

2.8 In vivo pressure-volume loop studies in mice (Kass Lab)

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A. OBJECTIVE

A comprehensive assessment of mouse cardiac hemodynamics in vivo can be essential to define the physiological significance of a given genetic or pharmacological modification. Pressure-volume relations have been the gold-standard approach for such assessment for more than 3 decades. Methods to obtain these relations in small rodent hearts including mice based on a miniature conductance catheter were developed by the Kass laboratory in the late 1990's, and have since been commercialized and are now widely used. Here we provide our protocol for using this method, which we have found provides high quality signals, accurate calibration, and physiologically meaningful results.

The PV catheter is available in a variety of sizes. In mice, the smallest diameter (1-1.2F or 0.33-0.4 mm) provides the least likelihood of interacting with the contracting endocardium to generate a compression artifact. These catheters have a single micromanometer sensor, and four electrodes – an outer pair that provides stimulating current, and an inner pair that senses changes in voltage. Voltage is inversely proportional to changes in chamber volume (e.g. blood conductivity), and is converted to provide the volume signal. Somewhat larger catheters (1.4-2 F) are available although we find anything over 1.4F is less useful in mice, and better for rat.

Several companies provide catheters and/or the electronic systems needed to measure PV relations in small rodents that are available through AD Instruments (partnering with Millar Instruments) or Transonics (partnering with Scisense). Analytical software is provided by AD Instruments (LabChart and PowerLab).

B. CAUTIONS

As with any technique, there are a number of features of the method that should be attended to carefully in order to obtain reliable, reproducible and physiologically meaningful data.

- The anesthetics often need to be adjusted for a given mouse/rat strain and/or genetic model, so that you do not generate cardiac depression, hypotension, bradycardia, or other features indicative of cardiovascular depression. The type of anesthesia used can make a difference. Ideally, the heart rate of an anesthetized mouse should be kept above 500 bpm.
- Temperature control in anesthetized mice and rats is important. Without proper heating, the heart rate in anesthetized mice can fall to almost half the normal rate (e.g. heart rates in the mid-upper 300-400's; instead of the more common 500-600/min range).
- Be careful not to damage carotid arteries and the vagus nerve during surgical procedures in the neck where you are inserting catheters into the jugular vein.

- We cannulate the left jugular vein to provide fluids during an open chest procedure by inserting a plastic tube (P10 in mice) or a microneedle. This is done early in the procedure to provide volume expansion with albumin and saline, and maintain fluid status.

C. MATERIALS

Reagents

- Injectable anesthesia: Urethane (800–1,200 mg kg⁻¹) + Etomidate (5–10 mg kg⁻¹); ket + Xyl?
- Inhalants: Isoflurane (induction 3–4%, maintenance 1.5% mixed with 100% oxygen)
- Analgesia: hydromorphone (1 mg kg⁻¹)
- Muscle relaxant: pancuronium (2 mg kg⁻¹)
- 0.9% (wt/vol) sodium chloride injection, USP
- Human Albumin 25%

Equipment

- Tabletop anesthesia machine, single (Harvard Apparatus, cat. no. 72-3012)
- Anesthetizing box, large (Harvard Apparatus, cat. no. 50-0116)
- MiniVent ventilator for mice (Harvard Apparatus, cat. no. 73-0043)
- Harvard ventilator model 683 for rats (Harvard Apparatus, cat. no. 55-0000)
- Tracheotomy cannula, 1.3 mm outer diameter (o.d.) for mice (Harvard Apparatus, cat. no. 73-2730)
- Tracheotomy cannula, 3.0 mm o.d. for rats (Harvard Apparatus, cat. no. 73-2733)
- Tubing kit (Harvard Apparatus, cat. no. 72-1049)
- Gaymar T/Pump circulating water blanket (Gaymar Industries, cat. no. TP-400) or Homeothermic Blanket (Harvard Apparatus, cat. no. 507214-16)
- Stereo microscope (Carl Zeiss Optical Inc., cat. no. Stemi 2000)
- Cole–Parmer Illuminator 50 W (Cole–Parmer, cat. no. 41720)
- Battery-operated Thermal Cautery Unit (Fine Science Tools Inc., cat. no. 18015-00)
- Dumont no. 55 Dumostar Forceps (Fine Science Tools Inc., cat. no. 11295-51)
- Graefe forceps, curved (Fine Science Tools Inc., cat. no. 11052-10)
- Moria MC31 forceps (Fine Science Tools Inc., cat. no. 11370-31)
- Mayo scissors (Fine Science Tools Inc., cat. no. 14512-15)
- Iris scissors (Fine Science Tools Inc., cat. no. 14041-10)
- Halsey needle holder (Fine Science Tools Inc., cat. no. 12501-13)
- Olsen–Hegar needle holder (Fine Science Tools Inc., cat. no. 12002-12)
- Vannas–Tubingen spring scissors, titanium (Fine Science Tools Inc., cat. no. 15610-08)
- Cotton-tipped applicators, sterile (Solon, cat. no. 368)
- Surgical suture, black braided silk, 5.0 (Surgical Specialties Corp., cat. no. SP116)
- Insulin syringe 1/2 cc (Becton Dickinson, cat. no. 329461)

- Millar PV system MPVS-300/400 or MPVS Ultra (Millar Instruments Inc.). The system includes calibration cuvette for mice and rats; MPVS Ultra includes resistivity calibration cuvette. **As noted, this is the system we have used, but other systems are available based on somewhat different electronic processing – e.g. Transonics provides the system first developed by Scisense**
- For mice: SPR-839, 1.4F (Millar Instruments Inc.) PowerLab 4/30 with Chart Pro (AD Instruments Inc., cat. no. ML866/P) (not required for MPVS-400 system)
- MiniARCO trimmer (animal clipper; Wahl, cat. no. 8787-450A)
- Intradermic tubing PE-10 (Becton Dickinson, cat. no. 427401)
- Intradermic tubing PE-50 (Becton Dickinson, cat. no. 427411)
- Needle assortment (18, 26, 27 and 30 gauge; Thomas Scientific)
- Various-sized syringes
- Perivascular flow module TS420 (Transonic Systems Inc.)
- 1-mm perivascular probes (Transonic Systems Inc., cat. no. 1PRB)
- Visual Sonic Echo system (optional; Vevo 770 High-Resolution *In Vivo* Imaging System; RMV 707B High Frame Rate Scanhead; RMV 716 Scanhead, Vevo Integrated Rail System III, Imaging Kit, Aquasonic ultrasound gel)

D. METHODS

Anesthesia, body temperature control and intubation

1. Deliver intraperitoneal injection of anesthesia containing urethane (800 mg kg^{-1}) + etomidate ($5\text{--}10 \text{ mg kg}^{-1}$) + morphine (2 mg kg^{-1}) in 0.2 ml of physiological saline solution; then place animal into the anesthesia chamber containing isoflurane 3–4% for induction.
2. When the animal is anesthetized, shave neck and chest area with a clipper and carefully move the animal to the heating pad, insert the temperature feedback probe into the rectum and set the desired body temperature to $36.5\text{--}37^\circ\text{C}$. **Note: temperature is extremely importance to the success of this procedure.**
3. Using surgical tape, tape down the front paws and one distal paw and tail of the mouse to the heating blanket, use the remaining distal paw to monitor the depth of anesthesia.
4. Following midline neck incision, pull the skin away from the underlying muscles and cut it off. Pull the pretracheal muscles apart gently with forceps and dissect around the trachea to isolate it from surrounding tissues.
5. Pass a 5-0 surgical silk suture behind the trachea, make a small cut onto the surface, insert the tracheotomy cannula (1.3 mm o.d. for mice and 3.0 mm for rats) and tie down with the suture. **Note: Alternatively, intubation can also be performed without a tracheal incision but this procedure requires more experience.**
6. Connect the tracheotomy cannula to the respirator providing 100% oxygen.
7. Calculate and set the recommended ventilation settings for mice or rats, depending on the animal weight. **Note: set respirator according the following**

**formulas: tidal volume (V_t , ml) = $6.2 \times M^{1.01}$ (M = animal mass, kg);
respiration rate (RR, min^{-1}) = $53.5 \times M^{0.26}$.**

8. Regularly monitor the depth of the anesthesia by checking the response to a tail pinch or toe pinch and make necessary adjustments if required.
9. Cannulate left jugular vein by inserting a plastic tube (P10 in mice) or cannulation needle connected to tubing for later possible fluid/drug or saline injections/infusions. **Note: Secure the tubing by tape to avoid accidentally pulling out of the tubing.**

Surgical procedures for LV or RV catheterization (procedures under a stereomicroscope)

Left ventricle closed-chest approach

1. In an immobilized anesthetized animal, make an inverted T-shaped middle-neck incision from mandible to the sternum under a stereomicroscope.
2. Move aside parotid glands and with forceps, bluntly dissect the thin muscle layer around the throat to expose and isolate the right carotid artery. **Note: avoid injuring the parotid glands and vagus nerves.**
3. Secure suture around the proximal end of the artery, gently pull it and tape it to the table and pass two additional sutures around the carotid artery. Place a very loose knot on the mid suture and gently pull the distal suture with a needle holder and clamp it to the skin of the animal to fix it in the desired position.
4. Make a small incision near the proximal end of the artery with a bent tip 27G needle and insert the catheter tip into the vessel followed by gently securing the middle suture.
5. Simultaneously, release the distal suture and quickly advance the presoaked catheter (for 30 min into physiological saline solution) into the left ventricle until the PV signal is displayed in the monitor.

Open-chest approach

1. In anesthetized intubated animal, make an incision over the xyphoid process and separate the skin from the chest wall by blunt forceps under a stereomicroscope.
2. Starting around the xyphoid process, cut through the chest wall moving laterally on both sides with the heat cautery dissector until the diaphragm is clearly visible from beneath.
3. Cut through the diaphragm to expose the apex of the heart.
4. Gently remove the pericardium from the heart with forceps.
5. Using a 27 gauge needle, make a stab wound at the apex of the heart into the left ventricle (can be done in the RV apex for RV insertion as well). **Note: this needle should never pass further than 2-4 mm into the chamber.**
6. Insert the catheter tip retrograde into the left or right ventricle and adjust the position of the catheter while monitoring the continue PV loops on your computer display. **The position of the catheter should be such that the most proximal electrode (furthest from the catheter tip) is no longer visible but just inside the cavity.** The loops should show a typical shape with straight vertical sides.

FOR BOTH PROCEDURES

7. Allow the PV signal to stabilize for 5-10 min.

8. During this period, we will generally provide 100-150 microliters of albumin and normal saline to offset insensible volume loss due to the surgery. This should be standardized, based on what you find provides normal range blood pressures (110-120 mmHg systolic), cardiac outputs (~15 ml/min), and dP/dt_{max} (~14,000 mmHg/s) in a healthy normal mouse. Then this is used for all animals that would be studied.
9. Record baseline PV loops at steady state or at varying preloads during the inferior vena cava occlusions. **Note: This latter procedure is used to derive various load-independent indices of systolic function.**
10. Vena cava inferior occlusions can be performed in open-chest ventilated animals by pulling of a suture placed around the vessel, by lifting the vessel with a small stick or compressing it with a forceps. **Note: We generally suspend ventilation for a few seconds while acquiring these data to avoid lung motion – inflation artifacts.**
11. Remove the catheter by gently pulling it back through the stab wound and euthanize the animal following approved protocols by each institution. **Note: Immediately place the tip of the catheter into a syringe filled with saline to prevent blood clotting.**

Volume signal calibrations, data analysis

The volume catheter signal is in relative units based on conductivity of blood and tissue. To a reasonable approximation, this is directly related to actual cavity volume by a linear equation, with a slope and offset. The offset is related to conductivity of structures surrounding the blood pool – e.g. myocardium, the other heart chamber, even lung. The slope is related to the current field configuration generated in the heart and is rarely 1.0. There are several ways these terms have been estimated. Commercial systems often provide small pre-drilled cylindrical chambers that can be filled with mouse blood, presumably matching the conductance of the blood relevant to when the heart was studied in vivo. Placement of the catheter into each volume cylinder cuvette and measuring a signal, then plotting signal versus known volume provides a calibration curve. Our experience with this method in the past has found the results generally underestimate real cardiac output, and we have not favored this approach. The in vivo geometry and conditions are not reliably mirrored by the ex vivo cylinder model. We prefer to directly measure cardiac output using an ultrasound probe, and to estimate the offset term (parallel conductance) using the classical hypertonic saline method.

Saline calibration

The current introduced by the outer excitation electrode pair on the catheter passes through any conductive material, and some of the signal is derived from the muscle wall and surrounding structures. These conductances are generally considered to be constant, whereas the blood pool is providing the primary source of time-varying conductance. In the Transonic/Scisense system, a method is used that attempts to calculate this non-blood pool generated conductance (or admittance) in real-time, though practically, accurate calibration often with external standards is required for this system as well. The classic method to assess the non-blood *parallel* conductance works by introducing hypertonic saline into the blood, effectively altering the conductivity

of blood without changing that of the surrounding structures. From such data, one can determine the parallel conductance, converted to a volume (V_p), and subtract this from the total signal to calibrate this offset.

1. To obtain a value for V_p , perform a saline bolus calibration with hypertonic saline (30%) bolus injection into the animal at the conclusion of the experiment as follows: firstly, cannulate jugular vein.
2. After turning off the respirator for a few seconds (and during the injection), inject 5–10 μl hypertonic (30% saline solution) i.v. into mice and 20–40 μl into rats to obtain visible shift to the right in PV loops (volumes and importantly relative stroke volume appears to rise even though pressures are unaltered). This is due to the conductance rise in the blood pool. **If you see loops shift rightward but the width (e.g. peak-to-trough or stroke volume) does not seem to increase – this is due to poor mixing of the saline. You will get an erroneous V_p estimate.** The parallel volume is calculated by data analysis software available in LabChart, and details of this method and theory have been reported².
3. Because of variability (like most dye-dilution methods), perform at least 3 saline calibrations in each animal, and either average all or the two that are closest to each other. Wait a few minutes after saline injection for the system to recover. If you inject too much hypertonic saline, blood pressure will decline due to contractile depression. This is because the hypertonicity leads to sodium uptake by myocytes which in turn results in calcium extrusion via the Na-Ca exchanger. Use only the data during the phase when volume appear to be increasing.
4. Enter the calculated V_p value from saline injection to PVAN together with the parameters derived from cuvette calibrations and convert volume to true volumes in microliters.

Aortic flow calibration

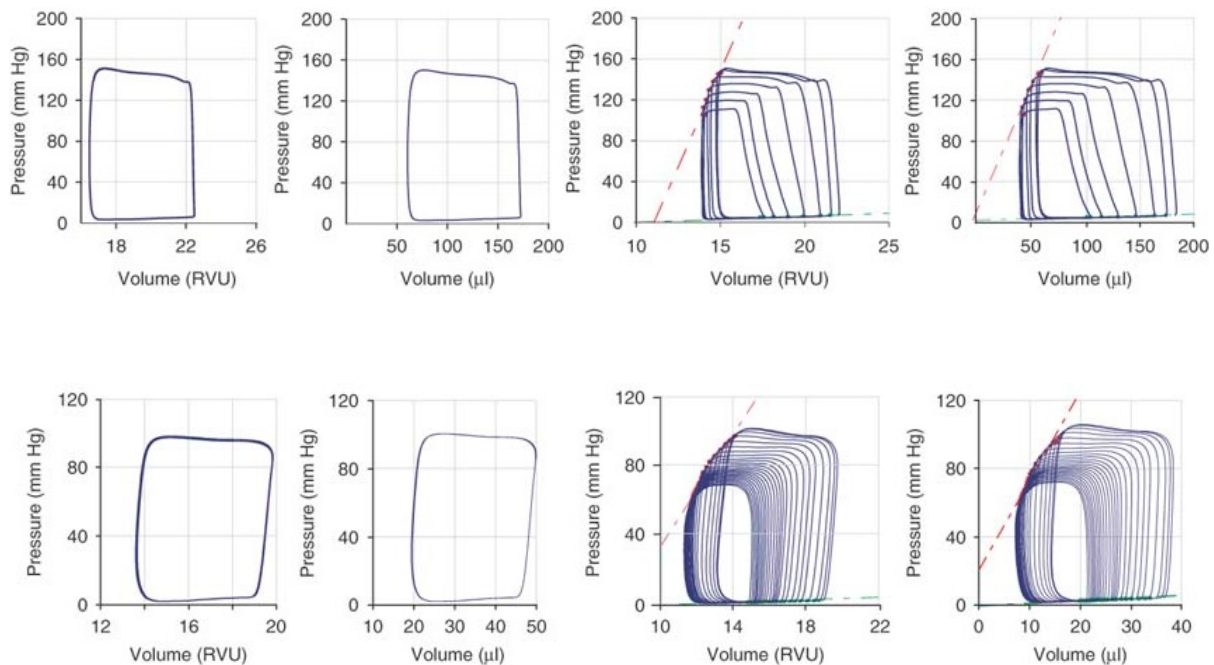
1. The other aspect of volume signal calibration involves the gain (e.g. slope of the relation between true volume and non-calibrated catheter signal). While the cuvette calibration and Scisense (Transonics) systems are supposed to correct for this automatically, we have found the most accurate approach is to determine cardiac output independently using an ultrasound flow meter. This is then matched to CO determined by the catheter to complete the calibration.
2. Turn the animal onto its left side slowly, paying attention to only minimally disturb the PV catheter signal. Make a lateral thoracotomy between T3 and T5 to create a small window and gently dissect a small part of the thoracic aorta running parallel to the spinal column with forceps.
3. Use either a perivascular flow probe (Transonics) placed around the aorta to assess cardiac output or a Doppler flow probe to determine cardiac output. This is then compared to CO from the catheter, the ratio yielding the calibration gain.

E. EVALUATION AND INTERPRETATION OF RESULTS

1. Following pressure volume loop signal calibration and analysis of signals from LV or RV by averaging at least 2 separate intervals of steady-state, many

hemodynamic parameters are generated to define ventricular performance. (e.g., ventricular pressure, stroke volume, cardiac output, stroke work, heart rate, end-systolic pressure, end-diastolic pressure, maximum or minimum dP/dt , dP/dt normalized to instantaneous developed pressure, relaxation time constants, etc.)

2. Pressure-volume loops acquired from vena cava occlusion induced preload reduction are the most widely used and flexible method for assessing pressure-volume relations. In particular, this load maneuver allows you to calculate more than the end systolic pressure volume relation (ESPVR), but also relations between dP/dt_{max} - and end-diastolic volume relation or maximal power and EDV, or stroke work and EDV. All are well established metrics for systolic chamber function that reduce the impact of loading conditions on the heart. Aortic constriction can also be used to generate a series of PV loops for such analysis, though beyond ESPVR, such data cannot be utilized to assess the other indexes. The capacity to assess multiple indexes is important – since some of these such as ESPVR itself, are chamber size dependent and calibration error sensitive. Others, such as PRSW are not (this has been extensively discussed in prior reviews).
3. Below are examples showing uncalibrated (in RVUs) and calibrated (in microliters) normal rat and mouse LV baseline PV loops (left two panels) and loops following inferior vena cava occlusions (right two panels).



F. REFERENCES

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