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Perfusion

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Works for me

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PROTOCOL CITATION

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1 WHOLE ANIMAL FIXATION VIA TRANSCARDIAL PERFUSION

List of Stuff Needed for Perfusion

- 4% Formaldehyde
- 0.9% Hep-saline
- Bottles & pump
- Surgical tool (scissors, clamps, bone rongeurs, spatula)
- Vials
- timer

2 Preparation

- Switch waste flow to deposit into chemical waste bucket
- Prepare perfusion pumps with 0.9% saline and 4% paraformaldehyde, check flow
- Test system; check for flow and air bubbles

3 Anesthesia

- Inject Clorohydrate 35 % into the left lower ventral abdomen
- Wait approximately 5 min for anesthetic to take effect
- Pinch the tip of the tail forcefully to test for reaction
- Add additional amount of anesthesia if necessary

4 Perfusion Rates:

Koob Lab—Pump runs in RPMS

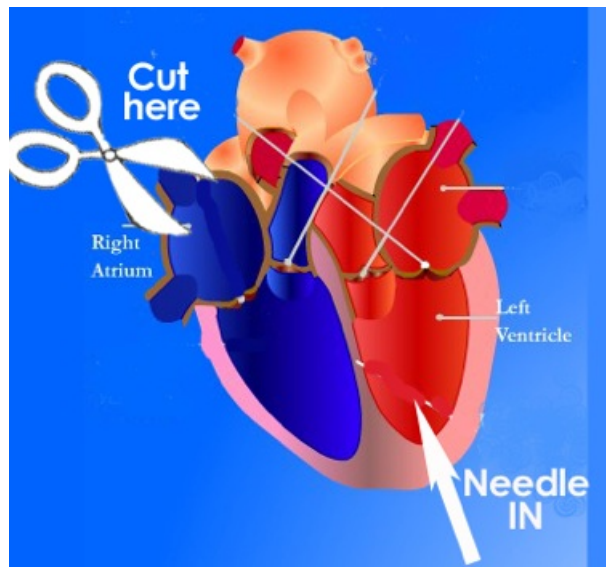
- Prefixative rinse-saline- 3 minutes @ max 90 RPM(i.e. ~24mls/min)

- Formaldehyde- 5 minutes @ max 90 RPM(i.e. ~24mls/min)
- Continue Form- 15 minutes @ 65 RPM(i.e. ~16mls/min)

5 Surgery and Fixation (do under hood or safe place)

- Place animal on the downdraft, make a "V" shape above the genital
- Lift skin upwards firmly using forceps, pinch a small hole into the abdomen, then cut along the "V" line
- Continue to cut upwards to expose the diaphragm, and then cut through the diaphragm
- Cut through the rib cage to expose the thoracic cavity
- Clamp open excess tissue for better access of the heart
- While holding the middle of the heart steady with forceps, make a cut slightly right to the apex of the heart (left ventricle), using one tip of a sharp scissors, then insert both tips of small scissors to make a bigger hole
- Insert catheter needle through the cut, up to the aorta. Clamp the entrance

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- Make a cut in the dark red-colored right atrium,
- Circulate 0.9% saline, make sure fluid is flowing freely, until the limbs of the rat are discolored (3 min)
- Turn off saline pump, and circulate 4% paraformaldehyde (approximately 250 ml) until neck is stiffened (3+15 min)

7 Brain Removal

- Use a pair of scissors to cut connective tissue under the skin, reaching in between the eyes
- Cut skin open, exposing muscles on top of skull to the eyes
- Use scalpel, cut muscle connecting to the skull bone on both sides
- Carefully place bone cutter against the inside of the skull, and make cuts following the scalpel incision
- Lift the occipital, parietal, and frontal bones using forceps, fully exposing the brain
- Cut membrane on top and scoop brain out of the skull; cut optical nerves and connective tissue
- Store brain in 4% fixative for 1 day.

8 Sucrose Treatment

- Take the brain out of the fixative, dab dry on utility towel
- Place brain in 20% sucrose solution (in distilled water) until it sinks to the bottom of the vial
- Place brain in 30% sucrose solution (in distilled water) for another day until it sinks to the bottom of the vial
- Freeze brains before cutting in dry ice.