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Golgi-Cox stain and neuronal morphological analysis

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We use this protocol and it's working

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Abstract

The Golgi-Cox technique allows for detailed study of neuronal morphology. With the help of optical microscopy and Sholl analysis, dendritic arborization, spine density, and neuronal organization can be analyzed. This allows us to identify structural changes in physiological or pathological conditions. Below we describe protocol for performing Golgi-Cox stain on rat brains and Sholl analysis.

Guidelines

The entire tissue preparation process must be carried out in a darkroom. Be sure to work in a laboratory fume hood when necessary.

Materials

Perfusion pump, Ketamine, Xylazine, Sterile saline solution, 0.9% saline solution, Syringes, Falcon tubes, Golgi-Cox solution, Vibratome, Gelatinized slides, Ammonium hydroxide, Kodak film fixer, Alcohol, Xylene, Light microscope equipped with camera lucida, Permount mounting medium, Cover slips.

Before start

Ensure you have all the necessary laboratory materials and equipment.

Perfusion

1 Anesthetize the rats by intraperitoneal injection of a ketamine/xylazine cocktail (0.75 ml ketamine + 0.25 ml xylazine + 5 ml sterile saline), administering a dose of 0.125 ml per 20 g of body weight.

2 Perfuse the rats intracardially with 0.9% saline solution and quickly remove the brain.

Tissue preparation

4w 5d

3 Place the brains in Golgi-Cox solution for 30 days. 4w 2d

4 Incubate 30% sucrose solution for 3 days. 3d

5 Using a vibratome (Leica, VT1000S microsystem, USA), obtain 200 µm coronal sections of the brain region of interest and mount them on 2% gelatinized slides. Keep the slides in a humid chamber.

Staining procedure (in darkroom)

1h 45m 25s

6 Rinse with distilled water 5 times. 5s

7 Treat sections with ammonium hydroxide for 30 minutes. 30m

8 Rinse with distilled water 10 times. 10s

9 Incubate for 30 minutes in Kodak Film Fixer. 30m

10 Rinse with distilled water 10 times and dehydrate in increasing concentrations of alcohol: 75% 5 minutes, 90% 5 minutes, and 100% 10 minutes twice. 30m 10s

11 Incubate with xylene for 15 minutes. 15m

12 Cover the slides with Permount mounting medium and coverslips.

Neuronal morphological analysis

13 Using an optical microscope (DM 2000 microscope, Leica Microsystems, USA) equipped with a camera lucida, reconstruct ten pyramidal neurons (5 in each hemisphere) from each brain in a two-dimensional plane.

14 Quantify dendritic tracing by Sholl analysis. Place a transparent grid with concentric rings spaced 10 µm apart over the dendritic drawing (centering the soma) and count the number of intersections. Calculate the total dendritic length by multiplying the total number of intersections in each ring by 10 µm. Calculate the dendritic arborization as the total number of dendritic branches present in each order of the dendritic axis.

15 To obtain the density of spines, count and draw the spines of ten distal and/or last-order dendrites with an approximate length of 10 µm (5 for each hemisphere) at 1000x magnification.

Protocol references

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