Modeling of the cardiac sympathetic nervous system and the contribution of epicardium-derived cells

Ge, Y.

8

General Discussion

The relevance of the superior cervical ganglion for cardiac autonomic innervation in health and disease: Systematic review - Part 1: Morphological considerations

Yang Ge[1,2], H. Sophia Chen[1,2], Jan Schoones[3], Marco C. DeRuiter[2], Monique R.M. Jongbloed[1,2]

1. Department of Anatomy & Embryology, Leiden University Medical Center, Einthovenweg 20, 2333 ZC Leiden, The Netherlands;

2. Department of Cardiology, Leiden University Medical Center, Albinusdreef 2, 2333 ZC Leiden, The Netherlands;

3. Walaeus library of the Leiden University Medical Center, Albinusdreef 2, 2333 ZC Leiden, The Netherlands

Adapted manuscript in preparation, with an additional discussion for this thesis

ABSTRACT

Cardiac autonomic innervation is of major importance for maintaining homeostasis, including the adaption to environmental conditions in healthy as well as in pathological states. The autonomic nervous system is divided in sympathetic and parasympathetic branches. Spinal branches of the sympathetic nervous system synapse in ganglia of the sympathetic chain, that is situated bilaterally in a paravertebral position. In humans and animal models, the heart receives cervical as well as thoracic sympathetic contributions. Bilateral cervical and thoracic ganglia, especially the stellate ganglia, are important components participating in cardiac sympathetic innervation. In disease states, such as cardiac ischemia, neuronal remodeling can occur, resulting in a sympathetic overdrive. In particular, the role of cardiac sympathetic hyperinnervation in arrhythmogenesis after myocardial infarction, has gained increasing attention over the past decades. The superior cervical ganglion is situated at the bifurcation of the common carotid arteries, that provides a relatively easily accessible anatomical landmark. This rather superficial position of the superior cervical ganglia, especially in comparison with the stellate ganglia, that are found at a deeper position within the thorax, likely facilitated the choice of these ganglia for many experimental studies on cardiac innervation in health and disease. However, the reports on the clinical relevance of contributions of cervical ganglia to cardiac innervation have been controversial, and these ganglia also contribute to the innervation of other structures in the head and neck, including the iris and submandibular gland. In this review, we aim to systematically investigate current evidence, as well as to expose current controversies, on the contribution of the superior cervical ganglia to cardiac innervation in health and disease in human and other animal models. This chapter encompasses part 1 of the review in preparation: Morphological data. In addition, a general discussion for this thesis is included.

INTRODUCTION

A balanced function of the cardiac autonomic nervous system is essential to maintain cardiovascular homeostasis. Cardiac innervation is provided by the autonomic nervous system, which is organised in sympathetic and parasympathetic branches. A balance between sympathetic and parasympathetic tone is mandatory to maintain a regular heartbeat. Parasympathetic innervation of the heart is provided by branches of the vagal nerve that synapse close to the target organ, i.e., in parasympathetic ganglia situated at the epicardial surface of the heart. For sympathetic innervation, preganglionic cardiac sympathetic axons synapse with postganglionic sympathetic neurons in the sympathetic chain (1). In contrast to nerve supply at the abdominal level, postganglionic axons towards the heart do not route via the gray ramus communicans and spinal nerve, but originate directly from the sympathetic chain ganglia. In humans, this innervation from the sympathetic chain is likely provided by both cervical and thoracic ganglia, although the exact level of ganglia contributing to the heart is still controversial (1).

Interest in cardiac autonomic innervation has increased in the past decades, as a myriad of studies have reported alteration in cardiac innervation, both morphologically as well as functionally, after cardiac damage. An especially intriguing phenomenon, is the so-called cardiac sympathetic hyperinnervation, that can occur after cardiac damage, such as myocardial infarction (2,3). This hyperinnervation is characterised by an increased amount of sympathetic nerve fibres in the area of damage and has been related to ventricular arrhythmias and sudden cardiac death after myocardial infarction (MI). Although several excellent mechanistic studies have been performed, to date the exact underlying relation between the occurrence of sympathetic hyperinnervation and ventricular arrhythmias after MI is still uncertain. Apparently sympathetic ganglia, which are renowned for their limited growth potential after birth, retrieve their potential for fast outgrowth after cardiac damage. These findings have prompted researchers in the field to study prerequisites for cardiac innervation in health and disease.

Although the stellate ganglion is generally accepted to provide the majority of cardiac innervation, also other ganglia have been proposed to provide their contributions in health and disease, including the thoracic and cervical ganglia (1,4). In addition, sidedness seems relevant in cardiac autonomic innervation (5), as well as the sex of the subject (6). In animal models, many studies have taken advantage of the relatively good accessibility of the superior cervical ganglion (SCG), that is located at a specific anatomical landmark location, at the bifurcation of the common carotid arteries (Figure 1).

In contrast, the stellate ganglia (consisting of the fused inferior cervical ganglion with the first thoracic ganglion) that are most renowned for their contribution to cardiac innervation, are located at a deeper location in the thorax, just below the subclavian artery at the level of the 7th cervical vertebra (Figure 1). Although cervical ganglia have indeed been shown to contribute to cardiac innervation both in animal models as well as in human, reports in literature differ (1), and these ganglia also contribute to innervation of other structures in the head and neck, including the iris, jaw, submandibular gland, the pineal gland and the carotid body (7–9). The SCG is in close spatial orientation with both the carotid body, a chemoreceptor sensitive organ that can respond to changes in blood O2, CO2 pH, and with the ganglion nodosum, the inferior ganglion of the vagal nerve (10,11).

As the SCG is used for studying cardiac innervation in a myriad of reports, including in our own studies (12), in this review, we aimed to systematically investigate current evidence, as well as to expose current controversies and gaps in knowledge, on the contribution of the SCG to cardiac innervation in health and disease in human and other animal models, considering potentially relevant aspects such as sex and sidedness.

MATERIALS AND METHODS

Research questions. Our research questions are defined as follows:

1. What is the morphological evidence that the SCG is involved in cardiac innervation in human and in other animal species, in health as well as in cardiac disease?
2. What is the functional evidence that the SCG is involved in cardiac innervation in human and in other animal species, in health as well as in cardiac disease?
3. Is sidedness relevant (i.e., using left or right sided SCG) to study cardiac innervation?
4. Have sex differences been studied and/or encountered?
5. Which controversies are encountered, and which questions are potentially unanswered by current data?
6. And finally, derived from these data: Is the use of the SCG in experimental setting an adequate structure to study cardiac innervation in health and disease?

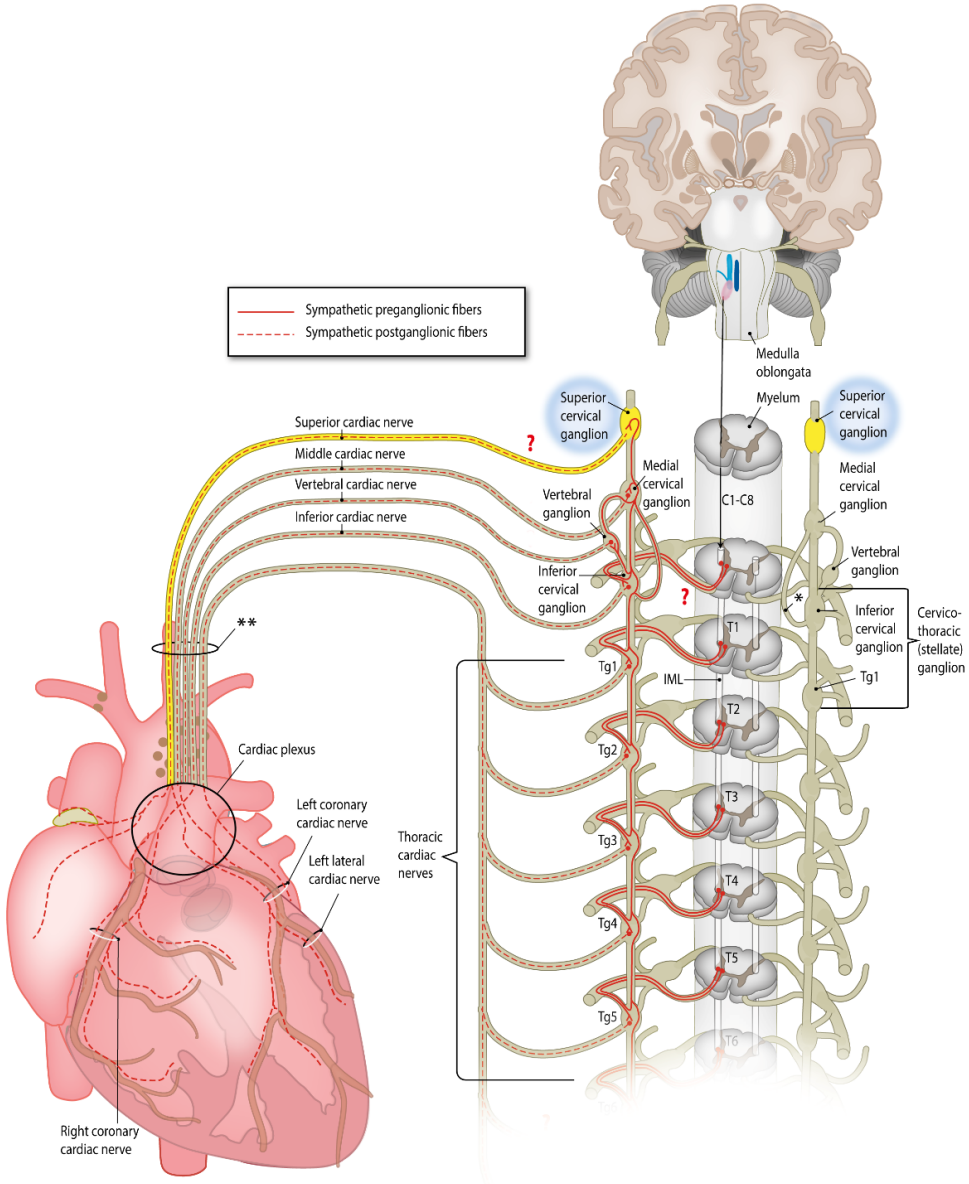
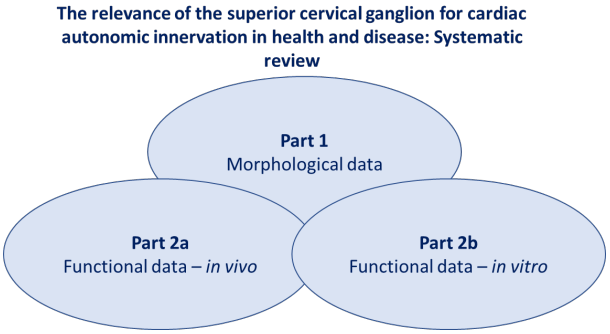


Figure 1: Anatomy of the cardiac sympathetic autonomic nervous system. The superior cervical ganglia are bilaterally indicated in bright yellow. (The figure is adapted from Wink et al. Auton Neurosci. 2020.)

We aim to provide the answer to these research questions in 3 parts:



Part 1: Morphological considerations (current review)

Part 2a: Functional considerations – in vivo studies

Part 2b: Functional considerations – in vitro studies

Search strategy. The systematic review was performed based on the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) checklist (http://prisma-statement.org/prismastatement/Checklist.aspx), using the same workflow as in previous systematic reviews from our group (1,13,14). A comprehensive literature search was performed in PubMed, Embase, Web of Science, and Cochrane Library. The search strategy was carried out by using key words for "superior cervical ganglion" and "heart", combined with "innervation" and "nerve growth factor". Duplicate articles were removed. The complete query can be found in Appendix A.

Selection of articles. Inclusion: Papers were considered eligible to be included when there was a combination of SCG with morphological or functional data in original research papers. All articles published after 1975 were included. Both studies in health and in disease models were included, and all animal species including human. Both prenatal and postnatal studies were included. In vitro studies were also included (Part 2b), as long as an interdependent effect of SCG and myocardium or vice versa was studied. Relevant articles found in the references of papers included via the query, were also included, as long as they were original articles and published after 1975. Retrograde labeling studies were considered as morphological studies.

Exclusion: Studies in which the heart and SCG were studied independently, i.e., when no morphological or functional relationship was studied, were considered not eligible to answer our research questions and were therefore excluded. For example, when the effect of certain substances was studied independently on the heart and on the SCG, but no causal effect of the alterations in SCG on the heart or vice versa was described, the paper was excluded. In addition, we excluded papers that contained only information on the heart, only information on ganglia (without any relation to the heart), or papers describing other ganglia than the SCG (unless the SCG was described as well). Papers describing the SCG in relation to other organs than the heart were excluded. We included only original papers; therefore, reviews and book

chapters were excluded. Other reasons to exclude the paper were: non-English papers, papers that could not be retrieved after significant effort and papers that were from journals that were listed as predatory journal (https://predatoryjournals.com/publishers/). With regard to date of publication, we excluded studies that were published before 1975, partly because of the difficulties to retrieve several older papers.

Data extraction and appraisal. All abstracts were screened for eligibility by two independent observers (H.S.C. and M.R.M.J.) taking into account the selection criteria described above. In case of disagreement, differences between the observers' judgements were discussed to seek consensus.

After selection, all included papers were evaluated in light of the 5 research questions described above and categorised based on 1. Morphological data; 2. Functional data, in each section taking the following parameters into account data on species, disease model (health or disease), sidedness and sex.

Quality assessment. Quality assessment of included articles was performed with the Quality Appraisal for Cadaveric Studies (QUACS scale) for morphological studies (Part 1). The original QUACX score is composed of 13 parameters (15). As statistical analyses were not performed in any of the included studies with regard to the SCG, we omitted this parameter and based our score on and evaluation of the other 12 parameters, rendering a score of 12/12 (100%) the maximum score that could be obtained. If only part of the criteria for a specific parameter was met, 0.5 point (instead of 1 point) was assigned. For functional studies, the SYRCLE's risk of bias tool and CAMARADES 10-item quality checklist will be used (Part 2a, in preparation and not described in this thesis). The methodological quality of the included studies was first rated independently by two authors (H.S.C. and M.R.M.J.). After comparing and discussing the individual scores for each article, a consensus score was achieved for each of the 21 articles included in this review (Table 2).

RESULTS

1. Study selection.

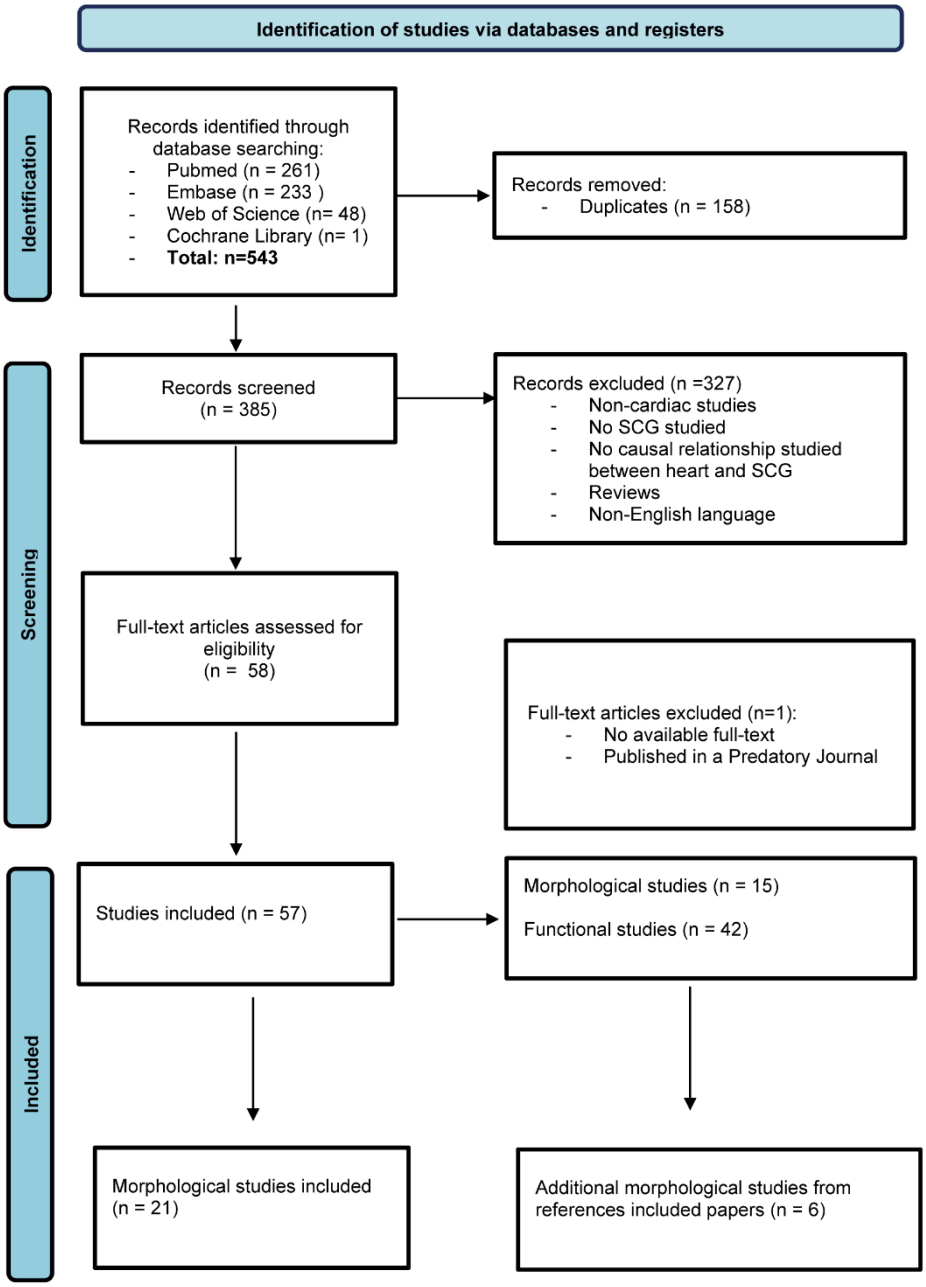
The search results and study selection flowchart are reported in Flowchart 1. From the initial 543 records identified through database searching and cited reference searching, 158 were duplicates and a total of 327 records were excluded because the studies did not meet our inclusion criteria, leaving 58 records available. One study was published in a predatory journal, leaving 57 studies considered eligible for inclusion in this review. Of these, 15 were morphological studies, and 42 were functional studies. As described above, the current review (Part 1) focusses on morphological papers. Next to the originally included 15 papers, we added 6 papers that fulfilled the inclusion criteria derived from screening references, leading to the 21 papers that were evaluated.

2. Overview of included studies.

Table 1 provides an overview of the 21 morphological studies included in part 1 of the systematic review. A total of 4 studies was performed in human, and 17 in other species: monkey (n=8), dog (n=3), cat (n=2), rat (n=1), shrew (n=1), chick (n=2). In one of the studies describing monkeys, also the primate Philippine Tarsier was included. No morphological studies were found in mice (*Mus musculus*). Two studies were performed in prenatal stages (17,18), both in chick, the remaining 19 papers concerned postnatal stages in mammals.

3. Quality assessment

Table 2 provides the quality assessment of the 21 included studies. Quality scores ranges from 46% to 67%. In the majority of morphological studies, a thorough description of results was presented, either with or without details on consistency of data with regard to number or percentages of cases in which observations were made. The far majority of studies adequately discussed findings in the context of contemporary evidence. Most studies adequately supported their description of data with photographs and/or drawings. The relatively low scores could be attributed largely to deficient data on education of dissecting researchers and on the number of observers, that was lacking in all of the included studies. In addition, distinctly indicated study limitations and clinical implications were lacking in the majority of the morphological manuscripts included. Approximately half of the included studies specified the sex of the specimen used (21,23,28,30,31,33–36).



Flowchart 1: PRISMA flow diagram (16).

Table 1: Results part 1: Included morphological papers

#	Author	Year	Species	Title paper	Original query or added from references
1	Kirby(17)	1980	Chick	*Developing innervation of the chick heart: a histofluorescence and light microscopy study of sympathetic innervation*	from references
2	Armour(20)	1981	Dog	*Localization of sympathetic postganglionic neurons of physiologically identified cardiac nerves in dog*	Original
3	Billman(21)	1982	Monkey	*A description of the upper thoracic autonomic nervous system in the rhesus monkey (Macaca Mulatta)*	Original
4	Hopkins(20)	1984	Dog	*Localization of sympathetic postganglionic and parasympathetic preganglionic neurons which innervate different regions of the dog heart*	Original
5	Shih(22)	1985	Cat	*Horseradish peroxidase localization of the sympathetic postganglionic neurons innervating the cat heart*	from references
6	Janes(23)	1986	Human	*Anatomy of human extrinsic cardiac nerves and ganglia*	Original
7	Wu(24)	1988	Cat	*Sympathetic postganglionic innervation of the cardiac coronary artery in cats*	from references
8	Pardini(25)	1989	Rat	*Organization of the sympathetic postganglionic innervation of the rat heart*	Original
9	Chuang(26)	1992	Monkey	*Localization of the sympathetic postganglionic neurons innervating cardiac coronary artery with horseradish peroxidase in monkeys*	from references
10	Hirakawa(27)	1993	Dog	*Sympathetic innervation of the young canine heart using antero- and retrograde axonal tracer methods*	Original
11	Verberne(18)	1999	Chick	*Contribution of the cervical sympathetic ganglia to the Innervation of the pharyngeal arch arteries and the heart in the chick embryo*	Original
12	Pather(28)	2003	Human	*The sympathetic contributions to the cardiac plexus*	Original
13	Chuang(29)	2004	Monkey	*Horseradish peroxidase localization of sympathetic postganglionic and parasympathetic preganglionic neurons innervating the monkey heart*	Original
14	Kawashima (1)(30)	2005	Human	*Topological changes of the human autonomic cardiac nervous system in individuals with a retroesophageal right subclavian artery: two case reports and a brief review*	Original
15	Kawashima (2)(31)	2005	Monkey	*Comparative anatomical Study of the autonomic cardiac nervous system in macaque monkeys*	Original

16	Kawashima (3)(32)	2005	Human	*The autonomic nervous system of the human heart with special reference to its origin, course, and peripheral distribution*	from references
17	Tanaka(33)	2007	Shrew	*Gross anatomical study of the sympathetic cardiac nerves in the house musk shrew (Suncus murinus)*	original
18	Kawashima(34)	2008	Monkey	*Systematic morphology and evolutionary anatomy of the autonomic cardiac nervous system in the lesser apes, gibbons (Hylobatidae)*	from references
19	Kawashima(35)	2009	Monkey	*Comparative anatomy and evolution of the cardiac innervation in new world monkeys (Platyrrhini, E. Geoffroy, 1812)*	original
20	Kawashima(36)	2011	Monkey	*Comparative morphological configuration of the cardiac nervous system in lorises and galagos (Infraorder Lorisiformes, Strepsirrhini, Primates) with evolutionary perspective*	original
21	Kawashima(37)	2013	Philippine Tarsier	*Evolutionary anatomy and phyletic implication of the extrinsic cardiac nervous system in the Philippine Tarsier (Tarsius syrichta, Primates) in comparisons with Strepsirrhines and New World Monkeys*	original

Table 2 Quality assessment of included papers

Study	Subjects					Methods				Limitations reported	QUACS score (%)
	*n**	*Species*	*Age (years)*	*Weigh t*	*Sex (M/F)*	*Type*	*Data on sidednes s*	*Condition and setting*	*Solution, processing and stain(ing)*		
Kirby et al (1980)	NR	Chick	4 days of incubation - 3 days after hatching	NR	NR	Histomorphology	Yes	Health: NR	Fixation: Cold neutral buffered formalin or frozen in liquid nitrogen slush, freeze-dried and treated with formaldehyde gas Embedding: paraffin Sections: 10um Stain(ing): glyoxylic acid for catecholamine histofluorescence	NR	50
Armour et al (1981)	38	Dog	NR	12-20 kg	Either sex, number NR	Retrograde labeling Macromorpholog y	Yes	Health: NR Setting: Unilateral thoracotomy with identification of a cardiac nerve by measuring cardiovascular responses to stimulation, injection of 30% aqueous HRP into the nerve, re-anesthesia on the third postoperative day by overdose of	Fixation: perfusion with 1.0% paraformaldehyde, 1.25%glutaraldehyde in phosphate buffer (pH7.4), ganglia stored in 10% sucrose in phosphate buffer until processing Sections: Frozen sections, 40um Stain(ing): HRP and neutral red	NR	63

								sodium pentobarbital, followed by fixation and removal of tissue			
Billman et al (1982)	10	Monkey	NR	NR	10/0	Macromorphology	Yes	Health: NR Setting: freshly killed or obtained no later than 24h post mortem	Fixation: No fixative used	NR	46
Hopkins et al (1984)	27	Dog	NR	12-18 kg	Either sex, number NR	Retrograde labeling Macromorphology	Yes	Health: NR Setting: Unilateral thoracotomy with injection of 30% aqueous HRP or wheat germ agglutinin-HRP in a variety of regions of the heart, re-anesthesia on the third postoperative day by overdose of sodium pentobarbital, followed by fixation and removal of tissue	Fixation: perfusion with 1.0% paraformaldehyde, 1.25%glutaraldehyde in phosphate buffer (pH7.4), ganglia stored in 10% sucrose in phosphate buffer until processing Sections: Frozen sections, 40um Stain(ing): HRP and neutral red	Leakage of HRP from the injection site could have been picked up by thoracic neuronal elements other than those in the heart	67
Shih et al (1985)	15	Cat	NR	2.0-4.0 kg	Either sex, number NR	Retrograde labeling Histomorphology	Yes	Health: NR Setting: Right thoracotomy with injection of 20% HRP in a variety of regions of the	Fixation:1.25% glutaraldehyde, 1% paraformaldehyde in 0.1M phosphate buffer (pH7.4), ganglia stored	NR	58

								heart, re-anesthesia after 72-96h with sodium pentobarbital followed by fixation and removal of tissue	in 10% sucrose-phosphate buffer Sections: Frozen sections, 40 um Stain(ing): HRP and neutral red		
Janes et al (1986)	23	Human	6-89	NR	15/8	Autopsy (n=10) and embalmed (n=13) cadavers Macromorphology Histomorphology	Yes	Health: NR	Fixation: Embalming fluid; 25% glycerine, methylhydrate, 6% phenol, 6% formaldehyde and water Embedding: ethanol-benzene-paraffin Sections: 10um Stain(ing): Cresyl violet or Cresyl violet with Luxol vast blue	NR	67
Wu et al (1988)	28	Cat	NR	2.0-4.0 kg	Either sex, number NR	Retrograde labeling Histomorphology	Yes	Health: NR Setting: Unilateral thoracotomy with dissection of a variety of coronary arteries and enclosement with a silicone tubing impregnated with 40% HRP in 1% dimethyl sulfoxide solution, re-anesthesia 4 days	Fixation: 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1M phosphate buffer (pH7.4), ganglia were stored in 10% sucrose-phosphate buffer Sections: Frozen sections, 40um Stain(ing): HRP and neutral red	NR	58

								postoperative with fixation and removal of tissue			
Pardini et al (1989)	69	Rat	NR	250-350 g	NR	Retrograde labeling Histomorphology	Yes	Health: NR Setting: Mid-sternal thoracotomy with injection of Diamidino Yellow into left or right ventricular free wall or surface of the heart, re-anesthesia 96-120 hours later followed by fixation and removal of tissue	Fixation: 10% buffered formalin Sections: Frozen sections, 20 um Stain(ing): no staining/Diamidino Yellow?	NR	54
Chuang et al (1992)	Monkey	Adult, exact age NR	4.0-7.0 kg	Either sex, number NR		Retrograde labeling Histomorphology	Yes	Health: NR Setting: Unilateral thoracotomy with dissection of a variety of coronary arteries and enclosement with a silicone tubing impregnated with 2mg HRP in 5ul 2% dimethyl sulfoxide solution, re-anesthesia after 84-96 hours with fixation and removal of tissue	Fixation: 1.25% glutaraldehyde plus 1% paraformaldehyde in 0.1M phosphate buffer (pH 7.4), ganglia were stored in 10% sucrose-phosphate buffer Sections: Frozen sections, 40 um Stain(ing): HRP	NR	67
Hirakawa et al	23	Dog	>2 months	2.5-3.5 kg	Either sex,	Retrograde labeling	Yes	Health: NR	Fixation: Perfusion with 2.5% formalin and 1%	NR	63

(1993)					number NR	Macromorphology Histomorphology		Setting: Unilateral thoracotomy with injection of 3-4% wheat germ agglutinin HRP into various regions of the heart	glutaraldehyde, ganglia stored in 30% sucrose in phosphate buffer until processing Sections: Frozen sections, 50um Stain(ing): HRP and neutral red		
Verberne et al (1999)	24	Chick (n=3) and Quail (n=21)	stage HH10-13	NR	NR	Histomorphology	No	Health: NR	Fixation: ethanol-acetic acid for 24-72hr Embedding: paraffin Sections: 5 um Staining: tyrosine hydroxylase and haematoxylin	NR	58
Pather et al (2003)	8 21	Human	18-55 <0	NR	4/4 11/10	Type cadavers NR Macromorphology Histomorphology	Yes	Health: NR	Fixation: formalized (Solution NR) Embedding: paraffin Sections: 0.1um*** Stain(ing): hematoxylin and eosin	NR	58
Chuang et al (2004)	16	Monkey	Adult, exact age NR	4.0-8.0 kg	Either sex, number NR	Retrograde labeling Macromorphology Histomorphology	Yes	Health: NR Setting: Unilateral thoracotomy with injection of HRP in a variety of regions of the heart, reanesthesia with sodium pentobarbital after 84-96 hours	Fixation: Perfusion with 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1M phosphate buffer (pH7.4) and subsequently 10% sucrose in 0.1M phosphate buffer (pH 7.4), ganglia stored in 10% sucrose-phosphate buffer for 24 hours	NR	63

								and removal of tissue	Sections: Frozen sections, 40 um Staining: HRP and neutral red		
Kawashima (1) et al (2005)	2	Human	80 and 84 years	NR	Female	Embalmed cadavers Macromorphology	Yes	Health: retroesophageal right subclavian artery (cause of death: case 1:gastric cancer, case 2: duodenal cancer)	Fixation 10% formalin, and preservation in 10% alcohol for more than 1 year	NR	63
Kawashima (2) et al (2005)	11	Monkey	10 adults 1 child, exact age NR	NR	7/4	Macromorphology	Yes	Health: NR	Fixation: 10% formaldehyde solution and preserved in 30% for >1 year	NR	58
Kawashima (3) et al (2005)	18	Human	adult, exact age NR	NR	NR	Embalmed cadavers Macromorphology	Yes	Health: clearly abnormal hearts and/or surrounding vessels were excluded Setting: 3 bodies were examined after decalcification	Fixation: 10% formalin, preserved in 30% ethyl alcohol over 1 year, subjected to normal Japanese embaling procedures for student anatomical practice Staining: Sudan black B	NR	58
Tanaka et al (2007)	10	Shrew	adult, exact age NR	40-50 g	5/5	Macromorphology	Yes	Health: NR Setting: anesthesia with injection of pentobarbital, perfusion through catheter in the intraabdominal	Fixation: 4% paraformaldehyde in 0.01M phosphate buffer (pH 7.2) Staining: Whole-Mount immunostaining	NR	58

								aorta with 0.01M phosphate buffer (pH 7.2), followed by 4% paraformaldehyde and labeling of the blood vessels by injection of neoprene latex, fixation and removal of tissues			
Kawashima et al (2008)	10	Monkey	5 adults, 2 subadult, 3 infants, exact age NR	NR	8/2	Macromorphology	Yes	Health: NR	Fixation: 10% formaldehyde solution and/or preserved in 70% alcohol for more than 10 years	NR	63
Kawashima et al (2009)	12	Monkey	7 adults, 1 juvenile, 2 subadult, exact age NR	NR	4/7 1 unknown	Macromorphology	Yes	Health: clearly abnormal heart, abnormal surrounding great vessels, and/or a heart position associated with a condition such as diaphragmatic hernia, thorax abnormality or pregnancy were excluded	Fixation: 10% formaldehyde solution and/or preserved in 70% for at least 3 years	NR	63
Kawashima et al (2011)	7	Monkey	3 adults, 2 subadult, 2 infant, exact age NR	NR	3/4	Macromorphology	No**	Health: clearly abnormal heart, abnormal surrounding great vessels, and/or a	Fixation: 10% formaldehyde solution and/or preserved with 30-70% alcohol for at least 3 years	NR	63

								heart position associated with a condition such as diaphragmatic hernia, thorax abnormality or pregnancy were excluded			
Kawashima et al (2013)	3	Monkey	adult, exact age NR	NR	NR	Macromorphology	Yes	Health: NR	Fixation: 10% formaldehyde solution and/or preserved in 70% alcohol for more than 10 years	NR	54

4. The relevance of the SCG for cardiac innervation: Morphological data

In many of the included studies, the evidence of morphological contributions of the SCG to cardiac innervation was not the primary objective of the study. In our analysis, we only included data that was specifically related to the SCG and/or superior cardiac nerve. Table 3 outlines the primary objectives/aims of the included papers, and provides a summary of the main findings related to SCG contributions to cardiac sympathetic innervation. Findings are discussed below, per developmental stage (prenatal versus postnatal) and per species.

Table 3: Objectives and summary of main results of included studies

	Study	Objective	Results
Chick/Quail			
	Kirby et al (1980)	To correlate the developing innervation of the chick heart with the known innervation of the adult chicken heart	No connections from SCG to the heart
	Verberne et al (1999)	To investigate in detail the contribution to cardiac innervation To study the neural crest origin of the SCG to show their relationship with the sympathetic neural crest	No connection between the SCG and the heart
Shrew			
	Tanaka et al (2007)	To elucidate the routes of the sympathetic cardiac nerves from their origin to the heart	The nerve originating from the SCG descended to reach the aortic arch and formed nerve plexuses after reaching the arterial pole supplying nerves to the ventral wall of the ventricle
Rat			
	Pardini et al (1989)	To investigate the organization of the cardiac sympathetic innervation of the rat	3% of the labeled somata were found bilaterally in the SCG after injection of the left ventricular free wall 1% of the labeled somata were found bilaterally in the SCG after injection of the right ventricular free wall
Cat			
	Shih et al (1985)	To investigate whether sympathetic innervation to the heart may be more specific with respect to the ganglionic origin of postganglionic neurons than previously believed To investigate if the sympathetic supply to the cardiac regions of the apex, to the sinoatrial nodes and to the right and left ventricles may differ from one another	No HRP-positive neurons where observed in the superior cervical ganglia after injections into the apex Occasionally labeled cells in the bilateral SCG after injection into the ventral wall of the RV Occasionally labeled cells in the bilateral SCG after injection into the dorsal wall of the LV Occasionally labeled cells in the right SCG after injection into the Sinoatrial nodal region No HRP-positive neurons where observed in the superior cervical ganglia after injections into pericardial sac
	Wu et al (1988)	To examine the retrograde axonal origin of the postganglionic innervation of the coronary arteries	Few cells in the SCG after application of HRP to the main trunk of the left coronaries

		To trace the innervation of the terminal branches of the coronary arteries	Occasionally some labeled cells in the SCG after application of HRP to the main trunk of the right coronaries Few cells in the LSCG, no neuron in the RSCG after HRP application to the terminal branch of the ventral descending vessel of the left coronary artery No neurons in the SCG after HRP application to the terminal branch of the dorsal descending vessel of the right coronary artery or pericardial sac
Dog			
	Armour et al (1981)	To determine the locations of the cells of origin of cardiac postganglionic sympathetic fibers	Occasional labeled neurons were located in the caudal regions of the ipsilateral superior cervical ganglion No labeled neurons were observed contralaterally No exclusive origin for any individual cardiac nerve
	Hopkins et al (1984)	To determine whether a topographical organization related to regions of the heart exists within cervicothoracic ganglia and whether specific regions of the heart with specific functions are innervated by neurons in specific locations in the ganglia	Innervation of a specific region of the heart does not arise from neurons in one locus of a ganglion. Very few sympathetic cardiac postganglionic neurons are found in the superior cervical ganglia.
	Hirakawa et al (1993)	To determine the origin of cardiac sympathetic postganglionic fibers and to demonstrate their distribution in the heart	A small number of labeled cells found in bilateral SCG ater injection of tracers into various regions of the heart
Monkey			
	Billman et al (1982)	To present a detailed description of the cervical and upper thoracic ANS anatomy	No branches from the SCG to the heart
	Chuang et al (1992)	To study the postganglionic neurons innervating the cardiac coronary artery	After application of HRP to the main trunk of the left coronary artery 98.3% labeled neurons were localized in the SCG (the right SCG (57.4%) appeared to be more densely labeled than the left (44.6%)) After application of HRP to the main trunk of the right coronary artery 97.1% labeled neurons were found in the SCG (the right SCG (61%) appeared more densely labeled than the left (36%)) After application of HRP to the terminal branch of the ventral descending vessel of the left coronary artery 62.6% labeled neurons were observed in the right and 36.1% in the left SCG After application of HRP to the terminal branch of the dorsal descending vessel of the left coronary artery, 96.2% labeled neurons were found in the SCG on both sides (the left SCG (54.6%) appeared more densely labeled than the right (41.6%))

			There were no HRP-labeled neurons in the SCG after application to the internal surface of the pericardial sac
	Chuang et al (2004)	To investigate the autonomic neurons innervating the heart	Sympathetic postganglionic neurons innervating the heart are located predominantly in the superior cervical ganglion
	Kawashima (2) et al (2005)	To clarify the general morphology of the autonomic cardiac nervous system	The superior cardiac nerve originating from the superior cervical ganglion is usually absent
	Kawashima et al (2008)	To study when the vertebral ganglion consistently appears in primate evolution To study how the composition of the cervicothoracic ganglion is associated with primate evolution To study when the middle cervical ganglia (MCG) developed the communicating branches with the spinal cervical nerves To study when the superior cardiac nerve originating from the SCG and thoracic cardiac nerve appear consistently	The superior cardiac nerve originated from the SCG in 65% (4 rights, 9 left) The superior cardiac nerve originated from the sympathetic trunk in 50% (5 right, 5 left)
	Kawashima et al (2009)	To report on the anatomy of the autonomic cardiac nervous system	SCG is consistently present The superior cardiac nerve never originated from the SCG, but from the sympathetic trunk between the SCG and MCG in 41.7%
	Kawashima et al (2011)	To describe the detailed systematic morphology of the autonomic cardiac nervous system To update and provide additional information on the surrounding nervous system To examine the intraspecific and interspecific variation in the autonomic nervous system To determine whether family-dependent morphology exists To examine the relationship between the autonomic cardiac nervous system and its surrounding structures To consider the common morphology from an evolutionary perspective within the lineage	SCG is consistently present The superior cardiac nerve never originated from the SCG, but from the sympathetic trunk between the SCG and MCG in 21.4%
	Kawashima et al (2013)	To elucidate the general morphology of and variations in the extrinsic cardiac nervous system To test the hypothesis that the morphology of the cardiac innervation is a conservative structure preserving its phylogeny	No cardiac nerves arising from the SCG

		To consider whether the tarsier's morphology is more similar to that of the strepsirrhini or new world monkeys To update and provide additional information on the relationship between the neglected cardiac nervous system and its surrounding structures	
Human			
	Janes et al (1986)	To describe the anatomy of the extrinsic cardiac nerves and ganglia	No cardiopulmonary nerves arising from the SCG
	Pather et al (2003)	To determine the cervical and thoracic sympathetic contributions to the cardiac plexus	100% incidence of SCG in both fetal and adult cases All sympathetic contributions from the cervical sympathetic trunks were found to arborize directly in the deep cardiac plexus
	Kawashima (1) et al (2005)	To investigate the topological changes of the human autonomic cardiac nervous system in retroesophageal right subclavian artery (a branchial arterial anomaly) compared to the normal autonomic cardiac nervous system	Case 1: the superior cardiac nerves arising from both the SCG and sympathetic trunk between SCG and MCG were observed on the right side, not on the left side Case 2: the superior cardiac nerves arising from the SCG and sympathetic trunk between SCG and MCG were observed on both sides
	Kawashima (3) et al (2005)	To clarify the detailed morphology of the entire autonomic cardiac nerves, including both sympathetic and parasympathetic nerves	The SCG was observed in all cases The superior cardiac nerve originating from the SCG was observed in 88.9% (15 right, 17 left) The superior cardiac nerve originating from the sympathetic trunk between the SCG and MCG was observed in 69.4% (11 right, 14 left)

***Prenatal studies*.**

Both prenatal studies that were included, were performed in chick. In the study of Kirby et al., aimed at studying the developing sympathetic innervation in the chick with regard to postnatal innervation, histomorphological techniques (catecholamine histofluorescence and silver preparations) were used. The authors report that all nerve branches from the superior cervical ganglia were directed cranially along the carotid arteries. No fluorescent postganglionic fibres could be traced directly from the SCG to the heart, nor accompanying the vagal nerves through the chick neck (17). In the study of Verberne et al. it was hypothesized that the cervical ganglia might contribute to cardiac sympathetic innervation. Both histo-morphological techniques (thyroxin hydroxylase (TH), marker for sympathetic nerve tissue; and HNK1, marker for migrating neural crest and nerve tissue) and heterospecific transplantation using quail chick-chimeras (visualisation of quail cells in chick with the quail nuclear antibody QCPN) were used to study specifically the contribution of the chick cervical sympathetic ganglia to the developing heart and great arteries. Of note, in the chick heart, sympathetic innervation is derived from the sympathetic neural crest (i.e. *trunk* neural crest arising from somite 1-20) (18). In this study both trunk neural crest chimeras and cardiac neural crest chimera were made. The chick cervical sympathetic chain consists of 14 sympathetic ganglia, and the authors showed that the sympathetic neural crest gives rise to cervical ganglia 4-14 in chick, as well as to carotid paraganglia. Tracing of cells of cardiac neural crest chimeras, showed QCPN positive cells in satellite cells of the SCG, however neuronal somata were negative. Three levels in the cervical sympathetic trunk (superior, middle and inferior) were observed to contribute TH^+ nerve fibres to the heart. The authors report that the SCG contributes to the carotid nerve (18). The carotid nerve, or carotid sinus nerve, is known as a branch of the glossopharyngeal nerve, involved in innervation of the carotid sinus and carotid body (38). Remarkably, Verberne at al. describe the *carotid nerve* as a thyroxin hydroxylase positive nerve, while the vagus and glossopharyngeal nerves are TH negative. The TH^+ carotid nerve along its course joined the parasympathetic nodose ganglion, via which it contributed to nerve fibres in cardiac vagal branches that enter the heart via both the arterial and venous poles (18). The carotid nerve also contributed to nerve fibres that were connected to the developing baro-and chemoreceptors in the outflow tract of the heart, thus suggesting a role of the SCG in sensory afferent innervation (18). No evidence for an contribution to sympathetic innervation was presented.

Postnatal studies.

Morphological data on SCG in relation to cardiac innervation in human. A total of 4 human studies were included, 3 in healthy subjects (23,28,32) and 1 in subjects with an aberrant right subclavian artery (30).

The position of the SCG in human is described behind the bifurcation of the common carotid artery and between the first to third cervical vertebrae (4). Nerve branches can originate from the SCG. In our previous papers, we have used the nomenclature as proposed by Kawashima who describes cardiac nerves as nerves connecting to the heart either "with direct connections or connections via the cardiac plexus" (1,5,32). The superior cardiac nerve is described as arising from the SCG and from the sympathetic trunk between the superior and middle cervical ganglia (4), and in its course usually follows the common carotid arteries. The cardiac plexus receives contributions from cardiac nerves and branches (30).
As previously observed, data in human on morphological observations of contributions of the SCG in humans is controversial (1). In 1986, Janes reported that no cardiopulmonary nerves were observed to originate from the SCG (23). In contrast, in 2003 Pather, based on data derived from 21 fetuses and 8 adults, reported the presence of the SCG in all specimen. The superior cardiac nerve (also present in all specimen examined) was reported to arise from the SCG in 53% of cases (31/58 sides, from which 16/29 on the right side and 15/29 on the left side) (28). All sympathetic contributions from the cervical sympathetic trunks were found to branch directly into the deep cardiac plexus. In line with this are the reports from Kawashima in 2005, where the SCG was also observed in all cases (32). It was found that the SCG communicates with spinal level C1-C3, and the SCG showed communicating branches with the spinal nerves on all sides. A superior cardiac nerve originating from the SCG was observed on 32/36 sides (88.9%: 15 right sides, 17 left sides). The superior cardiac nerve originated from the sympathetic trunk between the superior and the middle cervical ganglia on 25 sides (69.4%; 11 right sides, 14 left sides). Of note, in three right sides the superior cardiac nerve did not originate from the SCG or the cervical sympathetic trunk between the superior and the middle cervical ganglia. Therefore, although an apparent difference between the right and left sides was not observed, the frequency of the right superior cardiac nerve might be slightly less than the left. It was also reported that the SCG and stellate ganglia were consistent in size, position and communicating branches, whereas middle cervical and vertebral ganglia were variable (32). In 2 human cases with an aberrant right subclavian artery the superior cardiac nerve was also found to arise from the SCG. In the first case, a superior cardiac nerves arising from both the SCG and sympathetic trunk between SCG and MCG was observed on the right side, but not on the left side. In the second case, the superior cardiac nerves arising from the SCG and sympathetic trunk between SCG and MCG were observed on both sides (30).

Morphological data on SCG in relation to cardiac innervation in monkey. A total of 8 studies in different species of monkeys were included: one paper in the Rhesus Monkey (Macaca Mulatta) (21), one in Taiwan monkey (29), one in Macaque Monkey (31), one in the Gibbons (Hylobatidae) (34), in New World Monkeys (Platyrrhini) (35), Lorises and Galagos (36), in the Philippine Tarsier of the Tarsiidae family (37) and in one study the species of the monkey was not specified (26).

In 1982, Billman et al. described the position of the SCG in the monkey medial to the nodose ganglion, and dorsal to the carotid sinus area (21). Conform the situation described in mouse, a connection was observed between the SCG and the nodose ganglion (11). It was described in this study in the rhesus monkey that the carotid sinus receives nerves from the ipsilateral SCG as well as from the glossopharyngeal nerves, that are likely respectively efferent and afferent fibers (21). No direct sympathetic branches from the SCG to the heart were observed. Studies using retrograde labeling with horseradish peroxidase (HRP), however, supported nerve connections between the SCG and the heart (26). In 1992, Chuan et al. studied the localization of sympathetic postganglionic neurons innervating the coronary arteries, by injection of HRP to the proximal part of the right coronary artery (RCA) and left coronary artery (LCA), as well as to the distal branches of the ventral and dorsal descending arteries of the RCA and LCA. After sacrificing the animals, the paravertebral ganglia including the SCG were examined. Results show that after application of HRP in the proximal part of the RCA, and the proximal and distal part of the LCA, HRP positive cells could be localized mainly in the right SCG, and next in the left SCG. After HRP injection in the distal branches of the dorsal descending vessels of the RCA, labeled cells could be found mainly in the left SCG and next in the right SCG (26). In 2004, Chuang et al. examined several autonomic ganglia, including the SCG after injections of HRP in the subepicardial and myocardial layers in different cardiac regions. After injection in most locations, labeled cells could be found in the SCG, with exception of the posterior upper part of the left ventricle. Specifically, results showed that after injection of HRP in the cardiac apex, the region of the sino-atrial node, and in the right ventricle, HRP labeled sympathetic neurons were found only in the right SCG in 64.8% and in the left SCG in 35%. Also HRP injection in the left ventricle, lead to tracing of the labeled sympathetic cells mainly in the left SCG (51%) or right SCG (38.6%) (29). Between 2005 and 2013, 5 studies in monkeys were performed by Kawashima et al. in a variety of monkey species, that met the inclusion criteria of our review. As summarised in **Table 4**, several monkey interspecies-differences, a.o. regarding the presence of the superior cardiac nerve, were noted. For instance, a superior cardiac nerve arising from the SCG was absent in Rhesus monkeys (21), however could consistently be observed in New World Monkeys (35). An evolutionary summary figure based on several landmark studies in different monkey species, is provided in **Figure 2.**

Table 4: Evolutionary differences between different monkey species

Billman et al (1982)	Rhesus Monkeys	No branches from the SCG to the heart
Chuang et al (1992)	Monkeys, not specified	Labeling of the left coronary artery stem: → 98.3% labeled neurons localized in the SCG Labeling of the right coronary artery → 97.1% labeled neurons localized in the SCG No labeled neurons in the SCG after application to the internal surface of the pericardial sac
Chuang et al (2004)	Taiwan Monkeys	Sympathetic postganglionic neurons innervating the heart are located predominantly in the superior cervical ganglion
Kawashima (2) et al (2005)	Macaque Monkeys	The superior cardiac nerve originating from the superior cervical ganglion is usually absent
Kawashima et al (2008)	Lesser Apes, Gibbons	The superior cardiac nerve originated from the SCG in 65% (4 rights, 9 left) The superior cardiac nerve originated from the sympathetic trunk in 50% (5 right, 5 left)
Kawashima et al (2009)	New World Monkeys (Platyrrhini)	SCG is consistently present The superior cardiac nerve never originated from the SCG, but from the sympathetic trunk between the SCG and MCG in 41.7%
Kawashima et al (2011)	Lorises and Galagos	SCG is consistently present The superior cardiac nerve never originated from the SCG, but from the sympathetic trunk between the SCG and MCG in 21.4%
Kawashima et al (2013)	Philippine Tarsier (Comparisons With Strepsirrhines and New World Monkeys)	No cardiac nerves arising from the SCG

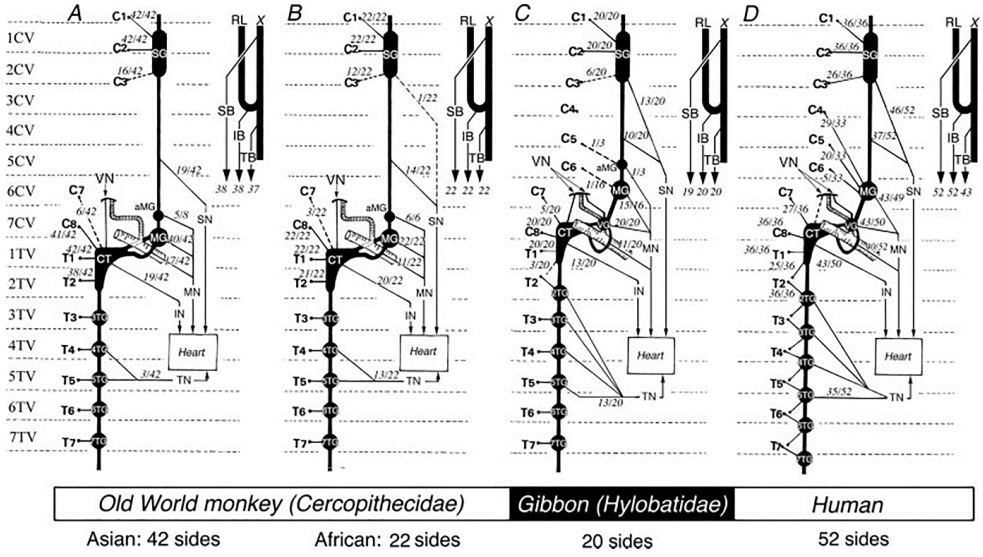


Figure 2: Evolutionary overview of cardiac sympathetic innervation. SG: Superior cervical ganglion. Source: Derived with the permission from Kawashima et al. Anat Rec. 2008 (Copyright Clearance Center 2021). The Figure represents a summary of data derived from studies in different monkey species.

Morphological data on SCG in relation to cardiac innervation in dogs. We included 3 studies in dogs (20,27,39). In all studies, retrograde labeling was performed, as well as micromorphology and/or histomorphology. In 1981, Armour et al. labeled cardiac nerves in dogs with HRP based on physiological identification, and found only sporadically retrogradely labeled neurons in the caudal pole of the ipsilateral SCG. No labeled neurons were observed on the contralateral side. Of note, in more than half of the SCG, no retrogradely labeled neurons could be traced back after injection of HRP into the cardiac nerves (39). In 1984, Hopkins et al. found corresponding data in dogs, with only very few sympathetic cardiac postganglionic neurons that could be traced back after HRP injection in the heart, intraventricular cavity, pericardial sac and aortic arch (20). Hirakawa et al, in 1993 reported similar findings after injecting HRP in various regions in the dog heart, with only a small number of labeled cells traced back into the bilateral SCG (27). After injections into the sino-atrial node, labeled cells could be traced back bilaterally in several ganglia, including the SCG (27). This was also the case after injection into the right or left atrium, although only few cells were observed in comparison to the other ganglia examined. No cells could be traced back into the left SCG after injection of HRP into the wall of the right ventricle (27).

Morphological data on SCG in relation to cardiac innervation in cats. We included 2 studies in cats (22,24). In all studies, retrograde labeling was performed, as well as micromorphology and/or histomorphology. Data in the cat show similar results as in dogs, with no or only very few cells that could be traced back into the SCG after injections of HRP into the heart (summarised in Table 3) (22,24)

Morphological data on SCG in relation to cardiac innervation in rat. In 1989, Pardini et al. described the organization of cardiac sympathetic innervation in rat, using both histomorphology and retrograde labeling with Diamidino Yellow injections into the right and left ventricle (25). Results show that labeled cells could be traced back bilaterally in an area ranging from the SCG until the 6th thoracic ganglion. However, only a small minority of cells of traced cells were found bilaterally in the SCG after injection of the left ventricular and right ventricular free wall (3% and 1% respectively).

Morphological data on SCG in relation to cardiac innervation in shrew. In shrew, Tanaka et al. found, based on macromorphological studies, that the nerve originating from the left SCG descended to reach the aortic arch and formed nerve plexuses after reaching the arterial pole, supplying nerves to the ventral wall of the ventricle (33).

No studies providing morphological data on morphological contributions of the SCG in mouse were included.

DISCUSSION

In this systematic review, we assessed the existing literature on the morphological relevance of the super cervical ganglion for cardiac innervation in health and disease stages. Key findings are 1. The superior cervical ganglion could be identified in all species examined; 2. Morphological evidence of a contribution of the SCG to cardiac innervation differs between species and even within species, such as evidence in monkeys; 3. A superior cardiac nerve innervating the heart could be found in the majority of human studies; 4. In the majority of retrograde labeling tracing studies, labeled cells could be found in the SCG after injections in the heart, however these cells formed a minority of cells as compared to contributions of other (cervical/thoracic/cervicothoracic) ganglia; 5. Functional studies will be needed to point out the functional consequences of these cells for cardiac sympathetic innervation.

Of note, although many experimental function studies are performed in mouse and rat, only 1 morphological study was found in rat, but none in the mouse (*Mus musculus*). Remarkably, in mice it has been shown that ganglionectomy of the SCG before MI leads to an almost entire loss of myocardial sympathetic innervation of the left ventricular anterior wall, in addition to a significantly reduction in chronic consequences of MI, such as myocardial inflammation, myocyte hypertrophy, and overall cardiac dysfunction (40).

Our query provided studies concerning both prenatal and postnatal stages. Prenatal SCG have the advantage that they are more prone to sprout during co-culturing *in vitro* than postnatal ganglia, at least in control ("healthy") settings. The embryological 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 (41), it has been speculated that cervical ganglia are generated from the thoracic sympathetic chain (4,42). 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 (43). The limited number of cervical ganglia could in this perspective be attributed to regression of most of the cervical intersegmental arteries, and subsequent remodeling and fusion of the corresponding ganglia. The upper 4 cervical ganglia would thus eventually form the superior cervical sympathetic ganglion, anatomically related or induced by the developing external carotid artery (43). In our review, 2 prenatal studies were included - both performed in chick - that did not show a clear contribution of the SCG to sympathetic innervation in the chick heart during the stages examined. However, as it was observed that the SCG contributes to the (TH+) carotid nerve during development (18), a contribution of the SCG to parasympathetic innervation of the heart, is not excluded.

The superior cervical ganglion (SCG) is a remarkable organ, that has an unique spatial anatomical localisation. It is situated at between the branching point of the common carotid arteries, in close proximity to the carotid body, which it innervates. The innervation pattern however, is much broader than the cardiovascular system alone. Nerve fibers originating from the SCG run along both carotid arteries, and will provide sympathetic input towards the head where it stimulates parts of the eye (iris), mouth and small blood vessels. In this respect, it may be relevant that the SCG is situated adjacent to the above mentioned carotid body (CB), by itself an intriguing structure, involved in oxygen, carbon and pH sensing, that has been shown to produce many neurotrophic factors (44). With regard to cardiovascular disease states, a role of the carotid body in hypertension has been indicated (45). In addition to the spatial relation with the carotid body, there are several studies in literature that indicate a connection of the sympathetic SCG with the parasympathetic nodose ganglion in mouse (11) (Figure 3), a finding that was also observed in monkeys in the current review (21). Given this evidence as well as the observation that the SCG contributes to the carotid nerve during development as described above (18), a contribution of the SCG to parasympathetic innervation of the heart, is possible.

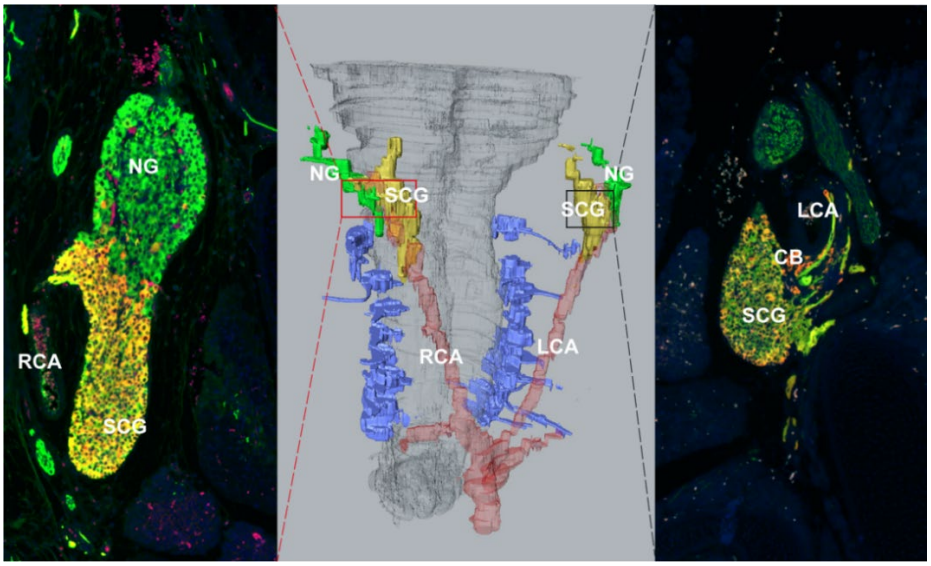
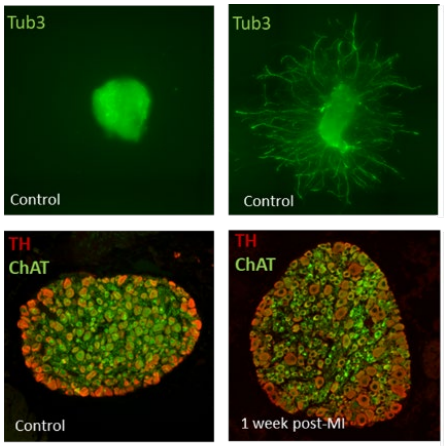


Figure 3. Connection of the sympathetic SCG with the nodose ganglion and carotid body in mouse. The middle panel shows a 3D reconstruction image of an adult mouse spinal cord, carotid arteries (indicated in red), SCG (yellow) and NG (green). SCG, superior cervical ganglion; NG, nodose ganglion; RCA, right carotid artery; LCA, left carotid artery; CB, carotid body.

General discussion of findings in light of the thesis. In Chapter 5 of this thesis, the presence of cholinergic cells in the SCG was described based on histopathological findings in healthy SCG as well as after MI, although the clinical/functional implications are at present unclear. The role of the autonomic nervous system in post-MI arrhythmogenicity has gained increasing attention over the past decades. Vagal innervation is considered as cardioprotective, whereas a sympathetic overdrive is associated with arrhythmias and sudden cardiac death (48,49). A myriad of studies in human, rat, rabbit and pig, have indicated neuronal and electrical remodeling in the stellate ganglia after MI (50–52). In contrast to the stellate ganglion, data on the time course of neural remodeling in the SCG – as indicated situated in close proximity to carotid body - after MI is still limited. It has been previously reported in rabbits that the response of the chemo sensitive cardiac reflex of the carotid body was enhanced in the acute phase of MI (53), and hypertonicity of the carotid has been linked with cardiac disease such as hypertension and chronic heart failure. In rats with induced chronic heart failure, denervation of the carotid body performed early after MI, resulted in improved survival due to reduction of ventricular remodeling, diminished fibrosis and reduction of arrhythmias(54). Whether this is only a transient phenomenon is unclear, as is the time-course of remodeling of the SCG and the carotid after MI. Therefore in Chapter 5, we assessed the neuronal remodeling of the murine SCG, including the carotid body, at several time points post-MI. Of interest, we found ganglionic remodeling not only in the SCG, but also in the carotid body, with overt upregulation of expression of several neurotrophic factors. Generally, a remodeling towards a more sympathetic phenotype was observed, which would fit the hypothesis of a higher sympathetic tone underlying cardiac arrhythmogenesis after MI.



At the left, sympathetic ganglion of sham-operated mice. At the right, sympathetic ganglion 7 days after occlusion of the left anterior descending coronary artery. An increase in neurite outgrowth, and remodeling of neuron size and expression (upregulation of TH) can be observed.

The finding of remodeling of SCG after MI, prompted the investigation of the molecular pathways involved in disease states. The exact molecular signature and cellular composition of the SCG in the control/healthy is however, also still largely unexplored. This inspired us to perform RNA sequencing at the single cell level. As our ultimate desire is to translate our findings in animal models to the human situation, we strived for applying a method that can ultimately also be applied on human tissues. In Chapter 6, we describe the development of a method for single-nucleus RNA sequencing (snRNA-seq) of murine ganglia using low-input

nucleus isolation and multiplexing with barcoded antibodies. The advantage of this method is, amongst other practical advantages, the possibility to also apply it to human neurons, that are larger than murine neurons and not suitable for single cell RNA seq. In Chapter 7, we took advantage for this method to study the molecular and genetic signature of the SCG and provide reference data for interpretation of our results of snRNA-seq after MI-R, that is currently ongoing.

Our aim to review the morphological contributions of the SCG to cardiac innervation was partly motivated by the findings of our *in vitro* studies, where we observed that murine embryonic SCG cultured with adult myocardium have a high tendency to sprout towards myocardium. Remarkably, as described in Chapter 2 of this thesis, we show that adding human epicardial derived cells (EPDCs) to our cell cultures, significantly increased this sprouting. Moreover, we found during subsequent studies that the sex of the donor of the EPDC, influenced the length and density of nerve sprouting, with a higher sprouting capacity observed in the presence of male EPDCs, as is described in Chapter 3. We propose the SLIT2/ROBO pathway as a potential candidate involved in these sex-differences observed. This also clinically relevant in light of the increasing attention for differences in presentation and outcome of cardiovascular disease between males and females (55).

Future perspectives. Our next step in studying the relevance of the SCG in health and disease, will be to systematically review the *functional* evidence of contributions of SCG ganglia in cardiac innervation, both *in vivo* and *in vitro*. With regard to the contribution of human EPDCs to cardiac innervation and the role of sex therein, we strive to further study the relevance of the SLIT2 /ROBO pathway. As a next step in the experiments described in Chapter 2 and 3, we aim to further validate our results by performing *SLIT2* knockdown and overexpression in male and female EPDCs and to evaluate the presence of the receptors of SLIT2 (ROBO receptors) on cardiac sympathetic autonomic ganglia, including the SCG. In addition, we strive towards increasingly progressing our experiments to human tissues. As a first step in this approach, taking into account the limited availability of human tissues as well as several other impediments described in Chapter 4, we have developed a human proliferative EPDC cell line (hiEPDCs), to facilitate their use in experiments. The Cardiology dept. of LUMC also houses a human proliferate atrial myocardial cell line (hiACMs), that together with the hiEPDCs provides a promising experimental model for our cardiac innervation studies in health and disease. We ultimately strive to also develop an inducible neuronal cell line (hiNeurons) which will be the focus of future studies. With regard to the experiments mentioned in Chapter 5, our next step will be to further explore the significance of expression of cholinergic factors, by performing i.a. qPCR of SCG and determining levels of catecholamines. With regard to Chapters 6 and 7, our next step will be to explore the molecular and genetic remodeling of SCG on the single

nucleus level after MI. Data presented in Chapter 7 will have an important function to serve as reference in interpretation of these data. In our future experiments with snRNA-seq, we will also take the sex of the donor into account, and aim to explore differential pathways involved in ganglion growth and remodeling in health and disease in both male and female. Finally, our next projects will aim at electrophysiological consequences of neuronal remodeling with regard to arrhythmogenesis.

REFERENCES

1. Wink J, van Delft R, Notenboom RGE, Wouters PF, DeRuiter MC, Plevier JWM, et al. Human adult cardiac autonomic innervation: Controversies in anatomical knowledge and relevance for cardiac neuromodulation. Auton Neurosci Basic Clin [Internet]. 2020;227(September 2019):102674. Available from: https://doi.org/10.1016/j.autneu.2020.102674
2. Li CY, Li YG. Cardiac Sympathetic Nerve Sprouting and Susceptibility to Ventricular Arrhythmias after Myocardial Infarction. Cardiol Res Pr. 2015;2015:1–8.
3. Zipes DP, Rubart M. Neural modulation of cardiac arrhythmias and sudden cardiac death. Hear Rhythm Off J Hear Rhythm Soc. 2006;3(1):108.
4. 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(6):425–38.
5. Zandstra TE, Notenboom RGE, Wink J, Kiès P, Vliegen HW, Egorova AD, et al. Asymmetry and Heterogeneity: Part and Parcel in Cardiac Autonomic Innervation and Function. Front Physiol. 2021;12(September):1–15.
6. Smetana P, Malik M. Sex differences in cardiac autonomic regulation and in repolarisation electrocardiography. Pflugers Arch Eur J Physiol. 2013;465(5):699–717.
7. Razipour SE, Zarrintan S, Mathkour M, Iwanaga J, Dumont AS, Shane Tubbs R. Review of the External Carotid plexus: Anatomy, Function, and Clinical Manifestations. Anat Cell Biol. 2021;54(2):137–42.
8. De Stefano ME, Leone L, Lombardi L, Paggi P. Lack of dystrophin leads to the selective loss of superior cervical ganglion neurons projecting to muscular targets in genetically dystrophic mdx mice. Neurobiol Dis. 2005;20(3):929–42.
9. Fedele MLM, Galiana MD, Golombek DA, Muñoz EM, Plano SA. Alterations in metabolism and diurnal rhythms following bilateral surgical removal of the superior cervical ganglia in rats. Front Endocrinol (Lausanne). 2018;8(JAN):1–10.
10. Brognara F, Felippe ISA, Salgado HC, Paton JFR. Autonomic innervation of the carotid body as a determinant of its sensitivity: Implications for cardiovascular physiology and pathology. Cardiovasc Res. 2021;117(4):1015–32.
11. Bookout AL, Gautron L. Characterization of a cell bridge variant connecting the nodose and superior cervical ganglia in the mouse: Prevalence, anatomical features, and practical implications. J Comp Neurol. 2021;529(1):111–28.
12. Ge Y, Smits AM, van Munsteren JC, Gittenberger-de Groot AC, Poelmann RE, van Brakel TJ, et al. Human epicardium-derived cells reinforce cardiac sympathetic innervation. J Mol Cell Cardiol [Internet]. 2020;143:26–37. Available from: https://doi.org/10.1016/j.yjmcc.2020.04.006
13. Engele LJ, Mulder BJM, Schoones JW, Kiès P, Egorova AD, Vliegen HW, et al. The coronary arteries in adults after the arterial switch operation: A systematic review. J Cardiovasc Dev Dis. 2021;8(9):1–18.
14. Koppel CJ, Jongbloed MRM, Kiès P, Hazekamp MG, Mertens BJA, Schalij MJ, et al. Coronary anomalies in tetralogy of Fallot – A meta-analysis. Int J Cardiol. 2020;306:78–85.
15. Wilke J, Krause F, Niederer D, Engeroff T, Nürnberger F, Vogt L, et al. Appraising the methodological quality of cadaveric studies: Validation of the QUACS scale. J Anat. 2015;226(5):440–6.

16. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ. 2021;372.
17. Kirby ML, McKenzie JW, Weidman TA. Developing innervation of the chick heart: A histofluorescence and light microscopic study of sympathetic innervation. Anat Rec. 1980;196(3):333–40.
18. Verberne ME, Gittenberger-De Groot AC, Van Iperen L, 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(4):407–19.
19. 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 Basic Clin. 2007;132(1–2):37–43.
20. Hopkins DA, Armour JA. Localization of sympathetic postganglionic and parasympathetic preganglionic neurons which innervate different regions of the dog heart. J Comp Neurol. 1984;229(2):186–98.
21. Billman GE, Dickey DT, Stone HL. A description of the upper thoracic autonomic nervous system in the rhesus monkey (Macaca mulatta). Am J Primatol. 1982;2(2):159–66.
22. Shih CJ, Chuang KS, Tsai SH, Liu JC. Horseradish peroxidase localization of the sympathetic postganglionic neurons innervating the cat heart. J Auton Nerv Syst. 1985;13(3):179–89.
23. Janes RD, Christopher Brandys J, Hopkins DA, Johnstone DE, Murphy DA, Armour JA. Anatomy of human extrinsic cardiac nerves and ganglia. Am J Cardiol. 1986;57(4):299–309.
24. Wu JJ, Chuang KS, Shih CJ, Liu JC. Sympathetic postganglionic innervation of the cardiac coronary artery in cats. J Auton Nerv Syst. 1988;24(3):215–20.
25. Pardini BJ, Lund DD, Schmid PG. Organization of the sympathetic postganglionic innervation of the rat heart. J Auton Nerv Syst. 1989;28(3):193–201.
26. Chuang KS, Liu JC, Liu NH, Lin MT. Localization of the sympathetic postganglionic neurons innervating cardiac coronary artery with horseradish peroxidase in monkeys. Chin J Physiol. 1992;35(3):219–26.
27. Hirakawa N, Morimoto M, Totoki T. Sympathetic innervation of the young canine heart using antero- and retrograde axonal tracer methods. Brain Res Bull. 1993;31(6):673–80.
28. Pather N, Partab P, Singh B, Satyapal KS. The sympathetic contributions to the cardiac plexus. Surg Radiol Anat. 2003;25(3–4):210–5.
29. Chuang KS, Liu WC, Liou NH, Liu JC. Horseradish peroxidase localization of sympathetic postganglionic and parasympathetic preganglionic neurons innervating the monkey heart. Chin J Physiol. 2004;47(2):95–9.
30. Kawashima T, Sasaki H. Topological changes of the human autonomic cardiac nervous system in individuals with a retroesophageal right subclavian artery: Two case reports and a brief review. Anat Embryol (Berl). 2005;210(4):327–34.
31. Kawashima T, Sato K, Akita K, Sasaki H. Comparative anatomical study of the autonomic cardiac nervous system in macaque monkeys. J Morphol. 2005;266(1):112–24.
32. 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(6):425–38.
33. Tanaka A, Tanaka S, Miyamoto K, Yi SQ, Nakatani T. Gross anatomical study of the sympathetic cardiac nerves in the house musk shrew (Suncus murinus). Anat Rec. 2007;290(5):468–76.
34. Kawashima T, Thorington RW, Kunimatsu Y, Whatton JF. Systematic morphology and evolutionary anatomy of the autonomic cardiac nervous system in the lesser apes, gibbons (Hylobatidae). Anat Rec. 2008;291(8):939–59.
35. Kawashima T, Thorington RW, Whatton JF. Comparative anatomy and evolution of the cardiac innervation in new world monkeys (Platyrrhini, E. Geoffroy, 1812). Anat Rec. 2009;292(5):670–91.

36. Kawashima T, Thorington RW. Comparative Morphological Configuration of the Cardiac Nervous System in Lorises and Galagos (Infraorder Lorisiformes, Strepsirrhini, Primates) with Evolutionary Perspective. Anat Rec. 2011;294(3):412–26.
37. Kawashima T, Thorington RW, Murakami K, Sato F. Evolutionary Anatomy and Phyletic Implication of the Extrinsic Cardiac Nervous System in the Philippine Tarsier (Tarsius syrichta, Primates) in Comparisons With Strepsirrhines and New World Monkeys. Anat Rec. 2013;296(5):798–806.
38. Porzionato A, Macchi V, Stecco C, De Caro R. The Carotid Sinus Nerve—Structure, Function, and Clinical Implications. Anat Rec. 2019;302(4):575–87.
39. Armour JA, Hopkins DA. Localization of sympathetic postganglionic neurons of physiologically identified cardiac nerves in the dog. J Comp Neurol. 1981;202(2):169–84.
40. Ziegler KA, Ahles A, Wille T, Kerler J, Ramanujam D, Engelhardt S. Local sympathetic denervation attenuates myocardial inflammation and improves cardiac function after myocardial infarction in mice. Cardiovasc Res. 2018;114(2):291–9.
41. Sadler TW. Embryology of neural tube development. Am J Med Genet C Semin Med Genet. 2005 May;135C(1):2–8.
42. Rubin E. Development of the rat superior cervical ganglion: Ganglion cell maturation. J Neurosci. 1985;5(3):673–84.
43. Kanagasuntheram R, Dharshini P. The cervical sympathetic trunk--a new hypothesis. Ann Acad Med Singapore. 1994 Nov;23(6):923–8.
44. López-Barneo J, Ortega-Sáenz P, Pardal R, Pascual A, Piruat JI. Carotid body oxygen sensing. Eur Respir J [Internet]. 2008 Nov 1;32(5):1386 LP – 1398.
45. Abdala AP, McBryde FD, Marina N, Hendy EB, Engelman ZJ, Fudim M, et al. Hypertension is critically dependent on the carotid body input in the spontaneously hypertensive rat. J Physiol. 2012 Sep;590(17):4269–77.
46. Debinski W, Kuchel O, Buu NT, Tremblay J, Hamet P. Atrial natriuretic factor constitutes an intrinsic functional unit within superior cervical ganglia of the rat. Neuroscience. 1990;36(1):21–6.
47. Debinski W, Kuchel O, Buu NT, Cantin M, Genest J. Atrial natriuretic factor partially inhibits the stimulated catecholamine synthesis in superior cervical ganglia of the rat. Neurosci Lett. 1987 Jun;77(1):92–6.
48. Fukuda K, Kanazawa H, Aizawa Y, Ardell JL, Shivkumar K. Cardiac innervation and sudden cardiac death. Circ Res. 2015 Jun;116(12):2005–19.
49. Hausenloy DJ, Bøtker HE, Ferdinandy P, Heusch G, Ng GA, Redington A, et al. Cardiac innervation in acute myocardial ischaemia/reperfusion injury and cardioprotection. Cardiovasc Res. 2019 Jun;115(7):1167–77.
50. Ajijola OA, Yagishita D, Reddy NK, Yamakawa K, Vaseghi M, Downs AM, et al. Remodeling of stellate ganglion neurons after spatially targeted myocardial infarction: Neuropeptide and morphologic changes. Hear Rhythm. 2015 May;12(5):1027–35.
51. Nguyen TP, Sovari AA, Pezhouman A, Iyer S, Cao H, Ko CY, et al. Increased susceptibility of spontaneously hypertensive rats to ventricular tachyarrhythmias in early hypertension. J Physiol [Internet]. 2016;594(6):1689–707.
52. Han S, Kobayashi K, Joung B, Piccirillo G, Maruyama M, Vinters H V, et al. Electroanatomic remodeling of the left stellate ganglion after myocardial infarction. J Am Coll Cardiol. 2012 Mar;59(10):954–61.
53. Rocha I, Rosário LB, de Oliveira EI, Barros MA, Silva-Carvallho L. Enhancement of carotid chemoreceptor reflex and cardiac chemosensitive reflex in the acute phase of myocardial infarction of the anesthetized rabbit. Basic Res Cardiol. 2003 May;98(3):175–80.
54. Del Rio R, Marcus NJ, Schultz HD. Carotid chemoreceptor ablation improves survival in heart failure: rescuing autonomic control of cardiorespiratory function. J Am Coll Cardiol. 2013 Dec;62(25):2422–30.

55. E. Davis, D.A. Gorog, C. Rihal, A. Prasad, M. Srinivasan, "Mind the gap" acute coronary syndrome in women: A contemporary review of current clinical evidence, Int J Cardiol 227 (2017) 840-849.

SUPPLEMENTAL FILES

Appendix A: Query

Date: 7-10-2021.

Databases:

PubMed

http://www.ncbi.nlm.nih.gov/pubmed?otool=leiden

Triple strategy:

- Superior cervical ganglion (main subject) & Heart

- Superior cervical ganglion & Heart innervation

- Superior cervical ganglion & Heart & Nerve Growth Factors

((("Superior Cervical Ganglion"[majr] OR "superior cervical ganglion"[ti] OR "superior cervical ganglia"[ti] OR "superior cervical gangl*"[ti] OR "ganglion cervicale superius"[ti] OR "ganglion cervical superior"[ti] OR "ganglion cervicale"[ti]) AND ("Heart"[mesh] OR "Cardiovascular System"[mesh] OR "Cardiovascular"[tw] OR "Cardiac"[tw] OR "myocardial"[tw] OR "Heart"[tw] OR "Heart Diseases"[mesh] OR "Cardiovascular Diseases"[mesh] OR "Arrhythmia"[tw] OR "Myocardial infarction"[tw] OR "cardiac overload"[tw] OR "cardiac damage"[tw] OR "heart failure"[tw] OR "cardiac damage"[tw] OR "myocardial reperfusion"[tw] OR "cardiac innervation"[tw] OR "arrhythmias"[tw] OR "atrial"[tw] OR "ventricular"[tw])) OR (("Superior Cervical Ganglion"[Mesh] OR "superior cervical ganglion"[tw] OR "superior cervical ganglia"[tw] OR "superior cervical gangl*"[tw] OR "ganglion cervicale superius"[tw] OR "ganglion cervical superior"[tw] OR "ganglion cervicale"[tw]) AND ("Heart/innervation"[majr])) OR (("Superior Cervical Ganglion"[Mesh] OR "superior cervical ganglion"[tw] OR "superior cervical ganglia"[tw] OR "superior cervical gangl*"[tw] OR "ganglion cervicale superius"[tw] OR "ganglion cervical superior"[tw] OR "ganglion cervicale"[tw]) AND ("Heart"[mesh] OR "Cardiovascular System"[mesh] OR "Cardiovascular"[tw] OR "Cardiac"[tw] OR "myocardial"[tw] OR "Heart"[tw] OR "Heart Diseases"[mesh] OR "Cardiovascular Diseases"[mesh] OR "Arrhythmia"[tw] OR "Myocardial infarction"[tw] OR "cardiac overload"[tw] OR "cardiac damage"[tw] OR "heart failure"[tw] OR "cardiac damage"[tw] OR "myocardial reperfusion"[tw] OR "cardiac innervation"[tw] OR "arrhythmias"[tw] OR "atrial"[tw] OR "ventricular"[tw]) AND ("Nerve Growth Factors"[Mesh] OR "Nerve Growth Factors"[tw] OR "Nerve Growth Factor"[tw] OR "Neurite Outgrowth Factor"[tw] OR "Neurite Outgrowth Factors"[tw] OR "Neuronal Growth Associated Protein"[tw] OR "Neuronal Growth Associated Proteins"[tw] OR "Neuronotrophic Factor"[tw] OR "Neuronotrophic Factors"[tw] OR "Neurotrophic Factor"[tw] OR "Neurotrophic Factors"[tw] OR "Neurotrophic Protein"[tw] OR "Neurotrophic Proteins"[tw] OR "Neurotrophin"[tw] OR "Neurotrophins"[tw] OR "Glia Maturation Factor"[tw] OR "Glia Maturation Factors"[tw] OR "Glial Cell Line-Derived Neurotrophic Factor"[tw] OR "Glial Cell Line-Derived Neurotrophic Factors"[tw] OR "Nerve Growth Factor"[tw] OR "Nerve Growth Factors"[tw] OR "Netrin"[tw] OR "Netrin-1"[tw] OR "Netrins"[tw]

OR "Neuregulin"[tw] OR "Neuregulin-1"[tw] OR "Neuregulins"[tw] OR "Neurotrophin 3"[tw] OR "Neurturin"[tw] OR "Neurturins"[tw])))

Embase. http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=main&MODE=ovid&D=oemezd

(((*"Superior Cervical Ganglion"/ OR "superior cervical ganglion".ti OR "superior cervical ganglia".ti OR "superior cervical gangl*".ti OR "ganglion cervicale superius".ti OR "ganglion cervical superior".ti OR "ganglion cervicale".ti) AND (exp *"Heart"/ OR exp *"Cardiovascular System"/ OR "Cardiovascular".ti,ab OR "Cardiac".ti,ab OR "myocardial".ti,ab OR "Heart".ti,ab OR exp *"Heart Disease"/ OR exp *"Cardiovascular Disease"/ OR "Arrhythmia".ti,ab OR "Myocardial infarction".ti,ab OR "cardiac overload".ti,ab OR "cardiac damage".ti,ab OR "heart failure".ti,ab OR "cardiac damage".ti,ab OR "myocardial reperfusion".ti,ab OR "cardiac innervation".ti,ab OR "arrhythmias".ti,ab OR "atrial".ti,ab OR "ventricular".ti,ab)) OR ((*"Superior Cervical Ganglion"/ OR "superior cervical ganglion".ti,ab OR "superior cervical ganglia".ti,ab OR "superior cervical gangl*".ti,ab OR "ganglion cervicale superius".ti,ab OR "ganglion cervical superior".ti,ab OR "ganglion cervicale".ti,ab) AND (exp *"Heart"/ OR exp *"Cardiovascular System"/ OR "Cardiovascular".ti,ab OR "Cardiac".ti,ab OR "myocardial".ti,ab OR "Heart".ti,ab OR exp *"Heart Disease"/ OR exp *"Cardiovascular Disease"/ OR "Arrhythmia".ti,ab OR "Myocardial infarction".ti,ab OR "cardiac overload".ti,ab OR "cardiac damage".ti,ab OR "heart failure".ti,ab OR "cardiac damage".ti,ab OR "myocardial reperfusion".ti,ab OR "cardiac innervation".ti,ab OR "arrhythmias".ti,ab OR "atrial".ti,ab OR "ventricular".ti,ab) AND (exp *"neurotrophic factor"/ OR *"Nerve Growth Factor"/ OR "Nerve Growth Factors".ti,ab OR "Nerve Growth Factor".ti,ab OR "Neurite Outgrowth Factor".ti,ab OR "Neurite Outgrowth Factors".ti,ab OR "Neuronal Growth Associated Protein".ti,ab OR "Neuronal Growth Associated Proteins".ti,ab OR "Neuronotrophic Factor".ti,ab OR "Neuronotrophic Factors".ti,ab OR "Neurotrophic Factor".ti,ab OR "Neurotrophic Factors".ti,ab OR "Neurotrophic Protein".ti,ab OR "Neurotrophic Proteins".ti,ab OR "Neurotrophin".ti,ab OR "Neurotrophins".ti,ab OR "Glia Maturation Factor".ti,ab OR "Glia Maturation Factors".ti,ab OR "Glial Cell Line-Derived Neurotrophic Factor".ti,ab OR "Glial Cell Line-Derived Neurotrophic Factors".ti,ab OR "Nerve Growth Factor".ti,ab OR "Nerve Growth Factors".ti,ab OR "Netrin".ti,ab OR "Netrin-1".ti,ab OR "Netrins".ti,ab OR "Neuregulin".ti,ab OR "Neuregulin-1".ti,ab OR "Neuregulins".ti,ab OR "Neurotrophin 3".ti,ab OR "Neurturin".ti,ab OR "Neurturins".ti,ab))) NOT (conference review or conference abstract).pt

Web of Science. http://isiknowledge.com/wos

((ti=("Superior Cervical Ganglion" OR "superior cervical ganglion" OR "superior cervical ganglia" OR "superior cervical gangl*" OR "ganglion cervicale superius" OR "ganglion cervical superior" OR "ganglion cervicale") AND (ti=("Heart" OR "Cardiovascular System" OR "Cardiovascular" OR "Cardiac" OR "myocardial" OR "Heart" OR "Heart Disease" OR "Cardiovascular Disease" OR "Arrhythmia" OR "Myocardial infarction" OR "cardiac overload" OR "cardiac damage" OR "heart failure" OR "cardiac damage" OR "myocardial reperfusion" OR "cardiac innervation" OR "arrhythmias" OR "atrial" OR "ventricular") OR ab=("Heart" OR "Cardiovascular System" OR "Cardiovascular" OR "Cardiac" OR "myocardial" OR "Heart" OR "Heart Disease" OR "Cardiovascular Disease" OR "Arrhythmia" OR "Myocardial infarction" OR "cardiac overload" OR "cardiac damage" OR "heart failure" OR "cardiac damage" OR "myocardial reperfusion" OR "cardiac innervation" OR "arrhythmias" OR "atrial" OR "ventricular"))) OR ((ti=("Superior Cervical Ganglion" OR "superior cervical ganglion" OR "superior cervical ganglia" OR "superior cervical gangl*" OR "ganglion cervicale superius" OR "ganglion cervical superior" OR "ganglion cervicale") OR ab=("Superior Cervical Ganglion" OR "superior cervical ganglion" OR "superior cervical ganglia" OR "superior cervical gangl*" OR "ganglion cervicale superius" OR "ganglion cervical superior" OR "ganglion cervicale")) AND (ti=("Heart" OR "Cardiovascular System" OR "Cardiovascular" OR "Cardiac" OR "myocardial" OR "Heart" OR "Heart Disease" OR "Cardiovascular

Disease" OR "Arrhythmia" OR "Myocardial infarction" OR "cardiac overload" OR "cardiac damage" OR "heart failure" OR "cardiac damage" OR "myocardial reperfusion" OR "cardiac innervation" OR "arrhythmias" OR "atrial" OR "ventricular") OR ab=("Heart" OR "Cardiovascular System" OR "Cardiovascular" OR "Cardiac" OR "myocardial" OR "Heart" OR "Heart Disease" OR "Cardiovascular Disease" OR "Arrhythmia" OR "Myocardial infarction" OR "cardiac overload" OR "cardiac damage" OR "heart failure" OR "cardiac damage" OR "myocardial reperfusion" OR "cardiac innervation" OR "arrhythmias" OR "atrial" OR "ventricular")) AND (ti=("neurotrophic factor" OR "Nerve Growth Factor" OR "Nerve Growth Factors" OR "Nerve Growth Factor" OR "Neurite Outgrowth Factor" OR "Neurite Outgrowth Factors" OR "Neuronal Growth Associated Protein" OR "Neuronal Growth Associated Proteins" OR "Neuronotrophic Factor" OR "Neuronotrophic Factors" OR "Neurotrophic Factor" OR "Neurotrophic Factors" OR "Neurotrophic Protein" OR "Neurotrophic Proteins" OR "Neurotrophin" OR "Neurotrophins" OR "Glia Maturation Factor" OR "Glia Maturation Factors" OR "Glial Cell Line-Derived Neurotrophic Factor" OR "Glial Cell Line-Derived Neurotrophic Factors" OR "Nerve Growth Factor" OR "Nerve Growth Factors" OR "Netrin" OR "Netrin-1" OR "Netrins" OR "Neuregulin" OR "Neuregulin-1" OR "Neuregulins" OR "Neurotrophin 3" OR "Neurturin" OR "Neurturins") OR ab=("neurotrophic factor" OR "Nerve Growth Factor" OR "Nerve Growth Factors" OR "Nerve Growth Factor" OR "Neurite Outgrowth Factor" OR "Neurite Outgrowth Factors" OR "Neuronal Growth Associated Protein" OR "Neuronal Growth Associated Proteins" OR "Neuronotrophic Factor" OR "Neuronotrophic Factors" OR "Neurotrophic Factor" OR "Neurotrophic Factors" OR "Neurotrophic Protein" OR "Neurotrophic Proteins" OR "Neurotrophin" OR "Neurotrophins" OR "Glia Maturation Factor" OR "Glia Maturation Factors" OR "Glial Cell Line-Derived Neurotrophic Factor" OR "Glial Cell Line-Derived Neurotrophic Factors" OR "Nerve Growth Factor" OR "Nerve Growth Factors" OR "Netrin" OR "Netrin-1" OR "Netrins" OR "Neuregulin" OR "Neuregulin-1" OR "Neuregulins" OR "Neurotrophin 3" OR "Neurturin" OR "Neurturins")))) NOT dt=(meeting abstract)

Cochrane. https://www.cochranelibrary.com/advanced-search/search-manager

(("Superior Cervical Ganglion" OR "superior cervical ganglion" OR "superior cervical ganglia" OR "superior cervical gangl*" OR "ganglion cervicale superius" OR "ganglion cervical superior" OR "ganglion cervicale") AND ("Heart" OR "Cardiovascular System" OR "Cardiovascular" OR "Cardiac" OR "myocardial" OR "Heart" OR "Heart Disease" OR "Cardiovascular Disease" OR "Arrhythmia" OR "Myocardial infarction" OR "cardiac overload" OR "cardiac damage" OR "heart failure" OR "cardiac damage" OR "myocardial reperfusion" OR "cardiac innervation" OR "arrhythmias" OR "atrial" OR "ventricular")):ti,ab,kw