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Research Article

RAPID GOLGI TECHNIQUE FOR STAINING PYRAMIDAL NEURONS IN RAT HIPPOCAMPUS

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ABSTRACT
The Golgi-Cox is a gold standard technique to stain the neurons. However the major limitations of the study are that it is time taking and inconsistent. In this paper we describe a modification of the Golgi–Cox method, in which the tissue blocks were immersed in rapid Golgi solution and silver nitrate for staining of neurons in rat brain. The method is simple, reproducible, rapid, inexpensive and provides uniform staining with very good resolution of neuronal soma, dendrites as well as spines.

Key Words: *Golgi - Cox Stain, Silver Nitrate, Neurolucida*

INTRODUCTION
The classical Golgi method for staining neurons in the brain was first developed by Camillio Golgi (Golgi, 1837 in Mazzarello, 1999). Subsequently, the remarkable neuroanatomist Ramon Cajal (1909a) applied the technique to demonstrate previously unimagined neuronal morphology virtually in all parts of the nervous system. However, the main drawback of the method is inconsistency of impregnation of the stain into the neurons resulting in reduced specificity, reproducibility, and success rate (Globus and Scheibel, 1966 a; Pasternak and Woolsey, 1975; Zhang *et al*., 2003). These modifications were aimed at faster staining process by decreasing the time for staining, reducing precipitation, promoting uniform crystallization, increasing reliability, and reproducibility of staining neurons by the Golgi method.
The Golgi–Cox method has been frequently used to stain neurons in both less myelinated, younger as well as in more myelinated, older rat brains. As compared to Golgi method the advantage of Golgi–Cox method include increased probability of staining more number of neurons (Scheibel and Tomiyasu, 1978).Thus, apart from inconsistency and lack of uniform, reproducible results, requirement of exceptionally long time to achieve neuronal staining is another major disadvantage of Golgi–Cox method, which practically limits the use of these methods.
One of the possibilities to overcome the shortcomings of the Golgi–Cox method mentioned above could be by increasing random motion of the metallic ions in the staining solution facilitating their influx and deposition within the neurons, thus reducing the time required as well as increasing the success rate of more stained neurons and reproducibility of results.

MATERIALS AND METHODS
Experiments were conducted on inbred male Wistar rats (180–250 g) maintained in standard home cages under 12:12 h light/dark cycle with food and water *ad libitum in central animal house* St.John's Medical College, Bangalore. The experiments were approved by the Institutional Animal Ethics Committee (IAEC) and every effort was made to minimize the use of number of animals and their suffering. Ten rats were sacrificed by cervical dislocation and decapitated.
The dissection was done and hippocampus was immersed en-block in rapid Golgi solution.

Preparation of Golgi Fixative
Potassium dichromate – 5g
Chloral hydrate - 5g
Glutaraldehyde – 8ml

Formaldehyde -6ml
Dimethyl sulphoxide (DMSO) – 6-8 drops
These chemicals were dissolved in 100 ml of distilled water.

Impregnation Solution (0.75% silver nitrate)

750mg of silver nitrate (AgNO3) was dissolved in 100ml of distilled water.

Procedure for Rapid Golgi Staining

Day 1*:* 50ml of the fixative was prepared and about 25ml of the fixative was poured into an amber coloured bottle and tissue blocks of the hippocampus were immersed in fixative. The bottle was closed tightly and kept in a dark chamber. The remaining 35ml of the fixative was stored in a refrigerator.

Day 2*:* The fixative in which the tissue was immersed on day 1 was slowly poured out. The tissue was rinsed by adding a small amount of fixative; the remaining fixative was poured into the bottle and kept in the dark chamber.

Day 3*:* 50 ml of fixative was prepared freshly. Tissue blocks were rinsed once or twice with fresh fixative. About 25ml of the fixative was poured into the bottle containing the tissue block and kept in the dark chamber.

Day 4*:* The tissue blocks remained undisturbed in the fixative.

Day 5*:* The tissue blocks were rinsed several times in 0.75% aqueous solution of silver nitrate (AgNO3) till the reddish brown colour of the potassium dichromate – silver complex disappeared. Tissue blocks were then placed in about 25ml of 0.75% (AgNO3) solution and kept in the dark.

Day 6*:* The tissue blocks remained undisturbed in the silver nitrate solution for 3 days.

Tissue Embedding

After 72 hours of impregnation in silver nitrate, tissue pieces were placed in a petri dish and silver deposits were brushed off gently. The tissue blocks were handled very gently and carefully at this stage and were not be treated with any aqueous solution. The tissue was bottled and then dehydrated in absolute alcohol for 10 minutes. After dehydration, the tissue blocks were carefully mounted onto block holders with required orientation and shell embedded with paraffin wax. The block holder was mounted onto a sliding/sledge microtome.

Sectioning

The hippocampus was cut into 120µm thick sections in horizontal plane. Care was taken to keep the exposed tissue surface moist with alcohol. The sections were collected in a petri dish containing 70% alcohol (Shankaranarayana Rao, 2004).

Cleaning and Mounting

The sections were lifted gently with a brush, blotted and transferred to xylene for clearing. Once the sections started sinking and became translucent they were mounted serially on slides with DPX and cover slipped. All the slides were coded to overcome the experimenter's bias.

The slides were allowed to dry at room temperature and were observed under a microscope (Olympus BX51, Japan) at low and high magnifications. Images were captured using a charged coupled device (CCD) digital camera (JVC, Tokyo, Japan and MBF CX9000) attached to the microscope using dedicated software Image-Pro Plus 5.1.1 (Media Cybernetics, Silver Spring, USA) and/or Neurolucida 9 (MBF Biosciences, USA).

RESULTS

The intensity of black staining of neurons in the sections was visually compared under microscope. The neurons were viewed randomly and the neurons, which fulfilled the following criteria were chosen (Figure 1and Figure 2).

The cell type must be identifiable
Dark and consistent silver impregnation throughout the extent of all the dendrites
The presence of untruncated dendrites

Relative isolation from the neighbouring impregnated neuron

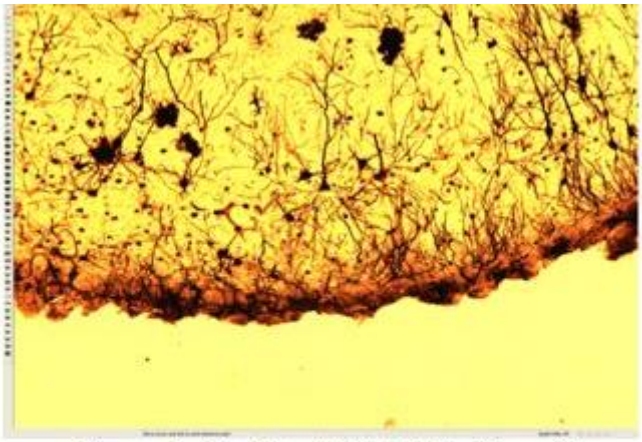

Figure 1: The Rapid Golgi Staining of Hippocampus showing the pyramidal neurons under 10X with the help of Neurolucida software

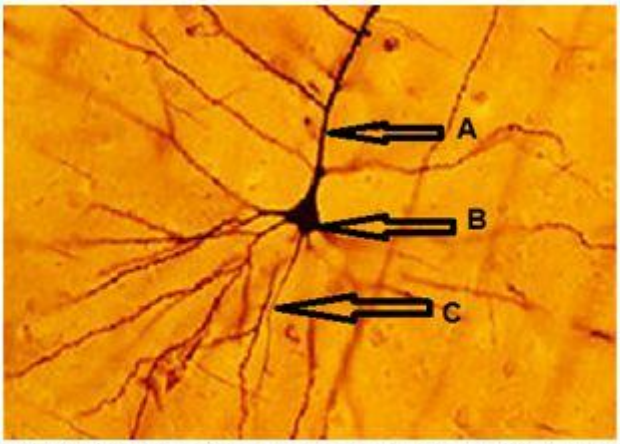

Figure 2: The pyramidal neuron under 40X showing the (A). Apical dendrite, **(B).** Cell body, **(C).** Basal dendrites

DISCUSSION

Golgi–Cox stain is one of the powerful techniques to study the neuronal morphology in brain sections (Ramon y Cajal, 1909 b). However, its major drawbacks are that it is much time consuming to get the results, taking several weeks to more than four months (Globus and Scheibel, 1966b).

The Golgi-Cox method resulted in impregnation of many neurons with rich dendritic plexuses and normal overall appearance. Occasional cells appeared grossly atrophic with irregular somata and apparent loss of apical and basilar dendritic segments. With the rapid Golgi method, the vast majority of impregnated neurons had rich dendritic plexuses or were otherwise normal in appearance. The major drawback of rapid Golgi technique is it stains of 20 to 30%of neurons (Amit Ranjan, 2010).

The Golgi silver impregnation technique gives detailed information on neuronal morphology of the few neurons it labels, whereas the majority remain unstained. In contrast, the Nissl staining technique allows

for consistent labelling of the whole neuronal population but gives very limited information on neuronal morphology. A rapid method combining Golgi silver impregnation with cresyl violet staining provides a useful and simple approach to combining cellular morphology with cytoarchitecture without the need for deimpregnating the tissue (Pilati, 2008).

Atrophy and lesions of the pyramidal neurons in subiculum, CA1 and CA3 regions were identified by using rapid Golgi technique (Shankaranarayana Rao, 2004).

The impregnated neurons with silver nitrate are typically examined as the postsynaptic neuronal components. The structural characteristics and the pattern of distribution of their synaptic connections with other groups of identified neurons are analyzed. Due to the high power of resolution of the Golgi-electron microscopic technique, the ultrastructural analysis of Golgi-impregnated neurons can be expanded to elucidate activity-dependent structural alterations in their cytoarchitecture (Benshalom, 1992).

The rapid Golgi method appears to be highly sensitive to postmortem delay or other factors which accompany studies involving human brain tissues obtained at autopsy. The Golgi-Cox method appears to be relatively insensitive to such factors (Buell, 1982).

The present study is mainly helpful in staining the brain tissues in short period for the morphometry of the neurons.

Conclusion

Rapid Golgi staining improves efficiency and reduces the time of staining to ten days. Thus procedure will allow the researchers to significantly increase their turnover of the slides. It will facilitate studying of anatomical connections such as arborizations and spine counting in the neurons.

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