RESEARCH ARTICLE | Cardiac Excitation and Contraction

Cardiac sympathetic innervation via middle cervical and stellate ganglia and antiarrhythmic mechanism of bilateral stellectomy

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Irie T, Yamakawa K, Hamon D, Nakamura K, Shivkumar K, Vaseghi M. Cardiac sympathetic innervation via middle cervical and stellate ganglia and antiarrhythmic mechanism of bilateral stellectomy. Am J Physiol Heart Circ Physiol 312: H392–H405, 2017. First published December 23, 2016; doi:10.1152/ajpheart.00644.2016.—Cardiac sympathetic denervation (CSD) is reported to reduce the burden of ventricular tachyarrhythmias [ventricular tachycardia (VT)/ventricular fibrillation (VF)] in cardiomyopathy patients, but the mechanisms behind this benefit are unknown. In addition, the relative contribution to cardiac innervation of the middle cervical ganglion (MCG), which may contain cardiac neurons and is not removed during this procedure, is unclear. The purpose of this study was to compare sympathetic innervation of the heart via the MCG vs. stellate ganglia, assess effects of bilateral CSD on cardiac function and VT/VF, and determine changes in cardiac sympathetic innervation after CSD to elucidate mechanisms of benefit in 6 normal and 18 infarcted pigs. Electrophysiological and hemodynamic parameters were evaluated at baseline, during bilateral stellate stimulation, and during bilateral MCG stimulation in 6 normal and 12 infarcted animals. Bilateral CSD (removal of bilateral stellates and T2 ganglia) was then performed and MCG stimulation repeated. In addition, in 18 infarcted animals VT/VF inducibility was assessed before and after CSD. In infarcted hearts, MCG stimulation resulted in greater chronotropic and inotropic response than stellate ganglion stimulation. Bilateral CSD acutely reduced VT/VF inducibility by 50% in infarcted hearts and prolonged global activation recovery interval. CSD mitigated effects of MCG stimulation on dispersion of repolarization and T-peak to T-end interval in infarcted hearts, without causing hemodynamic compromise. These data demonstrate that the MCG provides significant cardiac sympathetic innervation before CSD and adequate sympathetic innervation after CSD, maintaining hemodynamic stability. Bilateral CSD reduces VT/VF inducibility by improving electrical stability in infarcted hearts in the setting of sympathetic activation.

NEW & NOTEWORTHY Sympathetic activation in myocardial infarction leads to arrhythmias and worsens heart failure. Bilateral cardiac sympathetic denervation reduces ventricular tachycardia/ventricular fibrillation inducibility and mitigates effects of sympathetic activation on dispersion of repolarization and T-peak to T-end interval in infarcted hearts. Hemodynamic stability is maintained, as innervation via the middle cervical ganglion is not interrupted.

* T. Irie and K. Yamakawa contributed equally to this work.

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MYOCARDIAL INFARCTION (MI) increases the risk of sudden cardiac death due to ventricular tachyarrhythmias [ventricular tachycardia (VT)/ventricular fibrillation (VF)] (13) by causing pathological cardiac (16, 34, 41) and neural (15, 44, 53) remodeling. Sympathetic activation via the right or left stellate ganglion increases T-peak to T-end (Tp-Te) interval, a marker of sudden cardiac death (8, 33), and increases dispersion of repolarization (DOR), predisposing to VT/VF (27, 44, 45, 50). Left cardiac sympathetic denervation (CSD) has been reported to decrease the burden of VT/VF in patients with hereditary channelopathies, including long QT syndrome and catecholaminergic polymorphic ventricular tachycardia, who have structurally normal hearts (4, 17, 38). In a small series of patients with structural heart disease, bilateral CSD has shown promise in reducing the burden of internal cardioverter defibrillator (ICD) shocks. This study also suggested that bilateral CSD may lead to a greater ICD shock-free survival compared with left CSD in patients with cardiomyopathy (43). However, the electrophysiological mechanisms behind the reduction in life-threatening arrhythmias with CSD are not clear. In addition, it is unknown how much of cardiac sympathetic innervation, which may be required for beat-to-beat function, is preserved after bilateral CSD. The middle cervical ganglia (MCGs), located anatomically superior to the stellate ganglia, have been reported to contain cardiac sympathetic neurons (7, 21) and could provide sympathetic innervation after CSD. However, their electrophysiological effects have not been characterized. The purpose of this study was to delineate 1) sympathetic innervation of the heart via the MCG compared with the stellate ganglia, 2) electrophysiological and antiarrhythmic effects of CSD, including VT inducibility, and 3) alterations in functional cardiac sympathetic innervation after CSD and its antiarrhythmic mechanisms in a porcine model.

METHODS

Study procedures were approved by the UCLA Institutional Animal Research Committee and were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Creation of myocardial infarcts. Eighteen Yorkshire pigs were sedated (6–10 mg/kg Telazol and 2–5 μg/kg fentanyl), intubated, and placed under general anesthesia with inhaled isoflurane (0.5–1.5%). After femoral arterial access was obtained with an 8-F sheath, an
Amplatzer guide catheter (Boston Scientific, Marlborough, MA) was advanced over a J-tipped guide wire under fluoroscopic guidance and used to cannulate the left main coronary artery from the left femoral artery. A coronary angiogram was performed to delineate the branches of the left anterior descending coronary artery (LAD). A 3-mm luminal angioplasty balloon (Armada 35 PTA, Abbott Vascular, Temecula, CA) was advanced past the first diagonal branch of the LAD over a 0.014-in. coronary guide wire (Balance Middle Weight wire, Abbott Vascular) and inflated. Polystyrene microspheres (5.0–7.5 ml, Polysciences, Warrington, PA) were injected through the lumen of the percutaneous balloon catheter (26). Repeat coronary angiography after microsphere injection showed confirmed poor blood flow in the LAD after the first diagonal branch. Continuous electrocardiogram (ECG) monitoring was performed. ECG changes including ST segment elevation and or T-wave inversions were also monitored until they could ambulate without assistance. Electrocardioversion was performed. Animals were then extubated and monitored until they could ambulate without assistance.

**Surgical preparation.** Four to six weeks after MI, 18 infarcted (48 ± 2 kg) and 6 normal (43 ± 1 kg) animals were sedated (6–10 mg/kg Telazol and 2–5 μg/kg fentanyl) and placed under general anesthesia with isoflurane (0.8–1.5%). Normal animals had not undergone a percutaneous interventional procedure before terminal experiments. Median sternotomy was performed, and isolation of bilateral stellate ganglia and T2 thoracic ganglia (all animals) and bilateral MCG (12 of 18 animals) was performed. Presence and extent of scar tissue was assessed by gross inspection and voltage measurements. Regions within the heart with a voltage of 0.5–1.5 mV were defined as scar, those with a voltage of 0.5–1.5 mV were defined as border zone, and electrodes overlying regions with a voltage ≥ 1.5 mV were defined as viable/normal (14, 23). The location and extent of infarct were marked on the heart. The MCG were identified often above and behind the subclavian artery, at the inlet of the thorax (Fig. 1). After completion of the surgical portion of the procedure isoflurane was discontinued, and an intravenous infusion of α-chloralose (10–30 mg·kg⁻¹·h⁻¹) was begun (5, 10, 11). During anesthesia with both α-chloralose and isoflurane, heart rate (HR) as well as jaw tone and eyelid reflexes were monitored every 10–15 min to ensure adequate sedation.

**Stellate and middle cervical ganglion stimulation.** Bipolar needle electrodes (Fig. 1) were used for bilateral stellate ganglion stimulation and bilateral MCG stimulation with a Grass Stimulator (model S88, Grass Technologies, Warwick, RI) in both infarcted (n = 12) and normal (n = 6) animals. Threshold was defined as the current needed to increase HR or systolic blood pressure by 10% (at 4 Hz, 4 ms). Stimulation was performed at 1.5 times threshold for 30 s. In normal animals, the stimulation current used was 2.4 ± 0.5 mA for the right stellate ganglion and 2.3 ± 0.4 mA for the right MCG; the stimulation current was 5.6 ± 0.8 mA for the left stellate ganglion and 6.0 ± 0.7 mA for the left MCG. For infarcted animals, the stimulation current used was 6.7 ± 1.0 mA for the right stellate ganglion and 5.9 ± 1.2 mA for the right MCG; the stimulation current was

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**Fig. 1.** Isolation of the middle cervical ganglia (MCG) and Tp-Te interval and ARI recordings. A: anatomy of MCG and surrounding tissue is shown. The MCG sits just at the thoracic inlet and provides cardiopulmonary nerves that innervate the heart. B: customized bipolar needle electrodes were placed in the isolated MCG. C: method for the measurement of Tp-Te interval from surface ECG. The peak of the T wave was determined as the highest voltage of the T wave (T-p). T-end (T-e) was determined by the tangent of the T wave. D: the region of scar in infarcted hearts predominantly involved the LV apex, anterior, and anterolateral walls. E: 56-electrode sock is placed over the ventricles to obtain unipolar electrograms that are used for ARI analysis. F: template for the polar maps used to display regional ARIs from the sock electrodes. Ant, anterior; Lat, lateral; Post, posterior; RVOT, right ventricular outflow tract.
9.8 ± 1.0 mA for left stellate ganglion stimulation and 10.0 ± 1.0 mA for left MCG. In normal animals, a second stellate and MCG stimulation was performed before CSD for 60 s and electrophysiological effects were compared at 5, 10, 15, 20, 30, 45, and 60 s. A 30-min period was allowed for return of hemodynamic parameters to baseline in between stimulations. The timeline for the experimental protocol in normal animals is shown in Fig. 2.

**Hemodynamic and electrocardiogram recordings.** Hemodynamic parameters were continuously recorded with a 12-pole conductance pressure catheter in the left ventricle (LV), connected to a MPVS Ultra Pressure Volume Loop System (Millar Instruments, Houston, TX). ECG was continuously obtained with the GE Cardiolab System (GE Healthcare). $t$-Te interval was assessed in the inferior leads TX). ECG was continuously obtained with the GE Cardiolab System (GE Healthcare). $t$-Te interval was assessed in the inferior leads TX). ECG was continuously obtained with the GE Cardiolab System (GE Healthcare). $t$-Te interval was assessed in the inferior leads TX). ECG was continuously obtained with the GE Cardiolab System (GE Healthcare). $t$-Te interval was assessed in the inferior leads TX). ECG was continuously obtained with the GE Cardiolab System (GE Healthcare). $t$-Te interval was assessed in the inferior leads TX).

Table 1. Hemodynamic parameters in normal animals

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<th></th>
<th>BL (PreBSGS)</th>
<th>BSGS</th>
<th>BL (PreMCGS)</th>
<th>BMCGS (PreCSD)</th>
<th>PreCSD</th>
<th>PostCSD</th>
<th>BL (PreMCGS)</th>
<th>BMCGS (PostCSD)</th>
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<tr>
<td>HR, bpm</td>
<td>84 ± 17</td>
<td>98 ± 13*</td>
<td>89 ± 14</td>
<td>103 ± 10*</td>
<td>86 ± 12</td>
<td>89 ± 8</td>
<td>90 ± 12</td>
<td>104 ± 10*</td>
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<td>$dP/dt_{max}$, mmHg/s</td>
<td>1,178 ± 166</td>
<td>2,391 ± 724*</td>
<td>1,086 ± 116</td>
<td>2,364 ± 531*</td>
<td>1,032 ± 157</td>
<td>1,087 ± 296</td>
<td>1,007 ± 228</td>
<td>2,232 ± 467*</td>
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<td>$dP/dt_{min}$, mmHg/s</td>
<td>–1,126 ± 317</td>
<td>–1,149 ± 462</td>
<td>–1,172 ± 337</td>
<td>–1,174 ± 302</td>
<td>–1,121 ± 353</td>
<td>–1,094 ± 286</td>
<td>–984 ± 266</td>
<td>–1,149 ± 382</td>
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<td>LVESP, mmHg</td>
<td>103 ± 20</td>
<td>147 ± 23*</td>
<td>99.5 ± 21</td>
<td>134 ± 24*§</td>
<td>97.5 ± 22</td>
<td>94 ± 16</td>
<td>87 ± 13</td>
<td>125 ± 20†</td>
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Values represent mean ± SD % change in parameters from prestimulation value ($n = 6$). PreCSD represents hemodynamic values immediately before removal of the stellate ganglia. PostCSD values represent hemodynamic parameters after a 30-min period of stabilization after removal of stellate ganglia. HR, heart rate; bpm, beats per minute; $dP/dt_{max}$, $dP/dt_{min}$, maximum and minimum dP/dt; LVESP, left ventricular end-systolic pressure; BL, baseline; BSGS, bilateral stellate ganglion stimulation; BMCGS, bilateral middle cervical ganglion stimulation. *P value < 0.05 for % change from baseline (prestimulation). †P = 0.08 for % change in LVESP with BMCGS PreCSD vs. PostCSD, §P = 0.02 for comparison of % change in LVESP for BSGS vs. BMCGS.
Infarct pigs (IVC) 1.97 ± 0.54 2.53 ± 0.28 1.89 ± 0.46 3.78 ± 0.95*

Values (in ng/ml) are means ± SE. CS, coronary sinus; IVC, inferior vena cava; BSGS, bilateral stellate ganglion stimulation; BMCGS, bilateral middle cervical ganglion stimulation. *P < 0.05 compared with baseline.

period after CSD was allowed before repeat stimulation or inducibility testing. VT/VF inducibility was tested before and after CSD.

Inducibility testing for ventricular tachyarrhythmias. In 18 infarcted hearts, programmed ventricular stimulation up to triple extra-stimuli to a minimum coupling interval of 200 ms or an effective refractory period (ERP) was performed before and after CSD first from the right ventricular (RV) endocardium at the mid- to apical septum with an endocardial quadripolar electrophysiology catheter connected to a MicroPace stimulator (MicroPace EP, Santa Ana, CA). Current was set at two times the ventricular capture threshold in each animal with a pulse duration of 1 ms. If VT/VF was not induced from the RV endocardium at baseline before CSD, a second site on the LV epicardium close to the scar was used before CSD. The same or a very similar site was used for induction of VT/VF after CSD as had been used to induce VT/VF before CSD. The induction protocol was as follows: an extrastimulus was placed after 8 beats of drive cycle length (at 500 ms) at an interval of 400 ms (S2). The premature extrastimulus interval (S2) was reduced by 10 ms until either an interval of 200 ms or an ERP was reached or VT/VF was induced. If VT/VF was not induced, then S2 was fixed at 20 ms above the ERP or 220 ms (if ERP was <200 ms) and a second premature extrastimulus (S3) was added at an interval of 400 ms. The S3 interval was then reduced by 10 ms until an S2–S3 interval of 200 ms or ERP was reached or VT/VF was induced. Finally, if no VT/VF was induced, then the S3 interval was set at 220 ms (if ERP was <200 ms) or 20 ms above ERP and an S4 interval (triple extrastimulus) was added at an S3–S4 interval of 400 ms. This S4 interval was then reduced by 10 ms until a coupling interval of 200 ms or effective period was reached or VT/VF was induced. Inducibility was defined

Table 2. Norepinephrine levels in normal and infarcted animals

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>BSGS</th>
<th>Baseline</th>
<th>BMCGS</th>
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<tr>
<td>Normal pigs (CS)</td>
<td>0.38 ± 0.09</td>
<td>8.70 ± 2.28*</td>
<td>0.54 ± 0.16</td>
<td>13.73 ± 4.47*</td>
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<td>Normal pigs (IVC)</td>
<td>0.58 ± 0.22</td>
<td>1.52 ± 0.29*</td>
<td>0.55 ± 0.07</td>
<td>1.61 ± 0.29*</td>
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<tr>
<td>Infarct pigs (CS)</td>
<td>1.35 ± 0.41</td>
<td>7.47 ± 1.48*</td>
<td>1.79 ± 0.51</td>
<td>7.83 ± 2.16*</td>
</tr>
<tr>
<td>Infarct pigs (IVC)</td>
<td>1.97 ± 0.54</td>
<td>2.53 ± 0.28</td>
<td>1.89 ± 0.46</td>
<td>3.78 ± 0.95*</td>
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Fig. 3. A: the time course of ARI shortening with bilateral stellate ganglion stimulation (BSGS) was similar to that with bilateral MCG stimulation (BMCGS) (n = 6). B: there were no significant regional differences between BSGS and BMCGS in normal hearts (P values > 0.05 for all regions, n = 6). C: apical ventricular pacing before CSD demonstrated shorter global ARIs than right atrial (RA) pacing in normal animals (n = 6) at the same cycle lengths. However, after CSD there was no significant difference in ARI between ventricular and atrial pacing, suggesting that apical pacing may cause reflex sympathetic activation that is prevented by CSD. Ant, anterior; Lat, lateral; Post, posterior wall; RV, right ventricle; LV, left ventricle.
as hemodynamically tolerated VT that lasted >30 s or that degenerated into VF requiring direct cardioversion.

Measurement of norepinephrine levels. Norepinephrine (NE) levels in the inferior vena cava (IVC) and coronary sinus (CS) were measured to confirm adequate ganglion stimulation. Venous blood was obtained from a luminal catheter inserted in the IVC, superior to the adrenal veins and close to the right atrium. CS blood was obtained by cannulation of the CS with a luminal catheter (St. Jude Medical, St. Paul, MN) from the right external jugular vein. Blood samples at baseline and in the last 5 s of stellate ganglion and MCG stimulation before CSD were immediately centrifuged (3,000 rpm, 15 min) to separate the plasma portion. Quantification of NE was performed with an ultrasensitive enzyme-linked immunoassay (ELISA) (BA E-5200, sensitivity 1.3 pg/ml, Labor Diagnostika Nord, Nordhorn, Germany) and an ELISA microplate reader (Fisher Scientific, Waltham, MA).

Activation recovery interval recordings. In 12 infarcted and 6 normal animals, detailed electrophysiological mapping was performed. A 56-electrode sock (Fig. 1) was placed over the ventricles to assess activation recovery interval (ARI), a surrogate of local action potential duration. Unipolar electrograms were recorded (0.05–500 Hz) with the GE Cardiolab System. ARIs were calculated with customized software (ScalDyn, University of Utah, Salt Lake City, UT) as previously described (44, 46). Briefly, activation time (AT) was defined as the interval from electrogram onset to most negative

![Graphs and figures showing data](http://ajpheart.physiology.org/)

**Fig. 4.** Effects of CSD in normal animals. **A:** there was no difference in global ARI in normal heart before compared with after CSD. **B:** bilateral MCG stimulation after CSD had similar effects on global ARI compared with before CSD, with significant ARI shortening observed. **C:** there were no differences in the regional effects of MCG stimulation after CSD compared with before CSD. **D:** before CSD bilateral MCG stimulation increased dispersion of repolarization in normal hearts, and this effect was not modified by CSD. **E:** bilateral MCG stimulation in normal hearts also increased Tp-Te interval, with no differences observed in this parameter before compared with after CSD in normal hearts.

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dV/dt of the activation wave, and repolarization time (RT) was defined as the interval from electrogram onset to the most positive dV/dt of the repolarization wave. ARI was defined as the difference between RT and AT. Map3D software (University of Utah, Salt Lake City, UT, http://www.sci.utah.edu/cibc-software/map3d.html) was used to map the epicardial pattern of activation and ARI with a sock-electrode polar map template (Fig. 1F). Global DOR was calculated as the variance of ARIs recorded across all epicardial electrodes.

In this article, “anterior” refers to the ventral and “posterior” to the dorsal aspect of the heart. In normal animals, mean ARIs in the following regions were analyzed on the basis of electrode location: LV apex, LV anterior, LV lateral, and RV anterior, RV lateral, RV posterior wall, and RV outflow tract. A median of three electrodes (range 2–4) per region were used for regional ARI and AT analysis.

Atrial and ventricular pacing. To evaluate the effect of CSD on ARI during ventricular pacing compared with atrial pacing, ventricular pacing was performed at baseline and during MCG stimulation before and after CSD (pacing cycle length = 400 ms or 500 ms, depending on HR during stimulation) in six normal and five infarcted animals. To compare differences to ventricular pacing, right atrial pacing at the same cycle length was performed in normal animals at baseline and during bilateral MCG stimulation, before and after CSD. In addition, Map3D software (University of Utah) was used to map the epicardial pattern of activation and ARI during scar pacing in five infarcted hearts. The template, with sock electrode locations, used for creation of these polar maps is shown in Fig. 1.

Preganglionic vs. postganglionic neural fibers within MCG. To evaluate presence of pre- vs. postganglionic fibers within the MCG in normal animals after CSD, bilateral MCG stimulation was repeated after infusion of hexamethonium, a nicotinic receptor blocker. Right vagal nerve stimulation (VNS) was also performed before and after hexamethonium infusion to ensure nicotinic receptor blockade, as vagal efferent parasympathetic fibers are known to be preganglionic. The right cervical vagal trunk was isolated via a lateral neck cutdown, and bipolar needle electrodes (Cyberonics, Houston, TX) attached to a Grass stimulator were used for right VNS (10 Hz, 1 ms). Threshold was defined as the current at which HR decreased by 10%, and stimulation was performed at 1.2 times threshold for 20 s, before and after hexamethonium. Hexamethonium was infused for 30 min (0.025–0.2 mg·kg⁻¹·min⁻¹, dose titrated by response to right VNS in each animal).

Statistical analysis. Unless specified otherwise, data are presented as means ± SE. Global ARI was calculated as the mean ARI across all 56 electrodes. Global DOR was calculated as the variance in ARIs across all 56 electrodes. For comparison of paired variables, Wilcoxon signed-rank test or paired t-test was used. Sample size of infarcted animals was driven by the assumption of a 40% reduction in VT/VF inducibility after CSD with 80% power to detect this reduction at an α of 0.05. McNemar’s test was used to compare VT inducibility before and after CSD. Sample size of normal animals was driven by 80% power to detect a 10% difference in the effects of MCG vs. stellate ganglion stimulation on global ARI in the same animal at an α of 0.05. The Mann-Whitney test was used for comparison of
continuous variables in different groups. Percent change in regional ARIs for stellate ganglion vs. MCG stimulation were analyzed with repeated-measures ANOVA after controlling for false discovery rate at 5%. SPSS (version 22, IBM, Armonk, NY) was used for statistical analysis. P < 0.05 was considered statistically significant.

RESULTS

Thoracic sympathetic innervation in normal hearts. The timeline for the experimental protocol in normal animals is shown in Fig. 2. In normal hearts bilateral stellate ganglion and bilateral MCG stimulation significantly increased HR, maximum dP/dt (dP/dt_max) and LV end-systolic pressure (LVESP) (Table 1). Comparison of bilateral stellate ganglion to bilateral MCG stimulation demonstrated no differences in the HR or dP/dt_max increase. However, bilateral stellate ganglion stimulation had a greater effect on LVESP than bilateral MCG stimulation. CS NE levels increased in a similar fashion during bilateral stellate and bilateral MCG stimulation compared with prestimulation values (Table 2).

Both bilateral stellate ganglion and MCG stimulation decreased epicardial ARI (Fig. 2). There was no significant difference in the time course of ARI shortening during the 60 s of stimulation between the stellate ganglia and MCG (Fig. 3), and no differences in regional ARIs were detected between bilateral stellate ganglion and bilateral MCG stimulation (Fig. 3).

Compared with right atrial pacing, LV apical pacing at baseline and during MCG stimulation at the same cycle length led to significantly shorter epicardial ARIs (P < 0.05), suggesting that ventricular pacing alone led to cardiac sympathetic activation (Fig. 3).

CSD and residual sympathetic innervation in normal hearts. CSD did not cause hemodynamic deterioration in normal hearts (Table 1). No difference in global ARI was observed before compared with after CSD (Fig. 4).

MCG continued to exert significant sympathetic effects after CSD. Global ARI continued to decrease during MCG stimulation after CSD (Fig. 4). Effects of bilateral MCG stimulation after CSD on HR and dP/dt_max were unchanged compared with MCG stimulation (Table 1). Of note, comparison of atrial to ventricular pacing at the same cycle length showed that the differences in ARI shortening between right atrial pacing and LV pacing were no longer significant after CSD, with or without MCG stimulation (Fig. 3). DOR and Tp-Te interval, which increased during MCG stimulation, were unaffected by CSD in normal hearts (Fig. 4). Furthermore, CSD had no effect on activation time [pre-CSD: baseline 26.1 ± 1.5 ms vs. MCG stimulation 30.4 ± 4.1 ms (P = 0.3); post-CSD: baseline 26.1 ± 1.1 ms vs. MCG stimulation 28.2 ± 1.5 ms (P = 0.2)].

To evaluate presence of preganglionic fibers within the MCG, response to hexamethonium was evaluated. Right VNS was also performed to ensure adequate nicotinic receptor blockade. Global ARI significantly increased during right VNS before hexamethonium infusion (Fig. 5). After hexamethonium infusion, there was no change in global ARI during right VNS. However, the effects of MCG stimulation after hexamethonium were unchanged, suggesting the presence of predominantly postganglionic sympathetic fibers in the MCG (Fig. 5).

A Timeline of Creation of Myocardial Infarct and Mapping Experiments in Infarcted Animals

B

C

Fig. 6. Experimental protocol in infarcted hearts and effect of bilateral stellate ganglion stimulation (BSGS) compared with bilateral MCG stimulation (BMCGS). A: timeline of the experimental protocol in 12 infarcted animals. *BMCGS or BSGS was performed in a random order. B: in all infarcted animals BSGS significantly decreased ARI, as did BMCGS. BMCGS, however, caused greater ARI shortening than BSGS. C: polar maps from an infarcted animal show the effects of MCG and stellate ganglion stimulation on ARI. Black dashed circle indicates region of the infarct/scar on polar maps. Blue dashed lines indicate course of left anterior descending coronary artery (LAD). BL, baseline.
Thoracic sympathetic innervation in infarcted hearts. The timeline for the experimental protocol in infarcted animals is shown in Fig. 6. In infarcted animals, changes in ARI were greater during MCG stimulation compared with stellate ganglion stimulation (MCG stimulation: 24.7 ± 3.5% vs. stellate stimulation: 12.0 ± 2.8%; *P < 0.02; Fig. 6) The increase in HR was also greater during bilateral MCG stimulation compared with bilateral stellate ganglion stimulation (Table 3),

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<th>BL (PreBSGS)</th>
<th>BSGS</th>
<th>BL (PreBMCGS)</th>
<th>BMCGS (PreCSD)</th>
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<td>HR, bpm</td>
<td>85 ± 10.7</td>
<td>94 ± 21*</td>
<td>84 ± 11.8</td>
<td>112 ± 22²</td>
<td>83 ± 13</td>
<td>84 ± 13</td>
<td>84 ± 13</td>
<td>114 ± 24*</td>
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<td>dP/dtₘₐₓ, mmHg/s</td>
<td>1,246 ± 390</td>
<td>2,127 ± 315*</td>
<td>1,123 ± 332</td>
<td>2271 ± 9678*</td>
<td>984 ± 349</td>
<td>880 ± 337</td>
<td>878 ± 338</td>
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<td>−1,093 ± 635</td>
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<td>−1,027 ± 630</td>
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<td>LVESP, mmHg</td>
<td>90 ± 20</td>
<td>123 ± 28</td>
<td>86 ± 21</td>
<td>99 ± 21²</td>
<td>78 ± 25</td>
<td>69 ± 22</td>
<td>69 ± 22</td>
<td>86 ± 26</td>
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Values represent mean ± SD % change in parameters from prestimulation value (n = 12). PostCSD values represent hemodynamic parameters after a 30-min period of stabilization after removal of stellate ganglia. PreCSD represents hemodynamic values immediately before removal of the stellate ganglia. HR, heart rate; dP/dtₘₐₓ, dP/dtₘᵢₙ, maximum and minimum dP/dt; LVESP, left ventricular end-systolic pressure; BSGS, bilateral stellate ganglion stimulation; BMCGS, bilateral middle cervical ganglion stimulation. *P value < 0.05 for % change from baseline (prestimulation). †P = 0.06 for % increase in dP/dtₘₐₓ with BMCGS compared with BSGS. ‡P = 0.02 for comparison of PreCSD vs. PostCSD. §P < 0.01 for comparison of BMCGS vs. BSGS.

Thoracic sympathetic innervation in infarcted hearts. The timeline for the experimental protocol in infarcted animals is shown in Fig. 6. In infarcted animals, changes in ARI were greater during MCG stimulation compared with stellate ganglion stimulation (MCG stimulation: 24.7 ± 3.5% vs. stellate stimulation: 12.0 ± 2.8%; *P = 0.02; Fig. 6) The increase in HR was also greater during bilateral MCG stimulation compared with bilateral stellate ganglion stimulation (Table 3),
while the increase in LVESP was greater with stellate ganglion stimulation. Finally, the rise in CS NE levels was similar during bilateral MCG and bilateral stellate ganglion stimulation (Table 2).

CSD and residual sympathetic innervation in infarcted hearts. In infarcted hearts, CSD led to a prolongation of global ARI (pre-CSD: 385 ± 17 ms vs. post-CSD: 392 ± 17 ms; P < 0.05; Fig. 7). CSD had no effect on HR or dP/dt\text{max}. However, a decrease in LVESP was observed after CSD in infarcted hearts (P < 0.02; Table 3).

In 66.7% of MI animals (12/18), VT/VF was inducible before CSD. After CSD, VT/VF was inducible in 6 of these 12 animals, reducing VT inducibility by 50% (P < 0.05; Fig. 7). Of note, the six animals that were not inducible for VT/VF before CSD remained noninducible after CSD. Residual cardiac sympathetic pathways after CSD. In infarcted animals, global ARI continued to decrease during MCG stimulation (Fig. 8). No significant differences in the effects of MCG stimulation on hemodynamic parameters were observed before vs. after CSD (Table 3).

Of note, CSD mitigated the increase in DOR during bilateral MCG stimulation in infarcted animals. Before CSD, bilateral MCG stimulation increased DOR by 356 ± 222 ms² (P < 0.02; Fig. 8). However, after CSD the increase in DOR with MCG stimulation was no longer significant (Fig. 8). Before CSD, MCG stimulation increased Tp-Te interval from 44 ± 5 ms to 58 ± 6 ms. Therefore, the percent increase in this interval after CSD during MCG stimulation was significantly reduced (P < 0.05; Fig. 8). Given that premature ventricular contractions that cause ventricular arrhythmias in infarcted hearts often originate from the myocardium, we qualitatively assessed activation patterns during apical pacing in five infarcted hearts (Fig. 9), which suggested improvement in epicardial conduction/activation and decrease in functional block after CSD, or during MCG stimulation after CSD, in two of the five infarcted hearts.

DISCUSSION

Major findings. In this study the MCG provided significant innervation to the ventricles, and these sympathetic pathways remained intact in normal and infarcted animals after CSD.
regions of late activation are seen at the border zone of the infarct, creating an after CSD in a different infarcted animal. During MCG stimulation, 2 localized areas of activation were no longer observed during MCG stimulation after CSD. After CSD, the entire region is more uniformly activated. Black dashed circle indicates region of the infarct on polar maps.

Furthermore, bilateral CSD reduced VT inducibility acutely in the setting of chronic MI, without compromising hemodynamic stability, a finding that can have implications for cardiomyopathy patients with recurrent ICD shocks. An important mechanism behind the antiarrhythmic benefit of CSD in infarcted hearts was the mitigation of the effects of sympathetic activation on DOR and on prolongation of Tp-Te interval, a marker of sudden cardiac death.

**Cardiac sympathetic innervation.** Contribution of the MCG to cardiac sympathetic innervation has previously been over-

![AT (ms)](image)

**Fig. 9.** Modulation of propagation and activation time by CSD. A: polar maps of activation time/sequence obtained during scar/apical pacing in this infarct animal demonstrated a localized region at the septal border zone of the infarct that was activated late as compared with the surrounding area. This area of functional block (localized regions of late activation, delineated as “II”) before CSD was no longer observed after CSD, and the entire area was activated more homogeneously. B: polar maps of activation time/sequence during scar pacing at baseline (BL) and during bilateral MCG stimulation (BMCGS) before and after CSD in a different infarcted animal. During MCG stimulation, 2 localized regions of late activation are seen at the border zone of the infarct, creating a potential isthmus or area of slow conduction that could serve as the substrate for a reentrant circuits. Both of these regions of late myocardial activation are no longer observed during MCG stimulation after CSD. After CSD, the entire region is more uniformly activated. Black dashed circle indicates region of the infarct on polar maps.

Although this study was performed in the swine model, significant similarities between canine and human autonomic nervous systems exist. As in canines and in humans, the sympathetic chain on each side consists of a superior cervical, a middle cervical, and a stellate ganglion, followed by a ganglion located at the level of each rib (20). As in the canine model, sympathetic stimulation via the stellate ganglion increases DOR (45, 50). Direct and indirect sympathetic activation in humans increases DOR, similar to pigs and dogs (44). Vagal nerve stimulation and decentralization of cardiac sympathetic innervation to the heart are known. Furthermore, hemodynamic compromise with bilateral CSD remained a concern (43). In this study, hemodynamic parameters were not compromised, while arrhythmia inducibility decreased after CSD.

Antiarrhythmic effects of CSD. Previous reports had shown that left CSD can reduce ischemia-driven ventricular arrhythmias (30, 39). In this study, the rationale for performing bilateral rather than just left CSD was driven by emerging evidence in patients with structural disease and refractory VT/VF. It has been noted that, unlike patients with channelopathies, case series of patients who undergo bilateral CSD for refractory ventricular arrhythmias in the setting of structural heart disease (i.e., MI) have greater VT-free survival compared with patients who undergo left-side-only procedures (43). Furthermore, right stellate ganglion stimulation is also proarrhythmic, increasing DOR (50) and Tp-Te interval, an independent marker of sudden cardiac death in patients (29, 33). Therefore, in this model bilateral CSD was performed both to assess hemodynamic stability after bilateral (rather than just left-sided) CSD and to assess VT inducibility acutely after bilateral CSD. However, acute effects of CSD on ventricular arrhythmias in the setting of a chronic remodeled infarct were unknown. Furthermore, hemodynamic compromise with bilateral CSD remained a concern (43). In this study, hemodynamic parameters were not compromised, while arrhythmia inducibility decreased after CSD.

Cardiac sympathetic innervation. Contribution of the MCG to cardiac sympathetic innervation has previously been over-

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As in canines, the right stellate ganglion provides predominant innervation to the anterior aspect of the heart and the left stellate ganglion provides innervation to the posterior/dorsal aspect of the heart (45, 52). As in canines, sympathetic afferent fibers exist throughout the cardiac chambers and sense mechano-chemical stimuli (22). Disruption of these afferent fibers reduces sympathetic outflow and has been shown to decrease the number of ectopic beats that occur with brief episodes of ischemia (36). Ventricular pacing leads to activation of cardiac mechanoreceptors and increased sympathetic tone, and alteration of processing through the intrinsic cardiac ganglia can be observed (18, 31). Afferent fiber activation subsequently increases sympathetic efferent outflow (37) and can explain the shorter ARIs that were observed with apical ventricular pacing compared with right atrial pacing before CSD, despite a fixed pacing cycle length. This effect was eliminated after CSD. Therefore, a portion of the antiarrhythmic benefit of CSD is likely due to interruption of afferent neurotransmission, which then alters subsequent efferent neurotransmission via the stellate and middle cervical ganglia. Sympathetic activation during VT inducibility testing (18, 35, 42), which by its nature involves ventricular pacing, is reduced by CSD in infarcted hearts, leading to fewer observed arrhythmias. In fact, it has been shown that sympathetic afferent responses to noxious stimuli are enhanced in heart failure dogs and increase renal sympathetic outflow more than in normal animals (48). Furthermore, chemical cardiac sympathetic deafferentation by epicardial application of resiniferatoxin decreases cardiac sympathetic nerve activity and attenuates cardiac remodeling in rats with heart failure (47). Therefore, reduction in afferent transduction leading to decreased efferent outflow could play an important role in the antiarrhythmic benefit of CSD. A framework for the current understanding of the effect of CSD on cardiac neurotransmission via afferent and efferent fibers is shown in Fig. 10.

Although CSD prolonged global ARI in infarcted animals, this effect was not observed in normal animals. This may be due to the heightened sympathetic tone and neural remodeling that occurs in infarcted hearts (2), which can be improved by interruption of efferent and afferent fibers through the stellate ganglia and removal of these cardiac neurons.

It has been demonstrated both in this infarct model (26) and in patients with ischemic and nonischemic cardiomyopathy that scar regions are rarely composed of homogeneous and dense fibrotic tissue (which would not give rise to any electrical signal) and contain many islands of live myocardium (24, 26, 32, 49). These regions of live myocardium or channels within “scar” give rise to fractionated bipolar electrograms, which have been shown to be good targets of catheter ablation in patients with VT (19, 32, 40). However, these same scar regions with intermingling myocytes have heterogeneous innervation (9, 44) that can lead to regions of delayed activation and conduction block, allowing for reentry and ventricular arrhythmias to occur (3, 34). In this study, we noted that in two animals regions of slow activation along the septal border zones of infarcts were improved after CSD, either at baseline or during MCG stimulation, suggesting that CSD allowed for more uniform cardiac activation, an antiarrhythmic effect. More homogeneous activation could be either due to earlier activation of “late” areas or, more likely, due to slower activation of the surrounding regions as a result of disruption of sympathetic efferent fibers.

MCG stimulation after CSD. A theoretical concern with performing bilateral CSD in patients with cardiomyopathy had been the possibility of eliminating sympathetic pathways that may be necessary for day-to-day activities and exercise. In this study, efferent innervation from the MCG remained intact after

Fig. 10. Efferent and afferent cardiac sympathetic pathways. Afferent fibers from the myocardium that traverse through the MCG pass through the stellate ganglia before reaching the spinal cord, and some of these pathways are interrupted by CSD. In addition, preganglionic efferent fibers that pass from the spinal cord through the stellate ganglia and to the MCG and any postganglionic fibers that arise from the stellate ganglia and innervate the myocardium are also interrupted. However, efferent postganglionic fibers from MCG neurons to the myocardium remain intact despite CSD. Aff, afferent neurons; Eff, efferent neurons; DRG, dorsal root ganglion; IML, intermediolateral nucleus; DH, dorsal horn of the spinal cord.
CSD, and CSD did not lead to any hemodynamic compromise in the porcine model.

Stellate ganglion stimulation has been reported to increase DOR and Tp-Te interval (45, 50). Tp-Te interval represents both transmural and whole heart DOR and increases the risk of sudden cardiac death (8, 27, 33). In this study, MCG stimulation also increased DOR and Tp-Te interval in infarcted hearts. Importantly, CSD mitigated the effects of MCG stimulation on DOR and Tp-Te interval in the setting of chronic MI, a novel finding of this study. The electrical stability that results from CSD in infarcted hearts during sympathetic activation and the lack of hemodynamic compromise is reassuring.

Limitations. Electrogroms were recorded only from ventricular epicardium. Therefore, intramural and endocardial effects were not assessed. This study was performed in a porcine model, and given interspecies differences direct interpolation to humans requires additional studies. In addition, isoflurane, which can suppress autonomic activity, was used for general anesthesia during the surgical portion of the protocol. However, anesthesia was switched to α-chloralose during the stimulation and CSD portions of the protocol. In addition, ARI and Tp-Te interval were not corrected for increases in HR. Therefore, increases in Tp-Te interval observed with stimulation are likely a conservative estimate of the actual effects. Given different stimulation currents to achieve the same hemodynamic threshold, direct comparison of parameters in MI vs. normal hearts was not performed. However, stimulation currents for right and left stellate ganglia vs. MCG were similar. Finally, direct comparison of ARI values at baseline or after CSD in infarcted compared with normal hearts cannot be made, as normal animals did not undergo a sham MI procedure, did not have VT inducibility testing, and had an additional bilateral stellate ganglion and MCG stimulation where the time courses of effects of bilateral stellate ganglion stimulation and MCG stimulation were compared. Finally, VT inducibility was not tested during MCG stimulation. However, VT inducibility, by the nature of its ventricular pacing, does cause sympathetic activation (18, 35, 42), suggesting that the beneficial effects of CSD are most influential during a state of elevated sympathetic tone.

Conclusions. Both MCG and stellate ganglia contribute significantly to cardiac sympathetic innervation. In addition, VT inducibility in animals with chronic MI is significantly reduced by bilateral CSD, even acutely. A mechanism for this benefit is the improvement in the increase in DOR and Tp-Te interval prolongation observed with sympathetic activation. Furthermore, efferent innervation via the MCG remains intact after bilateral CSD and hemodynamic parameters remain stable.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
T.I., K.Y., D.H., K.N., and M.V. performed experiments; T.I., K.Y., and M.V. analyzed data; T.I., K.Y., and M.V. interpreted results of experiments; T.I. and M.V. prepared figures; T.I. and M.V. drafted manuscript; K.Y., D.H., K.N., K.S., and M.V. approved final version of manuscript; D.H., K.S., and M.V. edited and revised manuscript; M.V. conceived and designed research.

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