УДК 611.8:611.12
МРНТИ 34.41.35

NEUROANATOMY OF HEART IN HUMAN AND MAMMALIANS

NERINGA PAUZIENE, KRISTINA RYSEVAITE-KYGUOLIENE, DAINIUS H. PAUZA

Lithuanian University of Health Sciences, Lithuania

Neringa Pauziene – https://orcid.org/0000-0001-5005-3352
Dainius H. Pauza – https://orcid.org/0000-0001-8597-4370

For citing/
библиографиялық сілтеме/
библиографическая ссылка:

Neringa Pauziene, Kristina Rysevaite-Kyguoliene, Dainius H. Pauza
Neuroanatomy of heart in human and mammalians. West Kazakhstan Medical Journal 2019;61(4):203–211.

Neringa Pauziene, Kristina Rysevaite-Kyguoliene, Dainius H. Pauza. Адам мен сүтқоректілер жүрегінің нейроанатомиясы.West Kazakhstan Medical Journal 2019;61(4): 203–211.

Neringa Pauziene, Kristina Rysevaite-Kyguoliene, Dainius H. Pauza. Нейроанатомия сердца у человека и млекопитающих. West Kazakhstan Medical Journal 2019;61(4): 203–211.

Neuroanatomy of heart in human and mammalians
Neringa Pauziene, Kristina Rysevaite-Kyguoliene, Dainius H. Pauza
Lithuanian University of Health Sciences, Lithuania

On reaching the heart, extrinsic cardiac nerves relay their signal to a network of autonomic ganglia situated throughout the epicardial surface. These ganglia interconnect and form a complex intrinsic ganglionated neural plexus responsible for integrating central and local inputs and relaying this signal to the cardiac conduction system, coronary vessels, heart valves and contractile muscle fibres. Within the human heart this intracardiac plexus could be defined in terms of seven subplexuses located and innervating discrete areas of the heart. Despite some noticeable адам мен interspecific differences in the overall neuronal number and ganglionic morphology, this type of structural organization is conserved throughout the mammalian heart.
Intrinsic ganglionated plexus consists of neurons expressing various modulatory agents. It is widely accepted that most intracardiac neuronal somata are cholinergic, yet nearly half of them are biphenotypic for either tyrosine hydroxylase or neuronal nitric oxide synthase. Moreover exclusively tyrosine hydroxylase positive somata are found in some mammals and human hearts. Therefore in addition to relaying preganglionic vagal impulses, cardiac ganglia also integrate sensory and sympathetic inputs for rapid temporal reflexes and local regulation of heart rate on a beat-to-beat basis.
The epicardium, in addition to numerous ganglia, is the main milieu for distribution of nerves towards the heart apex. The myocardium contains both scarce nerves located in the vicinity of blood vessels and a meshwork of fine nerve fibers. The endocardium contains a dense network of nerve fibers and nerve bundles with only a small part of them coalescing into nerves.
The described structural organization of intracardiac nervous system provides an anatomical basis of the autonomic control of circulation.
Keywords: *heart, innervation, intrinsic cardiac plexus, ganglia, neuron immunohistochemistry, electron microscopy.*

Адам мен сүтқоректілер жүрегінің нейроанатомиясы
Neringa Pauziene, Kristina Rysevaite-Kyguoliene, Dainius H. Pauza
Литва денсаулық ғылымдары университеті, Литва

Жүрекке жеткенде жүректің ішкі жүйкелері эпикарданың тұтас бетінде орналасқан вегетативті ганглиялар желісіне сигнал береді. Біріге отырып, бұл ганглиялар орталық және жергілікті импульстеріне және жүректің өткізгіш жүйесіне, коронарлы тамырларға, жүрек клапандарына және қысқаратын бұлшықет талшықтарына жіберу интеграциясына жауап беретін күрделі ішкі ганглионарды жүйке плексусын түзеді. Адамда ішкі жүрек плексусы жүректің жекелеген аймақтарында орналасқан және сәйкесінше олардың иннервациясын қамтитын жеті бөліктен тұрады. Нейрондар мен ганглиондар морфологиясының жалпы санындағы кейбір айрықша тұраралық айырмашылықтарына қарамастан, құрылымдық ұйымның бұл түрі барлық сүтқоректілерде сақталады.
Ішкі ганглионарлық плексус әртүрлі модуляторларды білдіретін нейрондардан тұрады. Ішкі жүрек нейрондары денелерінің көбі холинергиялық болып табылады деген пікір кең тараған, бірақ азот оксидінің не тирозингидроксилазасы үшін, не нейрональды синтазы үшін олардың жартысы дерлік бифенотиптік. Сонымен қатар, кейбір сүтқоректілер мен адамда нейронның тек тирозингидроксилазды-позитивті денелері анықталады. Сол себептен, вагустан преганглионарлы импульстарды беруден басқа, жүрек ганглиялары жүрек соғысы арасындағы

Neringa Pauziene
e-mail: neringa.pauziene@lsmuni.lt

Received/
Келіп түсті/
Поступила:
28.11.2019

Accepted/
Басылымға қабылданды/
Принята к публикации:
18.12.2019

ISSN 1814-5620 (Print)

Published by West Kazakhstan Marat Ospanov Medical University

интервалдың өзгерісі жолымен жүрек ритмінің шұғыл уақытша рефлекстері мен жергілікті реттелуін қалыптастыру үшін сенсорлық және симпатикалық импульстерін де біріктіреді.
Эпикард нервтерді бағыт бойынша жүректің жоғарғы тұсына тарату үшін негізгі орта болып табылады және көптеген ганглияларды құрайды. Миокардта жұқа нерв талшықтарының желісі басым келеді және нервтің аз бөлігі қан тамырларына параллель өтеді. Эпикардта жүйке талшықтары мен жүйке түйіндерінің тығыз желісі орналасады, және олардың аз ғана бөлігі нервтерге біріктірілген.
Жүрекішілік жүйке жүйесінің аталмыш ерекшеліктері қанайналымды автономиялық бақылау үшін анатомиялық негіз болып табылады.
Негізгі сөздер: *жүрек, иннервация, ішкі жүрек өрімі, ганглиялар, нейронды иммуногистохимия, электронды микроскопия.*

Нейроанатомия сердца у человека и млекопитающих

Neringa Pauziene, Kristina Rysevaite-Kyguoliene, Dainius H. Pauza
Литовский Университет Наук о Здоровье, Литва

Достигнув сердца, внешние сердечные нервы передают сигнал в сеть вегетативных ганглиев, расположенных по всей поверхности эпикарда. Соединяясь, эти ганглии образуют сложное внутреннее ганглионарное нервное сплетение, отвечающее за интеграцию центральных и местных импульсов и передачу их в проводящую систему сердца, коронарные сосуды, сердечные клапаны и сократительные мышечные волокна. У человека внутрисердечное сплетение (plexus) состоит из семи частей (subplexus), расположенных в отдельных областях сердца, и соответственно обеспечивающих их иннервацию. Несмотря на некоторые значительные межвидовые различия в общем числе нейронов и морфологии ганглиев, этот тип структурной организации сохраняется у всех млекопитающих.
Внутреннее ганглионарное сплетение состоит из нейронов, экспрессирующих различные модуляторы. Широко распространено мнение, что большинство тел внутрисердечных нейронов являются холинергическими, но почти половина из них – бифенотипична либо для тирозингидроксилазы, либо для нейрональной синтазы оксида азота. Кроме того, у некоторых млекопитающих и человека обнаруживаются исключительно тирозингидроксилаза-позитивные тела нейронов. Следовательно, кроме передачи преганглионарных импульсов от вагуса, сердечные ганглии также объединяют сенсорные и симпатические импульсы для формирования быстрых временных рефлексов и локальной регуляции сердечного ритма путем изменения интервала между сердечными сокращениями.
Эпикард является основной средой для распределения нервов по направлению к верхушке сердца и содержит многочисленные ганглии. В миокарде преобладает сеть тонких нервных волокон и проходит небольшое количество нервов параллельно кровеносным сосудам. В эндокарде располагается густая сеть нервных волокон и нервных пучков, причем лишь незначительная их часть объединена в нервы.
Данные особенности внутрисердечной нервной системы являются анатомической основой для автономного контроля кровообращения.
Ключевые слова: *сердце, иннервация, внутреннее сердечное сплетение, ганглии, нейронная иммуногистохимия, электронная микроскопия.*

Accesses of Mediastinal Nerves into Heart.

In humans as well as in other mammalians, extrinsic cardiac nerves access the heart through the heart hilum (HH), in which they course by specific intracardial pathways [1-5]. From the arterial part of the HH (i.e. around the ascending aorta and pulmonary trunk) nerves extend predominantly onto the cardiac ventricles. From the venous part of the HH (i.e. around pulmonary veins and venae cavae) accessing nerves proceed on both the atria and ventricles [2, 4, 6-11]. The number of nerves in any access site as well as the thickness of the nerves on the HH level vary from heart to heart, but commonly 2-5 nerves proceed through any neural access site [2].

Structural Organization of Intrinsic Cardiac Nerve Plexus

For simplicity and anatomical clarity, intrinsic cardiac neural ganglionated plexus may be subdivided into the epicardial, myocardial and endocardial ones according to the heart wall layers [2, 3, 5, 8, 12, 13].

Accessed in the HH, intracardiac nerves do not penetrate directly into the myocardium. Commonly, nerves extend into the epicardial layer, in which their courses are more or less associated with grooves of the heart surface: coronary, interatrial and/or interventricular that affected clustering of the epicardial nerves into particular routes or pathways [2]. Very close to both parts of the HH, nerves

proceeding by these pathways reach the epicardial ganglia that, as ganglionated fields (GFs), are distributed in corresponding regions and interconnect complexly among themselves via thin nerves (Fig. 1) [2]. In the GFs, epicardial nerves branch out, became thin and pass into the ganglia (Fig. 1). In GFs, new epicardial nerves originate from numerous intrinsic ganglia and course away from GFs to pass directly into the structures they innervate, in which they gradually disappear from view in two ways: either via penetration into myocardium or becoming gradually thinner in the epicardium [2, 11]. Since the latter nerves are mostly devoid of ganglia, they are named postganglionated nerves (postGNs), while previous - preganglionated nerves (preGNs). Based on characteristic disposition, appearance and interrelations of the preGNs, GFs, and postGNs, it has been suggested that the entire epicardial neural plexus may be considered as being composed of seven ganglionated subplexuses, each of which contains its own preGNs, GF, and postGNs [2] nization of intracardiac nerve plexus is typical for the mammalian species investigated so far [3, 4, 9, 13, 14]. In hearts of the human and the mammalian species examined neuroanatomically until now, the right atrium is supplied by two subplexuses, the left atrium - by three, the right ventricle - by one, and the left ventricle - by three subplexuses [2-4, 9, 13, 14]. Two nerve subplexuses originating from the arterial part of the HH extend to the left side by the left ventral coronary sulcus as well as to the right side by the right ventral coronary sulcus. Nerves originating from the venous part of the HH proceed by five pathways: by the anterior interatrial sulcus and sulcus situated dorsally amid the roots of superior vena cava (RSVC) and right superior pulmonary vein nerves proceed mainly to the right atrium; while by the left atrial nerve fold to the lateral left atrial surface; and by the ventral, and dorsal surfaces of the left atrium epicardial nerves course to the left atrium and dorsal wall of the left ventricle [2].

Myocardium contains both scarce nerves located in the vicinity of blood vessels and a meshwork of fine neurofilaments (NFs) [15, 16].

Whole-mount preparations of the rabbit and piglet interventricular septum and ventricular walls exhibited that both plentiful NFs and thin nerves are distributed within the endocardial layer [16, 17].

Anatomy of Intrinsic Cardiac Ganglia

The functional importance of cardiac neurons in health and disease has been repeatedly highlighted [18, 19]. and the detailed description of the location, distribution, and projections of the intracardiac ganglia has been provided for the heart in several mammalian species and human [1-5, 8, 9, 13, 20–41]. The amount of epicardial ganglia vary significantly from heart to heart (individual variability) and depend on age and animal species. Commonly, the human heart contains from less than 600 to 1560 epicardial ganglia that involve 14,000-43,000 neurons in adults [2, 6] and 94,000 in young hearts [39]. The highest density of epicardial ganglia in the human heart is identified on the atrial walls, near the heart hilum, especially on the dorsal and dorsolateral surfaces of the left atrium, where up to 50% of all cardiac ganglia are persistently located [2, 6, 39, 41]. Similar number of epicardiac ganglia and neurons was identified in the dog heart [3]. Other investigated animal hearts contain less number of epicardiac ganglia and neurons residing in. Accordingly, the sheep heart contains 769±52 epicardial ganglia containing ~17,000 neurons [5]; the pig heart - 362±52 ganglia and ~12,000 neurons [4]; guinea pigs - 329±15 ganglia with averagely 2,500 nerve cells [13]. According data of different authors the mean number of intrinsic cardiac neurons in the rat heart varies from 1,000 [42,43] to 4,000 [33], while more recent studies report about 6,576 neurons in old rats and only 5,000 in the juvenile rats [8]. There are ~2,100 neurons located in the rabbit heart [10] including 11-220 located in the rabbit ventricles [15]. Typically, only 17-20 cardiac ganglia are distributed in the mouse heart that involve meanly 1,100 intrinsic cardiac neuronal perikarya [7, 44, 45].

Shape and size of intracardiac ganglia are also depended on animal species [2, 3, 6, 7, 22, 36, 39, 46]. The human epicardial ganglia are small, compared with ganglia from the canine right atrium. The mean size of the human epicardial ganglia is 0.07 mm2, while the ganglia larger than 0.2 mm2 are rare [2]. Medium-sized epicardial ganglia (larger than 0.25 mm2) of the dog, contain up to 2000 neurons [36], while the number of neurons in the human epicardial ganglia range from a few to more than 400. The number of neurons in the human epicardial ganglia depends on age and the average number of neurons per ganglia is 93 only. In contrast to atrial ganglia, ventricular ganglia are significantly smaller and consist of 5–40 neurons [2] . Ganglia in the rabbit heart mainly are located on the base of left atrium wherein they involve up to 700 neuronal somata [10] and therefore are similar to the dog ones. Only 4% of the rabbit neurons are found on the ventricles where they are disseminated singularly or assembled into small ganglia of 2-34 neurons [15].

Somata of intrinsic cardiac neurons can aggregate into globular or plain ganglia (Fig. 2) [47].

Usually, the plain ganglia are small in area and involve fewer neurons than globular ganglia. More than half of large globular ganglia are submerged in adipose tissue overspreding the heart grooves and hilum. Smaller ganglia are scattered more widely and, depending on species, might be found almost anywhere on the atria, ventricles, sinuses of the great cardiac vessels or/and interatrial septum [11, 16, 47, 48]. Within the heart hilum of the rats and guinea pigs, intracardiac neuronal somata are persistently aggregated in peculiar ganglia that may be considered to be of intermediate type, because their certain parts resemble the globular ganglia, while their other parts look like the typical plain ones [47]. Despite of wide variety of the shape of intracardiac ganglia, majority of them are more or less oval. However, some ganglia may be very irregular due to their tongue-like extensions [2, 10, 47]. The human ganglia contain evenly distributed neurons within gangli-

on [49], while neuronal somata of the porcine cardiac ganglia distributed in the periphery, adjacent to the capsule and interior of these ganglia is comprised of neuropil [16, 22]. According to the position of ganglia with respect to epicardial nerves, two types of epicardial ganglia may be discriminated: some of them situated along a nerve, while others - at an intersection of the nerves [2, 16, 22, 47].

Whereas most of the ganglia are epicardial, some ganglia are located endocardially on the inner surface of the atria and interatrial septum. In some species, individual neurons and small ganglia are scattered directly in the nodal areas of conductive system: in the sinoatrial (SA) nodal area of the rabbit and dog [47,50] and in the sinoatrial node (SAN) and atrioventricular node (AVN) areas of the pig heart [51]. It's acknowledged that cardiac ganglionic cells located on the dorsal right atrium in the area of the SA node are associated with control of the sinoatrial node and neurons on the left dorsal atrium close to the inferior vena cava (IVC) root modulate atrioventricular (AV) conduction [20]. Generally, a typical cardiac ganglion consists of neurons, satellite cells and small intensively fluorescent (SIF) cells. The mammalian cardiac ganglia contain unipolar, bipolar and multipolar neurons with differing dimensions and shapes [23] which are surrounded by basket endings around individual cardiac principal neurons [52]. On the electron microscopic level, the human intracardiac ganglia appear as solitary neuronal somata or their clusters surrounded by nerve processes together with the satellite cells (Fig. 3). Other non-neuronal structures in the ganglia are fibroblasts, macrophages, mast cells, collagen fibrils and blood vessels [49]. Human and porcine intrinsic cardiac ganglia have thick capsule, composed of fibroblasts and collagen fibers [6,22], while 1-5 layers of perineural cells sheet rabbit ganglia (Fig. 3) [53].

Inside the ganglia, both the unmyelinated and myelinated axons, as well as numerous synapses are distributed throughout the neuropil (Fig. 4) [21, 22, 49, 53]. All synaptic profiles contain spherical vesicles and, in general, mitochondria. The most common type of synaptic profiles contain a predominance of clear, spherical vesicles (30-60 nm in diameter) and a few larger, spherical dense-cored vesicles (80-100 nm in diameter). Another type of synaptic profiles, in addition to the mentioned components, has glycogen-like particles. Finally, the third type having vesicle-containing profiles are clearly different from the first and second profiles as they contained plentiful large (70-230 nm in diameter) pleomorphic clear or dense-cored vesicles together with small clear and larger dense-cored vesicles, mitochondria, dense and multivesicular bodies. On the average, third type profiles are the largest in diameter (up to 5 μm) [6, 29, 49]. Synaptic profiles are more common in the ganglion neuropil than on neuronal somata (Fig. 4).

Intrinsic cardiac neuronal somata vary in size and shape. All nerve cells in the cardiac ganglia, according to their three-dimensional morphology can be divided into three categories: (1) large unipolar neurons, (2) large unipolar or bipolar neurons, and (3) small multipolar interneurons [22, 35, 54–56]. The morphometric analysis of the rabbit, rat, guinea pig, dog and human intrinsic cardiac neurons shows statistically insignificant species-dependent differences in diameters of neuron somata [11, 47]. The neuronal somata of dog are 23 μm in width, 32 μm in length, and 615 μm2 in area [47] while mouse and rabbit neurons are smaller [11, 57]. The size of porcine neurons vary according data of different authors: ventricular

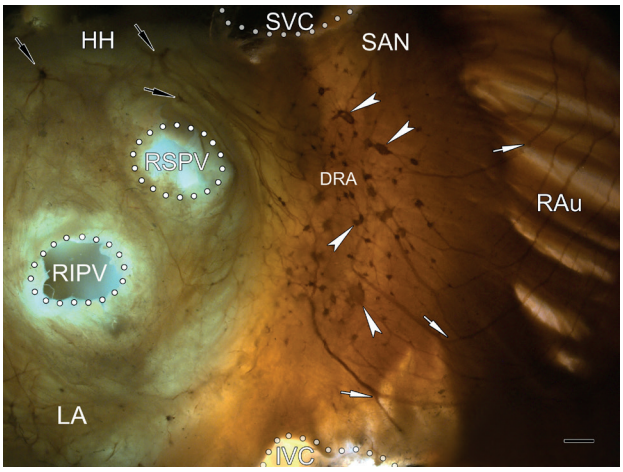

Fig. 1. Macrophotograph of the human dorsal right atrial ganglionated subplexus stained histochemically for acetylcholinesterase demonstrating the preganglionated (black arrows), postganglionated (white arrows) nerves and epicardiac ganglia (arrowheads point to some larger ganglia) distributed on the dorsal side of the right atrium. Abbreviations: SVC, superior vena cava; Rau, right auricle; HH, hilum of the heart; SAN, the region of sinoatrial node; IVC, inferior vena cava; DRA, dorsal right atrial subplexus; LA, left atria; RSPV, right superior pulmonary vein; RIPV, right inferior pulmonary vein. Scale bar: 2 mm.

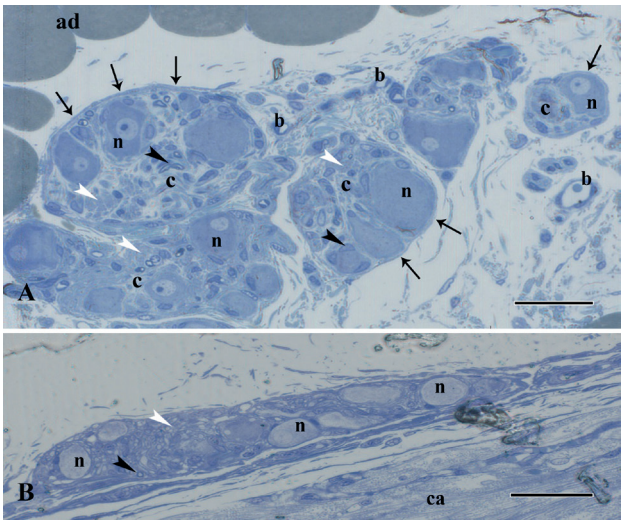

Fig. 2. Semithin sections of rabbit cardiac ganglia showing two types of organizations. A: Globular ganglion nearby the adipose tissue cells. Neurons in ganglion are clustered and perineurial cells (arrows) cover each cluster (c). Myelinated nerve fibers (black arrowheads) are located away from the neurons and unmyelinated nerve fibers (white arrowheads) located close to the neuron body. Abundant blood vessels (b) are located in the ganglion and nearby it. B: Plain epicardial ganglion (g) with neurons located peripherally and myelinated and unmyelinated nerve fibers centrally. Abbreviations: ad, adipocytes; ca, cardiomyocytes. Scale bar: 50 μm.

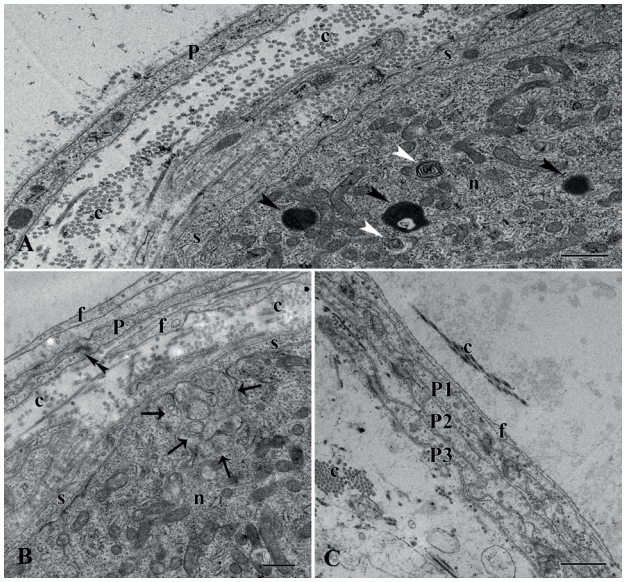

Fig. 3. Electron micrographs of rabbit intracardiac ganglia displaying perineurium and some neuron content. **A**: Ganglion sheathed by single layer of perineurial cells (P) containing numerous caveolae. Beneath the perineurium, large amount of collagen (c) is seen. Neuron body (n) with numerous mitochondria, plentiful ribosomes, droplets of lipofuscin (black arrowheads) and lamellated bodies (white arrowheads) are seen in the neuron cytoplasm. Thin satellite cell sheath (s) surrounds the neuron. **B**: Ganglion sheathed by single layer of perineurium (P). Fibroblasts (f) are seen above and below the perineurial layer. Note overlapping perineurial cells and intercellular junction between them (black arrowhead). Indentation of neuron surface filled by small processes (black arrows) is seen. **C**: Shows multiple perineurium layers (P1, P2, P3), fibroblast (f) and collagen (c). Scale bar: 500 nm in A and 1 μm in B and C.

neurons of the pig are shown being 22.2±5.3 μm in diameter, however large neuronal somata (33×46 μm) were described by R. C. Arora [16, 22]. Some of intracardiac neurons have multiple nucleoli. Canine cardiac neuronal somata contain up to 36% multiple nucleoli [29], whereas in the human they were found only in infants and only 15% [2].

Neurochemical Organization of Intrinsic Cardiac Ganglia

The neurochemical properties of intracardiac neurons are investigated in several mammalian species and it's summarized that the most of intacardiac neuronal somata are cholinergic ones [5, 10, 11, 18, 20, 22, 27, 31, 57–65]. However, specific combinations of neuropeptides and other potential neurotransmitters distinguish different functional types of neurons, demonstrating complex neurochemical anatomy of intracardiac ganglia [26, 66]. Therefore, it is highly likely that vagal transmission in the heart is modified by sympathetic, sensory and intrinsic neurons and those cardiac ganglia are complex integrators of convergent neuronal activity rather than simple relays [26]. In whole mount preparations of the guinea pig atria, 44% ganglia contain neuronal nitric oxide synthase (nNOS) possitive neuronal somata [67]. In the area of the rabbit SA node [50] and on the rabbit ventricles [15] there are two populations of nitrergic neurons – only nNOS possitive and biphenothypic with choline acetiltransferese (ChAT) (Fig. 5). The presence of a dual cholinergic/nitrergic, i.e. positive for ChAT and neuronal nitric oxide synthase (nNOS), phenotype for most of its neurons is characteristic for the human atrial ganglia as well [26].

Tyrosine hydroxylase-immunoreactive (TH-IR) cell bodies are determined in the intracardiac ganglionated plexuses of the pig [16, 68], guinea pig [69], mouse [46, 70] rabbit [15, 50], monkeys and human [26, 70, 71] (Majority of TH-IR neuronal somata were simultaneously positive for cholinergic markers [15, 16, 48, 55, 72, 73]. In the mouse heart, about 4% of neurons are exceptionally positive for TH (Fig. 5) [46], however such type of neuronal somata was not found in the hearts of rabbit and pig [50, 74, 75]. Presence of cholinergic and adrenergic biphenotypic neuronal somata in the heart also is confirmed by immunohistochemistry for neuronal transporters VMAT and VAChT [76]. Another type of the catecholamine-containing intrinsic cardiac cells is small intensively fluorescent or SIF cells (Fig. 5) [11, 18, 50]. Some authors interpret SIF cells as dopaminergic and serotonergic neurons, whereas large-diameter intrinsic cells positive for TH

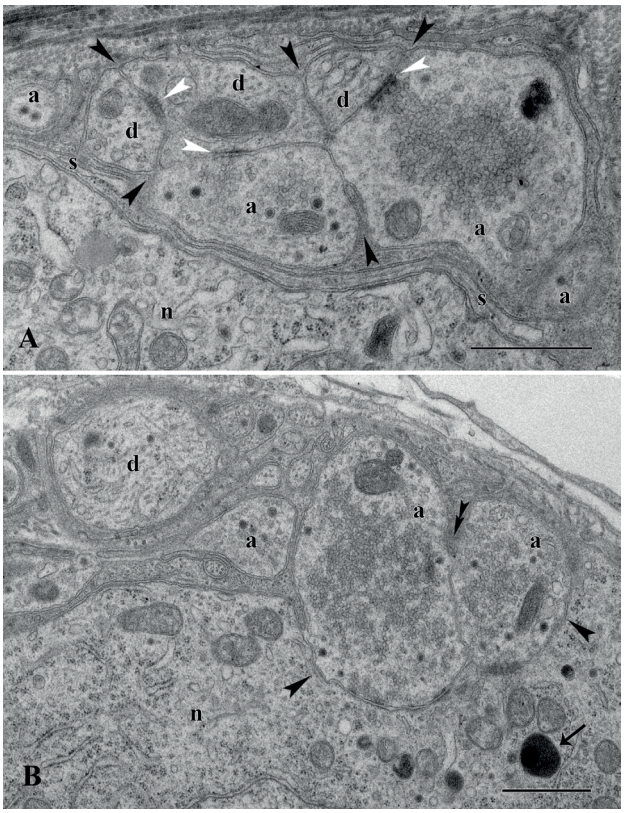

Fig. 4. Types of synapses located in the intracardiac ganglia. A. Complex of axodendritic synapses in the ganglion neuropil. Three profiles of dendrites (d) and two axon terminals (a) are in direct contact (black arrowheads) with each other. Synaptic membrane specializations (white arrowheads) are seen between axon terminals and dendrites, indicating axodendritic synapses, and between two dendrites, indicating dendrodendritic synapse. Numerous small clear vesicles with few dense cored vesicles are seen in the axoplasm. B. Axosomatic synapses. Two axon terminals (a) terminals making synapsis with the neuron soma. Note the direct contact without synaptic membrane specializations between axons (double arrowhead). Abbreviations: n, neuron soma; s, satellite cell; lipofuscin – arrow. Scale bar: 1 μm.

seem to represent a subpopulation of norepinephrine and/or epinephrine-secreting neurons [18]. However, small TH positive cells identified as SIF cells are negative for PGP 9.5 and, therefore, these cell bodies have not be identified as neuronal somata [17, 50].

In addition to cholinergic, adrenergic and nitrergic neuronal somata, peptidergic nerve fibers are also exposed in the intrinsic cardiac neural plexus (Fig. 5). Calcitonin gene-related peptide (CGRP) and substance P (SP) have been numerously identified in nerve fibers within cardiac ganglia. These fibers frequently form a basket surrounding several neuronal somata, but neuronal somata lack staining for these neuropeptides [16, 17, 26, 46, 50, 77]. SP is typically co-localized with CGRP, but many CGRP-IR nerve fibers are negative for SP [26]. Using a multiple labeling immunohistochemistry it is demonstrated that intrinsic cardiac neuronal somata contain the somatostatin in various combinations with SP and nNOS [56, 69], neuropeptide Y (NPY), and vasoactive intestinal peptide [27, 78]. The cell bodies of the rat intrinsic ganglion cells localized between the right and left branches of the His bundle are positive both for TH and dopamine beta hydroxylase (DBH) [64, 79].

Based on complex neurochemical organization of intrinsic cardiac ganglia it is accepted that, in addition to relaying preganglionic vagal impulses, cardiac ganglia also integrate sensory and sympathetic inputs for rapid temporal reflexes and local regulation of heart rate on a beat-to-beat basis [15].

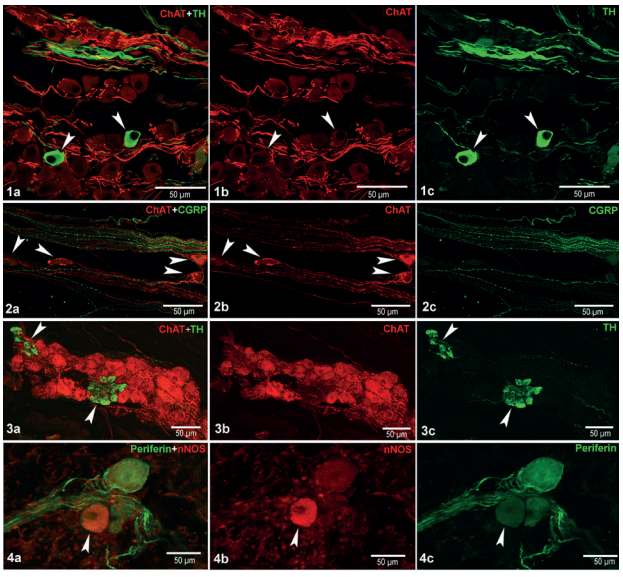

Fig. 5. Laser scanning microphotographs illustrating the neurochemical diversity of the intracardiac nerve cell bodies. Cholinergic neuronal somata (ChAT; in red) predominate in all ganglia (1a, b; 2a, b; 3a, b). Biphenotypic nerve cell bodies, i.e. immunoreactive simultaneously for ChAT and TH (in green) (1a-c), are rather common for the intrinsic cardiac nerve plexus. Numerous CGRP positive nerve fibers are located in the intracardiac nerves (2a, c). Note only ChAT positive neuron somata (arrowheads) located in the nerve (2a, c). Small intensively fluorescent (SIF) cells (arrowheads) clustered into small groups are intervened between ChAT positive neurons (3a, c). Nitriergic neuron is located in the small ganglion (4a, b). Abbreviations: ChAT, choline acetyltransferase; nNOS, neuronal nitric oxide synthase; TH, tyrosine hydroxylase; CGRP, calcitonin gene related peptide.

Innervation of Cardiac Conduction System

All regions of the cardiac conduction system (CCS) possess a significantly higher density of nerve fibers than the adjacent working myocardium [50, 68, 80-82]. The sinoatrial (SA) node area is defined as the most densely innervated region of the CCS [80, 81]. SA nodal innervation amid the cardiac pacemaker cells positive for Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4) is 3-4 fold higher than the surrounding atrial myocardium (Fig. 6) [14, 50, 68]. The density of nerve fibers and their chemical phenotypes varies between the zones of the SA node and this variability is dependent on animal species [25, 68, 80]. ChAT-immunoreactive and TH-immunoreactive nerve fibers are equally abundant in the SA node in mouse (Fig. 6) [14], however TH-immunoreactive fibers composed 51% and ChAT-immunoreactive - 37% in the rabbit SA node [50]. TH-IR nerves represent 40-45% of the total SA nodal innervation as displayed by PGP 9.5 immunoreactivity in the guinea pig heart, however unlike acetylcholinesterase- positive

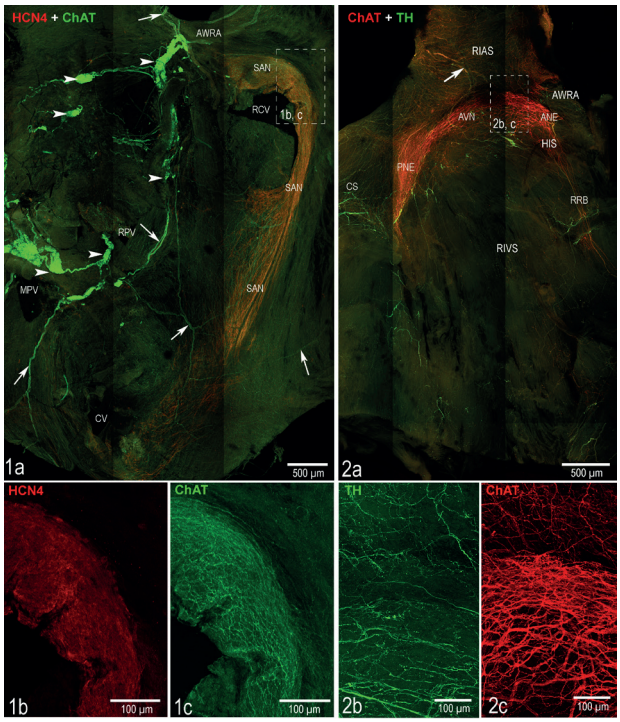

Fig. 6. Laser scanning microphotographs demonstrating morphologic patterns of innervation of the sinoatrial (SAN, panels 1a-c) and atrioventricular (AVN, panels 2a-c) nodal areas. Panels 1a-c display the whole mount preparations from the mouse atria (epicardial side), panels 2a-c demonstrate cardiac septa from the right side. Panels 1b-c demonstrate the significantly denser meshwork of the ChAT immunopositive nerve fibers overlapping HCN4 positive cells stained immunohistochemically in whole mount mouse atrial preparation. Panels 2b-c show the denser ChAT positive nerve fibers, compare to TH. White arrowheads indicate some ganglia, white arrows point to nerves. Abbreviations: ChAT, choline acetyltransferase; TH, tyrosine hydroxylase; HCN4, hyperpolarization activated cyclic nucleotide gated potassium channel 4; AWRA, anterior wall of right atrium; RCV, right cranial vein; RPV, right pulmonary vein; CV, caudal vein; RIVS, right side of interventricular septum; RBB, right branch of the His bundle; RIAS, right side of interatrial septum; CS, coronary sinus; AVN, atrioventricular nodal region; PNE, posterior extension of AVN; ANE, anterior extension of AVN; HIS, bundle of His.

nerves (AChE-positive nerves), a large number of TH-immunoreactive nerves were associated with perivascular plexuses both in and around the SA node [84]. The entire SA node in the guinea pig heart is densely innervated by sympathetic axons, the majority of which are immunoreactive for NPY [56]. In the human heart, the relative density of sympathetic nerve fibers immunoreactive for NPY and TH is significantly greater in the central region of the SA node compared with the periphery [81]. After NPY, the other predominant peptidergic nerve subpopulations are immunoreactive for the sensory peptides SP and CGRP [56, 84], Somatostatin (SOM) and VIP-immunoreactive nerves are very sparse both in the SA node and surrounding right atrium, exhibiting a percentage of stained area 10 - to 40 -fold less than that of NPY and TH -IR nerves, respectively [81, 84]. The great majority of CGRP-IR nerve fibers adjacent to the canine SA node course in numerous large nerve bundles [85].

The electron microscopic data conclusively demonstrate that all nerve fibers identified in the mouse SA node are exclusively composed of unmyelinated nerve fibers and involve axons with both cholinergic and adrenergic neurotransmitters. The axons within unmyelinated nerve fibers have varicosities with abundant round, small, clear, and a few dense-cored vesicles. A number of unmyelinated nerve fibers have axons that are incompletely enveloped by Schwann cells with a fragment of their plasma membrane in direct contact with the basal lamina surrounding the whole unmyelinated nerve fiber. These unmyelinated nerve fibers have varicosities and are distributed regularly in the vicinity of cardiac pacemaker cells. The density of nerve fibers amid the HCN4-immunoreactive cells around the root of superior vena cava are 3-4 folds higher in respect to neighboring atrial zones according to well corresponding data of fluorescent and electron microscopy. In the mouse SAN, the average distance between cardiac pacemaker cells and unmyelinated nerve fibers is less than 0.5 μm [14], whereas only about 80 nm in guinea pig [86]. SA nodal cells are closely associated by at least one unmyelinated nerve fiber or axon, but the majority of these cells regularly are in close proximity to 2-3 unmyelinated nerve fibers [14].

As staining for AChE, as well as immunohistochemical stainingfor PGP 9.5 demonstrates the highest density of thin nerves and NFs along the components of the atrioventricular conductive axis (AVCA) [16,17]. Electron microscopic studies revealed relatively specific endocardial nerves along the AVCA. These tiny nerves were ensheathed by fibroblast cells only, and the size of their axons varied from extremely tiny (<0.2 μm in diameter) up to enormously thick ones that reached 6 μm in diameter. However, thick axons were not frequent and composed only about 12% of all axons examined in this location. The number of axons per one NF varied greatly, but there were NFs containing up to 170 axons. Plenty of axons were not isolated from each other by Schwann cell processes, and 65% of them contacted other axons in the polyaxonal pocket of Schwann cell. Occasionally, polyaxonal Schwann cell pockets with more than 30 axons that were in direct contact with each other were observed [16, 17].

Conclusion. The intrinsic ganglionated neural plexus must be viewed as an intricate, three dimensional structure which is the basis of cardiac control. Its disproportional and discrete spatial distribution throughout the heart enables the intracardiac nervous system to have distinct and separate effects on specific cardiac tissue, thus integrating cardiac function in various mechanisms to meet the demands of the body.

Existing interspecific differences in neuronal abundance and neurochemical diversity may reflect the extent of cardiac adaptive capabilities in these species; likewise any alterations to this intricate circuit may result in impairment of cardiac function. Understanding the morphological basis of cardiac innervation is of uttermost importance in understanding the role of autonomic nervous system in control of circulation.

References:

1. Pauza DH, Pauziene N, Tamasauskas KA, Stropus R. Hilum of the heart. Anat Rec. 1997 Jul;248(3):322–4.
2. 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 Aug 1;259(4):353–82.
3. Pauza DH, Skripka V, Pauziene N. Morphology of the intrinsic cardiac nervous system in the dog: a whole-mount study employing histochemical staining with acetylcholinesterase. Cells Tissues Organs. 2002;172(4):297–320.
4. Batulevicius D, Skripka V, Pauziene N, Pauza DH. Topography of the porcine epicardiac nerve plexus as revealed by histochemistry for acetylcholinesterase. Auton Neurosci. 2008 Feb 29;138(1-2):64–75.
5. Saburkina I, Rysevaite K, Pauziene N, Mischke K, Schauerte P, Jalife J, et al. Epicardial neural ganglionated plexus of ovine heart: anatomic basis for experimental cardiac electrophysiology and nerve protective cardiac surgery. Heart Rhythm. 2010 Jul;7(7):942–50.
6. Armour JA, Murphy DA, Yuan BX, Macdonald S, Hopkins DA. Gross and microscopic anatomy of the human intrinsic cardiac nervous system. Anat Rec. 1997 Feb;247(2):289–98.
7. Rysevaite K, Saburkina I, Pauziene N, Noujaim SF, Jalife J, Pauza DH. Morphologic pattern of the intrinsic ganglionated nerve plexus in mouse heart. Heart Rhythm. 2011 Mar;18;8(3):448–54.
8. Batulevicius D, Pauziene N, Pauza DH. Topographic morphology and age-related analysis of the neuronal number of the rat intracardiac nerve plexus. Ann Anat. 2003 Oct;18;185(5):449–59.
9. Batulevicius D, Pauziene N, Pauza DH. Key anatomic data for the use of rat heart in electrophysiological studies of the intracardiac nervous system. Medicina (Kaunas). 2004 Jan;40(3):253–9.
10. Saburkina I, Gukauskiene L, Rysevaite K, Brack KE, Pauza AG, Pauziene N, et al. Morphological pattern of intrinsic nerve plexus distributed on the rabbit heart and interatrial septum. J Anat. 2014 May;224(5):583–93.
11. Pauziene N, Alaburda P, Rysevaite-Kyguoliene K, Pauza AG, Inokaitis H, Masaityte A, et al. Innervation of the rabbit cardiac ventricles. J Anat. 2015 Oct;
12. Marron K, Wharton J, Sheppard MN, Fagan D, Royston D, Kuhn

DM, et al. Distribution, Morphology, and Neurochemistry of Endocardial and Epicardial Nerve Terminal Arborizations in the Human Heart. Circulation. 1995 Oct;92(8):2343–51.
13. Batulevicius D, Pauziene N, Pauza DH. Architecture and age-related analysis of the neuronal number of the guinea pig intrinsic cardiac nerve plexus. Ann Anat. 2005 Jul;187(3):225–43.
14. Pauza DH, Rysevaite K, Inokaitis H, Jokubauskas M, Pauza AG, Brack KE, et al. Innervation of sinoatrial nodal cardiomyocytes in mouse. A combined approach using immunofluorescent and electron microscopy. J Mol Cell Cardiol. Elsevier; 2014 Oct;75:188–97.
15. Pauziene N, Alaburda P, Rysevaite-Kyguoliene K, Pauza AG, Inokaitis H, Masaityte A, et al. Innervation of the rabbit cardiac ventricles. J Anat. 2015 Oct 29;
16. Pauziene N, Rysevaite-Kyguoliene K, Alaburda P, Pauza AG, Skukauskaite M, Masaityte A, et al. Neuroanatomy of the Pig Cardiac Ventricles. A Stereomicroscopic, Confocal and Electron Microscope Study. Anat Rec (Hoboken). 2017 Oct;300(10):1756–80.
17. Pauziene N, Alaburda P, Rysevaite-Kyguoliene K, Pauza AG, Inokaitis H, Masaityte A, et al. Innervation of the rabbit cardiac ventricles. J Anat. 2016 Jan;228(1):26–46.
18. Slavíková J, Kuncová J, Reischig J, Dvoráková M. Catecholaminergic neurons in the rat intrinsic cardiac nervous system. Neurochem Res. 2003 Apr;28(3-4):593–8.
19. Kember G, Armour J a, Zamir M. Neural control of heart rate: the role of neuronal networking. J Theor Biol. Elsevier; 2011 May;277(1):41–7.
20. Armour J, Ardell J. Neurocardiology. 5th ed. Armour JA, Ardell JL, editors. New York: Oxford University Press; 1994. Structure and function of the mammalian intrinsic p.
21. Armour JA, Murphy DA, Yuan B-X, MacDonald S, Hopkins DA. Gross and microscopic anatomy of the human intrinsic cardiac nervous system. Anat Rec. 1997 Feb;247(2):289–98.
22. Arora RC, Waldmann M, Hopkins DA, Armour JA. Porcine intrinsic cardiac ganglia. Anat Rec. 2003 Mar;271(1):249–58.
23. Baptista CA, Kirby ML. The cardiac ganglia: cellular and molecular aspects. Kaohsiung J Med Sci. 1997 Jan;13(1):42–54.
24. Ellison JP, Hibbs RG. An ultrastructural study of mammalian cardiac ganglia. J Mol Cell Cardiol. 1976 Feb;8(2):89–101.
25. Hoover DB, Ganote CE, Ferguson SM, Blakely RD, Parsons RL. Localization of cholinergic innervation in guinea pig heart by immunohistochemistry for high-affinity choline transporters. Cardiovasc Res. 2004 Apr 1;62(1):112–21.
26. Hoover DB, Isaacs ER, Jacques F, Hoard JL, Pagé P, Armour JA. Localization of multiple neurotransmitters in surgically derived specimens of human atrial ganglia. Neuroscience. 2009 Dec 15;164(3):1170–9.
27. Horackova M, Armour JA, Byczko Z. Distribution of intrinsic cardiac neurons in whole-mount guinea pig atria identified by multiple neurochemical coding. A confocal microscope study. Cell Tissue Res. 1999 Sep;297(3):409–21.
28. Horackova M, Slavikova J, Byczko Z. Postnatal development of the rat intrinsic cardiac nervous system: a confocal laser scanning microscopy study in whole-mount atria. Tissue Cell. 2000 Oct;32(5):377–88.
29. Yuan BX, Ardell JL, Hopkins DA, Losier AM, Armour JA. Gross and microscopic anatomy of the canine intrinsic cardiac nervous system. Anat Rec. 1994 May;239(1):75–87.
30. King BYTS, Coakley JB. The intrinsic nerve cells of the cardiac atria of mammals and man. J Anat. 1958;92(3):353–76.
31. Leger J, Croll RP, Smith FM. Regional distribution and extrinsic innervation of intrinsic cardiac neurons in the guinea pig. J Comp Neurol. 1999 May;407(3):303–17.
32. Moravec M, Moravec J. Intrinsic innervation of the atrioventricular junction of the rat heart. Am J Anat. 1984 Nov;171(3):307–19.
33. Pardini BJ, Patel KP, Schmid PG, Lund DD. Location, distribution and projections of intracardiac ganglion cells in the rat. J Auton Nerv Syst. 1987 Aug;20(2):91–101.
34. Parsons RL, Neel DS, McKeon TW, Carraway RE. Organization of a vertebrate cardiac ganglion: a correlated biochemical and histochemical study. J Neurosci. 1987 Mar;7(3):837–46.
35. Pauza DH, Skripkiene G, Skripka V, Pauziene N, Stropus R. Morphological study of neurons in the nerve plexus on heart base of rats and guinea pigs. J Auton Nerv Syst. 1997 Jan 12;62(1-2):1–12.
36. Pauza DH, Skripka V, Pauziene N, Stropus R. Anatomical study of the neural ganglionated plexus in the canine right atrium: implications for selective denervation and electrophysiology of the sinoatrial node in dog. Anat Rec. 1999 Jul 1;255(3):271–94.
37. Pauziene N, Pauza DH, Stropus R. Morphological study of the heart innervation of bats Myotis daubentoni and Eptesicus serotinus (Microchiroptera: Vespertilionidae) during hibernation. Eur J Morphol. 2000 Jul;38(3):195–205.
38. Pauziene N, Pauza DH, Stropus R. Morphology of human intracardiac nerves: an electron microscope study. J Anat. 2000 Oct;197(3):437–59.
39. Saburkina I, Pauza DH. Location and variability of epicardiac ganglia in human fetuses. Anat Embryol. 2006 Nov;211(6):585–94.
40. Saburkina I, Pauziene N, Pauza DH. Prenatal development of the human epicardiac Ganglia. Anat Histol Embryol. 2009 Jun;38(3):194–9.
41. Singh S, Johnson PI, Lee RE, Orfei E, Lonchyna VA, Sullivan HJ, et al. Topography of cardiac ganglia in the adult human heart. J Thorac Cardiovasc Surg. 1996 Oct;112(4):943–53.
42. de Souza RR, Gama EF, de Carvalho CA, Liberti EA. Quantitative study and architecture of nerves and ganglia of the rat heart. Acta Anat. 1996 Jan;156(1):53–60.
43. Akamatsu FE, De-Souza RR, Liberti EA. Fall in the number of intracardiac neurons in aging rats. Mech Ageing Dev. 1999 Sep 8;109(3):153–61.
44. Tanaka A, Tanaka S, Miyamoto K, Yi S-Q, Nakatani T. Gross anatomical study of the sympathetic cardiac nerves in the house musk shrew (Suncus murinus). Anat Rec. 2007 May;290(5):468–76.
45. Rysevaite K, Saburkina I, Pauziene N, Vaitkevicius R, Noujaim SF, Jalife J, et al. Immunohistochemical characterization of the intrinsic cardiac neural plexus in whole-mount mouse heart preparations. Hear Rhythm. 2011 May:8(5):731–8.
46. Rysevaite K, Saburkina I, Pauziene N, Vaitkevicius R, Noujaim SF, Jalife J, et al. Immunohistochemical characterization of the intrinsic cardiac neural plexus in whole-mount mouse heart preparations. Heart Rhythm. 2011 May;8(5):731–8.
47. Pauza DH, Pauziene N, Pakeltyte G, Stropus R. Comparative quantitative study of the intrinsic cardiac ganglia and neurons in the rat, guinea pig, dog and human as revealed by histochemical staining for acetylcholinesterase. Ann Anat. 2002 Mar;184(2):125–36.
48. Inokaitis H, Pauziene N, Rysevaite-Kyguoliene K, Pauza DH. Innervation of sinoatrial nodal cells in the rabbit. Ann Anat. 2016;205.
49. Pauziene N, Pauza DH. Electron microscopic study of intrinsic cardiac ganglia in the adult human. Ann Anat. 2003 Apr;185(2):135–48.
50. Inokaitis H, Pauziene N, Rysevaite-Kyguoliene K, Pauza DH. Innervation of sinoatrial nodal cells in the rabbit. Ann Anat. Elsevier GmbH.; 2016;205:113–21.
51. Inokaitis H. Sinusinio prieširdžio mazgo ir jo inervacijos lyginamoji anatomija: daktaro disertacija: biomedicinos mokslai, biologija [Internet]. Kaunas; 2016. 107 p. Available from: https://library.lsmuni.lt/primo-explore/fulldisplay?docid=KMU02KMU02-000090286&context=L&vid=LSMU&lang=lt_LT&search_scope=LSMU&adaptor=Local Search Engine&tab=default_tab&query=any,contains,Hermanas Inokaitis&offset=0.
52. Ai J, Epstein PN, Gozal D, Yang B, Wurster R, Cheng ZJ. Morphology and topography of nucleus ambiguus projections to cardiac ganglia in rats and mice. Neuroscience. 2007 Nov 23;149(4):845–60.
53. Muthu P. No Title [Internet]. Lietuvos sveikatos mokslų universitetas; 2018. Available from: https://library.lsmuni.lt/primo-explore/search?query=any,contains,Prabhu Muthu&tab=default_tab&search_scope=LSMU&vid=LSMU&lang=lt_LT&offset=0.
54. Moravec M, Moravec J. 3-D characterization of ganglion cells of the terminal nerve plexus of rat atrioventricular junction. J Auton Nerv Syst. 1998 Nov 25;74(1):1–12.
55. Hoard JL, Hoover DB, Wondergem R. Phenotypic properties of adult mouse intrinsic cardiac neurons maintained in culture. Am J

Physiol Cell Physiol. 2007 Dec;293(6):C1875–83.
56. Steele PA, Choate JK. Innervation of the pacemaker in guinea-pig sinoatrial node. J Auton Nerv Syst. 1994 May 5;47(3):177–87.
57. Rysevaite K, Saburkina I, Pauziene N, Vaitkevicius R, Noujaim SF, Jalife J, et al. Immunohistochemical characterization of the intrinsic cardiac neural plexus in whole-mount mouse heart preparations. Heart Rhythm. 2011 May;8(5):731–8.
58. Crick SJ, Anderson RH, Ho SY, Sheppard MN. Localisation and quantitation of autonomic innervation in the porcine heart II: endocardium, myocardium and epicardium. J Anat. 1999 Oct;195(3):359–73.
59. Fregoso SP, Hoover DB. Development of cardiac parasympathetic neurons, glial cells, and regional cholinergic innervation of the mouse heart. Neuroscience. IBRO; 2012 Sep;221:28–36.
60. Hoover DB, Shepherd A V, Southerland EM, Armour JA, Ardell JL. Neurochemical diversity of afferent neurons that transduce sensory signals from dog ventricular myocardium. Auton Neurosci. 2008 Aug 18;141(1-2):38–45.
61. Parsons RL. Mammalian Cardiac Ganglia as Local Integration Centers: Histochemical and Electrophysiological Evidence. In: Dun NJ, Machado BH, Pilowsky PM, editors. Neural Mechanisms of Cardiovascular Regulation. Boston, MA: Springer US; 2004;335–56.
62. Brack KE. The heart's "little brain" controlling cardiac function in the rabbit. Exp Physiol. 2015 Apr 1;100(4):348–53.
63. Kandel E. Principles of Neural Science, Fifth Edition. McGraw Hill Professional; 2013. 1709 p.
64. Richardson RJ, Grkovic I, Anderson CR. Immunohistochemical analysis of intracardiac ganglia of the rat heart. Cell Tissue Res. 2003 Dec;314(3):337–50.
65. Standring S. Gray's Anatomy: The Anatomical Basis of Clinical Practice. Churchill Livingstone/Elsevier; 2008. 1551 p.
66. Hasan W. Autonomic cardiac innervation: development and adult plasticity. Organogenesis. 2013 Jan;9(3):176–93.
67. Calupca MA, Vizzard MA, Parsons RL. Origin of neuronal nitric oxide synthase (NOS)-immunoreactive fibers in guinea pig parasympathetic cardiac ganglia. J Comp Neurol. 2000 Oct 23;426(3):493–504.
68. Crick SJ, Anderson RH, Ho SY, Sheppard MN. Localisation and quantitation of autonomic innervation in the porcine heart II: endocardium, myocardium and epicardium. J Anat. 1999 Oct;195(3):359–73.
69. Mawe GM, Talmage EK, Lee KP, Parsons RL. Expression of choline acetyltransferase immunoreactivity in guinea pig cardiac ganglia. Cell Tissue Res. 1996 Jul;285(2):281–6.
70. Weihe E, Schütz B, Hartschuh W, Anlauf M, Schäfer MK, Eiden LE. Coexpression of cholinergic and noradrenergic phenotypes in human and nonhuman autonomic nervous system. J Comp Neurol. 2005 Nov;492(3):370–9.
71. Singh S, Johnson PI, Javed a, Gray TS, Lonchyna V a, Wurster RD. Monoamine- and histamine-synthesizing enzymes and neurotransmitters within neurons of adult human cardiac ganglia. Circulation. 1999;99(3):411–9.
72. Hoard JL, Hoover DB, Mabe AM, Blakely RD, Feng N, Paolocci N. Cholinergic neurons of mouse intrinsic cardiac ganglia contain noradrenergic enzymes, norepinephrine transporters, and the neurotrophin receptors tropomyosin-related kinase A and p75. Neuroscience. 2008 Sep 22;156(1):129–42.
73. Rysevaite K, Saburkina I, Pauziene N, Vaitkevicius R, Noujaim SF, Jalife J, et al. Immunohistochemical characterization of the intrinsic cardiac neural plexus in whole-mount mouse heart preparations. Hear Rhythm. 2011 May;8(5):731–8.
74. Pauziene N, Alaburda P, Rysevaite-Kyguoliene K, Pauza AG, Inokaitis H, Masaityte A, et al. Innervation of the rabbit cardiac ventricles. J Anat. 2016 Jan;228(1):26–46.
75. Pauziene N, Rysevaite-Kyguoliene K, Alaburda P, Pauza AG, Skukauskaite M, Masaityte A, et al. Neuroanatomy of the Pig Cardiac Ventricles. A Stereomicroscopic, Confocal and Electron Microscope Study. Anat Rec. 2017.
76. Hasan W, Smith PG. Modulation of rat parasympathetic cardiac ganglion phenotype and NGF synthesis by adrenergic nerves. Auton Neurosci. 2009 Jan 28;145(1-2):17–26.
77. Li L, Hatcher JT, Hoover DB, Gu H, Wurster RD, Cheng ZJ. Distribution and morphology of calcitonin gene-related peptide and substance P immunoreactive axons in the whole-mount atria of mice. Auton Neurosci. 2014 Apr;181:37–48.
78. Parsons RL, Locknar SA, Young BA, Hoard JL, Hoover DB. Presence and co-localization of vasoactive intestinal polypeptide with neuronal nitric oxide synthase in cells and nerve fibers within guinea pig intrinsic cardiac ganglia and cardiac tissue. Cell Tissue Res. 2006 Feb 12;323(2):197–209.
79. Moravec M, Moravec J, Forsgren S. Catecholaminergic and peptidergic nerve components of intramural ganglia in the rat heart. An immunohistochemical study. Cell Tissue Res. 1990 Nov;262(2):315–27.
80. Crick SJ, Sheppard MN, Anderson RH, Polak JM, Wharton J. A quantitative assessment of innervation in the conduction system of the calf heart. Anat Rec. 1996 Aug;245(4):685–98.
81. Crick SJ, Wharton J, Sheppard MN, Royston D, Yacoub MH, Anderson RH, et al. Innervation of the human cardiac conduction system. A quantitative immunohistochemical and histochemical study. Circulation. 1994 Apr 1;89(4):1697–708.
82. Steele PA, Gibbins IL, Morris JL. Projections of intrinsic cardiac neurons to different targets in the guinea-pig heart. J Auton Nerv Syst. 1996 Jan 5;56(3):191–200.
83. Crick SJ, Sheppard MN, Ho SY, Anderson RH. Localisation and quantitation of autonomic innervation in the porcine heart I: conduction system. J Anat. 1999 Oct;195(3):341–57.
84. Crick SJ, Sheppard MN, Anderson RH, Polak JM, Wharton J. A quantitative study of nerve distribution in the conduction system of the guinea pig heart. J Anat. 1996 Apr;188(2):403–16.
85. Ursell PC, Ren CL, Albala A, Danilo P. Nonadrenergic noncholinergic innervation. Anatomic distribution of calcitonin gene-related peptide-immunoreactive tissue in the dog heart. Circ Res. 1991 Jan;68(1):131–40.
86. Choate JK, Klemm M, Hirst GD. Sympathetic and parasympathetic neuromuscular junctions in the guinea-pig sino-atrial node. J Auton Nerv Syst. 1993 Jul;44(1):1–15.