Comparing three different staining methods to evaluate seizure-induced neuropathology in rats

Dhrumil Mehta
Mentored by Dr. John McDonough

Introduction

It is known that exposure to chemical warfare nerve agents such as soman can cause severe neuropathological damage in organisms. Staining methods are one way to identify neuropathological damage in the form of dead or dying neurons. The Hematoxylin and Eosin (H&E) stain results in bright red or purple coloration of degenerating cells, while the Fluoro-Jade B stain colors degenerating cells a fluorescent green. More recently, the Woelcke stain has been identified as perhaps an ideal stain to identify degenerating neurons because of the high contrast in the dark blue dying neurons and the gray background (Carpentier, Foquin, & Dorandeu, 2011). The purpose of this study was to distinguish degenerating neurons using the H&E, Fluoro-Jade B, and Woelcke stains to determine the most accurate method to identify dead neurons in soman-exposed rats.

Methods and Materials

A rotary microtome was used to cut 23 triplets of serial sections from brain tissue of both soman-exposed and unexposed rats. After being cut, each section of the triplet was mounted onto a slide, and then given a number corresponding to the stain that would be used on the tissue. Tissue 1 was stained with the Woelcke stain, tissue 2 with the Fluoro-Jade B stain, and tissue 3 with the H&E stain.

All slides were deparaffinized and rehydrated before the rest of each staining procedure which is listed below. The Woelcke staining procedure consisted of immersing slides in 5% iron alum overnight, washing in a distilled water bath, immersing in Weigert hematoxylin, rinsing in running tap water, submerging in 80% ethanol to differentiate between background and staining, and coverslipping in Permount™. The Fluoro-Jade B staining procedure involved pretreating slides in 0.06% potassium permanganate, immersing slides in 0.001% Fluoro-Jade B solution in the dark, dehydrating, and coverslipping in Permount™. The Fluoro-Jade B staining procedure consisted of immersing slides in 5% iron alum overnight, washing in a distilled water bath, immersing in Weigert hematoxylin, rinsing in running tap water, submerging in 80% ethanol to differentiate between background and staining, and coverslipping in Permount™. The H&E staining procedure was comprised of immersing in Mayer’s hematoxylin solution, counterstaining with eosin phloxine solution, dehydrating, and coverslipping in Permount™. Slides were scanned to create digital image files. Degenerating neurons were counted by outlining desired regions and counting dying neurons. The brain regions that were counted were the piriform cortex, amygdala, thalamus, and hippocampus. Data between brain regions was normalized by dividing the number of cells counted by the area of the region.

Results

The degenerating neuron counts of rats exposed to soman should generally be higher than rats that are not exposed to soman, which tends to be mostly the case in Graphs 1-4, since substantial brain damage should be present. A two-way ANOVA was conducted on the data prior to running the Bonferroni post-hoc test. The Bonferroni post-hoc test from Table 1 looks for significance between each type of stain to determine which stain can most accurately identify degenerating neuron counts.

Conclusions

The purpose of this study was to determine the most accurate staining method to identify degenerating neurons in soman-exposed rats. The Bonferroni test from Table 1 shows that the H&E and Fluoro-Jade B stains are significant for 2/4 brain regions, although the Fluoro-Jade B stain had a higher level of significance in the hippocampus, and was more significant in the amygdala than the H&E stain was in the thalamus. Thus, the most reliable method as found by this study was the Fluoro-Jade B stain, while the H&E stain was the second most reliable method. The Woelcke stain was not shown to be a reliable method for detecting neuropathological damage. Most notably, the Fluoro-Jade B stain showed the greatest ability to distinguish between control and exposed tissue in all four brain areas. All 3 stains are presented in Figure 1. More training and education on detecting degenerating neurons in each stain could have made the results of this study more accurate. Subsequent research would be needed to validate these findings by completing multiple trials of the same study, by using people trained to detect degenerating neurons.

References


Acknowledgements

This entire project and everything to do with it was possible with the help of the following people: Dr. John McDonough, Ms. Caitlin Karolenko, Dr. Jennifer Winkler, Dr. Jacob Skovira, Mr. Hunter Martin, Dr. Hilary McCarren, and Ms. Linda McDonough.