Premature Ventricular Contraction Coupling Interval Variability Destabilizes Cardiac Neuronal and Electrophysiological Control
Insights From Simultaneous Cardioneural Mapping

David Hamon, MD*; Pradeep S. Rajendran, BS*; Ray W. Chui, MS; Olujimi A. Ajijola, MD, PhD; Tadanobu Irie, MD, PhD; Ramin Talebi, BS; Siamak Salavatian, PhD; Marmar Vaseghi, MD, PhD; Jason S. Bradfield, MD; J. Andrew Armour, MD, PhD; Jeffrey L. Ardell, PhD; Kalyanam Shivkumar, MD, PhD

Background—Variability in premature ventricular contraction (PVC) coupling interval (CI) increases the risk of cardiomyopathy and sudden death. The autonomic nervous system regulates cardiac electrical and mechanical indices, and its dysregulation plays an important role in cardiac disease pathogenesis. The impact of PVCs on the intrinsic cardiac nervous system, a neural network on the heart, remains unknown. The objective was to determine the effect of PVCs and CI on intrinsic cardiac nervous system function in generating cardiac neuronal and electric instability using a novel cardioneural mapping approach.

Methods and Results—In a porcine model (n=8), neuronal activity was recorded from a ventricular ganglion using a microelectrode array, and cardiac electrophysiological mapping was performed. Neurons were functionally classified based on their response to afferent and efferent cardiovascular stimuli, with neurons that responded to both defined as convergent (local reflex processors). Dynamic changes in neuronal activity were then evaluated in response to right ventricular outflow tract PVCs with fixed short, fixed long, and variable CI. PVC delivery elicited a greater neuronal response than all other stimuli (P<0.05). The greatest cardiac electric instability was also observed after variable (short) CI PVCs.

Conclusions—Variable CI PVCs affect critical populations of intrinsic cardiac nervous system neurons and alter cardiac repolarization. These changes may be critical for arrhythmogenesis and remodeling, leading to cardiomyopathy.

Key Words: autonomic nervous system • coupling • intrinsic cardiac ganglia • neurocardiology • premature ventricular beats • PVC-induced cardiomyopathy

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remature ventricular contractions (PVCs) are common in clinical practice. In structurally normal hearts, despite being referred to as benign, PVCs may lead to cardiomyopathy or even to sudden cardiac death. Recent clinical studies have identified factors that predict worse outcomes in PVC patients. Particularly, patients with PVCs showing high coupling interval (CI) variability are at a greater risk for cardiac events, such as left ventricular (LV) dysfunction and sudden cardiac death.

Precise mechanisms underlying the adverse effects of PVCs remain unknown, but are likely multifactorial, including mechanical dyssynchrony, abnormalities in calcium handling and oxygen consumption, and autonomic imbalance. Of these mechanisms, the role of the autonomic nervous system (ANS) is not well understood. The ANS regulates all aspects of cardiac function. Afferent sensory neurons provide beat-to-beat information regarding the cardiac milieu. Processing and integration of this information at different levels of the ANS, including the intrinsic cardiac nervous system (ICNS), provides an elegant mechanism to ensure fine-tuned regulation of efferent neural signals to the heart. The ICNS, a distributed network of ganglia and interconnecting nerve fibers on the epicardial surface, represents the first level of the
WHAT IS KNOWN
- Variability in premature ventricular contraction (PVC) coupling interval increases the risk of cardiomyopathy and sudden death, yet the underlying mechanisms remain unknown.
- The autonomic nervous system regulates cardiac electric and mechanical indices, and its dysregulation plays an important role in cardiac disease pathogenesis. The ICNS, a neural network on the heart, is the first level involved in reflex control and impacted by cardiac injury.

WHAT THE STUDY ADDS
- PVCs are a powerful stressor for the ICNS, with the coupling interval being the predominant factor. Activation sequence is another important mechanism for the increase in dispersion of repolarization (ie, cardiac instability) on sinus beats after PVCs.
- PVCs with variable coupling intervals affect critical populations of ICNS neurons and alter cardiac repolarization, more than those with fixed short or long coupling intervals.
- We report, using a novel cardioneural mapping approach, that PVC-induced neural and electrophysiological changes may be critical for arrhythmogenesis and remodeling, leading to cardiomyopathy. Studies like ours, that provide a better understanding of neural control of cardiac function in healthy and disease states, will pave the way for neuroscience-based cardiovascular therapeutics.

Heart Rate Variability
Heart rate variability at baseline and after each of the PVC types was analyzed using the software Acknowledge (Biopac Systems, Goleta, CA).

Experimental Protocol
Once the animals stabilized after surgical preparation, cardiac neuronal activity was identified, and the following protocol was performed. Hemodynamic indices, cardiac electrophysiological data, and neuronal activity were recorded at baseline and during 5 minutes of PVCs and premature atrial contractions (PACs). PVCs and PACs were delivered every 10-sensed sinus beats (SBs) during a 5-minute sequence of short (effective refractory period +10 ms), long (80% of sinus rhythm cycle length), and variable CIs (random CIs generated between the short and long values). The order of sequences was chosen at random, and activity was allowed to return to baseline (minimum of 10-minute recovery interval) before proceeding to the subsequent intervention. Importantly, each intervention was compared with its own baseline. In addition, neurons were functionally classified as afferent, efferent, or convergent using the protocol outlined later.

Cardiac Electrophysiological Mapping
Epicaldic activation recovery intervals (ARIs), a surrogate for action potential duration, were derived from unipolar electrograms recorded from a 56-electrode sock array placed over the ventricles (Prucka CardioLab, GE Healthcare; Figure 1A). ARIs were calculated using a customized software ScalDyn (University of Utah, Salt Lake City, UT) as previously described. Global dispersion of repolarization (DOR) was calculated as the variance across all electrodes. ARIs and DORs were analyzed for the PVC and PAC beat delivered in the last minute, as well as the SBs following them (postextrasystolic SB [PES-SB]) that were compared with baseline SBs (average of 5 SBs before introduction of each extrasystolic subtype). To compare fixed with variable CI (short versus short and long versus long), at least 1 extrasystolic beat with a CI equal to the short and long CI subtypes was induced in the last minute of variable CI sequences. Thus, the electrophysiological impact of fixed versus variable CI type was not influenced by the immediate extrasystolic CI.

Intrinsic Cardiac Neuronal Recording
A 16-channel linear microelectrode array was used to record in vivo extracellular activity of cardiac neurons in the ventral interventricular ganglionated plexus (VIV GP), located at the origin of the left anterior descending coronary artery below the left atrial appendage, as previously described (Figure 1A and 1C).13,14

Functional Characterization of Intrinsic Cardiac Neurons
Cardiac neurons were functionally classified as afferent, efferent or convergent based on their responses to cardiovascular stimuli as previously described (Figure 1F and 1G).13,14 Afferent neurons were defined as those that received only mechanosensory inputs by epicardial mechanical stimuli or transduced changes in preload or afterload by transient occlusion of the inferior vena cava and descending thoracic aorta, respectively. Efferent neurons were defined as those that received only sympathetic or parasympathetic efferent inputs by bilateral stellate ganglia and cervical vagus nerve stimulation, respectively. Autonomic nerve stimulations were performed at low levels to assess direct inputs to the ICNS, independent of changes in hemodynamic indices (Table I in the Data Supplement). Neurons responding to both afferent and efferent stimuli were defined as convergent.13,14 A significant increase or decrease (P<0.05) in neuronal firing frequency during the intervention compared with baseline was considered as a response to that intervention (Figure 1F and 1G).13,14 Cardiac phase analysis (Figure 1E) and conditional probability analysis to assess ICNS network function was performed as previously described.13,14

Methods
Expanded version of Methods are presented in the Data Supplement.

Animals
Eight Yorkshire pigs (5 male and 3 female, weighing 57.1±2.5 kg) were used in this study. Animal experiments were performed in accordance with the National Institute of Health’s Guide for the Care and Use of Laboratory Animals and approved by the University of California, Los Angeles Chancellor’s Animal Research Committee.

Hemodynamic Assessment
LV cardiac mechanical indices were continuously recorded and analyzed at baseline and after interventions, including each of the PVC types.

ANS directly impacted by cardiac injury.12–14 Even in normal states, disruptions of ICNS function are arrhythmogenic.15 Moreover, neural remodeling within the ICNS after cardiac injury has been correlated with arrhythmias.13,14 Because both PVCs with variable CI and the ICNS have been linked to cardiomyopathy and life-threatening arrhythmias and premature atrial contractions (PACs), PVCs and PACs were delivered every 10-sensed sinus beats (SBs) during 5-minute sequences of short (effective refractory period +10 ms), long (80% of sinus rhythm cycle length), and variable CIs (random CIs generated between the short and long values). The order of sequences was chosen at random, and activity was allowed to return to baseline (minimum of 10-minute recovery interval) before proceeding to the subsequent intervention. Importantly, each intervention was compared with its own baseline. In addition, neurons were functionally classified as afferent, efferent, or convergent using the protocol outlined later.
PVC and PAC Delivery
A cardiac stimulator (EPS320, MicroPace, Canterbury, AU) was used to induce PVCs and PACs from the right ventricular outflow tract (RVOT) and right atrium, respectively, using a quadripolar pacing catheter (St Jude, St. Paul, MN). Atrial and ventricular pacing thresholds were measured, and PACs and PVCs were induced using the same current (1.2× RVOT threshold; 2 ms pulse width). In addition to PACs and PVCs, straight pacing was also performed at the same sites for 30 seconds, just overdriving the sinus rhythm cycle length (~20 ms). This was performed as a control to differentiate the effects of PVC from electrical stimuli and activation from the same site.
Statistics
Data are presented as mean±standard error of the mean. The significance level of changes in firing rate of each cardiac neuron between baseline versus stimulus interval was assessed using a statistical test developed for cortical neurons based on the Skellam distribution.18 This test has been previously validated for the study of cardiac neurons.13,14 A χ² test was used to compare the neuronal response between the different stimuli. A Wilcoxon rank–sum test or paired t test was used as appropriate to compare hemodynamic, ARI, and heart rate variability between baseline and each intervention. Pearson correlation was used to assess the strength of the relationship between local CI and repolarization time changes. A P<0.05 was considered to be statistically significant.

Results
Functional Characterization of Intrinsic Cardiac Neurons
The in vivo activity of cardiac neurons from the VIV GP was obtained at baseline and in response to cardiovascular stimuli, including PVCs and PACs. In 8 animals, the activity of 92 neurons (average: 11.5±2.6) was recorded. The basal firing frequency of the neurons was 0.11±0.02 Hz. Overall, based on their response to the cardiovascular stimuli, 44.6% of neurons were classified as afferent, 5.4% as efferent, and 23.9% as convergent (26.1% did not respond). A majority of neurons (92.8%) displayed activity clustered during a specific phase of the cardiac cycle, with 49.4% during systole, 25.3% during diastole, and the remaining 18.1% during both phases.

PVC as a Stimulus for Intrinsic Cardiac Neurons
The cardiac neuronal response to PVCs of any CI was compared with afferent and efferent cardiovascular stimuli, as well as cardiac pacing. With regards to afferent stimuli, a greater percentage of neurons were impacted by PVCs (66.3%) than by activation of mechanosensitive afferent inputs (39.1%), decrease in preload by inferior vena cava occlusion (32.6%), or increase in afterload by aortic occlusion (26.9%; P<0.001; Figure 2A). Similarly, in regards to efferent stimuli, the neuronal response to PVCs was greater than the response to either bilateral stellate ganglia (7.6%) or vagus nerve stimulation (28.3%; P<0.0001; Figure 2B). It is noteworthy that the vast majority of neurons that responded to afferent or efferent cardiovascular stimuli also responded to PVCs. In addition, PVCs induced a greater response from neurons when compared with straight RVOT (19.0%) and right atrial pacing (13.6%; P<0.0001; Figure 2C). Of all the neurons that responded to PVCs, 19.7% only responded to RVOT pacing and 14.8% did not respond to any other cardiovascular stimuli, including pacing. Therefore, neither hemodynamic changes (Table I in the Data Supplement) nor electric stimulation or dyssynchrony particularly explain PVCs’ impact on cardiac neurons.

Impact of PVC CI on Intrinsic Cardiac Neurons
The response of cardiac neurons to PVCs of short, long, and variable CIs was compared. Overall, 29.3% of neurons responded to short CI PVCs, 39.1% to long CI PVCs, and 43.5% to variable CI PVCs (P<0.05 for short versus variable CI; Figure 3A). The CI did not have a differential effect on afferent or efferent neurons. Twenty-seven percent of afferent neurons responded to short CI PVCs, 43.9% responded to long CI PVCs, and 34.1% to variable CI PVCs (Figure 3C). Forty percent of efferent neurons responded to short CI PVCs, 40.0% responded to long CI PVCs, and 60.0% to variable CI PVCs (Figure 3D). Interestingly, the CI did have a differential effect on convergent neurons. More convergent neurons responded to variable CI PVCs (72.7%) than either short (40.9%) or long CI PVCs (40.9%; P<0.05 for variable CI PVCs versus short and long CI PVCs; Figure 3E). Further, of the convergent neurons that responded to only 1 PVC CI, a vast majority (75%) responded to variable CI (Figure 3B). High neuronal responses (≥30%) were seen with PACs as well. For PACs, however, all 3 CI protocols evoked similar effects on ICNS function, whether considered as a whole or subsetted into afferent, efferent, or convergent populations (Figure 3A).

Impact of PVC CI on ICNS Processing of Efferent Inputs
We analyzed the subset of cardiac neurons that received sympathetic or parasympathetic inputs (efferent and convergent neurons) to determine whether the PVC CI had an impact
of the neurons receiving sympathetic input, a greater percentage responded to variable CI PVCs (100.0%) than either short (42.9%) or long CI PVCs (57.1%; \( P < 0.05 \) for variable CI PVCs versus short and long CI PVCs; Figure 3F). A similar trend was observed for neurons receiving parasympathetic input. Sixty-nine percent of neurons responded to variable CI, 42.3% to short CI, and 38.5% to long CI PVCs (\( P < 0.05 \) for variable CI PVCs versus short and long CI PVCs; Figure 3G). We also performed a spectral analysis of the heart rate variability, which showed that variable CI PVCs elicited the greatest increase in sympathovagal balance (low frequency/high frequency) compared with baseline (\( P < 0.05 \); Figure 3H).

**Impact of CI on Electromechanical Characteristics of PVCs and PACs**

We analyzed ARI, DOR, and hemodynamics during PVCs and PACs to determine whether CI had an impact on cardiac electric and mechanical indices. There was no significant
difference in CI between PVCs and PACs regarding short (524±39 and 549±37 ms, respectively) or long CI (749±56 and 765±56 ms, respectively).

Mean global ARIs of short CI PVCs (341.9±22.2 ms) and PACs (347.5±23.7 ms) were shorter than long CI PVCs (377.6±21.2 ms) and PACs (389.2±19.4 ms; \( P < 0.05 \) for short CI PVCs and PACs versus long CI PVCs and PACs). There was no significant difference between PVCs and PACs with short CI and a small borderline difference between PVCs and PACs with long CI (\( P = 0.05 \)). Thus, the mean global ARI seemed to be more closely related to the CI than the origin of the extrasystolic beat (Figure 4A).

The DOR (repolarization time variance), however, was greater in PVC beats (871±148 and 942±130 ms² for short and long CI, respectively) compared with PAC beats (550±49 and 498±69 ms² for short and long CI, respectively; \( P < 0.05 \) for PVC versus PAC). The CI did not significantly affect DOR in PVC or PAC beats, and thus, changes in DOR are likely explained by the activation sequence (Figure 4B).

Similarly, mean activation time and activation time dispersion, estimating duration and variability in ventricular activation, differed significantly between PVC and PAC beats of short and long CI (\( P < 0.05 \)), whereas CI had a minimal effect on activation time of the same extrasystolic origin. Consistent with this, the extrasystolic QRS width did not differ between short and long CI PVCs (143±4 and 141±4 ms) or PACs (83±3 and 80±3 ms). There was no significant difference in any of these parameters between fixed and variable PVCs having the same CI (eg, fixed short versus variable short).

Regarding hemodynamic indices (LV end-systolic pressure, LV +dP/dt and –dP/dt; Table) of extrasystolic beats, short CI PVCs had significantly lower values as compared with long CI PVCs and premature atrial contractions (PACs).

Table. Hemodynamics of Extrasystolic and Postextrasystolic Sinus Beat

<table>
<thead>
<tr>
<th></th>
<th>LV ESP, mm Hg</th>
<th>LV +dP/dt, mm Hg/s</th>
<th>LV–dP/dt, mm Hg/s</th>
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<tbody>
<tr>
<td><strong>ES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVC short coupling</td>
<td>53±13*</td>
<td>488±82*</td>
<td>−520±179*</td>
</tr>
<tr>
<td>PVC long coupling</td>
<td>81±11</td>
<td>1012±139</td>
<td>−919±180</td>
</tr>
<tr>
<td>PAC short coupling</td>
<td>66±16</td>
<td>722±180*</td>
<td>−834±271</td>
</tr>
<tr>
<td>PAC long coupling</td>
<td>83±12</td>
<td>1072±139</td>
<td>−1042±162</td>
</tr>
<tr>
<td><strong>PES-SB</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVC short coupling</td>
<td>88±12</td>
<td>1524±179†</td>
<td>−1040±166</td>
</tr>
<tr>
<td>PVC long coupling</td>
<td>84±11</td>
<td>1213±150</td>
<td>−1058±161</td>
</tr>
<tr>
<td>PAC short coupling</td>
<td>84±14</td>
<td>1308±203*</td>
<td>−929±220</td>
</tr>
<tr>
<td>PAC long coupling</td>
<td>85±13</td>
<td>1207±181</td>
<td>−991±181</td>
</tr>
</tbody>
</table>

For extrasystolic (ES) beats, left ventricular (LV) end-systolic pressure (LV ESP), maximum rate of LV pressure change (LV +dP/dt) and minimum rate of LV pressure change (LV–dP/dt) for ventricular and atrial ES beats of different coupling intervals. For postextrasystolic sinus beat (PES-SB), hemodynamic parameters of PES-SB following ventricular and atrial ES beats of different coupling intervals. PAC indicates premature atrial contraction; and PVC, premature ventricular contraction.

*\( P < 0.05 \) for short versus long coupling.
†\( P < 0.001 \) for short versus long coupling.
CI PVCs ($P < 0.05$ for all), while only the LV+dP/dt differed between long and short CI PACs ($P < 0.05$). Therefore, both the CI and the extrasystolic origin seemed to affect hemodynamic indices, with CI being the predominant factor.

**Impact of Extrasystolic CI on the Postextrasystolic SB Indices**

To assess the impact of extrasystolic beats on cardiac electric stability, ARI and DOR were analyzed for the 5 SB after extrasystolic beats (PVC and PAC) of each CI type and were compared with baseline SB before their introduction. Maximal changes in ARI were always seen on the PES-SB, returning progressively to baseline over the subsequent SBs. Maximal increase in DOR could be seen from the PES-SB to the following 4 SBs because recovery toward baseline ARI values was sometime heterogeneous across different heart regions. However, overall, the mean maximal increase in DOR was also seen on the PES-SB, and all comparisons were performed on this specific beat (Figure 4C and 4D). Overall, PVCs induced a greater increase in the PES-SB DOR than PACs ($P < 0.05$). A short CI also had a greater impact than long CI ES beat ($P < 0.05$), and finally, there was a trend for an increase in DOR with variable CI as compared with fixed CI ($P = 0.10$, paired for the exact same CI), which was driven by differences induced by PVCs ($P = 0.06$), but not PACs ($P = 0.8$). Finally, when impact of each extrasystolic subtype on PES-SB DOR was compared with its own baseline SB DOR, only the variable short CI PVCs, cumulating all aforementioned characteristics, reached statistical significance ($P < 0.05$).

Regarding the impact of extrasystolic beats on the PES-SB hemodynamics (Table), a PES potentiation was observed with an inverse relationship to the CI. The most affected index was LV+dP/dt, which was significantly greater after a short than a long CI PVC ($P < 0.001$) or PAC ($P < 0.05$). Of note, the PES pause was greater after a short than a long CI PVC (1208±97 versus 992±81 ms; $P < 0.05$). As a result of these compensatory effects between extrasystolic beats and PES-SB, no global heart rate or hemodynamic changes were seen during the 5 minutes of PVCs or PACs at any CI when compared with that in baseline (Table I in the Data Supplement).

**Local Coupling Interval Impact on Extrasystolic and Postextrasystolic SB Dispersion**

We further investigated whether some electrophysiological characteristics of PVCs and PACs could explain why at similar CIs (short) PACs and PVCs (and PES-SB) display similar mean global ARI, while they have a dramatically different effect on DOR. The activation sequence when a PVC depolarizes the heart is radically different than the SB activation sequence. Indeed, regions activated late during sinus rhythm but early during RVOT PVCs (eg, RVOT) have a shorter CI than regions activated early during sinus rhythm but late during PVCs (eg, apex; Figure 5). Regional differences ($\leq 127$ ms) in local CI were observed between different electrodes with RVOT PVCs. We found that electrodes with shorter local CI during PVCs displayed greater shortening in the local PVC repolarization time than electrodes with a longer local CI ($r = 0.83\pm 0.07$; $P < 0.001$). Interestingly, this local CI impact partially remained on the subsequent beat (PES-SB) with a greater shortening in repolarization in regions previously affected with a shorter local PVC CI ($r = 0.32\pm 0.05$; $P < 0.05$). On the contrary, because this local CI effect does not exist with PACs, they produce homogeneous changes in repolarization across the heart.

**Discussion**

**Main Findings**

This is the first study assessing the impact of PVCs, induced at different CIs, on cardiac neuronal and electric stability with concurrent in vivo cardioneural mapping. Our main findings are the following:

1. PVCs (even a modest burden of 10%) are a powerful stressor, altering the activity of critical cardiac neuronal populations.
2. The association of an abnormal timing (CI) and activation sequence characterizing PVCs triggered these changes, with the CI being the predominant factor.
3. Variable CI PVCs compared with those with fixed short or long CIs had a significantly greater impact on cardiac neurons, more specifically on convergent neurons, which are responsible for reflex processing at the level of the heart.
4. Variable CI PVCs also differentially affected a greater percentage of neurons receiving sympathetic and parasympathetic input than the fixed CI PVCs. These sympathetic/parasympathetic interactions, mediated within the ICNS, may contribute to the increase in low frequency/high frequency ratio after variable CI PVCs.
5. Mirroring IC neuronal changes, the greatest cardiac electric instability in the PES-SB (ie, increase in dispersion) was seen after variable (short) CI PVCs. Factors increasing PES-SB dispersion were PVCs as opposed to PACs (heterogeneity in local CI across the heart), a shorter CI, and a variable CI.

**PVCs as a Unique and Powerful Stressor: Mechanistic Implications**

RVOT PVCs affected a large proportion (66.3%) of neurons contained within the VIV GP. These neurons are primarily associated with control of ventricular function.14 Most neurons that responded to afferent and efferent cardiovascular stimuli also responded to PVCs, suggesting that PVCs preferentially engage convergent neurons (Figure 2). Similar to a previous study, 26% of neurons did not respond to afferent and efferent cardiovascular stressors used for classification of cardiac neurons.14 Interestingly, however, almost half of these neurons (46%) responded to PVCs, which indicates that PVCs pose a strong and unique stress to ICNS neurons. We also analyzed the concomitant responses of this specific subset of neurons to PACs and straight pacing from the RVOT. These data indicated that the mechanism involved in triggering the changes in neuronal activity was predominately related to timing (ie, neurons were also activated by PACs) and only a small percentage of neurons also responded to the same abnormal myocardial activation sequence (ie, RVOT pacing). The remaining neurons responded to either a combination of timing with activation
abnormalities or involved another mechanism (ie, concomitant activation by both PACs and RVOT pacing or neither).

Interestingly, ventricular dyssynchrony, which involves a combination of abnormal timing and activation sequence, can be measured using strain indices and is likely to be a trigger that impacts neuronal activity. Hamdan et al have shown that biventricular pacing was associated with lower muscle sympathetic nerve activity than right ventricular pacing alone. Similarly, muscle sympathetic nerve activity and coronary sinus catecholamine levels are correlated with the burden of PVCs (induced by pacing) in patients, highlighting a sympathetic neuro-humoral impact potentially involving the heart. These changes on muscle sympathetic nerve activity were subsequently confirmed in heart failure patients during spontaneous PVCs, providing further support for the validity of our experimental model involving pacing-induced PVCs.

A recent study provided important insight on PVC-induced dyssynchrony, showing that the timing (ie, CI) had the greatest impact, consistent with our data. Importantly, they reported that longer CI resulted in more pronounced LV dysynchrony. It is interesting to note that long CI PVCs tended to affect more neurons and, particularly, more afferent neurons than short CI PVCs in our study. However, our electric

Figure 5. Premature ventricular contractions (PVC) local coupling interval impact on repolarization. **A**, Representative trace showing a sinus beat followed by a PVC induced at a coupling of 496 ms and the subsequent postextrasystolic sinus beat on (1) surface ECG lead I, (2) a unipolar sock electrode recorded from the right ventricular outflow tract (RVOT), and (3) from the left ventricular (LV) posterior-apical wall. Overall, mean activation time (AT), repolarization time (RT), and activation recovery interval (ARI) across the heart and values recorded from RVOT and LV posterior electrodes are displayed under each respective trace. **B**, Polar map showing myocardial activation during the sinus beat and the subsequent PVC and postextrasystolic beats. White and black stars indicate location of RVOT and LV posterior-apical wall electrodes, respectively. Note that the RVOT electrode, which had a shorter local CI than the LV posterior-apical one, was characterized by a greater shortening in RT that remained on the postextrasystolic-sinus beat, while activation pattern was back to normal. Such PVC-induced arrhythmogenic substrate may increase the likelihood for subsequent critically timed PVCs to trigger re-entry-mediated ventricular arrhythmias.
data showed that PVC activation time dispersion and DOR did not differ between short and long CI, and our hemodynamic data showed that short CI had a greater impact on both PVC beat and PES-SB compared with long CI. Therefore, adverse effect of PVCs is not solely hemodynamically mediated (Table; Table I in the Data Supplement). Rather, a greater preload (with longer CI PVCs), although inducing a better overall hemodynamic profile, may exaggerate mechanical stretch on the myocardial wall, thereby increasing activity in sensory neurites that are locally present.

Finally, perturbations in atrioventricular relationship are known to increase muscle sympathetic nerve activity, especially during closely coupled atrial and ventricular systole. We observed similar features (close systolic coupling) during long CI PVCs, which could have had an additional impact on ICNS neurons.

Impact of Variable Coupling PVCs on ICNS Network Function
Our data demonstrates that variable CI PVCs had a significantly greater impact on cardiac neurons, especially on convergent neurons, the local reflex processors. We compared the functional connectivity of neurons that responded to variable CI PVCs versus those that did not. Interestingly, we observed that functional network connectivity was greater with neurons that responded to variable CI PVCs (Figure 6). Variable CI PVCs seem to have a more complex impact on cardiac neurons than just the addition of short and long CI PVCs. Indeed, we have shown that most (75%) convergent neurons affected by 1 CI PVC type were only activated by variable CI PVCs (Figure 3B). Similarly, variable CI PVCs differentially affected a greater percentage of neurons receiving sympathetic/parasympathetic inputs (Figure 3F and 3G). Finally, there was no difference in the percentage of afferent neurons affected (Figure 3C). Therefore, unpredictability in CI appeared to be a specific trigger that a subpopulation of convergent neurons can detect, further causing sympathovagal imbalance. This cardio-cardiac reflex, likely also involving higher centers in the neuraxis (Figure 7), may subsequently impact cardiomyocyte function and lead to electric instability.

Enhanced response of neurons to a variable compared with constant stimulus has been reported in sensory neurons in visual, auditory, and olfactory system, a concept known as neural adaptation. Similarly, in the cardiovascular system, it has been previously shown that sympathetic nerve activity measured by muscle sympathetic nerve activity was greater when the heart was paced irregularly, and these findings were independent of hemodynamic changes. We speculate that variability of PVC CI compared with fixed CI may prevent neural adaptation and play an important role in reflex activation of the ANS.

Impact of PVCs on Cardiac Electric Stability: PES-SB Dispersion of Repolarization
Increase in the SB DOR has been described as arrhythmogenic, being a requirement for electric reentry and lethal ventricular arrhythmias. Furthermore, DOR has been strongly correlated with the Tpeak–Tend interval, which is a predictor of sudden cardiac death risk in most cardiomyopathies, as well as in more heterogeneous populations. Similarly, other ECG parameters estimating the spatial or temporal DOR have been shown to improve sudden cardiac death prediction.

More specifically, a short–long sequence has been described as a major trigger for ventricular arrhythmias. Therefore, part of the arrhythmogenesis may be explained by the PES-SB DOR (after the short CI PVC) and another by the CI of the subsequent PVC. PES-SB dispersion was globally higher after PVC (versus same CI PAC) because of their nonuniform local CI across the heart. A malignant short–long–short sequence after a PAC (as first short) has never been reported. Heart regions that depolarize late during sinus rhythm and early during PVCs (shorter local CI) are more impacted (shorter repolarization) than regions having a longer local CI (Figure 5).

Interestingly, PVCs arising from late activated regions in sinus rhythm (eg, aortic cusps, epicardial) are associated with worse outcomes. Finally, variable CI PVCs increased PES-SB dispersion as compared with fixed CI PVCs, despite comparisons after similar CI. Therefore, impact of variable CI on neuronal stability was the last potential mechanistic component of the increase in PES-SB dispersion that we could identify in the present study. Indeed, sympathetic stimulation has been shown to increase DOR experimentally in porcine models, as well as in humans, and lead to lethal ventricular arrhythmias. By carrying all deleterious characteristics, only variable short CI PVC induced a statistically significant increase in the PES-SB dispersion as compared with baseline. The level of dispersion necessary to initiate ventricular arrhythmias remains unknown, and given the rare incidence of such event, additional stress-mediated autonomic involvement is likely necessary to provide sufficient functional arrhythmogenic substrate.

Interestingly, in addition to inducing a greater PES-SB DOR (ie, vulnerability), a greater CI variability would also increase the likelihood for a subsequent PVC to trigger an arrhythmia if a specific CI is required. Finally, intracellular calcium handling likely involved in acute changes in DOR may translate into heterogeneous ion channel remodeling, resulting in marked heterogeneity in action potential configurations and durations, as reported in a chronic canine model of PVC-induced cardiomyopathy, which may lead to a more sustained arrhythmogenic substrate.

Neurmodulation represents an attractive approach that has been shown to specifically inhibit deleterious activity within the ICNS or intrathoracic extracardiac ganglia, thereby, mitigating the substrate and preventing arrhythmias. Further, it has antifibrotic properties, and myocardial fibrosis has been characterized in a model of PVC-induced cardiomyopathy and could compromise recovery, even after successful PVC suppression.

Limitations
General anesthetics may suppress evoked responses in the ANS. However, after surgical preparation, we switched to α-chloralose, which has minimal effects on ANS reflexes. Neuronal recording was selectively performed in the VIV GP (1 of 11 GPs in porcine heart). However, GPs have been described to have spatially divergent receptive fields, capable of transducing information from widespread cardiac regions, and VIV GP is primarily associated with control of ventricular function. It is also noteworthy that there is a high degree of
communication at all levels of the ANS and changes in low frequency/high frequency after PVCs, believed to reflect global sympathetic-vagal balance and its effect on cardiac dynamics, seemed to mirror the local impact on VIV GP neurons. This unique set of data assessing acute changes would benefit from confirmation in a chronic PVC model, in the setting of heart disease, and prompt further assessment of functional and anatomopathological remodeling at different levels of the neuraxis. Additionally, whether the differential effect of the CI seen on cardiac electric stability and neuronal behavior is dependent on a specific burden or a PVC location remains unknown. Indeed, we studied PVCs from only 1 location (RVOT), which is the most commonly encountered clinically, and investigating different locations in this set of animals was not feasible. A 10% burden induced acutely was enough to have a significant impact, which has been previously described as the lowest burden inducing a reversible cardiomyopathy, particularly with an epicardial origin. Although our data suggested that part
of destabilizing cardiac repolarization changes were mediated through PVC-induced changes in IC neural activity, our study was not sufficiently powered to establish a direct temporal link between these 2 components.

Surrogate markers of arrhythmogenesis have been used in this study rather than inducibility testing, which would have compromised our model of PVC delivery subsequent to sensed SB. Moreover, cardioversion shocks required to resuscitate animals from a ventricular arrhythmia would have disrupted or dislodged our neuronal recording interface. An extensive literature has correlated these surrogates with sudden cardiac death.25–32

Clinical Implications
Currently, the clinical approach for PVC patients consists of ruling out a structural arrhythmogenic substrate and, thereby, classifying the PVC as benign. However, cardiac events are known to occur with benign PVCs,2,3,5 and we have yet to deci-

cide whether the mechanism behind this small, but real risk. This study provides important mechanistic insights into mechanoelectric feedback (mediated through cardiac neurons) that have the potential to contribute to a new avenue of investigation and potentially provide a mechanistic basis for PVC-induced cardiomyopathy/arhythmogenesis.

Figure 7. Autonomic control of the heart. DRG indicates dorsal root ganglia; and ICNS, intrinsic cardiac nervous system.

Disclosures
None.

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Premature Ventricular Contraction Coupling Interval Variability Destabilizes Cardiac Neuronal and Electrophysiological Control: Insights From Simultaneous Cardioneural Mapping

David Hamon, Pradeep S. Rajendran, Ray W. Chui, Olujimi A. Ajijola, Tadanobu Irie, Ramin Talebi, Siamak Salavatian, Marmar Vaseghi, Jason S. Bradfield, J. Andrew Armour, Jeffrey L. Ardell and Kalyanam Shivkumar

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SUPPLEMENTAL MATERIAL

Methods

Animals

This study was approved by the University of California, Los Angeles Chancellor’s Animal Research Committee and conforms to the National Institute of Health’s Guide for the Care and Use of Laboratory Animals. Eight Yorkshire pigs (5 male and 3 female, weighing 57.1 ± 2.5 kg) were used.

Surgical preparation

Animals were sedated with telazol (6 mg/kg, IM), intubated and mechanically ventilated. General anesthesia was maintained with isoflurane (1-2%, INH). Femoral vein was cannulated for fluid maintenance and drug administration. A median sternotomy was performed to expose the heart and isolate both stellate ganglia. Lateral neck dissections were performed to isolate both cervical vagal trunks. Following completion of the surgical preparation, general anesthesia was changed to α-chloralose (50 mg/kg bolus followed by 35 mg/kg/hr continuous intravenous infusion). Body temperature was continuously monitored and maintained via circulating water heating pads. Acid-based status was evaluated hourly; respiratory rate and tidal volume were adjusted and bicarbonate was infused as necessary to maintain blood gas homeostasis. At the completion of the experiment, animals were euthanized using sodium pentobarbital (200 mg/kg, intravenous) and potassium chloride (150 mg/kg, intravenous) to arrest the heart.

Hemodynamic assessment

Left ventricular (LV) cardiac mechanical indices (LV end-systolic pressure, maximum rate of LV pressure change (dP/dt+) and minimum rate of LV pressure change (dP/dt-)) were continuously obtained by using a pressure transducer catheter (Mikro-Tip, Millar Instruments,
Houston, TX, USA) that was ultrasound guided into the LV via the left carotid artery and connected to a control unit (PCU-200, Millar Instruments). Systemic arterial pressure was obtained by using a pressure transducer attached to a cannula in the femoral artery. In addition, a 12-lead surface electrocardiogram was obtained using a cardiac electrophysiology recording system (Prucka CardioLab, GE Healthcare, Fairfield, CT, USA). A minimum of 3 beats were averaged for these indices during each condition for hemodynamic analyses.

**Heart rate variability**

Five minute intervals of electrocardiogram recording at baseline and following each of the premature ventricular contraction types were analyzed for heart rate variability using the Acknowledge (Biopac Systems, Goleta, CA, USA) software. Normalized low frequency band was used to estimate sympathetic tone, normalized high frequency band for parasympathetic tone, and the ratio as an index of sympatho-vagal balance.\(^1\)

**Cardiac electrophysiological mapping**

Activation recovery intervals are a well-correlated surrogate for action potential duration.\(^2\) Epicardial activation recovery intervals were derived from unipolar electrograms recorded (Prucka CardioLab, GE Healthcare) from a custom 56-electrode sock placed over the ventricles. ARIs were calculated using a customized software ScalDyn (University of Utah, Salt Lake City, UT, USA) as previously described.\(^3,4\) Activation time was defined as the time interval from the beginning of the QRS complex to the most negative derivative of the activation wave front, and repolarization time as the time interval from the beginning of the QRS complex to the most positive derivative of the repolarization wave front. Activation recovery interval was calculated as the difference between the activation and repolarization times. Global dispersion of repolarization (DOR) was calculated as the variance across all electrodes. Activation recovery
intervals and DOR were analyzed for the premature ventricular and atrial contraction beat delivered in the last minute, as well as the sinus beats following them (postextrasystolic sinus beat) that were compared with baseline sinus beats (average of 5 sinus beats before introduction of each extrasystolic subtype). To compare fixed with variable coupling interval (CI) (short versus short and long versus long), at least one extrasystolic beat with a CI equal to the short as well as the long CI subtypes was induced in the last minute of variable CI sequences. Thus, electrophysiological impact of fixed versus variable CI type was not influenced by the immediate extrasystolic CI.

**Intrinsic cardiac neuronal recording**

A linear microelectrode array was embedded in the ventral interventricular ganglionated plexus to record *in vivo* extracellular activity of cardiac neurons as previously described. The array consisted of 16 platinum–iridium electrodes (25 μm diameter electrodes with an exposed tip of 2 mm; impedance 0.3–0.5 MΩ at 1 kHz). The array was attached to a flexible cable, making it semi-floating. The electrode wires, as well as ground and reference electrodes, were connected to a microelectrode amplifier with a headstage pre-amplifier (Model 3600, A-M Systems Inc., Carlsborg, WA, USA). For each channel, filters were set to 300 Hz to 3 kHz with a gain of 1000. Cardiac neuronal waveform, hemodynamic data, and electrocardiogram (ECG) were input to a data acquisition system (Power1401, Cambridge Electronic Design, Cambridge, UK). Data analysis including artifact removal and spike sorting to identify single units was performed offline using the Spike2 (Cambridge Electronic Design) software. It is noteworthy that each of the 16 electrodes on the array can record the extracellular action potentials of several single units (neurons), with each neuron being identified by its unique waveform using principle component analysis. The waveform of a given neuron remains constant throughout the experiment.
Functional characterization of intrinsic cardiac neurons

Cardiac neurons were functionally classified as afferent, efferent, or convergent based on their responses to cardiovascular stimuli as previously described (Figure 1F & G).\textsuperscript{5, 6} Afferent neurons were defined as those that only received mechanosensory inputs and/or transduced changes in preload or afterload. To determine whether neurons received mechanosensory inputs, epicardial mechanical stimuli was applied for 10 seconds at the following 6 sites: right ventricular (RV) outflow tract (RVOT), RV mid-anterior wall, RV apex, LV mid-anterior wall, LV lateral wall and LV apex. To determine whether neurons transduced changes in preload and afterload, transient (30 s) complete occlusions of the inferior vena cava and descending thoracic aorta were performed using balloon catheters (20 mm, Atlas, Bard PV, AZ, USA) inserted through the femoral vein and femoral artery, respectively. Efferent neurons were defined as those that only received sympathetic and/or parasympathetic efferent inputs. For efferent stimulation, bipolar needle electrodes were inserted into the stellate ganglia and bipolar spiral cuff electrodes were wrapped around the cervical vagal trunk (Cyberonics Inc., PerenniaFlex Model 304, Houston, TX, USA) and connected to a stimulator with an isolation unit (Grass S88 and PSIU6, Natus Medical Inc., Pleasanton, CA, USA). For each stellate ganglia, threshold was defined as the current necessary to evoke a 10% increase in heart rate or blood pressure (4 Hz frequency, 4 ms pulse width). For each vagus, threshold was defined as the current necessary to evoke a 10% decrease in heart rate or blood pressure (10 Hz frequency, 1 ms pulse width). Bilateral stellate ganglia and vagus nerve stimulation were then performed for 1 minute at threshold current and a frequency of 1 Hz. Low frequencies were used for stimulation to assess direct inputs to the ICNS independent of changes in hemodynamic indices. Neurons responding to both afferent and efferent stimuli were defined as convergent.\textsuperscript{5, 6}
For epicardial mechanical stimuli and autonomic efferent nerve stimulations, cardiac neuronal activity was compared one minute before the stimuli (baseline) versus during the stimuli. For vascular occlusions, PVCs, PACs and pacing, neuronal activity was compared at baseline versus during the stimuli, as well as at baseline versus one minute after the stimuli (recovery). After each stimulus, we waited for neuronal activity and hemodynamics to return to baseline levels before proceeding. A significant increase or decrease (P<0.05) in neuronal firing frequency was considered as a change in neuronal activity to a given intervention (Figure 1F & G).5, 6

**Cardiac phase analysis**

Cardiac phase analysis was performed to determine if neurons displayed cardiac cycle-related periodicity as previously described.5, 6 Based on an activity histogram, neurons that generated at least 10 action potentials at baseline were classified as being related to a specific phase of the cardiac cycle if more than 30% of their activity occurred during the given phase.

**Conditional probability analysis**

Conditional probability analysis to assess ICNS network function was performed as previously described.5, 6 The conditional probability (probability: response to Y | response to X) was estimated as the number of neurons that responded to both stimulus X and Y, divided by the number of neurons that responded to stimulus X.

**References:**


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<th></th>
<th>Heart Rate (bpm)</th>
<th>LV ESP (mmHg)</th>
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<td>Baseline</td>
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<tr>
<td>IVC occlusion</td>
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<td>Aortic occlusion</td>
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<th>LV -dP/dt (mmHg/s)</th>
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**Supplemental Table 1:** Hemodynamics of interventions. BSGS, bilateral stellate ganglia stimulation; BVNS, bilateral vagus nerve stimulation; IVC, inferior vena cava; LV ESP, left ventricular end-systolic pressure; PAC, premature atrial contraction; PVC, premature ventricular contraction. *, P<0.05 for intervention versus baseline.