Sasaki H. Morphological comparison of the cardiac autonomic nervous system between normal and abnormal great arterial branching pattern with a brief review of the literature. Auton Neurosci 2007;132:37-43.
61. Zhang B, Li Z, Yang X, Li G, Wang Y, Cheng J, Tang X, Wang F.. Anatomical variations of the upper thoracic sympathetic chain. Clin Anat 2009;22:595-600.
62. Ellison JP, Williams TH. Sympathetic nerve pathways to the human heart, and their variations. Am J Anat 1969;124:149-62.
63. Pather N, Partab P, Singh B, Satyapal KS. The sympathetic contributions to the cardiac plexus. Surg Radiol Anat 2003;25:210-215.
64. Saccomanno G. The components of the upper thoracic sympathetic nerves. J Comp Neurol 1943;79:355-378.
65. De Gama BZ, Lazarus L, Partab P, Satyapal KS. The sympathetic and parasympathetic contributions to the cardiac plexus: A fetal study. Int J Morphol 2012;30:1569-1576.

66. Seki A, Green HR, Lee TD, Hong L, Tan J, Vinters HV, Chen PS, Fishbein MC. Sympathetic nerve fibers in human cervical and thoracic vagus nerves. Heart Rhythm 2014;11:1411-1417.
67. Pauza DH, Skripka V, Pauziene N, Stropus R. Morphology, distribution, and variability of the epicardiac neural ganglionated subplexuses in the human heart. Anat Rec 2000;259:353-382.
68. Singh S, Johnson PI, Lee RE, Orfei E, Lonchyna VA, Sullivan HJ, Montoya A, Tran H, Wehrmacher WH, Wurster RD. Topography of cardiac ganglia in the adult human heart. J Thorac Cardiovasc Surg 1996;112:943-953.
69. Armour JA, Murphy DA, Yuan BX, Macdonald S, Hopkins DA. Gross and microscopic anatomy of the human intrinsic cardiac nervous system. Anat Rec 1997;247:289-298.
70. Petraitiene V, Pauza DH, Benetis R. Distribution of adrenergic and cholinergic nerve fibres within intrinsic nerves at the level of the human heart hilum. Eur J Cardiothorac Surg 2014;45:1097-1105.
71. Crick SJ, Wharton J, Sheppard MN, Royston D, Yacoub MH, Anderson RH, Polak JM. Innervation of the human cardiac conduction system. A quantitative immunohistochemical and histochemical study. Circulation 1994;89:1697-1708.
72. Coote JH. The organisation of cardiovascular neurons in the spinal cord. Reviews of Physiology, Biochemistry and Pharmacology. Springer-Verlag; 1988. p. 147-285.
73. Szulczyk A, Szulczyk P. Spinal segmental preganglionic outflow to cervical sympathetic trunk and postganglionic cardiac sympathetic nerves. Brain Res 1987;421:127-134.
74. Lichtman JW, Purves D, Yip JW. Innervation of sympathetic neurones in the guinea-pig thoracic chain. J Physiol 1980;298:285-299.
75. Randall WC, Mcnally H. Augmentor action of the sympathetic cardiac nerves in man. J Appl Physiol 1960;15:629-631.
76. Zhou W, Yamakawa K, Benharash P, Ajijola O, Ennis D, Hadaya J, Vaseghi M, Shivkumar K, Mahajan A. Effect of stellate ganglia stimulation on global and regional left ventricular function as assessed by speckle tracking echocardiography. Am J Physiol Heart Circ Physiol 2013;304:H840-H847.
77. Yanowitz F, Preston JB, Abildskov JA. Functional distribution of right and left stellate innervation to the ventricles. Production of neurogenic electrocardiographic changes by unilateral alteration of sympathetic tone. Circ Res 1966;18:416-428.
78. Ajijola OA, Howard-Quijano K, Scovotti J, Vaseghi M, Lee C, Mahajan A, Shivkumar K. Augmentation of cardiac sympathetic tone by percutaneous low-level stellate ganglion stimulation in humans: a feasibility study. Physiol Rep 2015;3.
79. Randall WC, Szentivanyi M, Pace JB, Wechsler JS, Kaye MP. Patterns of sympathetic nerve projections onto the canine heart. Circ Res 1968;22:315-323.
80. Schlack W, Thamer V. Unilateral changes of sympathetic tone to the heart impair left ventricular function. Acta Anaesthesiol Scand 1996;40:262-271.
81. Vaseghi M, Zhou W, Shi J et al. Sympathetic innervation of the anterior left ventricular wall by the right and left stellate ganglia. Heart Rhythm 2012;9:1303-1309.