

Journal of Visualized Experiments

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--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE57621R1
Full Title:	Real-Time Pressure-Volume Analysis of Acute Myocardial Infarction in Mice
Keywords:	Ischemia/reperfusion injury; myocardial infarction; left ventricular catheterization; Pressure-volume catheter; left ventricular function; ejection fraction; cardiogenic shock; hemodynamics.
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Author Comments:	
Additional Information:	
Question	Response
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TITLE:

Real-Time Pressure-Volume Analysis of Acute Myocardial Infarction in Mice

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KEYWORDS:

Ischemia/reperfusion injury (I/R injury), myocardial infarction, left ventricular catheterization, PV-catheter, left ventricular function, hemodynamic

SUMMARY:

Acute myocardial infarction in mice induces acute but incompletely characterized changes in left ventricular (LV) function. LV catheterization in mice undergoing coronary artery occlusion serves as a novel method for a real-time evaluation of LV function.

ABSTRACT:

Acute myocardial infarction can lead to acute heart failure and cardiogenic shock. The evaluation of hemodynamics is critical for the evaluation of any potential therapeutic approach directed against acute left ventricular (LV) dysfunction. Current imaging modalities (*e.g.*, echocardiography and magnetic resonance imaging) have several limitations since data on LV pressure cannot directly be measured. LV catheterization in mice undergoing coronary artery occlusion could serve as a novel method for a real-time evaluation of LV function.

At the beginning of the procedure, mice were anesthetized followed by endotracheal intubation. For LV catheterization, the right carotid artery was exposed *via* middle-neck incision. The catheter was introduced and placed into the LV cavity. Left thoracotomy was conducted and the left main coronary artery (LCA) was ligated. To induce reperfusion, the suture was released after 45 min. Pressure-volume data was recorded at all times.

Ligation of the LCA caused a decrease in LV systolic function as evidenced by a 30% reduction in stroke volume, LV ejection fraction (EF) and cardiac output. Maximum dP/dt as a parameter for LV contractility was also significantly reduced and diastolic function was severely impaired (minimum dP/dt -40%). Reperfusion over a period of 20 min did not lead to a complete recovery of LV function.

Real-time pressure-volume analysis served as a valid procedure for monitoring cardiac function during acute myocardial infarction in mice. Maintaining stable anesthesia and a standardized surgical approach was crucial to ensure valid results. As the early phase of acute myocardial infarction is critical for morbidity and mortality, the delineated method could be beneficial for preclinical evaluation of new strategies for cardioprotection.

INTRODUCTION:

Cardiovascular disease is the most common cause of death in western civilization¹. Acute myocardial infarction is a critical event, which is associated with high acute and chronic mortality². Even if revascularization is achieved *via* emergency percutaneous coronary intervention (PCI), mortality remains high, particularly within the first 48 h after onset of symptoms in patients with acute myocardial infarction³. Cardiogenic shock caused by acute reduction in left ventricular (LV) function is a major cause for in-hospital mortality in these patients³. This early reduction in LV function is caused by myocardial damage following ischemia and reperfusion. This so-called ischemia/reperfusion (I/R) injury is mediated by changes in cellular metabolome such as exaggerated generation of reactive oxygen species⁴⁻⁵.

To explore possible protective mechanisms leading to a decrease in myocardial damage in a preclinical setting, reliable mouse models are essential including methods for evaluation of post-I/R LV function⁶. In this setting, transthoracic echocardiography⁷ and magnetic resonance imaging (MRI)⁶ are widely used for functional phenotyping⁸⁻⁹. However, these methods are not suitable for the assessment of severe LV dysfunction and cardiogenic shock in an ongoing acute myocardial infarction and cannot directly show data on LV pressure. The Langendorff apparatus using isolated heart in an *ex vivo* assay provides information about the underlying pathomechanisms of early-phase I/R injury¹⁰. This method is limited due to its inability to reproduce *in vivo* adaptive mechanisms such as regulation of the autonomous nervous system or hormonal regulation and acid-base homeostasis. There is currently no method available for a complete functional phenotyping of cardiogenic shock and left ventricular dysfunction during an ongoing myocardial I/R injury.

A synchronized approach with combination of pressure-volume (PV) catheterization and transient surgical left main coronary artery (LCA) occlusion could be beneficial but technically challenging. Stable extracardiac hemodynamics during I/R injury are essential for valid results since unstable anesthesia or blood loss could heavily influence the results. A novel approach for hemodynamic phenotyping of I/R injury *via* LV PV catheterization and transient LCA occlusion could bring new insights on cardiogenic shock and LV dysfunction in acute myocardial infarction and serve as a method for future analysis on cardioprotection.

89 **PROTOCOL:**

90
91 All experiments were completed in accordance and compliance with all relevant regulatory
92 ('European Convention for the Protection of Vertebrate Animals used for Experimental and other
93 Scientific Purposes' (Directive 2010/63/EU) and institutional guidelines (Landesamt für
94 Naturschutz, Umwelt und Verbraucherschutz, Recklinghausen, Germany 84-02.04.2016A162). All
95 experiments have been performed with male C57BL/6JRj mice at the age of 6 months.

96 97 **1 Preparation**

98
99 1.1 Prepare a surgical microscope and a heating pad as well as a rectal probe to monitor the
100 body temperature. Clean and sterilize all surgical instruments.

101
102 1.2 Prepare 2 pieces of 10 cm 5-0 silk thread for vessel ligation, 5 cm of 6-0 polypropylene
103 thread and 2 mm silicon tube for ligation of the LCA.

104
105 1.3 Prepare the PV catheter calibration cuvette by pre-heating it to 37 °C and prepare a 100
106 µL Hamilton syringe filled with 15% sodium chloride (NaCl) in H₂O for saline calibration.

107
108 1.4 Place the PV catheter (3 cm, 1.4 F) in 37 °C pre-heated 0.9% NaCl in H₂O (saline) at least
109 30 min before measurement. Connect the catheter to the data acquisition device and connect
110 the device to an analog/digital converter. Connect both devices to a computer.

111
112 1.5 Set up the software. Perform software-guided pressure calibration and conductance
113 calibration as demanded by the software-guided workflow⁸.

114 115 **2 Anesthesia and Analgesia**

116
117 2.1 Anesthetize the mouse using ketamine 100 mg/kg body weight and xylazine
118 hydrochloride 10 mg/kg body weight by intraperitoneal injection. At the beginning of I/R surgery,
119 administer 0.05 mg/kg body weight buprenorphine intraperitoneally to maintain analgesia.

120
121 2.2 After 10 min, perform an endotracheal intubation using a 20 G intravenous (iv) catheter
122 and ventilate the mouse with 40% oxygen (O₂) and 2% (v) isoflurane. Set appropriate ventilation
123 parameters (*e.g.*, 220 µL stroke volume, 150/min for a 25-30 g C57BL/6JRj mouse).

124
125 2.3 Continuously monitor body temperature *via* rectal probe. Fasten mouse on the heated
126 plate with the head pointing towards the investigator.

127 128 **3 Left Ventricular Catheterization**

129
130 3.1 Disinfect chest and neck with an alcoholic skin disinfectant. Wait for skin disinfectant to
131 dry. Remove the chest hair by using a small animal shaving system.

132

3.2 Perform a 10 mm longitudinal median incision 5 mm beneath the bottom lip towards the sternum using small surgical scissors.

3.3 Dissect the left and right part of the submandibular gland *via* blunt preparation using a forceps. Separate muscle and fat tissue in the right paratracheal region to expose the right common carotid artery. Mobilize and separate the vessel for a total length of 5-10 mm from connective tissue via careful blunt preparation alongside the vessel with a bent forceps.

Note: Avoid mechanical manipulation of the vagus nerve or the carotid body at all time as this can cause severe hypotension and bradycardia.

3.4 Pass the two prepared silk threads under the vessel. Ligate the distal vessel with a tight knot and place a loose knot on the proximal exposed region that still allows passage of the catheter.

3.5 Fix the threads of the cranial (tight) knot next to the head of the mice to apply a light tension on the vessel as this will facilitate the introduction of the catheter. Place a hemostat vascular clamp on the proximal vessel proximal of the loose knot to reversibly block blood flow.

3.6 Perform a wedge-shaped incision 1 mm proximal to the cranial knot to open the vessel with micro scissors.

Note: A small drop of blood will indicate proper execution of this step.

3.7 Insert the catheter carefully for 10 mm. Start recording of catheter data.

Note: Stretching the incision with a forceps can make this process easier.

3.8 Extract the vascular clamp. Add 1-2 drops of saline to the incision to facilitate catheter movement. Continue introducing the catheter for approximately another 10 mm. After passing the proximal knot with the sensor tip, fasten the knot carefully just enough to prevent blood reflux alongside the thinner parts of the catheter without impairing catheter movement.

Note: The size of the sensor at the tip of the catheter prevents reflux of blood when extracting the vascular clamp.

3.9 Gently continue inserting the catheter until pressure analysis shows arterial blood pressure profile indicating that the catheter is placed in the aorta (**Figure 3A**).

Note: The catheter reaching the aortic valve will be indicated by light resistance and pulse-synchronized motion of the catheter.

3.10 When experiencing resistance trying to advance through the aortic valve, pull the catheter back 5 mm and advance again until LV catheterization will be indicated in a change in

PV analysis as diastolic pressure will reach 0-20 mmHg (**Figure 3B**). Note changes in volume monitoring to further confirm left ventricular placement of the sensor tip (**Figure 3C**). Fasten the proximal knot more tightly to prevent catheter movement.

4 Ischemia/Reperfusion Surgery

4.1 Perform skin incision from caudal sternum towards the left axilla for a total length of 15 mm. Proceed with blunt preparation of the two muscle layers until the ribs can be visualized.

4.2 Open the thorax *via* incision between the third and fourth left rib. Use surgical hooks to gain access to the pericardium. Resect the pericardium above the heart. Before continuing with LCA ligation, wait 30 s without touching the animal to record PV data for valid analysis.

4.3 Localize the LCA emerging under the left auricle and descending at the left side of the heart towards the apex. Use a 6-0 polypropylene suture to encircle the artery with a loop 2 mm underneath the left auricle. Place a small silicon tube under the loop and place a tight knot above.

Note: Distal myocardium turning grey serves as positive control for LCA occlusion⁶. I/R surgery should be performed within 5 min independently from the operating investigator.

4.4 Cut the suture at 1 mm length. Release the surgical hooks and manually close the muscle layers above the incision. Wait 45 min while continuously recording PV data.

4.5 After 45 min, re-open the incision and remove the silicon tube to induce reperfusion. Record data for another 20 min.

Note: A change to a red color as seen before ischemia indicates successful reperfusion.

5 Calibration

Note: The calibration of the PV catheter system consists of 4 mandatory steps, two of which have to be performed after the measurement. Calibration should be repeated after every experiment to ensure valid results.

5.1 Perform pressure calibration and conductance calibration before the experiment as described in Step 1.5.

5.2 Perform saline calibration when the catheter is still placed in the left ventricle after the experiment itself is finished. Localize the right jugular vein lateral of the carotid artery in the prepared area. Inject 10 μ L 25% NaCl in H₂O *via* a Hamilton syringe while recording data.

5.3 Calculate the calibration using the acquisition software by highlighting the ascending phase in volume curve (**Figure 5C**). Repeat this process for a total of 3 times. Avoid loss of blood after syringe extraction by using a vascular clamp to rapidly compress the puncture.

5.4 Perform volume calibration to calibrate volume data acquisition by analysis of standardized volumes. Obtain approximately 500 μL mouse blood from cardiac puncture with a slightly heparinized 1 mL syringe (e.g., 5 μL per 200 IE heparin). Pull back the PV catheter 10-15 mm to avoid damaging the catheter.

5.5 Fill the obtained blood into the 37 °C pre-heated calibration cuvette (**Figure 5A**). Avoid bubbles as it may interfere with the results. Add the catheter tip into each well and record data. Obtain a standard curve by software-guided analysis (**Figure 5B**). Repeat the process for a total of 3 times.

6 Data Analysis

6.1 After completing the calibration steps, perform software-guided data analysis. Therefore, highlight the appropriate section (at least ten cycles) within the **Analyze** section of the **PV Workflow** and perform baseline analysis. Exclude cycles with deviations due to ventilation or manipulation if necessary (**Figure 3D**).

6.2 Perform PV baseline analysis before passage of the aortic valve (arterial pressure only), immediately before and after LCA occlusion. Proceed by conducting PV baseline analysis and in intervals of 5 min. while ischemia and after reperfusion. At the end of the experiment, perform analysis of pressure data after retraction of the catheter from the left ventricle (arterial pressure).

6.3 Analyze at least 10 consecutive cycles to avoid sampling error. When experiencing strong interference of obtained values with the ventilation, transient interruption of the ventilation for a maximum of 5 s can be considered.

6.4 Use the following parameters that are calculated in **Baseline Analysis (Figure 3D)** to characterize LV function:

1. Stroke volume (μL)
2. Ejection fraction: Stroke volume / end-diastolic volume (%)
3. Cardiac output: Stroke volume * heart rate ($\mu\text{L}/\text{min}$)
4. Cardiac index: Cardiac output / body surface area ($\mu\text{L}/(\text{min}*\text{cm}^2)$)
5. Stroke work: Inner area of PV curve ($\text{mmHg}*\mu\text{L}$)
6. Maximum pressure (Pmax); Mean pressure (Pmean)
7. max dP/dt (mmHg/s) as a parameter of LV systolic function
8. min dP/dt (mmHg/s) as a parameter for LV compliance
9. Time constant of isovolumetric relaxation: Tau (ms)

REPRESENTATIVE RESULTS:

After LV catheterization, reversible LCA ligation was performed for 45 min followed by 10 min of reperfusion. PV data was recorded at all times (**Figure 1**).

Correct placement of the PV catheter was confirmed by obtaining the characteristic LV PV graph

(**Figure 2A**). LV catheter placement showed the typical ventricular pressure range with a minimum of 0-20 mmHg whereas false placement of the PV-catheter in the aorta would have shown a typical arterial pressure curve with a minimum pressure of 30-60 mmHg (diastolic blood pressure) and a small excursion at the end of systole indicating the aortic valve closing (**Figures 3 B and 3C**). Successful occlusion of the LCA was visually confirmed by blanching of the distal LV myocardium (**Figure 2B**).

After LCA occlusion, PV data was acquired in 5 min intervals. Pressure data analysis demonstrated no changes in maximum LV systolic pressure indicating preserved peripheral perfusion and stable anesthesia (**Figure 4A**). Analysis of LV volume revealed a significant decrease in both EF (52% vs. 40%, $p=0.008$) and absolute stroke volume (**Figures 4B and 4C**). These changes happened within the early phase of ischemia and LV functional data remained unchanged in the later phase of ischemia. Maximum dP/dt as a parameter of LV contractility showed a 30% reduction in mice undergoing myocardial ischemia. Stroke work was 30% reduced (**Figures 4D and 4E**). As a parameter for diastolic function, minimum dP/dt was significantly decreased indicating impaired LV compliance (**Figure 4F**). Reperfusion by extraction of the silicon tube was visually validated. Reperfusion did not show significant changes in PV data analysis within a period of 20 min (**Figures 4A-4D**). Sham-operated animals did not show a significant reduction in LV systolic or diastolic parameters (**Figures 4I-4J**).

At the end of data acquisition, cuvette calibration and saline calibration were performed (**Figure 5**).

FIGURE AND TABLE LEGENDS:

Figure 1: Scheme of the method. Sequence of left ventricular (LV) catheterization, left main coronary artery (LCA) occlusion and reperfusion.

Figure 2: Surgical procedures. (A) Left ventricular catheterization placed *via* the right common carotid artery. (B) Left main coronary artery occlusion with polypropylene suture and silicon tube.

Figure 3: Representative pressure-volume data. (A) Representative arterial pressure indicated by a minimal pressure of > 30 mmHg and a typical excursion at the end of systole indicating closing of the aortic valve. (B) Representative left ventricular pressure data showing diastolic values < 20 mmHg. (C) Representative left ventricular pressure-volume diagram. (D) Screenshot of software-based PV baseline analysis.

Figure 4: Pressure/volume data in mice undergoing ischemia/reperfusion. (A) Systolic left ventricular blood pressure (Pmax). (B) Left ventricular (LV) ejection fraction (EF) (%). (C) LV stroke volume (μ L). (D) Maximum dP/dT (max dP/dt) (mmHg/s). (E) LV Stroke work (SW). (F) Minimum dP/dt (min dP/dt) (mmHg/s). (G) Time constant of isovolumetric relaxation Tau (ms). (H) Pressure/volume diagram before and 45 min after induction of myocardial ischemia. (I-J) Stroke volume (SV) (μ L) and maximum dP/dt (max dP/dt) (mmHg/s) in sham-operated animals compared to animals after 15/30 min ischemia. Data (A-G) are presented as mean \pm SEM. * $p < 0.05$ *via* Student's t-test or ratio-paired t-test, $n=4$ mice/group (A+D-G)) or $n=3$ mice/group (B,

C). +45: 45 min ischemia; rep.: reperfusion.

Figure 5: Post-hoc calibration. (A) Schematic of calibration cuvette. Volumes in μL . (B) Representative linear regression analysis of obtained volume data to perform cuvette calibration. (C) Representative volume data after injection of 10 μL of 25% sodium chloride in H_2O into the right jugular vein to perform saline calibration.

DISCUSSION:

PV monitoring of LV hemodynamics in acute myocardial infarction serves as a novel method for real-time *in vivo* assessment of cardiogenic shock and impaired LV function in I/R injury. PV catheterization can provide a broad spectrum of parameters with regard to LV systolic and diastolic function. In addition to the LV volumetric parameters typically obtained by echocardiography or MRI (chamber volumes, EF, stroke volume and cardiac output), PV analysis yields a more complete profile of LV function by simultaneously providing measures of LV systolic performance (contractility dP/dt , stroke work) and LV compliance ($-dP/dt$, Tau) as a parameter for diastolic function.

As acute heart failure in patients with acute myocardial infarction is a critical predictor for early in-hospital morbidity and mortality², monitoring of acute hemodynamic impairment and cardiogenic shock in acute myocardial infarction could serve as a valuable tool for identifying possible protective mechanisms in an experimental setting.

Several factors turned out to be critical for successful data acquisition. Stable anesthesia was crucial for valid PV data since isoflurane showed a strong cardiodepressive effect with drops in pressure, LV EF and stroke volume. Atraumatic preparation of the carotid artery was important to avoid hypovolemia due to blood loss. Furthermore, compression or injury of the vagus nerve and the carotid body could result in severe impairment of hemodynamics.

Saline calibration and cuvette calibration appeared to be another critical step to maintain valid data. For saline calibration, injection of 15% NaCl solution led to increased conductance indicated by a temporary increase in volume level (**Figure 5C**). Maintaining the same speed when injecting was crucial for stable data. When conducting cuvette calibration, it was important to avoid bubbles within the cuvettes to ensure valid results.

The obtained PV data furthermore indicate the importance of a simultaneous acquisition of pressure and volume data for a valid hemodynamic characterization since pressure data alone did not show significant changes throughout the experiment (**Figure 4A**). The combined PV analysis offered both baseline parameters for LV systolic function (*e.g.*, ejection fraction) as well as parameters for LV contractility (dP/dt) and LV relaxation ($-dP/dt$, Tau).

Interestingly, acute occlusion of the LCA in patients usually causes a severe deficit of LV function with immediate necessity for mechanical hemodynamic support and is associated with a high mortality rate¹¹⁻¹². LCA occlusion in mice showed less hemodynamic impairment and LCA occlusion-associated death during the procedure was not observed. As a sign of persisting

hemodynamic stability during ischemia, systolic LV blood pressure was stable at all time (**Figure 4A**). However, this effect could be caused by more distal ligations in mice compared to LCA occlusions in humans.

Taken together, real-time hemodynamic monitoring of acute myocardial infarction in mice could serve as a new method for studying cardioprotective mechanisms in severe LV dysfunction aiming to improve early-phase treatment of patients undergoing acute myocardial infarction.

ACKNOWLEDGMENTS:

The authors acknowledge the following funding sources: Else Kröner-Fresenius-Stiftung (Tienush Rassaf); Hans und Gertie Fischer Stiftung (Tienush Rassaf), grant from the medical faculty, University Duisburg-Essen, Germany (Tienush Rassaf, Lars Michel); Ernst- und Berta Grimmke-Stiftung (Christos Rammos).

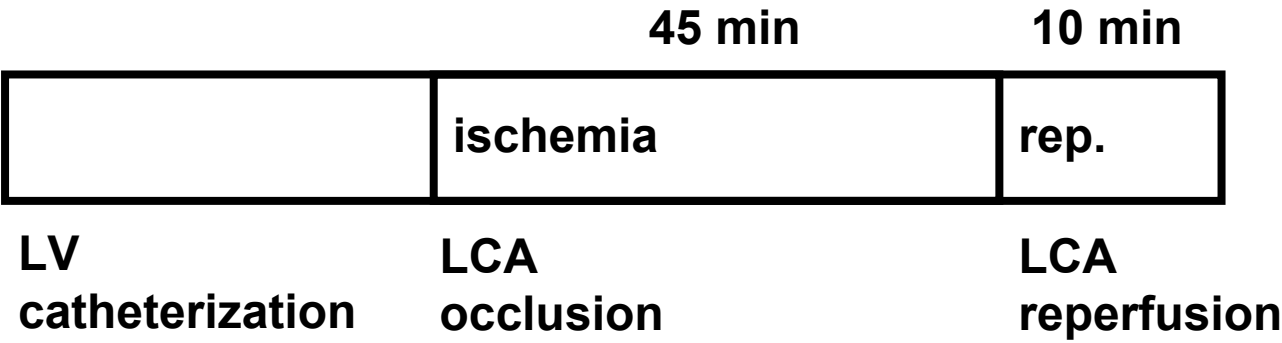
DISCLOSURES:

The authors have nothing to disclose.

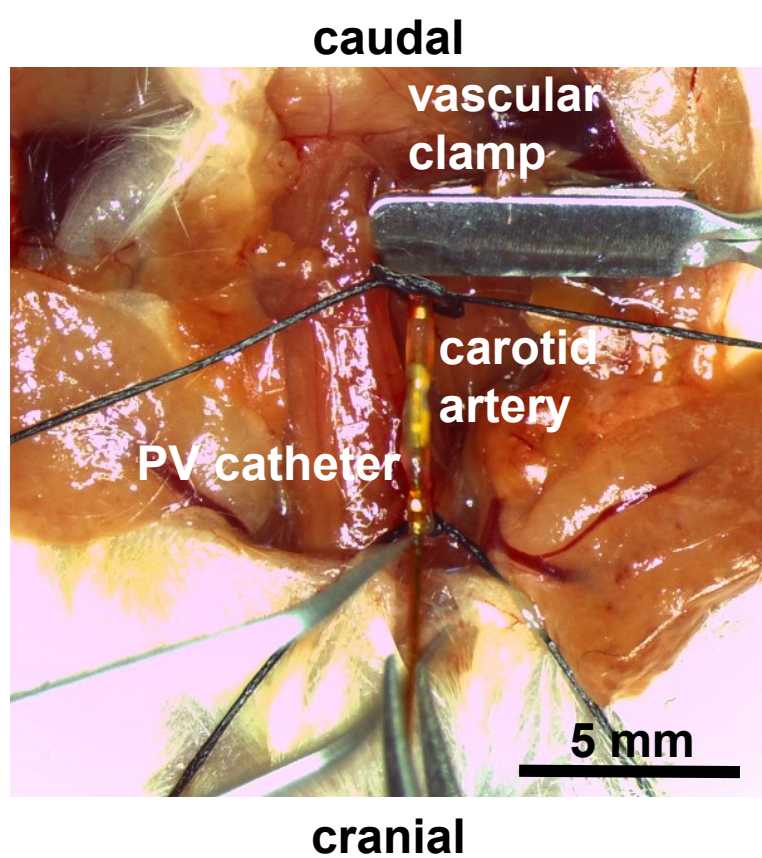
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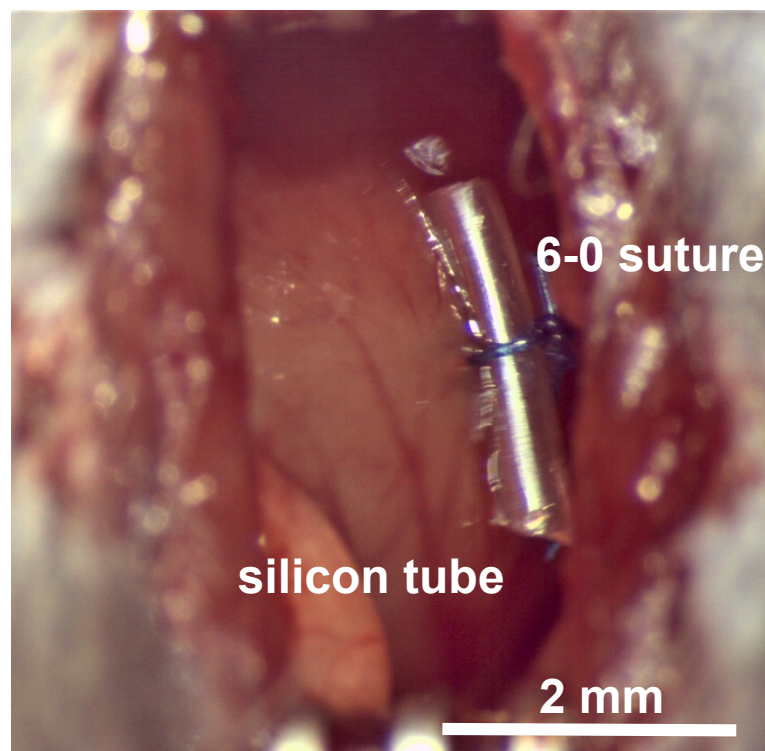
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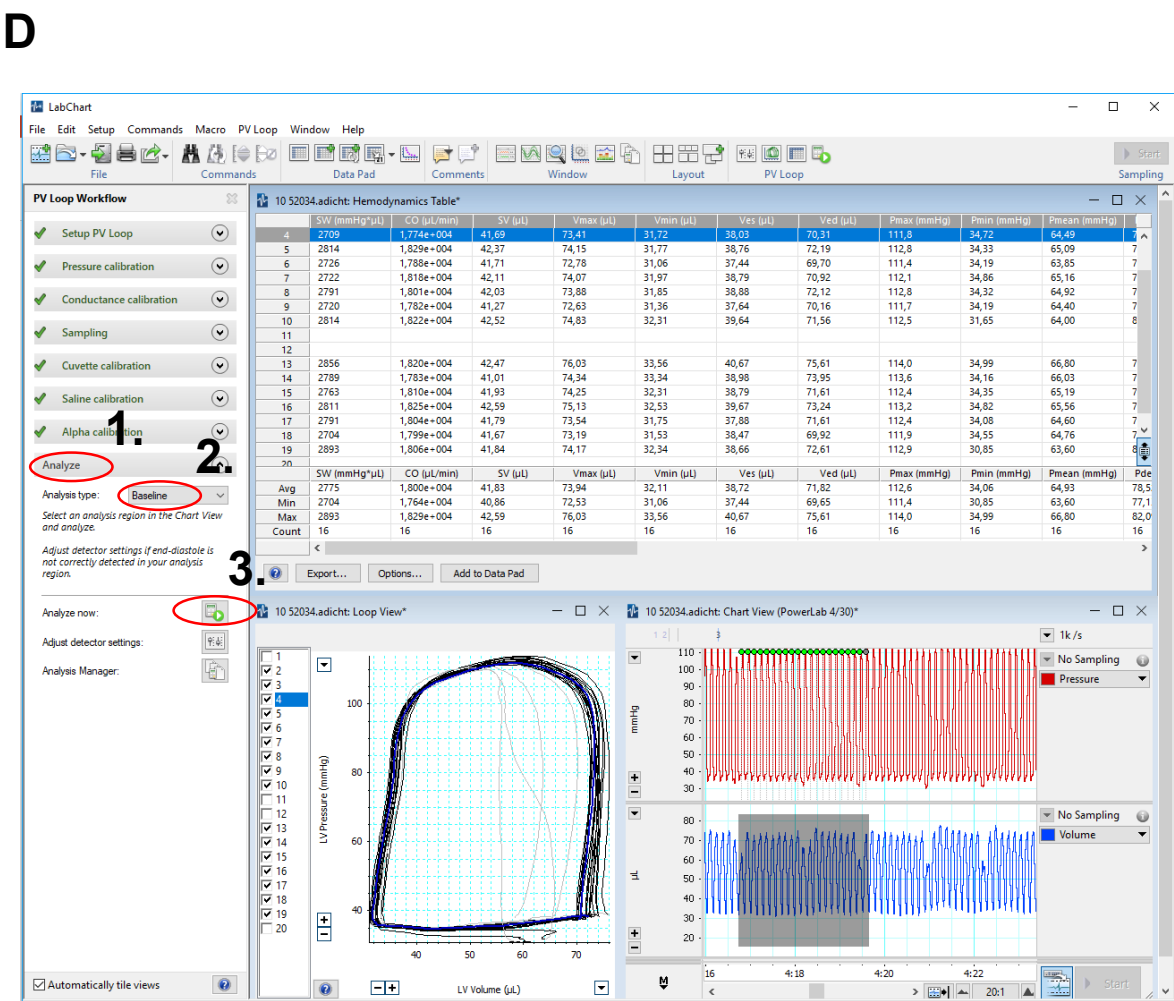
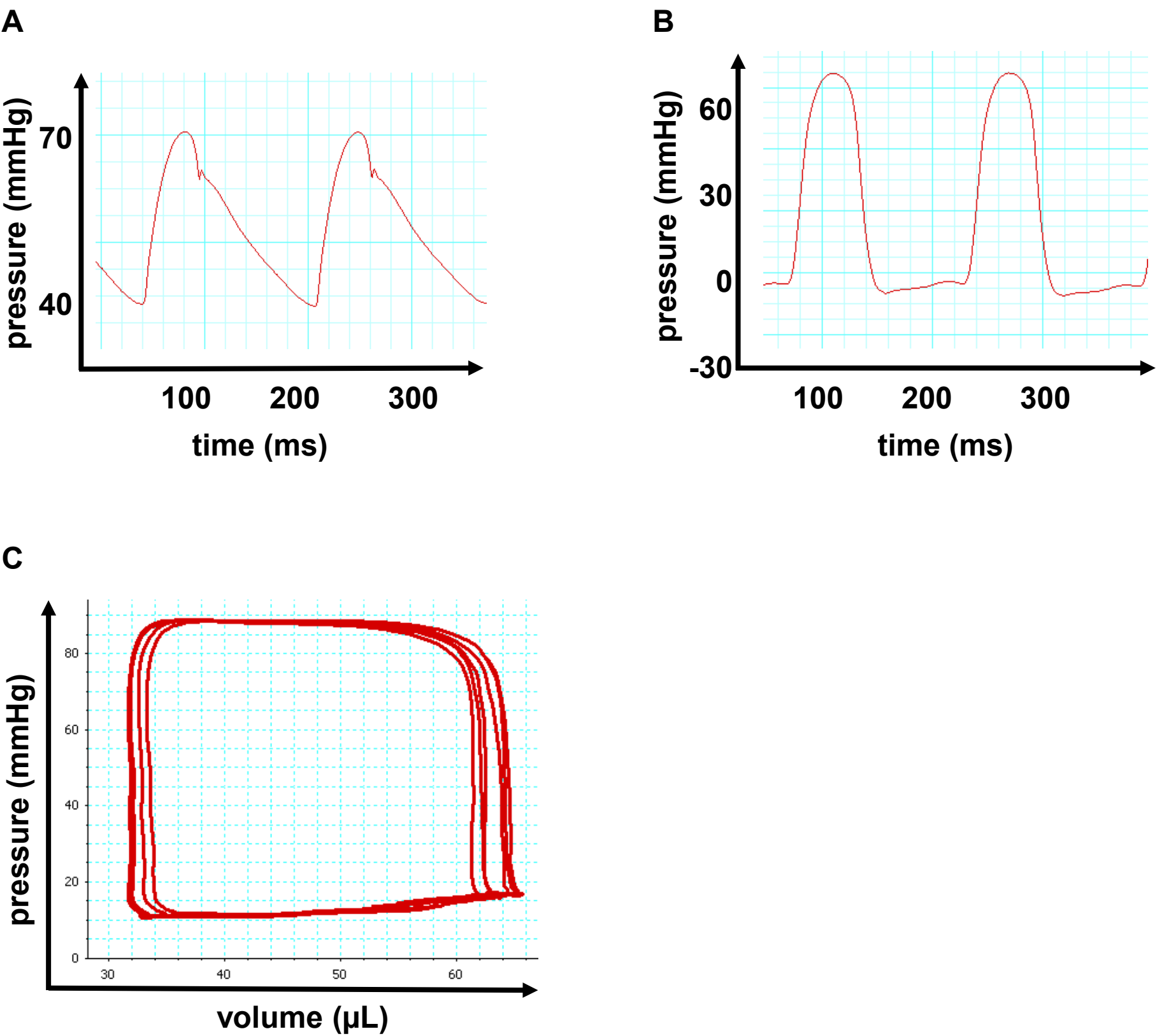


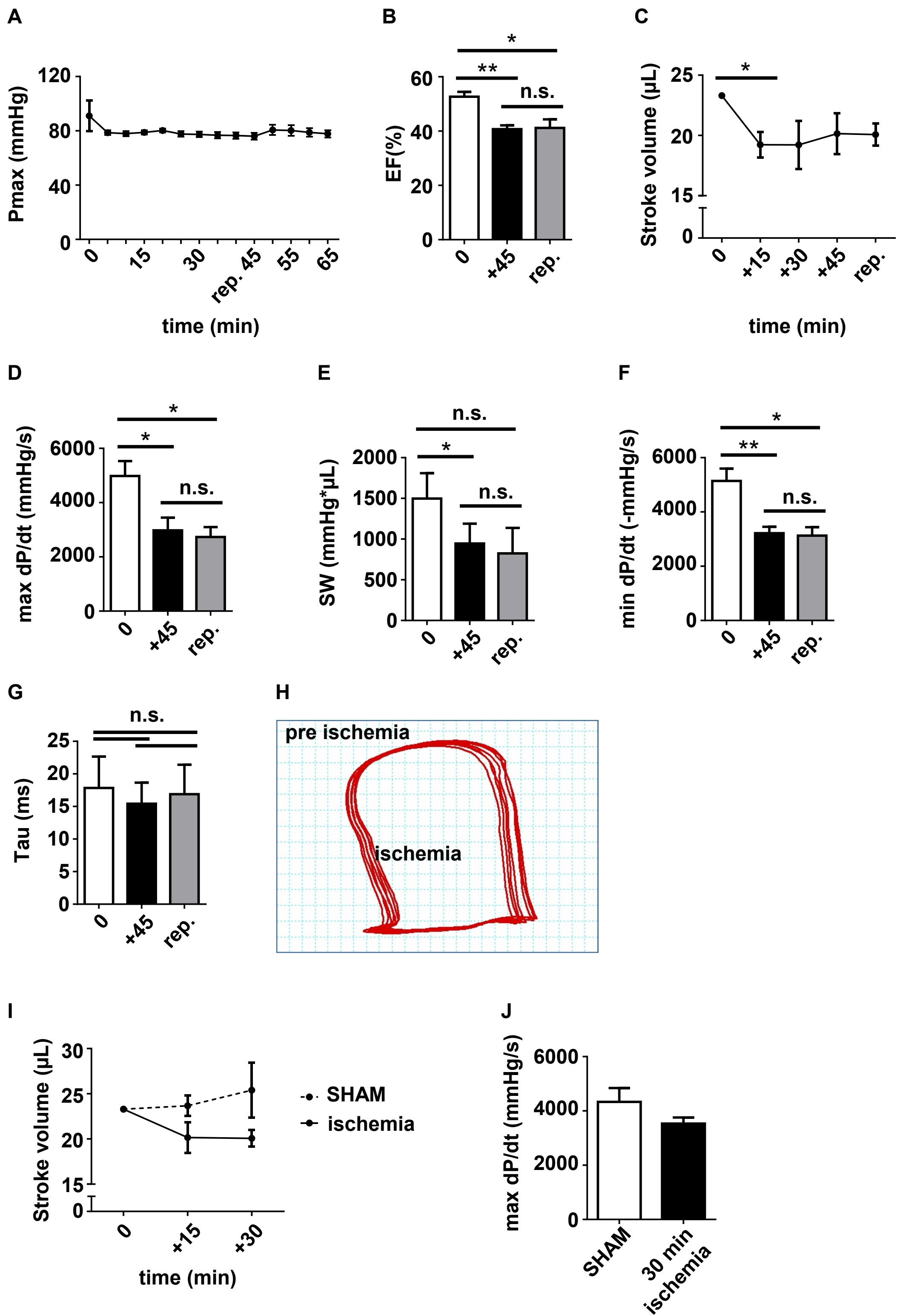
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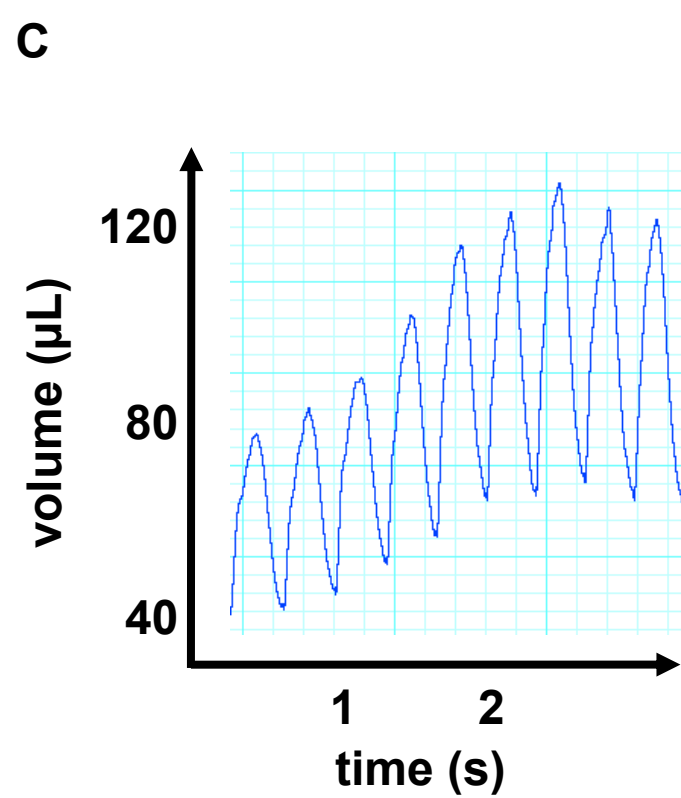
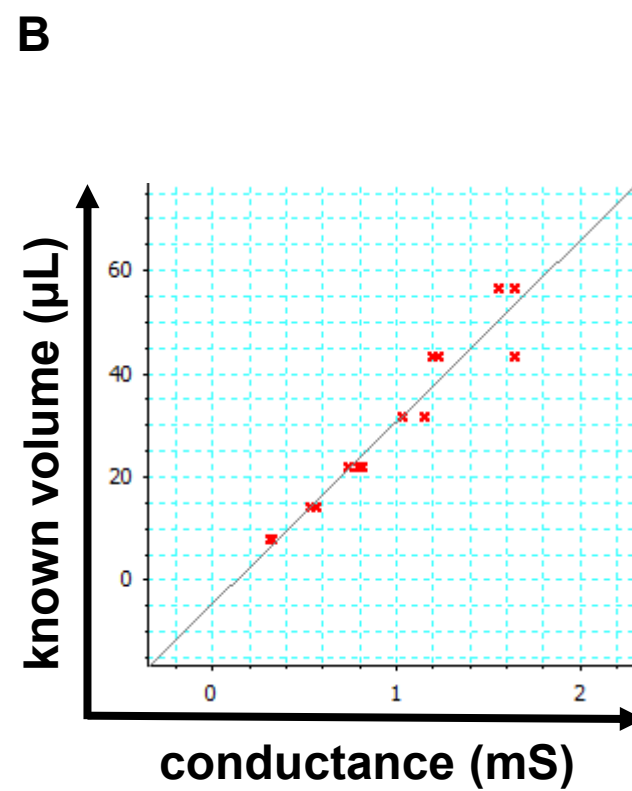
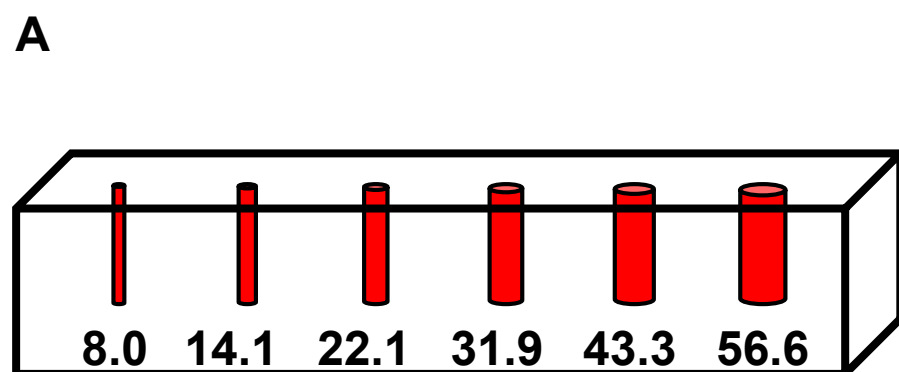


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Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Calibration cuvette	Millar instruments	910-1049	Calibration cuvette
Contura professional hair trimmer	Wella	HS-60	Small animal shaving system
Eclipse Needle 27G	BD	REF 305770	27G needle
Forceps	FST	11203-25, 11069-0	Surgical forceps
Forceps	Aesculap Braun	BN731R, BD 311R	Surgical forceps
Foris FS2434	Eizo	OFTD2033	Monitor
Hamilton Syringe 100 µl needle	Hamilton	80621	100µl syringe with needle
Heated Small animal OP table	Harvard Apparatus	15001	Heated OP table
Heparin-Natrium 25000	Ratiopharm	N68542.04	Heparin
Ketamin 10% 100 mg/ml	bela-pharm	FS1670041	Ketamin
Labchart Pro 8 + Pro modules	AD Instruments	MLS260/8	PV data analysis software
LAS EZ	Leica	LAS EZ	Microscope camera software
Leica IC80 HD	Leica	IC80 HD	Microscope camera
Leica M80	Leica	M80	Microscope
Micro-tip catheter transducer	Millar instruments	SPR-839	PV catheter
MiniVent	Harvard Apparatus	845	ventilation
MPVS Ultra	Millar instruments	PL3508B48/M	PV catheter data acquisition device
Octenisept	Schülke	20000832-A	disinfectant
Plastipak 1ml	PD	REF 303172	1ml syringe
PowerLab 8/35	AD Instruments	PL3508	analog/digital converter
Prolene 6-0	Ethicon	XNEH7814.P31	Polypropylene suture
Retraction Kit	FST	18200-20	retraction of surgical situs
Seraflex 5-0	Naila	IC108000	silk suture
Small and micro-scissors	FST Essen	08, 14064-11	Surgical scissors
Small silicon tube	Reichelt Chemietechnik		tube for LCA occlusion

Sodium Chloride	Sigma-Aldrich	S7653	Sodium Chloride
testo 108	testo	5631080	rectal thermometer
Thinkcentre desktop computer	Lenovo	PC0EJS2V	Computer
Vasofix Safety 20G	Braun	4269110S-01	intubation catheter
Windows 10	Microsoft	KW9-00240	Operating system
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Rebuttal relating to the editorial and reviewer comments regarding:

Real-Time Pressure-Volume Analysis of Acute Myocardial Infarction in Mice

by Michel *et al.*

MS: 57621 R0 112917

We would like to thank the editor for the cordial invitation to resubmit our revised manuscript. Furthermore, we would like to express our thanks to the editor and the referees for the thorough evaluation of our manuscript and their important comments. We have carefully considered their recommendations and incorporated the changes proposed in the manuscript.

In the following we respond in detail to the questions:

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

The manuscript was thoroughly proofread.

2. Please provide an email address for each author on the first page.

We added all email addresses to the first page (l. 11-13).

3. Please rephrase the Long Abstract to more clearly state the goal of the protocol in one paragraph with 150-300 word. Please attention that in the final form, Long Abstract will be used as the Abstract. The Short Abstract will be used as Highlight for the databases.

The headlines used in the abstract (Introduction, Methods,...) were removed. The abstract was rephrased and shortened to more clearly state the goal of the protocol (l. 26-46).

4. Please use SI units, e.g. please use “ μ L” instead of “ μ l”. Please leave a white space between the values and the units.

We checked the manuscript for correct use of SI units and corrected if necessary. We added white spaces (l. 305, 307, Figures).

5. Please define all abbreviations before use.

We checked all abbreviations and added definitions when necessary.

6. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

We removed all commercial language and modified the comments/description section to fit with the terms used in the manuscript (l. 96-98).

7. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

All text in the protocol section is now written in the imperative tense or as a “Note” (l. 132, 147, 151, 159, 165, 190, 201, 205)

8. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. The Protocol steps should contain only 2-3 actions per step and a maximum of 4 sentences per step.

Steps exceeding 3-4 sentences have been divided up into several smaller steps (l. 110-116, 120-172, 176-200, 203-228, 232-257).

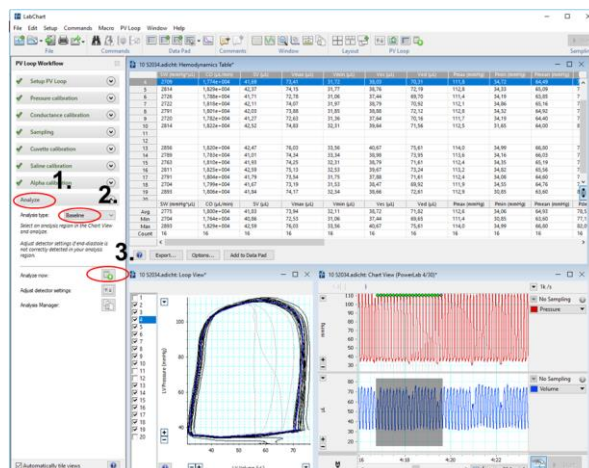
9. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

The protocol was checked for proper description of the steps. We added a reference to published material that provides additional information and schematic images of the procedure (l. 190).

10. For steps that involve software or analyzing tools, please make sure to provide all the details such as “click this”, “select that”, “observe this”, etc. Please mention all the steps that are necessary to execute the action item. Please provide details so a reader may replicate your analysis including buttons clicked, inputs, screenshots, etc. This is the level of detail we’re looking for. Please keep in mind that software steps without a graphical user interface cannot be filmed.

We added more details to steps involving the software (6.1). We added a screenshot of the software (Figure 3 D) and encircled the buttons that need to be activated (l. 232-235).

D



11. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

Personal pronouns were replaced with alternatives (l. 22, 116).

12. Please leave a blank line between all protocol steps as well as Notes.
Done.

13. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets or dashes.

The numbering has been adjusted. We removed dashes (l. 78-257 and 6.4, l. 247-257).

14. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution. Please mention the age, gender and strain of the animals.
The paragraph has been moved to the beginning of the protocol. Age, gender and strain of the mice have been added (l. 79-83).

15. Protocol: 2.2 (currently 2.b): Please avoid using any personal pronouns.

We changed "you" to "towards the investigator" (l. 116).

16. Protocol: 3.1 (3.a): Please clearly describe and explain each action. Please include all the instruments used.

We added details and used instruments (l. 120-121).

17. Protocol: 3.2 (3.b): How is each action done? Using what?

We added details on how the preparation is done using a forceps (l. 126-130).

18. Protocol: 3.6 (3.f): Please use the imperative tense for all the sentences of the protocol steps. Please move the discussion to the Discussion section.

Imperative tense was inserted and the last part has been re-edited to fit the "Protocol" section (l. 144-157).

19. Protocol: Text after 5: If that is a note, please indicate it as a Note.

Indicated as "Note" (l. 203-205).

20. Protocol: 5.2 (5.b), 5.3 (5.c): Please use the imperative tense for all the sentences of the protocol steps. Please move the discussion to the Discussion section.

Imperative tense was inserted. The part describing changes caused by ion concentration that did not fit the "protocol" section was changed and moved to "Discussion" (l. 210-223, l. 326-330).

21. Protocol: 6.1 (6.a) Please clearly describe each action in the imperative tense. If using a software or analyzing tool, please include all the buttons clicked, or refer to appropriate references.

We added details to the descriptions and inserted a screenshot (l. 232-235, Figure 3 D).

22. There is a 10-page limit for the Protocol, but there is a 2.75-page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

We consider steps 3, 4 and 6 to be the most important for the protocol and suitable as filmable content. We uploaded one version with highlighted protocol for filming and one version with highlighted changes since revision (l. 118-199, 232-257).

23. Please upload each Figure individually to your Editorial Manager account as a .png, .pdf, or a .tiff file. Please combine all panels of one figure into a single image file.

Figures were uploaded as separate files for each figure combining all panels of a single figure.

24. Figure 2: Please define and add the scale bars.

Scale bars were added (Figure 2).

25. Figure 3: (A) and (B): please add the values and units for the horizontal axis. (C) Please use “L” instead of “I”.

Values and units have been added. “I” was corrected (Figure 3 A+B).

26. Figure 4: (A) and (C): please define the horizontal axis. (C) Please use “L” instead of “I”. (H) please define each axis.

Horizontal axis defined (A+C), “I” corrected (Figure 4 A+C).

27. Figure 5: Please use “L” instead of “I”. (C): please include the values for each axis.

“I” corrected, values added (Figure 5 C).

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We did not reuse any figures.

29. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please list all the materials, equipment, instrument, and software used in your work.

We double-checked the table of supplies.

Reviewers' comments:

Reviewer #1: Manuscript Summary: The manuscript describes a very technical procedure for monitoring real time cardiac function during acute myocardial ischemia and reperfusion. This is a very important advancement in establishing relevant animal models to test therapeutic interventions that may translate to humans. Being able to capture real

time changes in hemodynamics and cardiac function is necessary in order to make these models relevant for human interventions. This research group are recognized experts in vascular biology and cardiology and are very well published in this area. This manuscript is well suited for JoVE due to technical and skillful procedures required for this model. Visualization of the procedure is necessary for others to replicate their teachings

We thank the reviewer for her/his comments.

Major Concerns: none

Minor Concerns:

It would be helpful to include an optimal time of stabilization and data collection prior to the LCA Ligation.

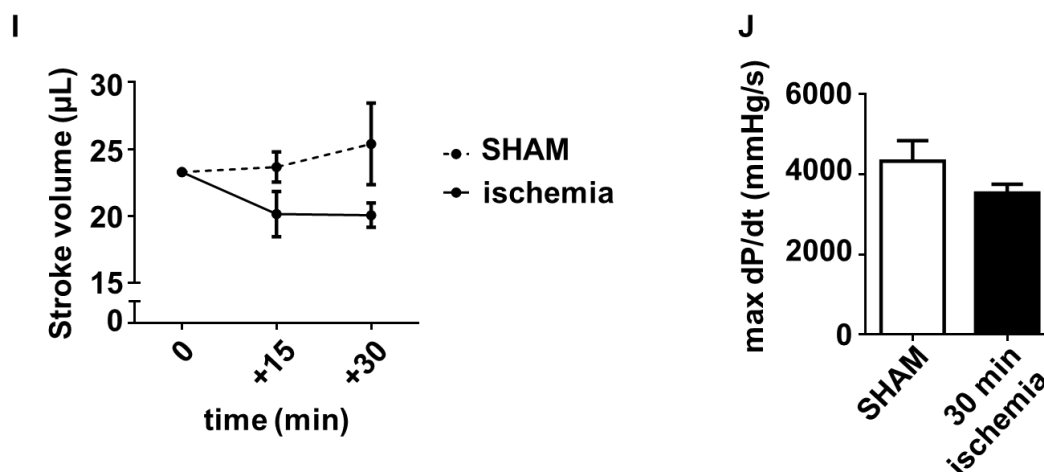
We thank the reviewer for raising this point. We added a description for optimal data collection after opening of the pericardium before LCA ligation (l. 181-183)

How much time is necessary and does this vary from one surgical technician to the next based on skill.

We added details on this topic (Step 4.3 “Note): I/R surgery should be performed within 5 min independently from the investigating operator (l. 190-191).

Showing changes in SHAM operated animals would be helpful to demonstrate that the acute changes during ischemia reperfusion are indeed that profound rather than loss of function from catheter implantation and anaesthesia over the course of the experimental protocol.

We thank the reviewer for this important issue. We added figures showing SHAM operated animals (Figure 4 I+J) and referred to them in the “results” section stating that SHAM did not induce LV systolic or diastolic dysfunction (l. 280-281).



Reviewer #2: Manuscript Summary:

The manuscript "Real-Time Invasive Hemodynamic Imaging of Acute Myocardial Infarction in Mice" by Michel et al. describes the use of conductance catheter technology to monitor the pressure/volume (PV) relation in mice during and after coronary occlusion. This animal study was very well planned and well executed by a laboratory that is widely-recognized for their work in myocardial ischemia as well as advanced laboratory methods for the study of myocardial ischemia. As such, the work reported here represents a valuable contribution to the cardiovascular research community (in general) and to JoVE (in particular).

Overall, the manuscript is extremely well-written, particularly considering that English may not be a first language for the authors. Only upon careful analysis can one detect a few typos or grammar issues. However, these issues are quite minor and could easily be overlooked.

Thus the manuscript could readily be accepted in its current form should the authors elect not to incorporate the minor suggestions recommended below.

We thank the reviewer for her/his comments.

Major Concerns: NONE

Minor Concerns:

Page 1, line 3 (Title): The authors might consider rephrasing the title to make it somewhat more precise. In particular, the majority of readers will equate "hemodynamics" with blood pressure monitoring and may therefore miss the fact that a far more sophisticated PV analysis is being conducted here. Further, the word "imaging" might be confused with "medical imaging," which of course is not the subject of this manuscript. Thus, one might prefer a title such as: "Real-Time Invasive Monitoring of Pressure-Volume Relationship during Acute Myocardial Infarction in Mice." Alternatively, one might consider: "Real-Time Conductance Monitoring of Pressure-Volume Relationship during Acute Myocardial Infarction in Mice" or "Real-Time Pressure-Volume Analysis of Acute Myocardial Infarction in Mice."

We thank the reviewer for this important issue. We changed the title to "Real-Time Pressure-Volume Analysis of Acute Myocardial Infarction in Mice" (l. 3).

Page 1, line 44: To be consistent with the reperfusion period specified later in the manuscript, recommend changing "20 minutes" to "10 minutes". Alternatively "10-20 minutes" could be specified throughout.

We apologize for the mistake. We changed the reperfusion period to 20 min in the protocol section (l. 198).

Page 3, lines 123-129: Steps (d) and (e) under "Cardiac Catheterization" contain elements that appear to be duplicated and overall read a bit less smoothly than the rest of this most excellent description of a complex surgical procedure. One might consider adopting something akin to the following:

We thank the reviewer for these helpful remarks. We adapted the changes to the manuscript.

d. Perform a wedge-shaped incision 1 mm proximal to the cranial knot to open the vessel with micro scissors. A small drop of blood will indicate proper execution of this step. Insert the catheter carefully for 10 mm. Stretching the incision with forceps can make this process easier. Start recording of catheter data.

l. 144-151.

e. Extract the vascular clamp. Add 1-2 drops of saline to the incision to facilitate catheter movement. Continue introducing the catheter for approximately another 10 mm. After passing the proximal knot with the sensor tip, fasten the knot carefully to prevent blood reflux alongside the thinner parts of the catheter.

l. 153-157.

In considering Steps (d) and (e), please note that the following sentence should either be removed or clarified: "The size of the sensor tip of the catheter should reflux of blood." This sentence seemed difficult to interpret, so maybe a line of text is missing here? Or perhaps what was meant was: "The sensor at the tip of the catheter should become refluxed with blood."

We apologize for the mistake. The word "prevent" was missing – we corrected the sentence to "The size of the sensor at the tip of the catheter prevents reflux of blood when extracting the vascular clamp." (l. 159-160).

Page 3, lines 123-129: The last sentence in step (e) seems to suggest that the proximal knot should be fastened tightly before the catheter reaches the LV. Perhaps the ligature is tightened just enough to prevent blood reflux and this point, then fastened more tightly in step (f) after the proper placement is optimized by the volumetric reading from the conductance catheter? If so, please clarify.

We thank the reviewer for this issue. We added details to this step: "...fasten the knot carefully just enough to prevent blood reflux alongside the thinner parts of the catheter without impairing catheter movement." (l. 155-157) [...] "Fasten

the proximal knot more tightly to prevent catheter movement." (l. 172).

Page 4, line 146: Under I/R surgery, it may be helpful to specify the location along the LCA where the suture is placed relative to the left auricle.

We thank the reviewer for this comment. We specified the location of the LCA and the location of the suture (4.3; l. 185-188).

Page 4, line 147: Under I/R surgery, a "small silicon tube" is specified, whereas Fig 2B indicates a polyethylene tube. Perhaps a soft silicon tube might offer advantages over a hard PE tube, but please consider making the figure consistent with the text.

We changed the description in the figure to "silicon tube" (Figure 2 B).

Page 4, line 157: Please consider "to ensure valid results".

Done (l. 206).

Page 5, line 187: Rather than "to avoid falsification by artefacts," please consider "to avoid sampling error".

Done (l. 244).

Page 5, line 211: Rather than "characteristically," please consider "characteristic".

Done (l. 263).

Page 5, line 215: Rather than "incision," please consider "excursion".

Done (l. 267).

Page 5, line 216: Rather than "paling," please consider "blanching".

Done (l. 268).

Page 6, line 235: Rather than "situs," please consider "sites" or "procedures".

Done ("Procedures") (l. 288).

Page 6, line 235: Rather than "commune," please consider "common".

Done (l. 288-289).

Page 6, line 236: With regard to the "silicon tube," please refer to the comment for line 147, above.

Done ("silicon") (l. 289).

Page 6, line 238: Rather than "incision," please consider "excursion".
Done (l. 291).

Page 6, lines 257-260: In order to be technically correct, it should probably be acknowledged that echo and MRI typically yield the parameters of EF, stroke volume and cardiac output. Please consider rephrasing to something like:

"In addition to the LV volumetric parameters typically obtained by echocardiography or MRI (chamber volumes, EF, stroke volume and cardiac output), PV analysis yields a more complete profile of LV function by simultaneously providing measures of LV systolic performance (contractility dP/dt , stroke work) and LV compliance ($-dP/dt$, Tau) as a parameter for diastolic function."

We thank the reviewer for this remark and adapted the suggestion (l. 313-317).

Page 7, line 272: Mortality is a relative term, but please consider deleting "very" since the in-hospital mortality rate associated with a coronary event (<7%) is actually pretty low as compared with other maladies such as pancreatic cancer.

"Very" has been deleted (l. 338-339).

Page 7, lines 275-277: Please consider deleting the following sentence since it rather undercuts the last sentence in the discussion (that occurs directly afterwards): "Differences in coronary anatomy and hemodynamic compensation of LCA occlusion are therefore possible limitations for transmission of the acquired data on patient's care." This is because the "differences in coronary anatomy and hemodynamic compensation of LCA occlusion" are probably very minor as compared to differences in the location of the coronary occlusion. This relates to the comment raised above regarding line 146. The mortality due to coronary occlusion is directly related to the area at risk, and patients with a complete occlusion of the left main will experience far more LV dysfunction (and higher acute mortality) than patients with a more distal coronary occlusion. Thus it may be prudent to explore more proximal ligations in mice (say proximal enough to incur 7% mortality) before concluding that a mouse is somehow more resistant to hemodynamic impairment than a human. Overall, one might argue that it is too early to make such a comparison given that so few hemodynamic studies have been conducted during MI in mice, or in humans. As approximately 20-fold more researchers have access to Millar pressure catheters as compared to Millar PV conductance catheters, a related (and perhaps more appropriate) subject for discussion might be the relative merits of monitoring LV

hemodynamics (pressure only) versus the more elegant and comprehensive PV analysis described in this manuscript.

We thank the reviewer for this important issue. We deleted the sentence as suggested. We added a new paragraph to the discussion describing the advantages of simultaneous acquisition of pressure and volume data compared to pressure data alone (l. 332-336).

We expended the paragraph concerning LCA ligation in mice compared to LCA occlusions in humans mentioning that this effect could be due to more proximal occlusions in humans (l. 342-343).