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Dual Optical Mapping of Action Potentials and Calcium Transients in the Mouse Heart during Optogenetic Stimulation of the ICNS

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Salama G, Hwang SM. Simultaneous optical mapping of intracellular free calcium and action potentials from langendorff perfused hearts. Curr Protoc Cytom. 2009;Chapter 12(Unit 12):17.

Protocol status: In development
We are still developing and optimizing this protocol.

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Troubleshooting

1 The animal is euthanized by a method approved by IACUC (Institutional Animal Care and Use Committee), the chest opened, the heart and lungs removed, and the aorta quickly cannulated on a Langendorff apparatus to perfuse the coronary vessels. The cannulation step should occur in ice-cold Tyrode's solution (112mM NaCl, 5mM KCl, 25mM NaHCO3, 1mM KH2PO4, 1.2mM MgSO4, 1.8mM CaCl2, 50mM glucose) and completed within 10 minutes of euthanasia.

2 With a Langendorff apparatus, hearts can be perfused at either constant coronary pressure or constant flow rate. Hearts were immobilized in a custom-built chamber to reduce motion artifact.

3 Hearts were stained with bolus injections of voltage-sensitive RH237 (30uL of 1mg/mL in DMSO, Thermo Fisher Scientific, S1109) and Ca indicator Rhod-2 AM (30uL of 1mg/ml in DMSO, Biotium, 50024) into the coronary perfusate.

4 Excitation-contraction uncoupling agents such as blebbistatin were not used.

5 Light from a 100-W tungsten lamp was collimated, passed through 530 ± 30 nm interference filters, split by a 560 nm dichroic mirror, and focused on the dorsal epicardial surface of the heart for excitation. Emitted fluorescence was collected with tandem camera lenses (50 mm f/1.2 mm, Nikon and 50 mm f/0.95, Navitar) and split with a 600 nm dichroic mirror.

6 The longer wavelength moiety, containing the V_m signal, was filtered between 610-750 nm and the shorter wavelength moiety, containing the intracellular Ca^{2+} signal, was filtered between 570-595 nm. The emitted fluorescence signals were recorded using 2 CMOS cameras (SciMedia, MiCAM ULTIMA) with a sampling rate of 2 kHz and 100 × 100 pixels with a 5 × 5 mm field of view. Pixel resolution of the images was 150 × 150 μm^2. All stimulations were performed on the dorsal heart at 10 Hz, 10 ms, and 221 mW for 10 s. Data were acquired in 40 s intervals with 15 s collected before and after the stimulation.