Sympathetic Modulation of Electrical Activation In Normal and Infarcted Myocardium: Implications for Arrhythmogenesis

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ABSTRACT

Background
The influence of cardiac sympathetic innervation on electrical activation in normal and chronically infarcted ventricular myocardium is not understood.

Methods and Results
Yorkshire pigs with normal hearts (NL, n=12) or anterior myocardial infarction (MI, n=9) underwent high-resolution mapping of the anteroapical left ventricle, at baseline, and during left and right stellate ganglion stimulation (LSGS & RSGS, respectively). Conduction velocity (CV), activation times (AT), & directionality of propagation were measured. Myocardial fiber orientation was determined using diffusion tensor imaging and histology. Longitudinal CV (CV_L) was increased by RSGS (0.98±0.11m/s vs. 1.2±0.14m/s, p<0.001), but not transverse CV (CV_T).

This increase was abrogated by β-adrenergic receptor and gap junction (GJ) blockade. Neither CV_L nor CV_T was increased by LSGS. In the peri-infarct region, both RSGS and LSGS shortened ARIs in sinus rhythm (423±37ms vs. 322±30ms, p<0.001, and 423±36ms vs. 398±36ms, p=0.035, respectively), and altered activation patterns in all animals. CV, as estimated by mean ATs, increased in a directionally dependent manner by RSGS (14.6±1.2ms vs. 17.3±1.6ms, p=0.015), associated with GJ lateralization. RSGS and LSGS inhomogeneously modulated AT, and induced relative or absolute functional activation delay in parts of the mapped regions in 75% and 67% respectively, in MI animals, and in 0% and 15%, respectively in control animals (p<0.001 for both).
Conclusion:

Sympathoexcitation increases CV in normal myocardium, and modulates activation propagation in peri-infarcted ventricular myocardium. These data demonstrate functional control of arrhythmogenic peri-infarct substrates by sympathetic nerves, and in part explain the temporal nature of arrhythmogenesis.

Key Words: Autonomic nervous system, sympathetic nerves, conduction velocity, electrical propagation, ventricular arrhythmias.

NEW AND NOTEWORTHY

This study demonstrates regional control of conduction velocity in normal hearts by sympathetic nerves. In infarcted hearts however, not only is modulation of propagation heterogeneous, some regions showed paradoxical conduction slowing. Sympathoexcitation altered propagation in all infarcted hearts studied, and we describe the temporal arrhythmogenic potential of these findings.
INTRODUCTION

The autonomic nervous system (ANS) influences cardiovascular physiology, and is strongly linked to ventricular arrhythmias (VAs), and sudden cardiac death (SCD)(62). Mechanistically, sympathetic innervation increases repolarization lability and heterogeneity(42, 57), and is associated with arrhythmogenesis(56). Following cardiac injury, there is also extensive structural and functional neural remodeling throughout the cardiac neural hierarchy (1, 18, 41), and is associated with increased sympathetic tone. In the heart, nerve sprouts have been identified in peri-infarct zones and associated with VAs, via poorly understood mechanisms(9).

Sympathetic innervation, while it directly affects APD in ventricular tissue(59), has not been shown to directly modulate muscle-to-muscle conduction velocity and propagation patterns in normal hearts. A prior study found no significant influence of sympathoexcitation on conduction velocity or propagation patterns(59). However, the preparation used was limited by a mismatch between the ganglion stimulated and region mapped. Electrical activity was recorded in the region innervated by right sympathetic nerves while the left nerves were stimulated.

We sought to examine whether sympathoexcitation significantly increases CV in normal hearts, and in peri-infarct zones of infarcted hearts. We assessed this by right and left stellate ganglion stimulation (RSGS and LSGS, respectively), with high resolution mapping of the anterior wall of the left ventricle.
in a porcine model with an intact ANS, under anesthetic conditions known to preserve autonomic neural activity and reflexes.
METHODS

Animal Subjects

Experimental procedures involving animal subjects were performed in accordance with guidelines set by both the University of California Institutional Animal Care and Use Committee (IACUC), and The National Institutes of Health Guide for the Care and Use of Laboratory Animals. Yorkshire pigs of either gender (50-70kg) were used for terminal experiments. Twelve control animals, and 9 infarcted animals (6-8 weeks post-infarct) were studied. The anesthetic regimen for terminal experimentation was sedation with Telazol (8-10mg/kg, intramuscular) and Dexmedetomidine (0.05-0.2mg/kg), intubation, and sternotomy was performed on isoflurane (1%-2%), and fentanyl 100mcg, intravenous bolus given once at the beginning of the preparation, and intermittently during surgery). Following completion of sternotomy, isoflurane was slowly transitioned to alpha-chloralose solution (6.25mg/125ml; bolus 1ml/kg; maintenance 25-35ml/kg titrated to effect). Temperature was maintained at 36-38°C, with the use of a heated surgical table, and a heated blanket. The sternotomy was also kept covered to minimize cardiac cooling and insensible losses. After completion of the experimental protocol, animals were euthanized by sodium pentobarbital (100mg/kg). Paralytic agents were not used.

Infarct Induction

Antero-apical infarcts were induced percutaneously. Animal subjects (35±4.5kg, n=9) were sedated with Telazol (8-10mg/kg, intramuscular) and Fentanyl (50-
100mcg, intravenous). To accommodate the weight gain during the post-infarct period (6-8 weeks), smaller animals were infarcted. As such, the weights of control and infarcted animals were uniform at terminal study. Following intubation, general endotracheal anesthesia and analgesia were maintained with inhaled isoflurane (1%-2%), and by boluses of fentanyl (50-100 mcg IV), respectively. With an 8-French Amplatz left (AL1) coronary guide catheter, a balloon tipped coronary angioplasty catheter (FoxCross™, Abbott Vascular (Temecula, CA, USA) was advanced over a guide-wire to the mid-LAD. The balloon was inflated to sub-occlusive pressures, and a 5-7.5mL suspension consisting 80% by volume polystyrene microspheres (Polybead® 90µm, Polysciences Inc., Warrington, PA, USA) and radiopaque contrast was slowly injected over 1-2 minutes through the angioplasty catheter. Transmural myocardial ischemia was confirmed by immediate ST-segment elevation in ECG leads I, II, and V1.

**Sympathetic Stimulation**

LSGS and RSGS were performed with repeated square-wave pulses at a frequency of 4Hz, 4ms duration for 30 seconds. Stimulation amplitude was set at the amplitude at which blood pressure and/or heart rate increased by 10% (generally 1-8milliamps (mA)). Cardiac sympathetic activation was also confirmed by surface T-wave changes, increases in heart rate, and blood pressure as previously described(2). Bipolar electrodes were placed around each stellate ganglion (superior and inferior) for stimulation, with the cathode being
superior. Over the course of the entire experimental protocol, hemodynamic
responses to LSGS and RSGS were maintained, and the preparations remained
intact.

Cardiac Electrical Mapping
A 64-electrode array (8x8 configuration, 2mm inter-electrode spacing) was
placed on the antero-apical epicardium of the left ventricle in normal animals, and
in the same region in the peri-infarct zone of infarcted animals. From these
arrays, epicardial electrograms (EGMs) were recorded at baseline and during
SGS. Following the surgical preparation, animal subjects were allowed to
stabilize for 40 minutes before experiments were commenced. Electrograms
were recorded with a customized multiplexer (University of Utah, Salt Lake City,
UT)(19, 32). Using a previously described customized program(2) to generate
the first derivative of the QRS, and second derivative of the T wave, activation
time (AT) was measured as the time interval between the onset of ventricular
activation and the minimum dV/dt of the QRS; and repolarization time (RT) as the
maximum dV/dt of the T wave. The activation recovery interval (ARI), a surrogate
for APD(27, 32), was taken as the difference between RT and AT (ARI = RT -
AT), as previously described.
Electrical maps depicting AT and ARI measured from all electrodes were
projected onto a 2-D geometry using publicly available software Map3D
(Scientific Computing and Imaging Institute, University of Utah).
Conduction Velocity

Bipolar pacing (2 mm bipole spacing resulting in point stimulation) was performed superficially on the epicardium adjacent to the plaque to avoid breakthrough conduction to the surface. Due to the tachycardia seen during RSGS, pacing was performed at 10% above the maximal heart rate seen during RSGS, ≈ 120 beats/minute. CV measured as the distance traversed across the multi-electrode plaque (in mm), divided by the difference in local activation times between the earliest activation (pacing site), and the earliest activation time at the opposite end of the plaque (in ms) i.e. CV = Distance traversed/[AT_end − AT_pacing site]; (Figure 2D). Data obtained in mm/ms were expressed as m/s for comparison. CV was averaged from 5 beats at baseline and at 30s of SGS, however, propagation maps show a single median beat (beat 3).

Diffusion Tensor Magnetic Resonance Imaging

Fiducial markers were placed on the hearts to indicate the exact location of the plaque as it was removed. Rapidly excised hearts were first rinsed with saline, and all cavities filled with a silicone rubber resin (Templet Polyvinylsiloxane, Microsonic Inc., Ambridge, PA) to maintain a near end-diastolic geometry during imaging. The hearts were then placed in a plastic cylindrical container filled with a susceptibility-matched fluid (Fomblin, Solvay Solexis, West Deptford, NJ) and held in place using open-cell foam. 3T (Siemens Prisma, Erlangen, Germany) MRI was then performed with T1 weighted imaging using a 3D Fast Low Angle
Shot (FLASH) sequence (0.5x0.5x0.5mm spatial resolution, scan time: 1.5hr).

The images were visualized using a 3D volume rendering in Osirix (Pixmeo, Geneva, Switzerland).

Diffusion Tensor MRI (DTI) were then acquired using a readout segmented diffusion weighted spin echo sequence (20) with b-value=1000s/mm$^2$ along 30 directions and 1.0x1.0x1.0mm spatial resolution with 5-10 signal averages to improve SNR (scan time: 8-10hrs). The DTI acquisition was oriented to the epicardial tangent plane in the region immediately below the plaque electrode using the implanted fiducial markers. Ten slices were acquired parallel to the prescribed epicardial plane and analysis was limited to only the most epicardial layer, hence the DT-MRI maps represent tissue that is within 1mm of the epicardial surface. Diffusion tensors were reconstructed from the diffusion weighted images using linear regression and custom Matlab (The Mathworks, Natick, MA) software. Care was taken to identify the fiducial markers at all planes examined. Fiber orientations were determined by calculating the primary eigenvector of the diffusion tensor in each voxel, and displayed with a color map. The colors correspond to the orientation of the primary eigenvector ("myofiber" long-axis) of the diffusion tensor. The x, y, and z components of the vectors are mapped to red, green and blue, respectively (i.e horizontal fibers are predominantly red while vertical vectors are green).

All data points in the region immediately below the plaque electrode are displayed. It is well known that myocardial scar leads to an increase in apparent
diffusivity compared with healthy tissue (37, 40) as well as a loss of directionality (i.e. anisotropy). In a map of fiber orientation, this will manifest as seemingly random orientations.

At each voxel, the cosine similarity coefficient – a measure of similarity between adjacent fiber orientations (i.e. voxels) – was calculated as the difference between each voxel and the average measure of the nine neighboring voxels. Fiber orientations and maps of fiber organization were plotted in the epicardial tangent plane in the region of the plaque electrode as determined by implanted fiducial markers.

**Histology and Immunohistochemistry.**

The region under the multielectrode plaque was excised with very tight margins, and placed immediately in 10% Phosphate buffered formalin for 5-7 days, and transferred to 70% ethanol. The epicardial and subepicardial layers were cut parallel to the surface for paraffin embedding. Samples were processed for embedding in cassettes with pieces of foam above and below the specimen to keep the tissue completely flat. Once embedded, the epicardial tissue layer was carefully sectioned away (approximately 50-75µm) until myocardium was reached. Four-micron thin sections of the myocardium were collected for hematoxyline and eosin (H&E), and trichrome elastic von Giesen (Tri-EVG) staining to examine the scar border-zone region mapped. Sympathetic nerves were identified by immunohistochemistry (IHC) antibodies against tyrosine
hydroxylase (Cat# ab112, 1:200 dilution; Abcam, Cambridge, MA, USA), and
detected by diaminobenzidine reaction (DAB, Life Technologies, Green Island,
NY, USA). Connexin density was examined by antibodies against Cx-43 (Cat#
C6219, 1:2000 dilution St Louis, MO, USA), and also detected by DAB reaction.
Histologic and IHC slides were digitally scanned, and electronically stored for
analysis (Scan Scope, Aperio, Vista, CA, USA). Immunohistochemical
quantifications were performed by computerized morphometry (TissueStudio,
Definiens Inc., Parsippany, NJ, USA).

**Statistical Analyses**

Data are reported as means ± standard deviation (SD) unless stated otherwise.
Multi-group comparisons were performed using a two-way analysis of variance
(ANOVA). If the $p$ value using the ANOVA was < 0.05, post hoc comparisons
were made using the Tukey-Kramer minimum significant difference (MSD) test.
Paired comparisons were performed with the Wilcoxon signed rank test if data
were not normally distributed, and with the Student T test for normally distributed
data. Comparisons of control and infarcted animal subjects were performed with
the Mann-Whitney test. Repeated tests of independence were performed with the
Cochran-Mantel-Haenszel test. For all comparison, a $p$ value < 0.05 was
considered statistically significant. Data were analyzed with JMP Pro 11 (SAS,
Raleigh, NC) or Systat 13 (Systat, San Jose, CA).
RESULTS

Sympathoexcitation increases conduction velocity in normal ventricular myocardium

The experimental preparation, and representative examples of electrograms recorded from the antero-apical LV in sinus rhythm, and during RSGS and LSGS are shown in Figure 1A. Hemodynamic responses to RSGS and LSGS in this preparation have been previously reported, and were not different between control and infarcted animals(2). Compared to baseline values, RSGS shortened ARIs on the anteroapical LV 11-fold greater than LSGS (16.3±6.5% vs. 1.5±2.9%, p<0.001) in sinus rhythm (mean heart rates at baseline and during RSGS and LSGS were 63.3±7bpm, 81.6±9.4bpm, and 66±8.1bpm, respectively, p< 0.001 for BL vs. RSGS and RSGS vs. LSGS, p>0.2 for BL vs. LSGS), and greater than 3-fold during ventricular pacing (12.4±4.8% vs. 4.3±2.3%, P<0.01)(Figure 1B-C). These data indicate that RSGS provides greater functional innervation of the epicardium mapped than LSGS.

Detailed sub-epicardial fiber orientation for each animal was determined by DT-MRI and histology, and the orientation of GJs, determined by connexin-43 (Cx-43) staining was confirmed. On the anterior subepicardium, myocardial fiber orientation was predominantly oriented horizontally (Figure 2A-B), and GJs were principally oriented longitudinally (Figure 2B). As expected, CV_T was slower than CV_L, and this relationship remained unchanged by RSGS and LSGS (Figure 2C-D). CV_L was increased by RSGS (0.98±0.11m/s vs. 1.2±0.14m/s, p<0.001), but
not LSGS (0.99±0.1 m/s vs. 0.95±0.13 m/s, p=0.11) (Figure 2D,3A). The time
course of the RSGS effect is shown in figure 2E. Neither RSGS nor LSGS had
an effect on CVT (0.75±0.06 m/s vs. 0.74±0.06 m/s, p=0.86, and 0.74±0.05 m/s
vs. 0.79±0.04 m/s, p=0.17 respectively for RSGS and LSGS)(Figure 2C,3B).
Anisotropy ratio (AR, CV_L/CV_T) at baseline, and during RSGS and LSGS were
1.4±0.2, 1.7±0.1, and 1.1±0.1, respectively (ANOVA p=0.05, p=0.035 for RSGS
vs LSGS).
Mean AT, another indicator of the rapidity of propagation in the mapped
epicardium, was similarly shorter in the longitudinal direction of propagation,
relative to transverse (11.5±0.7ms vs. 14.9±1.0ms, p<0.001) (Figure 3C), a
relationship unchanged by either SGS (10.4±1.0ms vs. 14.6±0.8ms, p<0.001 for
RSGS, and 11.3±0.7ms vs. 14.3±1.1ms, p=0.015 for LSGS). However, RSGS
shortened mean AT (p=0.035) in the longitudinal direction compared to baseline,
while LSGS did not (p=0.2). Spatial activation heterogeneity (AT variance per
cm²), a marker of dissimilarity in activation times over the area mapped, was
similarly lower with longitudinal than transverse propagation (12.4±1.3ms²/cm²
vs. 22.1±1.5ms²/cm², p=0.003). In these normal hearts, neither RSGS nor LSGS
significantly changed spatial activation heterogeneity (Figure 3D).
To ascertain whether increased CV was mediated by β-adrenergic signaling,
intravenous esmolol (2.0-2.2mg/kg) was slowly administered until > 10% drop in
heart rate and/or blood pressure were recorded. As shown in Figure 3E, β-
blockade eliminated the increase in CV_L seen with RSGS. Given the dependence
of RSGS-mediated CV increase on fiber orientation (and hence GJ distribution),
we examined whether the increase in CV_L was mediated via GJ modulation.

Cellular uncoupling was achieved with Carbenoxolone (1.4-1.6mg/kg), a GJ blocker(14, 26), resulting in complete abrogation of the CV_L response to RSGS 24.7±8.9%, -2.6±3.7%, and -2.8±3.9% (p<0.001) respectively for control, esmolol and carbenoxolone, respectively (Figure 3E-F). Mean CV_L and CV_T following carbenoxolone administration were 0.9±0.1m/s and 0.62±0.1m/s.

The impact of RSGS on excitation properties of the myocardium during pacing, as estimated by the magnitude of the maximal negative slope of the local activation complex (-dV/dt_max) was obtained from local unipolar electrograms(21).

As shown in figure 3G, -dV/dt_max values were not different between baseline and RSGS during pacing (-313.3±80.5mV/ms vs. -337±80.2mV/ms, p=0.2).

Influence of SGS on sinus rhythm activation and APD in peri-infarct regions

To examine how SGS modulates activation propagation around infarcts,
detailed structural characterization of the peri-infarct zone was performed. As previously reported, the peri-infarct zones are heterogeneous and patchy, with islands of surviving myocardium distributed within dense scar regions(10, 15, 44, 48, 59, 60) (Figure 4A-B). These peri-infarct zones demonstrate large and small nerve bundles and sprouts positive for tyrosine hydroxylase (TH) (Figure 4C-D).

Interestingly, compared to normal pigs in which myocardial fiber orientation on the epicardium is generally arranged horizontally in the anteroapical LV, myofiber orientation in the infarcted heart maintained similar orientation as it approached
the edge of the dense infarct (Figure 4C-D). At the scar edges, however, there was considerable anisotropy of peri-infarct fiber orientation (Figure 4E-F).

In the peri-infarct region, there was no significant difference in baseline sinus rhythm ARI between control and MI animals (397±65ms vs. 422±94ms, p=0.7).

Both RSGS and LSGS significantly shortened ARI in peri-infarct regions (423±37ms vs. 322±30ms, p<0.001, and 423±36ms vs. 398±36ms, p=0.035, respectively) (Figure 5A, C). Unlike control animals where LSGS did not result in ARI shortening in the anteroapical region, LSGS had a significant impact in infarcted animals, although this effect was modest compared to RSGS. Mean heart rates at baseline and during RSGS and LSGS were 68.7±5.6bpm, 98±8bpm, and 73.2±6.1bpm, respectively, p<0.001 for BL vs RSGS, p=0.035 for RSGS vs LSGS, and p=0.2 for BL vs. LSGS).

Mean AT of the peri-infarct region was not significantly different in sinus rhythm between control and MI animals (19.6±1.3ms vs. 21.7±1.9ms, p=0.2); however, MI animals demonstrated greater spatial activation heterogeneity compared to the same region in control animals (12.4±4ms²/cm² vs. 4.1±0.6ms²/cm², p=0.044) in sinus rhythm. RSGS significantly shortened mean AT in the peri-infarct zone (17.6±1.6ms vs. 21.0±1.8ms, p=0.035) in sinus rhythm, while LSGS did not result in significant mean AT shortening (19.7± 1.7ms vs. 21.8± 2.4ms, p=0.2)(Figure 5B,D). An example of a subject in which LSGS shortened mean AT is depicted in Figure 5B. It can also be easily noted that SGS non-uniformly modulated AT and ARI (APD) in the peri-infarct zone.
SGS and inhomogeneous activation propagation in peri-infarct regions

In the peri-infarct zone, the path taken by activation wavefront may not always be captured by the array, therefore, changes in AT in the mapped area were used to approximate the rapidity of propagation. Further, due to the loss of tissue architecture, and the presence of scar in the mapped area, longitudinal and transverse fiber orientations do not accurately describe propagation patterns. Therefore, to determine the impact of SGS on CV, we examined two pacing wavefronts in the peri-infarct zone as depicted in Figure 6A. Wavefronts I and II are directed along, and towards the dense scar border in MI.

Compared to baseline, RSGS but not LSGS significantly decreased mean AT in the peri-infarct zone (17.3±1.6ms vs. 14.6±1.2ms, p=0.015; and 15.5±1.0ms, p=0.075 respectively). Interestingly, this decrease was seen only for wavefront I but not II (Figure 6B-C). More importantly, regardless of the effect on mean AT, RSGS and LSGS inhomogeneously altered AT, frequently exposing relative (i.e. compared to adjacent regions after SGS) or absolute (compared to the same site at baseline) delay in activation in the peri-infarct zone (Figure 6B, asterisk). Further, in all animals studied, the pattern of electrical activation was changed by RSGS and/or LSGS. In control animals, spatially homogeneous changes in activation were induced by SGS. In the peri-infarct zone, ATs were unchanged, shortened, or paradoxically increased during SGS (Figures 6B&D). As shown in figure 6E, these spatial differences in activation were seen in 75% and 67% of RSGS and LSGS maps respectively in MI animals, and in 0% and 15% respectively in control animals (p<0.001 for both RSGS and LSGS).
To examine whether gap junction distribution was related to the direction influence of RSGS on propagation, we examined the distribution of Cx-43 immunoreactivity in peri-infarct zones mapped. As shown in figures 6F-H, compared to the longitudinal distribution of Cx-43 in control hearts, there was greater than three-fold (p<0.001) increase in GJ lateralization in the peri-infarct zone, along with decreased density (2.8-fold, p=0.03). Interestingly, this lateralized location was consistent with the direction of propagation of wavefront I, suggesting that these lateralized GJs were influenced by RSGS. Spatial heterogeneity of activation (figure 6I) for wavefronts I and II at baseline were 20.7±5.1ms²/cm² and 13.1±2ms²/cm² (p=0.075). During RSGS, these values were 14±4.1ms²/cm² and 15.7±4ms²/cm², respectively, and during LSGS, 15.1±4.4ms²/cm² and 19.1±7ms²/cm² respectively (p>0.2 when compared to baseline and between SGS types). In sinus rhythm, activation heterogeneity at baseline, and during RSGS and LSGS was 11.7±3.7ms²/cm², 10.4±4.5ms²/cm², and 10.7±3.1ms²/cm².

**SGS can alter propagation in putative ventricular tachycardia circuits.**

In diseased myocardium, late potentials and local abnormal ventricular activations are seen after or within the QRS respectively, and reflect regions with delayed or abnormal electrical propagation. Often, ventricular arrhythmias are induced by unidirectional block in such regions, and these sites become critical isthmuses for monomorphic ventricular tachycardia (MMVT). An example of a
late potential and the impact of SGS on propagation is shown in Figure 7A. Two adjacent electrodes (E1 and E9) mapping electrical activity in the upper left corner of the array are displayed. During SGS, conduction through this region exhibited alternating functional block (2:1 propagation). Mean AT in the region mapped was longer when the late potential was present than when absent (9.3±2.7ms vs. 8.6±2.8ms, p<0.001), indicating conduction block into that region, or more rapid propagation such that the LP is no longer present(Figure 7B-C, respectively). Local ARIs recorded in the focal region (0.12cm²) demonstrating alternating conduction was not significantly different between beats with and without the late potential (291.7±1.3ms vs. 293.7±3.0ms, p=0.2).
The major findings of the present study are: 1) CV\(_L\) is increased directly by excitation of sympathetic fibers innervating the ventricles, mediated partly by GJ conductance; 2) in peri-infarct zones, characterized by extensive connexin lateralization, rapidity of activation propagation is increased by sympathoexcitation parallel to the scar border in the apico-basal axis; 3) sympathoexcitation induces inhomogeneous changes on activation, and even paradoxically prolongs activation propagation at some peri-infarct sites; and 4) peri-infarct propagation in putative VT circuits can be altered by SGS. This represents the first demonstration of these electrophysiologic phenomena by direct cardiac sympathetic innervation, in an experimental preparation with intact cardiac neuraxial control, mapped in vivo, under an anesthetic regimen that does not suppress autonomic function.

The CVs obtained in normal animals in our study are in line with prior examinations of CV in various preparations(6). Although the CVs we report are at the higher end of the spectrum, they are within accepted values(10, 15, 44, 48, 60). The anisotropy ratios (AR) in this study (≈1.4 are also lower than prior studies (range 2-10)(10, 15, 44). The somewhat higher CV values and lower ARs are likely due to the differences in experimental preparations, which include myocardial strips in a bath(10), low temperature(36, 59), and a decentralized preparation(50). Further, compared to the intact beating heart in vivo, factors critical to conduction velocity such as cell geometry and size, connexin turn over and gap junction distribution, and cellular excitability are not completely
maintained in myocardial strips or langendorf-perfused hearts(46). Although pacing from the center of the array may have yielded different CV and AR values, other studies with different pacing wavefronts similar to this study had higher AR values(10, 15, 44). Differences may also be related to species, as differences in CVs have been reported across species(15). While Purkinje fibers in the pig are known to exist transmurally in the porcine ventricle(4) unlike some other species(50), it is unlikely that Purkinje conduction contributed to these values, due to its orientation in the ventricular wall(15), and CV values that are well below the 2-3m/s range recorded for Purkinje conduction(11).

**Modulation of propagation in normal hearts by sympathoexcitation**

The characterization of cardiac electrical responses to sympathoexcitation have largely been limited to repolarization dynamics(38). Effects of sympathetic excitation include APD shortening via modulation of the slow inward rectifying potassium channel (IKs), increased repolarization heterogeneity, increased intracellular calcium, and increased after-depolarizations(5), among other effects. The sodium channel, NaV1.4 (encoded by SCN5A), is known to be upregulated and modulated by ß-adrenergic stimulation(13, 31). However, this is on a longer time scale, and unlikely to have played a role on the short-term effects of SGS studied here. On short time scales, ß-adrenergic stimulation was shown in single cell experiments to increase the sodium current by recruiting sodium channels to the membrane(29). Gap junctions, which can be regulated via a variety of mechanisms represent a dynamic target of ß-adrenergic receptor activation(7).
This can occur via accumulation of GJs at the intercalated discs, increased GJ expression, and increased permeability or conductance(17), although controversy exists over this latter mechanism(43), occurring via cyclic AMP (cAMP)-protein kinase A (PKA)-mediated phosphorylation of connexin 43. There is substantial evidence from in vitro preparations that GJ function can be modulated by BAR activation, on short and long times scales(7). Whether the velocity of muscle-to-muscle propagation, directly under the influence of cardiac sympathetic nerves is increased, and the mechanisms underlying such increases remained unknown.

Wallace and Sarnoff(59) examined the effects of sympathetic nerve stimulation on ventricular conduction. In their studies, SNS was accomplished by LSGS, and the anterior aspect of the LV was mapped. The authors observed that sympathoexcitation by LSGS had no significant impact on myocardial CV, and in fact, some sites showed an increase in AT. These experiments were however limited by discordance of the ganglion stimulated and the region mapped, the decentralization of the LSG, and in some cases complete removal of the RSG. Using electrophysiologic indices to examine the functional innervation patterns of left and right sympathetic nerves on the heart, our group and others have demonstrated that the anterior wall of the heart is innervated predominantly by the RSG, while the posterior wall is innervated by the LSG(2, 53, 63). Cardiac sympathetic denervation is frequently performed for intractable arrhythmias, however, left cardiac sympathectomy is still widely performed. One implication of
the present study is that unilateral (left-sided) sympathectomy may be inadequate for arrhythmia control if the VT originates from the anterior wall. This may account for certain failures of left sympathectomy for arrhythmia control, and emphasizes the role of bilateral sympathectomy over unilateral (55).

In the present study, the anterior LV was mapped during RSGS, in a non-decentralized preparation (intact nerve connections), under an anesthetic regimen in which autonomic nerve activity and reflexes are maintained. We confirmed greater functional innervation of the mapped region by RSGS than LSGS, and demonstrated increased CV\textsubscript{L} but not CV\textsubscript{T} during sympathoexcitation. Using DT-MRI and histology, our findings on subepicardial myofiber orientation patterns are consistent with Streeter et al. (52), who similarly examined this in porcine. We further demonstrated that the differential effects of RSGS on CV\textsubscript{L} vs. CV\textsubscript{T} was mediated by the distribution of GJs, known to exist predominantly at end-to-end intercalated disks than side-to-side in normal hearts, by inducing cellular uncoupling (14, 26). This eliminated the increase in CV\textsubscript{L} during RSGS. A number of factors may account for the increase in CV\textsubscript{L} but not CV\textsubscript{T}. The relationship between CV and GJ conductance is not linear, and differs for CV\textsubscript{L} and CV\textsubscript{T}. For a specific GJ conductance, intercellular resistivity, a major determinant of CV is greater in the transverse than longitudinal direction, related to size of GJ plaques and connexin density (22). Equal modulation of GJ conductance may therefore yield different degrees of change in CV.

Further, while the effects of ß-adrenergic activation are myriad, how the relationships between GJ conductance, intercellular resistivity, and CV change
following adrenergic stimulation is not well understood, but may differ for \( CV_T \) and \( CV_L \). Another important factor is the differential impact of steady state pacing frequencies on \( CV_L \) and \( CV_T \). Spach et al, examined the CV at increasing steady state pacing frequencies, and found that \( CV_T \) decreased to a greater extent than \( CV_L \)(49). It is entirely possible that the pacing cycle length utilized here (500ms) may have affected the results seen with RSGS on \( CV_L \) and \( CV_T \). Since propagation is related to both cytoplasmic conduction (which is fast and exhibits low resistivity), and gap junctional conduction (which is relatively slow and exhibits high resistivity), one may expect that the elongated cell geometry would predict how many GJs per unit space the propagating wavefront needs to pass through. Such that propagation along the longitudinal axis of the cell travels through fewer junctions per unit space than in transverse, and as a result is subject to fewer barriers to conduction(54).

In a Langendorff rabbit heart preparation(36), Ng et al. indirectly assessed conduction times as the delay between an extra-stimulus impulse (S2), and the beginning of monophasic action potential (MAP) signal. In the rabbit preparation, SNS achieved by field stimulation of the outflow aspects of the spinal cord, shortened S2-MAP delay by 17%. The direct path taken by the impulse was not measured; hence direct assessment of CV could not be performed, and as such the shortened delay could not be directly ascribed to increased CV. In the present study, the SG were stimulated directly. As such low currents were used with reduced potential of activating other nearby ganglia or nerves with field stimulation.(36) Further, the increase in CV observed in our study (20%) is in
line with that seen by Ng et al (17%). The present study represents the first direct
demonstration of increased CV by excitation of cardiac sympathetic nerve fibers
in an intact neural preparation.

Conduction in Peri-Infarct Zones: Impact of Sympathoexcitation

The peri-infarct zone between dense scar and surviving myocardium is complex
structurally and electrophysiologically, with islands or strands of surviving
myocardium surrounded by or interdigitated with scar(33-35). This region is
known to harbor structural circuits that maintain monomorphic VT, myocardium
exhibiting decreased source-sink mismatch(61), with increased potential for
ectopic beats, and increased density of adrenergic nerve endings(9, 58).

We demonstrated that subepicardial fiber orientation remained arranged
parallel (“east-west”) to the epicardial surface, although there was increased
anisotropy just at the edge of the scar. Consistent with previous descriptions(30,
47), we identified decreased Cx-43 density, and extensive lateralization in the
peri-infarct zones mapped. CV in this region (as measured by changes in AT)
was increased by RSGS in a wavefront-dependent manner. Wavefronts directed
superiorly along the scar border demonstrated a significant decrease in AT. Both
RSGS and LSGS shortened ARIs in this region (although the effect was smaller
with LSGS). These finding highlight two important points, 1) despite scarring,
myocyte loss, and ion channel remodeling, there remains significant functional
regulation of electrical activation in the peri-infarct zone by cardiac sympathetic
nerves, and 2) there is remodeling of the laterality of sympathetic innervation in
the antero-apical region following MI, with increased functional control by LSGS compared to the control state where LSG has little functional effects. This may suggest that growth-associated protein 43 (GAP43) positive nerve sprouts may arise more from the LSG than RSG in this infarct model(9, 58). It may also reflect structural and functional changes in the LSG or within the intrinsic cardiac network, or downregulation of parasympathetic restraining influences(1, 18). Paradoxical increases in AT in the peri-infarct zone may be related to aberrant innervation patterns with preserved parasympathetic but not sympathetic nerve endings, and transdifferentiated neurons to cholinergic phenotype form adrenergic(25). At the level of the myocyte, alterations in ionic properties (e.g. depressed intracellular K+ and peak calcium transients(39)) and adrenergic signaling pathways (e.g. adrenergic receptor downregulation(28) and decreased G-protein receptor kinase-2 (GRK2) in cardiomyopathy(64)) in surviving peri-infarct myocytes induced by hyperinnervation or local tissue factors, may also play a role(16). These findings may underlie the proarrhythmic risk described in association with peri-infarct zone nerve sprouts(9).

**Modulation of Arrhythmia Circuits by Peri-Infarct Innervation**

Characteristics of peri-infarct tissue harboring potential circuits within which reentry could occur have been extensively studied, and form the basis for catheter ablation studies(23, 24, 51). Although these circuits may exist in many scars, most patients are not incessantly in monomorphic ventricular tachycardia (MMVT). Appropriately timed premature stimuli may modulate the electrical
properties of these circuits to initiate MMVT, however, this process is stochastic.

As such, functional electrical control of these circuits is critical. In the present study, we present the first direct example of sympathetic nerve modulation of such a circuit in an epicardial peri-infarct zone (Figure 7A-C). In this case, a putative circuit exhibiting a late potential on every depolarization developed functional block during RSGS, and permitted conduction on alternating ventricular depolarizations (2:1 conduction pattern). This form of block is prerequisite to make the circuit available for a premature ectopic stimulus propagating slowly within the circuit, and if the conditions are correct, to exit the circuit and capture ventricular myocardium. The well-described decrease in source-sink mismatch in peri-infarct myocardium increases the likelihood that such stimuli result in ventricular capture(39).

Functional modulation of such circuits likely occurs on a dynamic basis in vivo, depending on the sympathetic state of the subject. Parasympathetic neurotransmission, known to be present at all levels of sympathetic tone, likely plays a significant role in neurotransmission(45). During exercise, stress, general anesthesia, and sleep for example, neurotransmission (both sympathetic and parasympathetic) likely modulate the electrophysiologic properties of peri-infarct circuits capable of harboring VT. Therapeutic modulation may be achieved by sympathectomy, vagal stimulation, tragal stimulation, and/or spinal cord stimulation(3, 45). These interventions warrant study on how peri-infarct circuits are modulated.
Limitations

In this study, we mapped an equivalent region of the antero-apical epicardium in normal and infarcted hearts. Whether other regions of the peri-infarct zone (for example right ventricular apex or posterior LV) exhibit similar findings is unclear. Further, remodeling of sympathetic nerves and neurons has been described, the role of other neurotransmitters such as neuropeptide Y were not explored. In addition, whether sympathoexcitation-mediated changes in the peri-infarct zone are prevented by beta-blockade was not studied. While there is agreement on the use of local electrograms to estimate local activation(8), the use of the activation recovery interval to estimate local action potential duration has limitations. The orientation of the T wave (positive vs. negative) may result in error in estimation of the local APD(12). The model used in this study, with its intact neural connections, and preservation of neural reflexes by the anesthetic regimen has improved the sensitivity of detecting electrical perturbations by sympathoexcitation. Although no arrhythmias were induced for this work, the arrhythmogenic implications of these findings are significant.

Conclusions

Taken together, the data presented herein for the first time, demonstrate the impact of excitation of sympathetic fibers innervating the heart on electrical propagation in normal and infarcted hearts. These data highlight the important role adrenergic activation plays in altering propagation patterns (Figure 8), with
the critical potential of initiating and maintaining ventricular arrhythmias. The data also emphasize the dynamic nature of the peri-infarct substrate, and its functional modulation by sympathetic innervation, emphasizing the critical role the autonomic nervous system plays in arrhythmogenesis.
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REFERENCES:


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Figure 1. Influence of left and right sympathetic ganglia on activation recovery intervals in the anterior left ventricle.

A. The experimental preparation is shown, with the location of the left ventricle mapped. The high-density mapping array is shown in the inset. Representative examples of electrograms recorded at baseline and during left and right stellate ganglion stimulation (LSGS and RSGS, respectively) are also shown. B. Representative electrical maps showing of the impact of RSGS and LSGS on activation recovery intervals (ARIs), a surrogate for local action potential duration (APD) are shown. C. Quantifications of the differential effects of LSGS and RSGS on ARIs in sinus rhythm, and during ventricular pacing are shown (n=8, **p<0.01, ***p<0.001, Wilcoxon signed rank test).

Figure 2. Sympathoexcitation increases conduction velocity in normal hearts: influence of fiber orientation and gap junction distribution.

A. Representative diffusion tensor magnetic resonance (DT-MRI) image of the region mapped on the LV. The colors correspond to the orientation of the primary eigenvector (“myofiber” long-axis) of the diffusion tensor. The x, y, and z components of the vectors are mapped to red, green and blue, respectively (inset). B. Representative examples of fiber orientation (Trichrome elastic Von Giesen stain), and gap junction (GJ) distribution (black arrow heads) shown by connexin-43 immunoreactivity in normal myocardium are shown, respectively. Activation maps showing propagation in C. transverse and D. longitudinal
directions at baseline, and during right and left stellate ganglion stimulation (RSGS and LSGS, respectively). Electrical step symbol indicates pacing site. Electrograms from the sites indicated by the asterisks in panel D are also shown.

**E. Time course of RSGS on activation propagation (as estimated by activation time)** is shown. Arrows indicate the initiation and termination of RSGS.

**Figure 3. Sympathoexcitation and conduction velocity in normal hearts.**

**A-D.** Graphs showing the impact of RSGS and LSGS on longitudinal and transverse conduction velocity (CV), mean activation time, and activation heterogeneity (n=12, ***P<0.001, Wilcoxon signed rank test). **E.** Graphs showing the impact of esmolol (ESM), and carbenoxolone on change in CV during RSGS (n=3, **p<0.01, Wilcoxon signed rank test). **F.** Representative activation maps depicting the effect of carbenoxolone (CARBX) ± RSGS on longitudinal propagation. **G.** Maximal negative slope of activation waveform (-dV/dt\text{max}) of electrograms during pacing at baseline and during RSGS (n=5, **p=0.2, Wilcoxon signed rank test).

**Figure 4. Characterization of peri-infarct zones.** Shown are **A.** 3-D reconstructed magnetic resonance image of the peri-infarct zone and **B.** photograph of the same heart before mapping. The box indicates the regions mapped, while the black arrows indicated surviving islands of myocytes in the per-infarct region. **C.** Trichrome elastic Von Giesen stain of the peri-infarct region, with nerve bundles indicated by the arrowhead. The linear
arrangement of the surviving myocytes can be easily seen. **D.** Tyrosine hydroxylase immunoreactivity highlighting cardiac sympathetic nerves in the scar border is shown. Both large (long arrows) and small (short arrows) nerve bundles can be easily seen. **E.** Diffusion tensor magnetic resonance image showing fiber disarray in the peri-infarct zone (the colors of each vector bar represents three-dimensional orientation of the local myofiber), and accompanying heat map is depicted in **F.** Scale bar is in arbitrary units of dissimilarity between adjacent fibers as shown in **E.**

**Figure 5.** Sympathetic nerves exert functional control of sinus rhythm activation and repolarization in the peri-infarct zone.

Maps depicting a peri-infarct zone, and the influence of right and left stellate ganglion stimulation (RSGS and LSGS) respectively on activation recovery intervals (ARIs), a surrogate for action potential duration (**A.**), and activation in sinus rhythm (**B.**), black arrows in **A** and **B** highlight the same region for comparison across conditions. Heart rate (HR) for each map is shown in the top right corner of panel **A.** (bpm - beats per minute). Composite data is shown in **C-D.** for ARI and mean activation time (n=7, ***p<0.001, *P<0.05, Wilcoxon signed rank test).

**Figure 6.** Non-uniform modulation of activation in peri-infarct zones.

**A.** Illustration of the wavefronts used to assess propagation in the peri-infarct zone is shown in the left and middle image, while the fiber arrangement in the a...
trichrome stain is shown in the image on the right. B. The impact of right and left stellate ganglion stimulation (RSGS and LSGS, respectively) on two wavefronts is shown. Not only is the mean activation time decreased in wavefront I, the pattern of propagation is also altered. Wavefront II does not show a decrease in mean AT, however, the impact of SGS on propagation patterns can be appreciated, as well as the emergence of regions of relative or absolute delay (asterisk). C. Graphical summary of the data for wavefronts I & II are shown. D. Representative examples of the non-uniformity and paradoxical increases in peri-infarcted vs. control myocardium. E. Graphical summary of the percentage of animal subjects showing relative or absolute delay in response to SGS. F. Representative examples of connexin-43 (Cx-43) immunoreactivities indicating gap junction (GJ) distribution in the regions mapped electrically in control and infarcted animals. Longitudinal (arrowheads) and lateralized (arrows) Cx-43 can be appreciated. The distribution of Cx-43 at the scar border (top right) and further away (i.e. more lateral) from the scar border (middle left) in an infarcted heart is shown. Graphical quantifications of Cx-43 lateralization and overall density are shown (G. and H. respectively middle right and lower left) (n=7-9, ***p<0.001, *p<0.05, Mann-Whitney test). I. Spatial activation heterogeneity for wavefronts (WF) I and II at baseline, and during RSGS, and LSGS.

**Figure 7.** SGS can alter propagation in putative ventricular tachycardia circuits.
A. Electrograms recorded from the peri-infarct region of a subject, depicting a late potential (LP, red arrows) at baseline and during left sympathetic stimulation.

B. Mean activation time in the region mapped with and without local delay (i.e. LP) (p<0.001, Student’s T test). C. Propagation (top row) and activation recovery interval (bottom row) maps with and without local delay (LP). Dashed circle indicated the location of electrodes 1 and 9 (E1 and E9 respectively) recording the LP.

Figure 8. Cardiac electrical indices during sympathoexcitation in normal and infarcted hearts.

FIGURES
Figure 1. Influence of left and right sympathetic ganglia on activation recovery intervals in the anterior left ventricle.
Figure 2. Sympathoexcitation increases conduction velocity in normal hearts: influence of fiber orientation and gap junction distribution.
Figure 3. Sympathoexcitation and conduction velocity in normal hearts

A. Longitudinal Conduction Velocity (m/s)

B. Transverse Conduction Velocity (m/s)

C. Mean Activation Time (ms)

D. Activation heterogeneity (ms²/m²)

E. Change in Longitudinal Conduction Velocity (%)

F. Contour plots of BL, CARBX, and CARBX + RSGS

G. dV/dx (mV/m)

Legend:
- BL
- RSGS
- LSGS
- RSGS + ESM
- RSGS + Carbenoxolone
Figure 4. Characterization of peri-infarct zones.
Figure 5. Sympathetic nerves exert functional control of sinus rhythm activation and repolarization in the peri-infarct zone.

A

Activation Recovery Interval (ms)

472.0
462.8
452.8
442.5
432.3
422.0
411.7
401.4
391.1
380.8
370.5
360.2
350.0
349.7
339.4
329.1
318.8
308.5
298.3
288.0
278.0

HR 69 bpm
BL
RSGS
LSGS

B

Sinus Rhythm Activation Time (ms)

9.00
12.5
19.6
26.7
33.8
40.9
48.0

BL
RSGS
LSGS

4mm

C

Normal Sinus Rhythm

Activation Recovery Interval (ms)

500
400
300
200
100
0
BL
RSGS
BL
LSGS

***
*

D

Normal Sinus Rhythm

Mean Activation Time (ms)

30
20
10
0
BL
RSGS
BL
LSGS

*
Figure 6. Non-uniform modulation of activation in scar border zones.

A

Right Ventricle
Left Ventricle
Infarct Region

B

Wavefront I
Wavefront II

C

Wavefront I
Wavefront II

D

Control
MI

E

Delay
No Delay

F

CONTROL
INFARCT
INFARCT

G

Connexin Lateralization Index (A U) I II III IV V

H

CX43 Immunoactivity (uM/300 μm²)

I

Spatial Activation Heterogeneity (uM²/min²)
Figure 7. Insights into sympathetic modulation of potentially arrhythmogenic properties of peri-infarct zones.

A

Baseline

Sympathetic Stimulation

B

C

Mean Activation Time (ms)

AT (ms)

**

15.0

14.1

13.2

12.3

11.4

10.5

9.55

8.64

7.73

6.82

5.91

5.00

Local Delay Present

Absent Local Delay

Local Delay Present

Local Delay Absent

ARI (ms)

301.0

295.2

292.4

290.5

293.7

291.9

290.1

288.3

286.5

284.6

282.8

281.0
**Schematic Figure 8.** Cardiac electrical indices during sympathoexcitation in normal & peri-infarcted myocardium

**Normal Heart**

- **Activation:**
  - Increased longitudinal GJ conductance
  - Increased CV

- **Repolarization:**
  - APD Shortening

**Infarcted Heart**

- **Activation:**
  - Increased directionally dependent activation propagation
  - Spatially heterogeneous modulation of propagation including paradoxical prolongation
  - Modulation of late potentials

- **Repolarization:**
  - Increased spatial dispersion of repolarization
  - Increased LSG control of antero-apical LV