

ANESTHESIOLOGY

GABAergic Signaling during Spinal Cord Stimulation Reduces Cardiac Arrhythmias in a Porcine Model

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Neuraxial modulation, including spinal cord stimulation, has been shown to decrease cardiac sympathoexcitation and reduce ventricular arrhythmogenesis
- There is an incomplete understanding of the molecular mechanisms through which spinal cord stimulation modulates cardiospinal neural pathways

What This Article Tells Us That Is New

- This study of Yorkshire pigs found that spinal cord stimulation reduces myocardial ischemia–reperfusion–induced myocardial sympathetic excitation and ventricular arrhythmias through γ -aminobutyric acid–mediated pathways in the thoracic spinal cord

Autonomic nervous system imbalances play a major role in the pathophysiology of myocardial ischemia induced ventricular arrhythmias and sudden cardiac death.^{1–4} After

ABSTRACT

Background: Neuraxial modulation, including spinal cord stimulation, reduces cardiac sympathoexcitation and ventricular arrhythmogenesis. There is an incomplete understanding of the molecular mechanisms through which spinal cord stimulation modulates cardiospinal neural pathways. The authors hypothesize that spinal cord stimulation reduces myocardial ischemia–reperfusion–induced sympathetic excitation and ventricular arrhythmias through γ -aminobutyric acid (GABA)–mediated pathways in the thoracic spinal cord.

Methods: Yorkshire pigs were randomized to control ($n = 11$), ischemia–reperfusion ($n = 16$), ischemia–reperfusion plus spinal cord stimulation ($n = 17$), ischemia–reperfusion plus spinal cord stimulation plus γ -aminobutyric acid type A (GABA_A) or γ -aminobutyric acid type B (GABA_B) receptor antagonist (GABA_A, $n = 8$; GABA_B, $n = 8$), and ischemia–reperfusion plus GABA transaminase inhibitor (GABA_{in}, $n = 8$). A four-pole spinal cord stimulation lead was placed epidurally (T1 to T4). GABA modulating pharmacologic agents were administered intrathecally. Spinal cord stimulation at 50 Hz was applied 30 min before ischemia. A 56-electrode epicardial mesh was used for high-resolution electrophysiologic recordings, including activation recovery intervals and ventricular arrhythmia scores. Immunohistochemistry and Western blots were performed to measure GABA receptor expression in the thoracic spinal cord.

Results: Cardiac ischemia led to myocardial sympathoexcitation with reduction in activation recovery interval (mean \pm SD, $-42 \pm 11\%$), which was attenuated by spinal cord stimulation ($-21 \pm 17\%$, $P = 0.001$). GABA_A and GABA_B receptor antagonists abolished spinal cord stimulation attenuation of sympathoexcitation (GABA_A, $-9.7 \pm 9.7\%$, $P = 0.043$ vs. ischemia–reperfusion plus spinal cord stimulation; GABA_B, $-13 \pm 14\%$, $P = 0.012$ vs. ischemia–reperfusion plus spinal cord stimulation), while GABA_{in} alone caused a therapeutic effect similar to spinal cord stimulation ($-4.1 \pm 3.7\%$, $P = 0.038$ vs. ischemia–reperfusion). The ventricular arrhythmia score supported these findings. Spinal cord stimulation during ischemia–reperfusion increased GABA_A receptor expression with no change in GABA_B receptor expression.

Conclusions: Thoracic spinal cord stimulation reduces ischemia–reperfusion–induced sympathoexcitation and ventricular arrhythmias through activation of GABA signaling pathways. These data support the hypothesis that spinal cord stimulation–induced release of GABA activates inhibitory interneurons to decrease primary afferent signaling from superficial dorsal horn to sympathetic output neurons in the intermediolateral nucleus.

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activated and synapse in the dorsal horn of the thoracic spinal cord, initiating a complex cardiospinal neural circuit and reflex efferent sympathoexcitation.⁵ The increased spinal sympathetic nervous system output leads to acute physiologic changes in cardiac electrophysiology as well as long-term neuronal remodeling of the intrathoracic, extracardiac ganglia, and the intrinsic cardiac nervous system.^{6–8}

Neuromodulation therapy with spinal cord stimulation of the high-thoracic spinal cord has been shown to have cardiac antiarrhythmic effects.^{9–12} Spinal cord stimulation therapy is postulated to reduce sympathetic afferent neural signaling induced by myocardial ischemia in the dorsal horn and stabilize efferent outflows to cardiac tissues, thus reducing ventricular arrhythmias during ischemia.¹³ We and others have previously reported that spinal cord stimulation therapy can improve ventricular arrhythmias and cardiac function through a reduction in local sympathetic nerve activation in ischemic myocardium and reactive gliosis in the spinal cord.^{12,14} However, the mechanisms through which spinal cord stimulation modulates neural signaling and cardiac sympathoexcitation have not been elucidated.

One possible mechanism through which spinal cord stimulation may affect neural signaling in the spinal cord is γ -aminobutyric acid (GABA)–mediated pathways.^{15–19} GABA functions through activation of γ -aminobutyric acid type A (GABA_A) and γ -aminobutyric acid type B (GABA_B) receptors. Both receptor subtypes are found in the spinal cord; however, there are important differences in structure, anatomic location, and function between the receptor subtypes.^{20,21} In a rodent model of neuropathic pain, spinal cord stimulation was found to work through GABA release in the dorsal horn,²² and clinically, the use of intrathecal GABA_B receptor agonists enhanced the response to spinal cord stimulation for nonresponder subjects.²³ Further studies investigating the role of γ -aminobutyric acid–mediated (GABAergic) pathways in pain literature found differential effects of peripheral nerve injury and spinal cord stimulation models on GABA_A versus GABA_B receptors.^{21,24–26}

Thus, the goal of this study is to determine the role of GABA in the therapeutic effects of spinal cord stimulation to reduce sympathetic excitation and ventricular arrhythmias during myocardial ischemia–reperfusion. We hypothesize that spinal cord stimulation reduces ventricular arrhythmias through GABA–mediated pathways in the thoracic spinal cord. Our primary aim was to determine the effect of GABA on cardiac sympathoexcitation and arrhythmias during ischemia–reperfusion, with and without spinal cord stimulation, through a series of functional experiments in which GABA receptors in the spinal cord were pharmacologically blocked and augmented while the effects of spinal cord stimulation on cardiac sympathoexcitation and ventricular arrhythmogenesis were quantified in a translational large animal porcine model. Secondarily, given the possible differential effect of spinal cord stimulation on GABA receptor–mediated pathways, we investigated changes in

GABA_A and GABA_B receptor expression in the thoracic spinal cord with spinal cord stimulation. These data could provide mechanistic insight in the protective role of spinal cord stimulation on ventricular arrhythmias, thus helping clinical translation of spinal cord stimulation therapy.

Materials and Methods

The study protocol was approved by the Institutional Animal Research Committee at the University of Pittsburgh (Pittsburgh, Pennsylvania). All experiments were performed in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All experiments were performed between daylight hours 6:30 AM and 19:00 PM. Our report and study followed the appropriate Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines (Supplemental Document, <https://links.lww.com/ALN/D46>).

Experimental Protocols

An overview of experimental approach and timeline of experimental protocols are shown in figure 1. Yorkshire pigs (n = 68, 34 males and 34 females, mean age 4 months) were used in this study. In phase one of our experimental protocols, animals were first randomly assigned into three groups—control, ischemia–reperfusion, and ischemia–reperfusion plus spinal cord stimulation—to establish a model of acute ischemia with and without spinal cord stimulation. Then, in phase two, animals were randomized into all six experimental groups to yield the following final sample sizes: control (n = 11, mean \pm SD 46 \pm 9 kg), ischemia–reperfusion (n = 16, 41 \pm 5 kg), ischemia–reperfusion plus spinal cord stimulation (n = 17, 44 \pm 6 kg), Ischemia–reperfusion plus spinal cord stimulation plus GABA_A receptor antagonist (GABA_A, n = 8, 40 \pm 5 kg), ischemia–reperfusion plus spinal cord stimulation plus GABA_B receptor antagonist (GABA_B, n = 8, 40 \pm 4 kg), and ischemia–reperfusion plus GABA transaminase inhibitor (GABA_{in}, n = 8, 49 \pm 3 kg). There was one animal death in the ischemia–reperfusion plus spinal cord stimulation plus GABA_B receptor antagonist group before protocol completion. Data are reported on n = 7 animals in this group. Animals in the control group underwent the same surgical preparation and time course as experimental groups; however, no cardiac ischemia or spinal cord stimulation was performed. In the ischemia–reperfusion and ischemia–reperfusion plus spinal cord stimulation groups, the animals had a spinal cord stimulation catheter placed and cardiac ischemia performed, but only the ischemia–reperfusion plus spinal cord stimulation group had the catheter turned on during the protocol. In the GABA_A and GABA_B antagonist groups, the animals had intrathecal and spinal cord stimulation catheters placed, with spinal cord stimulation therapy on. GABA antagonists were applied, and cardiac ischemia was performed

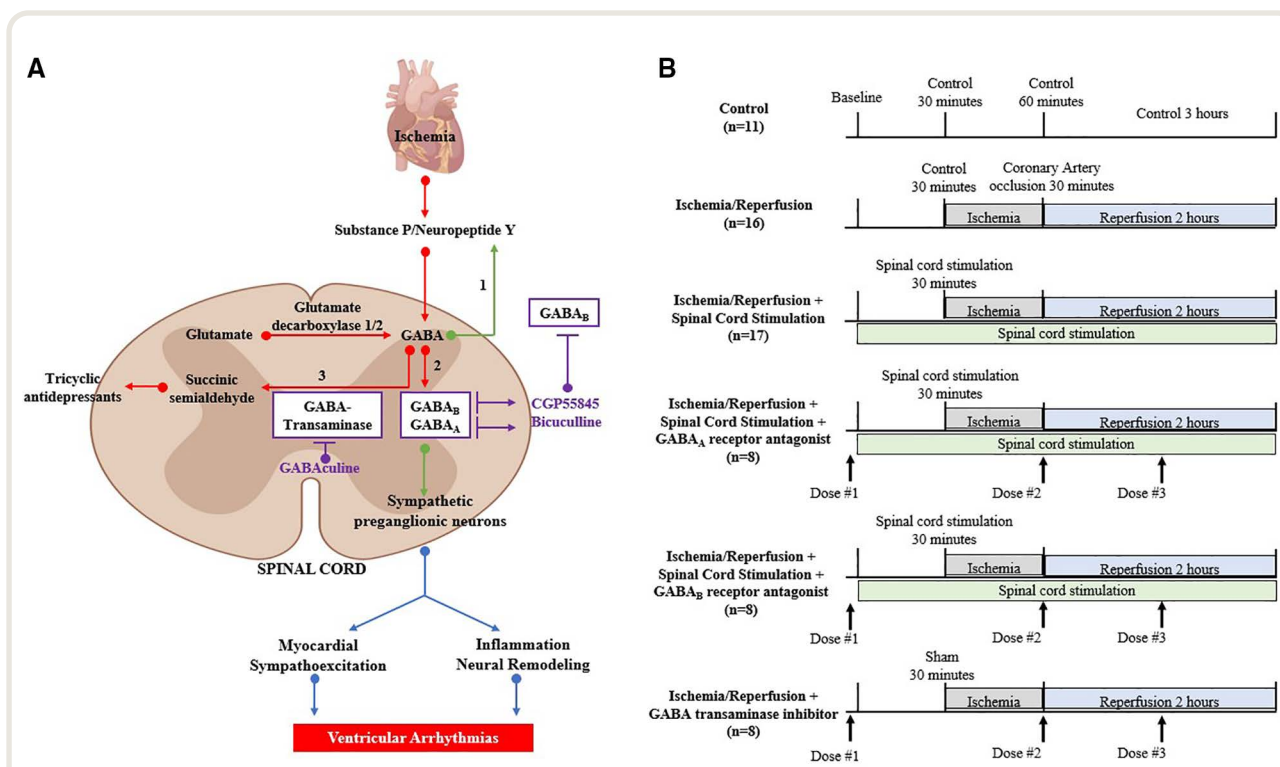


Fig. 1. Central illustration and timeline of experimental protocol. (A) Proposed pathway for ischemia-induced sympathoexcitation, and 1 to 3 are proposed sites for spinal cord stimulation γ -aminobutyric acid (GABA)-mediated inhibition of cardiac sympathoexcitation. (B) Experimental protocol and treatment groups for spinal cord stimulation mechanistic investigation Yorkshire pigs were randomized to six groups: control, ischemia–reperfusion, ischemia–reperfusion plus spinal cord stimulation, ischemia–reperfusion plus spinal cord stimulation plus γ -aminobutyric acid type A (GABA_A) antagonist bicuculline, ischemia–reperfusion plus spinal cord stimulation plus γ -aminobutyric acid type B (GABA_B) antagonist CGP55845, and ischemia–reperfusion plus GABA transaminase inhibitor groups. Spinal cord stimulation was initiated 30 min before ischemia. The intrathecal GABA antagonists were each applied 5 min before the start of spinal cord stimulation and reapplied every 60 min after that. Intrathecal GABA transaminase inhibitor was applied after baseline control measures and reapplied every 60 min.

as described. In the GABA_A group, the animals had intrathecal and spinal cord stimulation catheters placed, spinal cord stimulation was not turned on, GABA transaminase inhibitor was applied, and cardiac ischemia was performed as described.

Animal Preparation

Animal experimental preparation was conducted as previously described.¹² Animals were sedated with Telazol (Zoetis, USA; 4 to 8 mg/kg, intramuscular), intubated, and mechanically ventilated with oxygen. General anesthesia was induced and maintained with inhaled isoflurane (1 to 3%) during surgical preparation. Heart rate (HR) and surface electrocardiogram (ECG) were monitored throughout the experiment using a Prucka CardioLab recording system (GE Healthcare, USA). The carotid and femoral arteries were catheterized for blood pressure monitoring. In addition, jugular and femoral veins were cannulated for IV saline infusion (10 ml/kg) and drug administration. To maintain acid–base equilibrium, arterial blood gas was tested hourly with adjustment of

ventilation as necessary. Body temperature was maintained by an external warmer. Animals were placed in the prone position and underwent partial laminectomy to expose the spinal cord. They were then placed in the supine position for median sternotomy to expose the heart. After the completion of surgical preparation, animals were placed in the left lateral decubitus position, and general anesthesia was transitioned to IV α -chloralose (50 mg/kg initial bolus followed by a 20 mg \cdot kg⁻¹ \cdot h⁻¹ continuous infusion). Use of IV α -chloralose as an anesthetic has been previously shown to be least disruptive of autonomic nervous system activity and has been used extensively in investigational studies.²⁷ The depth of anesthesia was assessed throughout the experiments by monitoring corneal reflexes, jaw tone, and hemodynamic indices. In the end, animals were euthanized by injection of potassium chloride.

Acute Myocardial Ischemia

We created acute myocardial ischemia as previously described.^{27–29} Briefly, a Prolene suture (Ethicon, USA) was

placed around the left anterior descending coronary artery (LAD) below the second diagonal branch of the LAD. The suture was led through a short polyethylene tubing segment, which was then used to ligate the coronary artery to induce cardiac ischemia for 1 h. Ischemia was confirmed by the presence of ST segment elevations. After 1 h of ischemia or when the pig had nonresuscitable pulseless ventricular tachycardia or ventricular fibrillation (defined as the lack of conversion to a perfusing rhythm after defibrillation 10 times), the suture was removed, and reperfusion was permitted for 2 h. When pulseless ventricular tachycardia or ventricular fibrillation occurred during ischemia, resuscitation efforts were applied to the animal in accordance with Advanced Cardiac Life Support (ACLS) guidelines.

Spinal Cord Stimulation

A four-pole spinal cord stimulating lead was inserted in the epidural space, with the lead located at the thoracic spinal cord level 1 to 4 and the most cranial pole of the lead at thoracic spinal cord level 1. Current controlled stimulation (model S88 stimulator, Grass Instruments, USA) was delivered at 50 Hz and 0.4-ms pulse duration starting 30 min before ischemia and was continued throughout the ischemia–reperfusion protocol. Stimulation currents were set at 90% of motor threshold, which was determined by increasing stimulus intensity with 2 Hz of frequency and 0.4-ms pulse duration until muscle contractions were observed in the shoulder. The mean \pm SD motor threshold was 1.3 ± 1.0 mA.

Intrathecal Administration GABA_{A/B} Receptor Antagonists and GABA Transaminase Inhibitor

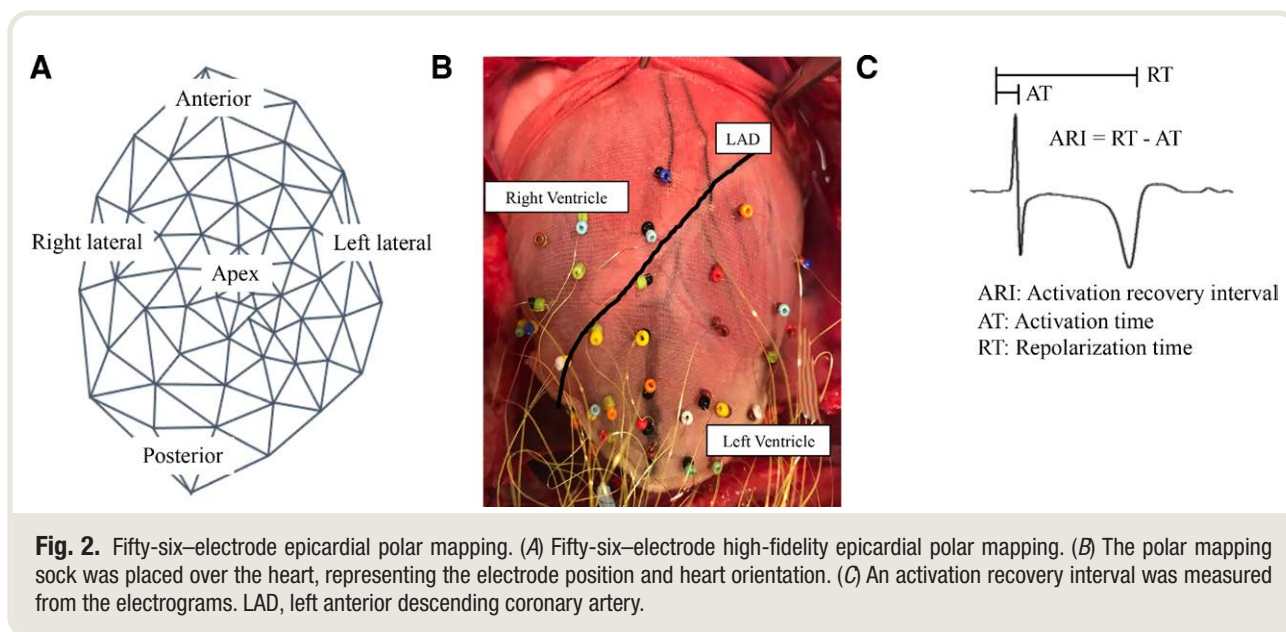
GABA receptor antagonists and GABA transaminase inhibitor were delivered *via* an intrathecal catheter placed at thoracic T1 to T4 spinal level inserted through a small incision in dura mater at thoracic spinal level 5. The lowest therapeutic dose was chosen based on the literature.^{30–33} For the GABA_{A/B} receptor antagonists, 1,000 μ g GABA_A antagonist Bicuculline (Sigma–Aldrich, USA) or 3,000 μ g GABA_B antagonist CGP55845 (Sigma–Aldrich) was dissolved in 2 ml normal saline and warmed to 37°C, and was infused more than 5 minutes using a syringe pump. Given that the peak dorsal horn drug concentration occurs 30 min after intrathecal administrations, each antagonist was applied 30 min before starting ischemia–reperfusion and reapplied at 60-min intervals. GABA_A was used as inhibition of GABA transaminase reduces the degradation of GABA leading to increased neuronal GABA concentrations.³⁴ GABA_A (5 mg; Enzo, USA) was dissolved in 1 ml dimethyl sulfoxide and 4 ml saline, and 2 mg GABA_A was infused more than 5 min using a syringe pump, 30 min before ischemia–reperfusion, with no spinal cord stimulation, and reapplied at 60-min intervals.

Hemodynamic Assessment and Surface ECG Recordings

We performed hemodynamic assessment and ECG recording as previously described.¹⁴ To measure left ventricular end-systolic and end-diastolic pressure throughout the experiment, a 12-pole conductance, high-fidelity pressure-monitoring pigtail catheter (5-French) was inserted into the left ventricle *via* the left carotid artery and connected to an MPVS Ultra Pressure Volume Loop System (Millar Instruments, USA). Left ventricular systolic function was evaluated by end-systolic pressure and maximum rate of pressure change (delta pressure/delta time maximum), and left ventricular diastolic function was evaluated by end-diastolic pressure and minimum rate of chamber pressure change (delta pressure/delta time minimum). ECG data were continuously recorded on Prucka CardioLab system. Precordial lead electrodes (V1 to V6) were positioned posteriorly in a manner that reflects standard anterior precordial lead electrode placement and records the horizontal plane.

Electrophysiologic Recordings and Analysis

A 56-electrode nylon mesh was placed around the heart, and unipolar electrograms (0.05 to 500 Hz) were measured using a Prucka CardioLab electrophysiology mapping system (fig. 2, A and B). All physiologic measures were recorded at baseline, during spinal cord stimulation, during acute ischemia (or until pulseless ventricular tachycardia or ventricular fibrillation requiring more than 10 internal cardiac defibrillation episodes), and throughout 2 h of reperfusion. We assessed the activation recovery interval, which has been shown to be a surrogate of local action potential duration (fig. 2C). Activation recovery intervals were calculated with customized software (iScalDyn, University of Utah, USA) as previously described.¹⁴ Sympathetic stimulation is associated with shortened activation recovery interval duration. In this study, activation recovery interval was analyzed by whole heart and regionally in the ischemic and nonischemic zones of the myocardium, as defined by whether the distribution of the left anterior descending coronary artery was perfused. The percentage of ischemic myocardium was calculated as the area at risk within the ventricles. To ensure accuracy of activation recovery interval measurement, each electrogram with ST segment changes was both measured by semiautomated accepted software and then checked by hand following the guidelines described by Haws and Lux for activation recovery interval measurement in ischemia and carefully measured across four to five beats.¹⁴ All electrophysiologic and hemodynamic measurements were made offline by investigators blinded to the experimental group. Measurements were calculated every 15 min from baseline to end of recording in blinded fashion.



ECG-based Arrhythmia Scoring System and Individual Arrhythmias

Ventricular arrhythmias, which include premature ventricular contractions, ventricular tachycardia, and ventricular fibrillation, were counted using the Prucka CardioLab system. Premature ventricular contractions were identified by the presence of a premature QRS complex, and ventricular tachycardia was classified as three or more consecutive premature ventricular contractions in accordance with the recommendations of the Lambeth Conventions.³⁵ An arrhythmia score was calculated for each animal throughout ischemia and reperfusion. To calculate the arrhythmia score, a clinically based ECG scoring method was used, which was adapted from Curtis and Walker.³⁶ We evaluated the following components and formulated the score as described: “0: no premature ventricular contractions, ventricular tachycardia, or ventricular fibrillation,” “1: premature ventricular contractions,” “2: one to five episodes of ventricular tachycardia,” “3: more than five episodes of ventricular tachycardia or one episode of ventricular fibrillation,” “4: two to five episodes of ventricular fibrillation,” and “5: more than five episodes of ventricular fibrillation.” The scoring system assigns a numeric value based upon the severity of arrhythmia, with larger scores representing greater severity.³⁷

Heart Staining and Measurement of Ischemic Region (Area at Risk)

To determine potential ischemic insults, Evans blue was used for the heart staining as previously described.³⁸ At the end of the experiment after the animal was euthanized, the LAD ligation was tightened again, and a cross clamp was placed on the aorta just above the base of the heart with the animal in the supine position. We made sure that the aorta was completely sealed in order to prevent the dye from

leaking out of the heart. Evans blue dye was injected *via* the needle punctured right below the cross clamp. The area at risk was defined as the area not stained by Evans blue.

Immunohistochemistry and Image Analysis

Sections from spinal thoracic segment T3 were used for double labeling with neuronal nuclei plus GABA_A and GABA_B receptor antibodies to measure the change in GABA plus neurons in the thoracic spinal cord during ischemia–reperfusion with and without spinal cord stimulation. Immediately upon collection, spinal cord tissues were placed in 4% paraformaldehyde (Thermo Scientific, USA) at 4°C for about 48 h, followed by a 30% buffered sucrose solution that contained approximately 0.01% sodium azide. After the tissues sank in sucrose, they were embedded in optimal cutting temperature compound (Fisher, USA) and stored in an –80°C freezer before use. The frozen samples were cut at 35-μm thickness by using a cryostat (CryoStar NX 50; Thermo Fisher Scientific, USA) and washed in phosphate-buffered saline (pH 7.4) before blocking in a 5% normal goat or donkey blocking serum (phosphate-buffered saline containing 0.3% Triton X-100) blocking buffer at room temperature for 1 h. The slices were first incubated in anti-GABA_A or anti-GABA_B antibody (Supplemental Table, <https://links.lww.com/ALN/D47>) in a phosphate-buffered saline containing 0.3% Triton X-100 solution overnight at 4°C, and then transferred to anti-neuronal nuclei antibodies solution overnight at 4°C before the secondary antibodies in phosphate-buffered saline solution were applied (1 h at room temperature; Supplemental Table, <https://links.lww.com/ALN/D47>). The slices were rinsed three or four times (5 min per time) after each incubation of antibodies. After they were mounted and coverslipped with mounting medium (4',6-diamidino-2-phenylindole; H-1500, Vector

Laboratories, USA), the slices were imaged by using a Nikon Eclipse Ti2 Inverted Microscope System and NIS-Elements AR Imaging Software v 5.10.01 (Nikon Instruments Inc., USA). The spinal cord sections were imaged with the $\times 20$ objective. All exposure times and processing procedures were identical across samples and treatment groups.

Image analysis was performed with the investigator blinded to the experimental group spinal cord section during the entire analysis, and protocols were standardized to avoid potential experimental bias. Analysis of images was completed using NIS-Elements AR Analysis Software v 5.10.01. A minimum of two spinal cord slices was used per animal. The spinal cord was divided into left and right regions of interest, and the number of immunoreactive cells was counted based on uniformly set thresholds across groups. Data were averaged for each animal and analyzed by group and anatomic region.

Western Blot

For further quantitative evaluation of GABA receptor expression in the spinal cord, we extracted the proteins from T3 dorsal horn and examined the expression of GABA receptors by subtypes—GABA_A receptor, GABA_B receptor, GABA_B receptor 1, and GABA_B receptor 2—using Western blot. The fresh spinal cord tissues of pig were dissected on wet ice, flash-frozen in liquid nitrogen, and stored in a -80°C freezer before use. The dorsal part of the frozen tissues was homogenized mechanically in ice-cold radioimmunoprecipitation assay lysis buffer (Thermo Fisher Scientific, USA) containing 1X Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific). Homogenates were centrifuged at 4°C for 10 min at 14,000 revolutions per minute, and the supernatant fraction was used to measure protein concentration with a Bradford Assay kit (Thermo Fisher Scientific) according to the manufacturer's instructions. A total protein concentration of $10\text{ }\mu\text{g}$ was applied to 4 to 20% Tris-Glycine eXtended precast protein gels (Bio-rad Laboratories, USA) using Tris-Glycine Sodium Dodecyl Sulfate running buffer (Thermo Fisher Scientific) at 200 voltage on ice for 1 h, and then transferred to polyvinylidene fluoride membranes (Thermo Fisher Scientific) at 100 voltage for 30 min at 4°C . Membranes were blocked with SuperBlock blocking buffer (Thermo Fisher Scientific) with Tween 0.05% for 1.5 h at room temperature, and then incubated at 4°C overnight with primary antibodies (Supplemental Table, <https://links.lww.com/ALN/D47>). To detect the primary antibody signals, horseradish peroxidase-conjugated secondary antibody and an enhanced chemiluminescence detection reagent (RPN2235; GE Healthcare, Buckinghamshire, United Kingdom) were applied before imaging. Membranes were then stripped by a Western blot stripping buffer (Thermo Fisher Scientific) for 10 min, blocked, and incubated for 1 h with glyceraldehyde-3-phosphate dehydrogenase antibody. The expression of glyceraldehyde-3-phosphate dehydrogenase was considered as a control and used to normalize the intensity levels of the target proteins. The membranes were

captured in an image analysis system (ChemiDoc XRS + System, Bio-rad), and the bands were quantified by densitometry using an image analysis program (Image Lab; Bio-rad).

Statistical Analysis

All data were examined for normality using the Shapiro-Wilk test. Data with normal distribution are expressed as mean \pm SD, and data with nonnormal distribution are presented as median and interquartile range. One-way repeated measures ANOVA with *post hoc* Tukey test was used for all within-group cardiac electrophysiologic (activation recovery interval) and hemodynamic measures. Mixed-effect models were used to assess cardiac electrophysiologic (activation recovery interval, arrhythmia score) and hemodynamic variables between experimental groups. We employed mixed effects models to examine the effect of time point (repeated measure) on raw values within testing conditions wherein subject number was treated as a random effect, and percent area at risk and sex were treated as fixed effects. We employed mixed effects models to compare the percent change in measures from baseline, between conditions, controlling for sex and percent ischemic area at risk, wherein condition was entered as a class variable in the model. For immunohistochemistry and Western blot analysis, one-way ANOVA with *post hoc* Tukey test was performed to compare the percentage of positive GABA_{A/B} neurons and GABA_{A/B} receptor subunit concentration between the groups. For all results, a *P* value < 0.05 was considered statistically significant. All figures were created using GraphPad Prism software (version 8; GraphPad Software Inc., USA). Calculation for sample size was based on preliminary data with a mean activation recovery interval of 450 ms, change of 20% from this mean, SD of 65, two-tailed alpha 0.05, and power 80% during acute ischemia between control and spinal modulation, which determined the sample size $n = 8$ per experimental group.

Results

Ischemia-Reperfusion Decreases Global Activation Recovery Interval

Cardiac electrophysiologic measures are reported at baseline, control 30 min, and control 60 min or spinal cord stimulation 30 min and LAD 30 min. Ischemia data are reported at 30 min as multiple animals had irretractable ventricular tachycardia/ventricular fibrillation requiring resuscitation as ischemia proceeded, resulting in incomplete data sets at 60 min. Comparing cardiac electrophysiologic measures across time points, within each group, myocardial ischemia led to expected cardiac sympathetic excitation as demonstrated by activation recovery interval duration shortening, in all experimental groups except the control group, which did not undergo cardiac ischemia-reperfusion (table 1). Comparing hemodynamic parameters within each group, HR increase was seen after ischemia in the GABA_A group, and spinal cord stimulation decreased maximal rate of rise of left ventricular pressure (delta pressure/delta time maximum)

Table 1. Global Activation Recovery Intervals

	Control	Ischemia–Reperfusion	Ischemia–Reperfusion Plus Spinal Cord Stimulation	GABA _A Receptor Antagonist	GABA _B Receptor Antagonist	GABA Transaminase Inhibitor
Baseline	376 ± 61	378 ± 65	405 ± 62	412 ± 101	437 ± 76	359 ± 27
Control 30 min or spinal cord stimulation 30 min	391 ± 66	389 ± 76	406 ± 71	407 ± 70	432 ± 75	360 ± 35
Coronary artery occlusion 30 min	395 ± 78	306 ± 63*	363 ± 71*	311 ± 36*	355 ± 69*	316 ± 43*
<i>P</i> value, baseline vs. LAD occlusion	0.353	<0.0001	0.002	0.029	0.036	0.008

Data are expressed as mean ± SD. Global activation recovery intervals in all groups, except control, reduced in ischemia compared to baseline. Control (n = 11), ischemia–reperfusion (n = 16), ischemia–reperfusion plus spinal cord stimulation (n = 17), GABA_A receptor antagonists: ischemia–reperfusion plus spinal cord stimulation with intrathecal GABA_A antagonist (n = 8), GABA_B receptor antagonists: ischemia–reperfusion plus spinal cord stimulation with intrathecal GABA_B antagonist (n = 7), GABA transaminase inhibitor: ischemia–reperfusion plus intrathecal GABA transaminase inhibitor (n = 8).

*Statistically significant, with each *P* value shown in the table.

GABA, γ -aminobutyric acid; GABA_A, γ -aminobutyric acid type A; GABA_B, γ -aminobutyric acid type B.

Table 2. Hemodynamics

		HR, beats/min	Systolic Blood Pressure, mmHg	Left Ventricular End Systolic Pressure, mmHg	Maximal Rate of Rise Left Ventricular Pressure (dP/dt max), mmHg/s
Control	Baseline	88 ± 26	110 ± 16	94 ± 18	2,308 ± 659
	Control 30 min	82 ± 18	109 ± 14	97 ± 21	2,250 ± 802
	Control 60 min	87 ± 22	112 ± 11	89 ± 19	2,144 ± 546
Ischemia–reperfusion	Baseline	88 ± 21	125 ± 17	102 ± 18	2,568 ± 919
	Control 30 min	86 ± 22	125 ± 17	100 ± 18	2,559 ± 959
	Coronary artery occlusion 30 min	92 ± 26	117 ± 18	95 ± 19	2,228 ± 775
Ischemia–reperfusion plus spinal cord stimulation	Baseline	82 ± 13	128 ± 21	106 ± 19	2,609 ± 1,207
	Spinal cord stimulation 30 min	82 ± 16	127 ± 22	103 ± 23	2,281 ± 708
	Coronary artery occlusion 30 min	78 ± 12	122 ± 18	104 ± 17	2,085 ± 682**
GABA _A receptor antagonist	Baseline	87 ± 17	125 ± 19	92 ± 5	2,475 ± 413
	Spinal cord stimulation 30 min	97 ± 19	125 ± 25	90 ± 3	2,326 ± 302
	Coronary artery occlusion 30 min	107 ± 16*	107 ± 23	84 ± 9	2,013 ± 438
GABA _B receptor antagonist	Baseline	79 ± 13	112 ± 11	94 ± 9	2,116 ± 435
	Spinal cord stimulation 30 min	75 ± 17	108 ± 10	99 ± 19	2,712 ± 1,595
	Coronary artery occlusion 30 min	78 ± 8	105 ± 12	106 ± 18	3,020 ± 1,265
GABA transaminase inhibitor	Baseline	84 ± 7	144 ± 15	123 ± 15	1,923 ± 642
	Sham 30 min	82 ± 8	148 ± 16	117 ± 18	1,902 ± 460
	Coronary artery occlusion 30 min	85 ± 9	141 ± 21	120 ± 18	1,689 ± 337

Data are expressed as mean ± SD. Within-group analysis showed that HR decreased during ischemia–reperfusion compared to 30 min into spinal cord stimulation in the GABA_A receptor antagonist group (**P* = 0.029), and maximal rate of rise on left ventricular pressure reduced in the ischemia–reperfusion plus spinal cord stimulation group (***P* = 0.038). All other *P* > 0.05 for within-group analysis. Control (n = 11), ischemia–reperfusion (n = 16), ischemia–reperfusion plus spinal cord stimulation (n = 17), GABA_A receptor antagonists: ischemia–reperfusion plus spinal cord stimulation with intrathecal GABA_A antagonist (n = 8), GABA_B receptor antagonists: ischemia–reperfusion plus spinal cord stimulation with intrathecal GABA_B antagonist (n = 7), GABA transaminase inhibitor: ischemia–reperfusion plus intrathecal GABA transaminase inhibitor (n = 8).

dP/dt max, delta pressure/delta time maximum; GABA, γ -aminobutyric acid; GABA_A, γ -aminobutyric acid type A; GABA_B, γ -aminobutyric acid type B; HR, heart rate.

during ischemia as compared to baseline. All other hemodynamics did not have any significant changes (table 2).

Ischemia–Reperfusion Decreases Percent Change in Activation Recovery Interval in Ischemic Myocardium

Activation recovery interval was analyzed regionally in the ischemic and nonischemic zones of the myocardium, and the changes in cardiac electrophysiological measures during cardiac ischemia–reperfusion were compared between

the five experimental groups: ischemia–reperfusion alone, ischemia–reperfusion plus spinal cord stimulation, ischemia–reperfusion plus spinal cord stimulation plus GABA_A receptor antagonist (GABA_A), ischemia–reperfusion plus spinal cord stimulation plus GABA_B receptor antagonist (GABA_B), and ischemia–reperfusion plus GABA_A aculine. The magnitude of cardiac ischemic insult was measured by the area at risk in the heart. There were no differences in area at risk between the groups (ischemia–reperfusion, 22 ± 12%; ischemia–reperfusion plus spinal cord stimulation, 26 ± 13%;

GABA_A, $31 \pm 10\%$; GABA_B, $25 \pm 8\%$; GABAculine, $22 \pm 9\%$; all $P > 0.207$; data presented as mean \pm SD). The magnitude of ischemia-induced sympathoexcitation, as determined by the change in activation recovery interval from baseline to 30-min LAD ligation, was compared between groups to see the effects of spinal cord stimulation alone as compared to spinal cord stimulation plus GABA antagonists and GABAculine during cardiac ischemia. Cardiac ischemia decreased activation recovery interval in the ischemic region, and this activation recovery interval reduction was mitigated by spinal cord stimulation (fig. 3A). The effect of spinal cord stimulation on activation recovery interval reduction in the ischemic myocardium was abolished by both intrathecal GABA receptor antagonists (GABA_A, $P = 0.043$ vs. spinal cord stimulation; GABA_B, $P = 0.012$ vs. spinal cord stimulation). While application of GABA transaminase inhibitor alone produced a reduction in activation recovery interval shortening that was similar in magnitude to that of spinal cord stimulation (fig. 3A). No activation recovery interval changes were seen in the nonischemic region between groups (fig. 3B).

For hemodynamic parameters, systolic blood pressure and maximal rate of rise of left ventricular pressure (delta pressure/delta time maximum), there were no differences between groups at 30 min after cardiac ischemia. However, the HR in the GABA_A group was greater than in the spinal cord stimulation and GABA_B groups ($P = 0.018$ vs. spinal cord stimulation; $P = 0.019$ vs. GABA_B). In addition, maximal rate of rise of left ventricular pressure (delta pressure/delta time maximum) in GABAculine was greater than in the control and GABA_A groups ($P = 0.043$ vs. control; $P = 0.022$ vs. GABA_A; table 1).

Ventricular Arrhythmia Score Changes during Ischemia-Reperfusion

Ventricular arrhythmia scores were calculated throughout ischemia and reperfusion and compared across all experimental groups. A higher arrhythmia score indicates greater arrhythmia severity. Ischemia-reperfusion was associated with an elevation in arrhythmia score, whereas spinal cord stimulation during ischemia decreased cardiac arrhythmias.

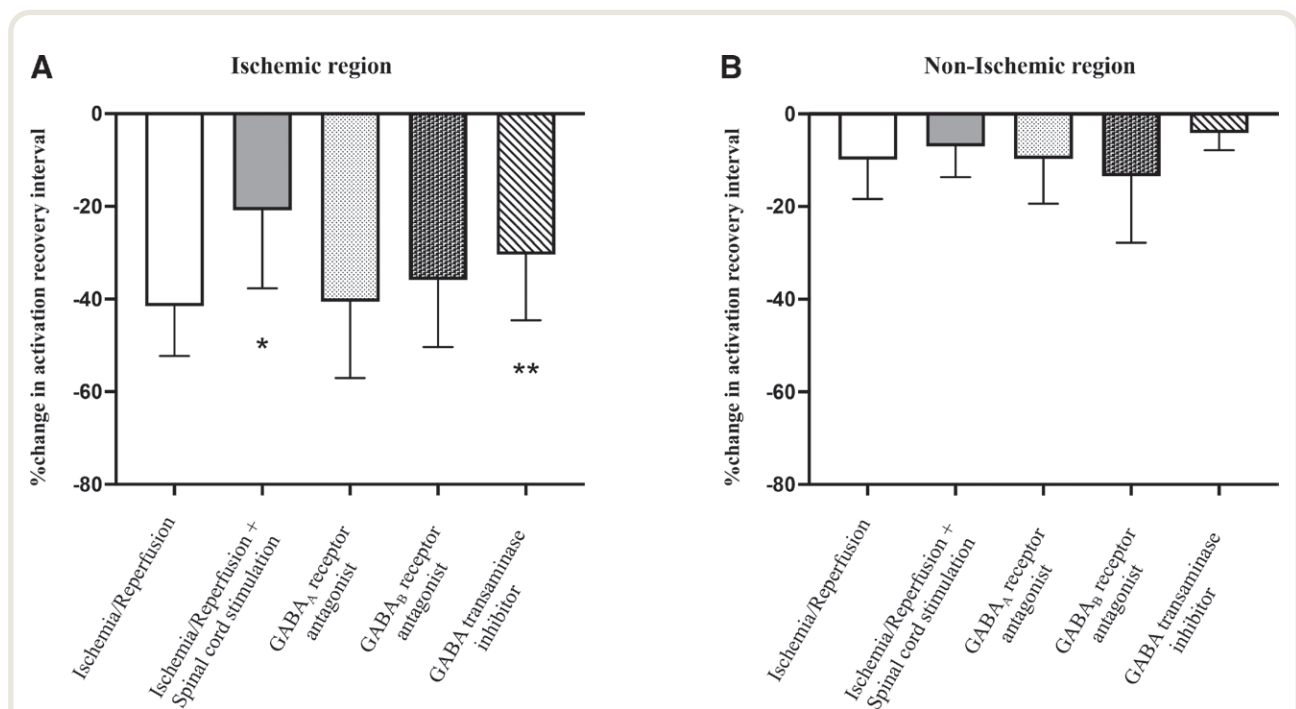


Fig. 3. Ischemia-reperfusion decreases percent change in activation recovery interval in ischemic myocardium. Within the ischemic region (A) ischemia-reperfusion plus spinal cord stimulation and GABA transaminase inhibitor (GABAculine) reduced percent activation recovery interval change, as compared to ischemia-reperfusion alone (* $P = 0.001$ ischemia-reperfusion -42 ± 11 percent vs. ischemia-reperfusion plus spinal cord stimulation -21 ± 17 percent; ** $P = 0.038$ ischemia-reperfusion -42 ± 11 percent vs. GABAculine -30 ± 14 percent; $P = 0.523$ ischemia-reperfusion -42 ± 11 percent vs. GABA_A -41 ± 17 percent; $P = 0.261$ ischemia-reperfusion -42 ± 11 percent vs. GABA_B -36 ± 15 percent). Within the nonischemic region (B), no significant differences were seen across the groups (all $P > 0.05$. Ischemia-reperfusion -10 ± 8 percent, ischemia-reperfusion plus spinal cord stimulation -7 ± 7 percent, GABA_A -10 ± 10 percent, GABA_B -13 ± 14 percent, GABAculine -4 ± 4 percent). Values expressed as means \pm SD. Ischemia-reperfusion ($n = 16$). Ischemia-reperfusion plus spinal cord stimulation ($n = 17$), GABA_A, ischemia-reperfusion plus spinal cord stimulation with intrathecal GABA_A antagonist ($n = 8$), GABA_B, ischemia-reperfusion plus spinal cord stimulation with intrathecal GABA_B antagonist ($n = 7$). GABAculine, ischemia-reperfusion plus GABAculine ($n = 8$).

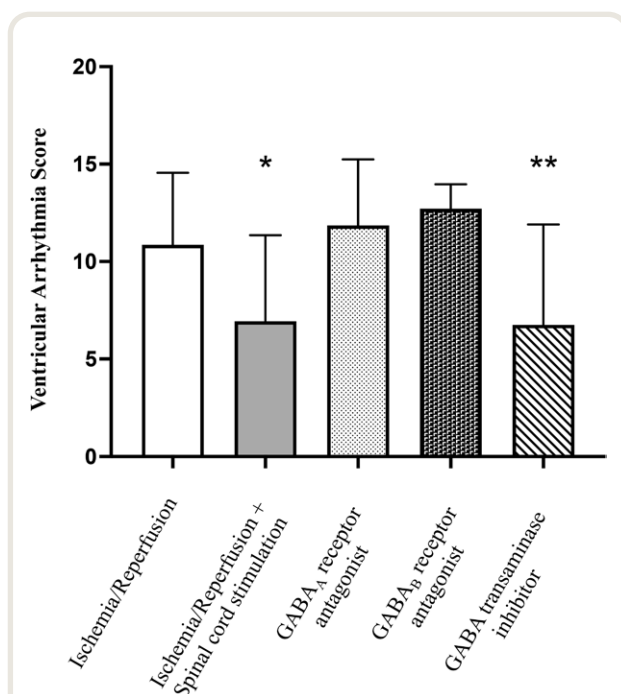


Fig. 4. Ventricular arrhythmia score changes during ischemia and reperfusion. Ischemia–reperfusion plus spinal cord stimulation plus intrathecal γ -aminobutyric acid (GABA) transaminase inhibitor GABAculine reduced ventricular arrhythmias as compared to ischemia–reperfusion alone. Values are expressed as median and interquartile range. * $P < 0.001$, ** $P = 0.003$. Ischemia–reperfusion ($n = 16$). Ischemia–reperfusion plus spinal cord stimulation ($n = 17$). γ -Aminobutyric acid type A (GABA_A): ischemia–reperfusion plus spinal cord stimulation with intrathecal GABA_A receptor antagonist ($n = 8$). γ -Aminobutyric acid type B (GABA_B): ischemia–reperfusion plus spinal cord stimulation with intrathecal GABA_B receptor antagonist ($n = 7$). GABAculine: ischemia–reperfusion plus GABA transaminase inhibitor ($n = 8$).

GABAculine treatment during ischemia–reperfusion also reduced the arrhythmia score, similar to that of spinal cord stimulation. On the other hand, spinal cord stimulation plus both intrathecal GABA receptor antagonists abolished the spinal cord stimulation reduction in cardiac arrhythmia score during myocardial ischemia–reperfusion (fig. 4).

Ischemia–Reperfusion Decreases GABA_A Receptor Expression, and This Reduction Was Less with Spinal Cord Stimulation

Immunohistochemistry. As demonstrated in figure 5, cardiac ischemia–reperfusion significantly reduced GABA_A receptor plus neurons (quantified as the percentage of neuronal nuclei-positive cells colocalized with GABA_A receptor). Spinal cord stimulation during ischemia–reperfusion showed a greater percentage of GABA_A plus neurons than ischemia–reperfusion alone; however, the expression was still less than in the control condition. In contrast, there was no change in expression of GABA_B receptor plus

neurons during ischemia–reperfusion with or without spinal cord stimulation (fig. 6). The anatomical distribution of GABA receptors was also investigated in the three regions: superficial dorsal horn laminae (I to II), deep laminae (III to VII, X), and the intermediolateral cell column as shown in figure 7. There was greater expression of GABA_A plus neurons with ischemia–reperfusion plus spinal cord stimulation, as opposed to ischemia–reperfusion alone, in all anatomical regions (fig. 7). No differences were seen in percentage of GABA_B plus neurons per anatomical region in ischemia–reperfusion *versus* ischemia–reperfusion plus spinal cord stimulation (ischemia–reperfusion: superficial, 25% [15–51]; deep, 14% [4–37]; intermediolateral nucleus, 11% [5–35] *versus* ischemia–reperfusion plus spinal cord stimulation: superficial, 26% [12–35]; deep, 18% [4–31]; intermediolateral nucleus, 9% [5–14]; all $P > 0.05$; data presented as median and interquartile range).

Western Blot. Myocardial ischemia alone did not affect the expression of GABA receptor subtypes. During ischemia–reperfusion with spinal cord stimulation, however, the expression of GABA_A receptor, GABA_B receptor subtypes was greater than the control and ischemia–reperfusion (fig. 8). Spinal cord stimulation did not affect the expression of either GABA_B receptor subtype.

Discussion

In this preclinical translational porcine model of cardiac ischemia–reperfusion injury with thoracic spinal cord stimulation, we show that (1) spinal cord stimulation therapy during cardiac ischemia reduced myocardial sympathoexcitation and ventricular arrhythmias, (2) intrathecal GABA_A and GABA_B receptor blockade during spinal cord stimulation therapy abolished the protective myocardial effects of spinal cord stimulation and increased sympathetic excitation and arrhythmias, (3) intrathecal administration of GABA transaminase inhibitor (GABAculine) reduced myocardial sympathoexcitation and ventricular arrhythmias during cardiac ischemia–reperfusion with similar magnitude to spinal cord stimulation, and (4) spinal cord stimulation neuromodulation during cardiac ischemia was associated with a significant increase in GABA_A receptor expression with no significant change in GABA_B receptor expression. Thus, these results importantly show that spinal cord stimulation is likely reducing ischemia–reperfusion–induced sympathoexcitation and cardiac arrhythmias through activation of spinal GABAergic pathways.

The cardiac electrophysiologic results from this study support our model of ischemia-induced activation of cardiospinal neural reflexes and the therapeutic effect of thoracic spinal cord stimulation in modulating sympathetic output and reducing cardiac arrhythmias. The cell bodies of ischemia-sensitive cardiac neurons are located in the thoracic dorsal root ganglion and project back to the dorsal

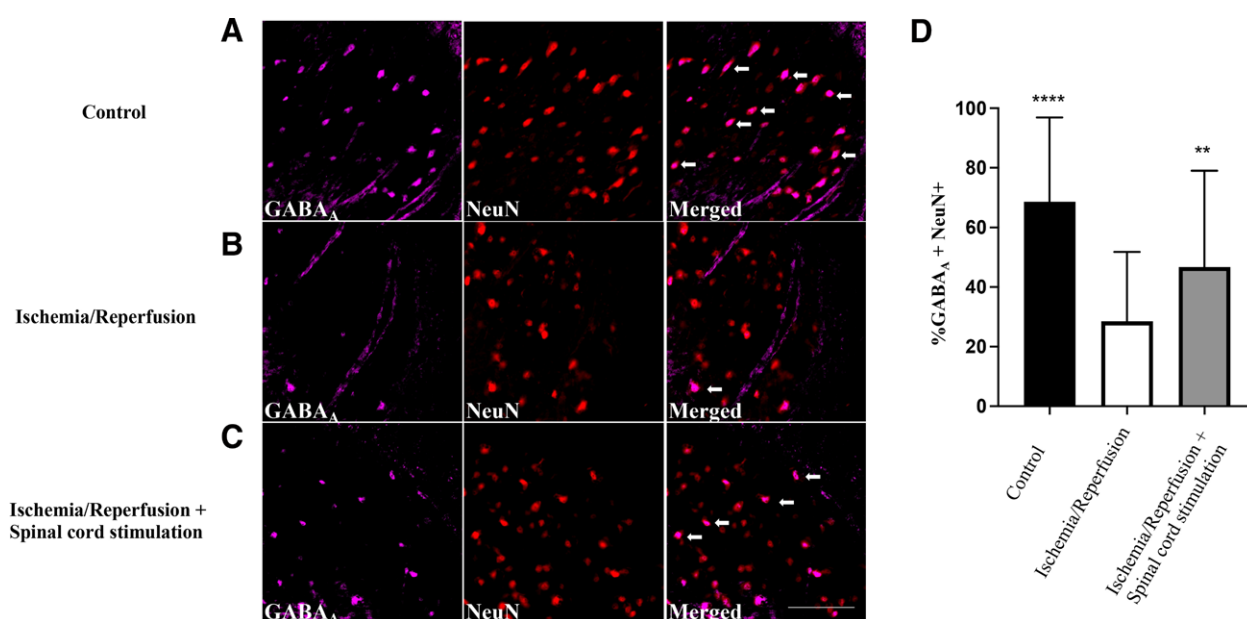


Fig. 5. Ischemia–reperfusion decreases γ -aminobutyric acid type A (GABA_A) receptor expression in the thoracic spinal cord, and this reduction was lesser in the presence of spinal cord stimulation. (A to C) Representative images from superficial dorsal horn laminae segment thoracic spinal cord T3. Magnification is 20 \times , and scale bar is 50 μ m. (D) Ischemia–reperfusion reduced the percentage of GABA_A plus neuronal nuclei plus neurons as compared to the control group with no injury; this reduction was lesser in the presence of spinal cord stimulation. Values expressed as mean \pm SD. ** P = 0.003, **** P < 0.001. Control (n = 5). Ischemia–reperfusion (n = 5). Ischemia–reperfusion with spinal cord stimulation (n = 5).

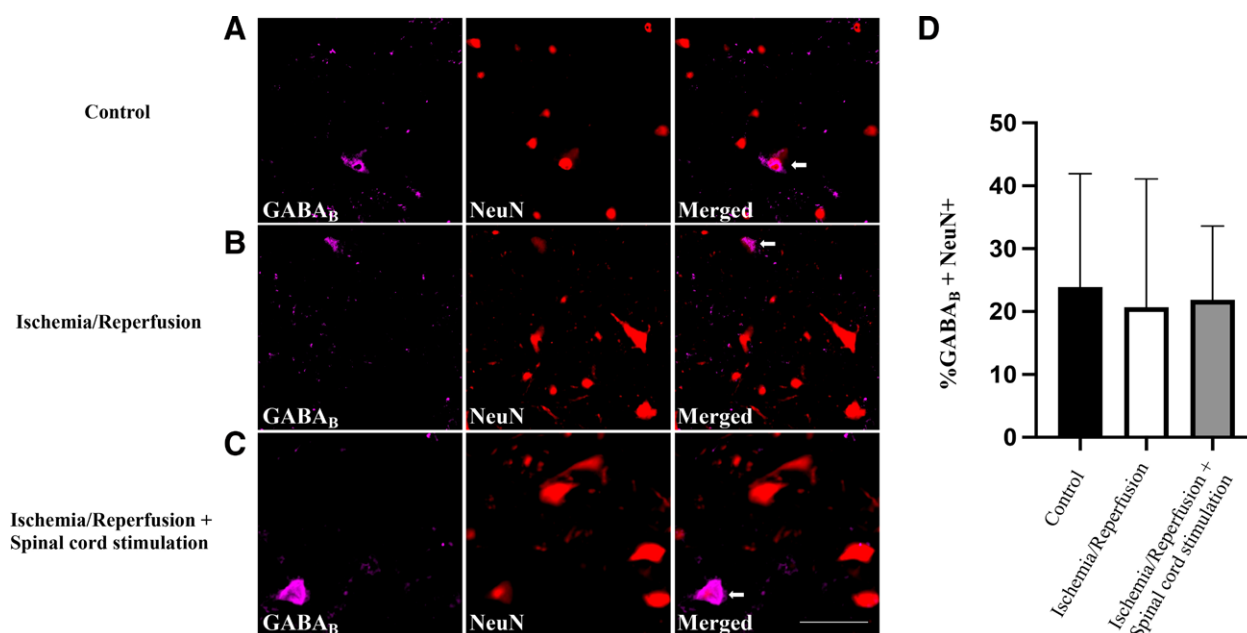


Fig. 6. γ -Aminobutyric acid type B (GABA_B) receptor expression is unchanged in ischemia–reperfusion with or without spinal cord stimulation. (A to C) Representative images from superficial dorsal horn laminae segment T3. Magnification is 20 \times , and scale bar is 50 μ m. (D) There was no change in percentage of GABA_B plus neuronal nuclei plus neurons during ischemia with or without spinal cord stimulation. Values are expressed as mean \pm SD. Control (n = 5). Ischemia–reperfusion (n = 5). Ischemia–reperfusion plus spinal cord stimulation (n = 5).

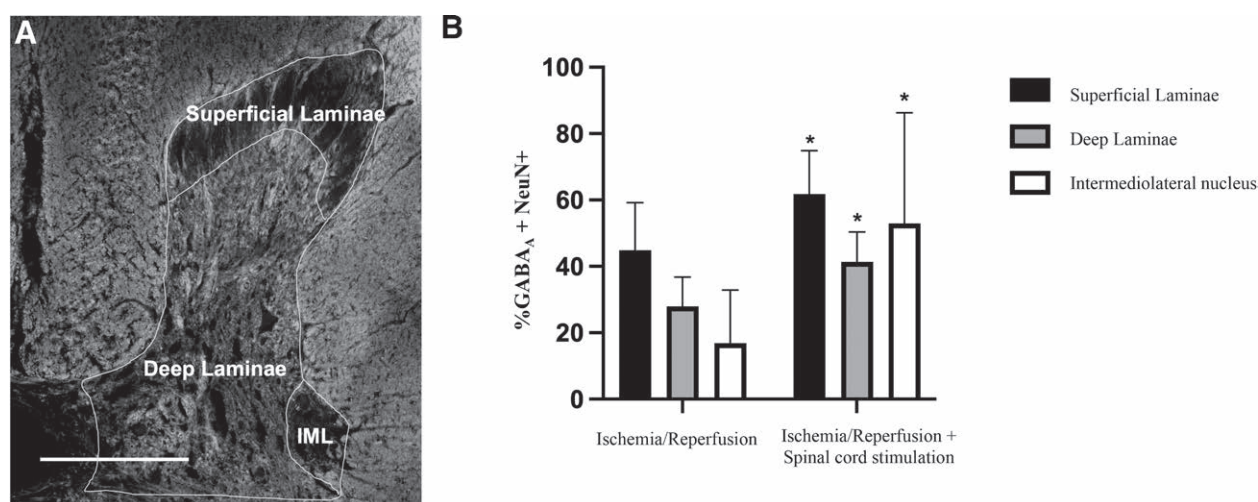


Fig. 7. Regional analysis of γ -aminobutyric acid type A ($GABA_A$) plus neurons in the thoracic spinal cord. (A) Representative image of spinal cord regions of interest; superficial laminae (I to II), deep laminae (III to VII, X), and intermediolateral nucleus. Magnification is 4 \times , and scale bar is 100 μ m. (B) Percentage of $GABA_A$ plus neurons (expressing neuronal marker neuronal nuclei) in each regional area for ischemia–reperfusion versus plus spinal cord stimulation. Data displayed as mean \pm SD. * $P < 0.05$ for regional comparison of ischemia–reperfusion versus ischemia–reperfusion plus spinal cord stimulation. Control (n = 5). Ischemia–reperfusion (n = 5). Ischemia–reperfusion plus spinal cord stimulation (n = 5).

column of the thoracic spinal cord, where they activate a complex cardiorespiratory neural reflex circuit, which results in increased efferent output from sympathetic preganglionic neurons.^{1,4,5,39} As demonstrated by the results of this study and previous reports, neuromodulation *via* spinal cord stimulation can interrupt the cardiorespiratory reflex circuit, thus reducing local sympathoexcitation in ischemic myocardium and decreasing lethal ventricular arrhythmias.^{14,39}

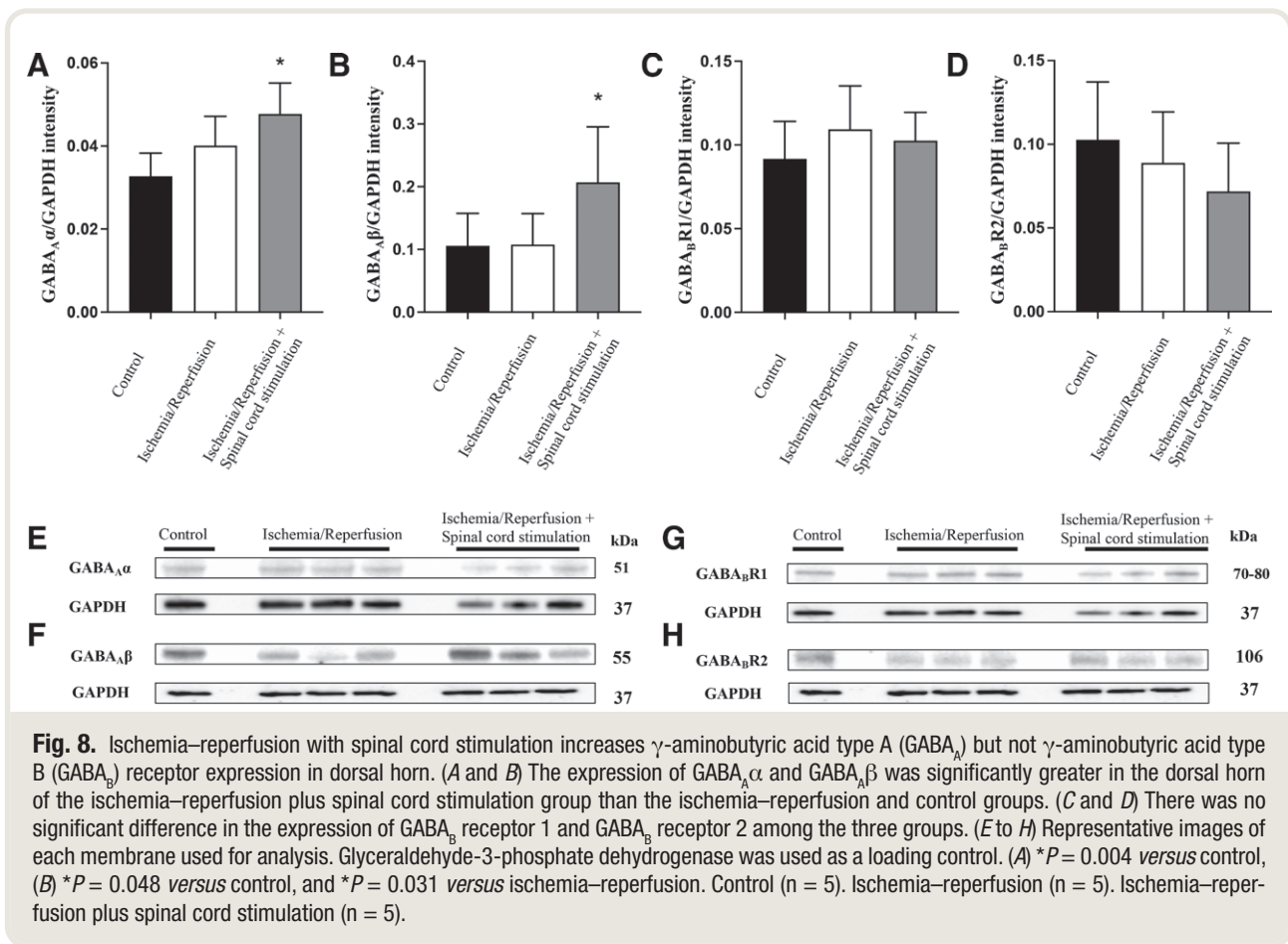
While GABA mediated pathways have been implicated in spinal cord stimulation's analgesic mechanisms, far less is known about the role of GABA signaling in spinal cord stimulation therapy for the reduction of myocardial ischemia-induced sympathoexcitation and cardiac arrhythmias. GABA inhibitory signaling in the spinal cord is primarily achieved through activation of either $GABA_A$ or $GABA_B$ receptors, which have important differences in structure, anatomic location, and function.^{24,40,41} Structurally, the $GABA_A$ receptor is a ligand-gated chloride channel, while the $GABA_B$ receptor is a G-protein-coupled receptor. Anatomically, $GABA_A$ receptors are evenly distributed throughout the spinal cord, while $GABA_B$ receptors are concentrated in dorsal horn laminae I to III and can function as autoreceptors, presynaptic to GABA containing interneurons synapsing on primary afferent fibers^{21,24,41} (fig. 1).

Functionally, both receptor types mediate presynaptic inhibition of primary afferent fibers and interneuron regulation of spinal cord reflexes. $GABA_A$ receptors have been found to mediate shorter-duration components of GABA-induced inhibition, while $GABA_B$ receptors

mediate the longer-duration spinal reflexes. Additionally, there is evidence of differential $GABA_A$ and $GABA_B$ receptor expression in response to nerve injury and spinal cord stimulation.^{18,21,26,41} Therefore, to determine the role of GABA signaling pathways in spinal cord stimulation, we evaluated (1) the functional effect of pharmacologic blockade of $GABA_A$ and $GABA_B$ receptors, as well as (2) individual changes in GABA receptor subtype expression during spinal cord stimulation.

Our results show that the reduction in myocardial sympathoexcitation and ventricular arrhythmias seen with spinal cord stimulation during cardiac ischemia–reperfusion was abolished by intrathecal administration of both $GABA_A$ and $GABA_B$ receptor antagonists. Additionally, intrathecal administration of the GABA transaminase inhibitor (GABAcuine) alone provided cardiac protection similar to spinal cord stimulation therapy. Bicuculine is a GABA transaminase inhibitor, and as such, reduces the degradation of GABA, leading to increased neuronal GABA concentrations.³⁴ These results showing loss of spinal cord stimulation therapeutic effect with GABA antagonists and gain of therapeutic effect with a GABA transaminase inhibitor provide strong evidence that spinal cord stimulation neuromodulation is working through activation of GABAergic signaling mechanisms within the spinal neural network to attenuate ischemia-induced sympathoexcitation.

Interestingly, while we found no difference in the functional effect of pharmacologic blockade of $GABA_A$ versus $GABA_B$ receptors, immunohistochemistry analysis showed a difference in $GABA_A$ versus $GABA_B$ receptor



protein expression with cardiac ischemia and spinal cord stimulation. We found that cardiac ischemia was associated with a reduction in GABA_A plus neurons, whereas ischemia plus spinal cord stimulation resulted in an increase in GABA_A plus neurons. No differences were seen in GABA_B plus neurons with either ischemia–reperfusion alone or ischemia–reperfusion plus spinal cord stimulation. Additional quantification of GABA_A and GABA_B receptor subtype expression was performed using Western blot analysis, and the results further supported the differences seen with immunohistochemistry. Ischemia–reperfusion plus spinal cord stimulation increased both GABA_Aα and GABA_Aβ subunits expression, while there was no difference seen in either GABA_BR1 or GABA_BR2 during ischemia–reperfusion with or without spinal cord stimulation.

Investigation into the anatomic distribution of upregulated GABA_A neurons revealed that GABA_A neurons were increased throughout the superficial and deep laminae of the dorsal horn, as well as in the intermediolateral cell column, during ischemia with spinal cord stimulation as compared to ischemia alone. GABA_A receptors in the superficial dorsal horn laminae likely inhibit presynaptic cardiac ischemia-sensitive primary afferent neurotransmitter release.^{19,21,40} While the upregulation of GABA_A neurons

in the deeper laminae and intermediolateral cell column may represent activation of inhibitory GABAergic interneurons that are presynaptic to sympathetic preganglionic neurons.^{17,19,40} This study builds upon our previous work using Cfos for neuronal activation, where we reported that spinal cord stimulation activates interneurons in the deep laminae of the thoracic dorsal horn.¹² Inhibitory interneurons in these deep laminae (V, VIII, and X) have been shown to synapse on sympathetic preganglionic neurons regulating efferent sympathetic outflow to the heart.^{19,42}

Our finding of differential GABA receptor subtype expression with no difference in functional effects of GABA_A or GABA_B receptor antagonists is similar to findings of previous studies investigating GABA signaling in nociceptive pathways. While Castro-Lopes *et al.* reported a downregulation in GABA_B receptor binding and an upregulation in GABA_A receptor binding in a rodent model of peripheral injury,²¹ follow-up studies by Gwak *et al.*²⁵ and Malan *et al.*³¹ found similar functional responses to both GABA_A and GABA_B receptor agonists and antagonists. Both GABA subtype receptor agonists induced analgesia, and both GABA_A and GABA_B antagonists caused hyperalgesia and allodynia during nerve injury.

The ability of both GABA_A and GABA_B receptor antagonists to reverse the therapeutic effect of spinal cord

stimulation in ischemia suggests that spinal cord stimulation may be decreasing efferent sympathetic output through an increase in endogenous GABA tone in the spinal cord. This is further supported by our finding that GABA_A alone, which inhibits GABA degradation and increases neuronal GABA concentrations, caused a reduction in ischemia-induced cardiac sympathoexcitation and ventricular arrhythmias similar to that seen with spinal cord stimulation. Alternatively, the similar functional response with both GABA receptor subtype antagonists could also be due to one of the following: (1) intrathecal pharmacologic receptor antagonists work to block GABA receptors that are already expressed, and as such, the effect of spinal cord stimulation on GABA receptor expression may be independent from the effect that blockade of GABA receptors may have; or (2) GABA receptor expression is affected by changes in neurotransmitter levels, and therefore, we cannot determine if the changes seen in GABA receptor expression are due to a direct effect of spinal cord stimulation on receptor upregulation, or changes in GABA neurotransmitter release/uptake.

Clinical Implications

The data from this study provide important new mechanistic insight into how spinal cord stimulation is reducing cardiac ischemia–reperfusion–induced sympathoexcitation and ventricular arrhythmias. The majority of work investigating the mechanisms underlying spinal cord stimulation has focused on nociceptive pathways.^{15,16,22,26} Our study uniquely focuses on the mechanisms through which spinal cord stimulation is decreasing cardiac autonomic sympathoexcitation since the mechanisms through which spinal cord stimulation reduces pain may not be the same as the those for reduction in cardiac sympathoexcitation. In fact, several studies suggest that spinal cord stimulation for analgesia in peripheral nerve injury has a greater effect *via* GABA_B receptors, whereas in this study, we are showing opposing results in that the autonomic modulation effects of spinal cord stimulation may be having a greater effect through GABA_A receptors.^{16,33}

While studies in animal models have demonstrated the cardiac protective effects of neuraxial modulation, the clinical application of spinal cord stimulation in humans with heart disease has been equivocal.⁴³ The limited clinical translation is likely due to our incomplete understanding of the mechanisms through which spinal neural signaling controls cardiac sympathoexcitation, and how spinal cord stimulation modulates cardiospinal neural pathways. Therefore, these data have possible wide-reaching clinical implications as they can allow future studies to be aimed at maximizing the therapeutic effects of spinal cord stimulation specifically on autonomic modulation and reduction of sympathoexcitation-induced cardiac arrhythmias. It is

important to understand how we can best optimize spinal cord stimulation as it is an invasive therapy that carries its own procedural risks, especially in the setting of acute cardiac ischemia and revascularization procedures where patients may be anticoagulated.

Limitations

Although this study provides new insights into the mechanisms behind spinal cord stimulation neuromodulatory effects during cardiac ischemia, there are limitations. In this study, acute ischemia was performed on normal hearts to determine the impact of GABA signaling pathways on spinal cord stimulation in a structurally normal central nervous system. These results may not be applicable in hearts with chronic infarction or heart failure in which there may be adverse remodeling in the cardiospinal neural network.^{28,29} Additionally, given the open chest model that was used, continuous anesthesia was necessary throughout experimental protocols. As previously reported, many general anesthesia drugs are mediated by the GABA pathways, including α -chloralose, which is known to have less impact on the autonomic nervous system and is used in many animal experiments.⁴⁴ Therefore, while it may be possible that α -chloralose affected GABA receptor expression, this study was conducted with α -chloralose used in the same concentration during surgical preparation across all experimental groups, so any effects seen would be similar for all experimental groups, and the differences shown between groups for GABA receptor expression changes would likely be unaffected.

In conclusion, we demonstrate that thoracic spinal cord stimulation during acute cardiac ischemia–reperfusion reduces myocardial sympathoexcitation and ventricular arrhythmias through activation of GABA_A signaling pathways, which may function to reduce primary afferent signaling in the superficial dorsal horn and activate inhibitory interneurons to decrease sympathetic output from sympathetic preganglionic neurons in the thoracic spinal cord. These findings help shed light on the pathways through which spinal cord stimulation neuromodulation reduces cardiac ischemia-induced sympathetic output and can aid in future studies to improve the efficacy of clinical spinal cord stimulation to reduce myocardial arrhythmogenesis.

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Competing Interests

The authors declare no competing interests.

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Supplemental Digital Content 2. Supplemental Table: Primary and Secondary Antibodies, <https://links.lww.com/ALN/D47>

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ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

Dr. Silas Weir Mitchell: The Nightmare of War and the Death of Pain



Born into a family of physicians in Philadelphia, Silas Weir Mitchell, M.D. (1829 to 1914), spent his youth in a reverie, writing poetry and getting lost in *The Arabian Nights*. “You have brains, but no industry,” his father chided. As a student at Jefferson Medical College, Mitchell developed an interest in scientific research. After taking over his father’s practice in 1858, he spent his evenings studying the effects of curare and snake venom in animals. His love of the theoretical had found a home, and he dreamed of becoming a famous physiologist. But the Civil War (*image above*) would disrupt his plans. As a Union Army surgeon, Mitchell received “the awful harvest of Gettysburg” at Turner’s Lane Hospital. He treated countless soldiers with complex nerve injuries and coined the term “causalgia” from the Greek *kausos* (burning heat) and *algos* (pain). He published a haunting short story in *The Atlantic Monthly* that described the suffering, both physical and existential, of an amputee with phantom limbs. For his experience with pain syndromes and his skill with the pen, Mitchell was invited to speak at the Massachusetts General Hospital on the 50th Anniversary of Ether Day. At the podium, he recited a poem that marveled at the “Death of Pain”—the powerful moment at which anesthesia triumphed, and all suffering in the world seemed stilled. (Wood engraving by Winslow Homer, *Harper’s Weekly*, 12 July 1862.) (Copyright © the American Society of Anesthesiologists’ Wood Library-Museum of Anesthesiology. www.woodlibrarymuseum.org)

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