Extracardiac Neural Remodeling in Humans With Cardiomyopathy

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Background—Intramyocardial nerve sprouting after myocardial infarction is associated with ventricular arrhythmias. Whether human stellate ganglia remodel in association with cardiac pathology is unknown. The purpose of this study was to determine whether cardiac pathology is associated with remodeling of the stellate ganglia in humans.

Methods and Results—Left stellate ganglia were collected from patients undergoing sympathetic denervation for intractable ventricular arrhythmias and from cadavers, along with intact hearts. Clinical data on patients and cadaveric subjects were reviewed. We classified ganglia from normal, scarred, and nonischemic cardiomyopathic hearts without scar as NL (n=3), SCAR (n=24), and NICM (n=7), respectively. Within left stellate ganglia, neuronal size, density, fibrosis, synaptic density, and nerve sprouting were determined. Nerve density and sprouting were also quantified in cadaveric hearts. Mean neuronal size in normal, scarred, and nonischemic cardiomyopathic hearts without scar groups were $320\pm4$ μm$^2$, $372\pm10$ μm$^2$, and $435\pm10$ μm$^2$ ($P=0.002$), respectively. No significant differences in neuronal density and fibrosis were present between the groups. Synaptic density in ganglia from SCAR and NICM groups were $57.8\pm11.2$ μm$^2$/mm$^2$ ($P=0.084$) and $44.5\pm7.9$ μm$^2$/mm$^2$ ($P=0.039$), respectively, compared with the normal group, $17.8\pm7$ μm$^2$/mm$^2$ (overall $P=0.162$). There were no significant differences in left stellate ganglia nerve sprouting or myocardial nerve density between the groups.

Conclusions—Neuronal hypertrophy within left stellate ganglia is associated with chronic cardiomyopathy in humans. Ganglionic and myocardial nerve sprouting and nerve density were not significantly different. These changes may be related to increased cardiac sympathetic signaling and ventricular arrhythmias. Further studies are needed to determine the electrophysiological consequences of extracardiac neuronal remodeling in humans. (Circ Arrhythm Electrophysiol. 2012;5:1010-1016.)

Key Words: cardiomyopathy • autonomic nervous system • sympathetic nervous system • ventricular arrhythmia

Clinical Perspective on p 1016

The sympathetic nervous system exerts profound influence on cardiac function and electrophysiology. The sympathetic nervous system is associated with sudden cardiac death, increased dispersion of repolarization, and ventricular arrhythmias (VAs) in ischemic and nonischemic myocardial substrates. Pharmacological and nonpharmacological modulation of adrenergic signaling remains a focal point in managing myocardial ischemia and VAs.

Intramyocardial neural remodeling (nerve sprouting) occurring at the border zones of myocardial scar and normal tissue has been associated with VAs and sudden cardiac death in animal models and humans. Data regarding physiological function and pathological evidence of extracardiac neural remodeling after myocardial injury have been reported in animal models. However, there are minimal data on extracardiac neural remodeling in humans. Of the available studies, evidence for extracardiac neural remodeling includes transdifferentiation of sympathetic nerves to cholinergic within stellate ganglia of rats with heart failure and stellate ganglion neuronal hypertrophy in chronically exercise-trained rats. Recently, in a rabbit model of ischemia-reperfusion injury, nerve sprouting and hyperinnervation within bilateral stellate ganglia were observed up to a month after myocardial injury. Patients with cardiopulmonary disease have been reported to have greater fibrosis and neuron density within their stellate ganglia than those without such conditions, although the differences were marginal. Whether extracardiac neurons undergo physical remodeling because of cardiac pathology remains unknown in humans.

The purpose of this study was to perform an in-depth study to determine whether the presence of cardiac pathology and severe VAs is associated with extracardiac neural remodeling in humans.
Methods

Specimen Collection

Cadaveric Specimens
Whole intact hearts and left stellate ganglia (LSG) were collected from cadavers (Donated Body Program, University of California-Los Angeles, and West Virginia University). Use of preserved human specimens was in accordance with institutional guidelines. All available clinical information regarding cause of death and medical history of the cadavers was collected. Hearts were grossly dissected by a cardiac pathologist to identify any cardiac pathology, including epicardial coronary artery disease, myocardial infarction, valvular pathology, and other abnormalities. Any surgical interventions performed previously on the hearts were also noted and correlated with clinical history as available. Posteroventral and anterolateral papillary muscles at the midventricular level were harvested from all specimens for histological analyses. All regions with or suspected to have myocardial pathology, including infarctions, were also sampled for histological analysis. The ganglia were marked for superoinferior orientation and sectioned for histological analyses.

Clinical Specimens
LSG were collected from patients with VAs undergoing thoracic sympathectomy for control of arrhythmias. These were patients with normal or abnormal myocardial function but with severe VAs recalcitrant to conventional therapies, including invasive catheter ablation. Use of these human pathological specimens was in accordance with institutional guidelines and was approved by the UCLA institutional review board. Detailed clinical information on the patients was also collected. These included coronary angiography, nuclear myocardial perfusion studies, positive emission tomography, echocardiography, cardiac computed tomography, and magnetic resonance imaging. Electrophysiological mapping and electrophysiological details of patients’ hearts and arrhythmias were collected. Health records were also reviewed for clinical and anatomic information, and retrospective review of this data was approved by the institutional review board.

Arrhythmias
Ventricular tachycardia (VT) storm was defined as 20 episodes of VT per hour. Recurrent VT referred to frequent VTAs not meeting the above criteria. Recurrent implantable cardioverter defibrillator shocks were used as a designation for patients with implantable cardioverter defibrillator shocks not meeting criteria for VT storm.

Classification
Based on cardiac substrate, LSG from cadaveric and surgical pathological sources were segregated into normal (NL), scarred (SCAR), and nonischemic cardiomyopathic hearts without scar (NICM; absence of scar but presence of a nonischemic cardiomyopathy). NL samples were obtained from cadavers without any evidence of gross or histological cardiac pathology. The SCAR group consisted of stellate ganglia from subjects with documented myocardial scars. This included cadaveric specimens with healed infarcts and patients with ischemic and nonischemic cardiomyopathy with intramyocardial scar documented by a combination of the imaging modalities listed above. NICM samples were obtained from patients with no evidence of myocardial scar but with a nonischemic cardiomyopathy. These patients had severe VAs that were refractory to medical and ablative strategies.

Histological and Immunohistochemical Studies
Stellate ganglia were serially sectioned, and representative histological sections from the middle of the ganglia were used for analyses. All slides were scanned, and digital images were electronically stored for analysis (Scan Scope, Aperio, Vista, CA). Entire ganglia, excluding nerve tracts, were analyzed. Histological and immunohistochemical quantifications were performed by computerized morphometry (Tissue Studio, Definiens Inc, Parsippany, NJ). Slides were analyzed in a numerically blinded fashion to avoid bias in data analysis. Intensity of staining was measured during the computerized analysis and was standardized across the studied samples.

Neuronal size was quantified by thionin ( Fisher Scientific, Pittsburgh, PA) and Geske’s modification of Verhoeff elastic von Giessen and Masson trichrome (EVG-trichrome). Fibrosis within stellate ganglia was quantified by EVG-trichrome staining. A blinded observer scored the severity of fibrosis within ganglia on a scale of 0 to 5. A value of 0 indicated no fibrosis present. A value of 1 was assigned for perivascular fibrosis only. A value of 5 was given for extensive fibrosis throughout the entire ganglia. Values of 2, 3, and 4 were assigned, respectively, for progressive degrees of fibrosis more severe than perivascular but not covering the entire stellate ganglion.

Diamino benzidine (Life Technologies, Green Island, NY) immunostaining was used to quantify neuronal growth (growth-associated protein 43 [GAP43], 1:2000, Life Technologies) and nerve synaptic density (Synaptophysin, 1:200, Life Technologies). Neuronal size is expressed in square microns (μm²). Immunoreactivity is expressed as immunostained area in μm²/total tissue area studied in mm² (μm²/mm²). Positive controls (using neuronal cancer tissue) were performed in parallel with all immunostaining experiments to confirm antibody immunoreactivity.

Myocardial tissue was stained with hematoxylin and eosin and EVG-trichrome to histologically distinguish scar from the normal myocardium. Nerve fiber density was quantified using S100 immunostaining. S100 immunostaining was expressed as described above.

Statistical Analyses
Means for continuous variables were compared using a nonparametric 1-way ANOVA model (Kruskal-Wallis), where P values were computed using exact permutational methods. The 3 post hoc pairwise mean comparisons under this ANOVA model were judged significant using the Fisher least significant difference criterion, which controls the overall type I error rate when there are 3 groups.

The mean percentages of small, medium, and large neurons were compared using a 1-way multivariate ANOVA model because the percentage of small, medium, and large must equal 100% for any subject, making these 3 variables nonindependent.

Mean and SEM are reported, or individual data points in each group are displayed in jitter plots, with lines connecting means across the 3 groups. An adjusted P≤0.05 was considered statistically significant.

Results
The Table shows characteristics of the cadaveric and patient subjects included in the study. The mean age of the subjects in the study was 63±14 years. Twenty-four percent of the study subjects were women. A total of 34 ganglia were obtained from 10 cadaveric subjects and 24 patients undergoing denervation. Based on the cardiac pathology, cadavers were classified as NL, SCAR, and NICM. All the cadaveric hearts in the SCAR group contained healed infarcts; no acute infarctions were noted histologically. Shown in Figure 1 are representative gross and histological images of a heart in the (A) NL group and (B) SCAR group (black arrows). Figure 1C to 1F shows some of the various imaging modalities used to confirm the presence of myocardial scar (white arrows) in study patients (SCAR group).

Neuronal Size and Distribution
Examples of mean neuronal size in stellate ganglia from NL controls (n=3), ganglia from SCAR hearts (n=24), and NICM (n=7) after thionin and EVG-trichrome staining are shown in Figure 2A. Thionin staining showed that neurons in SCAR ganglia were significantly larger than NL (371.9±10.2 μm² versus 320.1±4 μm²). Surprisingly however, neurons from NICM ganglia were the largest of the 3 groups (435±10 μm²; overall P=0.002; Figure 2B).
The distribution of small (<350 μm²), medium (350–500 μm²), and large (>500 μm²) neurons observed within thionin-stained ganglia is shown in Figure 2C. The majority of neurons in all 3 groups are <350 μm²; however, compared with NL, the percentage of small neurons is decreased in SCAR and NICM. The percentage of large neurons is increased in SCAR and NICM versus NL (multivariate ANOVA P<0.0182, exact Wilks lambda). There was no significant difference among the groups in the percentage of medium-sized neurons.

Ganglion Fibrosis and Neuronal Density

The degree of fibrosis observed in each stellate ganglion was scored by an observer (M.C.F.), blinded to the group assignment of each ganglion. A grading scale of 0 to 5 was used for fibrosis, as described in the Methods section. Mean fibrosis grades in NL, SCAR, and NICM were 2.7±0.7, 1.8±0.2, and 2.0±0.4, respectively (overall P=0.423; Figure 3A).

Neuronal density (cell number/tissue area) was not significantly different among the 3 groups (0.039±0.01 cells/μm² versus 0.028±0.005 cells/μm² versus 0.024±0.004 cells/μm²).

### Table. Characteristics of Patients and Cadaveric Subjects

<table>
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<tr>
<th>Subject</th>
<th>Age/Sex</th>
<th>Ganglia Source</th>
<th>Cause of Clinical Presentation or Death</th>
<th>Arrhythmias</th>
<th>History of Coronary Artery Disease</th>
<th>Myocardial Substrate</th>
<th>Myocardial Scar</th>
<th>Left Ventricular Ejection Fraction</th>
<th>Clinical Outcome</th>
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<td>ICM</td>
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<td>Yes, 3v</td>
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<td>No</td>
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<td>Inf, inf-lat</td>
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<tr>
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<tr>
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<td>NICM</td>
<td>None</td>
<td>15% Alive</td>
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</table>

3v indicates 3-vessel coronary artery disease; Ant, anterior; CHF, congestive heart failure; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter defibrillator; ICM, ischemic cardiomyopathy; Inf, inferior; LAD, left anterior descending coronary artery; Lat, lateral; LCX, left circumflex coronary artery; LSG, left stellate ganglion; LVOT, left ventricular outflow tract; LTFU, lost to follow-up; MV, mitral valve; NICM, nonischemic cardiomyopathy; NSCLC, non–small cell lung cancer; PDA, posterior descending artery; post, posterior; RCA, right coronary artery; RV, right ventricle; RVOT, right ventricular outflow tract; sep, septal; VF, ventricular fibrillation; VT, ventricular fibrillation; n/a, not applicable.
Synaptic Density and Nerve Sprouting
Neuron synaptic density was measured by synaptophysin immunostaining in stellate ganglia from NL, SCAR, and NICM and is shown in Figure 4A. Synaptic densities were 17.8±7.0 μm²/mm² versus 57.8±11.2 μm²/mm² (P=0.084) versus 44.5±7.9 μm²/mm² (P=0.039), respectively (overall P=0.162).

GAP43 is incorporated into growing neurons and is a marker of neuronal growth. Figure 4A shows GAP43 staining in stellate ganglia from NL, SCAR, and NICM groups. There was no significant difference in GAP43 immunoreactivity between the groups (2696.2±1004 μm²/mm² versus 3992±614 μm²/mm² [P=0.939] versus 2564.7±881 μm²/mm² [P=0.210], respectively; overall P=0.194).

Myocardial Nerve Density
Intramyocardial nerve density was assayed by S100 immunostaining. As shown in Figure 5, S100 immunoreactivity was similar between NL and SCAR hearts (33±12 μm²/mm² versus 28±6 μm²/mm², respectively; P=0.903), indicating no increase in nerve density in SCAR hearts with healed infarcts compared with NL. Nerve sprouting as assessed by GAP43 immunoreactivity was not different between NL and SCAR (data not shown).

Discussion
Major Findings
The major findings of the present study are as follows: (1) LSG neurons from patients with abnormal hearts are significantly larger compared with those from normal hearts. Furthermore, neurons from ganglia of patients with nonischemic cardiomyopathies are larger than those associated with scar-based pathology. (2) The degree of nerve sprouting and synaptic density in these chronically diseased hearts was not different from normal. This study represents the first evidence of extracardiac neural remodeling associated with cardiac pathology in humans.

Neuronal Characteristics and Cardiac Pathology
Hypertrophy of neurons in response to injury is a recognized phenomenon and has been described in animal models of neural injury. Neuronal hypertrophy within the stellate ganglia after myocardial infarction has not been previously reported in humans. The myocardium is highly innervated by sympathetic and parasympathetic nerves, as well as sensory C fibers that convey nociceptive stimuli to dorsal root ganglia. Myocardial injury, such as infarction or scarring, results in axonal injury. Neurotrophic signals, including nerve growth factor, are transmitted to the soma (via retrograde axonal transport and circulation) to signal axonal injury. Within the tissue, the nerve growth factor signaling is important for the hyperinnervation that results after myocardial injury. The resulting process of chromatolysis, of which soma hypertrophy is a component, ensues. This process may explain the pathogenesis of neuronal hypertrophy observed in our study.

Neurons may also hypertrophy in response to chronic signaling. Cavalcanti et al showed in a rat model of exercise training that neuronal size was increased within bilateral stellate ganglia. The mechanism of hypertrophy in this model is likely different from that of myocardial and axonal injury, although similar signals may be involved. It is likely that chronic sympathetic signaling occurring with chronic exercise training may contribute to neuronal hypertrophy. This finding may, in part, explain our finding that stellate ganglion neurons from NICM hearts showed significantly greater hypertrophy compared with those from SCAR hearts. It is consistent with the suggestion that in nonischemic cardiomyopathies, neurohormonal activation involving the stellate ganglia is a major component of the pathophysiological process resulting in
progressive cardiomyopathy. Furthermore, that these stellate ganglia were obtained from patients with refractory VAs may also suggest the involvement of these hypertrophied ganglia in ventricular arrhythmogenesis. This hypothesis underscores the rationale for a landmark randomized trial comparing placebo to $\beta$-adrenergic receptor blocker therapy and cardiac sympathetic denervation. The trial showed a profound decrease in the incidence of VAs and sudden death in patients after myocardial infarction. A potential mechanism for this benefit is the removal of remodeled (and possibly hyperactive) stellate ganglia.

Nerve Sprouting and Synaptic Density in Cardiac Pathology

Nerve sprouting in the myocardium and within stellate ganglia occurs after myocardial injury in animal models; however, nerve sprouting has not been documented in human stellate ganglia. In our study of chronic myocardial injury, synaptic density (synaptophysin immunostaining) within stellate ganglia appeared qualitatively greater in subjects with cardiac pathology (NL 17.8±7 $\mu$m$^2$/mm$^2$ versus SCAR 57.8±11.2 $\mu$m$^2$/mm$^2$ $P=0.084$ versus NICM 44.5±7.9 $\mu$m$^2$/mm$^2$ $P=0.039$; overall $P=0.162$); however, no differences were observed in stellate ganglion nerve sprouting (GAP43) between normal and pathological hearts. This finding in our study is consistent with previous studies on the dynamics of neural remodeling in animal models. In a rabbit and canine model of myocardial infarction, levels of synaptic density and nerve sprouting were measured at 1 week and 1 month after infarction. Synaptic density was greater at 1 month compared with 1 week postinfarct. This is contrasted with nerve sprouting, which was greatest at 1 week but decreased by 1 month. This pattern suggests that nerve sprouting may be a transient process, whereas increases in synaptic density are a more permanent adaptive process. Our study included healed infarcts, with levels of synaptic density persistently elevated compared with controls, whereas levels of nerve sprouting were similar to normal levels. Similarly, nerve density in the myocardium was similar among NL, SCAR, and NICM hearts.

Limitations

Because of the nature of our study, there are several limitations to consider. Our study associates cardiomyopathy with neuronal hypertrophy and synaptic density. It is not possible to tease
out whether the stellate ganglion changes are reactive or contribute to the development of a cardiomyopathy or arrhythmias. Furthermore, the timing of cardiac pathology may be different in the SCAR versus NICM groups. The differences noted in neuronal size between SCAR and NICM may be related to this temporal difference. The age and sex of subjects also differed between the groups (with NICM patients being generally younger and NL being mostly women). Although animal studies have not shown sex differences in neuronal size, the possibility of such differences in this data set is unknown. Furthermore, our results are in line with existing publications in animal models in which these factors have been controlled.\cite{16,30} Another limitation to note is that immunohistochemical assays are not directly quantitative, and differences are not translatable into fold differences. Although the objective values obtained from morphometric analyses for synaptophysin did not meet statistical significance, there was a trend toward significance, consistent with qualitative observations. Lastly, there are no physiological data (such as sympathetic nerve signaling) to correlate with the anatomic findings in this study. Obtaining such data in patients is, however, difficult, because it would involve an invasive procedure with significant risks in an already compromised patient population.

**Conclusions**

In summary, this study demonstrates that human cardiac pathology is associated with remodeling of neurons within LSG, including increased neuronal size and synaptic density. These persistent anatomic changes within stellate ganglia may suggest the presence of pathological signals between the heart and neuraxis. Further studies are warranted to elucidate the physiological consequences of neural remodeling in response to cardiac pathology.
**Acknowledgments**

We thank Jacob N. Fox, BS, Wei Zhou, PhD, Shelly Cote, RN, MSNP, Jean Gima, RN, MSNP, and Long Sheng Hong for significant contributions to this project. In addition, we acknowledge Clara Magyar, PhD, and the University of California-Los Angeles (UCLA) Translational Pathology Core Laboratory. We also thank contributors to the UCLA and West Virginia University Donated Body Programs, whose generosity allowed us to study cadaveric specimens. We also thank Dr Jeffrey Gornbein from the UCLA BioMathematics Department for statistical assistance.

**Sources of Funding**

This work was made possible by support from the National Heart, Lung, and Blood Institute grant (R01HL084261) to Dr Shivkumar. This work was also made possible by support from the National Heart, Lung, and Blood Institute grant (R01HL084261) to Dr Shivkumar. The authors also thank Dr Jeffrey Gornbein from the UCLA BioMathematics Department for statistical assistance.

**Disclosures**

None.

**References**


**Clinical Perspective**

This article reports our findings on neuronal remodeling within the stellate ganglion, in association with cardiomyopathy in humans. This is the first report of this phenomenon in humans. Our study demonstrates that in both ischemic and nonischemic cardiomyopathy patients (with arrhythmias) there is intraganglionic neuronal hypertrophy. Animal studies have shown remodeling within the stellate ganglia after myocardial injury. However, remodeling of human stellate ganglia remained unknown. Our study shows that there is profound enlargement of these control structures (the stellate ganglion neurons) in patients with both types of cardiomyopathy. This finding also suggests that these nerve bodies may serve as a pathophysiological link between neuroendocrine hyperactivation and arrhythmias that occur in patients with cardiomyopathy.
Extracardiac Neural Remodeling in Humans With Cardiomyopathy
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Circ Arrhythm Electrophysiol. 2012;5:1010-1116; originally published online August 23, 2012; doi: 10.1161/CIRCEP.112.972836
Circulation: Arrhythmia and Electrophysiology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3149. Online ISSN: 1941-3084

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