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Simultaneous Electrocardiography Recording and Invasive Blood Pressure Measurement in Rats

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TITLE:**Simultaneous Electrocardiography Recording and Invasive Blood Pressure Measurement in Rats****AUTHORS, AFFILIATIONS:**

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KEYWORDS:

Femoral arterial cannulation, intra-arterial blood pressure, hypertension, rat, invasive, electrocardiography

SUMMARY:

Here, we describe a setup for simultaneous recording of electrocardiography and intra-arterial blood pressure (BP) in experimental rats, which can be done with standard equipment in animal facilities and can be applied to physiological or pharmacological studies to investigate pathogenic or therapeutic mechanisms in cardiovascular medicine.

ABSTRACT:

For studies related to cardiovascular physiology or pathophysiology, blood pressure (BP) and electrocardiography are basic observational parameters. Research focusing on cardiovascular disease models, potential cardiovascular therapeutic targets or pharmaceutical agents requires assessment of systemic arterial pressure and heart rhythm changes. In situations where radio telemetry systems are not available or affordable, the technique of femoral artery cannulation is an alternative way to obtain intra-arterial pressure waveform recordings and systemic BP measurements. This technique is economical and can be performed with standard equipment in

animal facilities. However, invasive arterial pressure recording requires cannulation of small arteries, which can be a challenging surgical skill. Here, we present step-by-step protocols for femoral artery cannulation procedures. Key procedures include the calibration of the data acquisition system, tissue dissection and femoral artery cannulation, and setup of the arterial cannulation system for pressure recording. Surface electrocardiography recording procedures are also included. We also present examples of BP recordings from normotensive and hypertensive rats. The effects of two anesthesia regimens, intraperitoneal and inhalation anesthesia, on BP is demonstrated and discussed. This protocol allows reliable direct recordings of systemic BP with simultaneous electrocardiography.

INTRODUCTION:

Blood pressure (BP) and electrocardiography (ECG) are basic parameters for cardiovascular physiology and medicine. Experimental animal models have been widely applied in biomedical research for various cardiovascular diseases such as hypertensive heart failure¹ and procedures for ECG recording and BP measurement can be performed in experimental rats.

There are three methods for BP measurement in rats: intra-arterial cannulation (invasive)², tail cuff plethysmography (noninvasive)³, and radio telemetry (invasive). The reliability of BP measurement by tail cuff plethysmography can be affected by animal handling during the recording. For example, the tail cuff underestimates the core BP changes that occur simultaneously during the restraint and measurement phases⁴. Radio telemetry is considered the best “gold standard” technique for monitoring BP and heart rate in awake and freely moving animals⁵. However, since radio telemetry hardware and software are costly, intra-arterial cannulation is also widely used as an economical alternative.

Intra-arterial cannulation requires considerable microsurgical skill but yields the real waveforms of arterial pressure. BP can be recorded through a saline-filled catheter inserted in the radial, femoral, or brachial artery. This method of direct invasive BP measurement requires pre-surgical animal preparation, anesthesia, immobilization of laboratory animals, surgical skill in tissue dissection and arterial cannulation, and proper calibration before acquiring the measurement.

Rodent surface ECG is similar to human ECG. A rat ECG has sequences of P waves, QRS complexes, T waves, and QT intervals⁶. The P wave, PR interval, QRS complex, and T waves reflect atrial depolarization, impulse conduction from the atrial to the AV node, ventricular depolarization, and repolarization, respectively. The QT interval is defined as the period from the initiation of the Q wave to the end-point of the T wave where it returns to the iso-electrical baseline¹.

The ECG indicates the cardiac systole and diastole phases; therefore, the simultaneous recording of the surface ECG correlates with the invasive BP measurement. By using a combination of methodologies, it is possible to elucidate pathophysiological changes in a disease model or the pharmacological effects of a drug or therapy in cardiovascular medicine.

A spontaneous hypertensive rat (SHR) strain had been obtained by inbreeding of Wistar rats with high BP in Japan. The BP rises from 5 to 10 weeks of age and becomes stationary from 30 to 35

weeks of age⁷. Wistar-Kyoto rats (WKY) have systolic BP about 130 mmHg⁷ and are commonly used as normo-tensive control. We used SHR and WKY to demonstrate the result of intraarterial cannulation BP and ECG recording.

PROTOCOL:

All the animal experiments described were approved by the Institutional Animal Care and Use Committee of Kaohsiung Medical University.

1. Animal Care

1.1. To avoid difficult cannulation due to small arterial size, use rats with body weight over 200 g.

1.2. Remove chow from the cage and fast rats overnight.

1.3. Offer water ad libidum unless there is a special experimental design.

2. Experimental Preparation

2.1. Obtain the following materials: forceps with teeth (**Figure 1A**), surgical scissors (**Figure 1B**), forceps with fine tips (**Figure 1C, 1D**), forceps with angled tips (**Figure 1E**), bulldog vascular clamp (**Figure 1F**), silk string approximately 20 cm in length (**Figure 1G**), micro scissors (**Figure 1H**), intra-arterial cannula, a sterile polyethylene (PE) tube with an internal diameter of 0.5 mm and an outer diameter of 0.9 mm, 25-30 cm in length, connected to a 26G x 1/2" needle (**Figure 1I**), two three-way stopcocks (**Figure 1J**) for connecting the intra-arterial cannula and the pressure transducer (**Figure 1K**), and 1 mL syringes filled with heparinized saline (100 IU/mL).

2.2. Animal Preparation

2.2.1. Anesthetize the rat by inhalation of isoflurane (placing the rats in a 25 cm x 25 cm x 14 cm induction chamber saturated with 4% isoflurane, followed by nosecone use with 3% isoflurane).

NOTE: Additional sedation can be used with intra-peritoneal injection of 100 µL of 50 mg/mL Zoletil (Tiletamine + Zolezepam) after the complete of isoflurane induction.

2.2.2. Test the pain reflex by pinching the toes.

2.2.3. Place the rat in a supine position on a pre-cut Styrofoam board (or thick cardboard). Fix the four legs with rubber bands to immobilize the body (**Figure 2A**).

NOTE: To avoid potential noise during the ECG recording, the placement surface must not be electrically conductive.

2.3. Prepare instruments for BP and ECG recording. Include an analog input unit to acquire signals, a pressure transducer with a compatible hub, three bipolar needle-tipped electrocardiogram leads, and a computer with suitable software.

3. Pressure Transducer Calibration

3.1. Before initiation of BP recording, calibrate with a standard mercury sphygmomanometer (**Figure 1L**).

3.2. Remove the pressure cuff from the sphygmomanometer and connect the three-way stopcock on the inflation tube to the pressure transducer (**Figure 1K**) of the data acquisition system.

3.3. Screw the air-release valve clockwise. Keep eyes on the gauge and keep pumping the inflation bulb. When the gauge shows 100 mmHg, switch the three-way stopcock to connect to the pressure transducer. Use 100 mmHg pressure for the calibration. The conversion factor for calculating BP will be determined automatically.

3.4. Release the pressure by screwing the air-release valve counterclockwise until the pressure of the sphygmomanometer is back to zero.

3.5. Repeat step 3.2 with pressure of 200 mmHg.

3.6. Detach the pressure transducer from the mercury sphygmomanometer.

3.7. Connect the pressure transducer to the three-way stopcock of the PE catheter (**Figure 1I**).

4. Mini-surgery for Cannulation of the Femoral Artery

4.1. Surface landmark identification and excision of the skin (**Figure 2**)

4.1.1. Identify the location of inguinal crease (indentation at the junction between abdomen and thigh) (dashed line in **Figure 2A**).

4.1.2. Pinch the full layer of skin at the center of the inguinal crease.

4.1.3. Lift the skin and cut it off with the surgical scissors at an orientation approximately parallel to the ipsilateral thigh (**Figure 2B**). The femoral nerve and vessels are underneath the exposed subcutaneous tissue (**Figure 2C**).

4.2. Dissection of the tissue to expose the femoral artery

4.2.1. Dissect the tissue using forceps with fine tips, layer by layer. Stop the dissection at the level of femoral vessels. Make sure the dissection is not injuring the vessels underneath.

4.2.2. Carefully use forceps (**Figure 1C** or **1D**) to clear out the soft tissue along the femoral nerve and vessels to obtain good observation. The nerve is fiber-like in texture. The vein is dark purple and the artery is pulsatile (**Figure 3A**).

4.2.3. Use forceps with angled tips (**Figure 1E**) to extend the exposed length of the femoral artery and vein (**Figure 3B**).

4.3. Cannulation of the femoral artery (**Figure 3**)

4.3.1. Use forceps to separate the femoral vein from the artery and apply a bulldog clamp at the femoral artery as cranially as possible (**Figure 3C**).

4.3.2. Make a loose tie of the two silk strings, one just below the bulldog clamp and the other at the caudal terminal of the exposed femoral artery (**Figure 3D**).

4.3.3. Make a small hole over the ventral side of the femoral artery (**Figure 3E**) using the micro scissors (**Figure 1H**).

4.3.4. Insert the tip of the PE catheter through the small hole and advance the catheter cranially.

NOTE: Do not apply torsion while advancing the PE catheter. The torsion force may twist the femoral artery and result in luminal stenosis.

4.3.5. Remove the bulldog clamp after the PE catheter is securely advanced into the femoral artery lumen.

NOTE: Keep eyes on the PE catheter while removing the bulldog clamp from the femoral artery. The observation of a backflush of blood into the PE catheter confirms its placement in the arterial lumen.

4.3.6. Tighten the upper silk string to secure the position of the PE catheter.

NOTE: Any accidental pull or dislocation of the PE catheter can result in major bleeding.

4.3.7. Tighten the lower silk string to prevent bleeding from the caudal side of the femoral artery.

4.4. Confirmation of the success of femoral artery cannulation

4.4.1. Use a 1 mL syringe to inject 0.1-0.2 mL of heparinized saline into the femoral artery. The resistance on injection must be trivial. If any obvious resistance upon injection is noticed, check the whole cannulation again.

NOTE: Make sure the three-way stopcocks are appropriately switched before applying any

negative pressure or saline injection into the femoral artery cannula. At this time point, any injection into the pressure transducer will need a re-calibration.

4.4.2. Check if there is any oozing around the cannulation site. If not, cover the surgical site with a wet cotton ball.

5. Recording of Blood Pressure

5.1. After a smooth flush for the PE catheter, attach the PE catheter three-way stopcock to the one on the pressure transducer (**Figure 4**).

5.2. Make sure that there are no air bubbles in the cannulation system. Also check the connection junctions of the three-way stopcock.

5.3. Start the data acquisition system with sampling frequency of 1000 Hz to record the BP. Arterial pressure waves will be demonstrated (**Figure 6**).

5.4. Allow the whole setup to stabilize for at least 3-5 minutes. In cases with unsteady signals, stabilization time can be extended to 15 minutes.

5.5. Check the cannulation site periodically to make sure there is no bleeding.

6. Surface ECG

6.1. Check the three leads of the bipolar ECG to make sure the positive, negative, and reference platinum electrodes are intact.

6.2. Insert the leads subcutaneously at the left foreleg, right foreleg, and right hindleg (**Figure 5**).

6.3. Attach the electrode hubs to a custom-built ECG amplifier with sampling frequency of 1000 Hz and filter frequency of 3-500 Hz. Keep ECG leads steady during recording. Motion of the ECG leads can produce unsteady baselines and artifacts.

7. Animal euthanasia after completing of the experiment

7.1. After completing of the BP and ECG recording, stop the acquisition system. Remove the electrodes. Ligate the femoral artery by tightening up the previously placed silk string right after withdrawing the PE catheter.

7.2. Place the rats individually in the visible euthanasia chamber connected to compressed carbon dioxide (CO₂) gas cylinders. Seal the top securely.

7.3. Introduce 100% CO₂ with a fill rate of about 10% to 30% of the chamber volume per minute.

7.4. Keep your eyes on the rat; lack of respiration and faded eye color should appear within 2–3 minutes.

NOTE: If unconsciousness does not occur after 3 minutes, the system should be examined for the chamber fill rate, CO₂ supply, or leaks.

7.5. Maintain CO₂ flow to the chamber for 1 more minute after respiration ceases.

7.6. Ascertain cardiac and respiratory arrest and note fixed and dilated pupils to confirm death.

NOTE: If the rat is not dead but in CO₂ narcosis, use a secondary method of euthanasia such as cervical dislocation.

REPRESENTATIVE RESULTS:

We purchased SHR and normotensive Wistar-Kyoto WKY rats from the National Laboratory Animal Center (Taipei, Taiwan). All animals were housed in a temperature-controlled facility (20–22 °C) with free access to water and standard chow on a 12 h light/dark cycle.

We used six 47-week-old rats and they were weighed before the BP and ECG measurement. The representative tracings from simultaneous recording of ECG and BP in SHR and WKY are shown in **Figure 6**. **Table 1** shows parameters for BP and heart rate. Statistical (*t*) tests revealed significantly higher systolic BP and mean BP in SHR (124.5 mmHg ± 15.1 mmHg and 84.3 mmHg ± 5.0 mmHg) than in WKY (90.0 mmHg ± 7.5 mmHg and 67.5 mmHg ± 5.0 mmHg) (*P* < 0.05) (**Table 1**).

The parameters of P wave, PR intervals, QRS width, and QT intervals can be measured from the ECG recordings (**Table 2**). Choice of right vs. left femoral cannulation in relation to ECG electrode placement (at the right hind limb) did not affect the BP or ECG signals.

FIGURE AND TABLE LEGENDS:

Figure 1. Materials. (A) Forceps with teeth, (B) surgical scissors, (C) tissue forceps, (D) tissue forceps with fine tips, (E) angle tip forceps, (F) bulldog vascular clamp, (G) silk string, (H) micro scissors, (I) polyethylene catheter connecting with a 26G x 1/2" needle and a three-way stopcock (J), (K) pressure transducer connecting with a three-way stopcock, (L) mercury sphygmomanometer with a stopcock (green arrow) and inflation bulb with an air-leak valve (white arrow).

Figure 2. Surface landmark for femoral artery dissection. (A) Supine rat with left inguinal crease highlighted with a dashed line. (B) Groin skin picked up by forceps with teeth to be cut off by surgical scissors. (C) The surgical zone for femoral artery dissection.

Figure 3. Tissue dissection and cannulation of the femoral artery. (A) Exposed femoral nerve (yellow arrow), femoral vein (blue arrow), and femoral artery (red arrow) after tissue dissection.

(B) Femoral vein and artery after the nerve cut-off. (C) Application of a bulldog vascular clamp over the cranial terminal of the dissected femoral artery. (D) Femoral artery with a bulldog clamp and two silk strings with loose ties. (E) A small hole on the ventral side of femoral artery, created using micro scissors. (F) Insertion of the polyethylene catheter into the femoral artery.

Figure 4. Femoral artery cannulation for pressure recording. The intra-arterial cannula (highlighted by a dashed green line) is connected with two three-way stopcocks (blue arrows) with syringes filled with heparinized saline. The pressure transducer (red arrow) is linked to the cannulation through the three-way stopcocks.

Figure 5. The whole experimental setup for invasive femoral arterial pressure and electrocardiography. Three electrocardiographic leads (red arrows) with platinum needle electrodes subcutaneously inserted over bilateral fore legs and right leg (yellow arrows). The exposed right femoral artery with cannulation (blue arrow).

Figure 6. Representative tracings. Simultaneous recordings of ECG and BP in WKY (left) and SHR (right) for the ECG (top), and arterial pressure waves (bottom).

Table 1. Blood pressure levels measured by invasive femoral artery cannulation. Data are presented as mean \pm standard deviation; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; BP, blood pressure.

Table 2. Measurements of electrocardiographic parameters. Data are presented as mean \pm standard deviation; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

DISCUSSION:

Invasive arterial cannulation allows highly accurate measurement of BP. It can be done with a PE tube without requiring an expensive catheter. Invasive BP measurement can also be performed simultaneously with a recording of the surface ECG.

The major learning curve for this method is the experimental skill required to cannulate small blood vessels. In experienced hands, the successful rate for femoral artery cannulation can approach 100%. Practice is recommended before performing true experiments. Some noteworthy points during the procedure: (1) make sure animals are completely anesthetized before starting the surgery; (2) use a bulldog clamp at the high position of the exposed artery before cutting the arterial wall to prevent extensive bleeding; (3) make the incision only over the ventral wall of the artery and avoid damaging the dorsal wall; and (4) steady placement of the cannulation system and electrocardiographic leads helps avoid motion artifacts during the recording.

The potential complications with this procedure include hemorrhage from the surgical dissection. The hemorrhage in a successful and smooth cannulation of an artery is very trivial. Major hemorrhage can be caused by accidental damage to the femoral artery or vein or the major branches during the tissue dissection. The hemorrhage can be stopped by local compression with

a clear cotton ball. Another cause of major hemorrhage is dislocation of the femoral cannulation. In this case, the blood loss is usually large enough to result in a significant drop in systemic BP. At this point, BP recording can be terminated early.

Telemeter implantation also is an established technique for invasive BP recording⁸. The advantage of telemetry is the ability to obtain a high-fidelity continuous recording over relatively long periods of time in conscious, freely moving animals without the limitations of restraint or anesthesia. Direct BP recordings can be done with successful subcutaneous implantation of radio-transmitters and carotid artery cannulation⁹. However, it can be challenging to implant the telemeter successfully and the hardware and software for radio telemetry are costly.

There are some limitations for invasive BP measurement by femoral artery cannulation. First, it cannot be performed in unrestrained and conscious rats. Second, BP in the femoral artery may be higher or lower than BP in the central aorta. Third, arterial cannulation can be difficult for small rodents, where femoral arteries may be too small to insert the PE tube. Fourth, the cannulated artery should be ligated after completing the BP recording and ligation of femoral artery will result in hind leg ischemia. Due to this limitation, the animals are usually euthanized following invasive BP recording.

Invasive femoral artery cannulation requires restraining and anesthetizing the animals, which potentially introduces stress and influences both BP and electrocardiography data¹⁰. The stress can be alleviated by appropriate anesthesia. Anesthesia includes injection or inhalation protocols. In our past experience, high elevated BP in I.P. anesthetized WKY rats suggests that the animals were still under stress or in pain. Normal BP in conscious WKY rats is 130/80 mmHg; this dropped to below 100 mmHg in our inhalation-anesthetized WKY rats (**Table 1**). Inhalational anesthesia (isoflurane) has several advantages over injectable agents: quick onset, minimal animal handling, ease of control, no controlled drugs, and a quick recovery. The disadvantages are the cost of the equipment, the hazard of human exposure if gas leaks into the working environment, and a significant suppressive effect on BP. Isoflurane-induced BP reduction should be considered when choosing the anesthesia regime.

Further applications are possible for invasive BP measurement. According to the fluid dynamics principles for pulse wave velocity (PWV), the stiffer artery propagates the pulse wave faster¹¹. When applying double cannulations over the carotid artery and femoral artery, the aortic PWV can be determined. The length of aortic pulse propagation can be measured as the distance between the tips of the cannula at the carotid (proximal) and distal (femoral) arteries. The PWV is the ratio between the aortic length and the difference between the time at the minimal values of the proximal and distal arterial pulses¹².

The setup described above for simultaneous electrocardiography and intra-arterial BP recording in experimental rats is an inexpensive and highly accessible technique for physiological or pharmacological studies investigating pathogenic or therapeutic mechanisms in cardiovascular medicine.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

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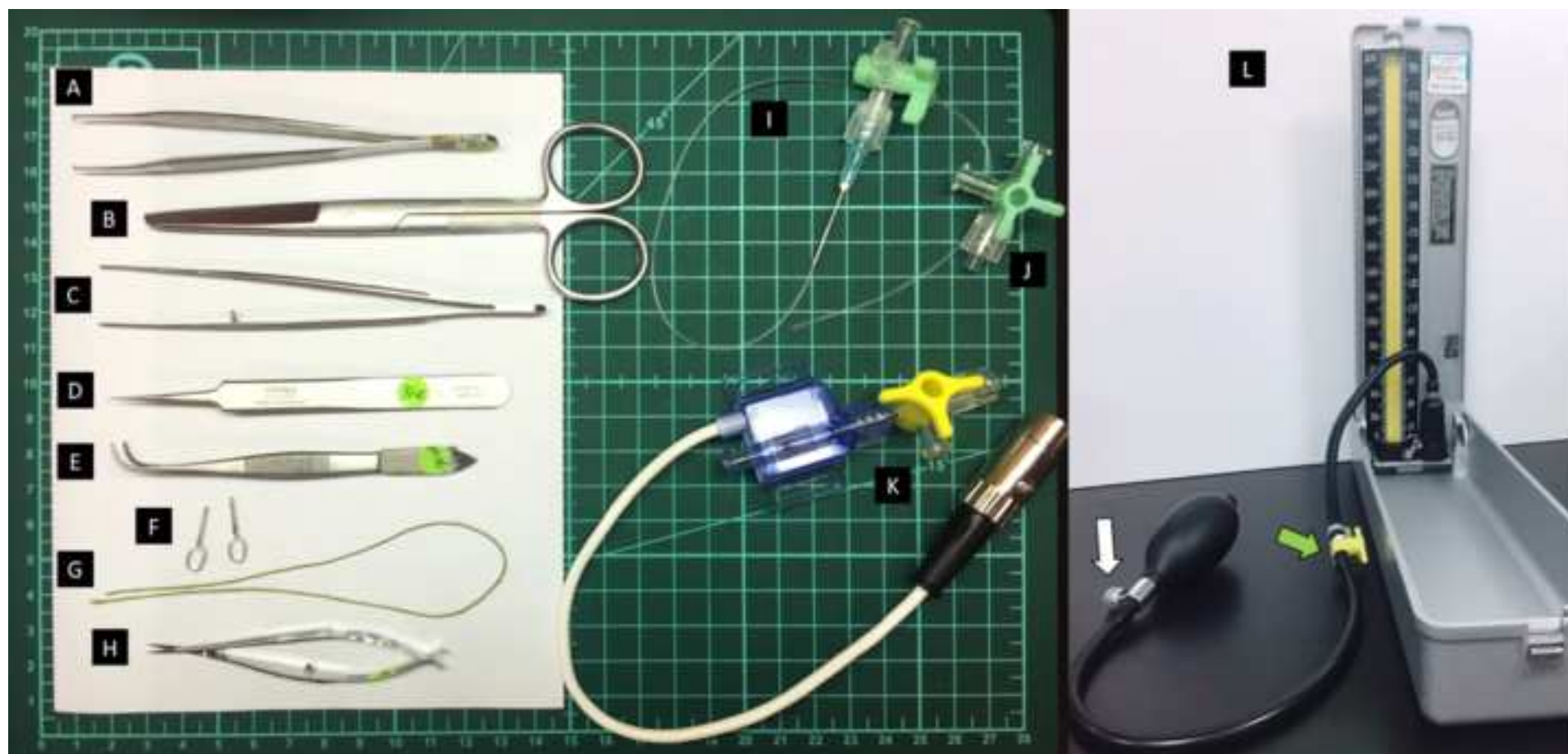


Figure 2

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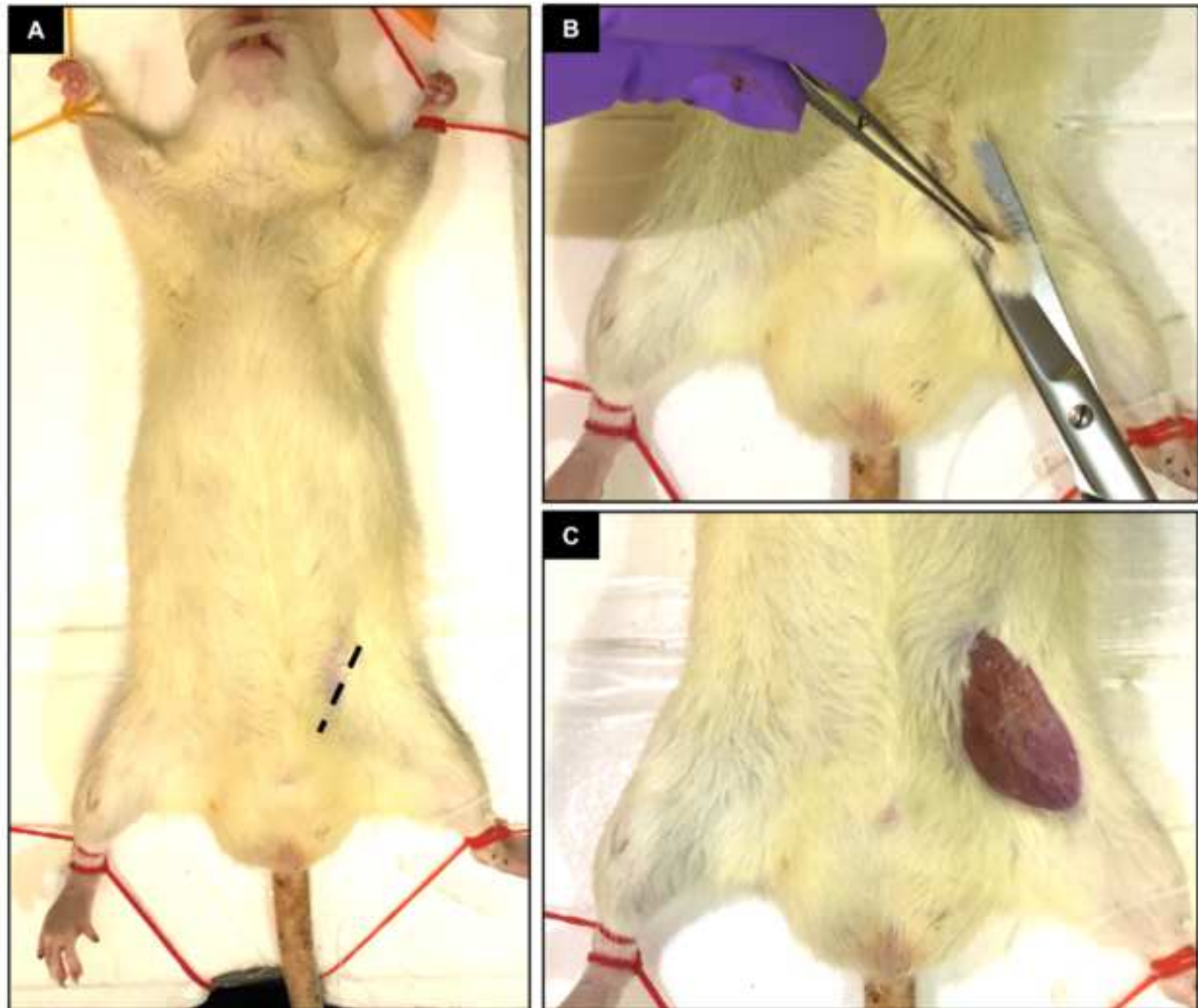


Figure 3

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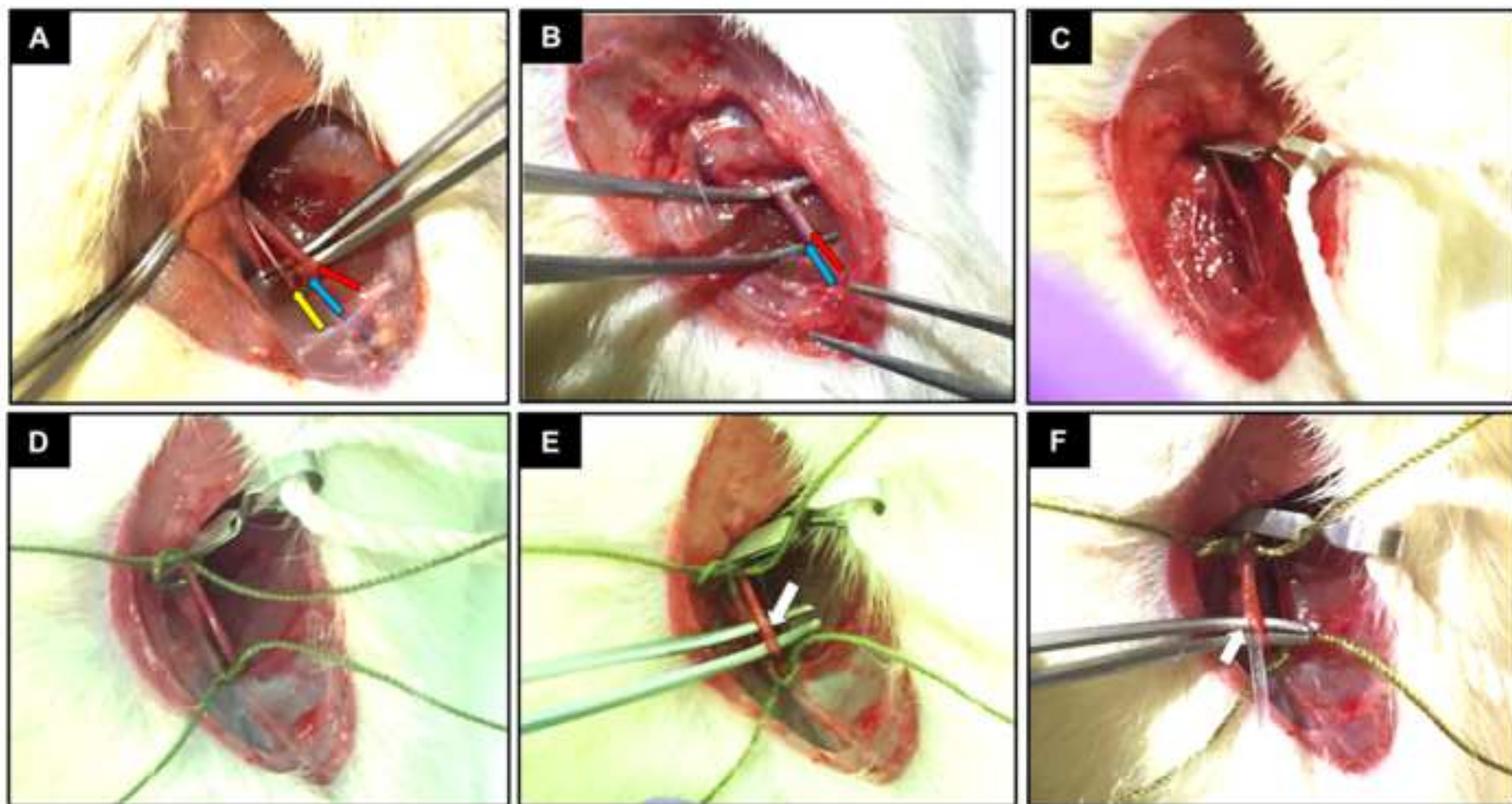


Figure 4

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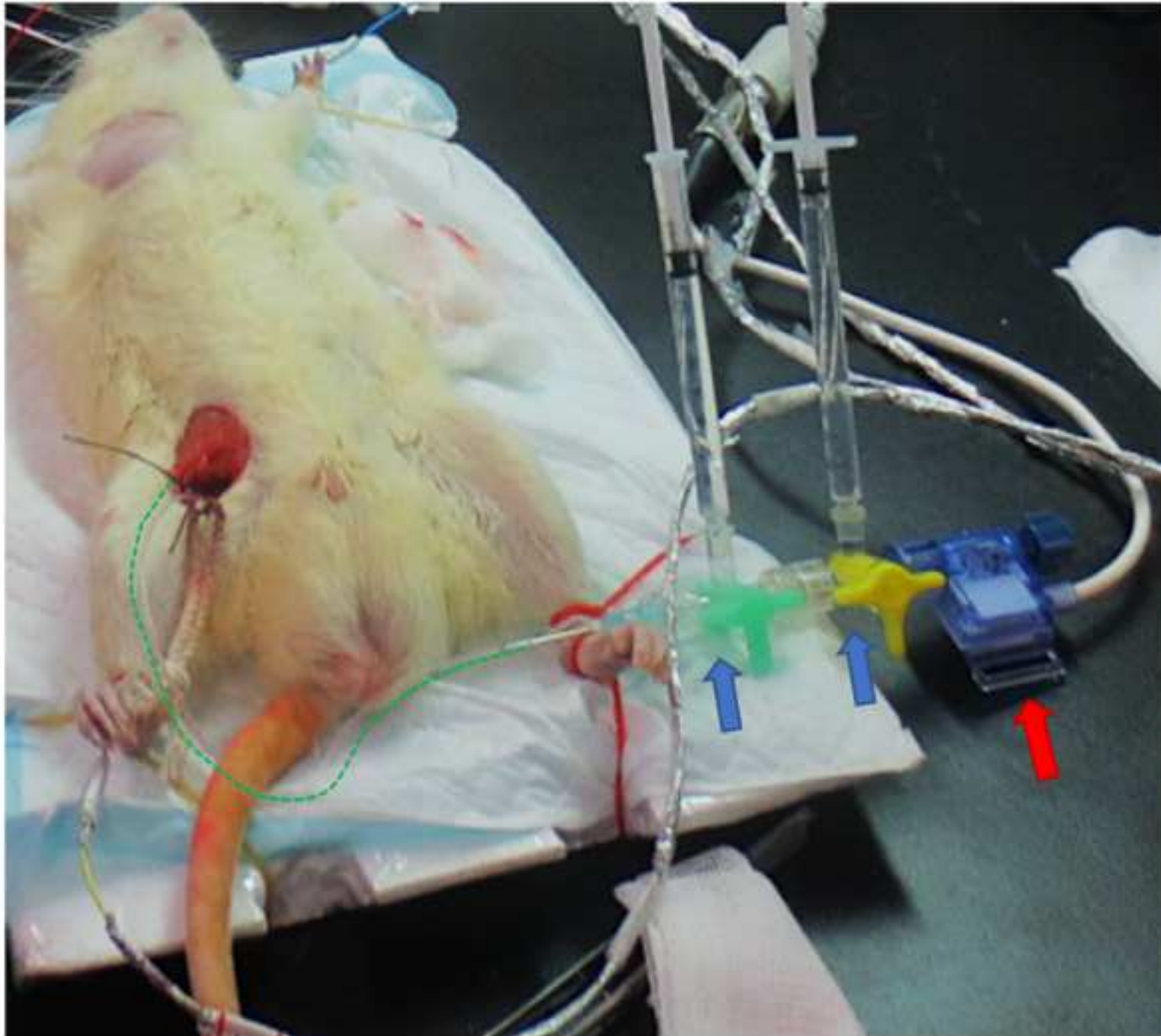


Figure 5

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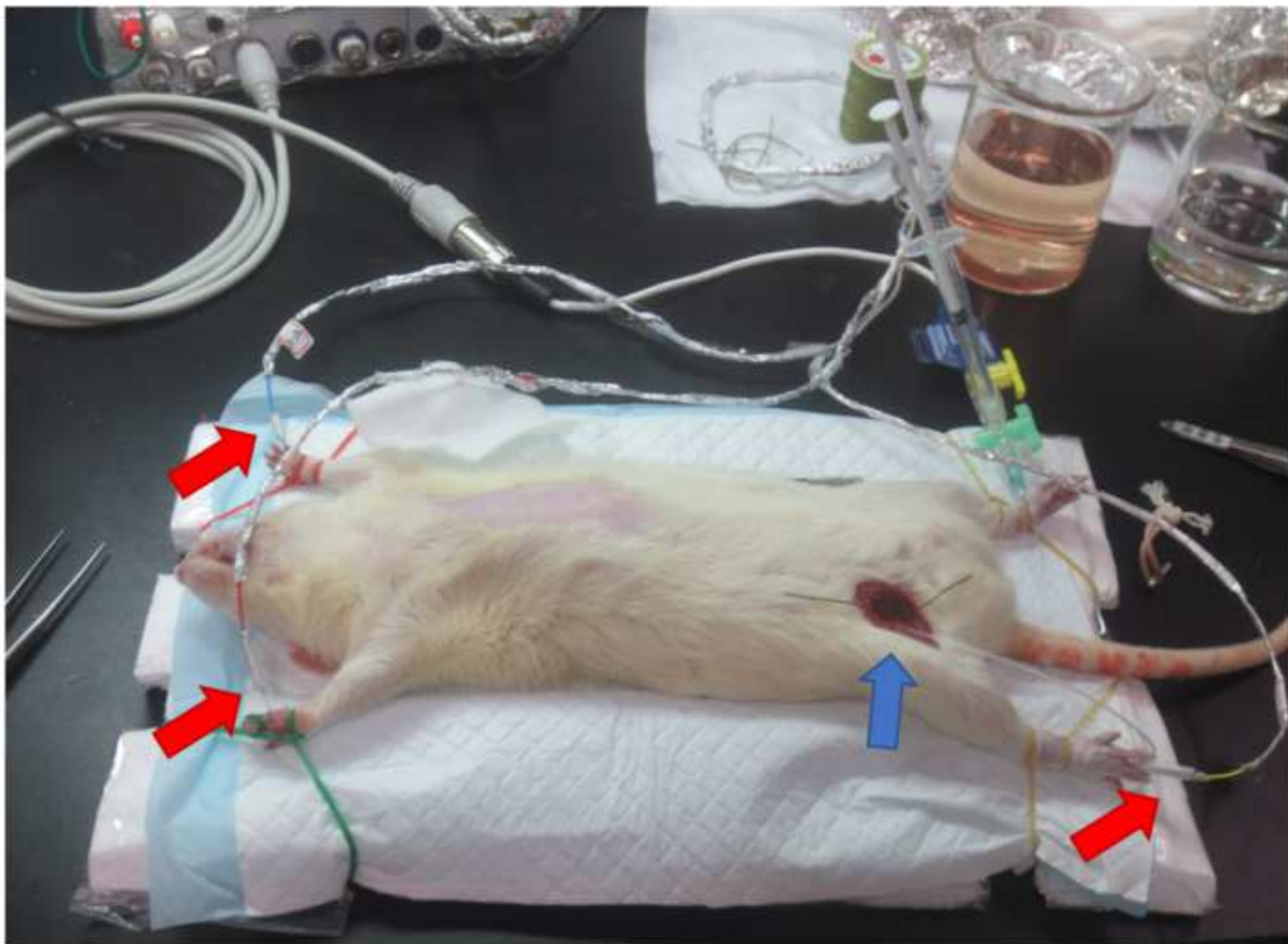
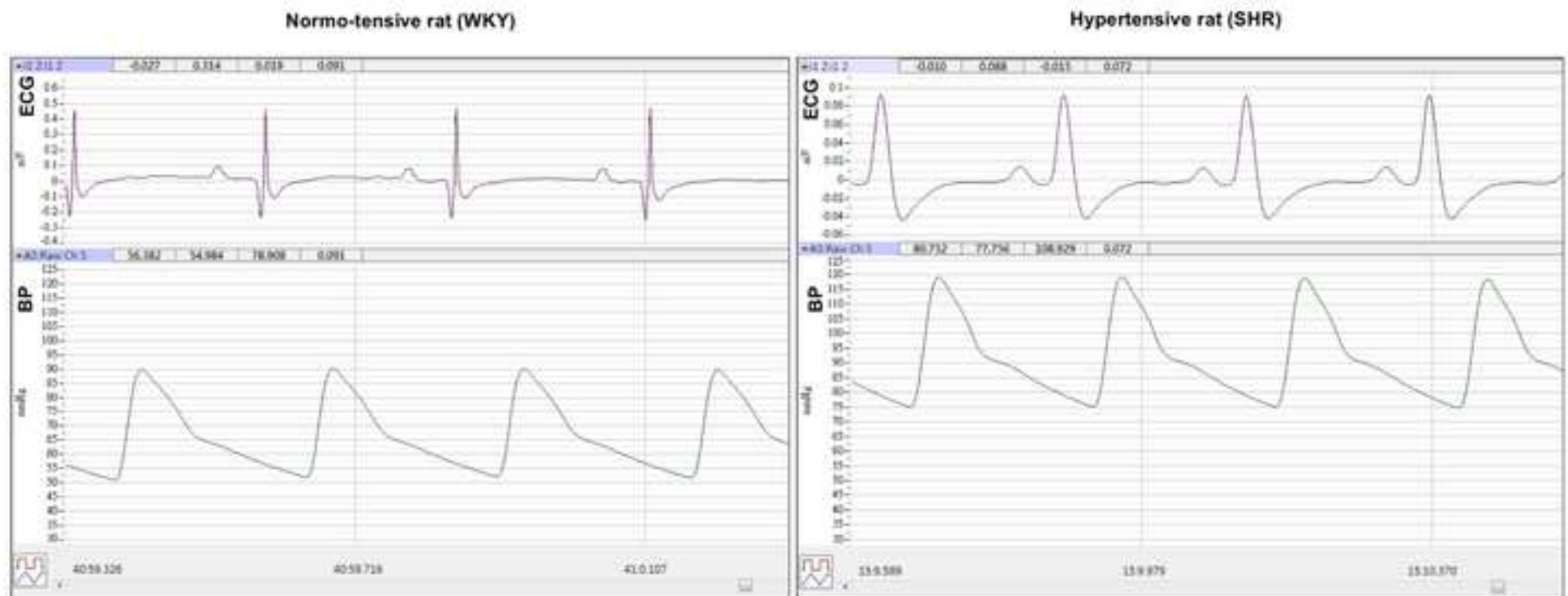


Figure 6

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	WKY (n= 3)	SHR (n= 3)	P value
Weight, g	407.7 ± 6.4	353.7 ± 10.3	<0.01
Heart rate, /min	241.3 ± 15.0	262.0 ± 15.7	0.18
Systolic BP, mmHg	90.0 ± 7.5	124.5 ± 15.1	<0.05
Diastolic BP, mmHg	56.3 ± 4.0	64.2 ± 0.6	0.08
Mean BP, mmHg	67.5 ± 5.0	84.3 ± 5.0	<0.05
Pulse pressure, mmHg	33.6 ± 4.4	60.4 ± 15.1	0.08

	WKY (n= 3)	SHR (n= 3)	P value
Heart rate, /min	241.3 ± 15.0	262.0 ± 15.7	0.18
P wave, msec	38.7 ± 9.3	43.0 ± 4.6	0.52
PR interval, msec	59.0 ± 7.8	61.3 ± 4.7	0.69
QRS width, msec	43.0 ± 11.3	50.0 ± 2.6	0.4
QT interval, msec	112.0 ± 12.8	116.7 ± 9.5	0.15

Name of Material/ Equipment	Company	Catalog Number
Polyethylene tube	BECTON DICKINSON	427401
26G x 1/2"" needle	TERUMO	160426D
Adson Forceps	TOP Line	12-540
Bulldog vascular clamp	Teleflex	357581
Computer	AUSUS	X453M
External analog signal recording device	iWorx	T5141538
Graefe Forceps	AESCLAP Surgical Instruments	BD312R
Mercury sphygmomanometer	Spirit	CK-101
Pressure transducer	iWorx	IworxBP100
Semken Forceps	MEDE TECHNIK	10-104
Software	LabScribe3	
Surgical scissors	HEBU	1714
Syringe (1 mL)	TERUMO	160426D
Three-way stopcocks	Cole-Parmer	EW-30600-23
Tipped forceps	World Precision Instruments	504506
Vannas Scissors	World Precision Instruments	500086

Comments/Description

internal diameter of 0.5 mm, outer diameter of 0.9 mm

12 cm (4.75") Long, Straight, 1 x 2 Teeth
8 mm

This allows the recording of up to three channels of ECG, EMG or EEG as well as GSR (skin conductance) from a single iWire input or
MICRO DRESSING FORCEPS, CURVED, SERRATED, 105 mm, 4 1/8

100 mm

14.5 cm long

11 cm long, 0.1x0.06 mm Tips

8.5 cm long, Straight, 0.025 x 0.015 mm Tips, 7mm super fine Blades

n the recording Module.



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Editor-in-Chief

Journal of Visualized Experiments

Dear Editors:

We would like to submit the revised manuscript entitled “**Simultaneous Electrocardiography Recording and Invasive Blood Pressure Measurement in Rats**” for your consideration for publication in your journal. First, we would like to thank the editor for giving this opportunity for making a revision and all reviewers’ comments for us to improve the manuscript. The editorial comments have been addressed for both requirements for video production and formatting of the article for publication. The spelling and grammar issues have been solved by consulting an English editor.

Along with uploading revised manuscript, a rebuttal document that addresses each of the editorial and peer review comments individually is also uploaded.

All authors have read and approved submission of the manuscript. All authors are involved in the experimental data analysis/interpretation. All authors have declared no commercial association, such as consultancies, stock ownership or other equity interests or patent-licensing arrangements. Thank you.

Sincerely,

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