A Modified Golgi Impregnation method for In Vitro MR Microscopy

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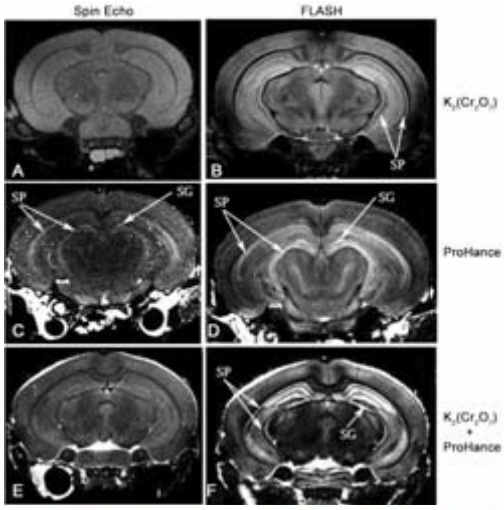
In the late 19th century Camillo Golgi revolutionize the budding discipline of neuroscience with his Black Reaction [1, 2], now known as the Golgi stain. This reaction stains a small eclectic, likely random, portion of neurons and processes in the tissue of interest. The Golgi stain consists of pretreatment with potassium dichromate and other additives [3] followed by 'development' with silver nitrate resulting in black deposits clearly delineating soma, axons, and dendrites against a yellow background. More recently, Johnson and colleagues have pioneered efforts in microMR histology [4] where different weighting schemes (T_1-weighted, T_2-weighted, proton density weighted, etc.) as well as contrast agents are used in an effort to highlight specific details in a manner analogous to Golgi's Black Reaction. Contrast in the MR image arises from physio-chemical heterogeniety in the underlying tissue structure that modulate concentration and relaxation characteristics of the water protons within the tissue. Although there is much speculation as to what precisely the Black Reaction does, potassium chromate, $K_2(Cr_2O_7)$;, is a powerful oxidizer. We hypothesized that just as pretreatment with $K_2(Cr_2O_7)$ alters tissue properties in a dramatic way for subsequent silver staining, these altered tissue properties (and perhaps locally high concentrations of the Cr(III) paramagnetic) would give rise to different MRI contrast and/or differential absorption of the gadolinium contrast agent ProHance. Enhanced or different contrast would lead to better definition of anatomical features and more facile identification of abnormalities.

Materials and Methods

Adult male mice (C57LB/6) were used in the study. Fixation in 4% PFA was accomplished by cardiac perfusion with 4% PFA, decapitation, and soaking of the head in 4% PFA for 24 hours. The skin, lower jaw, ears, cartilagenous nose tip were then removed, and the brains with the intact skull were processed by soaked for 5 days at 4°C in one of three different solutions (each containing 0.01% sodium azide):

1). 6% potassium dichromate solution ($K_2(Cr_2O_7)$);
2). 10 mM ProHance (Gd-HP-DO3A from Bracco Diagnostics);
3). 10 mM ProHance in 6% potassium dichromate solution.

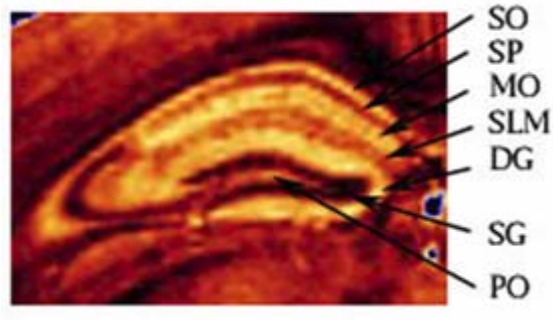


MR Microscopy

Imaging was performed with an 11.7T Bruker Avance imaging spectrometer with a microimaging gradient insert and 20 mm birdcage RF coil, samples immersed in fomblin. Imaging parameters were as follows: T_1-weighted 3D spin echo (SE) imaging protocol (TR/TE, 50ms/4.2ms, 512x256x256, 25mm x $(12.5mm)^2$; NA=12) and 3D FLASH (TR/TE, 25ms/4.2ms; flip angle: 30^o; 512x256x256, 25mm x $(12.5mm)^2$; NA=18).

Results and Discussion

Coronal slices through the hippocampal region from 3D MR images of samples subjected to one of the three soaking procedures are shown in the top figure. In the right column it is apparent that in SE T_1 weighted images the three procedures afford qualitatively different contrast but that the images exhibit relatively little anatomical detail. In the Prohance soaked sample, we do observe hyperintensity in the stratum pyramidale (SP) and Granule Cell Layer (SG) attributable to selective partitioning of ProHance into these structures. The FLASH sequence employed to obtain the left column provides both T_1 and T_2* weighting. Each soak procedure provides qualitatively different contrast with a wealth of anatomic detail is apparent in these images. The bottom figure shows an expanded view of the hippocampus of the ProHance plus $K_2(Cr_2O_7)$ treated sample. The detailed layers of the hippocampus are easily identified: SO, Stratum Oriens; SP, Stratum Pyramidale; MO, Molecular Layer; SLM, Stratum Lacunosum Moleculare; DG, Dentate Gyrus; SG, Granule Cell Layer. PO, Polymorphic Layer.



Conclusion

We present a method for in vitro MR histology that combines 19th & 21st century technology. With this new method, neuroanatomical contrast is dramatically improved compared to conventional fixation. Further studies are needed to optimize the fixatives, fixation time and MR parameters with this technique. Open questions remain about the underlying contrast mechanisms. Possibilities under investigation include tissue structure modifications, Cr(III) localization, and preferential localization of Prohance.

1. Golgi, G. *Gazzetta Medica Italiana: Lombardia*, 1873. 33, 244.
2. Pannese, E., The Golgi Stain: Invention, Diffusion and Impact on Neurosciences, in *Journal of the History of the Neurosciences*, M. Bentivoglio and A. Vignolo, Editors. 1999, Taylor and Francis: New York, NY. p. 132.
3. Rosoklija, G., B. Mancevski, B. Ilievski, T. Perera, S.H. Lisanby, J.D. Coplan, A. Duma, T. Serafimova and A.J. Dwork *Journal of Neuroscience Methods*, 2003. 131(1-2), 1.
4.Johnson, G.A., H. Benveniste, R.D. Black, L.W. Hedlund, R.R. Maronpot and B.R. Smith *Magnetic Resonance Quarterly*, 1993. 9(1), 1.