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Anesthesiology. Author manuscript; available in PMC 2022 March 01.

Published in final edited form as:

Anesthesiology. 2021 March 01; 134(3): 405–420. doi:10.1097/ALN.0000000000003662.

Spinal Anesthesia Reduces Myocardial Ischemia-Triggered Ventricular Arrhythmias by Suppressing Spinal Cord Neuronal Network Interactions in Pigs

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Abstract

Background: Cardiac sympathoexcitation leads to ventricular arrhythmias. Spinal anesthesia modulates sympathetic output and can be cardioprotective. However, its effect on the cardio-spinal reflexes and network interactions in the dorsal horn (DH) cardiac afferent neurons and the intermediolateral nucleus (IML) sympathetic neurons that regulate sympathetic output is not known. We hypothesize that spinal bupivacaine reduces cardiac neuronal firing and network interactions in the DH-DH and DH-IML that produce sympathoexcitation during myocardial ischemia, attenuating ventricular arrhythmogenesis.

Methods: Extracellular neuronal signals from the DH and IML neurons were simultaneously recorded in Yorkshire pigs (N=9) using a 64-channel high-density penetrating microarray electrode inserted at the T2 spinal cord. DH and IML neural interactions and known markers of cardiac arrhythmogenesis were evaluated during myocardial ischemia and cardiac load-dependent perturbations with intrathecal bupivacaine.

Results: Cardiac spinal neurons were identified based on their response to myocardial ischemia and cardiac load-dependent perturbations. Spinal bupivacaine did not change the basal activity of cardiac neurons in the DH or IML. After bupivacaine administration, the percentage of cardiac neurons that increased their activity in response to myocardial ischemia was decreased. Myocardial ischemia and cardiac-load dependent stress increased the short-term interactions between the DH and DH (324 to 931 correlated pairs out of 1189 pairs, $p<0.0001$), and DH and

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Conflicts of Interest: None

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IML neurons (11 to 69 correlated pairs out of 1135 pairs, p<0.0001). Bupivacaine reduced this network response and augmentation in the interactions between DH-DH (931 to 38 correlated pairs out of 1189 pairs, p<0.0001) and IML-DH neurons (69 to 1 correlated pairs out of 1135 pairs, p<0.0001). Spinal bupivacaine reduced shortening of ventricular activation recovery interval and dispersion of repolarization, with decreased ventricular arrhythmogenesis during acute ischemia.

Conclusion: Spinal anesthesia reduces network interactions between DH-DH and DH-IML cardiac neurons in the spinal cord during myocardial ischemia. Blocking short-term coordination between local afferent-efferent cardiac neurons in the spinal cord contributes to a decrease in cardiac sympathoexcitation and reduction of ventricular arrhythmogenesis.

Introduction

Imbalances in the autonomic nervous system (ANS) and cardiac sympathoexcitation can lead to cardiac dysfunction, including ventricular arrhythmias associated with sudden cardiac death.[1–5]

The peripheral and central components of the ANS coordinate the cardio-spinal reflexes, in which spinal cord neurons give continuous feedback to the intrinsic cardiac neuronal network to regulate, beat-by-beat, regional cardiac function.[1,2,5–7] Acute myocardial ischemia significantly increases spinal sympathoexcitation and intrinsic cardiac neuronal activity contributing to arrhythmias.[4,5,8–10] Spinal interventions (pharmacologic and electrical), not only provide anesthesia, but they also modulate spinal cord sympathetic output and have been shown to be cardioprotective in animals and humans.[11–17] Thus, the spinal cord, by receiving and integrating afferent cardiac signals and then regulating efferent output, plays a critical role in the cardiac reflex loop and moderation of response to sympathoexcitation.[2–4,9]

We have previously demonstrated that spinal bupivacaine attenuates the proarrhythmic consequences of sympathetic stimulation, however, its effect on the spinal cord cardiac neuronal activity and network interactions between the afferent the dorsal horn (DH) neurons and the sympathetic intermediolateral (IML) nucleus neurons, which are the key integration sites of the cardiac spinal sympathetic reflex pathway,[18–22] remains poorly understood. Providing insights into the coordination and integration of neural signals in the spinal cord during myocardial ischemia is essential to the understanding of spinal regulation of cardiac sympathoexcitation that produces lethal arrhythmias. Additionally, drug and neuromodulation therapies are being increasingly employed for sympathetic-driven cardiac disorders and their effectiveness can be potentially optimized by modeling specific neural network activity. To understand these complex neural interactions, we have recently developed novel techniques for simultaneous recordings of cardiac electrophysiology/function and spinal cord neuronal network activity in the DH (afferent) and IML (efferent) regions with high-resolution, high-fidelity electrode microarrays.[7] Prior work has focused primarily on recordings of the single-unit activity of spinal neurons that do not describe neural network interactions.[23,24]

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The primary outcome of this study was to determine the alteration in spinal cord neuronal network interactions associated with the cardioprotective effects of spinal anesthesia during acute myocardial ischemia. We hypothesize that spinal bupivacaine will decrease the neural firing rate and short-term network interactions in the afferent DH-DH pairs of neurons and the afferent-efferent coordination between the DH-IML pairs of neurons during acute myocardial ischemia and reduce ventricular excitability. This study was performed on the porcine model, as the cardiac electrophysiology and cardiac nervous system of the pig are very close to those in humans. [7,11,25–28]

Materials and Methods

Nine Yorkshire pigs (38.1 ± 2.5 kg, 7 males, 2 females) were used in this study. All animal studies followed the recommendations of the Institutional Animal Care and Use Committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85–23, Revised 1996). All animal experiments were approved by the University of California, Los Angeles Chancellor's Animal Research Committee and performed in our laboratories at UCLA. Animals were euthanized by inducing terminal ventricular arrhythmia under general anesthesia

Animal preparation

Animals were sedated with intramuscular telazol (4–6mg/kg), intubated, and mechanically ventilated. General anesthesia was maintained with inhaled isoflurane (1.5–2.5%) and intravenous boluses of fentanyl (total 20 μg/kg) for analgesia during surgical preparation. [7,11,29] Continuous intravenous saline was infused throughout the procedure. During the surgical preparation, a partial T1-T5 laminectomy was performed, followed by the sternotomy. T2 paravertebral ganglia and the heart were exposed after the sternotomy. T2 electrodes were impaled into the ganglia, and the epicardial electrodes were placed around the heart. Laminectomy was completed in the lateral position, and a small part of the dura was cut to advance the intrathecal and place the neural recording probe. After surgical preparation was completed, inhaled isoflurane was changed to intravenous α-chloralose (50 mg/kg initial bolus followed by a 20 mg/kg/hr continuous infusion). Myocardial ischemia and cardiac load-dependent stressors were performed before and after bupivacaine injection (Figure 2A). ECG data (12-lead) was continuously recorded via a Prucka CardioLab system (GE Healthcare, Fairfield, CT). Left ventricular (LV) systolic pressure (LVSP), and maximum and minimum rate change of LV pressure (dP/dt max, dP/dt min) were also measured using a 12-pole conductance, high-fidelity pressure monitoring pigtail catheter (5 Fr) inserted into the LV via the left carotid artery and connected to an MPVS Ultra Pressure-Volume Loop System (Millar Instruments, Houston, TX). Arterial blood gas was tested every hour and corrected as necessary by adjusting the ventilation.

Recording of spinal cord neuronal activity

The activity generated by neural soma in the T2 level of the spinal cord was directly recorded *in situ*. The T2 level of the spinal cord was accessed following laminectomy and the opening of a small window in the spinal cord dura. A high-density 2D penetrating microarray (64 electrode recording sites; Neuronexus, Ann Arbor, MI), mounted on a

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micromanipulator, was inserted 2mm into the spinal cord at the T2 level, lateral to the midline (Figure 1A). This position allowed the recording sites to capture both DH and IML neurons. Neural signals were recorded directly from the thoracic spinal cord, amplified and digitized (SmartBox acquisition system, NeuroNexus, Ann Arbor, MI). Using the high impedance headstage and the filter setting, the 2D microarrays were only able to record the extracellular action potential from the adjacent soma and not axons of passage.[30,31] Electrocardiogram (ECG) and left ventricular pressure (LVP) were recorded in the same file as the neural data.

Cardiac excitability and Electrophysiologic recordings

The cardiac electrophysiological parameters in this porcine model have been extensively studied and well-characterized by our lab, thus providing us with a promising model for studying myocardial sympathoexcitation and arrhythmogenesis[7,11,29]. In order to acquire unipolar ventricular epicardial electrograms, a high-fidelity 56-electrode sock array was placed around the heart (Figure 1B), and electrograms (EGMs) were recorded by Prucka CardioLab system (GE Healthcare, Fairfield, CT). Whole heart activation recovery interval (ARI), a well-validated measure for action potential duration, was analyzed as previously described.[29,32] A few supraventricular tachycardia episodes occurred during the experiments and were removed from the analysis. ARI measurement was in accordance with guidelines described by Haws and Lux.[33] Shortening of ARI is seen with an increase in myocardial sympathetic activity. To ensure accuracy, each electrogram with ST-segment changes was measured by software and then checked manually across five beats. The variance of all ARIs in the whole heart was used to calculate the epicardial dispersion of repolarization. Epicardial dispersion is correlated to the heterogeneity of repolarization time and is a measure of the increased risk for ventricular arrhythmias.[29] Acute ischemia is associated with shortened ARI duration and increased dispersion of repolarization. We recorded whole heart ARI and epicardial dispersion of repolarization to measure the electrophysiological effects elicited by cardiac stressors and the effect of intrathecal bupivacaine on these electrophysiologic parameters. The customized software iScalDyn (University of Utah, Salt Lake City, UT) was used to analyze ARI and epicardial dispersion data.[11,29,31,34] Manual classification of premature ventricular contractions (PVCs) during acute ischemia was performed using a continuous 12-lead ECG. Abnormal heartbeats containing an irregularity in QRS complex morphology, a decrease in R wave-to-R wave time interval, and an absence of a P wave were classified as PVCs. There were no episodes of sustained ventricular tachycardia or fibrillation.

Acute myocardial ischemia and cardiac load-dependent inputs

Animals underwent median sternotomy to expose the anterior surface of the heart. Umbilical tapes were placed around the base of the inferior vena cava and descending thoracic aorta to occlude the inferior vena cava and the descending aorta, respectively. To create acute myocardial ischemia, a 4–0 Prolene suture was placed around the left anterior descending (LAD) coronary artery near the third diagonal branch. The suture was led through a short polyethylene tubing segment for occlusion of the LAD. Inferior vena cava (1 min), descending aorta (1 min) and the LAD coronary artery (5 mins) were occluded before (control) and after thoracic intrathecal bupivacaine (Figure 2A). The above cardiac stressors

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were applied to functionally delineate spinal cord neuronal subpopulations responsive to acute myocardial ischemia and/or cardiac load-dependent perturbations [10]. There was a waiting period of 15 minutes between cardiac load-dependent stressors and 30 minutes after acute ischemia to allow hemodynamic and electrophysiologic indices to return to baseline.

Thoracic spinal anesthesia

Local anesthesia with 0.5% isobaric bupivacaine bolus of 1 mL and continuous infusion of 0.1% bupivacaine 1mL/hr was administered via an intrathecal catheter at the T2 level. The catheter position was confirmed during and at the end of the protocol. Previous studies have shown that the onset, spread, and duration of single-injection isobaric bupivacaine spinal anesthesia, used at various concentrations and volumes, is primarily related to dosage.[35,36] Based on these reports, we used 5 mg of intrathecal bupivacaine, waited 10 minutes to begin our protocol to allow for the onset of bupivacaine, and finished all cardiac stressor procedures within 60 minutes of bolus thoracic intrathecal bupivacaine injection.

Neuronal activity analysis

Spike sorting—Neuronal activity was identified as action potentials with a signal to noise ratio > 2:1.[31] The activity generated by individual neurons was identified using available spike sorting tools (principal component analysis and cluster on measurements techniques) in the Spike2 software program (Cambridge Electronics Design, Cambridge, England) (Figure 2B). Simultaneously occurring activities displaying a similar appearance across all electrode channels were interpreted as being artifactual. Artifacts arising from exogenous stimuli and electrical stimuli were removed from the analysis. Following artifact removal, the activity created by individual neuron somata from each of the 64 electrode channels was characterized by their specific amplitudes and waveforms.[30] Using these methods, action potentials produced by individual soma, rather than axons of passage, can be recorded for prolonged periods of time.[31]

Assessment of spinal neural response to cardiac interventions—To investigate the intra-spinal neuronal mechanisms contributing to the cardioprotective effects of bupivacaine, we first defined the response characteristics of T2 spinal DH and IML neurons, which were functionally delineated into subpopulations responsive to acute ischemia and cardiac load-dependent perturbations. Statistically significant increases or decreases in activity for each neuron between a 1 minute baseline period prior to each intervention and the neural activity during the intervention were analyzed.[45,46] Neurons with significant changes in activity were characterized as cardiac-related neurons.[10] We then evaluated the effect of bupivacaine on the modulation of neural activity and interactions in the spinal cord to such destabilizing inputs. Cardiac electrical responses were also evaluated.

Neural network interactions—Mathematical methods characterizing single neuron activities are typically based on the measurement of spike times or inter-spike intervals in spike trains. However, spike trains are discrete rather than continuous functions. Therefore, kernel-estimation techniques are often used. A well-validated method is convolving the spike train with a continuous function as the kernel to achieve a smooth and continuous signal, termed *spike-density function*.[37,38] In this study, we utilized the Gaussian kernel to

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calculate the spike density function. Correlation analysis was used to evaluate the potential correlation of neural firing between DH and IML neurons. The occurrence of a spike at one time-point is not independent of the occurrence of spikes at other times, both within spike trains from a single neuron and across spike trains from multiple neurons. The presence of these correlations has led to the proposal that such correlations might form a key element of the neural code. In this study, Pearson's linear correlation coefficient was used to measure the similarity between the spike density functions of a pair of neurons. Correlated neurons were defined as a pair of neurons with a correlation coefficient of higher than 0.7.[39]

Identification of IML neurons—Sympathetic efferent preganglionic neurons related to cardiac control originate in the IML cell column of the spinal cord and project their axons into the paravertebral chain.[21,22] Antidromic stimulation of the T2 paravertebral chain was used to identify the sympathetic preganglionic neurons located in the IML nucleus of the thoracic spinal cord.[18] Left T2 paravertebral chain stimulation was performed using bipolar needle electrodes. Stimulation current threshold was defined as the stimulation current amplitude, which elicited a 10% increase in systolic blood pressure at 4 Hz and 4 ms pulse durations. Stimulation pulses were delivered at 1 ms durations and 1 Hz at stimulation current threshold (7.0±1.0 mA) using a Grass S88 Stimulator (Grass Co., Warwick, RI). IML neurons were identified as those that had more than 60% one-to-one firing within 50 ms after T2 paravertebral chain stimulation pulses. IML neural activity was recorded on electrode sites that were clustered together in one region on the electrode that entered the IML. DH neurons were classified, putatively, as all neurons outside of the identified IML region.

Statistical analysis

Statistical power calculation was not conducted prior to the study. The sample size was selected based on our previous published work in which similar extracellular neural recordings were performed and neural network interactions in response to different cardiac stressors were compared.[7,30,40] To determine statistically significant neural activity changes and, thus, functional identity for each recorded neuron during bupivacaine application and cardiac perturbations, a statistical test derived from the Skellam distribution[41] was utilized as used previously in other peripheral ganglia.[30,31] The null hypothesis of this analysis is that the two firing rates are equal, and the assumption is that the number of action potentials identified follows a Poisson distribution. Based on this assumption, the difference in the activities follows a Skellam distribution[42], and the probability that the difference in the number of firings is larger than the observed value provides the desired P-value (unilateral test) using the Skellam cumulative distribution function. This analysis compares one minute of neural activity before the cardiac perturbation (baseline) to neural activity during the perturbation. A two-way chi-square test was used to compare the percentage of neurons that responded to each intervention and the number of correlated DH-IML neurons before and after bupivacaine administration. Shapiro-Wilk normality test was used to test if the data is normally distributed. ARIs, dispersion in ARIs and hemodynamics measures passed the Shapiro-Wilk normality test and therefore paired t-test was used to compare these measures. A Wilcoxon matched-pairs signed-rank test was used to compare the firing responses, correlation coefficients and number of PVCs that did not pass the Shapiro-Wilk normality

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test. Two-tailed testing was used for all statistical tests, and a P-value of 0.05 was considered statistically significant. P-values were corrected using the Bonferroni correction method due to multiple comparisons for correlation data analysis. Statistical analyses were performed using Prism (GraphPad Software, La Jolla, CA). Data are reported as median [25th, 75th percentiles] or mean ± SD.

Results

Hemodynamic responses

To evaluate the change in hemodynamics associated with intrathecal administration of bupivacaine, hemodynamic parameters were recorded at baseline and 10 minutes post-bupivacaine injection into the intrathecal space. Bupivacaine injection led to a decrease in LVSP (87±19 to 75±18 mmHg; p=0.015 (Table 1). Heart rate (79±16 to 80±12 bpm), dP/dt max (1826±500 to 1669±544 mmHg/s), and dP/dt min (−1900±667 to −1543±473 mmHg/s) were not significantly changed from baseline to 10 minutes after thoracic intrathecal bupivacaine placement (Table 1). Hemodynamic responses to the cardiac stimuli were measured by calculating the percentage difference in the hemodynamic parameters before the onset of a cardiac stimulation to during the cardiac intervention. Heart rate, LVSP, dP/dt max and dP/dt min responses to LAD coronary artery, aortic and IVC occlusions did not change before and after bupivacaine administration (Table 1).

Effect of spinal anesthesia on electrophysiologic changes and ventricular arrhythmias during acute myocardial ischemia

Whole heart electrophysiological measures were recorded throughout the entire protocol. 7 of 9 animals had full data sets with good signal quality and 2 animals were removed due to artifactual/noisy electrophysiological signal. The global ventricular mean activation recovery interval (ARI) shortened during acute ischemia at control and bupivacaine application (control: 448 ± 113 ms to 396 ± 93 ms, p=0.012; bupivacaine: 426 ± 86 ms to 393 ± 85 ms; p=0.028; Figure 3B). However, after thoracic spinal anesthesia, the magnitude of global ventricular ARI shortening during acute ischemia was attenuated (−11 ± 7 vs. −8 ± 6%; p=0.032; Figure 3C). Dispersion of repolarization (DOR) also increased during acute ischemia both at control and bupivacaine application (control: 582 ± 229 to 3743 ± 1945 ms^2, p=0.015; bupivacaine: 540 ± 169 to 2582 ± 1008 ms^2, =0.008; Figure 3D). However, the magnitude of increase in DOR during acute ischemia was suppressed after thoracic intrathecal anesthesia (677 ± 560% at control vs. 468 ± 391% at bupivacaine; p=0.030; Figure 3E). Ventricular arrhythmias were measured in episodes of PVCs. Acute ischemia was associated with fewer PVCs in the bupivacaine state (2.5 [1.75 – 5.25] PVCs during ischemia pre-bupivacaine vs. 1 [0 – 1] PVCs during ischemia post-bupivacaine, p=0.031, Figure 3F), indicating a functional, anti-arrhythmogenic benefit of thoracic spinal anesthesia.

Neuronal response to cardiac stressors and spinal bupivacaine

Neural recordings were obtained in all 9 animals and all animals were used for neural analysis. Out of 1184 spinal cord neurons identified, 824 cardiac-related neurons (DH: 791, IML:33) were classified based on their statistically significant response to cardiac stimuli.

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Twenty-nine (3.5%) of cardiac-related neurons responded to ischemia only, 57.5 % responded to cardiac load-dependent stimuli only, and 39.0 % responded to both ischemia and cardiac load-dependent stimuli. All further analysis has been performed on the 824 cardiac-related neurons.

Bupivacaine administration alone did not change the activity of DH and IML neurons prior to the cardiac interventions (DH: from 0.00 [0.00 0.02] Hz at baseline to 0.00 [0.00 0.02] Hz during bupivacaine; IML: from 0.00 [0.00 0.02] Hz at baseline to 0.00 [0.00 0.02] Hz during bupivacaine

Effects of acute myocardial ischemia and spinal bupivacaine on spinal cord neuronal activity

(a) Firing response—The number of spinal cord neurons that responded to the ischemia was decreased by bupivacaine application (DH: from 43% to 18%, $p<0.0001$, IML: from 39% to 9%, $p=0.004$) (Figure 4A, B).

The absolute value of the neuronal response to the ischemia was changed by bupivacaine. For DH neurons, bupivacaine suppressed the neuronal response to ischemia from 0.03 [0.00, 0.13] Hz, 95% CI [0.02 to 0.04] Hz pre-bupivacaine to 0.01 [0.00, 0.02] Hz, 95% CI [0.00 to 0.01] Hz post-bupivacaine ($p<0.0001$) (Figure 4C). Spinal bupivacaine application also decreased the IML neurons' response to ischemia from 0.03 [0.01, 0.14] Hz pre-bupivacaine to 0.01 [0.00, 0.02] Hz post-bupivacaine ($p=0.001$) (Figure 4D). IML neurons' response to reperfusion did not change after bupivacaine application (from 0.02 [0.00, 0.14] Hz, 95% CI [0.01 to 0.14] Hz pre-bupivacaine to 0.02 [0.00, 0.07] Hz, 95% CI [0.00 to 0.02] post-bupivacaine ($p=0.357$) (Figure 4D).

The temporal response of DH and IML neurons to myocardial ischemia were also evaluated in the absence of bupivacaine and after bupivacaine administration (Figure 5). DH and IML neurons show periodic spikes in activity with a correlated temporal response between DH and IML neurons during myocardial ischemia (Figure 5A, B). This correlation in the temporal response of DH and IML neurons to myocardial ischemia was abolished after bupivacaine administration (Figure 5C, D). This observation led us to investigate the neuronal activity correlation between the DH and IML neurons and within the DH neurons during the ischemia before and after spinal anesthesia.

(b) Neuronal network response—The number of correlated pairs of DH-DH and DH-IML neurons increased from baseline to during ischemia (DH-DH:324 correlated pairs of neurons to 931 correlated pairs of neurons out of 1189 pairs, $p<0.0001$; DH-IML:11 correlated pairs of neurons to 69 correlated pairs of neurons out of 1135 pairs, $p<0.0001$). This network response to ischemia was blunted after bupivacaine administration (DH-DH: 931correlated pairs of neurons vs. 38 correlated pair of neurons out of 1189 pairs, $p<0.0001$; DH-IML: 69 correlated pairs of neurons vs. 1 correlated pair of neurons out of 1135 pairs, $p<0.0001$) (Figure 6A, C). The magnitude of correlation of the correlated neurons in the control state also increased (DH-DH: from 0.19 [0.07, 0.73], 95% CI [0.16 to 0.20] before ischemia to 0.81 [0.71, 0.92], 95% CI [0.79 to 0.82] during ischemia ($p<0.0001$);DH-IML: from 0.14 [0.06, 0.41], 95% CI [0.09 to 0.25] before ischemia to 0.80 [0.75, 0.88], 95% CI

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[0.77 to 0.83] during ischemia (p<0.0001). This myocardial ischemia-induced augmentation in DH-DH and DH-IML correlation was suppressed after bupivacaine (DH-DH: 0.81 [0.71, 0.92], 95% CI [0.79 to 0.82] pre-bupivacaine to 0.08 [0.03, 0.18], 95% CI [0.07 to 0.08] post-bupivacaine, p<0.0001;DH-IML:0.80 [0.75, 0.88], 95% CI [0.77 to 0.83] pre-bupivacaine to 0.06 [0.03, 0.13], 95% CI [0.03 to 0.08] post-bupivacaine, p<0.0001) (Figure 6B, D).

Effects of cardiac load-dependent stress and spinal bupivacaine on spinal cord neuronal activity

Similar to the responses seen during acute myocardial ischemia, spinal bupivacaine reduced the activity of cardio-spinal neurons in the thoracic spinal cord.

(a) Firing response—Bupivacaine administration reduced the number of spinal cord neurons that responded to the aortic occlusion (DH: from 70% to 34%, p < 0.0001; IML: from 70% to 24%, p < 0.001) and IVC occlusion (DH: from 63% to 53%, p < 0.0001; IML: from 39% to 9%, p =0.014).

Spinal bupivacaine suppressed the DH and IML neuronal response to aortic occlusion (DH: from 0.15 [0.02 0.42] Hz pre-bupivacaine to 0.02 [0.00 0.05] post-bupivacaine, p < 0.0001; IML: from 0.23 [0.02 0.97] Hz pre-bupivacaine to 0.02 [0.00 0.07] post-bupivacaine, p=0.003) and release of the aortic occlusions (DH: from 0.03 [0.00 0.15] Hz pre-bupivacaine to 0.00 [0.00 0.02] post-bupivacaine, p < 0.0001; IML: from 0.02 [0.00 0.38] Hz pre-bupivacaine to 0.00 [0.00 0.02] post-bupivacaine, p=0.060). Spinal cord neuronal response to IVC occlusion was also mitigated by thoracic spinal bupivacaine application (DH: from 0.13 [0.03 0.42] Hz pre-bupivacaine to 0.05 [0.00 0.17] post-bupivacaine, p <0.0001; IML: from 0.44 [0.13 1.40] Hz pre-bupivacaine to 0.04 [0.01 0.23] post-bupivacaine, p<0.0001). The evoked spinal cord neuronal response to release of IVC occlusion was diminished after application of thoracic intrathecal anesthesia (DH: from 0.06 [0.02 0.22] Hz pre-bupivacaine to 0.03 [0.00 0.17] post-bupivacaine, p=0.016; IML: from 0.32 [0.10 2.40] Hz pre-bupivacaine to 0.06 [0.03 0.36] post-bupivacaine, p=0.017).

(b) Neural network response—DH-DH and DH-IML interactions increased during aortic and IVC occlusions. The number of correlated DH-DH neurons increased from baseline to during aortic and IVC occlusions (aortic: 192 correlated pairs of neurons to 957 correlated pairs of neurons out of 1111 pairs, p < 0.0001; IVC: 69 correlated pairs of neurons to 304 correlated pairs of neurons out of 368 pairs, p <0.0001). This increase in the number of correlated afferent neurons during great vessels occlusion was suppressed by bupivacaine administration (aortic:957 correlated pairs of neurons vs. 81 correlated pair of neurons out of 1111 pairs, p<0.0001; IVC: 304 correlated pairs of neurons vs. 39 correlated pair of neurons out of 368 pairs, p<0.0001). The number of correlated DH-IML neurons also increased from baseline to during great vessels' occlusions (aortic: 0 correlated pairs of neurons to 99 correlated pairs of neurons out of 844 pairs, p < 0.0001; IVC: 9 correlated pairs of neurons to 28 correlated pairs of neurons out of 1041 pairs, p =0.000). The number of DH-IML interactions during the cardiac load dependent stressor was suppressed after bupivacaine administration (aortic: 99 correlated pairs of neurons vs. 12 correlated pairs of

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neurons out of 844 pairs, p<0.0001; IVC: 28 correlated pairs of neurons vs. 5 correlated pairs of neurons out of 1041 pairs, p<0.0001).

In the control state, aortic and IVC occlusions augmented the interactions between DH neurons and the magnitude of the correlation of neural firing between DH-DD pairs of neurons was increased during great vessels' occlusions (aortic: from 0.13 [0.06, 0.48] at baseline to 0.77 [0.72, 0.84] during aortic occlusion, p <0.0001; IVC: from 0.15 [0.07, 0.31] at baseline to 0.77[0.72, 0.84] during IVC occlusion, p<0.0001). This increase in the correlation magnitude of neural firing between DH-DH pairs neurons was suppressed by bupivacaine (during aortic: from 0.77 [0.72, 0.84] pre-bupivacaine to 0.11 [0.06, 0.27] post-bupivacaine,, p <0.0001; during IVC: from 0.77[0.72, 0.84] pre-bupivacaine to 0.19[0.09, 0.39] post-bupivacaine, p <0.0001). the magnitude of correlation between DH and IML neuronal activity also increased from baseline to during aortic and IVC occlusions (aortic: from 0.13 [0.08, 0.33] at baseline to 0.82 [0.76, 0.86] during aortic occlusion, p <0.0001; IVC: from 0.14 [0.08, 0.58] at baseline to 0.75[0.57, 0.81] during IVC occlusion, p=0.002). Thoracic spinal anesthesia reduced the magnitude of correlation of the DH-IML correlated neurons in response to aortic and IVC occlusions (during aortic: from 0.82 [0.76, 0.86] pre-bupivacaine to 0.08 [0.05, 0.14] post-bupivacaine,, p <0.0001;during IVC: from 0.75[0.57, 0.81] pre-bupivacaine to 0.16[0.07, 0.33] post-bupivacaine, p <0.0001).

Discussion

Neural networks involved in cardiac control can generate exaggerated sympathetic reflex responses in the presence of cardiovascular dysfunction and lead to lethal arrhythmias.[4,9] The primary aim of this study was to investigate spinal bupivacaine suppression of the cardio-spinal neuronal network responses to myocardial ischemia. To characterize the neural interactions at the level of spinal cord, we simultaneously recorded the activity of the network of IML and DH neurons with high-density microelectrode silicon arrays during myocardial ischemia and spinal anesthesia, while assessing sympathoexcitation induced the cardiac arrhythmogenesis. Our major findings are: 1) at baseline, spinal anesthesia had no significant effect on the DH and IML neuronal activity; 2) during myocardial ischemia and cardiac-load dependent stressors, there was a significant increase in DH and IML cardiac neuron firing rate; 3) during myocardial ischemia, DH-DH cardiac neuron network interactions increased more than three-folds and this afferent activity synchronization during ischemia was diminished by bupivacaine; 4) DH-IML cardiac neuron network coordination was increased during ischemia and this short-term coordination between afferent and efferent network was suppressed by bupivacaine; 5) spinal anesthesia prevented the sympathetically mediated shortening of ventricular ARI during myocardial ischemia and suppressed the dispersion of repolarization (arrhythmogenic substrate); and 6) reduced the episodes of PVCs during myocardial ischemia.

Clinical and Physiological Implications of Spinal Regulation of Cardiac Sympathoexcitation during Myocardial Ischemia

Cardiac sympathoexcitation is one of the leading causes of cardiac arrhythmias and sudden cardiac death. Spinal or Stellate ganglion blocks are being used to treat sympathetically

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driven ventricular arrhythmias that are resistant to conventional antiarrhythmic drug therapy and novel spinal neuromodulation therapies are being developed to reduce cardiac arrhythmia and heart failure burden.[11,27,43–45] There exists a need to understand how these sympathetically driven arrhythmias develop and how best to optimize therapies for the patients. Providing insight into the coordination and integration of neural signals in the DH and IML neurons during myocardial ischemia is critical to the understanding of the spinal cord control of cardiac pathophysiology and optimizing new pharmacologic and bioelectronic neuromodulation therapies which can be tailored to the regulation of specific network activity.[4,46] While the myocardial substrate electrophysiologic changes underlying the antiarrhythmic effects of spinal anesthesia were recently described,[11] the neural network interactions in the spinal cord, through which the reduction in sympathetic output from the preganglionic neurons in the IML occurs, has not been characterized. In this study, we have investigated a possible mechanism through which spinal anesthesia reduces cardiac sympathoexcitation and thus provide important new insight into cardiac autonomic modulation at the level of the spinal cord.

Our results provide a foundation for future studies that may delineate complex neuronal interactions and network coordination in the spinal cord with other pharmacologic therapeutic agents. Administration of specific antagonist pharmacologic agents while recording neuronal activity can help identify the neurochemical signaling involved in the excitatory or inhibitory neural circuits in different spinal cord laminae. Further, alterations in spinal cord gene expression, including those involved in stress response and inflammation, is observed in chronic myocardial infarction and heart failure.[47] Impact of these changes on neural network interactions and their contribution to exaggerated sympathetic response in chronic heart diseases, are areas of future investigation.

Spinal neural network responses during myocardial ischemia: We show that a critical aspect of the cardio-spinal reflex response (to cardiac stress) is the significant increase in short-term coordination between DH to DH and DH to IML sites, indicating that local afferent-afferent and afferent-efferent neuronal network interaction at the level of spinal cord contributes significantly to amplification of cardiac sympathetic activity. This network interaction is blocked by bupivacaine, reducing sympathetically mediated cardiac arrhythmogenesis. Our results showing bupivacaine induced reduction in DH-DH and DH-IML correlations during myocardial ischemia suggests that the anti-arrhythmic effect of spinal bupivacaine is linked to its ability to disrupt the heightened spinal cord neural interactions and synchronization during sympathoexcitation. Our observations are also supported by a recent investigation by Ardell et al. that demonstrates that ischemia-induced neuronal transmission in the spinal cord amplified sympathoexcitation with increase in cardiac norepinephrine release that triggered ventricular arrhythmias.[48]

All applied cardiac stimuli in our study increased the number of correlated DH-DH and DH-IML pairs of neurons and the correlations of these neurons in the control state, suggesting that cardiac stimuli induce a hyper-excitatory state that increases neural interactions within the afferent and between the afferent and the efferent sites of the spinal cord. In this study, we have not tested the response to ischemia after the anesthesia wore off; however, this could be an area of investigation in the future to assess the memory effect of the bupivacaine.

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Attenuation of cardiac ischemia-induced excitatory neuronal pathways: Our results show that bupivacaine did not alter basal spinal neuronal activity (unchanged firing rate) but notably, it suppressed the cardiospinal neuronal response to myocardial ischemia and cardiac-load dependent stressors. This result sheds light on the underlying mechanisms in our prior study that showed thoracic epidural anesthesia did not impact cardiac electrophysiology at baseline, but rather reduced arrhythmogenicity during myocardial ischemia induced sympathetic hyperactivity.[11] DH and IML neurons show periodic spikes in activity in response to transduction of sensory signals from myocardial ischemia, with a correlated temporal response between DH and IML. This was abolished after bupivacaine administration. Our study specifically focused on the effect of bupivacaine on cardiac spinal neuronal activity. Previous single-unit studies show that noxious stimuli, such as myocardial ischemia, leads to the release of neuropeptides, including excitatory neurotransmitter glutamate and substance P, in the dorsal laminae of the thoracic spinal cord.[49–51] In response to cardiac ischemia, TRPV1 receptors in the thoracic spinal DH mediate neuronal activation and release of substance P from the central terminal endings of cardiac ischemic-sensitive afferent neurons.[52] Bupivacaine has been shown to act on a multitude of channels and receptors to reduce neurotransmission.[53–55] Yanagidate et al. demonstrated that bupivacaine inhibits TRPV1-induced excitatory transmission in the spinal DH during myocardial ischemia.[53] Our result that bupivacaine attenuates ischemia-induced neuronal firing in the DH and IML may indicate that, in part, the attenuated neurons in the DH could be related to an ischemia-induced excitatory pathway such as the TRPV1-mediated SP pathway.

Compared to the action of bupivacaine on DH neurons, its direct action on the neurons in the IML is less clear. We identified 33 efferent sympathetic preganglionic neurons through T2 paravertebral stimulation and observed that the firing pattern of these neurons in response to myocardial ischemia changed after bupivacaine application. Decreased cross-correlation between DH and IML neurons indicates a reduction in network interactions and suppression of afferent-efferent ‘cross-talk’ that is responsible for sympathetic hyperactivity in the preganglionic neurons. Though further studies are necessary to identify whether bupivacaine has a direct effect on neurons in the IML nucleus, our findings suggest that bupivacaine acts on both afferent signaling and efferent sympathetic outflow, likely changing the interactions between the DH and IML during excess sympathoexcitation.

Sympathoexcitation and ventricular arrhythmogenesis during myocardial ischemia

Sympathoexcitation leads to increased myocardial excitability leading to an arrhythmogenic substrate is characterized by shortening of activation recovery interval (ARIs) and dispersion of repolarization (DOR).[11,27] From an arrhythmogenicity standpoint, the difference of 15–20ms in ARI and greater than 500ms change in dispersion of repolarization is electrophysiologically very significant to create clinical arrhythmias.[1] Studies evaluating spinal neural activity modulation have also demonstrated equivalent changes in APD¬90 and ERP in conjunction with the prevention of arrhythmias.[56,57] Increased DOR creates electrical heterogeneity in the myocardium and leads to cardiac reentry mechanism for ventricular arrhythmias.[10,11,29]

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We have previously shown that thoracic epidural anesthesia selectively modulates the sympathetic driven cardiac excitability in the ischemic myocardium as compared to the remote myocardium, by preventing reduction of ARIs and decreasing DOR.[11] Similar results were seen in the current study with spinal bupivacaine. Bupivacaine suppressed the magnitude of increase in DOR during acute ischemia from a mean of 677 % in the controls to 468 % with bupivacaine (p=0.030) and reduced PVC burden during the brief ischemia period from a mean of 2.5 to 1. The short duration of ischemia triggered PVCs, which, if the ischemia was to be further prolonged, would lead to lethal VTs due to reentry.

The attenuation of ARI shortening, reduction in dispersion of repolarization and reduction in PVCs, were included to validate the model and demonstrate the effect of bupivacaine on cardiac sympathoexcitation, as previously described[11,14,58], and confirm that the recorded neural responses were due to the effects of bupivacaine. The primary focus of this study was to show that one of the mechanisms behind the suppression of the arrhythmogenicity by bupivacaine could be due to altering the spinal neural network and interactions between spinal afferent and efferent sites. We showed that spinal bupivacaine blocked the integration and processing of afferent neural signals in the DH during myocardial ischemia and prevented coordinated activity of DH-IML to reduce the cardiac sympathoexcitation.

Limitations

Both sympathetic and vagal influences work together in the autonomic nervous system towards the reflex control of the heart. Our studies were designed to study the impact of alternations in afferent signaling on the spinal sympathetic output in an *in-vivo* model of intact vagal control. In our model, we investigated neural interactions solely within the T2 level of the spinal cord. Multi-level functional assessment will entail simultaneous insertion at two or more levels. It is known that bupivacaine blocks sodium channels on afferent root fibers as well as ventral root preganglionic sympathetic fibers.[59] Since this study did not focus on recording from the DRG and sympathetic trunk ganglion, it is not apparent whether root fibers were affected by bupivacaine administration. The extent to which sodium channel blockage in these fibers may have affected the attenuation of signals observed in the DH and IML is also unknown. Additionally, there are supra-spinal inputs from higher centers that synapse onto the DH and IML regions.[23,60] Our study was not designed to delineate the influence of the higher centers on spinal cord processing and integration of cardiac signals. Also, the suppression in the neural interaction to the ischemia by bupivacaine that is reported in this study might be partially due to the ischemia preconditioning, although prior studies in this model suggest a limited effect with the brief periods of myocardial ischemia [31]. In this study we did not test the control ischemia after the bupivacaine wears off, however based on our other studies with a similar experimental model [26,30,31,40] the time controls during model validation did not show a significant difference in the neural response to multiple stressors. We acknowledge that there are other novel approaches for cardio-protection from myocardial ischemia, such as hypoxia signaling, circadian rhythm or adenosine signaling. We did not evaluate them in the current study.

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Conclusion

Thoracic spinal anesthesia with bupivacaine suppresses the cardio-spinal neuronal network responses to myocardial ischemia and cardiac stressors at the level of the spinal cord. Increased short-term coordination between DH to DH and DH to SPN at the level of spinal cord contributes significantly to increase in cardiac sympathetic activity. This increased cardio-spinal network interaction during myocardial ischemia is blocked by bupivacaine, reducing sympathetically mediated cardiac arrhythmogenesis. Future characterization of types of responsive neurons, neurochemicals involved in network signaling and synchronization, and their responses to various modulatory approaches can allow more specific drugs and bioelectric therapies to be developed.

Acknowledgments

Funding Sources: The study is funded by NIH RO1 HL136836.

Dr. Mahajan is supported by NIH RO1 HL136836 and NIH R44 DA049630. Dr. Howard-Quijano is supported by NIH K08 HL135418

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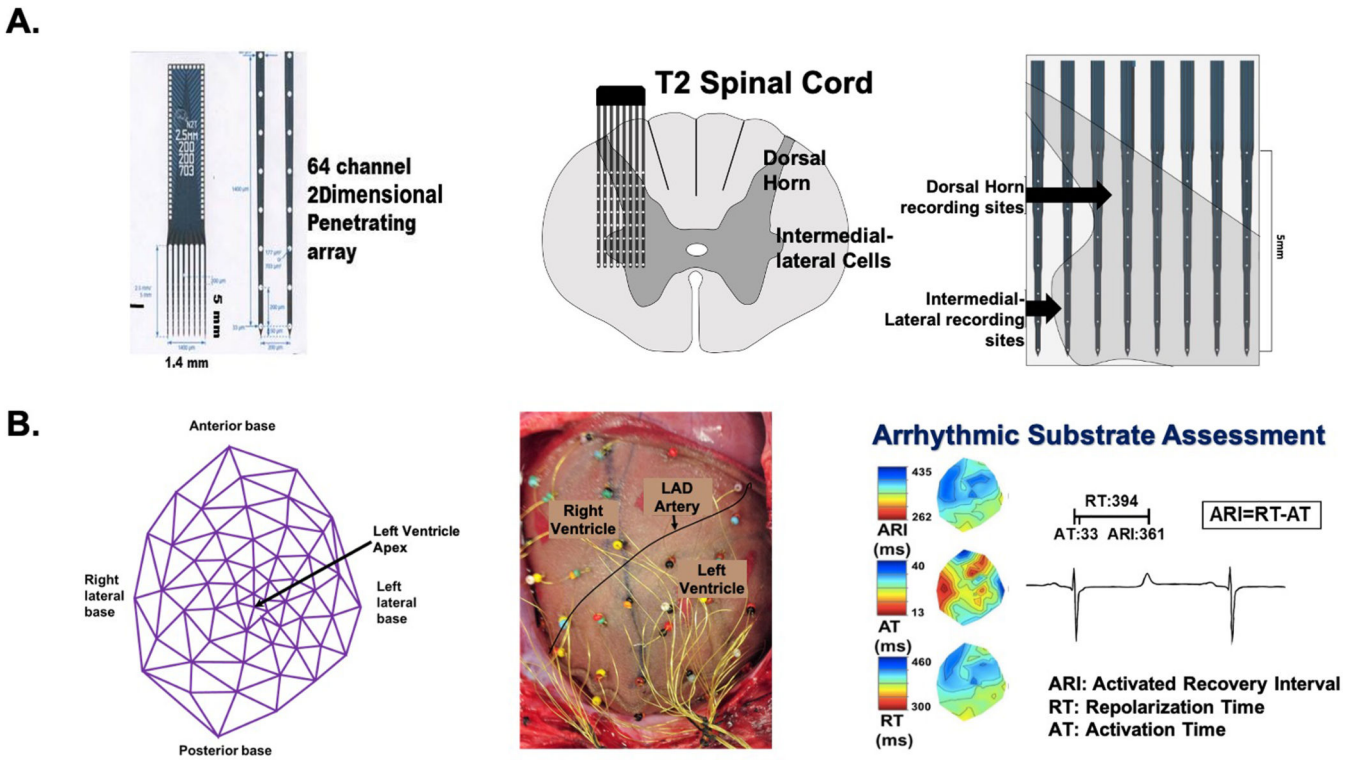

Figure 1. Spinal cord neuronal activity recording and cardiac electrogram.

(A) Extracellular activity in the spinal cord was recorded using a customized 64-channel silicon microelectrode array that penetrated the spinal cord at T2 level (Left and Middle panel). Right panel shows the location of the electrode in the spinal cord in the sensory (afferent) dorsal horn (DH) and the sympathetic (efferent) intermediolateral nucleus (IML) regions. **(B)** A 56-electrode high-fidelity epicardial sock array was placed around the heart to measure ventricular epicardial electrograms. Left and Middle panel show the electrode positioning and cardiac orientation of the arrays. Right panel shows a Polar ARI map demonstrating dispersion of repolarization recorded dynamically from the high-fidelity arrays and quantification of the ARIs from the electrograms. LAD: left anterior descending coronary artery,

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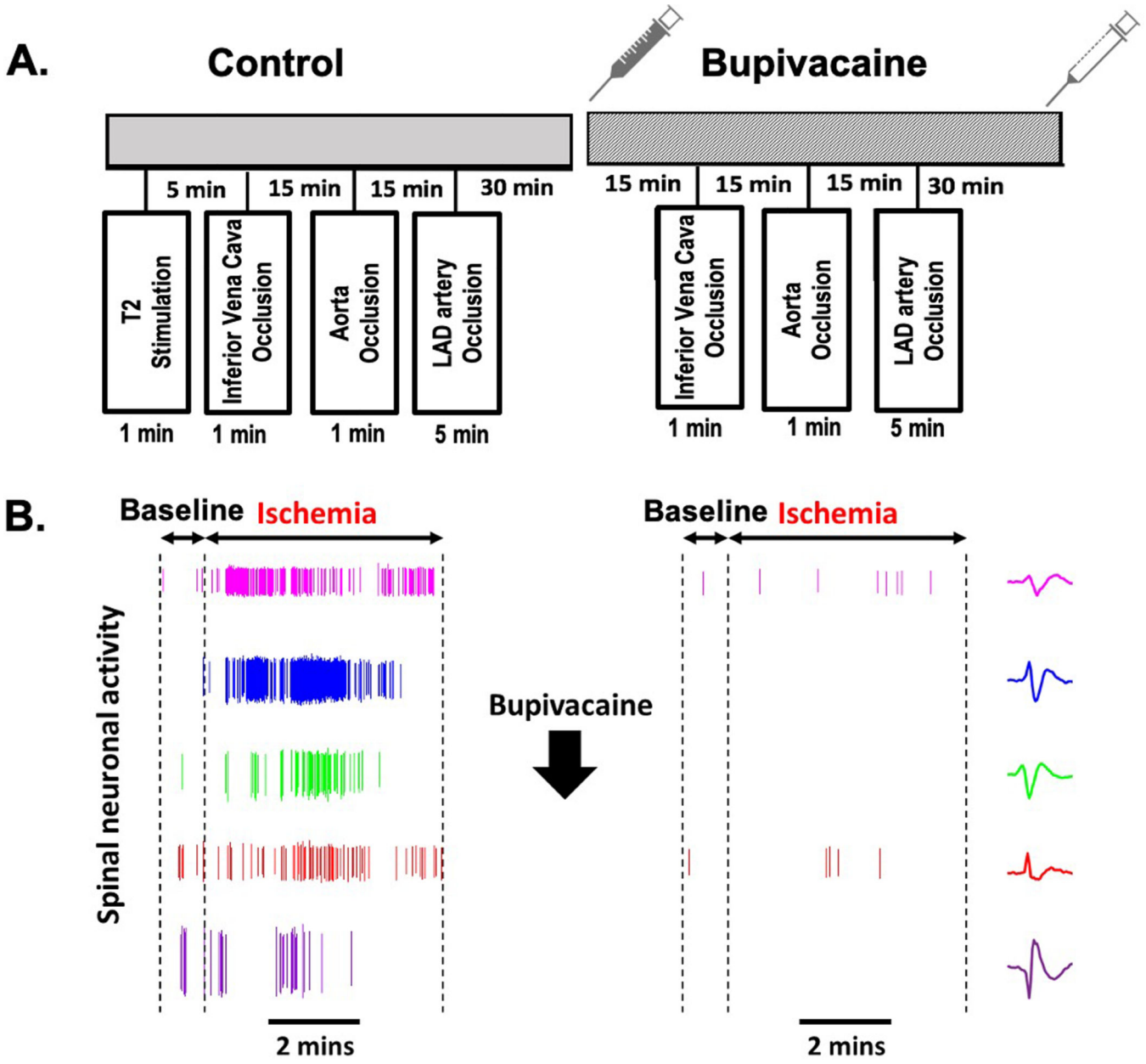

Figure 2. Experimental protocol.

(A) Cardiac stressors were applied before and during thoracic spinal bupivacaine administration. T2 paravertebral chain stimulation was performed to reliably identify the intermediolateral nucleus neurons. LAD: left anterior descending coronary artery **(B)** Representative response of spinal cord neuronal activity to 5-minute ischemia before and during bupivacaine administration.

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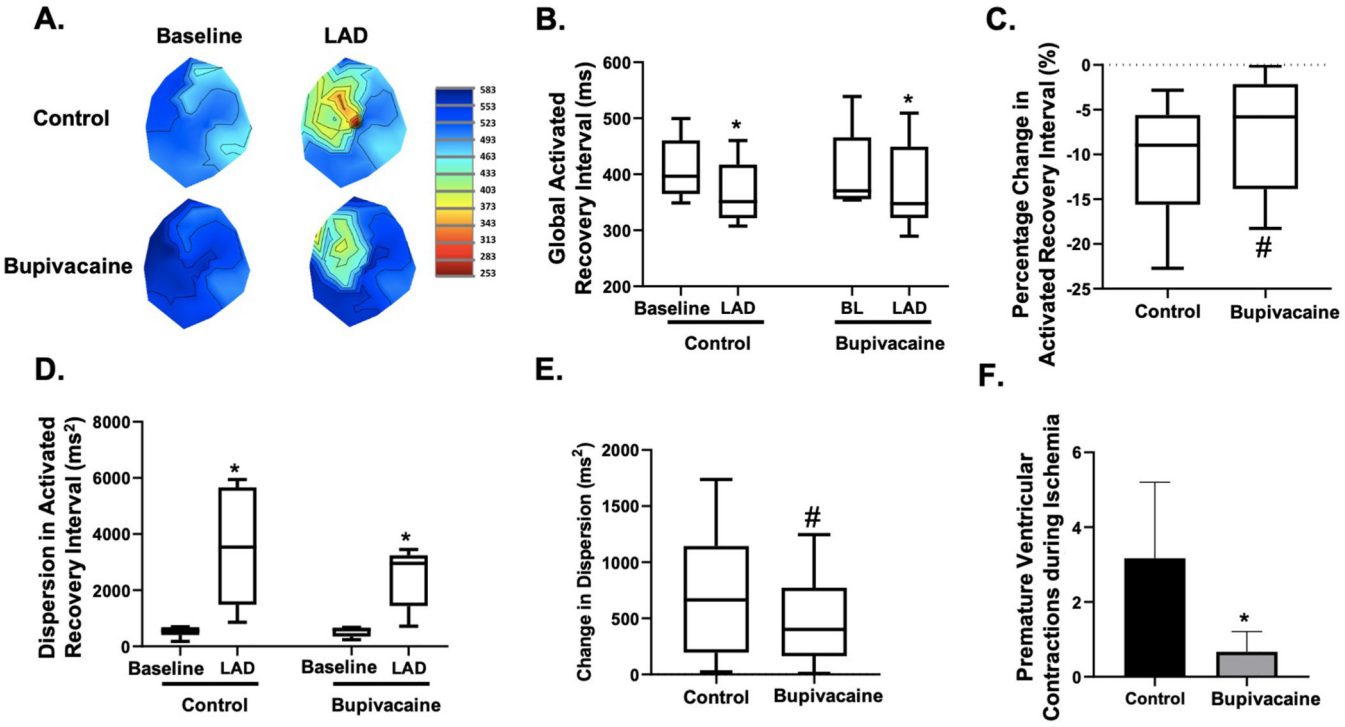

Figure 3. Effect of bupivacaine on electrophysiological responses to acute myocardial ischemia: Bupivacaine reduces myocardial excitability

(A) Representative polar Activated Recovery Interval (ARI) maps comparing the effect of bupivacaine on ventricular ARI and DOR changes during the left anterior descending (LAD) occlusion. **(B-C)** The global ventricular ARI was reduced during acute ischemia in both control and spinal anesthesia due to cardiac sympathoexcitation; however, the magnitude of ARI reduction was attenuated with thoracic intrathecal anesthesia (N=7). **(D-E)** Global dispersion of repolarization (DOR) increased during acute ischemia in both control and thoracic spinal anesthesia; however, the magnitude of DOR increase was greater with the control treatment (N=7). **(F)** Number of premature ventricular contractions (PVC) during ischemia was decreased after bupivacaine application (N=7), LAD: left anterior descending artery occlusion, * $P < 0.01$ vs. Baseline, # $P < 0.05$ vs. control. For B-D, the boxes show the 25th to 75th percentiles and the median and the whiskers show 5th and 95th percentiles. For F, mean with 95% confidence interval is shown on F.

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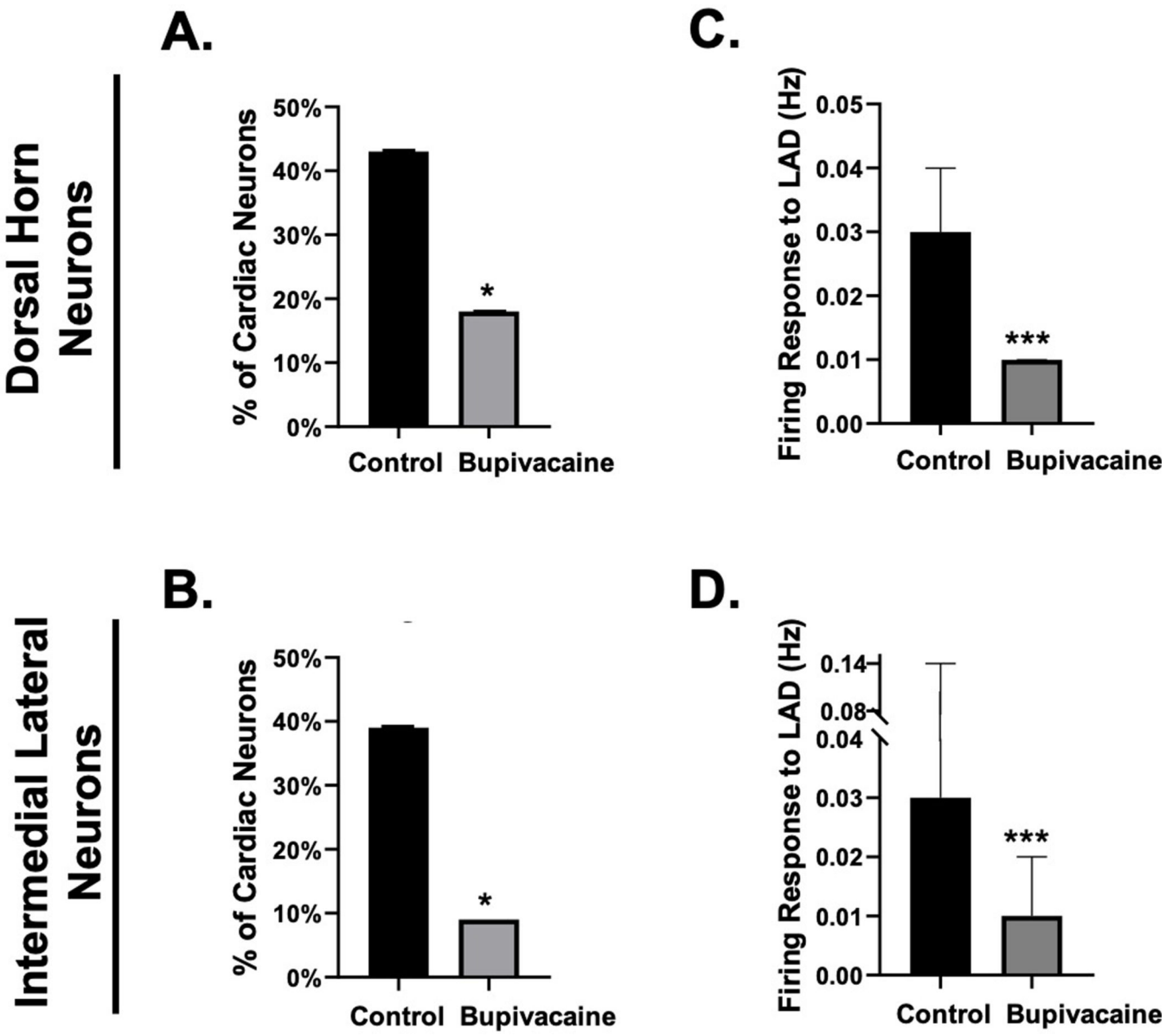

Figure 4. Effect of bupivacaine on the response of spinal cord cardiac-related neurons to ischemia.

Thoracic spinal bupivacaine administration decreased the number of cardiac-related **(A)** dorsal horn (DH)- and **(B)** intermediolateral nucleus (IML)- neurons that responded to left anterior descending coronary artery occlusion (LAD) (N=9). Firing response of cardiac-related **(C)** DH (n=791) and **(D)** IML neurons (n=33) to LAD was suppressed by bupivacaine administration. *P <0.05, ***P<0.001 vs. control. Bars and the error bars represent the median with 95% confidence interval.

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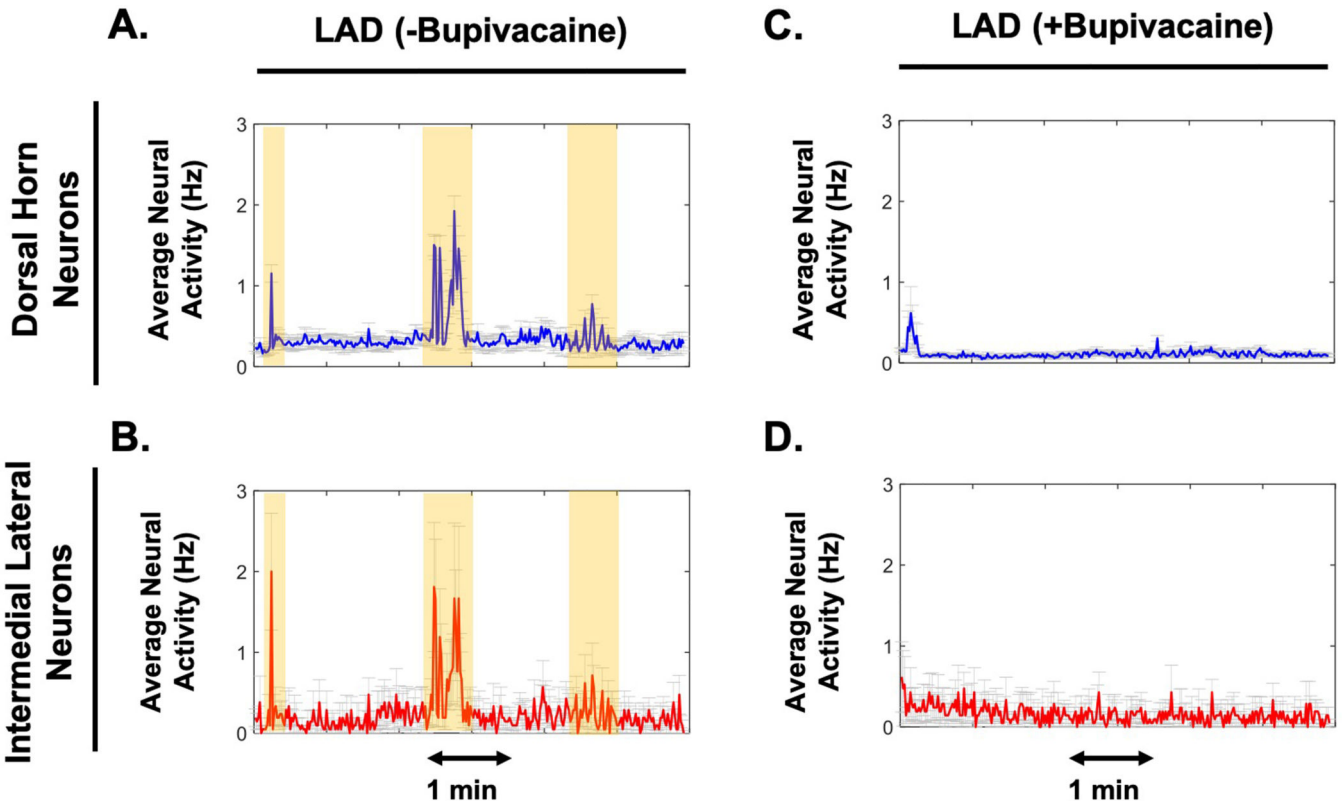

Figure 5. Effect of bupivacaine on the temporal response of dorsal Horn (DH) and (IML) neurons to myocardial ischemia.

(A) Average neural activity of the DH (n=791) (with SEM of neural firing at each time-point shown in grey) and **(B)** IML (n=33) neural activity during 5 mins left anterior descending (LAD) coronary occlusion without Bupivacaine administration. The shaded yellow area highlights the periods during LAD occlusion in which DH and IML neurons demonstrated correlated activity. **(C)** Average DH (n=791) and **(D)** IML (n=33) neuronal activity during LAD occlusion with bupivacaine administration. Error bars show the standard deviation.

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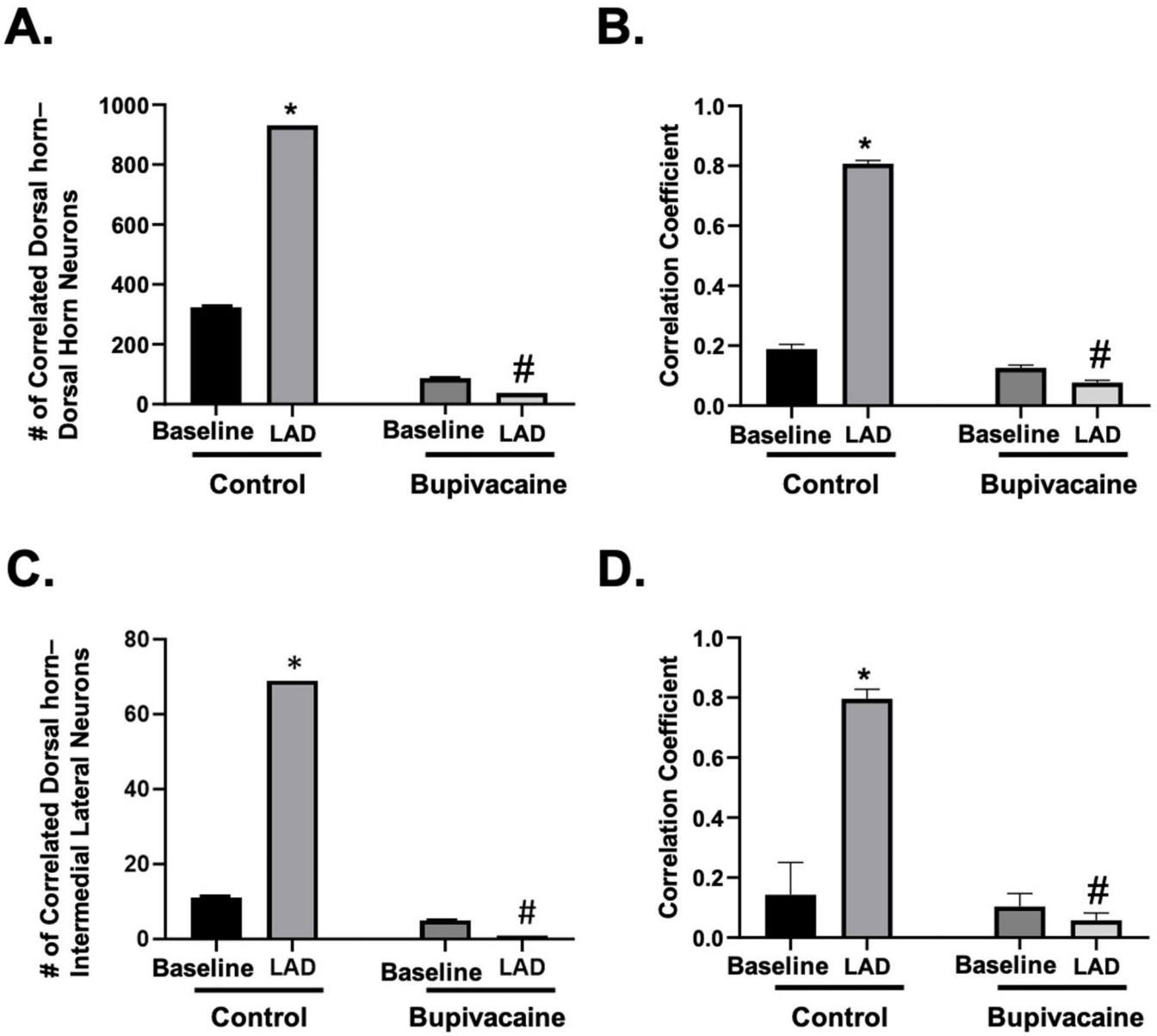

Figure 6. Effect of bupivacaine on cardiac spinal neural network response to myocardial ischemia.

Spinal bupivacaine decreased the interactions between the dorsal horn (DH) (n=791) and intermediolateral nucleus (IML) (n=33) by decreasing the number of correlated **(A)** DH-DH and **(C)** DH-IML neurons and the amount of correlation between the activity of correlated **(B)** DH-DH and **(D)** DH-IML neurons in response to the left anterior descending coronary artery (LAD) occlusion. *P <0.05 vs. Baseline, # P<0.05 vs. control. P-values in this figure have been corrected for multiple comparisons using the Bonferroni correction method.

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Table 1.

Hemodynamic Responses

	Baseline	10 min Post-Bupivacaine
HR (bpm)	79 ± 16	80 ± 12
Left Ventricle Systolic Pressure (mmHg)	87 ± 19	75 ± 18 *
dP/dT maximum (mmHg/s)	1826 ± 500	1669 ± 544
dP/dT minimum (mmHg/s)	−1900 ± 667	−1534 ± 473

	LAD Ischemia		Inferior Vena Cava Occlusion		Aorta Occlusion	
% Change	Pre-Bupivacaine (Control)	Post-Bupivacaine	Pre-Bupivacaine (Control)	Post-Bupivacaine	Pre-Bupivacaine (Control)	Post-Bupivacaine
HR	4 ± 10	1 ± 3	6 ± 12	0 ± 3	−2 ± 2	0 ± 4
LVSP	−11 ± 9	−10 ± 10	−62 ± 10	−50 ± 12	56 ± 35	57 ± 27
dP/dT maximum	1 ± 18	−12 ± 12	−47 ± 22	−33 ± 14	9 ± 11	32 ± 91
dP/dT minimum	0 ± 12	−12 ± 13	−57 ± 17	−44 ± 13	83 ± 38	55 ± 41

HR: heart rate, LAD: Left Anterior Descending Artery

* $p < 0.05$ vs. baseline