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# A method for vibratome sectioning of Golgi–Cox stained whole rat brain

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**Abstract**

A method for impregnating the whole rat brain with Golgi–Cox stain and sectioning with the vibratome is described. The method is simple, inexpensive and provides good resolution of dendrites and spines. © 1998 Elsevier Science B.V.

*Keywords:* Vibratome; Golgi–Cox staining; Dendrite; Neuroanatomical method; Spine

## 1. Introduction

There have been many variations in Golgi's method of impregnating nerve cells since its publication in the late 1800's (Golgi, 1873). It was Cajal, however, who applied the technique to demonstrate previously unimagined morphology in virtually every part of the nervous system (Cajal, 1909). Although Cajal's use of the Golgi technique was largely for descriptive purposes, in the past three decades there has been a resurgence in interest in using Golgi methods to investigate behavioural–morphological relationships (e.g. Globus and Scheibel, 1967; Coleman and Riesen, 1968; Woolsey and van der Loos, 1970; Valverde, 1971; Greenough, 1976; Juraska, 1984; Withers and Greenough, 1989; Kolb and Gibb, 1991; Steward, 1991; Jacobs and Scheibel, 1993; Steward and Rubel, 1993; Jones and Schallert, 1994).Such studies normally involve significant behavioral manipulation and/or training prior to tissue analysis and this carries two important requirements. First, it is desirable to have a staining protocol that is reliable since it is often difficult, or even impossible, to replace individual animals or groups after the experiment. Capricious staining can mean that the entire experiment must be repeated, which is both time consuming and expensive. Second, since it is not always obvious a priori where one would like to search for morphological changes, it is ideal to have a method that allows for simple embedding of the entire brain so that every section can be saved for later analysis. In this case it is desirable too, that there be even shrinkage of the tissue so, at least for small brains, it is best to stain without blocking the brain. We have been experimenting with various Golgi procedures over the past decade and have developed a modified Golgi–Cox procedure that meets these requirements.

There has been a recent increase in the use of the vibratome for the sectioning of fresh tissue, especially for immunohistochemical procedures, which has resulted in an increased availability of the vibratome in neuroscience laboratories (Landas and Phillips, 1982). We have found that the vibratome can easily be used for sectioning of Golgi-impregnated tissue and allows us to impregnate and section the whole rat brain without the costly celloidin-embedding and a special microtome. The method below is based upon Glaser and van der Loos (1981) Golgi–Cox procedure that we had previously modified for cryostat-sectioning (Kolb and McLimans, 1986).

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## 2. Materials and methods

Rats are administered an overdose of sodium pentobarbital and perfused with 0.9% saline. Brains are removed and placed in 20 ml Golgi–Cox solution (Glaser and van der Loos, 1981). The brains are stored in the dark for 14 days after which the Golgi–Cox solution is replaced with 30% sucrose. The brains are allowed to sit in the dark for 2–5 days before sectioning. The sucrose step allows the tissue to remain more pliable and less prone to fracture when it dries. Storing the brains in the dark reduces the background staining. We have found that the time in sucrose is not critical and have left brains in sucrose for a maximum of 2 months with no adverse effects on staining. The brains are blocked if needed, blotted dry and mounted on sectioning stages with cyanocacrylic glue. Care must be taken to ensure the entire block of tissue is firmly secured to the stage to prevent uneven sections or chunks tearing off the block during sectioning.

A Schick injector razor blade is prepared for sectioning by immersion in xylene for five minutes to remove any traces of oil. It is then wiped dry. The vibratome reservoir is filled with a 6% sucrose solution to a level that covers the sectioning blade. The vibratome speed and amplitude are both set at 5, which is the midpoint on both scales. Sections (200 $\mu$m) are saved and placed on 2% gelatinized microscope slides. The sections are kept wet during the course of sectioning. Once all sections of interest are collected and placed on the slides, the sections are pressed onto the slides by applying pressure to the slides with moistened bibulous paper. The blotting is best done by applying the heel of the palm to the moist blotting paper and applying direct, downward pressure, taking care not to move laterally. (A similar procedure is used with celloidin-embedded tissue.) The blotting step is necessary because sections are prone to coming off the slides if they are not blotted. Blotted slides are kept in a humidity chamber until they are ready to be stained. Slides are placed in a glass staining tray and then processed in the following manner.

1. Rinse in distilled water for 1 min.
2. Place in ammonium hydroxide for 30 min in the dark.
3. Rinse in distilled water for 1 min.
4. Place in Kodak Fix for Film for 30 min in the dark.
5. Rinse in distilled water for 1 min, 50% alcohol for 1 min, 70% alcohol for 1 min, 95% alcohol for 1 min, $2 \times 100\%$ alcohol for 5 min, a solution of 1/3 chloroform, 1/3 HemoDe (or xylene), and 1/3 100% alcohol and xylene (or HemoDe) for 15 min.
6. Coverslip with permount or cover sections with Canada Basalm.

We have found that in order to ensure consistent staining quality it is necessary to use fresh solutions. If this precaution is not taken the background staining tends to darken over time, making examination of the sections more difficult. It is also necessary to keep the slides exposed to the air for several months until the slides are absolutely dry or there can be darkening of the tissue. We note, parenthetically, that this was first noted by Cajal (1909) nearly 100 years ago but this wisdom seems to have been lost!

## 3. Results

Using this procedure we have found that dendrites and spines are evenly and consistently stained, as illustrated in Fig. 1. The method can be used effectively for brains of rats ranging in age from postnatal day 0 until old age, with only minor modifications of the impregnation time. In particular, small brains (less than 1 g) should be left in the Golgi–Cox solution for 6 days. We have also found the procedure to work well with other rodent brains including voles, gerbils and mice as well as cats.

## 4. Discussion

Our procedure offers the advantages that: (1) the whole brain is impregnated and cut at once, which eliminates the problems of unequal shrinkage of blocks of a given brain; (2) the sections are stained after mounting on the slides, which obviates the need to handle the individual sections; (3) coverslipping is much easier than for celloidin-embedded tissue in which the sections tend to curl; (4) the results appear to provide more extensive staining of fine branches than celloidin-embedded material; and (5) sections ranging from 90–200 $\mu$m thick are equally satisfactory for analysis. The use of a vibratome offers several other advantages including: (1) a lower initial cost than a cryostat; (2) ease of maintaining a sharp 'knife' since razor blades do the cutting; (3) less shrinkage than with paraffin or celloidin treatment; (4) no need for embedding as in paraffin or celloidin; (5) no need for special protective procedures as in frozen techniques; and; (6) less tissue fragmentation than occurs with frozen techniques.

We have compared our staining with this procedure to our staining with both celloidin-embedded and cryostat-sectioned tissue and found there to be far more extensive staining of distal branches of cortical pyramidal neurons (Table 1). For example, layer III pyramidal cells in somatosensory cortex have 20–30% more dendritic branches visible in both the apical and basilar fields in the vibratome tissue than in celloidin or frozen tissue (e.g. frozen sections in Kolb and Gibb (1991) vs vibratome sections in Kolb et al., 1996). A similar difference can be seen in similarly treated animals cut in

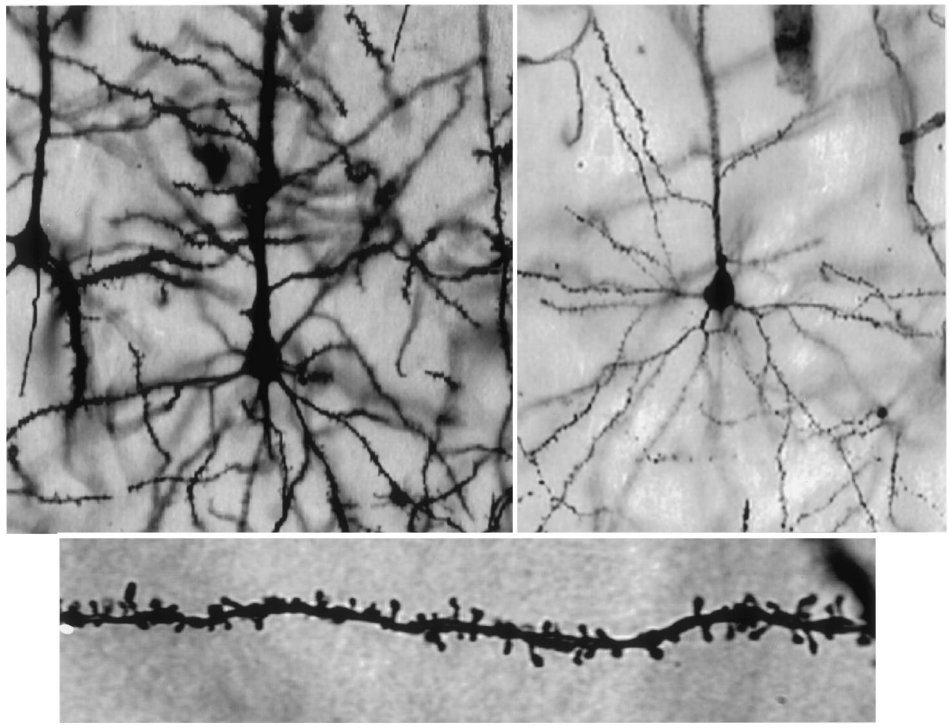

Fig. 1. Photomicrographs (200 × ) illustrating examples of staining from pyramidal neurons in layer III of somatosensory cortex of a rat (top left) and vole (top right). The piece of basilar dendrite at the bottom illustrates the clear staining of dendritic spines (630 × ).

celloidin (Jones and Schallert, 1994) versus our vibratome method (Forgie et al., 1996).

It could be asked, of course, whether we are now staining the complete dendritic field. This is difficult to determine but we have compared our estimates of dendritic branching to those reported in studies using in vitro intracellular injection techniques (e.g. Prusky and Whishaw, 1996; Tseng and Prince, 1996). Quantification of cells stained using our vibratome procedure appears to produce similar, or slightly higher, values than those reported the in vitro procedures. Our procedure therefore appears to produce staining that is comparable to the newly developed in vitro methods and may allow visualization of dendritic fields that are less easily seen in some methods that rely on tissue embedding.

Table 1
Comparison of dendritic branching in sections prepared using vibratome, frozen, or celloidin procedures

| Pyramidal cells | Basilar branches |
|---|---|
| Layer III | |
| Vibratome (Kolb et al., 1996) | 42 |
| Frozen (Kolb and Gibb, 1991) | 22 |
| Celloidin (Kolb and Gibb, 1997) | 18 |
| Layer V | |
| Vibratome (Forgie et al., 1996) | 54 |
| Celloidin (Jones and Schallert, 1994) | 19 |

The vibratome technique allows visualization of many more dendritic branches.
Numbers represent means of dendritic branches of pyramidal cells in parietal cortex (layer III cells) or motor cortex (layer V cells).

The main difficulty that we have encountered is that in some brains there are patches of heavy blood vessel artefact or occasional tiny crystals, especially in the pyriform cortex. (Similar crystal artefact was noted by (Landas and Phillips, 1982) in their vibratome method for single sections.). Neither of these artifacts conceal cellular detail, but they do make photography more difficult. A second problem we have seen is that since the whole brain is impregnated at once, there is often poor straining of central regions of brain, such as the thalamus. Staining of other subcortical regions such as the striatum and hippocampus is excellent, however.

In summary, we have found this technique to provide more reliable staining of fine branches and spines than more traditional celloidin-embedding procedures and it has proven useful in a variety of applications in behavioral–anatomical studies (e.g. Kolb et al., in press).

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