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Consistent and reproducible staining of glia by a modified Golgi–Cox method

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HIGHLIGHTS

- Visualization of entire glial cytoarchitecture is possible with our modified Golgi–Cox method.
- Intensive fixation is crucial to achieve pure glial staining.
- Impregnation time (<16 days) and temperature (26 °C ± 1) are critical to attain uniform staining.

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ABSTRACT

Background: Golgi–Cox staining is a powerful histochemical approach which has been used extensively to visualize the morphology of neurons and glia. However, its usage as a first-choice method is hindered by its uncertain nature, diminished consistency and lengthy staining duration. The FD Rapid GolgiStain™ Kit (FD Neurotechnologies, Inc., USA) has been developed by employing the Golgi–Cox approach. It is a simple, reliable and reproducible way of performing Golgi impregnation for the analysis of neuronal morphology.
New method: We report here simple modifications to the manufacturer's protocol which enable reproducible and reliable staining of glial cells.
Results: Exposure of brain tissue to 4% paraformaldehyde (PFA) during perfusion followed by postfixation with 8% glutaraldehyde in 4% PFA led to only glial cells being stained, whereas in the absence of postfixation both neurons and glia were stained with unclear morphology. Additionally, we found that impregnation at 26 °C ± 1 was critical to attain uniform staining.
Comparison with existing method: Our modified Golgi–Cox approach is consistent and reproducible and affords uniform glial staining throughout the brain.
Conclusion: As this protocol stains only a small percentage of cells, it is suitable for the analysis of individual cells.

1. Introduction

To quantitatively assess the morphology and spatial arrangement of glial cells, commonly used approaches employ immunohistochemistry, dye filling and genetic labelling by expression of fluorescent proteins. These methods depend on cellular markers which are specifically expressed in distinct subpopulations of glia. Further, the dye filling approach requires intensive manual work. Golgi impregnation is one of the most powerful and effective histochemical approaches used for studying neuroanatomical connections and morphology of neurons as well as glia. One of the main strengths of the Golgi method is that it stains only a small percentage of cells in any one selected region (Spacek, 1989). This allows a panoramic visualization of virtually all parts of an individual cell including the soma, axon, dendrites and dendritic spines. Golgi staining was pioneered by Italian physician and scientist Camillo Golgi in 1873 (Golgi et al., 1873) and famously utilized by Spanish neuroanatomist Santiago Ramón y Cajal to uncover a number of novel facts about the organization of the nervous system (Ramón and Cajal, 1888a,b). Golgi staining is achieved by impregnating nervous tissue with potassium dichromate and silver nitrate. Cells thus stained are filled by microcrystallization of silver chromate. Although Golgi staining has been used extensively for more than a century, its uncertain nature, diminished

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consistency and lengthy staining duration (Buell, 1982; Friedland et al., 2006; Globus and Scheibel, 1966; Williams, 1983; Zhang et al., 2003) hinder its usage as a first-choice method. Several modifications have been made to the staining protocol, e.g. the use of osmium (Armstrong and Parker, 1986; Dall'Oglio et al., 2010; Marin-Padilla, 1990; Williams, 1983), addition of Triton X-100 to the Golgi solution (Tokuno et al., 1990), replacement of mercuric chloride for silver nitrate, as in the Golgi–Cox variant (Gibb and Kolb, 1998; Landas and Phillips, 1982) and variations in some other constituents in the staining solution for glia staining (Pessacq, 1970). Additionally, the development of Golgi-aldehyde methods (Golgi–Kopsch and Golgi–Colonnier) allowed the staining of freshly removed brains as well as brains kept fixed for several years (Colonnier, 1964; D'Amelio, 1983; Kopsch et al., 1896; Riley, 1979). Further, modifications of other conditions have been implemented to overcome its major drawbacks, viz. prefixation in formalin (Dall'Oglio et al., 2010; Williams, 1983) and postfixation in glutaraldehyde (Grosche et al., 2013) or picric acid (Dall'Oglio et al., 2010), altered temperatures (Angulo et al., 1994; Berbel, 1986; Ranjan and Mallick, 2010, 2012), lowering the pH of the chromating solution (Angulo et al., 1994; Bertram and Ihrig, 1957; Davenport and Combs, 1954), application of vacuum (Friedland et al., 2006) and use of microwave energy (Armstrong and Parker, 1986; Marani et al., 1987; Zhang et al., 2003). Some studies have reported that when brain tissue was exposed to fixatives prior to staining, glial cells were also stained along with neurons (Adams, 1979; Dall'Oglio et al., 2010; D'Amelio, 1983; Howard and Barnes, 1979; Zhang et al., 2003), while in the absence of fixatives only neurons were stained (Marin-Padilla, 1990; Ranjan and Mallick, 2010). Using Golgi–Cox staining, Ranjan and Mallick (2012) recently reported that a temperature of 37 °C plays a vital role in the improved glial staining within 48 h. Moreover, they showed that fixation of rat brains with 4% formaldehyde solutions resulted in uniform staining of glia.

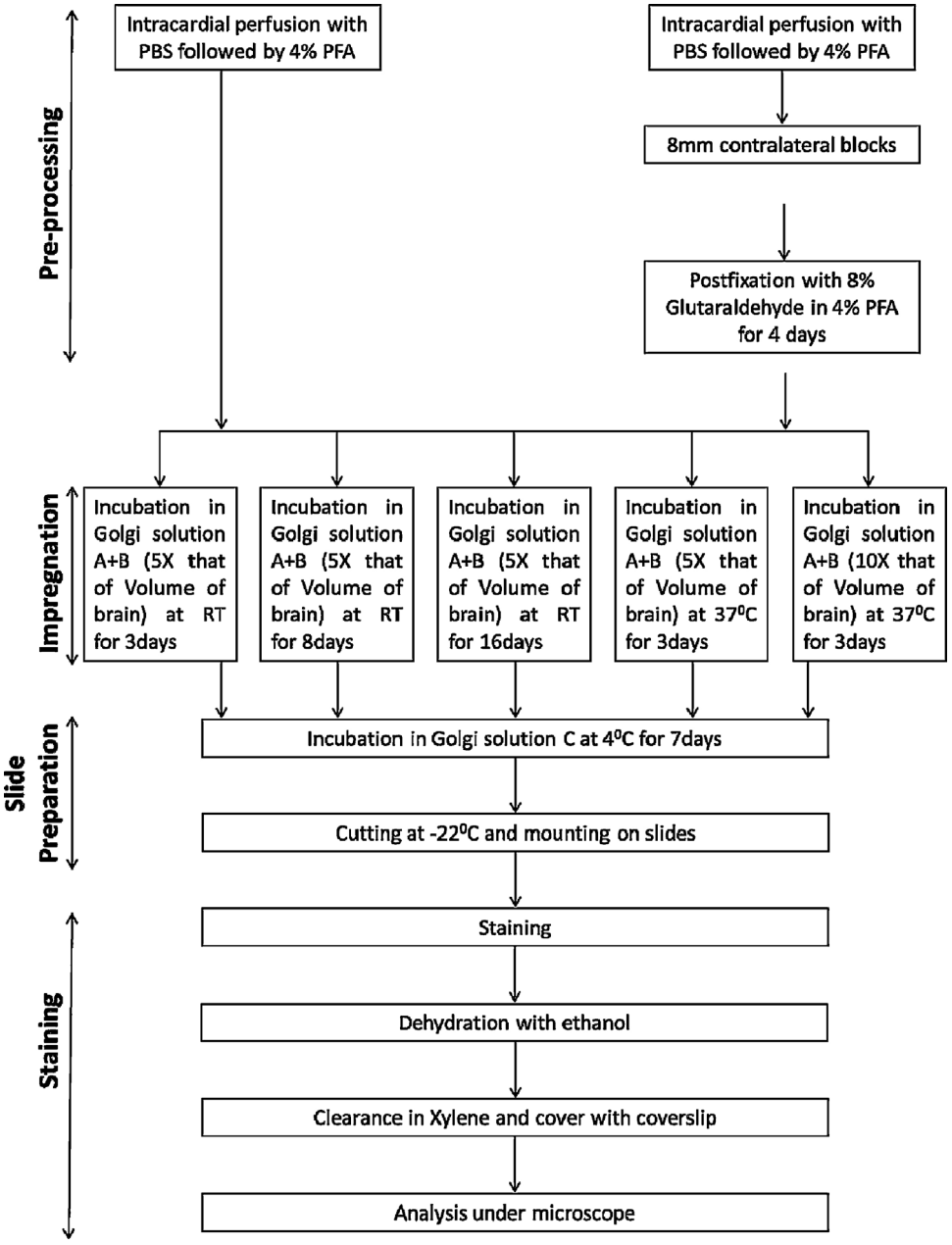

Fig. 1. Schematic representation of the methods used in this study.