Effect of organophosphate chemical exposure on REM sleep

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Introduction

Organophosphates (OPs) are a class of toxic chemicals that disrupt the mechanisms of the body (Bajgar, 2004). OP chemicals irreversibly bind to the enzyme, acetylcholinesterase (AChE), which is responsible for muscle contractions. During exposure, OPs cause numerous unpleasant symptoms such as salivation, convulsions, and seizures that can result in death. Previous research has shown that chemicals similar to OPs cause an increase in episodes of paradoxical or rapid eye movement (REM) sleep in rats (Gnadt et al., 1985). REM sleep is a stage of the sleep cycle when intense dreaming occurs. Increased episodes of REM sleep causes decreased sleep quality which is associated with poor immune function, depression, anxiety, and poor recovery after physical trauma. The electroencephalogram (EEG) signal, which records brain activity, and the electromyography (EMG) signal, which measures muscle activity, can be seen in Figure 1 for each stage in the sleep cycle. The purpose of this research was to determine how exposure to an OP chemical affects REM sleep. It was hypothesized that the mean difference in percent time spent in REM sleep between pre- and post-OP exposure would not equal zero. This research is part of a larger experiment that will eventually test the effect of anticholinergic medications on REM sleep.

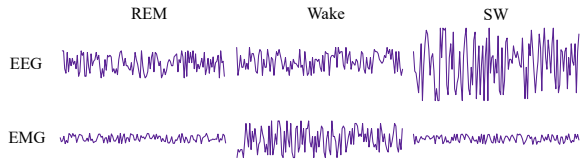


Figure 1 (above): Examples of REM sleep, wakefulness, and slow-wave (SW) sleep measured from −1 to 1 mV (*y*-axis) over a period of ten seconds (*x*-axis). NeuroScore™ software used this type of data to assign the stage of the sleep cycle a rat is in during a given epoch of time.

Materials and Methods

Materials included EEG and EMG transponders, Ponemah recording software, Sprague-Dawley rats, sucrose water, scopolamine hydrobromide, NeuroScore™ software, Excel software, Minitab software, and a computer.

The procedure consisted of surgically implanting EEG and EMG electrodes into the rat's skull and neck muscles, respectively (Figure 2). The rats were then given a four-day recovery period. Baseline EEG and EMG data of the time spent in each stage of the sleep cycle were then collected over three days.

On day seven, the rats were exposed to an OP chemical by subcutaneous injection (Figure 2) and then followed for ten days after exposure, during which post-exposure sleep cycle data was collected. Dosages of the OP chemical were varied to produce observable behavioral signs of intoxication, which is typical protocol for this type of experiment.

Once all the data was collected, the rats were euthanized, perfused and dissected. NeuroScore™ identified time spent in each stage of sleep during two-hour epochs. The data was exported to Excel for processing, where mean percent time in each stage was calculated for all epochs. In this research, a paired *t*-test compared means in pre- and post-exposed rats for REM sleep, slow-wave sleep, and wakefulness.

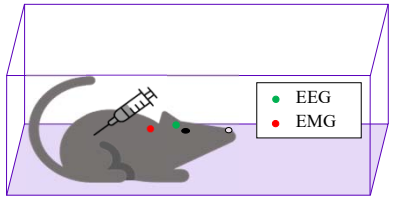


Figure 2 (left): Shown is a depiction of the rats being exposed to an OP on the seventh day through a subcutaneous injection. The dosage of OP chemical varied depending on the rat and when the rat showed behavioral signs of intoxication.

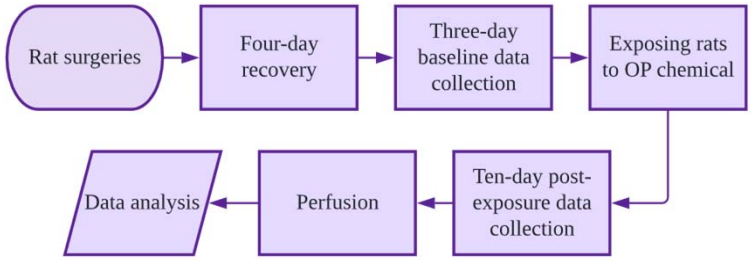
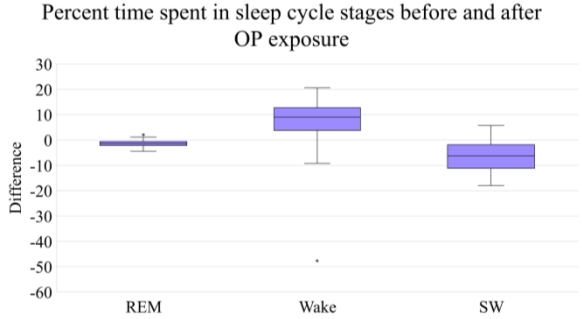


Figure 2 (above): Flowchart depicting the methods of this research

Results

In this research, thirty-five rats were used. A paired *t*-test was conducted for each of the three stages in the sleep cycle (Graph 1). The paired *t*-test for REM sleep showed a significant difference between percent time spent in REM sleep before and after OP exposure. The paired *t*-test for the wakefulness showed a significant difference between percent time spent in the wake stage before and after OP exposure. Lastly, the paired *t*-test for the SW sleep stage showed a significant difference between percent time spent in SW sleep before and after OP exposure.

Results (continued)



Graph 1 (above): A box and whisker plot of the differences in percent time for the three stages of the sleep cycle. The percent time spent in REM sleep significantly decreased from baseline (M = 6.97) to post-exposure (M = 5.58), $t(34) = 5.56$, $p < .001$. The percent time spent in wakefulness significantly increased from baseline (M = 50.55) to post-exposure (M = 56.93), $t(34) = -3.22$, $p = .003$. The percent time spent in SW sleep significantly decreased from baseline (M = 32.08) to post-exposure (M = 25.52), $t(34) = 7.02$, $p < .001$.

Discussion

The purpose of this research was to determine how exposure to an OP chemical affects REM sleep. This experiment showed significant difference in pre- and post-exposure REM sleep, meaning the hypothesis was supported. However, in previous research, REM sleep was increased after OP exposure, but in this experiment, REM sleep was decreased. The reason for this observation is unknown and could lead to further research. Additional research could also include testing the effect of an over-the-counter anticholinergic drug on REM sleep after OP exposure. These results can be used to understand health impacts on individuals who have been exposed to OP chemicals.

References

Bajgar, J. (2004). Organophosphates/nerve agent poisoning: Mechanism of action, diagnosis, prophylaxis, and treatment. *Advances in Clinical Chemistry*, *38*(12), 151–216. https://doi.org/10.1016/s0065-2423(04)38006-6

Gnadt. J. W., Pegram, G. V., & Baxter, J. F., (1985). The acetylcholinesterase inhibitor di-isopropyl-fluorophosphate increase REM sleep in rats. *Physiology & Behavior*, *35*(6), 911–916. https://doi.org/10.1016/0031-9384(85)90259-8

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