# MODIFICATION OF AGNOR STAINING TO REVEAL THE NUCLEOLUS IN THICK SECTIONS SPECIFIED FOR STEREOLOGICAL ASSESSMENT OF DOPAMINERGIC NEUROTOXICITY IN SUBSTANTIA NIGRA, PARS COMPACTA

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## ABSTRACT

Silver stains have been developed and modified to identify cellular components of the nervous system including neurons, astrocytes, and microglia, and subcellular structures including nucleic acids and proteins, in both normal and pathological states. Modified stains have been devised to reveal degenerative or reactive cell phenotypes, or the disintegrative and/or neuropathic lesions associated with Alzheimer's, Parkinson's, and Pick's diseases, Down's syndrome, or chemical toxicity. The nucleolus is the site of ribosome biosynthesis. Chromosomes contain a nucleolar organizing region, or NOR, that contains ribosomal genes and associates with the nucleolus. NORs contain argyrophilic, acidic proteins referred to as "AgNORs". Stains developed to reveal AgNORs in paraffin sections have been used extensively as diagnostic tools in oncology. The use of stereology as a tool to estimate cell number has become increasingly prevalent in neuroscience experiments. Requirements for experimental tissue have been refined to use thicker sections, between 40 to 80 microns, to increase the number of optical planes available for analysis; however, thick sections require modified protocols to assure complete penetration of stains. There has also been a shift in the 'counting unit' from the nucleus to the nucleolus to quantify cells that come into focus in the counting frame. The AgNOR staining protocol was modified to stain nucleoli in thick sections prepared for stereological evaluation of the dopaminergic neurons of the substantia nigra, pars compacta. The modifications included incubation in HCl to permeabilize the tissue, a bleaching step to reduce non-specific background silver staining, and counterstaining with reduced-strength tyrosine hydroxylase immunohistochemistry. Nucleoli appeared as black dots against a pale amber background. Dilutions of TH primary antibody were evaluated to maximize identification of neurons contained within the regional nucleus, but reduce the overall density of staining to prevent the stained nucleolus from being obscured. This optimal counterstain dilution was one-quarter normal strength. The silver nucleolar stain was compared to immunohistochemistry for nucleolin and found to be superior. Our modified protocol was used to visualize the nucleolus in several mammalian species (mouse, rat, monkey, and human) and was used in stereological assessments of compounds that cause dopaminergic neurotoxicity.

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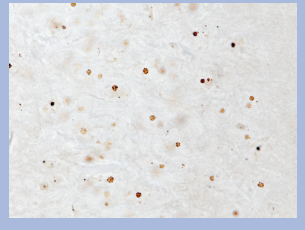
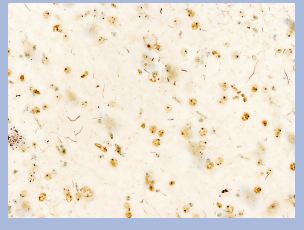
## RESULTS

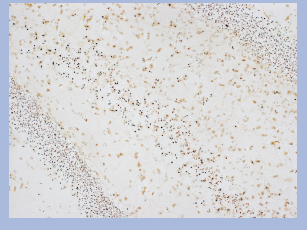


AgNOR particles - 80X



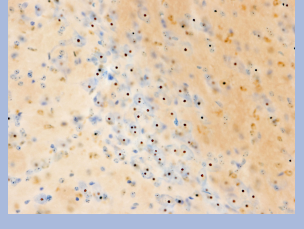
Rat cortex

CA1 - CA3 transition



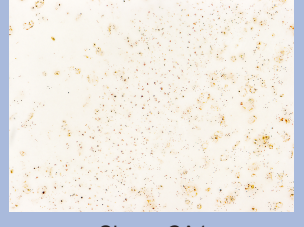
Rat dentate gyrus

SNpc no counterstain

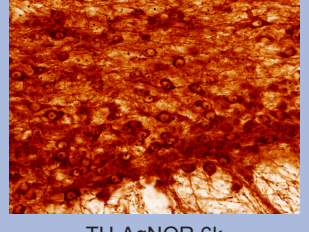


SNpc Nissl counterstain

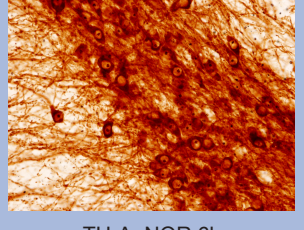
Monkey dentate gyrus



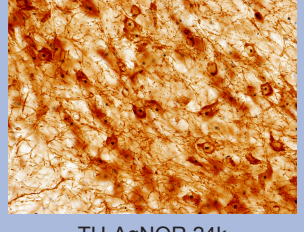
Sheep CA1



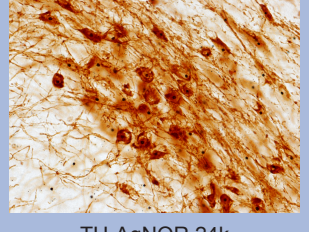
TH-AgNOR 6k



TH-AgNOR 6k



TH-AgNOR 24k



TH-AgNOR 24k

## CONCLUSIONS

1. The AgNOR silver staining method was modified to stain the nucleolus in thick sections.

2. Staining was evaluated across several mammalian species.

3. AgNOR staining revealed heterogeneity among nucleoli in different cell populations.

4. AgNOR staining was combined with Nissl and IHC for tyrosine hydroxylase.

5. TH staining intensity was optimized for thick sections.

6. AgNOR-TH dual staining enhances estimation of neuron number in stereological studies of dopaminergic neurotoxicity.