Video Article

Erratum: Specimen Preparation, Imaging, and Analysis Protocols for Knife-edge Scanning Microscopy

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Abstract

A correction was made to Specimen Preparation, Imaging, and Analysis Protocols for Knife-edge Scanning Microscopy. Important modifications were made to the Nissl staining protocol.

Protocol

A correction was made to Specimen Preparation, Imaging, and Analysis Protocols for Knife-edge Scanning Microscopy. The Nissl staining protocol was modified from:

2. Specimen preparation: Nissl

1. The mouse is deeply anaesthetized using ketamine and xylazine injected intraperitoneally and then perfused transcardially using 50 mL of room temperature phosphate-buffered saline (pH 7.4), followed by 250 mL of room temperature 10% neutral buffered formalin (pH 7.4). and finally with 3.0 cc of undiluted India ink.
2. Whole body perfusion with saline and fixative is necessary to clear the blood from the cardiovascular system and to fix the tissues. Perfusion with India ink is necessary to completely fill the vasculature of the cardiovascular system.
3. The resulting brain is then dehydrated through a series of graded ethyl alcohols (25%{}-100%) and then embedded in araldite plastic following the protocol in 1.8-1.9 above. If LR white is the embedding medium then the protocol in 1.10 above is followed.
4. The mouse is deeply anaesthetized using ketamine and xylazine injected intraperitoneally and then perfused transcardially using 50 mL of room temperature phosphate-buffered saline (pH 7.4), followed by 250 mL of room temperature 10% neutral buffered formalin (pH 7.4). and finally with 3.0 cc of undiluted India ink.
5. Whole body perfusion with saline and fixative is necessary to clear the blood from the cardiovascular system and to fix the tissues. Perfusion with India ink is necessary to completely fill the vasculature of the cardiovascular system.
6. The resulting brain is then dehydrated through a series of graded ethyl alcohols (25%{}-100%) and then embedded in araldite plastic following the protocol in 1.8-1.9 above. If LR white is the embedding medium then the protocol in 1.10 above is followed.
7. The mouse is deeply anaesthetized using ketamine and xylazine injected intraperitoneally and then perfused transcardially using 50 mL of room temperature phosphate-buffered saline (pH 7.4), followed by 250 mL of room temperature 10% neutral buffered formalin (pH 7.4). and finally with 3.0 cc of undiluted India ink.
8. Whole body perfusion with saline and fixative is necessary to clear the blood from the cardiovascular system and to fix the tissues. Perfusion with India ink is necessary to completely fill the vasculature of the cardiovascular system.
9. The resulting brain is then dehydrated through a series of graded ethyl alcohols (25%{}-100%) and then embedded in araldite plastic following the protocol in 1.8-1.9 above. If LR white is the embedding medium then the protocol in 1.10 above is followed.

to:

2. Specimen preparation: Nissl

1. This protocol closely follows the protocol of Mayerich et al.[7]
2. The mouse is deeply anaesthetized using ketamine and xylazine injected intraperitoneally and then perfused transcardially using 50 mL of room temperature phosphate-buffered saline (pH 7.4), followed by 250 mL of cold 4% phosphate-buffered paraformaldehyde (pH 7.4).
3. The mouse is then perfused with 100 mL of a solution of 0.1% thionin dye in deionized water, and the body placed in the refrigerator (4 °C) for 24 hours.
4. After 24 hours, the brain is removed from the calvaria and placed in a fresh solution of 0.1% thionin and left at 4 °C for 7 days. The lengthy duration of this procedure was to ensure full infiltration of thionin into the en bloc preparation.
5. The brain is then destained and dehydrated through a graded series of ethanols starting with 50% ethanol and water and increasing to 100% ethanol over a time period of 6 weeks. The ethanol changes from 50% to 70% are stored in the refrigerator (4 °C) and all changes of ethanol above 70% are stored at room temperature.
6. After three changes of acetone (2-4 days in each solution at room temperature), the brain is then embedded in araldite plastic following the protocol in 1.8-1.9 above. If LR white is the embedding medium then the protocol in 1.10 above is followed.

Disclosures

No conflicts of interest declared.