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# Intracardiac perfusion with fixative for ultrastructural neuroanatomical studies

Intracardiac perfusion with fixative for anatomical studies

Janet R Keast<sup>1</sup>, Peregrine Osborne<sup>1</sup>

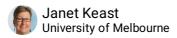
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#### **SPARC**

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This protocol is suitable for preserving tissues for ultrastructural neuroanatomical studies of peripheral nerves, ganglia, spinal cord or brain in adult rats. The protocol is performed under anesthesia and should incorporate all local requirements for standards of animal experimentation.

DOI

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#### **MATERIALS**

- **Xylazine Lyppard Catalog #L10605** Xylazine Lyppard Catalog #L10605

## Aldrich Catalog #441244

⊠Glutaraldehyde (25% aqueous solution EM

grade) ProSciTech Catalog #C001

# Preparation for perfusion

1 Make up the following solutions:

**Perfusion prewash solution:** To 300 ml 0.9% sodium chloride (w/v) add 3.75 ml 1% sodium nitrite (w/v) and 0.11 ml heparin (5000 IU/ml). This is made up immediately prior to use.

**Perfusion fixative:** 2% paraformaldehyde and 1.25% glutaraldehyde in 0.1M phosphate buffer, pH 7.4. The buffered paraformaldehyde solution is made up no longer than 48h prior to use, stored at 4C and brought to room temperature on the day of perfusion. Glutaraldehyde is added to this solution on the day of the perfusion.

## Perfusion

- 2 Induce anesthesia by an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg).
- 3 After opening the chest cavity, inject the left ventricle with mixture of 0.25 ml heparin (5000 IU/ml) and 0.5 ml 1% sodium nitrite.
- 4 A needle connected to tubing (T-connector to prewash and perfusion solutions), and a peristaltic pump (setting: 50 ml/min) is then inserted into the left ventricle and clamped into place using hemostats. Make a small incision in the right atrium to drain blood and perfusate during the procedure.
- 5 Perfuse with pre-wash solution until the fluid flowing from the right atrium is clear, the liver and extremities are pale (typically 2-3 min).

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6	Perfuse with fixative for 1	5-20 minutes,	by which tir	ne the org	ans have s	tiffened a	nd the
	neural tissues will be well	oreserved.					

-	7 Discost tissues	required for anal	veic and place i	n fivativo for a	further 18-24h at 4C.
	/ Dissect dissues	required for affai	iysis allu place i	II IIXalive ioi a	10111161 10-2411 at 40.

# Post-perfusion

8 Wash tissues with phosphate-buffered saline (PBS; 0.1 M, pH7.2), 3 x 30 min washes.

9 Store in PBS at 4C until processed further for embedding and electron microscopy.