Effects of Maternal Chlorpyrifos exposure on Guinea Pig Neurodevelopment

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Introduction

Maternal exposure to organophosphorus pesticides is a critical concern in modern countries where their use is common. Through interaction with the maternal blood supply, a developing fetus is exposed to many of the same environmental contaminants that the mother faces. One of these contaminants, chlorpyrifos (CPF), is a common agricultural pesticide primarily known for its action as an acetylcholinesterase (AChE) inhibitor. Both human and animal studies have revealed that neurodevelopmental deficits can stem from exposure to this organophosphorus compound[1]. The standard correlates of typical neurodevelopmental deficits include reduced axonal integrity and gross anatomical changes. MR imaging using advanced techniques such as diffusion tensor imaging are powerful tools to examine these correlates. In this study, we use these methods in a guinea pig model to examine the effects of maternal exposure to chlorpyrifos on the neurodevelopment of their offspring.

	Group	N	Mean ± Std. Dev	P
Mean Diffusivity	CPF	10	0.00086 ± 0.00002	**0.020**
	PO	10	0.00084 ± 0.00001	
Fractional Anisotropy	CPF	10	0.19619 ± 0.01209	**0.006**
	PO	10	0.21361 ± 0.01297	
Axial Diffusivity	CPF	10	0.00104 ± 0.00002	0.842
	PO	10	0.00104 ± 0.00002	
Radial Diffusivity	CPF	10	0.00077 ± 0.00002	**0.003**
	PO	10	0.00074 ± 0.00001	
Mean Kurtosis	CPF	10	0.68324 ± 0.02767	0.377
	PO	10	0.69592 ± 0.03454	
Axial Kurtosis	CPF	10	0.65349 ± 0.03563	0.686
	PO	10	0.66116 ± 0.04708	
Radial Kurtosis	CPF	10	0.75216 ± 0.02865	**0.048**
	PO	10	0.77727 ± 0.02418	

Figure 1: Diffusion imaging values for the Striatum.

Materials and Methods

Animal model

Pregnant Hartley guinea pigs were purchased from Charles River Lab. Starting at approximately 50 days gestation, the dams were given a subcutaneous injection between the shoulder blades consisting of either CPF dissolved in peanut oil (25 mg/kg) or peanut oil vehicle (0.5 ml/kg) for 10 days. Female pups born to these mothers were divided into those who were prenatally exposed to CPF (10 animals) or peanut oil vehicle ("PO," 10 animals). Starting on postnatal day (PND) 35-40, these animals were tested in the Morris water maze[2] (MWM). MRI/MRS experiments took place at approximately PND 70.

Guinea pigs are the preferred rodent model for this experiment due to their similarities to humans in both neurodevelopmental timing and systemic sensitivity to chlorpyrifos.[3] All experiments were carried out in accordance with the rules and regulations set forth by the University of Maryland School of Medicine Institutional Animal Care and Use Committee regarding the care and use of animals under a protocol approved by the committee, and complied with the principles of the '1996 Guide for the Care and Use of Laboratory Animals.

MRI/MRS experiments

All experiments were performed on a Bruker Biospec 7.0 Tesla 30 cm horizontal bore scanner equipped with a BGA20S gradient system capable of producing pulse gradients of 100 mT/m in each of the three axes, and interfaced to a Bruker Paravision 5.1 console. A Bruker four-element 1H surface coil array was used as the receiver and a Bruker 154 mm circular coil as the transmitter. The guinea pig was anesthetized in an animal chamber using a gas mixture of O_2 (1 L/min) and 4% isoflurane. The animal was then placed prone in an animal holder and the RF coil was positioned and fixed over the cranium. Fast spin echo based T2-weighted MR images with repetition time/effective echo time (TR/TE_{eff}) = 6197/60 msec, echo train length = 8, field of view (FOV) = 35×35 mm2, matrix size = 256×256, slice thickness = 1 mm, # of slices = 24, and # of averages = 2, in the axial plane were obtained for anatomical and segmentation analysis. Diffusion weighted images were acquired with a single shot spin echo EPI sequence with TR/TE_{eff} = 8500/45 msec, matrix size 96×96, 30 gradient directions, A_0=5, and two b-values (1000 s/mm^2 and 2000 s/mm^2). Other parameters identical to T2 above. ROIs for diffusion analyses were drawn with FSLview and included the whole brain, cortex, hippocampus, thalamus, striatum, and amygdala.

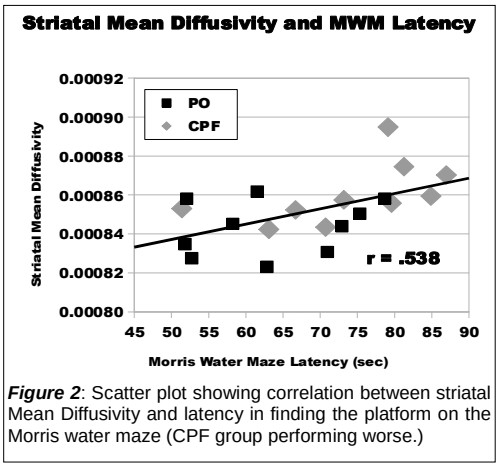

Figure 2: Scatter plot showing correlation between striatal Mean Diffusivity and latency in finding the platform on the Morris water maze (CPF group performing worse.)

Results

Animals prenatally exposed to CPF had lower body weight (441.7 ± 63.4 g) than those in the PO group (496.7 ± 47.4 g) at the time of the experiment [t(18) = -2.20, p < .05]. Brain parenchymal volume was also lower in the CPF group (2603.02 ± 102.05 mm^3) than the PO group (2734.01 ± 80.88 mm^3) [t(18) = -3.18, p < .01]. On a whole brain level, the CPF group had a lower fractional anisotropy (FA) value than the PO group [CPF: 0.258 ± 0.011, PO: 0.272 ± .017 [t(18) = -2.22, p < .05]. Within specific brain areas, the striatum was different between groups in most measures (Fig.1). Animals in the CPF group took longer to find the hidden platform in the MWM than animals in the PO group animals [CPF = 73.7 ± 11 s, PO = 63.7 ± 10.2 s, t(18) = 2.11, p < .05]. This latency score was significantly correlated with striatal mean diffusivity (MD, r = .538, p < .05, Fig. 2) and radial diffusivity (RD, r = .505, p < .05) measures, as well as fractional anisotropy (FA, r = -.547, p < .05) in the thalamus.

Discussion and Conclusions

The most striking and consistent finding in this study was the effect of maternal exposure to CPF on the striatum. DTI measures were significantly affected in this brain area and clearly correlated with the behavioral deficits observed in these offspring. This is in accord with other studies on more direct CPF exposure that revealed cholinergic and dopaminergic signaling alterations as well as overt mitochondrial dysfunction in the striatum[4,5]. Given the role of the striatum in movement disorders such as Parkinson's and Huntington's disease, as well as motivation and reward[6], this is not a finding that one can ignore in regards to human neurodevelopment and welfare. Further research along these lines can elaborate more precisely the affected mechanisms of striatal dysfunction in the offspring of CPF-exposed animals. To our knowledge this is the first demonstration of the structural correlate of the toxicity of CPF in the developing brain of a precocious species.

References

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