Award Number: W81XWH-12-2-0122

TITLE: Pediatric Susceptibility to Organophosphate-Induced Seizures and Effectiveness of Anticonvulsant Treatments

PRINCIPAL INVESTIGATOR: Francis Edward Dudek

CONTRACTING ORGANIZATION: University of Utah
Salt Lake City, UT 84112

REPORT DATE: January 2017

TYPE OF REPORT: Final Addendum

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
January 2017	Final Addendum	26 Sept 2014 - 25 Sept 2016

4. TITLE AND SUBTITLE

Pediatric Susceptibility to Nerve Agent-Induced Seizures and Effectiveness of Anticonvulsant Treatments

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-2-0122

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Francis Edward Dudek
Erika A. Scholl

E-Mail: Erika.scholl@hsc.utah.edu, ed.dudek@hsc.utah.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Utah
201 S Presidents Circle Rm 408
Salt Lake City, UT 84112-9023

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Organophosphate (OP) poisoning can result in status epilepticus (SE), and constitutes a medical emergency. Little data exist on OP-induced SE in immature animals, even though the immature brain is likely to respond differently to OPs, and the optimal therapies are also likely to differ from adults. Our previous results in designing a pediatric model to study the effects of the OP diisopropylfluorophosphate (DFP) showed that postnatal day 21 (P7) and P28 rats developed a robust, hours-long SE in response to the OP, which was a goal of the grant. However, P7 and P14 animals had few, if any, electrographic SE events and these events were all less than 1 hr. In order to further understand what may be occurring in P7 animals, we tested a 6-channel transmitter that could prove useful in determining whether electrographic seizures were focal in origin rather than generalized in these very young animals. We found that we could record EEG activity from P7 rat pups with very little noise, and the device was well tolerated. To examine whether P7 pups were even capable of sustaining electrographic seizures, we used kainic acid (KA), a direct glutamate agonist, to attempt to provoke SE. We found that while KA resulted in increased EEG activity, it was unclear whether this was indeed electrographic seizure activity. Further experiments are needed to examine any age-related effects of KA with our results from DFP.

15. SUBJECT TERMS Status Epilepticus, seizure, organophosphate, DFP, pediatric, neuronal injury, anticonvulsant, phenobarbital, propofol, midazolam, diazepam					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U	UU	10	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Introduction

Organophosphate (OP) poisoning often leads to status epilepticus (SE), a medical emergency. Often, the seizures associated with status epilepticus gradually become pharmacoresistant. Few if any studies have been undertaken on immature animals as models of OP-induced SE in the pediatric population, even though the immature brain is likely to respond differently to OPs than the adult. In addition, the optimal therapies for the pediatric population may well be different than for adults. Our ongoing work supported by this contract has shown that postnatal day 7 (P7) rat pups did not show clear, readily discernable electrographic or behavioral seizures.

One possibility is that OP-induced seizures are not generalized in the immature animals, and thus a EEG system with only 1 or 2 channels (as used in our experiments) is inadequate for detecting seizures in the P7 and P14 pups. Furthermore, even in cases where DFP (a cholinergic OP compound) induced abnormal electrical activity in P7 rat pups, the EEG patterns did not appear to be clear seizures, as might be expected based on previous published research concerning administration of other more traditional chemoconvulsive compounds, such as kainic acid (KA). Therefore, it is unclear which of the following possibilities is true: (1) OP's (i.e., DFP) do or do not induce electrographic and/or behavioral seizures in immature animals, (2) DFP-induced seizure activity is present in the immature brain, but with atypical waveforms, and thus not recognized as seizure activity, or (3) DFP-induced seizures are more focal than with adult animals, and thus not detected when only one or two electrodes are used. To explore solutions to these problems as we attempt to develop animal models of OP-induced seizures and brain damage in the pediatric population, we performed additional experiments.

Statement of Work – this section is the original SOW for the 2014-2016 contract

1. To determine whether DFP induced seizures that were focal in nature and thus possibly not detected with one or two recording channels, we (i.e., Epitel, Inc.) developed a six-channel recording system that would cover a greater proportion of the immature rodent brain. Thus, we tested the efficacy of a six-channel transmitter in recording different regions on the brain surface of a P7 rat pup.
2. To insure that our failure to detect DFP-induced seizures in the P7 and P14 age range, we tested whether the glutamate-receptor agonist, kainate (KA), could evoke electrographic seizures in a P7 rat pup.

Body

1. Our data from 2012-2014 (see final report for W81XWH-12-2-0122) suggested that P7 and P14 rat pups generated very few if any DFP-induced seizures. Since we previously used one- or two-channel transmitters, it was possible that seizures were not fully generalized and were occurring at other sites at a distance from the electrodes. We recently took advantage of a prototype, six-channel, wireless transmitter available from Epitel, Inc. for alpha testing. We found that the transmitter recorded EEG activity with very little noise from a P7 pup (Figure 1). Furthermore, due to the small size of this

device, the pup tolerated the transmitter even better than the previous two-channel transmitter, and we were able to record from P6 up to P15 (Figure 2). The recordings across this age range also appeared to show a discernable increase in baseline activity, which is a classical finding concerning the effects of increasing maturity on baseline EEG across this age range. These studies highlight the potential utility of the new six-channel transmitter for future use in rat pups as young as P6. In the future, this transmitter system will allows us to detect heterogeneity in electrographic seizures as a function of location in the immature rat brain.

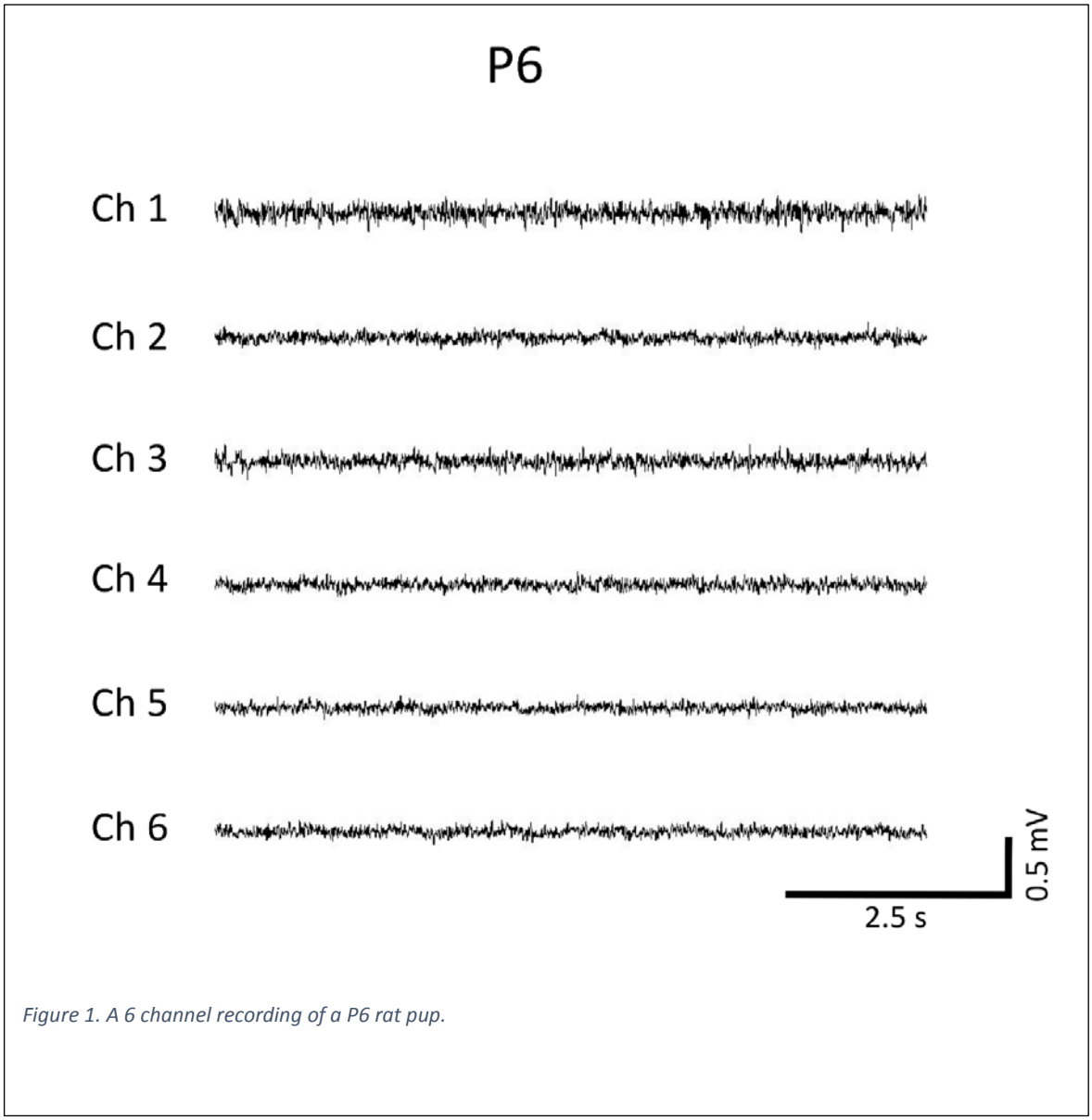

Figure 1. A 6 channel recording of a P6 rat pup.

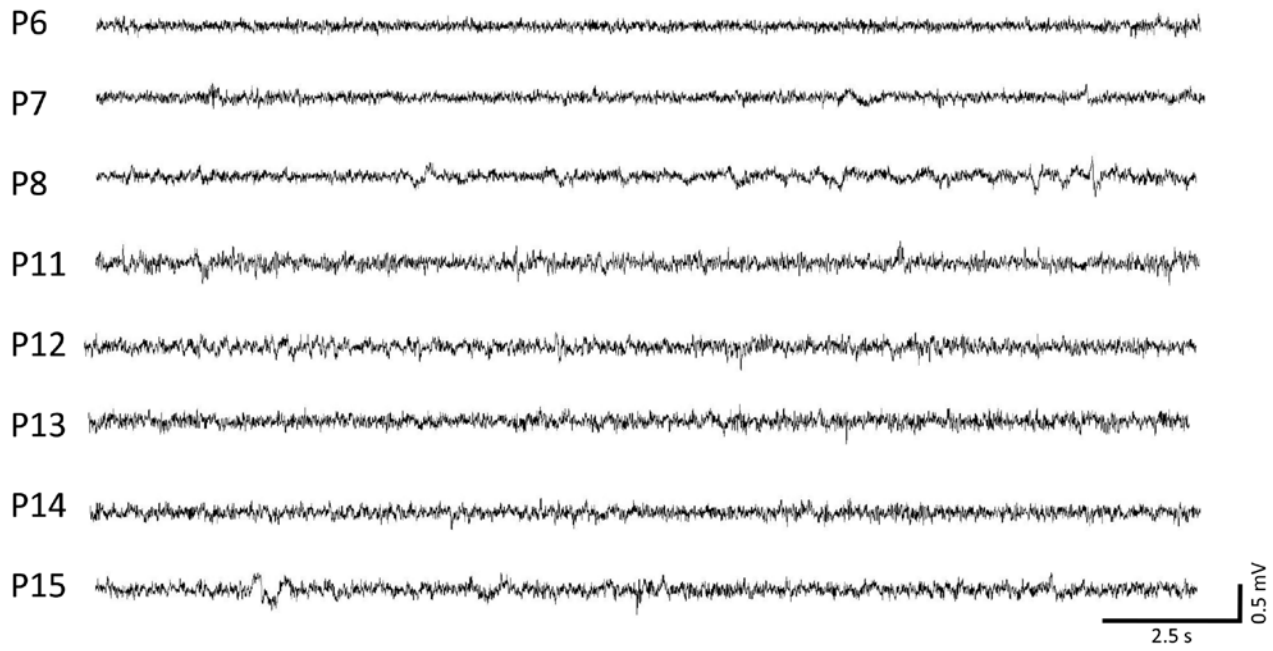

Figure 2. Record from a P7 rat pup over 11 days. The figure shows 1 channel from a 6 channel transmitter over the course of 11 days.

2. Because the cholinergic system in a week-old rat pup contains only about 30% of the M1 acetylcholine receptor, which is linked to seizure induction (Lee et al., 1990; Hamilton, et al., 1997), it is possible that seizure initiation in P7 rat pups is difficult to achieve using DFP, which has a cholinergic mechanism of action. To test if a chemoconvulsant with a glutamatergic mechanism might evoke electrographic seizures, we treated P7 rat pups with KA and recorded EEG activity via a two-channel transmitter. Figure 3 shows EEG activity after vehicle treatment, where spike-like events were present before and after vehicle treatment, but no injection-dependent increase in EEG activity was observed. Figure 4 shows the effect of KA treatment on EEG activity. Although KA administration appeared to cause increased EEG spike activity, there was a lack of rhythmicity in the EEG record, which is a hallmark of typical seizure activity. Therefore, while KA appeared to increase the overall activity of the brain, it was unclear whether this was true seizure activity. Additional experiments are required to compare KA-induced activity across different ages with our data on DFP.

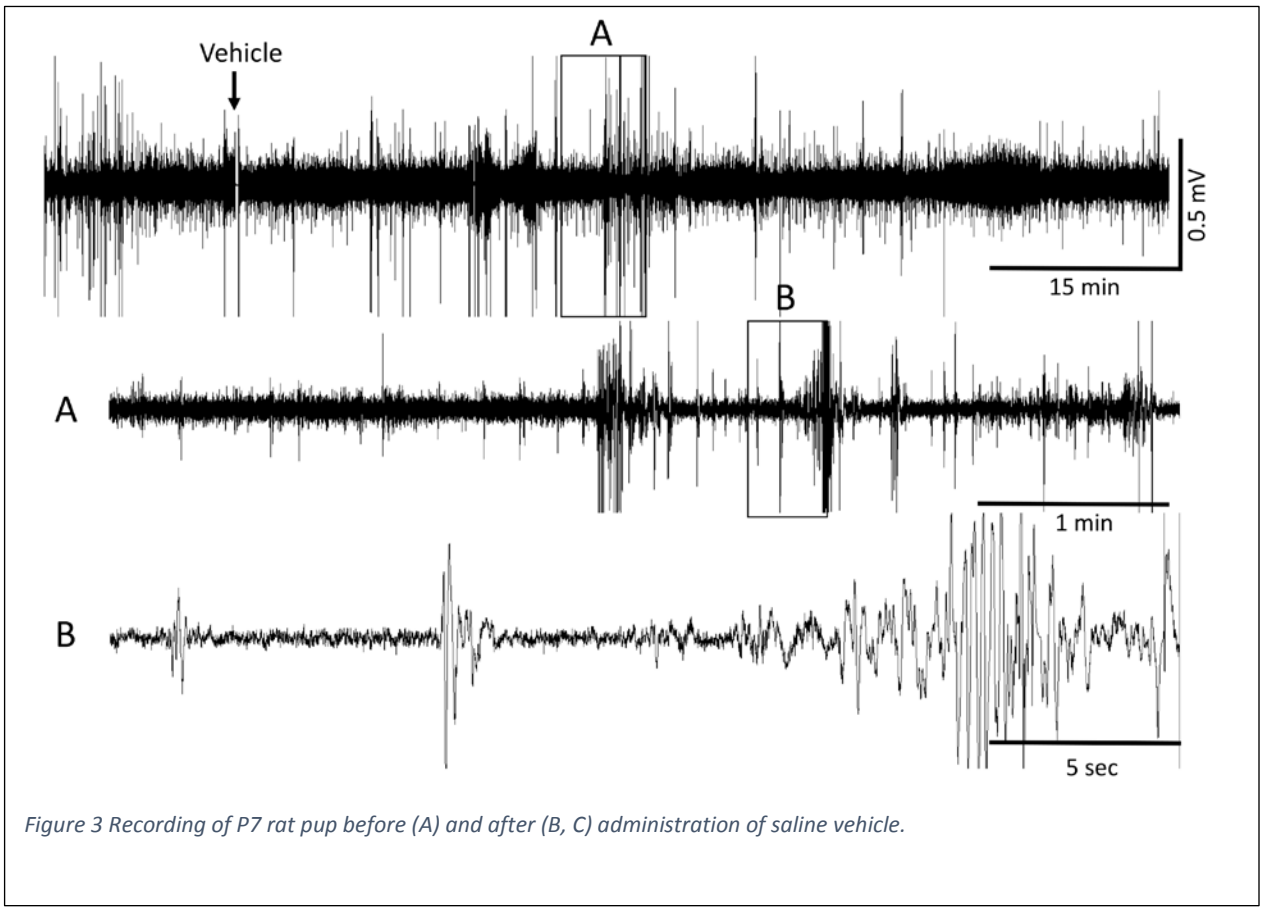

Figure 3 Recording of P7 rat pup before (A) and after (B, C) administration of saline vehicle.

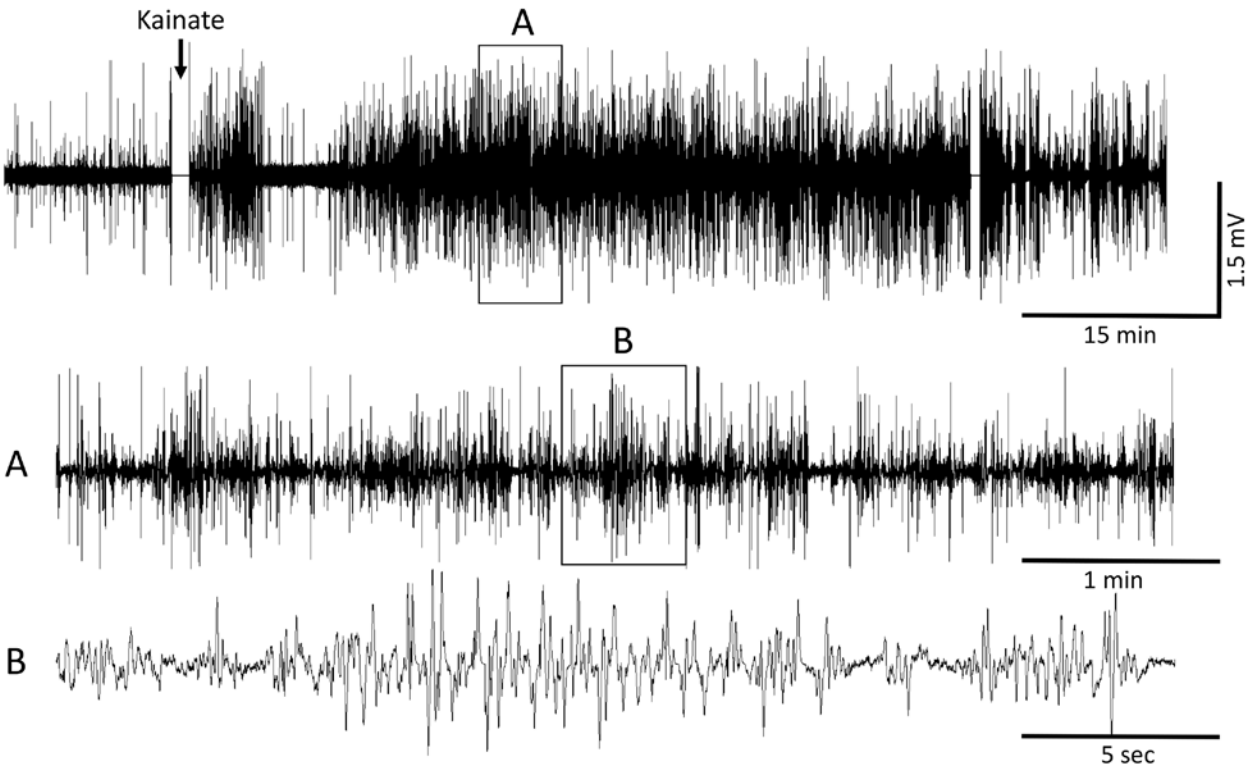

Figure 4. Recording of P7 rat pup before (A) and after (B, C) administration of 0.1 mg/kg KA.

Key Research Accomplishments

- Validated the use of a 6-channel transmitter device for P7 rat pups for future examination of possible focal origin of EEG seizure activity
- Tested the ability of KA to elicit electrographic seizure activity in P7 rats

Reportable Outcomes

No data has been presented to this time.

Conclusion

The 6-channel recordings highlight the potential utility of the new transmitter for future use in rat pups as young as P6. In the future, this transmitter system will allows us to detect heterogeneity in electrographic seizures as a function of location in the immature rat brain. Our attempts to induce electrographic seizure activity in P7 rats using KA were inconclusive. While it appeared to increase the overall activity of the brain, it was unclear whether this was true seizure activity. Additional experiments are required to compare KA-induced activity across different ages with our data on DFP.

References

J.E. Larsson and G. Wahlstrom, 1998. The influence of age and administration rate on the brain sensitivity to propofol in rats. Acta Anaesthesiol Scand, 42(8): 987-94.

Smith, I., White, P.F., Nathanson, M., and Gouldson, R. 1997. Propofol. An update on its clinical use. Anesthesiology, 81 (4): 1005-43.