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Appraisal of the effect of brain impregnation duration on neuronal staining and morphology in a modified Golgi–Cox method

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HIGHLIGHTS

- A modified, fast, and reliable Golgi–Cox staining method is described.
- It is less expensive and can be executed in labs where infrastructure is limited.
- 7 days impregnation, modifications in tissue and section processing yield good results.
- Tissue impregnated beyond 10 months is not suitable for morphometric evaluation.

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ABSTRACT

Background: Golgi–Cox staining method is considered as one of the best neurohistological and fascinating staining techniques to reveal the cytoarchitecture of the brain. Requirement of longer time (more than a month), laborious section processing steps, requirement of sophisticated equipment's and costly ready to use kits limits extensive use of this technique.
New method: The need for a modified staining technique is to overcome some of these hurdles. Here we describe a modification of Golgi–Cox staining involving reduced impregnation time (7 days), omitting tissue dehydration steps, and alterations in section processing steps. Different impregnation duration (7 days, 14 days, 1 month, 6 months and 10 months) effects on optimized staining of dorsal hippocampus and basolateral amygdala were investigated.
Results: Modified Golgi–Cox staining method was found to be effective in staining rat hippocampus and amygdala. Impregnation for 7 days, 14 days and 1 month resulted in giving good results and they were comparable. However, artifacts were slightly elevated with 6 months group but not extensively. Impregnation for 10 months negatively affected the staining process.
Comparison with existing method(s): Compared to existing methods the current method was found to be cost effective, fast, reliable and can be executed in labs where infrastructure is limited.
Conclusions: Current modification considerably benefitted in obtaining better results (good clarity and lesser artifact) in a short time. Longer impregnated brain sections were found to be unsuitable for morphometric evaluation due to more stain precipitation and artifact. The modified technique can be used to study cellular architecture in other brain regions.

1. Introduction

The "black reaction" a special histological staining method developed by Golgi (1873) allowed for the first time, a full view of the entire architecture of a neuron. This discovery laid the foundation for the modern neuroscience and nowadays this technique is popularly known as Golgi staining method. With the help of Golgi's method, Santiago Ramón y Cajal extensively studied and described the cellular architecture of the vertebrate nervous system (Cajal, 1909, 1910). More than 139 years since its discovery, it is still considered as one of the best neurohistological and fascinating staining techniques to reveal the cytoarchitecture of the brain (Bolton, 1898; Zhang et al., 2003). The Golgi method is not only used

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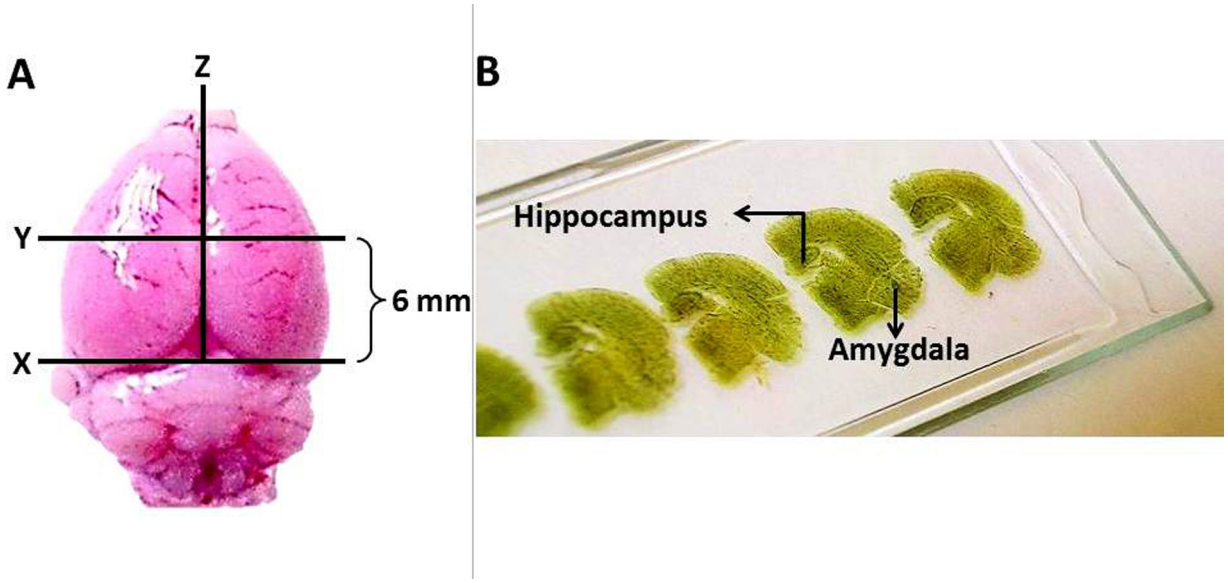

Fig. 1. Picture depicting various planes at which brain tissue was cut and processed for modified Golgi–Cox staining (A), coronal sections of right hemisphere stained with modified Golgi–Cox technique (B).

for neuroanatomical studies but also for exploring the relationships between brain morphology and behavior (Williams et al., 1980; Gibb and Kolb, 1998; Kolb et al., 2003; Ivy et al., 2010; Bustamante et al., 2010). The enticing feature of Golgi stain is not what tissue it stains, but the amount of tissue which is left untouched or unstained. It stains a random number of cells (3–5%) in the brain (Spacek, 1989) by an unexplained mechanism leaving the rest of the tissue untouched.

Golgi staining is based on the principle of metallic impregnation of neurons, allowing visualization of the entire architecture of a neuron. Depending on the processing duration and the type of metallic salts used, the Golgi staining is grouped into two major categories. Those leading to the deposit of silver chromate (Ag_2CrO_4) are usually referred to as "Rapid Golgi" method and those producing a deposit of metallic mercury such as mercuric sulphide (HgS) are categorized into "Golgi–Cox" methods (Windhorst and Johansson, 1999).

Golgi–Cox staining method developed by Cox (1891) is superior to traditional rapid Golgi staining, as the former stains more neurons (Scheibel and Tomiyasua, 1978) and gives excellent and clear results (Buell, 1982). Studies have also shown that the Golgi–Cox method is a potentially useful tool to identify and study the morphological characteristics of autonomic innervation in peripheral tissues (Gómez-Villalobos et al., 2009). However, there are some shortcomings of this fascinating technique including inconsistency (Buell, 1982; Williams, 1983), lack of uniformity, reproducibility (Globus and Scheibel, 1966; Pasternak and Woolsey, 1975; Zhang et al., 2003) and the requirement of a long time (several months) for staining (Windhorst and Johansson, 1999). These problems limit the extensive use of this technique. Poorly stained or inconsistent staining often hinders the tracing as well as the analysis of various components of neurons. These difficulties have lead to the exploration of several methodological modifications to improve reliability (Ranjan and Mallik, 2010) as well as sensitivity (Zhang et al., 2011a,b; Orlowski and Bjarkam, 2009) of the Golgi–Cox staining method.

There might be several factors which influence the optimal staining process in Golgi–Cox method. One of these is the time in which the tissue has to be kept submerged in the staining solution (duration of impregnation). At present the duration of impregnation using Golgi–Cox method has been reported to be between 14 and 80 days (Rutledge et al., 1969; Glaser and Van der Loos, 1981; Zhang et al., 2003). The effect of different brain impregnation duration and effectiveness of neuronal staining has not been investigated in detail. Another major confounding factor influencing the staining process is the processing of sections and method with which the section processing is done. Processing of cut sections in many methods reported earlier is found to be time consuming (Paul et al., 1997; Shankaranarayana Rao et al., 2004). However, in the current report we describe a simple modified Golgi–Cox staining method which is less expensive, fast, reliable and can be executed in labs where infrastructure is limited. Along with describing a modification in Golgi–Cox method, in the current study we investigated the effects of shorter and longer brain impregnation on staining quality of sections from dorsal hippocampus and basolateral amygdala (BLA).

2. Materials and methods

2.1. Animals

Healthy male albino rats of Wistar strain (3 months of age), weighing 200–220 g were obtained from Central Animal Research Facility (CARF), Manipal University. Rats (3 per cage) were housed in polypropylene cages measuring 41 cm × 28 cm × 14 cm. They were maintained in 12:12 h L:D environment, in an air-conditioned room in the central animal house and were fed with water and standard food ad libitum. Institutional Animal Ethics Committee (IAEC) has approved all the procedures used in the study.

2.2. Chemicals

Except sodium carbonate, all chemicals used for the study were obtained from Sigma and Aldrich, USA. Sodium carbonate was obtained from Merck, India.

2.3. Experimental design

A total of 20 rats were used for the study. Animals were sacrificed by cervical dislocation and the whole brain was dissected out carefully. According to the duration at which the tissue was impregnated in Golgi–Cox Staining (GCS) solution (before taking sections on a sliding microtome); they were divided into five groups having four rats per group. Group-I: brain impregnated for 7 days, Group-II: brain impregnated for 14 days, Group-III: brain impregnated