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Pro-arrhythmic Effects of Sympathetic Activation Are Mitigated by Vagal Nerve Stimulation in Infarcted Hearts

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Abstract

Background: Sympathoexcitation increases risk of ventricular tachyarrhythmias (VT). Vagal nerve stimulation (VNS) has been anti-arrhythmic in the setting of ischemia-driven arrhythmias, but it is unclear if it can overcome the electrophysiological effects of sympathoexcitation in the setting of chronic myocardial infarction (MI). The goal of this study was to evaluate whether intermittent VNS reduces electrical heterogeneities and arrhythmia inducibility during sympathoexcitation.

Methods: In Yorkshire pigs after chronic MI, a sternotomy was performed, a 56-electrode sock was placed over the ventricles (n=17), and a basket catheter was positioned in the left ventricle (n=6). Continuous unipolar electrograms from sock and basket arrays were obtained to analyze activation recovery interval (ARI), a surrogate of action potential duration. Bipolar voltage mapping was performed to define scar, border zone, or viable myocardium. Hemodynamic and electrical parameters, and VT inducibility were evaluated during sympathoexcitation with bilateral stellate ganglia stimulation (BSS) and during combined BSS with intermittent VNS.

Results: During BSS, global epicardial ARIs shortened from 384±59 to 297±63 ms and endocardial ARIs from 359±36 to 318±40 ms. Dispersion in ARIs increased in all regions, with the greatest increase observed in scar and border zone regions. VNS mitigated the effects of BSS on border zone ARIs (from −18.3±6.3% to −2.1±14.7%) and ARI dispersion (from 104 ms^2 [1, 1108] to −108 ms^2 [−588, 30]). VNS reduced VT inducibility during sympathoexcitation (from 75% to 40%, p<0.05).

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Relationship with Industry: Dr. Vaseghi has shares in NeuCures Inc. and patents on neuromodulation at University of California, Los Angeles. Other authors report no relationship with industry.

Conclusions: After chronic myocardial infarction, VNS overcomes the detrimental effects of sympathoexcitation by reducing electrophysiological heterogeneities exacerbated by sympathetic stimulation, decreasing VT inducibility.

CONDENSED ABSTRACT

Sympathetic activation plays a significant role in occurrence of ventricular arrhythmias. In this study, sympathetic stimulation significantly worsened global and regional ventricular electrophysiological heterogeneities in chronically infarcted porcine hearts. Notably, vagal nerve stimulation significantly mitigated the electrophysiological effects of sympathoexcitation on scar and border zone regions, increased ventricular effective refractory period during sympathetic stimulation, and reduced ventricular arrhythmia inducibility by 50%. This study highlights the efficacy of VNS in acutely reducing incidence of VT even during states of elevated sympathetic tone.

Tweet:

Vagal nerve stimulation mitigates sympathetically mediated pro-arrhythmic effects on border zones and scar regions in chronically infarcted porcine hearts. #EPPeeps #JACCCEP #CardioTwitter

Keywords

sympathetic; ventricular arrhythmias; vagal nerve stimulation; dispersion; myocardial infarction; neuromodulation

INTRODUCTION

Sympathetic activation due to pathological neural remodeling resulting from heart disease is known to contribute to ventricular arrhythmias.(1,2) Sympathoexcitation exacerbates existing electrophysiological heterogeneities in the myocardium and increases the occurrence of early and delayed after depolarizations.(3–5) *In-vivo* in healthy hearts, vagal nerve stimulation (VNS) has been shown to increase ventricular action potential duration and ventricular fibrillation (VF) threshold, as well as reduce the burden of ventricular arrhythmias if initiated prior to or at the time of ischemia.(6–9) However, in chronically infarcted animals, ventricular tachyarrhythmias (VT) often occur in the setting of stress and sympathoexcitation without ischemia. It is unclear if in chronically remodeled and diseased hearts, VNS can overcome the detrimental electrophysiological effects of sympathoexcitation by reducing electrophysiological heterogeneities exacerbated by sympathetic stimulation and decrease VT inducibility.

Early *in-vivo* studies by Levy *et al.* suggested that in healthy canine hearts, vagal nerve stimulation may blunt myocardial norepinephrine levels. In addition, the parasympathetic nervous system is also regulated by the sympathetic nervous system. Cardiac sympathetic activation causes release of norepinephrine, which can bind to parasympathetic neurons and inhibit release of acetylcholine.(10) Thus, while acetylcholine levels may be preserved throughout the heart following infarction, they may be functionally suppressed.(11) The ventricular electrophysiological effects of vagal nerve stimulation during

sympathoexcitation in structurally diseased hearts is thereby unclear. It is possible, that even during states of elevated sympathetic tone, such as electrical storm, implementation of vagal nerve stimulation in diseased hearts may reduce arrhythmias. In this study, we hypothesized that vagal nerve stimulation can be anti-arrhythmic even during sympathetic activation and reduce inducibility of VT that occurs with sympathetic stimulation. We tested this hypothesis in a chronic large animal porcine infarct model using detailed electrophysiological mapping.

METHODS

Ethical approval

Seventeen Yorkshire pigs (*Sus scrofa*; S&S Farms) weighing 48–56 kg were used for this study. Care of animals conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The study protocol was approved by the University of California, Los Angeles Institutional Animal Care and Use Committee.

Creation of myocardial infarcts

Percutaneous MI in the region of left anterior descending (LAD) coronary artery was created in 17 pigs (30–35 kg) as previously described under general anesthesia (isoflurane, 1–2%). (11,12) Briefly, an AL2 guide catheter (Boston Scientific, Marlborough, MA) was advanced from the femoral artery to the left main coronary artery. A percutaneous transluminal angioplasty (PTA) balloon catheter (Abbott, Chicago, IL) was advanced over a coronary guidewire into the LAD and positioned after the take-off of the first diagonal branch (Figure 1b). The balloon was inflated, and 3–5 mL of polystyrene microspheres (Polybead® 90.0μm, Polysciences, PA) followed by 5 mL normal saline were injected through the lumen of the catheter. The balloon was then deflated and removed. MI was confirmed by the presence of ST-elevation or T-wave inversion on ECG and coronary angiography showing a lack of flow in the artery (Figure 1c–d). After the procedure, ECG and blood pressure were monitored for 20 min prior to extubation. Immediate external defibrillation was performed if the animal developed VT or VF. After extubation, animals were monitored until they could ambulate.

Surgical preparation

Six to eight weeks post-MI, animals were sedated, intubated, and placed under anesthesia with isoflurane (1–2% INH). After completion of the surgical procedures, including sternotomy, anesthesia was transitioned to α-chloralose (Sigma-Aldrich; 50 mg/kg initial bolus, then 20–35 mg/kg/hr IV). Depth of anesthesia was adjusted based on hemodynamic indices, corneal reflex, and jaw tone. Arterial blood gases were monitored throughout the experiments; ventilation adjusted, or sodium bicarbonate was administered to maintain normal pH. The CardioLab System (GE Healthcare) was used to record continuous 12-lead electrocardiograms. Ventral precordial leads were placed posteriorly given sternotomy. The femoral and carotid arteries were cannulated to measure blood pressure continuously and obtain access to the left ventricle (LV) for basket catheter placement, respectively. Sheaths were placed in the femoral veins for delivery of medications and saline. Fentanyl boluses (20–30 mcg/kg) were used during sternotomy to reduce discomfort. Sodium pentobarbital

(Med-Pharmex Inc.; 100 mg/kg IV) followed by saturated KCl (Sigma-Aldrich; 1–2 mg/kg IV) was used for euthanasia.

Stellate ganglia and vagal nerve stimulations

For bilateral stellate ganglia stimulation (BSS), the stellate ganglia were isolated behind the parietal pleura (Figure 1g), as previously described and stimulated by bipolar needle electrodes using a Grass stimulator (Grass Technologies Model S88).(3,12) Stimulation current was set at an amplitude that led to a greater than 10% increase in heart rate (HR) and/or systolic blood pressure (SBP) for each ganglion.(3)

For bilateral VNS, after neck cutdown, the vagi were isolated in the carotid sheaths. The vagus was carefully dissected away from the sympathetic chain and insulated from the surrounding tissue. Bipolar cuff electrodes were placed around each vagi (Figure 1g). Threshold current for each vagus was defined as the amplitude that led to a 10% decrease in heart rate (10 Hz, 1 ms pulse-width).(7) Intermittent bilateral VNS (15 sec ON, 15 sec OFF) was performed at 1.2 times this current during BSS.

BSS was performed continuously at threshold current (4 Hz and 4 ms pulse-width) for both electrophysiological/hemodynamic recordings and during VT inducibility testing (described below). Similarly, BSS was performed continuously with concomitant intermittent VNS to determine the effects of VNS on mitigating the electrophysiological effects of BSS.

Electrophysiological study and mapping

Epicardial unipolar electrograms were continuously recorded using a 56-electrode sock placed over the ventricles ($n = 17$, Figure 1), *in-vivo* (Cardio Lab, GE Healthcare). In 6 animals, the 56-electrode sock was attached to customized multielectrode recording system (University of Utah, Salt Lake City, Utah) and a Constellation basket catheter (Boston Scientific, Marlborough, MA) was placed via a retrograde aortic approach from the left carotid artery into the LV, under fluoroscopic and ultrasound guidance (Figure 2) to record endocardial unipolar electrograms. Bipolar voltage electroanatomic mapping was performed, and location of each sock electrode overlying scar (defined as bipolar voltage < 0.5 mV), border zone (defined as bipolar voltage between 0.5 and 1.5 mV), or viable myocardium (defined as bipolar voltage > 1.5 mV) was meticulously measured using a duodecapolar catheter (2-2-2 interelectrode spacing, Abbott Medical, IL) and regional epicardial ARIs (scar, viable, border zone) quantified in 12 of 17 hearts. Very dense scar regions (voltage < 0.05 mV) with little to no definable bipolar recordings were excluded from analysis. Electroanatomic mapping of the heart was performed using the Ensite system (Abbott).

Activation recovery intervals (ARI) were analyzed from unipolar electrograms of each electrode using iScaldyn software (University of Utah, Salt Lake City, UT).(13,14) Activation time was defined from the origin to the minimum dV/dt of the activation wavefront of the unipolar electrogram and recovery time was defined as the start of activation to the maximum dV/dt of repolarization wavefront. ARI was calculated by subtracting activation time from repolarization time (Figure 1). ARI has been previously validated as a faithful correlate of local action potential duration.(13–16)

ARIs and hemodynamic variables including heart rate and blood pressure were analyzed before and during nerve stimulations (before and during BSS and before and during BSS+VNS). A minimum of 30 minutes was allowed between stimulations and the order of stimulations was randomized. After 45 seconds of BSS or BSS+VNS, during which electrical and hemodynamic data were obtained, effective refractory period was evaluated and VT inducibility testing performed as described below. The duration of nerve stimulations ranged between 3 to 5 minutes depending on the number extra-stimuli required to induce VT.

Effective refractory period measurement and VT inducibility testing

Effective refractory period (ERP) was measured by placement of a quadripolar catheter in the right ventricular (RV) apex. Pacing was performed at 3–5 mA and 2 ms pulse width, and capture confirmed by ventricular morphology on the surface ECG. A drive cycle length 10–20% faster than the baseline heart rate was used and a single extra-stimulus placed and decremented by 10 ms interval to measure ERP at baseline (prior to any nerve stimulation), during BSS, and during concomitant BSS and VNS from the same RV apical position.

VT inducibility was also assessed during BSS alone and during concomitant BSS and VNS by programmed ventricular stimulation (Micropace, New South Wales, Australia) with the same pacing parameters. Inducibility was determined at two different drive cycle lengths with up to three extra-stimuli (10 ms decrement, down to 200 ms or ERP), from two different sites (RV endocardium and LV anterior epicardium). If VT was induced from one specific site, this same site was used to induce VT during subsequent stimulations. If sustained VT or VF was inducible at baseline, a minimum wait period of 30 minutes was allowed after cardioversion before repeat stimulation/inducibility testing. Both VT inducibility (defined as occurrence of sustained VT or VF requiring anti-tachycardia pacing or defibrillation) or non-sustained VT (defined as occurrence of greater than 3 premature ventricular contractions) was quantified during each nerve stimulation.

Statistical analysis

Data are reported as means ± standard deviation (SD) for normally-distributed data, unless stated otherwise. Non-normal data are reported and shown as median [1^{st} quartile, 3^{rd} quartile]. Normality of data distributions was assessed using the Shapiro-Wilk test. Global ventricular ARIs were calculated as the mean ARI across all electrodes. Regional dispersion in ARI was defined as variance across electrodes from a specific region (i.e., scar, border zone, or viable regions). Paired Student's *t*-test was performed to compare responses between BSS and BSS+VNS in each animal. Paired Wilcoxon signed-rank test was performed to compare changes in regional dispersion between BSS and BSS+VNS. Changes in regional ARIs and dispersion in ARI from baseline were compared using one-way repeated measure analysis of variance and the Friedman test, respectively, with the false discovery rate corrected by the Benjamini-Hochberg procedure. Percentage change in ARI or change in dispersion in ARI in each region with nerve stimulation were compared by paired Student's *t*-test and the Friedman test, respectively. Inducibility for VT during each stimulation were compared by the exact binomial test. A p value ≤ 0.05 was

considered statistically significant. All statistical analyses were performed with GraphPad Prism software v8.

RESULTS

Hemodynamic effects of BSS and BSS combined with VNS

In chronically infarcted pigs ($n = 17$), BSS significantly increased all hemodynamic indices, including heart rate, systolic blood pressure, diastolic blood pressure and mean arterial pressure (Table 1). On the other hand, concomitant VNS with BSS mitigated the increases in all hemodynamic parameters.

Global ventricular electrophysiological effects

BSS shortened ventricular ERP from 297±36 ms at baseline to 239±22 ms ($p < 0.001$). During combined stimulation, ventricular ERP only decreased to 265±39 ms ($p < 0.05$), Figure 1. However, ERP was still shortened as compared to baseline ($p < 0.05$).

In a similar fashion as ERP, BSS shortened ventricular epicardial global ARIs (defined as the mean ARI across all 56 electrodes) from 384±59 ms at baseline to 297±63 ms ($n = 17$, $p < 0.001$, Figure 1). ARI did not significantly change compared to pre-stimulation during BSS+VNS (382±41 ms at baseline to 366±60 ms during BSS+VNS, $p = 0.1$, Figure 1). Therefore, VNS during BSS markedly blunted sympathetically-induced ARI shortening (−22.7±10.4% during BSS *vs* −4.5±9.2% during BSS+VNS, $p < 0.001$, Figure 1). Global epicardial dispersion in ventricular ARIs (variance across all electrodes) significantly increased during BSS from 648 ms^2 [509, 826 ms^2] at baseline to 992 ms^2 [594, 1400 ms^2] ($p < 0.01$). VNS added to BSS significantly mitigated these effects of sympathoexcitation on dispersion (baseline pre-stimulation global dispersion in ARI: 609 ms^2 [493, 747 ms^2], BSS+VNS: 743 ms^2 [592, 1000 ms^2], $p = 0.1$).

The endocardium followed the same trend as the epicardium, and during BSS, ventricular endocardial ARIs decreased from 359±36 ms at baseline to 318±40 ms during stimulation ($n = 6$, $p < 0.05$, Figure 2). Conversely, BSS+VNS did not elicit any significant shortening of global ARIs, (Figure 2).

Regional differences in ventricular electrophysiological effects

The genesis of ventricular arrhythmias in ischemic cardiomyopathies is known to be initiated and propagated within the scar and border zone regions.(1) While this is in part due to altered excitability of the border zone combined with the anatomic substrate of these regions allowing for reentrant circuits, the distinct pattern of sympathetic denervation and hyperinnervation in these regions augments these heterogeneities,(17,18) and the complex interaction of the sympathetic and parasympathetic nervous systems in these discrete functional domains is yet to be identified. To this end, the scar, border zone, and viable regions of the myocardium underlying the epicardial sock arrays were identified by epicardial voltage mapping and validated by electroanatomic mapping (Figure 3).

Detailed analysis of regional changes ($n = 12$) revealed that the effects of BSS on global epicardial ARI were paralleled by significant shortening in scar, border zone, and viable

regions of the epicardium (Figure 3). Notably, benefits of VNS in blunting the effects of BSS were not restricted to any particular region; scar, border zone and viable regions alike showed a blunting of sympathetic effects during VNS (Figure 3). Despite a sustained background of BSS, ARI shortening was significantly less during BSS+VNS than BSS alone in the scar (−13.6±5.3% with BSS *vs* −4.9±12.4% with BSS+VNS; $p < 0.05$), border zone (−18.3±6.3% with BSS *vs* −2.1±14.7% with BSS+VNS; $p < 0.01$), and viable regions (−14.3±6.2% with BSS *vs* −1.0±10.2% with BSS+VNS; $p < 0.01$, Figure 3).

BSS also increased local electrophysiological heterogeneities as reflected by variances in regional ARIs; these changes in dispersion were also stabilized by VNS. The regional dispersion of scar was significantly increased from 72 ms^2 [12, 177 ms^2] at baseline to 232 ms^2 [23, 1276 ms^2] during BSS ($p < 0.01$, Figure 3) as was dispersion in border zone (194 ms^2 [66, 399 ms^2] at baseline *vs* 355 ms^2 [100, 1457 ms^2] during BSS; $p < 0.05$) and viable regions (124 ms^2 [30, 337 ms^2] at baseline *vs* 432 ms^2 [107, 927 ms^2] during BSS; $p < 0.05$). On the other hand, VNS during BSS significantly reduced dispersion in all regions, Figure 3, and mitigated BSS-induced increases in local dispersion of viable (Δ regional dispersion: 297 ms^2 [22, 639 ms^2] with BSS *vs* 2 ms^2 [−111, 248 ms^2] with BSS+VNS; $p < 0.05$) and scar regions (Δ regional dispersion: 110 ms^2 [5, 1146 ms^2] with BSS *vs* −18 ms^2 [−111, 6 ms^2] with BSS+VNS; $p < 0.05$). Notably, in the border zone regions, effects of VNS during BSS reduced dispersion to levels even below baseline (pre-stimulation) values (Δ regional dispersion: 104 ms^2 [1, 1108 ms^2] with BSS *vs* −108 ms^2 [−588, 30 ms^2] with BSS+VNS; $p < 0.01$, Figure 3).

Effects of concomitant stimulation on susceptibility for ventricular arrhythmias

During BSS alone, 13 of 17 animals (>75%; Figure 4d) were inducible for any sustained or non-sustained VT with up to triple extra-stimuli (Figure 4). NSVT only was observed in 3 of these 13 animals. Animals with sustained VT exhibited repeated bouts of NSVT at less aggressive programmed stimulation before being inducible for sustained VT, which required cardioversion, with one animal experiencing spontaneous degeneration of sinus rhythm to VT/VF during BSS alone (i.e., without any ventricular pacing having been performed), Figure 4b.

However, VT inducibility was significantly reduced in the setting of BSS+VNS, with only 7 of 17 animals (~40%) still inducible for VT/VF. In addition, a decrease in episodes of NSVT observed during BSS were observed during combined BSS+VNS (Figure 4).

DISCUSSION

Major findings

The major findings of this study are as follows:

1. Despite a high background of sympathetic activation, vagus nerve stimulation is capable of mitigating the effects of bilateral stellate ganglia stimulation on global and regional ventricular ARI shortening.

2. Vagal nerve stimulation can reduce electrophysiological heterogeneities in regional ARIs across viable, border zone, and scar regions, as reflected by dispersion in ARI.

3. Vagal nerve stimulation reduces VT inducibility during sympathoexcitation.

Neuromodulatory approaches aimed at augmenting the parasympathetic nervous system (e.g., vagal nerve stimulation) are currently under intense investigation for the treatment of cardiovascular disease.(19) Previous studies have suggested that vagal nerve stimulation may reduce the burden of ventricular arrhythmias through the stabilization of existing heterogeneities in peri-infarct border zone action potential duration.(11) However, it is yet unknown if and how these interventions may act in the context of elevated sympathetic tone, a known trigger for ventricular arrhythmias where elevated norepinephrine levels, for example, may reduce acetylcholine release.(1)

This is the first study to evaluate detailed electrophysiological effects of concomitant stimulation of the stellate ganglia (sympathetic nervous system) and vagal nerves (parasympathetic nervous system). In this study, a porcine model of chronic myocardial infarction was used as it closely recapitulates both electrophysiological and autonomic changes seen in humans with structural heart disease.(20) While previous studies have suggested that vagal nerve stimulation may be antiarrhythmic in the setting of structural heart disease,(11) it remained unclear whether these effects may persist and be sufficient to combat the effects of sympathoexcitation.

Pro-arrhythmic mechanisms of sympathoexcitation

Stimulation of the bilateral stellate ganglia evoked significant increases in ventricular excitability, causing shortening of ERP as well as epicardial and endocardial action potential duration as reflected by ARIs. Importantly, electrophysiological heterogeneity of scar and border zone regions of the myocardium were markedly increased by sympathetic stimulation. Notably, sympathetic stimulation alone was sufficient to induce premature ventricular contractions, non-sustained ventricular tachycardia (NSVT) and ventricular tachyarrhythmias (VT/VF) in some animals.

Cardioprotective effects of vagal nerve stimulation

VNS has been shown to decrease the incidence of sudden cardiac death.(19) In addition in normal and infarcted porcine hearts, intermittent moderate levels of vagal nerve stimulation was shown to increase action potential duration.(7,11) The mechanism by which VNS increases action potential duration is an ongoing area of investigation and may occur through direct release and action of acetylcholine on muscarinic receptors, via inhibition of norepinephrine and neuropeptide Y release at the nerve-myocyte interface, or via interactions at the stellate ganglia.(21–23) In addition, chronic myocardial infarction and heart failure have been shown to be associated with significant neural remodeling in the intrinsic cardiac, vagal (nodose), and stellate ganglia.(24–26) Interestingly, while chronic VNS has been shown to decrease stellate ganglion nerve activity,(23,27) other studies have suggested that acute VNS may paradoxically increase stellate ganglion nerve activity, potentially through anastomosing sympathetic fibers and/or afferent fiber

activation, responses that may be frequency dependent.(28–30) In this study, the frequency of stimulation was similar to where cardiomotor vagal efferent fibers were shown to be engaged, close to the neural fulcrum, in a canine model.(24) The same frequency of stimulation was used in the ANTHEM-HF study, which showed improvement in echocardiographic and New York Heart Association class parameters in patients with heart failure,(31,32) and was also previously demonstrated to increase action potential duration in normal porcine hearts.(7) However, whether the beneficial electrophysiological effects of VNS would persist during states of sympathoexcitation in diseased hearts, in whom pre-existing baseline electrophysiological heterogeneities are exacerbated by sympathetic activation, remained unknown. The present study shows that VNS caused nearly a 70 ms prolongation of global ARIs (80% reduction in effects of BSS) during sympathoexcitation, with even more prominent effects observed in border zone regions (90% reduction in effects of BSS), areas that are specifically implicated in ventricular arrhythmogenesis. Moreover, VNS stabilized the cardiac electrophysiological heterogeneity within the border zone regions despite concomitant sympathetic stimulation. Border zone regions are known to serve as pro-arrhythmic regions responsible for both triggers and maintenance of ventricular arrhythmias. This is potentially related to both their structural heterogeneity consisting of areas of fibrosis intermixed with viable myocardium,(33–35) but also due to neural remodeling which leads to sympathetic nerve-sprouting after MI in this region.(17,36,37) This underlying structural and neural heterogeneity can further contribute to occurrence of arrhythmias, particularly in the setting of sympathoexcitation. Therefore, the potential beneficial effects of VNS in stabilizing these particular regions have significant electrophysiological implications.

Clinical significance

In this study, inducibility of ventricular arrhythmias was reduced by nearly 50% despite sustained sympathetic activation. These results, taken together with the global and regional electrical data suggest that vagal nerve stimulation can increase electrical stability, even during states of high sympathetic tone (Figure 5, Central Illustration), and may present a promising therapeutic option for treatment of ventricular tachyarrhythmias, even if acutely.

Limitations

General anesthesia with isoflurane is known to blunt autonomic responses. To limit this effect, anesthesia was switched to α-chloralose to reduce anesthetic effects on autonomic responses.(29) In addition, our results reflect the effects of acute sympathetic and vagal nerve stimulation. Chronic effects of intermittent vagal nerve stimulation during states of sympathoexcitation require further study.

Moreover, while scar and border zones are known to play important roles in harboring circuits that cause VT in ischemic heart disease, transmural gradients between these sites may compound myocardial heterogeneities.(38) In this study, however, accurate pairing of electrodes was not possible. Therefore, the role of transmural gradients in exacerbating arrhythmias could not be assessed.

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ABBREVIATION LIST

ARI	activation recovery interval
BSS	bilateral stellate ganglia stimulation
HR	heart rate
LV	left ventricle
RV	right ventricle
MI	myocardial infarction
SBP	systolic blood pressure
VNS	vagal nerve stimulation
VT	ventricular tachyarrhythmias
VF	ventricular fibrillation

REFERENCES

1. Vaseghi M, Shivkumar K. The role of the autonomic nervous system in sudden cardiac death. Progress in cardiovascular diseases 2008;50:404–19. [PubMed: 18474284]
2. Zipes DP, Rubart M. Neural modulation of cardiac arrhythmias and sudden cardiac death. Heart Rhythm 2006;3:108–13. [PubMed: 16399065]
3. Yagishita D, Chui RW, Yamakawa K et al. Sympathetic nerve stimulation, not circulating norepinephrine, modulates T-peak to T-end interval by increasing global dispersion of repolarization. Circ Arrhythm Electrophysiol 2015;8:174–85. [PubMed: 25532528]
4. Priori SG, Mantica M, Schwartz PJ. Delayed afterdepolarizations elicited in vivo by left stellate ganglion stimulation. Circulation 1988;78:178–85. [PubMed: 3383403]
5. Ben-David J, Zipes DP. Differential response to right and left ansae subclaviae stimulation of early afterdepolarizations and ventricular tachycardia induced by cesium in dogs. Circulation 1988;78:1241–50. [PubMed: 3180380]
6. Yamakawa K, Rajendran PS, Takamiya T et al. Vagal nerve stimulation activates vagal afferent fibers that reduce cardiac efferent parasympathetic effects. Am J Physiol Heart Circ Physiol 2015;309:H1579–90. [PubMed: 26371172]
7. Yamakawa K, So EL, Rajendran PS et al. Electrophysiological effects of right and left vagal nerve stimulation on the ventricular myocardium. Am J Physiol Heart Circ Physiol 2014;307:H722–31. [PubMed: 25015962]
8. Brack KE, Coote JH, Ng GA. Vagus nerve stimulation protects against ventricular fibrillation independent of muscarinic receptor activation. Cardiovasc Res 2011;91:437–46. [PubMed: 21576131]
9. Myers RW, Pearlman AS, Hyman RM et al. Beneficial Effects of Vagal Stimulation and Bradycardia During Experimental Acute Myocardial Ischemia. Circulation 1974;49:943–947. [PubMed: 4828616]

10. Xu ZJ, Adams DJ. Alpha-adrenergic modulation of ionic currents in cultured parasympathetic neurons from rat intracardiac ganglia. J Neurophysiol 1993;69:1060–70. [PubMed: 8098358]
11. Vaseghi M, Salavatian S, Rajendran PS et al. Parasympathetic dysfunction and antiarrhythmic effect of vagal nerve stimulation following myocardial infarction. JCI Insight 2017;2.
12. 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 2017;312:H392–H405. [PubMed: 28011590]
13. Haws CW, Lux RL. Correlation between in vivo transmembrane action potential durations and activation-recovery intervals from electrograms. Effects of interventions that alter repolarization time. Circulation 1990;81:281–8. [PubMed: 2297832]
14. Millar CK, Kralios FA, Lux RL. Correlation between refractory periods and activation-recovery intervals from electrograms: effects of rate and adrenergic interventions. Circulation 1985;72:1372–9. [PubMed: 4064279]
15. Chinushi M, Tagawa M, Kasai H et al. Correlation between the effective refractory period and activation-recovery interval calculated from the intracardiac unipolar electrogram of humans with and without dl-sotalol treatment. Jpn Circ J 2001;65:702–6. [PubMed: 11502045]
16. Yue AM, Paisey JR, Robinson S, Betts TR, Roberts PR, Morgan JM. Determination of human ventricular repolarization by noncontact mapping: validation with monophasic action potential recordings. Circulation 2004;110:1343–50. [PubMed: 15353505]
17. Cao JM, Fishbein MC, Han JB et al. Relationship between regional cardiac hyperinnervation and ventricular arrhythmia. Circulation 2000;101:1960–9. [PubMed: 10779463]
18. Habecker BA, Anderson ME, Birren SJ et al. Molecular and cellular neurocardiology: development, and cellular and molecular adaptations to heart disease. J Physiol 2016;594:3853–75. [PubMed: 27060296]
19. Brack KE, Winter J, Ng GA. Mechanisms underlying the autonomic modulation of ventricular fibrillation initiation--tentative prophylactic properties of vagus nerve stimulation on malignant arrhythmias in heart failure. Heart Fail Rev 2013;18:389–408. [PubMed: 22678767]
20. Nakahara S, Vaseghi M, Ramirez RJ et al. Characterization of myocardial scars: electrophysiological imaging correlates in a porcine infarct model. Heart Rhythm 2011;8:1060–7. [PubMed: 21354335]
21. Herring N, Cranley J, Lokale MN et al. The cardiac sympathetic co-transmitter galanin reduces acetylcholine release and vagal bradycardia: implications for neural control of cardiac excitability. J Mol Cell Cardiol 2012;52:667–76. [PubMed: 22172449]
22. Hoang JD, Salavatian S, Yamaguchi N, Swid MA, David H, Vaseghi M. Cardiac sympathetic activation circumvents high-dose beta blocker therapy in part through release of neuropeptide Y. JCI Insight 2020;5.
23. Shen MJ, Shinohara T, Park HW et al. Continuous low-level vagus nerve stimulation reduces stellate ganglion nerve activity and paroxysmal atrial tachyarrhythmias in ambulatory canines. Circulation 2011;123:2204–12. [PubMed: 21555706]
24. Li W, Knowlton D, Van Winkle DM, Habecker BA. Infarction alters both the distribution and noradrenergic properties of cardiac sympathetic neurons. Am J Physiol Heart Circ Physiol 2004;286:H2229–36. [PubMed: 14726300]
25. Rajendran PS, Nakamura K, Ajijola OA et al. Myocardial infarction induces structural and functional remodelling of the intrinsic cardiac nervous system. J Physiol 2016;594:321–41. [PubMed: 26572244]
26. Salavatian S, Hoang JD, Yamaguchi N et al. Myocardial Infarction Alters Cardiac Nociception in the Vagal Ganglia: Implications for Parasympathetic Dysfunction in Heart Disease. JCI Insight 2022; in press.
27. Shen MJ, Hao-Che C, Park HW et al. Low-level vagus nerve stimulation upregulates small conductance calcium-activated potassium channels in the stellate ganglion. Heart Rhythm 2013;10:910–5. [PubMed: 23357541]
28. Seki A, Green HR, Lee TD et al. Sympathetic nerve fibers in human cervical and thoracic vagus nerves. Heart Rhythm 2014;11:1411–7. [PubMed: 24768897]

29. Ardell JL, Nier H, Hammer M et al. Defining the neural fulcrum for chronic vagus nerve stimulation: implications for integrated cardiac control. J Physiol 2017;595:6887–6903. [PubMed: 28862330]

30. Rhee KS, Hsueh CH, Hellyer JA et al. Cervical vagal nerve stimulation activates the stellate ganglion in ambulatory dogs. Korean Circ J 2015;45:149–57. [PubMed: 25810737]

31. Anand IS, Konstam MA, Klein HU et al. Comparison of symptomatic and functional responses to vagus nerve stimulation in ANTHEM-HF, INOVATE-HF, and NECTAR-HF. ESC Heart Fail 2020;7:75–83. [PubMed: 31984682]

32. Premchand RK, Sharma K, Mittal S et al. Autonomic regulation therapy via left or right cervical vagus nerve stimulation in patients with chronic heart failure: results of the ANTHEM-HF trial. J Card Fail 2014;20:808–16. [PubMed: 25187002]

33. Schmidt A, Azevedo CF, Cheng A et al. Infarct tissue heterogeneity by magnetic resonance imaging identifies enhanced cardiac arrhythmia susceptibility in patients with left ventricular dysfunction. Circulation 2007;115:2006–14. [PubMed: 17389270]

34. Yan AT, Shayne AJ, Brown KA et al. Characterization of the peri-infarct zone by contrast-enhanced cardiac magnetic resonance imaging is a powerful predictor of post-myocardial infarction mortality. Circulation 2006;114:32–9. [PubMed: 16801462]

35. Rutherford SL, Trew ML, Sands GB, LeGrice IJ, Smaill BH. High-resolution 3-dimensional reconstruction of the infarct border zone: impact of structural remodeling on electrical activation. Circ Res 2012;111:301–11. [PubMed: 22715470]

36. Chen PS, Chen LS, Cao JM, Sharifi B, Karagueuzian HS, Fishbein MC. Sympathetic nerve sprouting, electrical remodeling and the mechanisms of sudden cardiac death. Cardiovasc Res 2001;50:409–16. [PubMed: 11334845]

37. Cao JM, Chen LS, KenKnight BH et al. Nerve sprouting and sudden cardiac death. Circ Res 2000;86:816–21. [PubMed: 10764417]

38. Srinivasan NT, Orini M, Providencia R et al. Prolonged action potential duration and dynamic transmural action potential duration heterogeneity underlie vulnerability to ventricular tachycardia in patients undergoing ventricular tachycardia ablation. Europace 2019;21:616–625. [PubMed: 30500897]

PERSPECTIVES

Competency in Medical Knowledge:

While beta-blockers remain a part of standard anti-arrhythmic therapy for patients with ventricular arrhythmias, sympathoexcitation may amplify arrhythmogenicity through non-adrenergic pathways, leaving patients refractory to traditional therapies. Detailed electrophysiological mapping in a chronically infarcted porcine model in this study demonstrated that modest levels of vagal nerve stimulation mitigated sympathetically-driven shortening of action potential duration and exacerbation of electrical heterogeneity, particularly in border zone and scar regions, significantly reducing VT during sympathoexcitation.

Translational Outlook:

This study demonstrates the acute beneficial effects of vagal nerve stimulation in mitigating the pro-arrhythmic effects of sympathoexcitation in a large animal model of chronic myocardial infarction. These findings add to the growing body of literature on the safety and efficacy of vagal nerve stimulation for treatment of ventricular arrhythmias. Further studies are needed to determine whether these effects are also observed in patients with ventricular arrhythmias, and whether vagal nerve stimulation can reduce burden of ventricular arrhythmias in the setting of ischemic cardiomyopathy.

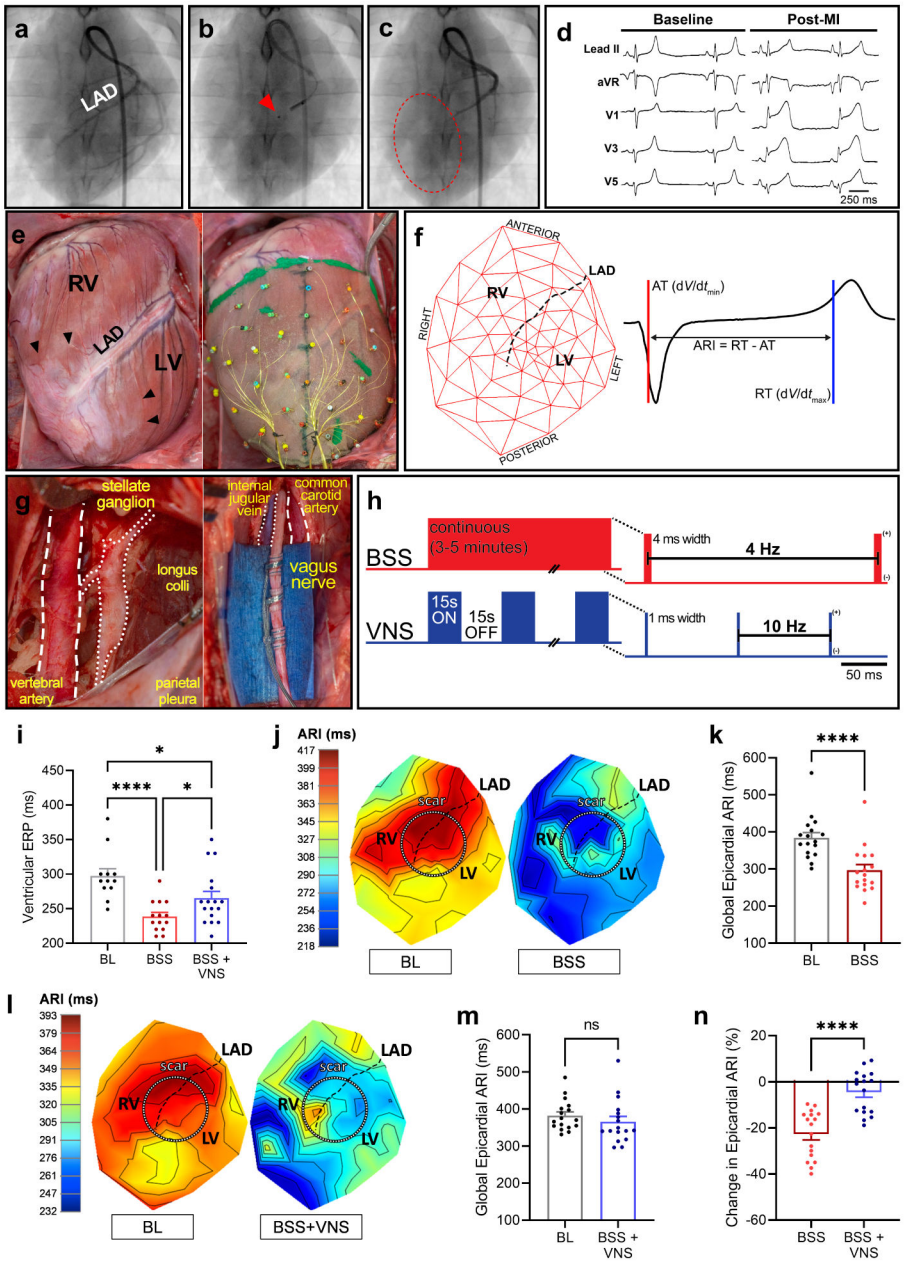

Figure 1. Ventricular epicardial effects of sympathetic stimulation and its attenuation by VNS following MI.

(a-b) Percutaneous creation of MI in region of the LAD coronary artery via injection of microspheres through a transluminal angioplasty catheter (red arrowhead indicates tip of catheter). **(c-d)** Following injection of microspheres, lack of flow was observed in the distal portion of the LAD (red circle), accompanied by ST-segment elevation. **(e)** Six to eight weeks after MI, prominent scarring of the anterior RV and LV was observed along with regions of patchy scar (black arrowheads) and a 56-electrode sock is placed around the ventricles to record unipolar electrograms. **(f)** Electrograms were then mapped onto a 2-D polar map for the assessment of regional electrophysiological differences. The difference in time between activation and recovery time (AT and RT, respectively) was

defined as ARI and used a surrogate of local action potential duration at each electrode/site. **(g-h)** The stellate ganglia and cervical vagus nerves were isolated for bipolar stimulation. Stimulation of the bilateral stellate ganglia are performed at 4 Hz for 3–5 minutes with and without concomitant 10 Hz VNS (50% duty cycle). **(i)** ERP from the RV endocardium was significantly shortened by BSS. Concomitant bilateral VNS reduced effects on ERP. **(j-k)** Representative polar maps at baseline (BL) and during BSS indicate a shortening of ARIs in infarct and remote regions of the RV and LV. Accordingly, a significant shortening of global epicardial ARIs across animals was observed. **(l-m)** Representative polar map at BL and during BSS+VNS suggests attenuation of BSS-induced ARI shortening. **(n)** Changes in epicardial ARIs from baseline (pre-stimulation) with BSS were significantly attenuated by concomitant VNS. BSS=bilateral stellate stimulation, VNS=bilateral vagus nerve stimulation, ERP=effective refractory period. Data are shown as mean ± SEM (n = 17). *p<0.05, ****p<0.0001, ns=not significant.

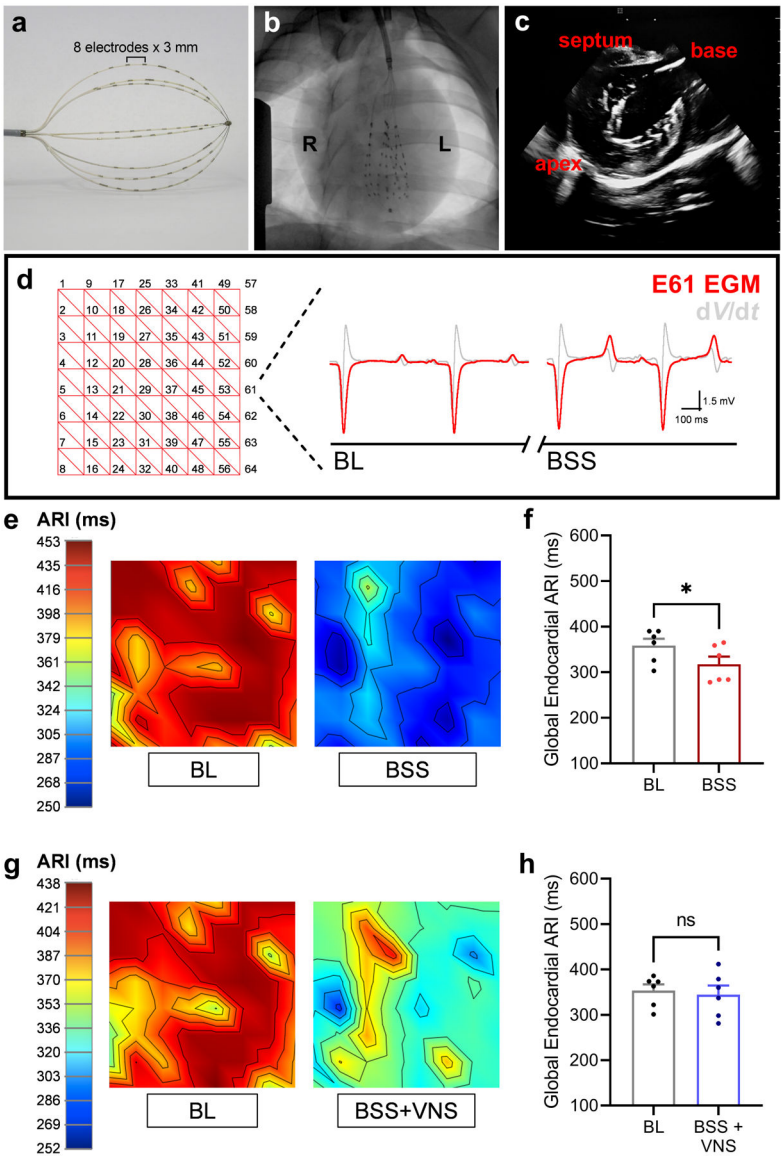

Figure 2. Ventricular endocardial effects of sympathetic stimulation and attenuation by VNS.

(a-c) A 64-electrode Constellation catheter was advanced into the LV for assessment of local endocardial unipolar electrograms. Sufficient contact of the basket catheter with the endocardium was confirmed by echocardiography. **(d)** Local unipolar electrograms from the endocardium were mapped onto a 2-D polar map. **(e)** A representative polar map of ARIs at baseline and during BSS indicates significant shortening of endocardial ARIs. **(f)** There was no significant ARI change during BSS+VNS relative to baseline. **(g-h)** BSS-induced shortening in endocardial ARI was significantly blunted by concomitant bilateral VNS. BSS=bilateral stellate stimulation, VNS=bilateral vagus nerve stimulation. Data are shown as mean ± SEM ($n = 6$). *$p<0.05$.

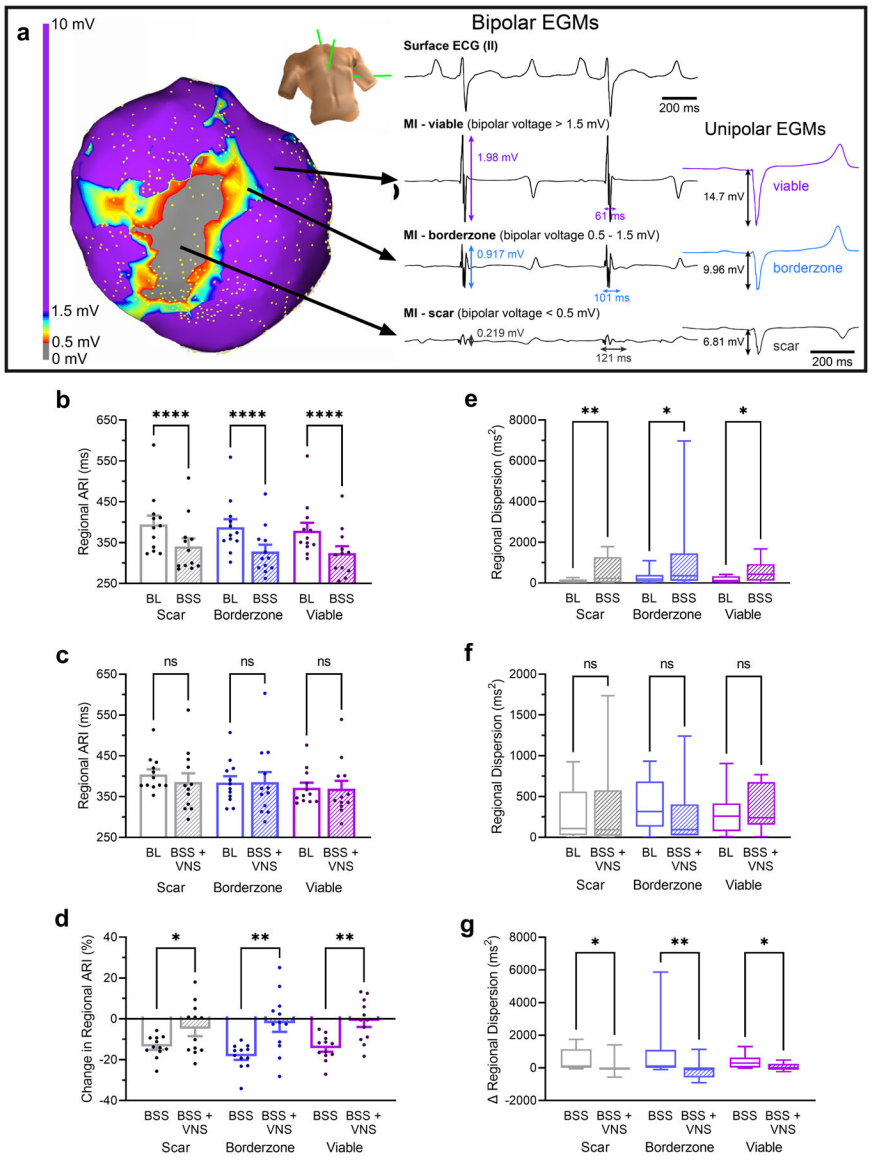

Figure 3. VNS mitigates ventricular ARI shortening and heterogeneities induced by sympathetic activation.

(a) Representative electroanatomic map confirms the presence of a dense antero-apical scar encircled by broad regions of heterogenous electrical border zone and remote, healthy myocardium. Based on the bipolar voltage at each site, electrodes were designated as viable (> 1.5 mV), border zone (0.5 – 1.5 mV) or scar (< 0.5 mV) with sample electrograms shown. **(b)** BSS induced significant shortening of ARIs in all regions **(c-d)** and these effects were significantly attenuated by concomitant VNS. **(e)** Importantly, BSS led to significant increases in regional dispersion, a measure of local heterogeneity in ARIs. **(f-g)** Increases in regional dispersion with BSS were attenuated by concomitant bilateral VNS. BSS=bilateral stellate stimulation, VNS=bilateral vagus nerve stimulation. Regional ARI data are shown as mean ± SEM and regional dispersion as median and interquartile range ($n = 12$). *p<0.05, **p<0.01, ****p<0.0001, ns=not significant.

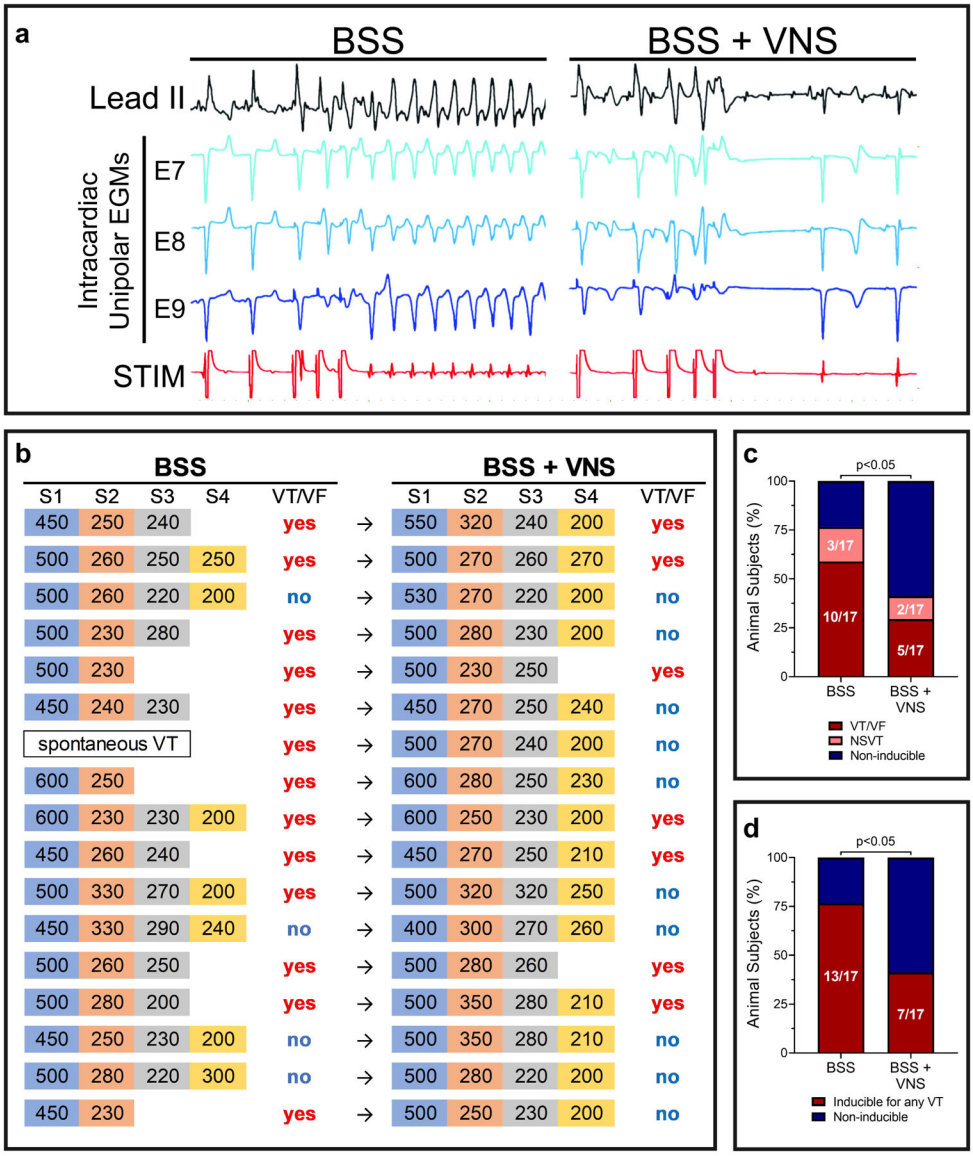

Figure 4. VNS reduces ventricular arrhythmias despite sympathoexcitation.

(a) Representative examples of programmed electrical stimulation during BSS alone or with concomitant VNS. **(b)** Pacing protocols used to induce VT/VF in each animal are shown. **(c-d)** During BSS alone, 13 of 17 animals were inducible. Concomitant VNS caused a significant reduction in VT inducibility with only 7 animals remaining inducible for VT. One animal had spontaneous VT during BSS without any extra-stimulus pacing. BSS=bilateral stellate stimulation, VNS=bilateral vagus nerve stimulation, NSVT=non-sustained VT.

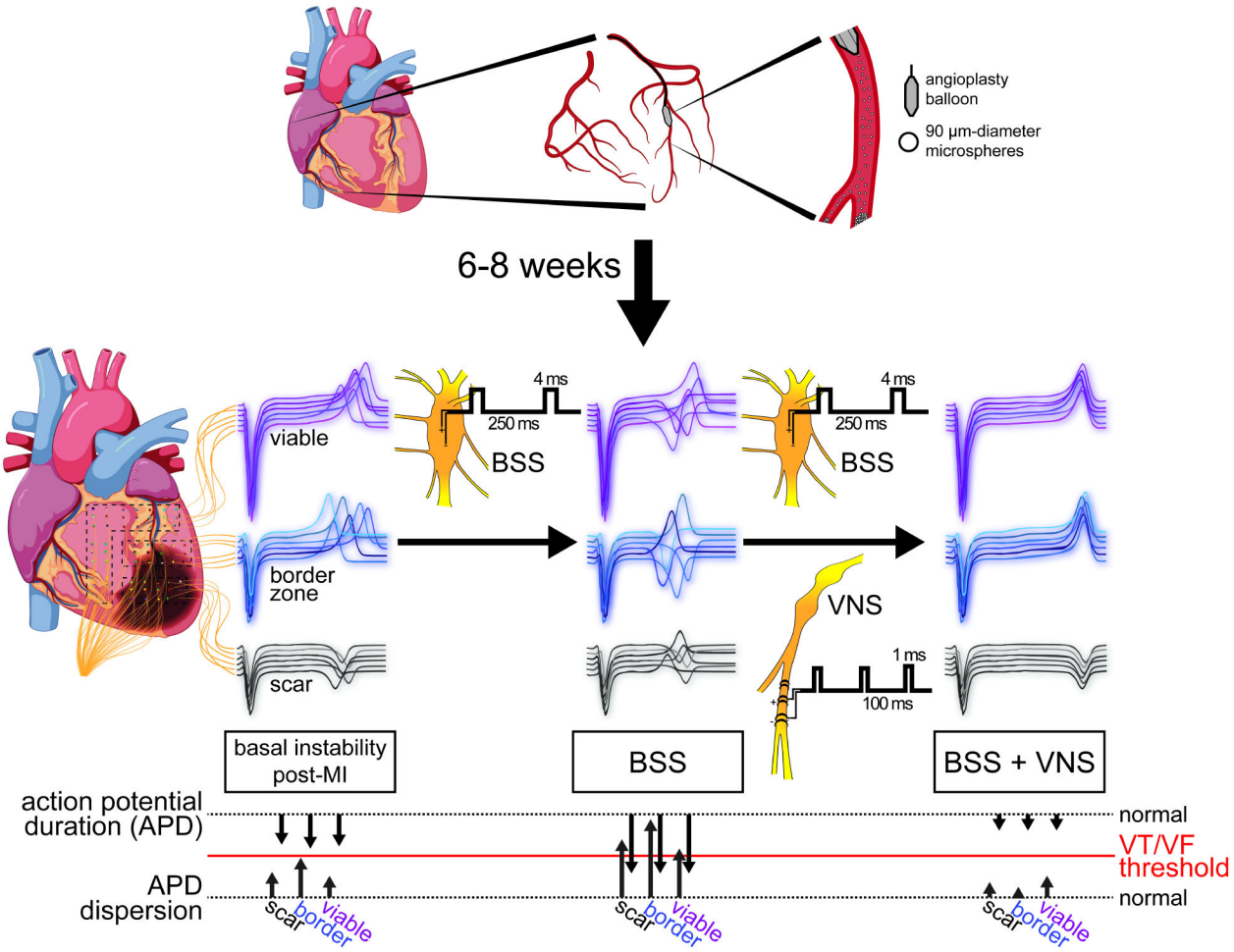

Figure 5. Central Illustration. Summary Figure:

Sympathoexcitation exacerbates baseline electrophysiological heterogeneities in chronically infarcted hearts, predisposing to VT/VF. These heterogeneities are reduced back to baseline, and even further mitigated below baseline in border zone regions, by intermittent vagal nerve stimulation. Vagal nerves stimulation reduces VT inducibility during states of elevated sympathetic tone.

Table 1.

Hemodynamic Responses to Sympathetic Stimulation Alone and Concurrent Vagal Nerve Stimulation With Sympathetic Stimulation

	BL	BSS	%	BL	BSS + VNS	%
Heart rate, beats/min	79.6 ± 13.1	**104.7 ± 21.2** [b]	32.0 ± 20.1	80.3 ± 9.0	77.4 ± 14.5	*–3.2 ± 17.2* [d]
Systolic pressure, mm Hg	113.0 ± 18.0	**154.6 ± 39.0** [b]	36.1 ± 31.8	110.7 ±18.7	**131.1 ± 34.5** [b]	*17.1 ± 17.9* [c]
Diastolic pressure, mm Hg	73.5 ± 18.8	**106.1 ± 27.4** [b]	54.8 ± 73.3	74.4 ± 18.3	84.5 ± 28.9	*11.4 ± 27.7* [c]
Mean arterial pressure, mm Hg	87.1 ± 18.0	**122.6 ± 20.3** [b]	44.1 ± 24.2	86.5 ± 17.5	**100.3 ± 29.9** [a]	*13.0 ± 20.9* [d]

Values are shown as mean ± SD. **Bold** values denote a significant increase; and *italic* values denote a significant decrease.

[a] $P < 0.05$ and

[b] $P < 0.001$ *vs* BL.

[c] $P < 0.05$ and

[d] $P < 0.001$ *vs* BSS alone.

BL = baseline; BSS = bilateral stellate ganglia stimulation; VNS = bilateral vagus nerve stimulation.