1390
doi:10.1017/S1431927622005669
Microsc. Microanal. 28 (Suppl 1), 2022

Microwave Brain Tissue Processing: Making Life Easier for Ultrastructural Immunohistochemical Analysis of Synapses in Rodent Models of Neurodegeneration

Cynthia Moore[1] and Charles K. Meshul[1,2,*]

[1.] Neurocytology Lab, VA Medical Center/Portland, Oregon, USA
[2.] Department of Behavioral Neuroscience and Pathology, Oregon Health & Science University, Portland, OR, USA
[*] Corresponding author: meshulc@ohsu.edu

There is a fine line between obtaining both sufficient immunohistochemical labeling and satisfactory ultrastructural brain tissue preservation. With the increased use of valuable and limited genetic rodent models, carrying out both light and electron microscopic analysis in the same brain tissue samples is essential. In addition, because of the complex synaptic connections within the brain, localizing multiple antigens using several antibodies in the same tissue has now become necessary. A significant advance in this area has come with the development of microwave technology to significantly decrease the time of tissue processing and hopefully leading to increased depth of tissue penetration of the antibody in the absence of detergents. With the use of the PELCO Biowave Pro® (Ted Pella, Inc), we have developed protocols for both light and electron microscopic immunohistochemistry, in which we can single, double or triple label the same slice of brain tissue for ultrastructural analysis, as detailed below: (https://www.facebook.com/MeshulSpecial). In our lab, the normal bench time to process tissue for electron microscopy (EM) takes about 22 hours. Using the Biowave Pro®, the total time for similar EM tissue processing is only about 41 minutes. To carry out single or double labeling for immunohistochemistry, bench top tissue processing in our lab takes between 27 and 39 hours to complete. By using microwave technology, that time is decreased to only 85 minutes. However, the most significant advance using the Biowave Pro® is post-fixing the intact/whole brain in the microwave to complete the tissue fixation process without over-fixing and compromising the antigenicity of the protein(s) of interest. Instead of the need to typically post-fix the whole brain overnight at 4^{o} C in the fixative, we have found that such a procedure can be carried out in about 45 minutes using the Biowave Pro®. However, the most important feature is the use of the Pelco SteadyTemp, which recirculates the fixative at a constant temperature of 25^{O}C for the 45-minute post-fixation time.

Using a toxin-based rodent model to mimic the progressive loss of dopamine as observed in patients with Parkinson's disease, we have reported changes in the density of nerve terminal glutamate immuno-gold labeling within identified nerve terminals making an asymmetrical/excitatory contact onto a dendrite that originates from dopamine neurons (Figure 1) [1]. Using either another toxin-based Parkinson's disease model [3] or a Huntington's disease rodent model [2], we have reported alterations in nerve terminal glutamate density that may be associated with the diseased brain. By carrying out essentially triple labeling to identify the origins of the nerve terminal, the postsynaptic dendrite/spine, and the relative density of the glutamate neurotransmitter within both the terminal and spine, this provides more detailed characterization of the changes in glutamate synapses in these disease models that will be important in terms of possible future therapy [4].

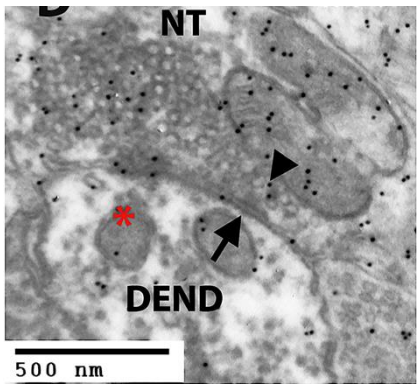

Figure 1. Diaminobenzidene (DAB)(darkened reaction product)-labeled nerve terminal (NT), immunolabeled using an antibody against the vesicular glutamate transporter 2, is seen making an asymmetrical synaptic contact (arrow) onto a DAB-labeled (*) dendrite (DEND), that has been immunolabeled using an antibody against tyrosine hydroxylase. Within the NT are 12-nm gold particles, showing the localization of an antibody against the neurotransmitter, glutamate.

References:

[1] C Moore, M Xu, JK Bohlen, and CK Meshul. European Journal of Neuroscience, **53**, (2021), 2061–2077. doi: 10.1111/ejn.14894
[2] A Parievsky, C Moore, T Kamdjou, C Cepeda, CK Meshul, and MS Levine. Neurobiology of Disease, **108**, (2017), 29-44. doi: 10.1016/j.nbd.2017.07.020.
[3] RH Walker, C Moore, G Davies, LB Dirling, RJ Koch, and CK Meshul. PLoS One, **7(3):**e32919 (2012). doi: 10.1371/journal.pone.0032919.
[4] The authors acknowledge funding from the Department of Veterans Affairs Merit Review Program. A very personal thanks to Ted Pella, a true gentleman, and his company for their continued support of not only my lab but of our local Pacific Northwest Microscopy Society. He is truly missed but his legacy continues.