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.[5, 6] 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.[5, 6] 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).[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.[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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	Heart Rate (bpm)		LV ESP (mmHg)	
	Baseline	**Intervention**	**Baseline**	**Intervention**
PVC short coupling	68 ± 5	70 ± 5	97 ± 13	91 ± 13
PVC long coupling	69 ± 6	70 ± 6	91 ± 10	89 ± 12
PVC variable coupling	67 ± 5	70 ± 5	90 ± 14	87 ± 14
PAC short coupling	65 ± 4	66 ± 4	87 ± 13	85 ± 13
PAC long coupling	64 ± 4	66 ± 3	84 ± 14	84 ± 14
PAC variable coupling	64 ± 4	66 ± 4	77 ± 15	79 ± 16
IVC occlusion	73 ± 6	72 ± 5	89 ± 13	55 ± 9*****
Aortic occlusion	70 ± 6	67 ± 7	84 ± 8	135 ± 14*****
BVNS	67 ± 5	65 ± 5	89 ± 15	85 ± 13
BSGS	71 ± 6	72 ± 6	96 ± 11	103 ± 12
	LV +dP/dt (mmHg/s)		**LV –dP/dt (mmHg/s)**	
PVC short coupling	1265 ± 172	1207 ± 158	-1246 ± 209	-1137 ± 207
PVC long coupling	1216 ± 142	1189 ± 151	-1057 ± 142	-1056 ± 176
PVC variable coupling	1226 ± 157	1195 ± 146	-1157 ± 189	-1110 ± 205
PAC short coupling	1156 ± 155	1174 ± 168	-1005 ± 208	-965 ± 191
PAC long coupling	1162 ± 189	1138 ± 192	-970 ± 210	-962 ± 223
PAC variable coupling	1107 ± 198	1122 ± 195	-875 ± 200	-927 ± 232
IVC occlusion	1238 ± 169	805 ± 107*****	-1069 ± 201	-539 ± 101*****
Aortic occlusion	1154 ± 105	1055 ± 111	-998 ± 143	-1454 ± 325
BVNS	1206 ± 194	1175 ± 187	-1030 ± 242	-902 ± 207
BSGS	1143 ± 167	1237 ± 140	-1204 ± 175	-1193 ± 152

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.