Animal: Yorkshire Pig

Surgical Prep:
- Sedation Telazol (8mg/kg)
- Anesthesia isoflurane (1-2% inhalation) concomitant with intermittent boluses of fentanyl (1–3 μg/kg iv)

Vascular access: femoral artery (pressure) veins (fluid)
- mid-sternotomy: access to heart, great vessels

Nodose Neuronal output recording of spike output and frequency (see Neural recording and analysis-workflow in protocols.io)

The pig is placed in dorsal recumbency. A midline incision is made at the neck and the skin is reflected back to form a window viewing the larynx and soft tissues surrounding the trachea. The soft tissues (muscles, adipose tissue) ventral and lateral to the trachea are removed so as to see the neurovascular tracts containing nerve, artery and vein. The nerve is carefully dissected and followed cranially (1–3+ cm) to find the bulge associated with the nerve – which is the nodose ganglion. The nodose ganglion is morphologically defined as a "bulge" along the vagus nerve.
Custom made linear microelectrode arrays developed to overcome the problem of difficulty in penetrating the nodose ganglia for recordings were utilized for functional recordings in the nodose ganglia. (Data analytics in protocol Work flow) protocol

To determine whether identified right atrial neuronal populations transduce mechanosensory inputs from select cardiac tissues, the right ventricular conus and the left ventricular lateral wall were sequentially touched gently by a finger during 10 s intervals with at least 2 min baseline data obtained between stimulus applications. A length of saline-soaked umbilical tape was placed around the base of the inferior vena cava and another one around the descending thoracic aorta. Silk ligatures were placed around the left anterior descending coronary artery about 1 cm from its origin. Enabling repeated occlusion of the inferior vena cava (for 20 s), the descending aorta (for 20 s) and the left anterior descending coronary artery (for 1 min) while recording evoked changes in IC neuronal activities. At least 5 min separated each of these stressors, thereby allowing for return to basal activities.

6 Cardiac Electrophysiology:
ECG recording
Surface ECG recordings. Continuous 12-lead ECG data were recorded using a holter monitoring system (H12digital monitor; Mortara Instruments, Milwaukee, WI). Frontal plane lead electrodes were placed in standard positions. To accommodate the open-chest surgical procedure, precordial lead electrodes V1 through V6 were placed posteriorly in the positions of V6 through V11 to mirror standard, anterior, precordial lead electrode placement and record the horizontal plane. ECGs were analyzed manually. T-wave vector changes in the ventral-dorsal (horizontal plane) and superior-inferior (frontal plane) were assessed using posteriorly placed leads as well as limb leads. Time from peak to end of T-wave (Tp-e) was measured from maximal T-wave voltage to the end of T-wave in limb leads with the clearest T-wave recording

7 ARI Recordings
A custom 56-electrode sock, placed over both ventricles, was attached to a Prucka CardioLab (GE Healthcare, Fairfield, CT) to identify regional activation recovery intervals (ARI). Global ventricular ARIs were calculated via customized software ScalDyn M (University of Utah, Salt Lake City, UT), as described previously. Localized ventricular epicardial activation times (ATs) were measured from the beginning of the QRS complex to the first minimal dV/dt in the QRS complex. Localized epicardial recovery times (RT) were computed from the beginning of the QRS complex to the first maximal dV/dt of the T wave. Activation recovery intervals were derived from subtracting ATs from these RTs. This parameter has been shown to correlate with local ventricular action potential durations. Global dispersion in ARI was calculated using the variance of all 56-electrode ARIs to identify spatial dispersion of regional ventricular epicardial

8 Hemodynamic
Systolic LV pressures were assessed by using a 5-F pigtail, 12-pole conductance-pressure catheter connected to an MPVS Ultra processor (Millar Instruments, Inc, Houston, TX) placed in the left ventricle via carotid artery sheath under ultrasound guidance. Proper catheter position was confirmed by the examination of segmental volume signals. Pressure was continuously monitored and recorded throughout experiments. Increases in LV pressures were noted at stimulation onset, confirming successful stimulation capture. Furthermore, a femoral arterial catheter was also placed and used for systemic arterial pressure monitoring.

9 Blood gases: monitored recorded