Introduction

Soman (pirbuterol methylphosphonofluoridate) is a highly toxic nerve agent. Like all other nerve agents, soman attacks the mammalian nervous system by inhibiting the acetylcholinesterase enzyme (AChE) so that it can no longer hydrolyze the neurotransmitter acetylcholine (ACh) into choline and acetic acid. Because the ACh is not being destroyed an excess builds up within the body and triggers cholinergic over-activity which can lead to a cholinergic crisis, the signs of which are nasal and bronchial secretions, difficulty breathing, muscle fasciculations, and, in cases of severe poisoning, seizures. All nerve agents are potent convulsant compounds that produce prolonged epileptic-like seizures in both experimental animals and humans (McDonough and Shih, 1997). If not rapidly treated, these seizures can progress to a condition of continuous seizure known as status epilepticus, a condition that by itself is considered a medical emergency. Prolonged seizures elicited by nerve agents can be malicious, causing cardiac dysfunction and producing lesions in vulnerable brain areas (Siedel, 1997).

Development of improved medical countermeasures to treat nerve agent-induced neuropathology have been studied and described in many test rodent animals but little research has been conducted in the area of non-human primates (Britt et al., 2000; Hayward et al., 1990). Non-human primates, in particular the African green monkey, are known for their similarities to the physiology of man and are often used in drug testing (Hayward et al., 1990). It is the purpose of this project to provide a thorough description of nerve-agent-induced lesions in the brain of an African green monkey that had been exposed to the nerve agent soman, developed prolonged seizures and presumably should display seizure-induced damage in multiple neural areas (Collombet et al., 2000, Baillie et al., 2005).

Materials and Methods

African green monkey brains from a previously performed study in which the animals had been exposed to the nerve agent soman were used. The brains were preserved in 10% paraformaldehyde, embedded in paraffin wax and then sectioned on a microtome at five microns. These sections were transferred onto slides for staining. The histopathological stains thionin and hematoxilyn and eosin (H&E) were chosen to be optimized. A test brain was utilized for several trials, these trials were to ensure the staining of the experimental brain was consistent, structure, and microscopic. A scanner was used to capture a slide of every section, these slides then were stained. The stains finished, analysis of the brain began. This step consisted of three parts; contrast, structural, and microscopic. A scanner was used to capture a picture of the slide, then Adobe Photoshop manipulated the image, creating contrast. Structure analysis depends on the contrast without which the structures are unidentifiable. A brain atlas was used and brainmaps.com were references used while identifying structures (Paxinos et al., 2000). Microscopic analysis was then completed using a microscope and digital software.

Results

Contrast and structural analysis showed differentiation between brain structures (Fig. 1); the gyri, sulci, and hippocampus were well defined. And though some smaller midbrain components were not differentiable, the project was not hindered by this (Fig. 2).

Figure 1. Progression of five micrometer anterior brain section through contrast analysis.

Figure 2. Five micrometer cross-section of anterior brain to compare contrast analysis (left) and H&E stain (right).

Microscopically, the entorhinal cortex and subiculum subdivision of the hippocampus displayed a pattern of neuronal damage. H&E highlights main cellular material and is a tool to separate living cells from dead, the dyning process yielding its trademark pink stain evident in the 4X magnification of Fig. 3. The 10X magnification allows for observation of cellular tissue, texture, and cell death.

Figure 3. Microscopic images of hippocampus at 4x, and a magnification of the entorhinal cortex and subiculum subdivision from indicator box at 10x and 20x.

Conclusions

Several sections of the experimental brain were mounted, stained, and analyzed, but the entire brain must be evaluated before any final conclusions can be drawn as to all the brain areas affected.

Sections of the entorhinal cortex and subiculum subdivision of the hippocampus displayed notable neuropathology. Hippocampal damage due to soman-induced seizures has been reported in rats and guinea pigs (Baillie et al., 2005; Collombet et al., 2006) and similar damage has now been localized in the same areas in this non-human primate.

Scanning in the slide images using a standard document scanner did not yield images with the sufficient contrast needed to identify all brain structures. A new, high-contrast, microscopic slide scanner is due to be received soon and will be utilized in further research.

Works Cited


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