Regional distribution of neuropathology in the rat brain following nerve agent exposure

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Introduction

Nerve agents are chemical warfare weapons that bind to the enzyme acetylcholinesterase (AChE) to prohibit the enzyme from breaking down the neurotransmitter acetylcholine (ACh). The resulting excess of ACh in the synapse causes excessive neuron firing, resulting in seizures (Aroniadou-Anderjaska et al., 2016). When these seizures become continuous, the body enters a state of status epilepticus (SE) that results in neural damage. Examination of brain damage in nerve agent exposed animals is typically limited to specific brain regions, such as the hippocampus, amygdala and piriform cortex (Petras, 1994). Understanding the extent and location of neuronal cell death throughout all areas of the brain is essential to relate the damage to post-exposure changes in behavior. The purpose of this project is to create a map of the neuropathological damage throughout the rat brain following exposure to the nerve agent soman.

Materials and Methods

Four male Sprague-Dawley rats previously implanted with cortical electrodes were exposed to the nerve agent soman. All animals developed continuous seizures. Four hours later the animals were deeply anesthetized and then perfused with saline followed by 10% neutral buffered formalin. The brains were blocked anterior to posterior with three cuts—one at the infundibulum, one 3 mm above and one 3 mm below. The blocks were embedded in paraffin and then sectioned at 5 µm with sections taken every 300 µm. The sections were labeled, put on slides and stained with hematoxylin and eosin (H&E) to show morphology (Figure 1). There were approximately thirty slides per brain, resulting in 180 slides total. The main regions of the brain were then microscopically assessed for damage to create a pictorial map of the neuropathology. The damage was then marked in atlas plates of a normal rat brain (Paxinos & Watson, 2004) with an "x" marking damaged areas.

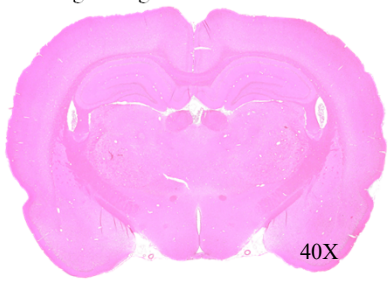


Figure 1 (left): A section at -1.75 mm from bregma of an exposed rat; H&E stained neurons appear a light pink.

Results

The damage (Figure 2) in each brain region was examined at four anterior-posterior (A-P) levels to create a combined map of damage marked in each region (Figures 3-6). Neural death was the most prevalent in deep cerebral white matter (DCW) throughout the entire brain. The damage was scattered throughout almost all regions of the brain, with more severe clusters in the hippocampus, piriform cortex, amygdala, striatum and around the DCW. The most severe damage was seen in the piriform cortex.

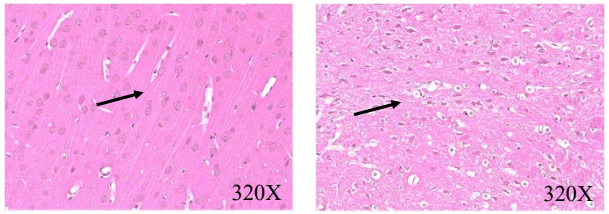


Figure 2 (above): Healthy tissue, left, from the rat brain shows healthy neurons (arrow) that. Damaged tissue, right, shows necrotic neurons (arrow) that are condensed darker cells.

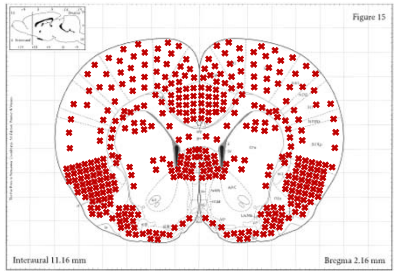
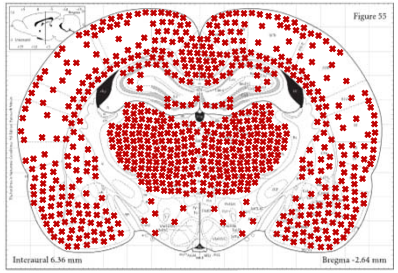


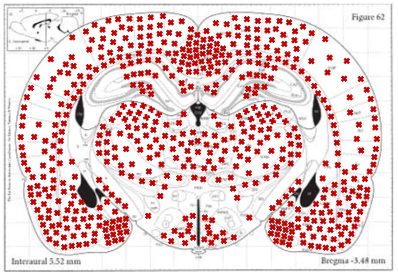
Figure 3 (left): Amalgamated map of the neural damage in the two brains at A-P 2.16 mm. There is damage throughout most of the brain with severe damage in the septal nuclei, cingulate cortex and insular cortex.

Figure 4 (right): Amalgamated map of the neural damage throughout all four brains at A-P -2.64 mm. Damage was severe throughout the putamen, thalamic nuclei, piriform cortex, and amygdala.



Results

Figure 5 (right): A combined map of all four brains at A-P -3.84 mm. Damage was extensive to many structures with heightened damage in the retrosplenial granular cortex and medial amygdala.



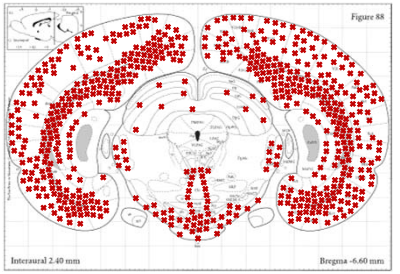


Figure 6 (left): An amalgamated map of neural damage at A-P -6.60 mm. Damage is most extensive in the dentate gyrus, other posterior parts of the hippocampus, entorhinal cortex, and around the caudal raphe nuclei.

Conclusions

The creation of an atlas that maps damage throughout the rat brain shifts the focus of analysis from specific regions to a holistic assessment of damage, thus creating an understanding of neural injury throughout the brain. The regions damaged can also correspond to changes in behavior after exposure (Aroniadou-Anderjaska et al., 2016). The analysis of the entire brain highlights areas of damage such as the DCW, peduncular cortex, retrosplenial granular cortex and other regions not typically analyzed which could prompt further research.

References

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Petras, J. M. (1994). Neurology and Neuropathology of Soman-Induced Brain Injury: An Overview. *Journal of the Experimental Analysis of Behavior*, 61, 319-329.