A Qualitative Study of Neuronal Staining with a Modified Golgi Cox Method

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Introduction

The Golgi method has been described as the *'only histological technique with personality'* (Scheibel & Scheibel, 1978). The classic Golgi method for staining neurons, first developed by Camillio Golgi in 1873 is unique in that it stains only a few cells (1-10%) in their entirety, thus making it possible to visualize all the processes of the stained neurons and a detailed study thereof. It consists of two steps—chromation of the tissue in potassium dichromate followed by impregnation of cells by immersion of the tissue in silver nitrate solution. Several variants of the classic Golgi method (Golgi, 1873) have been developed. The greatest drawbacks of the classic Golgi method and its variants are the inconsistency of impregnation and high failure rate (Zhang et al., 2003).

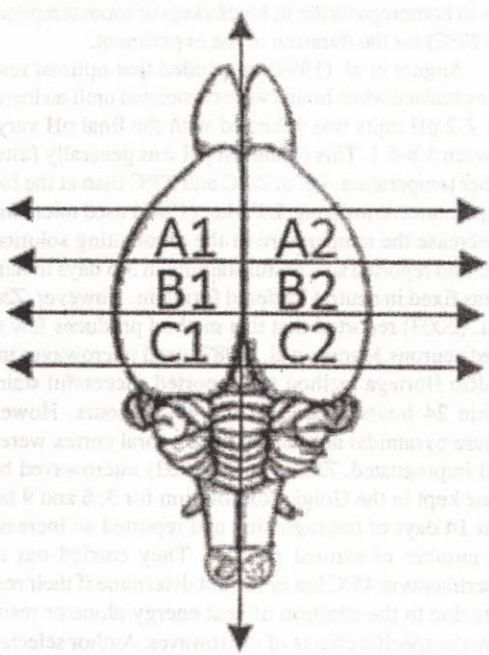

Fig. 1. Four coronal cuts and 1 sagittal cut to obtain 6 blocks from one rat brain.

The Golgi-Cox method is one of the more actively utilized Golgi methods (Zhang et al., 2003). In this method both chromation and impregnation are achieved simultaneously by immersing the specimens in a single solution containing both potassium dichromate and the impregnating metal ions (mercuric chloride). The advantages include an increased probability of staining a large number of neurons (Scheibel & Scheibel 1978), better dendritic morphology (Buell, 1982) and excellent contrast.

Besides inconsistency, the exceptionally long duration of time required to achieve neuronal impregnations is an important disadvantage shared by all Golgi methods.The duration of impregnation using the Golgi-Cox method has been variously reported to range between 14 days (Glaser & Van der Loos, 1981; Zhang et al., 2003) to 80 days (Rutledge et al., 1969, 1974), compared to 5 – 8 days if the rapid Golgi method is used (Globus & Schiebel, 1966). This study was conducted with the aim of reducing the time required for successful staining of neurons using the Golgi-Cox method in 5 mm blocks of brain. Author tested the effect of temperature on the dynamics of neuronal impregnation.

Materials and Methods

Experiments were conducted on inbred male wistar rats (250- 300 g) maintained in standard home cages under 12-h light/dark cycle with food and water *ad libitum*. Rats were sacrificed by cervical dislocation followed by decapitation. Brains were removed, washed with distilled water and then freshly prepared Golgi-Cox solution (Rutledge et al., 1969). Three coronal blocks: A, B and C, each 5 mm thick were prepared using a brain slicer (WPI). Each block was divided sagitally into two equal halves. The six blocks were labeled A1, A2, B1, B2, C1 and C2 respectively (Fig. 1) and placed in separate cotton lined amber bottles containing 30 ml fresh Golgi-Cox solution. Bottles of blocks A1, B1 and C1 were placed in an incubator maintained at 37°C. Bottles containing blocks A2, B2 and C2 (controls) were kept at room temperature (26°C to 27°C).

Impregnation and staining of neurons was checked after 6 hours, 12 hours and 24hours. At each time point one block (e.g. A1) was removed from the bottles kept at 37°C and the contralateral block (e.g. A2) from the bottles kept at

Table 1. Comparison of staining between brain blocks kept at 37°C and at room temperature after incubation for 6, 12 and 24 hours.

Block/ Section Thickness	Duration of Incubation	Results after impregnation at 37°C	Results after impregnation at 26 °C
5mm / 100-120 micron	6 hours	Nucleation centres, filled cell bodies, few stained branches seen.	None of the sections in any animal tested show evidence of beginning of impregnation.
5mm/ 100-120 micron	12 hours	Completely filled neurons in cortical region though not in deeper brain areas.	Similar to sections obtained after 6 hours impregnation except for the outline of a few dendrites seen in one section.
5mm/ 100-120 micron	24 hours	Completely filled neurons in cortical and sub cortical region.	Some nucleation centers, very few partially filled cell bodies in some cortical areas.

26°C (Table 1). Sections (250-300 microns) were prepared from each block.Sections were rinsed twice in distilled water (five minutes each). They were then kept in ammonia solution (1part ammonia: 3 parts distilled water) for 20 minutes each. Thereafter, they were rinsed twice in distilled water (five minutes each) and kept in 2% sodium thiosulphate for 20 minutes in the dark. The sections were then rinsed twice in distilled water (five minutes each), differentiated in grades of alcohol, cleared in toluene and mounted in DPX on gelatinized slides.The slides were allowed to dry at room temperature and were observed under a microscope at low and high magnification. Images were captured using a digital camera attached to the microscope Three sets of experiments were conducted. Qualitative analysis of sections was carried out to study if all the branches of neurons were filled and if dendritic spines could be visualized.To be taken as successfully filled, neuronal profiles had to satisfy two criteria,namely,the presence of untruncated dendrites and consistent and complete filling of dendrites.

Results

Complete impregnation of neurons was achieved by incubation at 37°C and stained dendritic spines could be seen against a clear background, in contrast hardly any neurons were stained in blocks kept at room temperature. The differences in the rate of filling of neurons were evident as early as 6 hours after the start of impregnation. By 12 hours numerous completely filled neurons could be seen in the cortical region of blocks incubated at 37°C and within 24 hours, all regions of the section (cortical and sub cortical regions) showed several successfully impregnated neurons.

Discussion

The Golgi-Cox solution used by author in this experiment is the same as reported by Rutledge et al. (1969). Using 5 mm thick slices of cat brain, they reported the duration of impregnation to be 40-80 days. This paper describes a modification of one of the physical parameters i.e., temperature, at which Golgi-Cox impregnation of neurons is carried out. In our study the temperature was the only variable between two contralateral blocks.While excellent staining was achieved within 24 hours in blocks incubated at 37°C, very few, if any, stained neurons could be seen in homotopic areas in blocks kept at room temperature (26-27°C) for the duration of the experiment.

Angulo et al. (1994) concluded that optimal results were obtained when brains were chromated until an increase of 1.7-2 pH units was achieved with the final pH varying between 5.8-6.1. This change of pH was generally faster at higher temperature, i.e. at 20°C and 23°C than at the lower temperature. Armstrong & Parker (1986) used microwaves to increase the temperature of the chromating solution to 55°C and reported successful staining in 3-5 days in human brains fixed in neutral buffered formalin. However, Zhang et al. (2003) reported that this method produces few well filled neurons.Marani et al. (1987) used microwaves in the del Rio Hortega method and reported successful staining within 24 hours instead of the 48-72 hours. However, mature pyramidal neurons in the cerebral cortex were not well impregnated. Zhang et al. (2003) microwaved brain tissue kept in the Golgi-Cox solution for 3, 6 and 9 hours after 14 days of impregnation and reported an increase in the number of stained profiles. They carried out their experiments at 45°C but could not determine if their results were due to the addition of heat energy alone or resulted from the specific effects of microwaves. Author selected an incubation temperature of 37°C because it allows to avoid the negative effects, if any, of gentle cooking at higher

temperature (Marani et al., 1987) on neural architecture. Besides, author did not observe any significant increase in precipitation of salts at this temperature.

Conclusion

Incubating brain blocks at 37°C dramatically reduced the time required to achieve complete staining of neurons. This method will allow researchers to increase their turnover and will be especially valuable in applications like tissue biopsy. This modification will also make the process more reliable and avoid wastage of precious tissue samples. An additional advantage is that it does not require any specialized equipment or chemicals.The reason behind the better results in present study may be due to the effect of temperature on the pH of the impregnating solutions. Further studies are needed to probe the underlying mechanism.

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Disastrous Effect of Interlinking Rivers
Alien Fish Tore the Nets

When Venkat Ramana, a fisherman from Tadepalli in Amaravati along the Krishna river, netted a big catch recently, the joy did not last long — his net was damaged.

Later, he heard others too complaining of the problem. The culprit turned out to be an alien variety of fish with sharp teeth and spikes.

Ramana and many others in the Vijayawada-Amaravati belt, whose livelihood is fishing, are discovering a new variety identified as Sailfin Catfish. Of no commercial value, this species is believed to have made its way into Krishna from the Godavari river through the Polavaram canal which was built to link the two rivers.

Besides damaging nets, the fish preys on commercially viable varieties, affecting overall catch and livelihood of fishermen. Invasion of such alien species is emerging as a major ecological challenge for the State, which built the interlinking canal two years ago to divert excess floodwater from Godavari to Krishna.

Researchers had then warned of collateral damage. "Invasion of non-native species will cause extinction of native species. Krishna's unique fish biodiversity will be lost due to interlinking," said an expert from the Acharya Nagarjuna University.

Interlinking has also increased turbidity and mineral content of Krishna water, a study by MVR College of Engineering and Technology, near Vijayawada, found.

"River linking is fraught with environmental dangers. It should be tackled by building reservoirs en route the link canal with sieves to hold back any alien species," says environmentalist V. Satyanarayana.