The golden days are back: modern application of gold impregnation technique on rat brain cryosections

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Introduction

Around 150 years ago, the silver impregnation method for histological sections of nervous tissue was published by Camillo Golgi (1). Different impregnation methods were used extensively for some time, and they contributed significantly to the development of neuroscience. With the development of new methods, such as electron and confocal microscopy, silver and gold impregnation methods were used less. However, they can still produce useful data, especially when used in combination with modern image analysis software.

Materials & methods

In this research, the impregnation technique was used to visualize nerve fibers in the areas of interest in the brain of Wistar female rats. The animals were divided into 5 groups: 1) sham-operated control group, 2) ovariectomized untreated group, 3) ovariectomized and treated with 40 mg/kg alendronate, 4) with 60 mg/kg hop extract Xanthoflav (generously donated by Hopsteiner, NY, USA) and 5) with a combination of the two. Rat brains were fixed in 4% paraformaldehyde, cryoprotected and frozen in pre-chilled isopentane. Coronal 35 µm-thick sections were prepared on a cryostat. 0.2% gold chloride solution in 0.02 M neutral phosphate buffer and 0.9% sodium chloride was used for impregnation. After the impregnation, the slides were dehydrated and coverslipped. Samples were photographed and analyzed in free and open-source program ImageJ (2). For staining intensity quantification, images were separated into 8-bit channels and the blue channel was used for analysis. Parameters chosen for measurement in ImageJ were area, standard deviation, min and max grey value, integrated density and mean grey value.

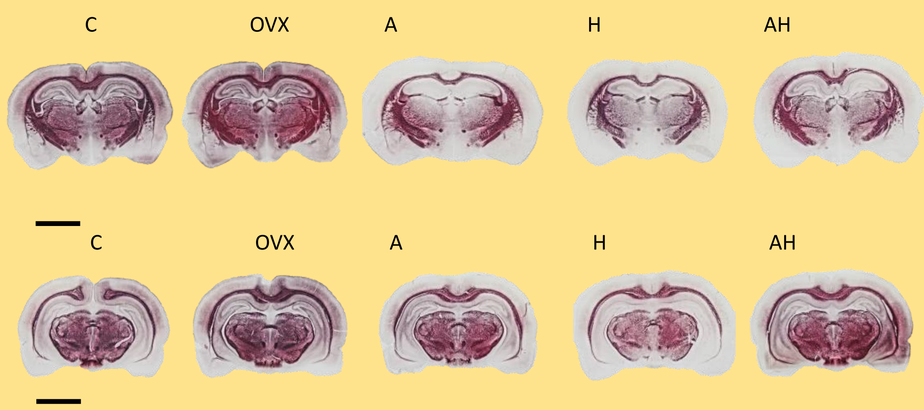

Figure 1. Photomicrographs of brain sections of 5 different animal groups stained with gold. Brain section thickness = 35µm; C=control group, OVX=ovariectomised control group, A=alendronate treated ovariectomised group, H=hop extract treated ovariectomised gorup, AH=Alendronate and hop extract treated ovariectomised group;; Scale= 4 mm

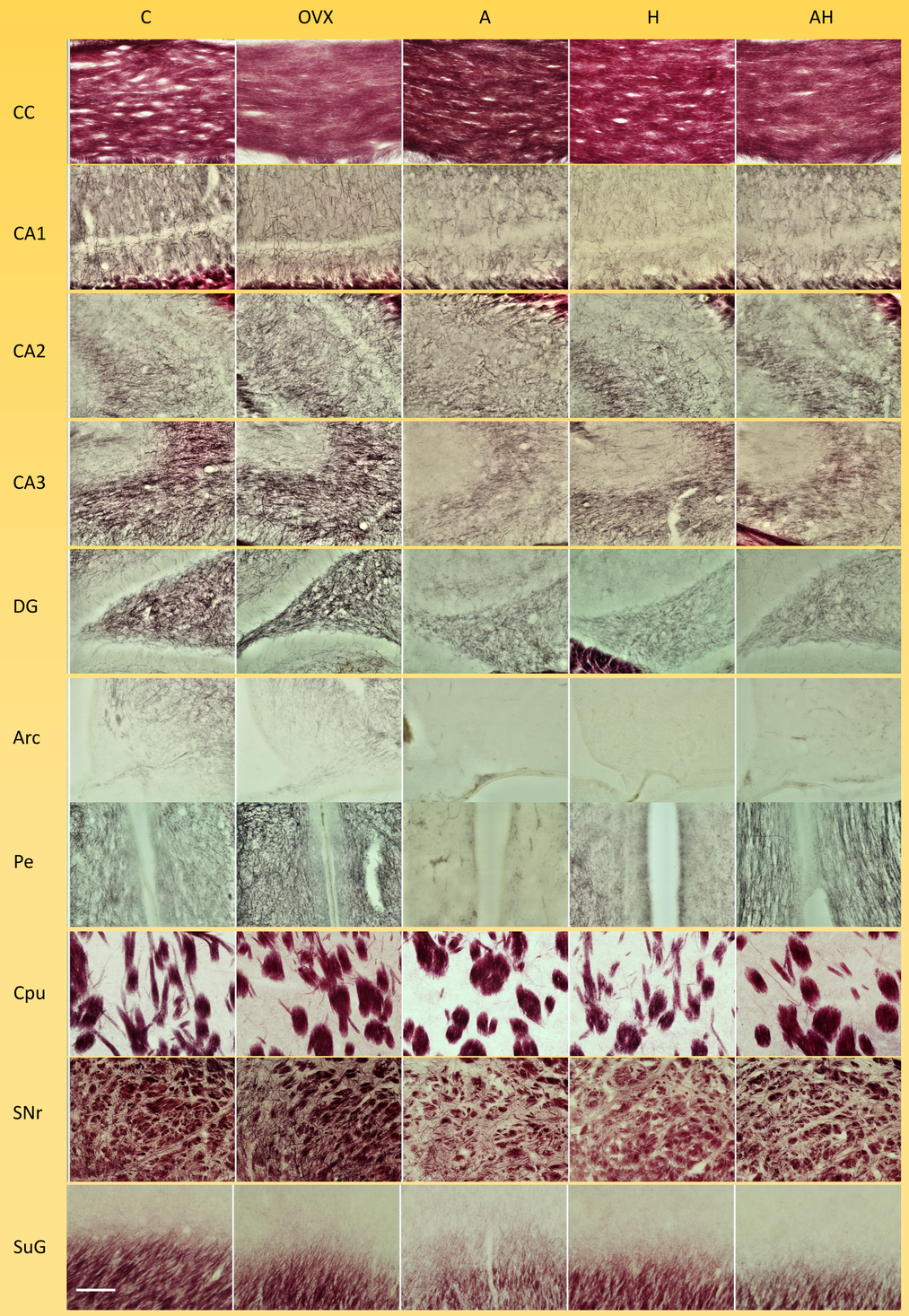

Figure 2. Photomicrographs of different areas of brain sections of 5 different animal groups stained with gold. Brain section thickness: 35µm; C: control group, OVX: ovariectomised control group, A: alendronate-treated ovariectomised group, H: hop extract-treated ovariectomised gorup, AH: alendronate and hop extract-treated ovariectomised group; CC: corpus callosum, CA1: cornu ammonis 1, CA2: cornu ammonis 2, CA3: cornu ammonis 3, DG: dentate gyrus, Arc: Arcuatus, Pe: periventricular nuclei, Cpu: Caudate putamen, SNr: Substantia nigra reticular part, SuG: Superficial grey layer of the superios collicullus; Scale: 200 µm

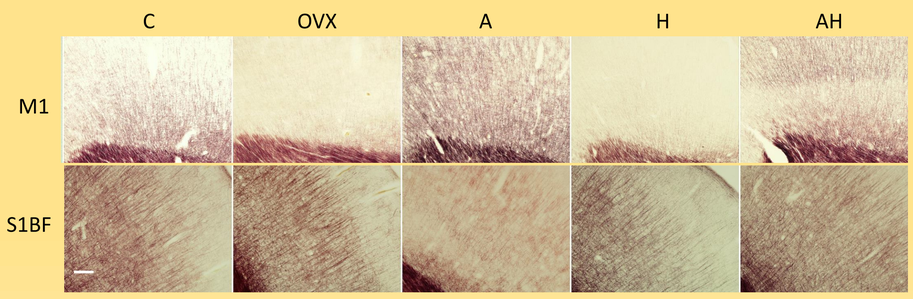

Figure 3. Photomicrographs of brain primary motor cotrex (M1) and primary somatosenzory cortex (S1BF) of 5 different animal groups stained with gold. Brain section thickness = 35µm; C=control group, OVX=ovariectomised control group, A=alendronate treated ovariectomised group, H=hop extract treated ovariectomised gorup, AH=Alendronate and hop extract treated ovariectomised group; Scale= 200µm

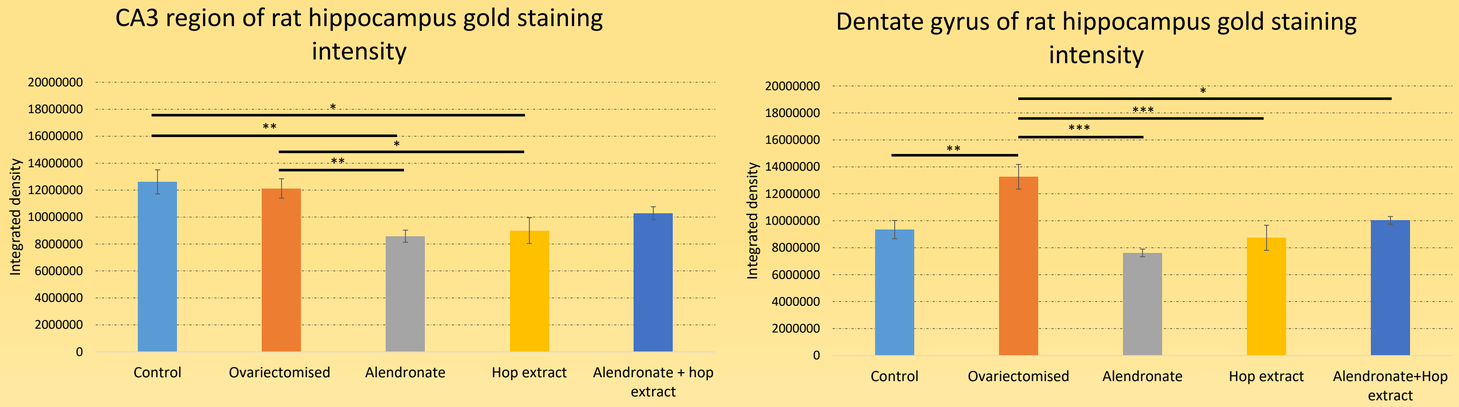

Figure 4. Quantification of gold stain intensity in CA3 and DG areas of brain sections of 5 different animal groups. Data is presented as integrated density of colour measured in region of interest. CA3: cornu ammonis 3. One way ANOVA for CA3 region $F_{(4,41)}=6.394$, $p=5.136\times10^{-4}$. One way ANOVA for dentate gyrus $F_{(4,41)}=10.37$, $p=9.796\times10^{-6}$; Post hoc Tukey HSD test *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$

Results and Conclusion

Brightfield microscope examination showed excellent quality of the nerve fiber impregnation. Significant changes in the staining intensity were found in several brain regions of interest between examined groups (primary motor cortex, dentate gyrus and cornu ammonis 3). The impregnation technique was very useful for the quantification of nerve fibers when combined with ImageJ image analysis software. Although impregnation methods in neuroscience are 150 years old, gold impregnation can be effectively used in modern research for the quantification of nerve fibers on brain cryosections using freely available image analysis software.

References

1. Golgi, C., 1873. Sulla struttura della sostanza grigia del cervelo. Gazzetta Medica Italiana. Lombardia 33, 244–246.
2. Schindelin, J., Rueden, C. T., Hiner, M. C., & Eliceiri, K. W. (2015). The ImageJ ecosystem: An open platform for biomedical image analysis. Molecular Reproduction and Development, 82(7–8), 518–529. doi:10.1002/mrd.22489