Degeneration and Normal Neuron Staining Method to Aid in Quantitative Analysis

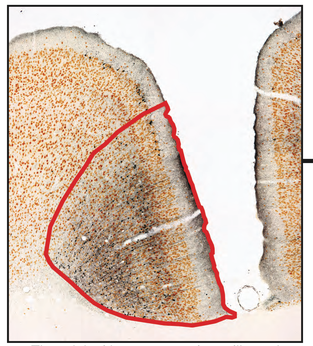
Tipton, B., York, T., Zurhellen, C., Baun, J., Switzer III, R.C.

NeuroScience Associates, 10915 Lake Ridge Drive, Knoxville, TN 37934

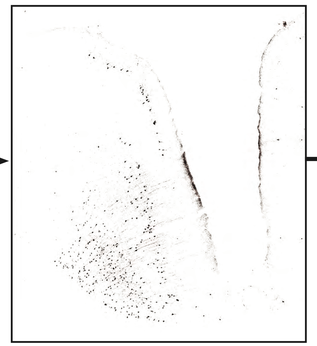
Abstract

The Amino Cupric Silver (ACS) stain of deOlmos reveals neurons in the degenerative (disintegrating) state in the brain and spinal cord. These neurons stain intensely black, while normal neurons do not take up the stain. To assess the degree or the amount of degeneration, a numbered score (0,1,2,3,4, with 4 being highest level of degeneration) is usually assigned. Here we describe a more quantitative means for this type of evaluation that can be achieved by applying a second stain "over" the ACS stain. The antibody NeuN reveals a marker expressed in all normal neurons over 2 weeks old. When combining the ACS stain with NeuN IHC on the same tissue, degenerative neurons stained with the ACS protocol can be counted against "live" neurons that stain positive for NeuN.

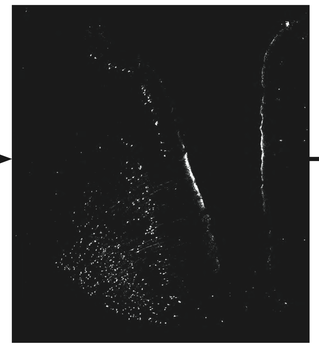
Using imaging software (FIJI or "Fiji Is Just ImageJ") the numbers of degenerating (black) neuron and NeuN positive (brown) neurons can be determined for any given region of interest. Using "batch processing" methods, captured images are pre-processed using Adobe Photoshop to make them more amenable for image analysis. Further batch processing using brightness, contrast, selection and thresholding tools, black degenerating neurons and brown "live" neurons are isolated for quantification using the "particle analysis" tool in FIJI. Although this method does not replace quantitative determinations provided by the more time consuming and expensive stereologic analysis, it does provide a numeric index of observables rather than a subjective assessment.



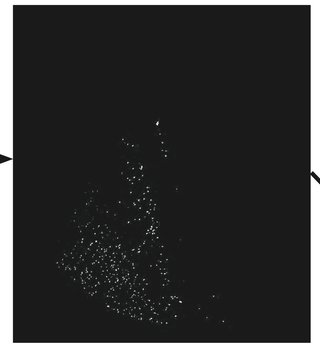
The original image was color-calibrated using Chromacal from Datacolor to ensure consistency throughout the analysis process. The top series of images shows the segmentation and thresholding techniques used to quantify degenerating neurons in cingulate cortex of rat. The bottom series shows the same steps for quantifying NeuN-positive cells. The region of interest outlined above was used for both processing series.



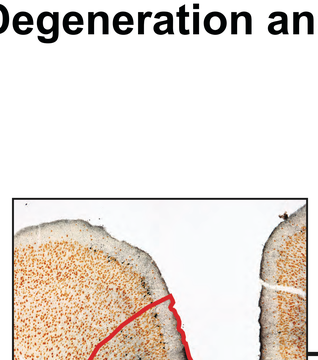
In Photoshop, the "Color Select" tool was used to isolate degenerating neurons (above) and NeuN-positive cells (below).

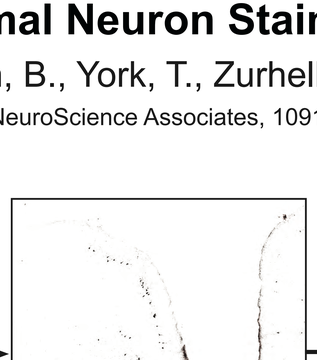


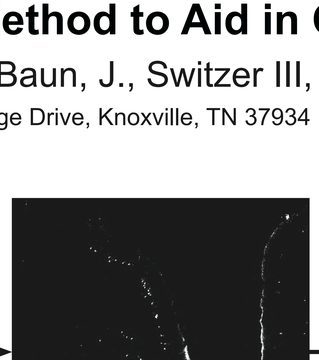
In FIJI ("Fiji Is Just ImageJ"), the image was converted to gray-scale and then thresholded to make a binary image. Using a series of noise filters (Despeckle and Remove Outliers), extraneous minute particles were erased while the cells were preserved. A watershed filter was applied to separate clumps of cells.

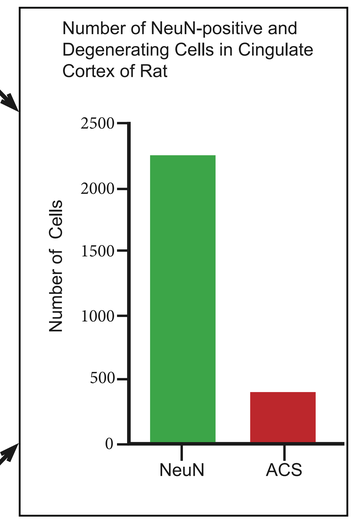


The region of interest (ROI) was traced on the original image. This outline selection was applied to the binary image. Everything outside of the selection was erased so as not to interfere with the quantification of cells.

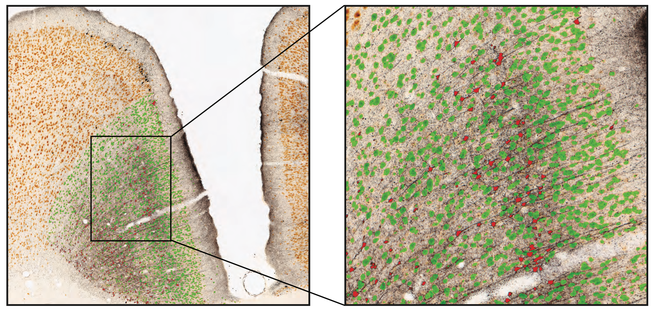








False color Look-Up Tables (LUT) were applied to the binary ROI images from the series above and overlaid onto the original image. Degenerating neurons are shown in red and NeuN-positive cells are shown in green. This creates a nice visualization comparing the two cell populations of interest.



Fluorescent markers can also be used to visualize and analyze this data. Images to the right show NeuN-positive cells (red channel) and degenerating neurons (green channel). FluoroJade-B was used to reveal the degenerating neurons. Similar steps to the ones described above can be used to quantify NeuN-positive cells and degenerating neurons in the fluorescent images.