

## Simultaneous Measurement of Right Ventricular and Pulmonary Artery Pressures in a Rat to Hemodynamically Characterize Pulmonary Artery Hypertension

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Received: January 12, 2020; Published: February 12, 2020

### Abstract

**Introduction:** Pulmonary Artery (PA) hypertension is disease of pulmonary vasculature with final constrictive remodeling of pulmonary arterioles leading to (RV) dysfunction and right sided HF failure. Pre-clinical animal models have been developed to mimic this disease. In this methodology article, surgical access, ventilator set up and direct open chest measurements of PA hypertension are described in a rat model. As acute vascular access to the rat's PA is complicated, mainly due to the anatomy of the right ventricle and the right ventricular outflow tract, to assure good hemodynamic data collection, open chest model was carefully designed using controlled mechanical volume ventilation (CMVV).

**Methods and Aims:** Setting of CMVV was adjusted to limit its influence on RV preload and LV afterload in the instance of chronic pulmonary disease. Volume-ventilation setting (tidal volume and respiration rate) was based on body weight. Isoflurane mono-anesthesia was used without any premedication. Chronic PA injury was induced by monocrotaline (MCT). PA pressures were compared using single and dual pressure catheter at 3-weeks post injury. Initially, single pressure catheter was positioned in the PA to assess data quality, while advanced data comparison (RV and PA pressures) during PA hypertension were made using dual pressure catheter. PA access was performed using "high" RV needle-stab, adjacent to the anatomical area of the PA outflow.

**Results:** Introduction of single pressure catheter was successful and collected data during RV systole and diastole did not produce any major pressure artefacts. Final position of the pressure catheter, in the main PA was guided by using visual cues i.e. distance of pressure sensor on the catheter, accompanied by simultaneous data recording from that location. In case of dual pressure catheter, insertion was also successful, and RV and PA pressure data were able to be recorded. In case of PA hypertension, systolic ranges were (41 - 52 mmHg) as compared to (25 - 30 mmHg), and diastolic (21 - 27 mmHg) vs. (9 - 14 mmHg); n = 4. Additionally, in case of hypertension, high afterload pressures complicated RV ejection, with PAP cresting about 1 mmHg higher than the maximal RVP, when overlaid. During further assessment, RV ejection was additionally complicated by higher PA diastolic notch pressures, at the end of systole; for hypertension (37 - 41 mmHg) vs. naïve (16 - 21 mmHg), n = 4.

**Keywords:** Pulmonary Artery (PA) Hypertension; Open Chest Access; Acute Measurement; Solid-State Pressure Sensor; 1.6F Pressure Catheter

## Abbreviations

CMVV: Controlled Mechanical Volume Ventilation; EDP: End Diastolic Pressure; Mv: Minute Ventilation; MCT: Monocrotaline; MCTP: Monocrotaline Pyrrole; PA: Pulmonary Artery; PAP: Pulmonary Artery Pressure; PE: Polyethylene; RR: Respiration Rate; RV: Right Ventricle; RVP: RVP: Right Ventricular Pressure; TBW: Total Body Weight; Vt: Tidal Volume.

## Introduction

PA hypertension is disease of pulmonary vasculature that progresses through stages with final constrictive remodeling of pulmonary arterioles leading to (RV) dysfunction and right sided HF failure. Right-heart failure (HF) is the main cause of death due to pulmonary arterial hypertension (PAH) [1]. Pulmonary arterial hypertension contributed to but did not directly caused death in (44%) patients [1], Some patients however were able to adapt to PA hypertension [2]. Measurement of RV pressure and load along with precise quantification of PA hypertension is a key determinant of clinical prognosis. Hemodynamic measurement allows to better predict the stage of right HF and need of e.g. heart transplantation, and in extension it improves overall chances of patient's survival. Preclinical animal model that fully imitates stages of human PA hypertension are currently non-existent. Injection(s) of dose or multiple doses of Monocrotaline (MCT) along with chronic hypoxia (CH) are two well- published animal models. Both models have its advantages and limitations. For more detailed animal pre-clinical models, please see recent review by [3]. This article is set out to describe in detail surgical approach and methodology of good measurement and PA hypertension. If used well, it should be helpful to answer some of the not published nuances about capturing good PA hypertensive data from the main PA, along with showing comparisons of PA pulse and dicrotic notch pressures. Additionally, by capturing simultaneous data from RV and PA one could clearly appreciate the role of afterload PA pressure in RV hypertrophy.

## Materials and Methods

### Surgical steps and ventilator set up

Male Sprague Dawley rats of (300 - 350g, Charles River, QC), were used for this acute procedure. Animals were housed with enrichment and provided with *ad libitum* rodent chow and reverse osmosis water via an automatic watering system. The light: dark cycle was 12:12 hours and maintained at  $23 \pm 0.5^\circ\text{C}$  and at 30 - 70% relative humidity. During this acute measurement, it was important to adhere to good laboratory practices by using pre-surgical preparations and general anesthesia.

These steps included, preparation of scrubbing area in a separate location from surgical field (performing aseptic prep), setting up surgical microscope, adjusting mechanical ventilator, pre-warming surgical table and isotonic fluids. For cardiac surgery, areas with low-traffic and relatively quiet (from open windows, elevator noise, duct ventilation overhead etc.) were selected. All hard surfaces were disinfected by Chlorox, but other disinfectants e.g. (Glutaraldehyde-Cide wipes, Phenolics-Lysol, Chlorhexidine-Nolvasan) can also be used.

Afterwards, set up of surgical microscope was performed. Before pre-anesthetizing, weight, age, sex and strain, and health status of each rat was recorded and checked whether the animal have had enough acclimatization time in the facility. Rat basic physiology values were written into surgical record document, including respiratory rate are (65 - 110 breaths/min), heart rate (305 - 500 b/min) and temperature ( $38.1 - 38.5^\circ\text{C}$ ) before selecting rat for anesthesia. The best was to also ensure that animals were at least twice handled by facility staff, including e.g. (cage change, enrichment and socializing) before this cardiovascular procedure.

Animals were anesthetized in Plexiglas induction chamber using 4% of Isoflurane with oxygen flow rate of 1- 1.2 l/min. Later, animals were trans-tracheally intubated and connected to control mechanical ventilator (CMV). Each rat weight was taken to calculate the setting of CMV SAR 1000 (CWE, Geneq, Montreal, QC).

Ventilator was used in volume-controlled ventilation setting. Calculated values for respiration rate (RR) and (Vt), tidal volume can be found in table 1 [4].

As Isoflurane is a profound respiratory depressant, respiration had to be closely monitored. When e.g. anesthetic dose (percentage of Isoflurane in oxygen) was increased, Vt has decreased while RR respiratory rate was unchanged. This depression was partially reversed by surgical stimulation during surgical anesthesia.

Post-induction, isoflurane anesthesia was decreased to 2% with oxygen flow 1 liter/min. Animal was at this time placed on the warming pad in a supine position, with the upper and lower extremities attached to the heating pad using surgical tape. Following intubation, inspection of basic clinical characteristics e.g. breathing pattern RR and color of mucous membranes were detected. Furthermore, the volume ventilation was set to I:E ratio 30/70, PEEP was set to 3 - 6 ml H<sub>2</sub>O. The body temperature was maintained at 38°C through a water circulated heat pump Gaymar, T-pump (Braintree Scientific, Inc., Braintree, MA).

Pre-operative analgesia was administered subcutaneously using i.p dose of ketorolac, 10 mg/kg. Ophthalmic ointment to both eyes was applied to prevent corneal desiccation. The lower thoracic wall and the abdominal surgical area were shaved and prepared for surgery. The body temperature was maintained at 38°C. Heat loss was protected by not wetting larger areas of skin when scrubbing. Chlorhexidine surgical scrub was used with clean gauze moved in a circular fashion starting at the surgical incision site and rotating outward. Surgical scrub was removed by using 70% alcohol.

Adequate surgical anesthesia was detected by loss of muscle tone and loss of reflexes e.g. (corneal, and pedal), and was measured before surgical procedure commenced.

PA injury was induced by monocrotaline (MCT) in saline by i.p single injection of 60 mg/kg [5]. As previously described *in vivo* by [6], activation of the toxic pyrrolizidine alkaloid Monocrotaline pyrrole (MCTP), leads to PA vascular injury in a rat.

- Respiratory rate (RR, min<sup>-1</sup>) = 53.5 \* Mb<sup>-0.26</sup>
- Tidal volume (Vt, ml) = 6.2 \* Mb<sup>1.01</sup>

Rat TBW (g)	RR (min <sup>-1</sup> )	Vt (ml)
250	77	1.53
270	75	1.65
285	74	1.75
300	73	1.84
325	72	2.00
350	70	2.15
400	68	2.45
450	66	2.77
500	64	3.1

**Table 1:** Pre-calculated values to set RR and tidal volume based on animal's body weight (Mb in Kg) to operate ventilator in volume mode.

### Pressure catheter set up

Prior to surgery, solid-state pressure sensor(s), located on the tip of the dual or single 1.6F pressure catheter (Transonic Scisense Inc., London, ON) were soaked in 0.9% saline for ~ 20 minutes and then balanced to zero mmHg against atmospheric pressure. Briefly, 20 ml syringe with saline was warmed to animal's body temperature 36 - 38°C. Then each of the catheter was connected to amplifier SP 200 (Transonic Scisense Inc., London, ON) and to data acquisition software. After soaking in warm saline, each pressure sensor was positioned just under the saline meniscus, and crude and fine calibration buttons on the amplifier was used to balance pressure sensor as close to zero mmHg, signal seen and recorded by data acquisition software as a baseline. Then until insertion, the catheter tip was left in the warm saline solution.

Experiments adhered to the guidelines set forth in the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85 - 23, Revised 1996) and were performed under protocols approved by local Institutional Animal Care and Use Committee University of Toronto, Canada).

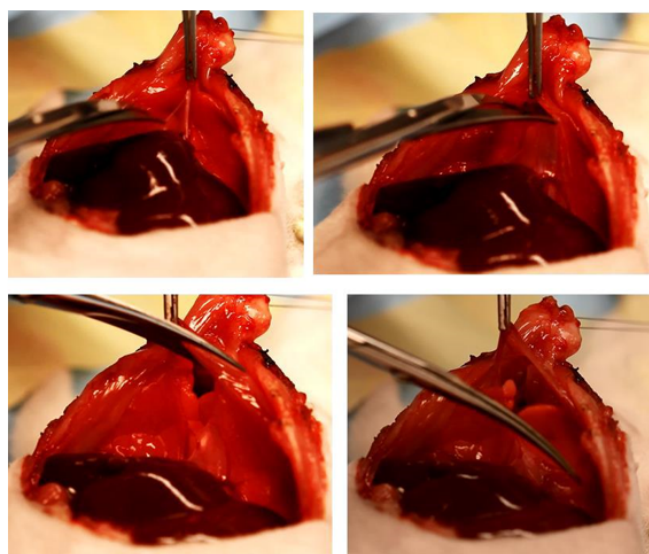
### Data collection and analysis

LabScribe3 recording and analysis software (iWorx/CB Sciences Dover, NH) was used to collect hemodynamic data. Pressure module was selected to report pressure wave parameters.

### PA pressure measurement using open chest using catheter with single pressure sensor

Animals were secured for surgery on water-heated blanket in a dorsal recumbence. Using scalpel and Adson tissue forceps, 4 - 6 cm skin cut was made, starting immediately below the xiphoid process across the lower thorax/upper abdomen area. Later, Metzenbaum scissors helped to enlarge the area while skin was lifted by forceps. Abdominal wall was opened in the area of xiphoid. Xiphoid was held by the Adson forceps while Metzenbaum scissors further bluntly opened the abdominal wall. Later, bipolar coagulator was used to cut through areas of both mammary arteries to limit bleeding into the chest cavity. Subsequently, the Adson's tissue forceps lifted the cartilage portion of xiphoid and diaphragm was checked for herniation while 4-0 silk was run through cartilaginous portion of xiphoid. This maneuver helped to lift xiphoid cranially. At the same time, suture tension was adjusted allowing good inspiration and expiration. At this point diaphragm was grasped by tissue forceps and cut was made by scissors across the diaphragm following the costal arc (steps on Figure 1). To better expose the beating heart and access to RV outflow, cut through the cartilaginous portion of the ribs was made (see Figure 2).

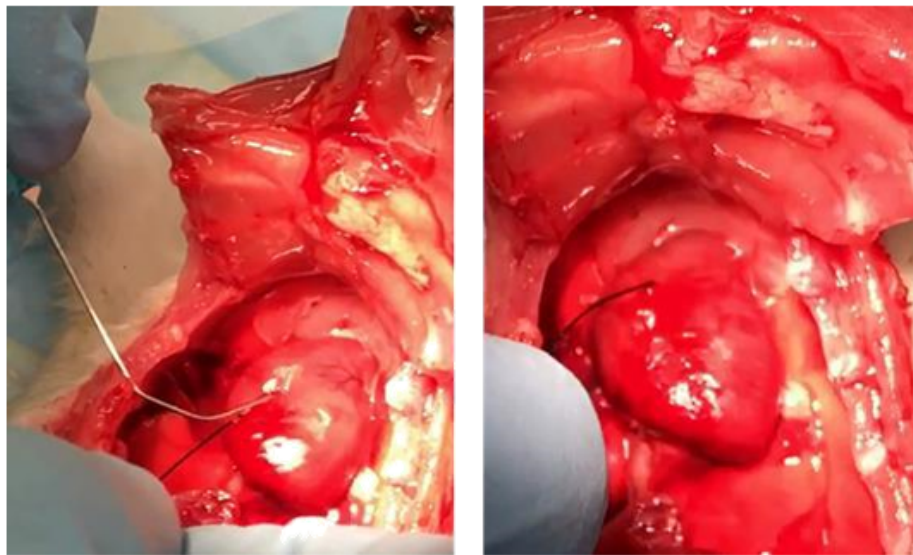
Blood was meticulously emptied from bottom of the chest cage using gauze or cellulose surgical spears. Note: on many occasions, it was observed that during hemodynamic assessment a little pooling of blood in the chest cavity restrained heart's diastolic filling, influencing overall data quality.



**Figure 1:** Steps of diaphragm opening using tissue forceps and Metzenbaum scissors. To avoid injury to the vital organs and structures in the chest cavity, lifting the xiphoid process and grasping diaphragm when first inserting scissors and cutting has to be ensured.

### **Pulmonary artery pressure catheter insertion**

Pressure catheter insertion through the RV free wall using antegrade access into the main pulmonary artery was used for the “high” RV stab, adjacent to the anatomical area of the PA outflow tract. The 23G needle (0.58 mm) was to fit (1.6F = 0.53 mm) catheter tip. Needle end was bent to better adjust the tip towards the plane of RV wall. After stabbing, blood was found in the needle conus. At this time, needle was slowly withdrawn, while catheter was inserted into RV outflow and pushed towards the conus of main PA, using dominant hand. Later, catheter was further advanced into the main pulmonary artery through pulmonary valve. In cases of an immediate resistance on entry, catheter was pulled back and needle was used again to make larger entrance. Slow maneuvering and patience were both exercised to cannulate and to properly position the catheter’s pressure sensor in the main PA. When making first needle puncture, surgeon was making sure to aim towards the RV outflow, while carefully watching movement of the curvature of the common PA trunk during cardiac cycles.



**Figure 2:** Steps of catheter insertion through the RV free wall using antegrade access into the main pulmonary artery through PA valve. On the left picture, 23G needle was bent allowing better hand maneuvering and final puncture of the area of conus arteriosus. Picture on the right shows full insertion of the catheter into the stab opening.

At this step using this open chest technique, it was important to achieve good catheter position in the main PA in order to record physiological PA pressures and to compare them with hypertensive pressure data. When catheter has passed through pulmonary valve, the signal has changed from ventricular to arterial pressure with classical notching and higher diastolic pressures (see Figure 3). But before that, the catheter needed to be stabilized in the RV for about 1 - 2 min while observing RV pressure signal and then careful advancement of the tip was performed ensuring proper pressure sensor location, limiting its entry into major side branches. Also, percentage of Isoflurane was temporarily reduced to 1%. Occasionally, pressure artifacts were observed when sensor was in direct contact with vessel wall, usually when heart was in systole. For that reason, pressure data needed to be constantly monitored on computer’s screen. In this case, sensor needed to be repositioned or whole catheter shaft needed to be pulled back (see Figure 3), with arrows predicting location of catheter’s tip during systole (right image) and diastole (image on the left). To assure good quality of hemodynamic data, constant live-feed data recording at physiological HR needed to be accumulated to control for repositions such as transitions from RV to PA, catheter advancements and pullbacks. In this model still X-ray or other imaging technique could have been used to further locate the pressure sensor; however the transition from RV to PA using pressure tracings was decided to be a sufficient marker to collect and to compare the main PA pressures.

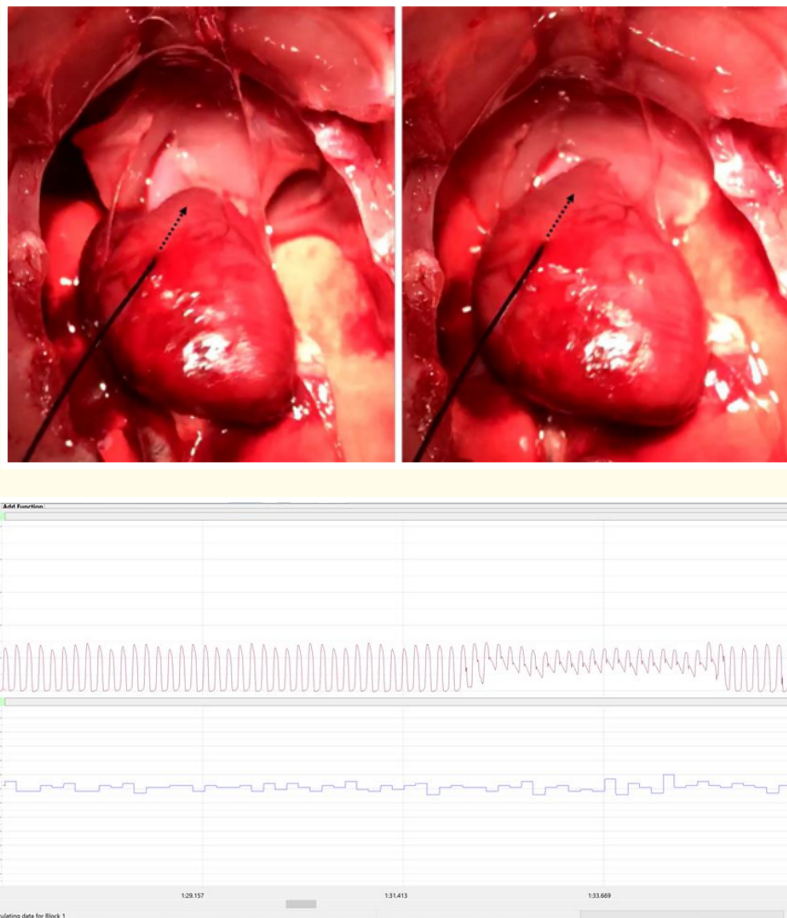


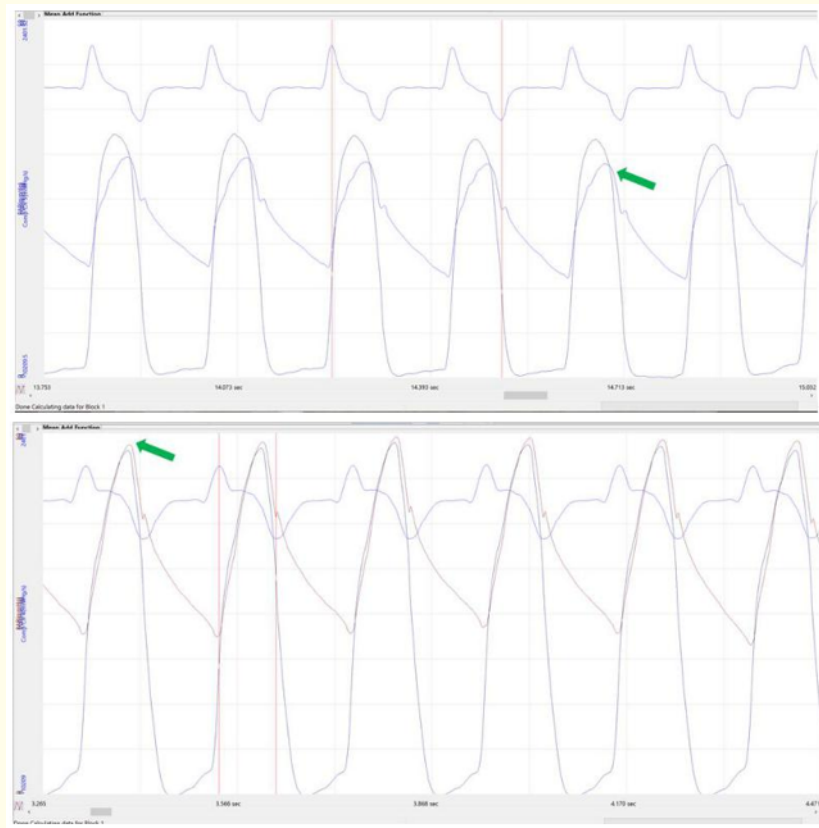
Figure 3: Quality of captured data was dependent on the position of the pressure catheter.

Catheter needed to be repositioned multiple times to get into the main pulmonary artery without pressure sensor touching arterial wall, otherwise creating signal artifacts and pressure spikes. To find an approximate direction of the catheter tip during data collection (during systole and diastole on the images above), surgeon needed to carefully push and pull on the catheter's shaft while data were simultaneously recorded on the computer screen (arrows point to the area of estimated presence of the pressure tip, screenshot shows the pressure data at these locations).

### PA hypertension, comparison of pressure traces using dual pressure catheter

Dual pressure catheter was used to collect RV and PA pressure data simultaneously. Open chest exam was done to compare the PA hypertension at 3 weeks post-MCT delivery. During pulmonary artery hypertension as seen on the figure 4, captured pressures in the PA, but as well in the RV had multiple characteristics. RV pressure wave in case of PA hypertension, had typical slender peak originated at the opening of the PA valve, while the valve closed much earlier than in case of normotension (typical wave pattern when afterload pressure is increased in cases of using e.g. direct-acting sympathomimetic amines). To compare waves again, the right ventricle pressure trace (RVP)

at the bottom of each image at Figure 4, were overlaid by pressure wave coming from the main PA (PAP). Bottom images depict 6 cardiac cycles. Bottom image is from animal suffering from hypertension and the image on the top is from healthy naïve animal of same sex and similar weight. To help approximate values during cardiac cycle RV dp/dt(s) are displayed on the top of each image.



**Figure 4:** Comparison of healthy naïve animal at the top vs. PA hypertensive animal (6 cardiac cycles for both). Scale of both images is 0 to 50 mmHg for an easier comparison. Right ventricle pressure trace (RVP) at the bottom of each image was overlaid by pressure wave coming from the pulmonary artery (PAP). At the top of both images is RV dp/dt.

Both, the RVP and PAP trace(s) were cresting much higher in hypertensive rat. Additionally, when both pressure traces were overlaid, images demonstrated that during hypertension the PAP was peaking higher than the maximal RVP, classical path-physiological demonstration of high afterload pressures, existing behind the PA valve. These pressures were complicating RV ejection at every cardiac cycle (green arrow on both images for comparison). RV ejection was also complicated by higher PA diastolic pressures, at the end of systole.

Additionally, Table 2 has selected value ranges from 4 animals, which further helped to characterize PA hypertension. Specifically, in case of systolic and diastolic pressures, which further categorized amount of pressure resistance in the PA that RV had to overcome in order to eject blood into hypertensive PA tree.

Pulmonary artery pressure rat	Range	Range
Hemodynamic parameter	Healthy	Pulmonary hypertension
Heart Rate (BPM)	306-357	277-312
Systolic Pressure (mmHg)	25-30	41-52
Diastolic Pressure (mmHg)	9-14	21-27
Dicrotic Notch Pressure (mmHg)	16-21	37-41
Mean Pressure (mmHg)	17-22	31-37
Pulse Pressure (mmHg)	11-19	17-31
Mean Diastolic Pressure (mmHg)	13-18	34-37

Table 2: Data ranges from 4 healthy and hypertensive animals.

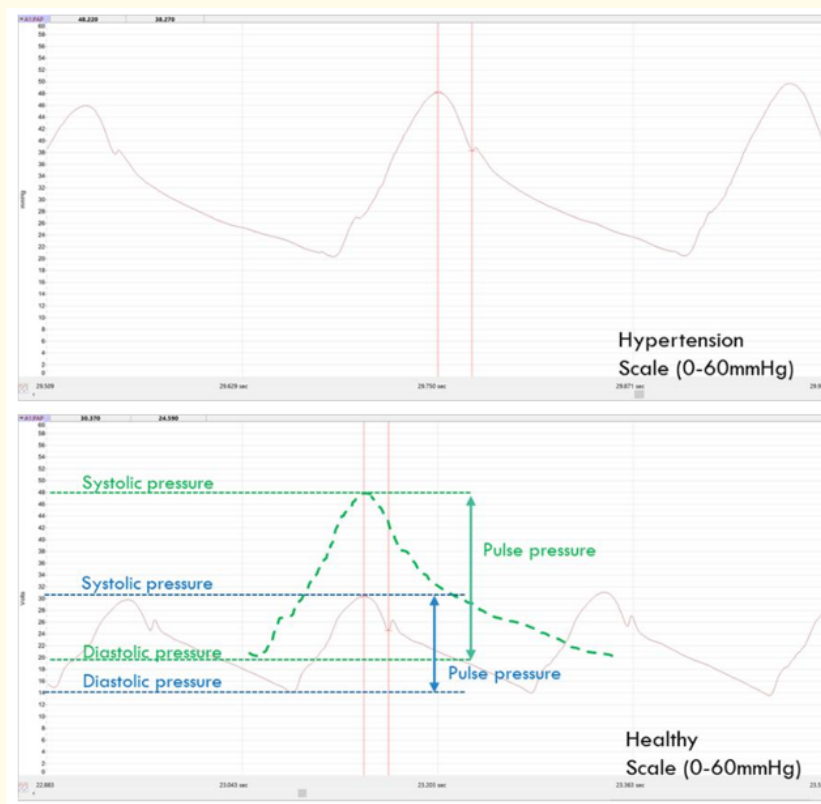


Figure 5: Graphical comparison of one pressure wave collected from hypertensive animal (top) vs. normotensive (bottom). Direct comparison of both waves is by using hand trace of hypertensive wave superimposition on top of normotensive allows comparison of e.g. pulse pressure.



Later, graphically constructed comparison of single pressure wave during hypertension and compared to normotensive pressure trace could be seen at Figure 5. Scale of both images was adjusted to 0 to 60 mmHg, to enable quick and easy visual comparison of e.g. pulse pressure or systolic and diastolic pressures. Bottom image was overlaid by hand-traced pressure wave (in green), generated based on high fidelity pressure trace from hypertensive animal.

### Discussion

Rodent animal models of pulmonary artery hypertension are well-established in the literature. Pathomorphological and pathophysiological changes post-monocrotaline injection are also well-defined and reviewed by many [3]. In this article, description of surgical approach with careful attention to accurate pressure data collection, captured at 3-weeks after the initial injury, was described. It was very difficult at the beginning to ensure that catheter position is in the main PA trunk (to compare pressure data using the same location). Additionally, to diligently position the catheter to limit pressure wave artifacts during RV systole and diastole, needed to be practiced at the beginning on normotensive animals. Likewise, to successfully place dual pressure catheter required some diligence to depict RV and PA pressure waves simultaneously. Overall, quality of data and comparison of PA hypertension was comparable to recent rat MCT study by Zhuang, *et al.* [7].

Research groups describing treatment or pathomorphological changes after different doses of MCT [5,7]. They are using fluid-filled polyethylene (PE) pressure catheter to account for pressure changes. It is important to note that PE catheters have better maneuverability through venous, RV, and RV outflow tract [7]. Using these catheters, it allows to perform closed chest measurements to fully embrace the effect of right pressure gradient (pressure gradient between right atrium RA/RV and large extra-thoracic veins). As described by Konecny [8], pressure gradient in case of closed chest is important as it plays key role in augmentation of preload pressure and volume, which leads to increase of the RV stroke volume and cardiac output, when compared during multiple cardiac cycles to open chest CMV. When collecting pressure data using PE fluid-filled catheter, low fidelity and in most cases not enough pressure-wave resolution complicates the analysis, especially in cases of dicrotic notch, pulse pressures and diastolic pressure wave. Moreover, due to the size of PE tubing, when fluid-filled catheter enters the PA from the RV, pulmonary valve could be held open, interfering capturing dicrotic notch pressure. Lastly, localization of PE catheter's measuring tip in vascular bed could be also difficult, as the tip of the tubing might not be able to be fully discerned by e.g. an X-ray imaging. For these reasons, solid-state pressure catheter was selected using the open chest setting. Open chest setting was used due to the limitation of current solid-state pressure catheter shaft (limited ability to manipulate shaft in and out of the RV). Shaft e.g. would need to be made from such material that would allow to relieve forces on the tubing and would withstand all multi-dimensional bending forces before reaching the PA. Additionally, there are inner cardiac structures that might prohibit otherwise an easy access to pulmonary artery that are commonly accessed by catheters in larger animals. These structures include e.g.: Tricuspid's valve moderator band, anterior papillary muscle, the septal papillary muscle or pulmonary valve. In this article, pressure data were collected from the main trunk of PA using an open chest approach supported by CMVV. Gathered normotensive data were successfully compared to chronic hypertension. In closing, it was also possible to collect pressure data from the RV and PA simultaneously, data that could be later used for other investigations.

### Conclusions

This study revealed that good pressure data could be collected from the main trunk of PA using an open chest approach supported by CMVV. Gathered normotensive data were successfully compared to chronic hypertension. In future, hemodynamic influence of respiratory pump in close chest and its influence on chronic PA hypertension needs to be analyzed using solid state pressure catheter. Using imaging technique, catheter location in pulmonary arterial tree needs to be assessed and standardized when collecting data. To collect artifact-free high-fidelity RV and PA pressure data, improvements of catheter's design needs to be made based on the anatomy of the rat's right ventricle and its outflow.

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**Volume 7 Issue 3 March 2020**

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