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Whole Rat Fixation Via Transcardial Perfusion

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details the procedure for performing whole rat fixation via transcardial perfusion. Following fixation, the rat brain can subsequently be removed and stored for further processing and analysis.

Materials

Perfusion Station:

Watson-Marlow 205S peristaltic pump with 12 channel cassettes

Peristaltic pump tubing

Y-shaped tubing with shutoff valve

Table stand

Perfusion box

Perfusion grid

20G x 1in needle

Beakers (for reagents)

Reagents:

500 mL 1X phosphate buffered saline (PBS)

300mL 4% paraformaldehyde (PFA) in 1X PBS

Tools:

Large surgical scissors

Locking forceps

Small surgical scissors

Rongeurs

Guillotine

Spatula

50mL conical tube

Tape



Safety warnings

⚠ Paraformaldehyde (PFA) is a toxic, irritant, and a potentially carcinogenic chemical that releases formaldehyde gas upon dissolution. All procedures involving PFA must be performed with appropriate personal protective equipment (PPE) and in a certified chemical fume hood to prevent inhalation of vapors. In case of a spill, follow the safety procedures outlined by your institution and contact the appropriate personnel to receive assistance.

Ethics statement

All procedures involved were conducted per the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, San Diego.

Preparation of Perfusion Station

20m

- 1 Prepare perfusion station inside of a fume hood as shown in setup image.

15m

Set up the perfusion box with a grid. Connect disposal tubing from the perfusion box to the waste bucket. Ensure the disposal tubing is firmly connected to the perfusion table. Ensure the waste bucket is covered with a lid, with a hole for the waste tubing. Use parafilm to cover up any gaps in the hole around the waste tubing.

Prepare reagents in beakers. For each reagent, use a separate tubing. Submerge one side of the tubing in the reagent, then connect the other side for both tubes to the Y-shape tubing shutoff valve. In the third exit of the Y-shape tubing, connect it with another tube that has a 20G x 1in needle secured at the end. Ensure the 20 gauge hypodermic needle is firmly inserted.

Thread this tubing through a **peristaltic pump** cassette, which opens on one side. Lift one cassette and adjust underneath to fit the tubing inside the slot. Then, snap the cassette back in place, pushing the lefthand tab on the cassette to lock into place. This may take several attempts as the cassette will not stay in place unless properly aligned with the tubing.

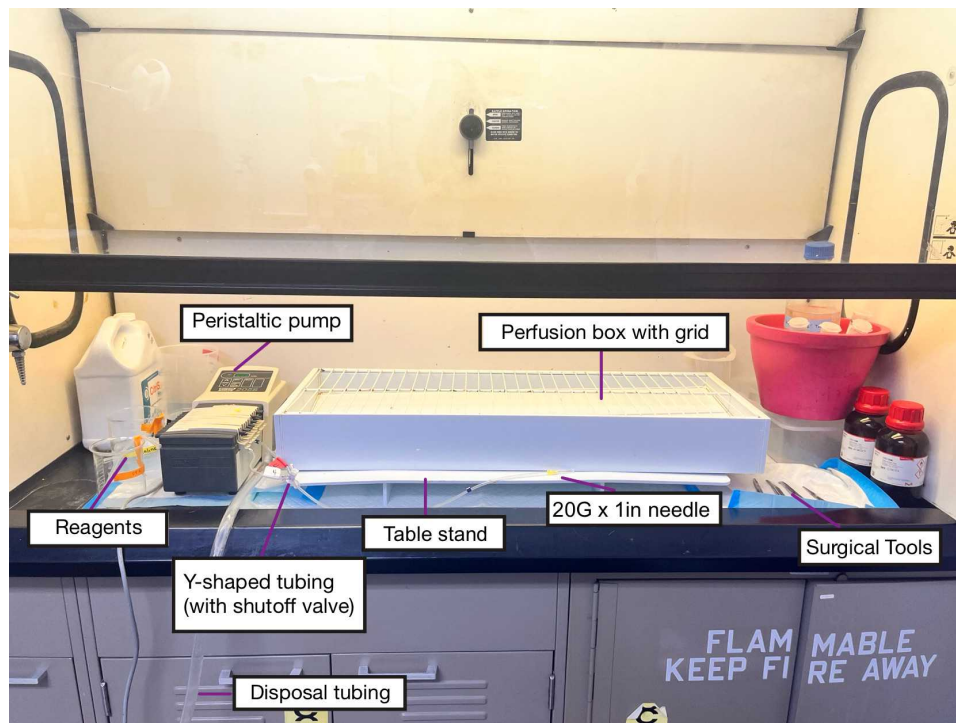


Figure of perfusion setup.



Closer look at peristaltic pump with y-shaped tubing threaded into cassettes.



2 **Note: PFA and PBS need to be stored on wet ice, keeping temperatures between 0°-4°C. All PFA conical tubes to store harvested brains need to be 20x the volume of the brain and also stored on ice until the brain is harvested.**



3 Ensure you have proper tools and chemical reagents. Approximate volumes are listed, but may be adjusted based on rat size.

2m

Reagents:

1. 500 mL 1X phosphate buffered saline (PBS)
2. 300mL 4% paraformaldehyde (PFA) in 1X PBS

(Use ~250-300mL of each reagent per rat)

Tools:

1. Large surgical scissors
2. 3 Locking Forceps
3. Small surgical scissors
4. Rongeurs
5. Guillotine
6. Spatula
7. 50mL conical tube
8. Tape

4 Ensure large disposal tubing is secured to the perfusion table to allow for hazardous waste flow to deposit into a chemical waste bucket without spillage.

1m

5 Turn on the perfusion pump. There are two settings to control fluid flow, clockwise and counterclockwise. Prime the pump by flowing fluid until no air bubbles are in the tubing, making sure that the clockwise setting is on.

2m

Prepare perfusion pumps with 1X PBS (90 RPM) and 4% PFA (90 RPM) and prime the system by checking for consistent flow and lack of air bubbles through the needle tip.

Rat Anesthesia

8m

6 Place rat in CO2 chamber and turn on CO2. Make sure to put male and female rats in separate chambers.

1m

7 Wait between 3-5 minutes, visually observing until the rat has taken its last breath and stops breathing.

5m



Note: It is important to monitor the rat's breathing to ensure you start the procedure immediately after it takes its last breath. This ensures that the heart is still beating and

allows for a cleaner perfusion.

- 8 Turn off CO2 and remove rat from chamber. 1m
- 9 Pinch the tip of the tail or hind paw forcefully to test for pedal reaction. If the rat reacts, place the rat back in CO2 chamber. 1m

Surgery and Fixation 29m

- 10 Splay out the rat's front and back limbs with ventral side facing up, and secure it to the perfusion grid using tape. 2m
- 11 Using surgical scissors, make an incision to expose the abdominal cavity. Example incisions are shown in the figure below. 2m

Example 1. Cut horizontally above the genital. Lift skin upwards using forceps and cup upwards on both sides of initial incision to expose the abdominal cavity.

Example 2. Cut a "V" shape above the genital. Lift skin upwards using forceps and cut upwards to expose the abdominal cavity.

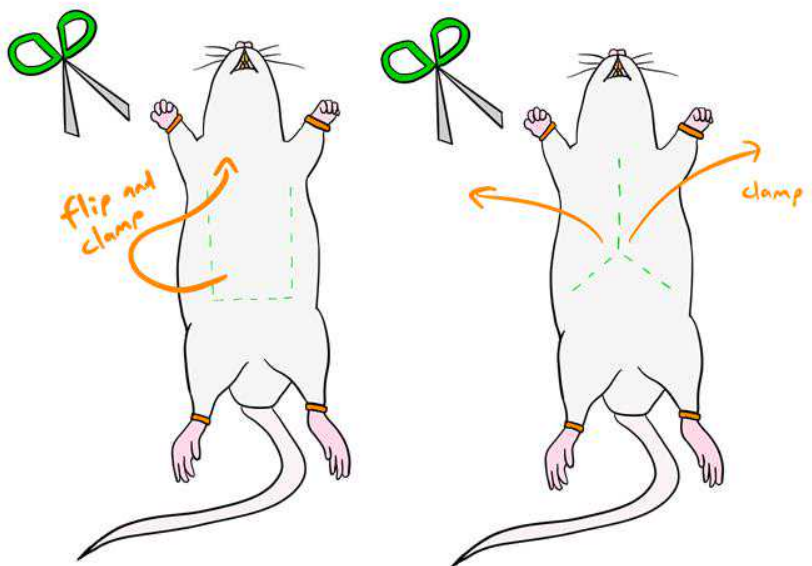
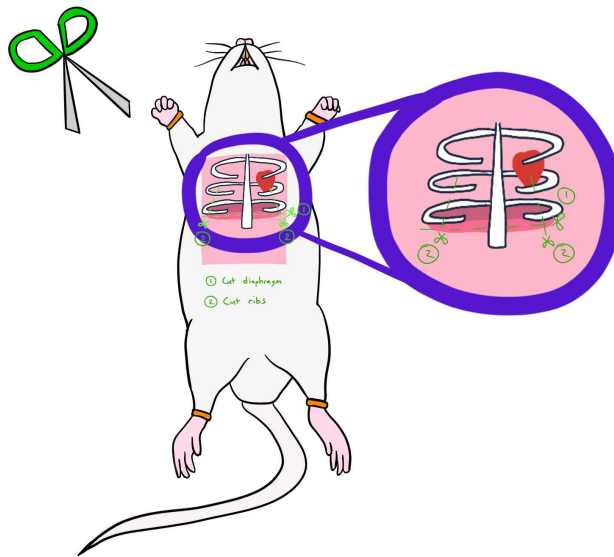


Diagram of incisions to expose heart for rat perfusion.

- 12 Once inside the abdominal cavity, the diaphragm can be identified as superior to the liver. Gently cut the diaphragm to collapse the lungs, exposing the thoracic cavity. Using an upward cut on both sides, the ribs can be cut and clamped out of the way for better access to the heart.



Note: It is important not to damage the lungs to avoid the lungs from filling with fluid.



Expanded diagram of incisions to expose heart.

- 13 Once the heart is exposed, use the forceps to hold the middle of the heart to keep it steady then insert the 20 gauge catheter needle through up to the aorta, pointing towards the left atrium, and then clamp the entrance.

1m



Note: Make sure not to hit the lungs when piercing the needle through the left ventricle. Make sure to not insert the needle too deep into the heart to avoid puncturing the heart which results in leakage.

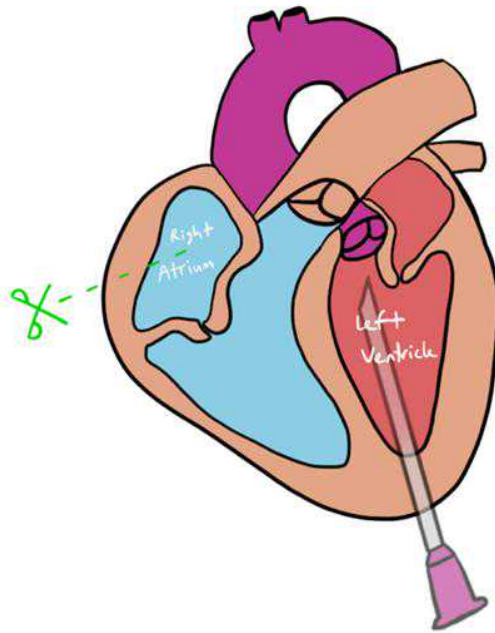


Diagram of incisions and needle placement for rat perfusion.

- 14 Make a cut in the right atrium, which will appear in a very dark red color. As soon as the atrium is cut, **immediately** turn on the PBS pump.

1m

An alternative technique is to clamp the abdominal aorta to restrict PBS/PFA only to the brain. This will shorten the duration of perfusion by more than half of its time.

- 15 Pump a prefixative rinse of 1X PBS for 3 minutes at 90 RPM (i.e. ~24mL/min), until the liver of the rat are discolored from a dark red to a light brown.

3m

Expect to use ~150mL per rat.

- 16 Then pump 4% PFA for 5 minutes at 90 RPM, before reducing the pump flow rate to 65 RPM and continue pumping 4% PFA for 15 minutes, until neck is stiffened. (Time measurements indicated here are overestimates of time and PFA required.)

20m

Visual indication of a complete perfusion can also be observed through the paling and stiffening of the liver. Perform the tail flick test for fixation confirmation.

Expect to use ~300mL per rat.

Brain Removal

25m

- 17 Carefully remove the rat from the perfusion grid. Using large surgical scissors or guillotine, remove the head of the rat by cutting at the neck. 2m
- 18 Working under the skin layer, cut down the midline of the head and peel away skin to expose the skull. 10m
- 19 Locate the foramen magnum, visualized by a hole in the occipital region of the skull. Using rongeurs, begin chipping away the skull in small segments, being careful to avoid nicking the brain tissue. Work from the back to the front of the skull nearing the nasal bridge. 10m

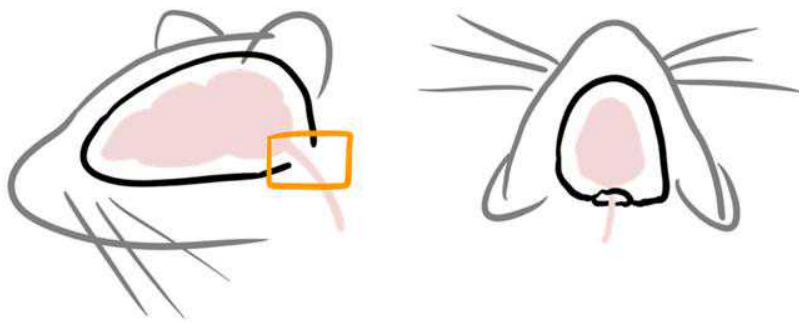


Diagram displaying location of the foramen magnum.

- 20 When the brain is fully exposed, use a small spatula to turn the head upside-down and with small surgical scissors cut the tethering nerves and connective tissues. Gently pull to remove. 2m
- 21 Place the removed brain into a 50mL conical tube filled with 4% PFA at 4°C for 24 hours to allow for post-fixation of brain tissue. 1m

Sucrose Treatment

2d



- 22 After 24 hours, remove brain from 4% PFA fixative. 1d
- 23 Place brain in 30% sucrose solution (in 1x PBS) until it sinks to the bottom of a storage vial at 4 degrees Celsius, approximately 24-36 hours. This brain can now remain in storage at 4°C until future use. 1d
- 24 If the brain is not used for over 3 weeks, sodium azide must be added to prevent bacterial growth. Use 0.02% w/v sodium azide in 1x PBS, for whole brains and 0.1% sodium azide in 1x PBS w/v for brain sections.

Acknowledgements

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