EEG analysis of two anticonvulsant treatments for nerve agent seizures in pediatric and adult rats

Ben Richardson
Mentored by Dr. John McDonough and Dr. Hilary McCarren

Introduction

Nerve agents are organic chemicals containing phosphorous that bind to and inhibit acetylcholinesterase, an enzyme that aids in the breakdown of the neurotransmitter acetylcholine. This interruption causes a continued signal from one nerve to another, resulting in seizures. The seizures produced by these agents are combated using anticonvulsants, such as benzodiazepines. Benzodiazepines interact with the receptors for the neurotransmitter γ-aminobutyric acid, or GABA, by increasing brain-wide inhibition (Lossin, Loya, Reddy, Rogawski, & Zolkowska, 2013). Individuals of different ages and sexes have different quantities of nerve connections based on their stage of development. The purpose of this project was to determine if there were differences between pediatric and adult animals in the electroencephalographic (EEG) development of seizures and the response of the seizures to benzodiazepines.

Methods and Materials

Two hundred and nine male and female Sprague-Dawley rats of different post-natal day (PND) ages - PND21, PN28, or PND70 - served as subjects. The rats had been surgically prepared with cortical electrodes to record EEG brain electrical activity. During testing, normal baseline activity was first recorded and then the animals were administered their assigned nerve agent: sarin (GB) or VX. Five minutes after seizure onset, the rats received their assigned benzodiazepine anticonvulsant, diazepam (DZP) or midazolam (MDZ), and EEGs were recorded for three more hours. All of this data was retrieved and organized into a single spreadsheet.

The EEG recording file for each rat was analyzed with Neuroscore™ software to output power values for the delta, gamma, and total power EEG bands (Figure 1) in five minute epochs to a spreadsheet. Data was grouped based on age, gender, anticonvulsant and if seizures stopped or not after treatment; average power and standard errors were calculated for each group. This data was graphed (Graph 1) and if there were no differences between sexes they were collapsed into a single data set. These final, collapsed data sets were graphed against one another and one-way ANOVA tests were performed at each of five designated epochs - baseline, seizure onset, 1, 2 and 3 hours after treatment.

Results

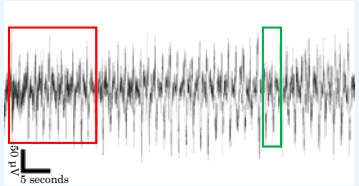
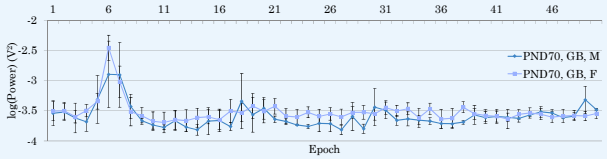


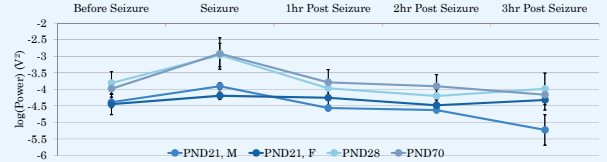
Figure 1 (above) is an example EEG trace from a PND70 rat exposed to GB. Power analysis was performed to determine how much of the signal was comprised of slow (delta, red) and fast (gamma, green) frequency waves at different time points relative to nerve agent exposure.

PND70s Total Power; GB Seizures Stopped with DZP

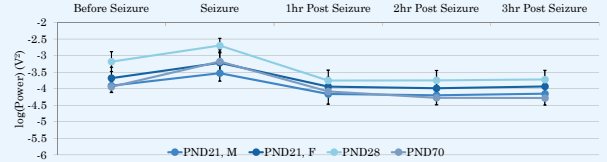


Graph 1 (above) displays total EEG power values for male and female PND70 animals that had GB-induced seizures that were successfully stopped with DZP. The standard error bars represent variability between individual animals within a sample group. Note the large increase in EEG power at seizure onset and quickly returns to baseline following DZP. There were no sex differences in the response to either GB or DZP.

Delta Power: GB Seizures Stopped with DZP



Delta Power: GB Seizures Stopped with MDZ



Graphs 2 (above, top) and 3 (above, bottom) display the final comparisons made between collapsed groups. The graphs were narrowed down into five important time points: before the seizure, during the seizure, and 1/2/3 hours after the initial seizure. This trend was representative of all three power types.

Results (cont'd)

There were no differences between sexes within the PND28 or PND70 age groups while female rats had lower power values than males in the PND21 age group. Graph 1 shows the typical response of nerve agent-induced seizures to an effective anticonvulsant; EEG power increases sharply at seizure onset and then decreases just as rapidly following successful diazepam treatment. Power analysis for groups of animals that stopped seizing after treatment with diazepam (Graph 2) displayed a clear increase in power at seizure onset and a decrease after treatment, but with a high variance between age groups. Again the PND70 and PND28 rats had higher power values than both PND21 groups. Power analysis for groups of animals that stopped seizing after treatment with midazolam (Graph 3) also displayed a clear change in power at onset and offset of seizures but had lower variance between the four groups than the other data sets.

Conclusions

From the one-way ANOVA tests at each time point, at the $\alpha = 0.05$ value, it was concluded that across all power types, the four age and gender groups were significantly different from each other at the vast majority of times regardless of anticonvulsant response. PND21 males had higher power values than females but these differences were not seen at older ages. Younger animals had lower power values, on average, than older animals, across all power types. Treatment with midazolam led to much smaller variance in power values than treatment with diazepam for all groups. Thus, midazolam may be a better treatment option for nerve agent induced seizures because it produces a more predictable seizure control across all ages.

References

Lossin, C., Loya, C. M., Reddy, K., Rogawski, M. A., & Zolkowska, D. (2013). Neuroactive steroids for the treatment of status epilepticus. *Epilepsia*, *54*(6), 93-98.

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