

## Pig ICN recording

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mid-sternotomy	
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Animal: Yorkshire Pig

Surgical Prep:

Sedation Telazol (8mg/kg)

Anasthesia isoflurane (1-2% inhalation), concomitant with intermittent boluses of fentanyl (1-3 μg/kg iv).

Vascular Access: femoral artery (pressure), vein (fluids)

Following completion of the surgery, anesthesia was changed to  $\alpha$ -chloralose (50 mg/kg intravenous bolus administration followed by continuous infusion at 10 mg·kg<sup>-1</sup>·h<sup>-1</sup> iv)

- Anasthesia for surgerey; 1-2% isoflurane
- Vascular access: femoral artery (pressure) veins (fluid)
- mid-sternotomy:access to heart, great vessels
- 5 Activity generated by neurons in the RAGP (or other ICN) is recorded in situ with a linear micro-electrode array. The multichannel linear microelectrode array (MicroProbes Inc., Guithersberg, MD,USA), consists of 16 platinum/iridium electrodes (25µm diameter electrode with an exposed tip of 2 mm; impedance 0.3-0.5 Mat1 kHz), is embedded in the right atrial fat that containing the RAGP such that its tip is placed adjacent to right atrial musculature
- The probe was attached to a flexible lead, allowing the probe to be semi-floating. The density of tissue in the ventral right atrial fat helped to maintain position stabilityover prolonged periods of time (6-8 h of recording)
- The connecting wires of the multichannel electrode, along with earth and reference wires, were attached to a 16-channel microelectrode amplifier with headstage pre-amplifier (A-M systems, Inc., model 3600; Carlsborg, WA,USA).
- neFor each channel, filters were set to 300 Hz to3kHz, and gain to 5K.A hook electrode was sewn to the atrial myocardium close to the RAGP

to provide a reference right atrial electrogram.

- 9 This atrial electrogram was utilized for determination of atrial rate, duration and characterization of atrial arrhythmias, including atrial fibrillation, and for identification of the timing of atrial electrical artifacts contained within the neural recording data.
- The 16 microelectrode array signals, along with recorded cardiovascular indices (ECG, right atrium electrogram and haemodynamic data see below), were digitized via a Cambridge Electronics Design (Cambridge, UK; model1401) data acquisition system for off-line analysis. The sampling frequency for neuronal data was 5.26 kHz; it was six times lower (0.877 kHz) for all other signals.
- Neuronal activity was identified as action potentials with signal-to-noise ratios greater than 3:1. The activity generated by individual neuronal somata was identified by the amplitude and configuration (waveform recognition) of these recorded action potentials, using the Spike 2 program. By using these techniques and criteria, action potentials generated by individual somata and/or dendrites, rather than axons of passage, can be recorded for extended periods of time
- 12 anasthesia for recording: alpha-chloralose (50 mg/kg iv bolus, 10 mg/kg/hr iv continuous)
- Hemodynamic recording
  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

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