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14. ABSTRACT
Organophosphate (OP) poisoning can lead to seizures and even status epilepticus (SE), which is associated with high morbidity and mortality. 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 recently published paper (Scholl, et al., 2018) details our results in designing an immature-rat model of OP-induced SE for the pediatric population using diisopropylfluorophosphate (DFP). We found that postnatal day 21 (P21) 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 rats had few, if any, electrographic SE events and these events were all less than 1 hr. One possible strategy to increase the duration of seizures in these very young rats would be to modify the pharmacological pretreatment that is given prior to DFP to make the brain more permissive for SE. When we replaced pyridostigmine bromide with scopolamine in P14 rats, both the occurrence of seizures and their duration was actually decreased, and mortality was increased; therefore, scopolamine was not a suitable replacement for the pyridostigmine bromide. Further experiments would be needed to further probe the utility of pharmacological adjuvants for eliciting sustained seizures. We also returned to the question of whether a P7 pup was even capable of generating and maintaining seizures. Because of our limited success using direct cholinergic (DFP) and glutamatergic (kainic acid) agonists, we tested picrotoxin, which blocks the $GABA_A$-receptor chloride channel. Treatment of P7 pups with picrotoxin resulted in robust and frank seizure activity, which lasted up to minutes, confirming that very immature rats can have sustained seizures.

15. SUBJECT TERMS Status Epilepticus, seizure, organophosphate, DFP, pediatric, neuronal injury, anticonvulsant, scopolamine, pyridostigmine bromide					
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Introduction

Exposure to an organophosphate poison (OP) can initiate seizures that rapidly progress to status epilepticus (SE). Often, the seizures associated with SE gradually become pharmacoresistant. While several groups have studied OP intoxication in adult rodents, very few studies have examined the effects on immature animals, 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 in the P7 and P14 rat pups, the immature brain is unable to sustain seizures with the specific pre- or post-treatments given prior to and immediately after DFP administration. The use of other pharmacological agents pre- or post-DFP might be enough to support proper initiation and or maintenance in these very young rats. 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, but still seemed to be abnormal electrical activity nonetheless. Our attempt to utilize kainic acid to generate a typical electrographic seizure profile in P7 rats resulted in a similarly unclear electrographic phenotype, as with DFP (see Addendum from 2017). It is possible that using a chemoconvulsant that acts on the GABAergic system rather than the glutamatergic system may give us a more recognizable electrographic profile and allow us to determine if P7 pups are capable of sustained seizure activity. To explore solutions to these problems as we attempt to develop rat models of OP-induced seizures and brain damage in the pediatric population, we performed additional experiments.

Statement of Work

1. To determine whether changing the type of pharmacological adjuvants delivered to the rat might extend the duration of SE, we will first replace the pretreatment of pyridostigmine with scopolamine.
2. To determine if the absence of DFP-induced seizures in the P7 and P14 age range was due to a lack of reactivity to DFP specifically or an inability of the very young brain to initiate and sustain seizures, we will test whether the $GABA_A$ chloride channel blocker, picrotoxin (PTX) 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. Because we saw more consistent seizure activity in P14 pups, we chose them for our adjuvant study. The pretreatment we have been using is pyridostigmine bromide (0.026 mg/kg, i.p.), a reversible acetylcholinesterase inhibitor which stays in the periphery and is necessary to avoid mortality due to bronchoconstriction and bronchorrhea. We tested this against

scopolamine methyl bromide (1 mg/kg, i.p.), which is a peripherally acting acetylcholine receptor inhibitor, and is used as a pretreatment in the pilocarpine model of status epilepticus to limit mortality. We found that seizures were elicited in 8/14 (57%) of rat pups pretreated with scopolamine, and this was lower than in pups pretreated with pyridostigmine (9/12; 75% had seizures). Rats treated with scopolamine also had a slightly higher mortality; 6/14 (43%) pups compared to 3/12 pups (33%) in pyridostigmine-treated pups. There was no statistical difference in either the number of rats that had seizures or in mortality, by Fisher's exact test. We also found no difference in the two groups in latency to seizure onset (Fig 1A). However, when we examined the total time spent in electrographic seizures, scopolamine-treated rats spent significantly less time in seizures than pyridostigmine-treated rats.

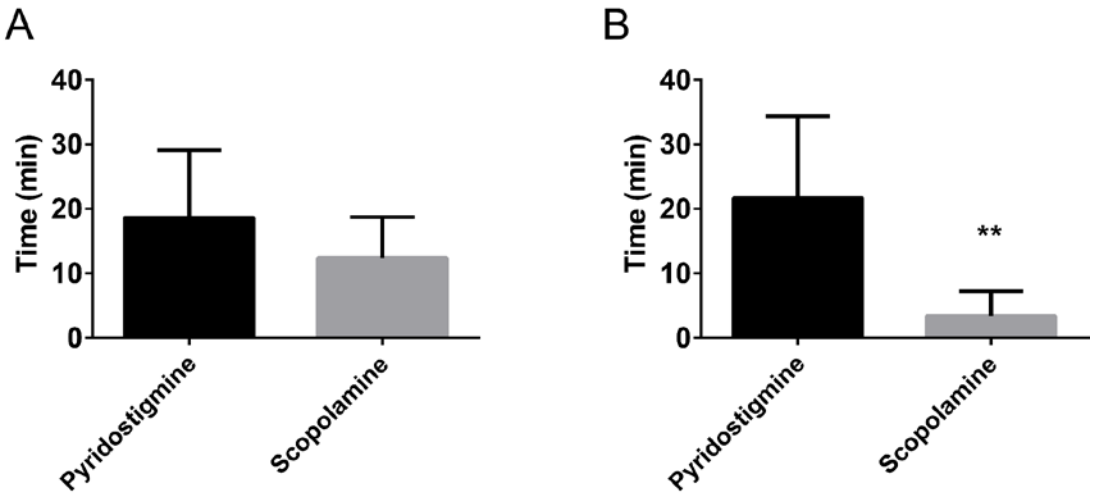

Figure 1. A., Latency to onset of electrographic seizures in P7 rat pups pretreated with 0.026 mg/kg pyridostigmine bromide (n=9) or 1 mg/kg scopolamine methyl bromide (n=6). B., Total duration of seizure events in each pretreatment group. *p<0.01, by Student's t-test.

2. Since the cholinergic system in a P7 rat pup contains only about 30% of the muscarinic receptor critical for seizure induction (Lee et al., 1990; Hamilton, et al., 1997), it is possible that seizure initiation in P7 rats is difficult to achieve using a cholinergic-acting agent like DFP. Our previous work showed that kainic acid, a chemoconvulsant with a glutamatergic mechanism, resulted in behavioral seizures and an increase in EEG spike activity, but no stereotyped rhythmicity, which is a hallmark of a typical electrographic seizure. Because both indirect (DFP) and direct (kainic acid) attempts to stimulate the excitatory glutamatergic system yielded unclear results, we chose to instead inhibit the GABAergic system to probe the ability of P7 pups to develop and sustain seizure

activity. PTX blocks the chloride channel in the $GABA_A$ receptor family, thus inhibiting inhibition and allowing normal excitability to go unchecked. Older studies showed that immature animals, including P7 rat pups, displayed rhythmic spiking pattern when exposed to $GABA_A$ receptor antagonists (Mares and Velisek, 1986; Velisek, et al., 1992). We found a similar EEG behavior (Fig 2). These are frank electrographic seizures, which continued for up to 40 min. Therefore, P7 rats have the capability to generate repetitive seizures, thus suggesting that the cholinergic system may be underdeveloped to easily sustain seizures at P7.

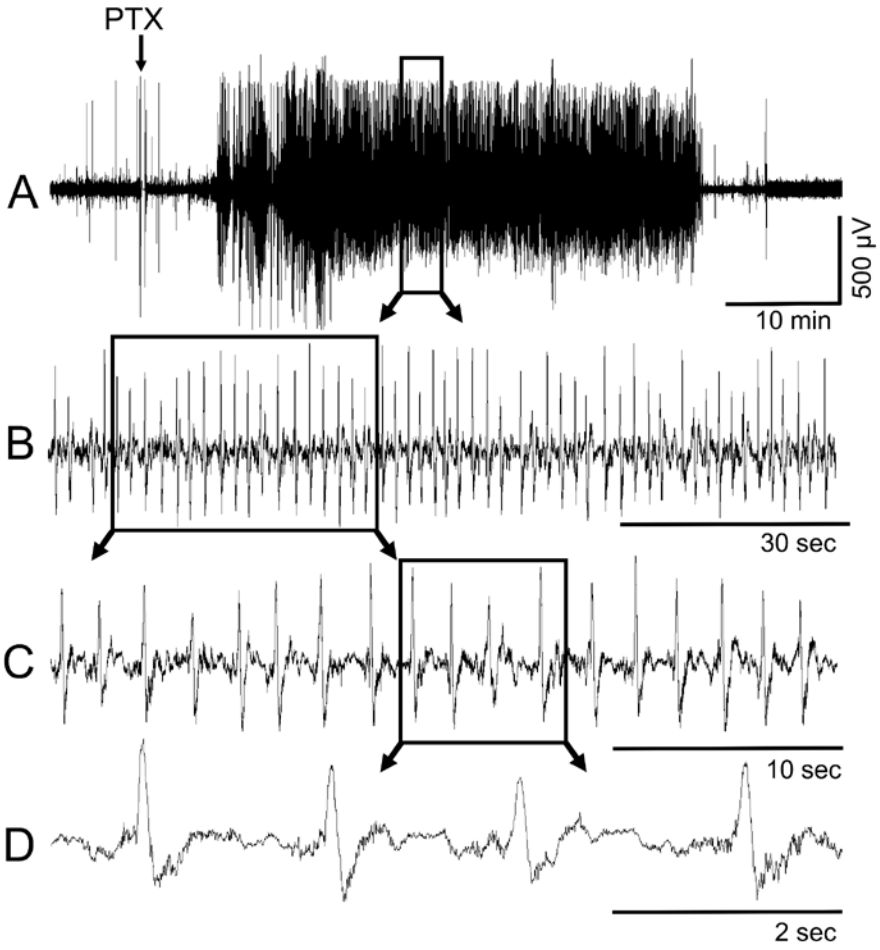

Figure 2 Representative trace from a P7 rat pup treated with 4 mg/kg PTX. The boxes in each trace are expanded in the trace below it.

Key Research Accomplishments

- Determined whether scopolamine methyl bromide promotes longer electrographic seizures in P14 rats
- Determined whether PTX induced electrographic seizures in P7 rats

Reportable Outcomes

None.

Conclusion

In P7 rat pups, the substitution of scopolamine methyl bromide as a pretreatment for pyridostigmine bromide did not increase the propensity for longer seizures; rather, it resulted in a trend towards fewer seizures with increased mortality. Furthermore, seizure duration was significantly shorter of in scopolamine-treated rats than in the pyridostigmine-treated rats. Together, these results indicate that scopolamine is not a suitable replacement for pyridostigmine as a pretreatment. In the future, other compounds in both the pretreatment and posttreatment period should be explored. The use of the GABAergic chloride-channel inhibitor, PTX, in P7 rat pups caused typical electrographic seizures, which consisted of repetitive spikes. This suggests that direct (kainic acid) or indirect (DFP) activators of the glutamatergic system in very immature rats may result in a more disorganized electrographic seizure activity than is observed in older but still immature rats.

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