Neuropathology in pediatric rat brains following nerve agent-induced seizures

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Introduction

Nerve agents used in chemical warfare cause seizures by inhibiting acetylcholinesterase. If seizures are not stopped quickly, then long term problems can occur such as neuronal death and cognitive decline (White et al., 2012). In the event of a chemical attack with nerve agent, children will be among the civilian victims that are affected. Although studies for neuronal damage have been conducted for adult populations, sufficient studies for younger populations have not been conducted (Ardinger et al., 2016). In this study, the brains of pediatric rats were analyzed and neuronal damage was assessed and mapped to determine if there are differences in damage between age groups.

Methods

Sprague-Dawley rats that were age postnatal day (PND) 21, 28, and 70 were used in this study. Each subject was exposed to the nerve agent sarin or VX and needed to develop a continuous seizure lasting at least four hours. Potential subjects were found in the experimental notebooks and then confirmed by viewing their EEG files. If the amplitude of the EEG graph exceeded twice the baseline amplitude for the entire experiment, then these subjects were analyzed. In total, the brains of 23 subjects were analyzed: nine PND 21 subjects, seven PND 28 subjects, and seven PND 70 subjects. For each subject, ten µm sections of the anterior, middle and posterior parts of the brain were stained with Hematoxylin and Eosin to identify necrotic neurons. Two regions of interest were demarcated in each section and were analyzed by performing cell counts using FIJI (newest version of ImageJ). The figures to the right are rat brain atlas pages that have the regions of interest outlined in red. For the anterior section, the regions of interest were a 4,000 by 2,200 pixel box in the piriform cortex, and the left side of the septum (Figure 1). For the middle section, the regions of interest were a 2,700 by 2,200 pixel box in the amygdala, and the left side of the hippocampus (Figure 2). For the posterior section, the regions of interest were a section of the entorhinal cortex and a section of the retrosplenial cortex (Figure 3). After cell counts were performed in each region of interest, a one-way ANOVA test was completed for each region of interest to determine if there was a significant difference in damage between age groups.

Results

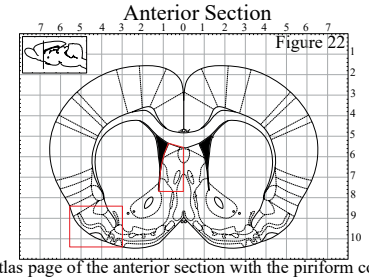


Figure 1: Rat atlas page of the anterior section with the piriform cortex and septum outlined in red.

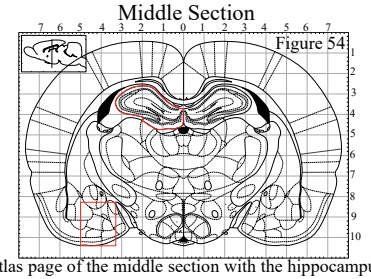


Figure 2: Rat atlas page of the middle section with the hippocampus and amygdala outlined in red.

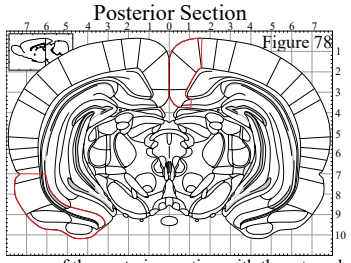
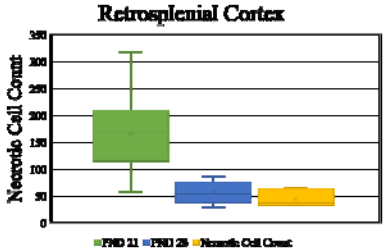


Figure 3: Rat atlas page of the posterior section with the retrosplenial cortex and entorhinal cortex outlined in red.

For each region of interest, the difference in the number of necrotic neurons was evaluated between the three age groups by one-way ANOVAs. For the septum, piriform cortex, amygdala, hippocampus and entorhinal cortex there were no significant differences in cell counts between age groups. For the retrosplenial cortex there was a significant age group effect ($F(2, 22) = 14.46$; $p < 0.0001$); Tukey's multiple comparison test showed the PND 21 animals had significantly greater damage than either the PND 28 or PND 70 groups, which were not different from one another (Graph 1).

Results (continued)



Graph1: Necrotic cell counts of the retrosplenial cortex for PND 21, 28,and 70 rats.

Conclusions

The purpose of this study was to determine if there was a significant difference in brain damage between rats of age PND 21, 28, and 70 after exposure to the nerve agents sarin or VX. The results of the one-way ANOVA tests after performing necrotic neuron counts show that there was a significant difference in damage between the age groups for only one region of interest, the retrosplenial cortex. The other regions of interest did not have significant differences in neuronal damage between age groups. PND 21 aged rats had significantly greater damage than PND 28 or PND 70 rats, while there was no difference in damage between the PND 28 and the PND 70 rats. The results from this study can be a starting point for understanding the pathology of pediatric rat brains after exposure to a nerve agent, VX or sarin. This may eventually help researchers to develop anticonvulsant drugs to treat children that have come into contact with a nerve agent during a chemical attack.

References

Ardinger, C., Dunn, E., Gyenai, K., McCarren, H., Miller-Smith, S. M., & McDonough, J. H. (2016, June). *The ability of common anticonvulsants to treat nerve agent-induced seizures in pediatric rats.* Poster session presented at the USAMRICD 2016 Bioscience Meeting, Aberdeen Proving Ground, MD.

White, H. S., Alex, A. B., Pollock, A., Hen, N., Shekh-Ahmad, T., Wilcox, K. S.,…Bialer, M. (2012). A new derivative of valproic acid amide possesses a broad-spectrum antiseizure profile and unique activity against status epilepticus and organophosphate neuronal damage. *Epilepsia, 53*(1), 134-146.