Functional Consequences of Repeated Organophosphate Exposure: Potential Non-Cholinergic Mechanisms

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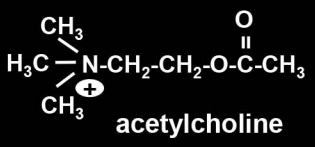
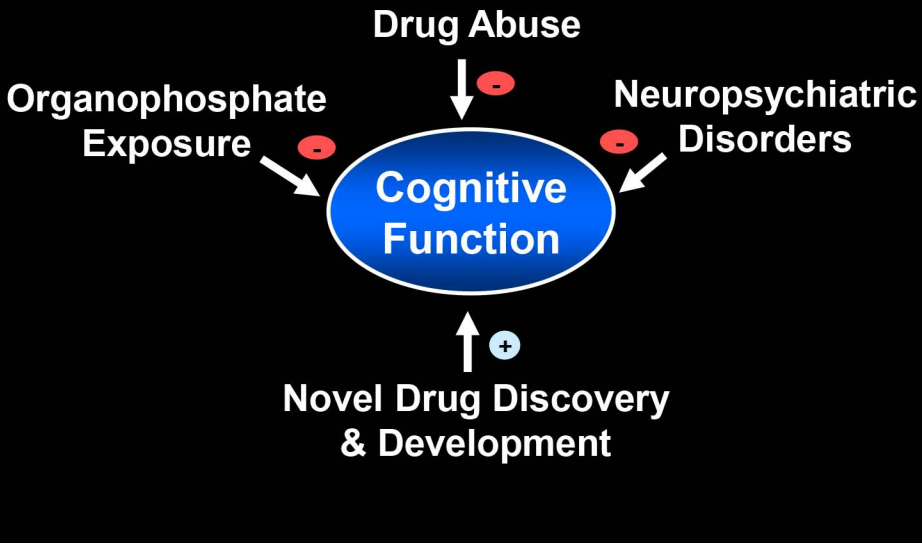
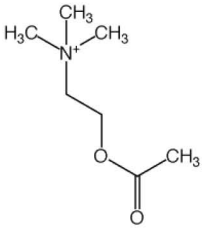
Georgia Health Sciences University

Terry Lab Interests

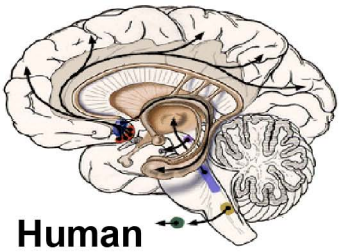




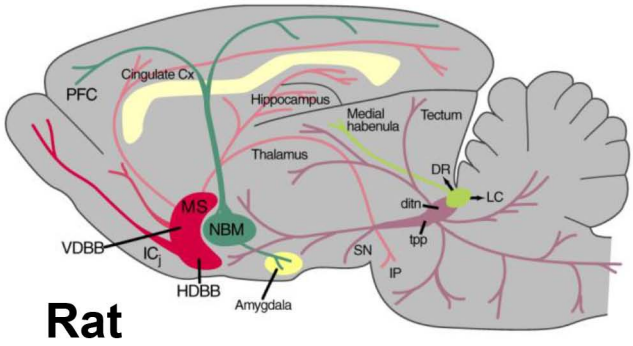


Acetylcholine

Central Cholinergic Pathways



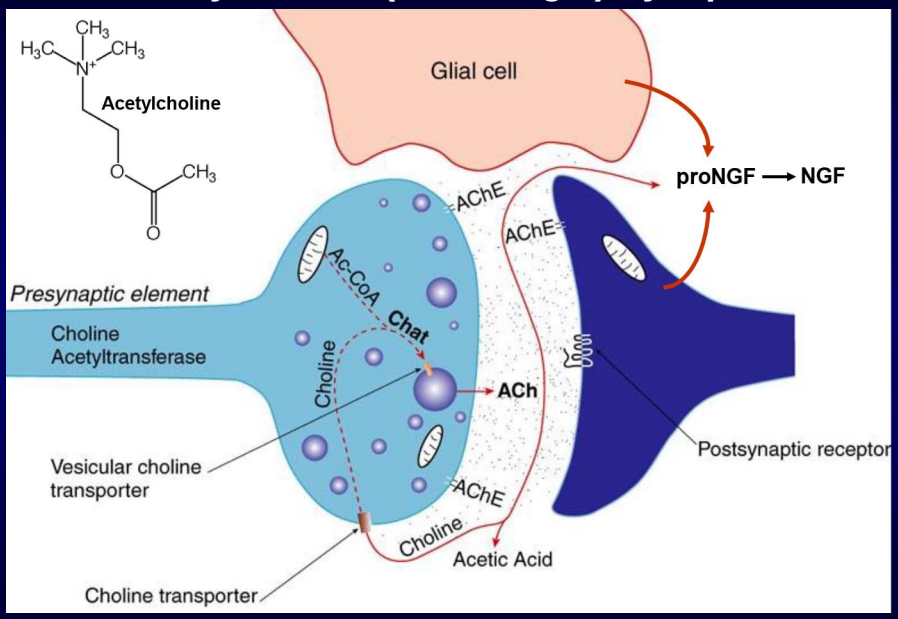
Human



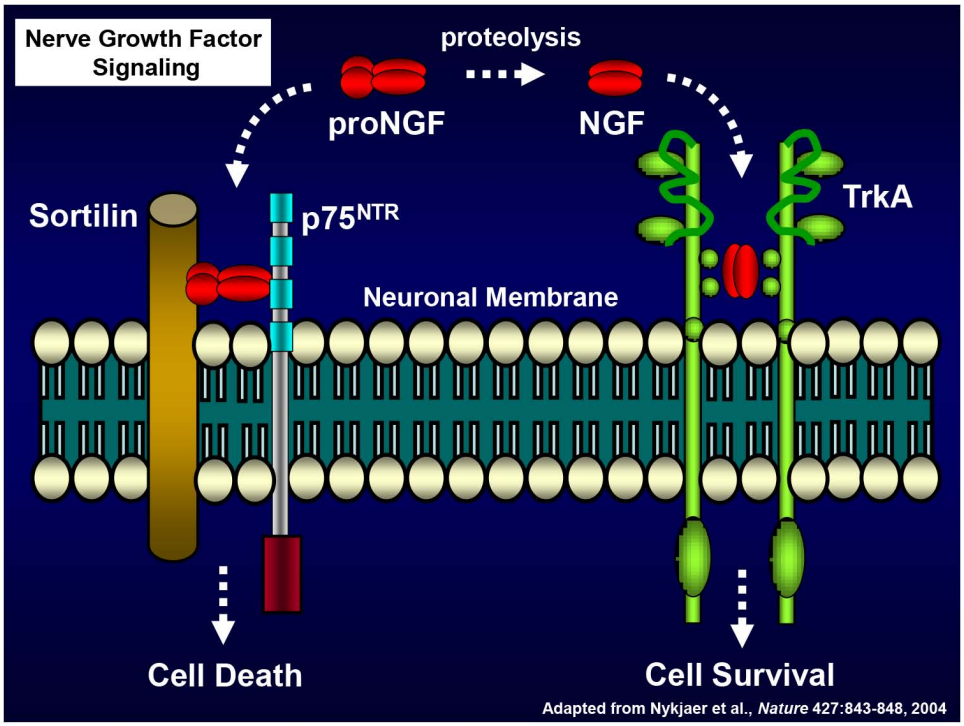
Rat

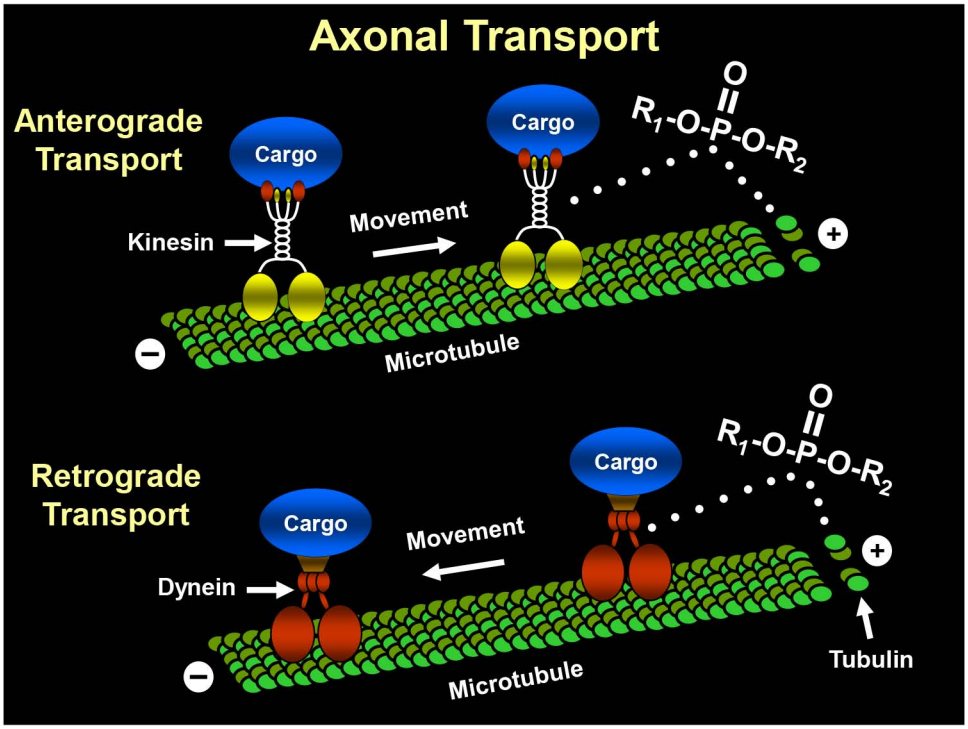
Source: "Fundamental Neuroscience", Second Edition, Copyright, 2003, Academic Press

Acetylcholine (cholinergic) Synapse



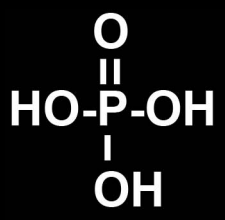
Source: "Fundamental Neuroscience", Second Edition, Copyright, 2003, Academic Press


Nerve Growth Factor Signaling
proteolysis
proNGF
NGF
Sortilin
p75NTR
TrkA
Neuronal Membrane
Cell Death
Cell Survival
Adapted from Nykjaer et al., Nature 427:843-848, 2004

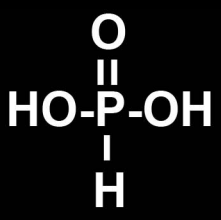

Axonal Transport
Anterograde Transport
Cargo
Cargo
O
R1-O-P-O-R2
Kinesin
Movement
Microtubule
Retrograde Transport
Cargo
Cargo
Movement
Dynein
Tubulin
Microtubule

Organophosphates

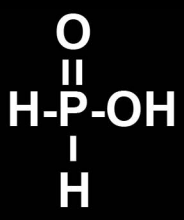
Chemicals Derived From:



Phosphoric Acid



Phosphonic Acid



Phosphinic Acid

Organophosphate-Based Chemicals Found in:

- Insecticides (e.g., malathion, parathion, diazinon, chlorpyrifos)
- Chemical Warfare ("nerve") Agents (e.g., soman, sarin, tabun, VX)
- Ophthalmic Agents (e.g., echothiophate, isoflurophate)
- Antihelmintics (e.g., trichlorfon)
- Herbicides (e.g., tribufos, merphos)
- Solvents, Plasticizers, and Extreme Pressure Additives for Lubricants

Reviewed, Katz and Brooks, 2010

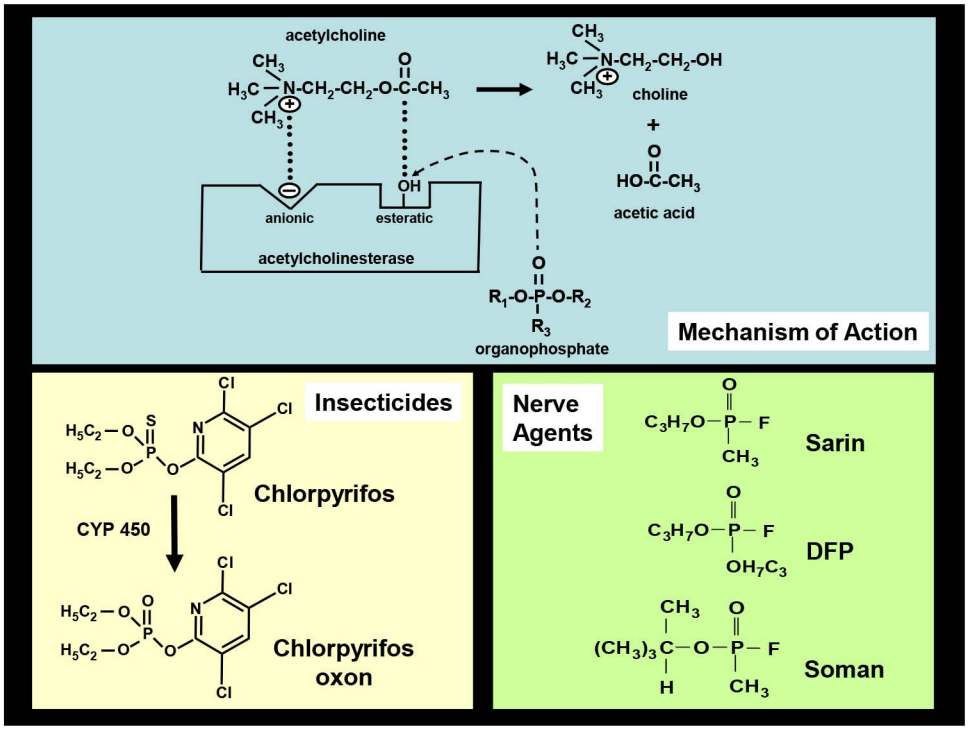
Gulf War Illness and OPs

- Exposure to one or more acetylcholinesterase inhibitors appears to offer a particularly plausible explanation for several of the neurological-based symptoms of GWI (Golomb et al., 2008)
- An estimated 41,000 military personnel in the first gulf war were exposed to insecticides that contained either carbamate or OP-based AChEIs (Fricker et al., 2000; US Department of Defense, 2003)
- As many as 100,000 military personnel may have been exposed to low (i.e., non-acutely toxic) levels of sarin/cyclosarin following the destruction of an Iraqi munitions storage complex at Khamisiyah, Iraq, in March 1991 (Berardocco, 1997).

OP-Pesticide Use in the First Gulf War

- Fly Bait
  - azamethiphos
- Pest Strips
  - dichlorvos
- Sprayed Liquids
  - chlorpyrifos, diazinon, malathion
- Fogs
  - chlorpyrifos, malathion

Source: http://www.gulflink.osd.mil/pest_final/index.html.



Organophosphate Toxicity

- **Acute**
  - Muscarinic (postganglionic parasympathetic) "DUMB-BELS": diaphoresis and diarrhea, urination, miosis, bradycardia, bronchospasm, emesis, lacrimation, salivation.
  - Nicotinic (neuromuscular junction)- muscle fasciculations, weakness, paralysis, respiratory failure; (CNS)- seizures or CNS depression/coma.
- **Chronic and/or Repeated Low-Level Exposures***
  - Anxiety, depression, psychotic symptoms, deficits in short-term memory, learning, attention, information processing, eye-hand coordination and reaction time, and extrapyramidal symptoms.

* Data primarily from case reports and retrospective epidemiological studies.

Overall Objectives

- Determine the consequences of repeated, "subthreshold" exposures to representative organophosphates on cognitive function in animal models.
  - Information processing and attention
  - Spatial Learning
  - Recognition Memory
  - Working Memory
- Determine the consequences of repeated, low-level exposures to representative organophosphates on neurobiological substrates of cognitive function
  - Cholinergic Markers
  - Neurotrophins
  - Axonal Transport
- Identify therapeutic targets for drug development

Study Algorithm

OP-Free Washout

Cognitive Function
- Water Maze
- Novel Object Recognition
- Prepulse Inhibition
- 5C-SRTT
- Radial Arm Maze

Cholinergic Markers
- Enzyme Assays
- Receptor Autoradiography
- Western Blot

Neurotrophins (NGF)
- Western Blot
- Immunohistochemistry

Axonal Transport
- Mitochondrial Movement
- βAPP Immunohistochemistry
- Mn^{2+} Enhanced MRI

Summary of Previous Chlorpyrifos Studies (repeated Subthreshold exposures)

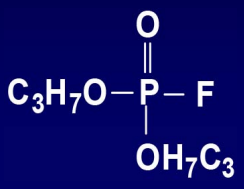
- Impairments in spatial learning
- Impairments in Prepulse Inhibition of the auditory startle response
- Decreased expression of cholinergic marker proteins in the brain
- Decreased expression of neurotrophin-related proteins in the brain
- Impairments of anterograde and retrograde axonal transport ex vivo

Terry et al., *J. Pharmacol Exp Ther* 305: 375-384, 2003.

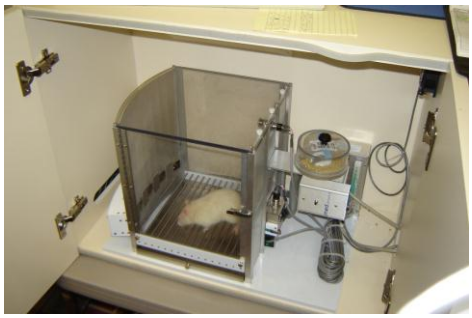
Terry et al., *J. Pharmacol Exp Ther* 322: 1117-1128, 2007.

Recently Published Studies

Chlorpyrifos



DFP



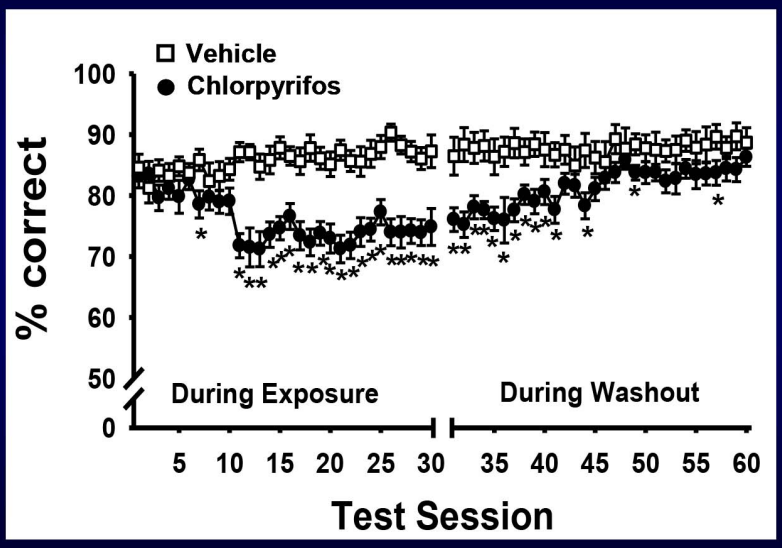
The Rat, Five Choice Serial Reaction Time Task (5C-SRTT)

Continuous Performance Task (CPT)
AX Type

Hit Lever ↓

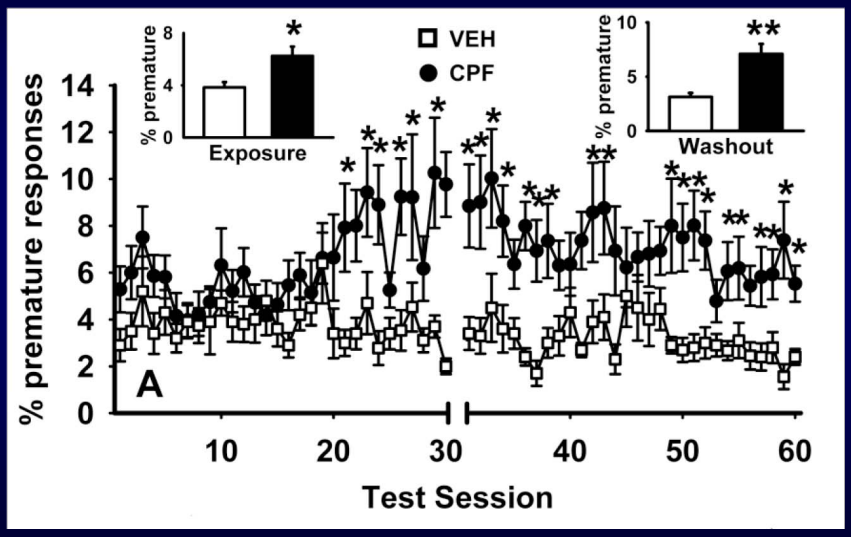
A H X J A K XO I Y A U B A X

5C-SRTT-Chlorpyrifos (Alternate Day Exposures)



Middlemore Risher et al., Neurotoxicology and Teratology 32: 415–424, 2010

5C-SRTT-Chlorpyrifos (Alternate Day Exposures)



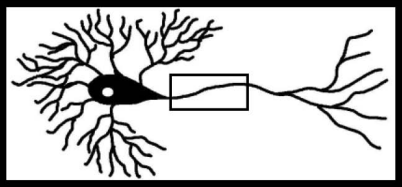
Middlemore-Risher et al., Neurotoxicology and Teratology 32: 415–424, 2010

5C-SRTT-Chlorpyrifos Experiments Conclusion

- Repeated exposures to subthreshold levels of chlorpyrifos lead to protracted impairments of sustained attention and an increase in impulsive behaviors in rats.

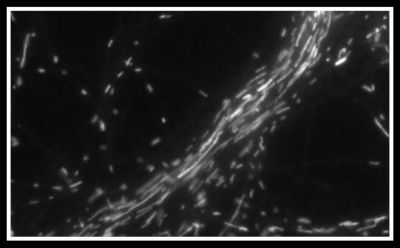
Middlemore-Risher et al., Neurotoxicology and Teratology 32: 415–424, 2010

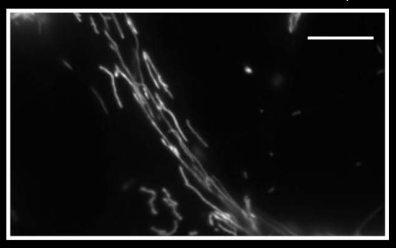
MitoTracker® Imaging Measurements



Movement = mean # moving/μm
Length= average length in the ROI
Number= # of mitochondria/μm

Scale bar= 100 μm

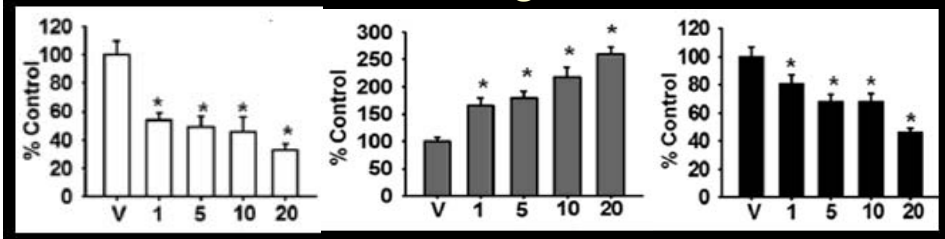




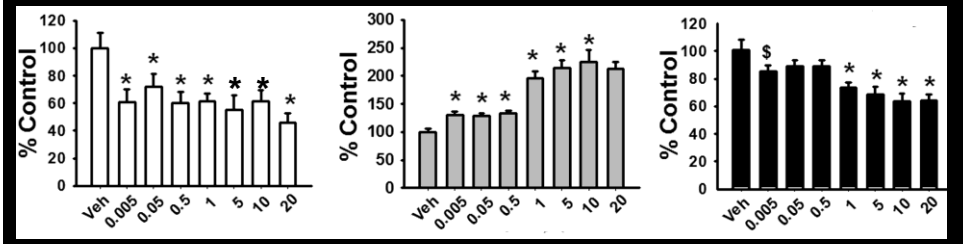
Vehicle **CPF oxon (5.0 nM)**

Middlemore-Risher et al., *J Pharm Exp Ther* 339:341-349, 2011

Movement Length Number

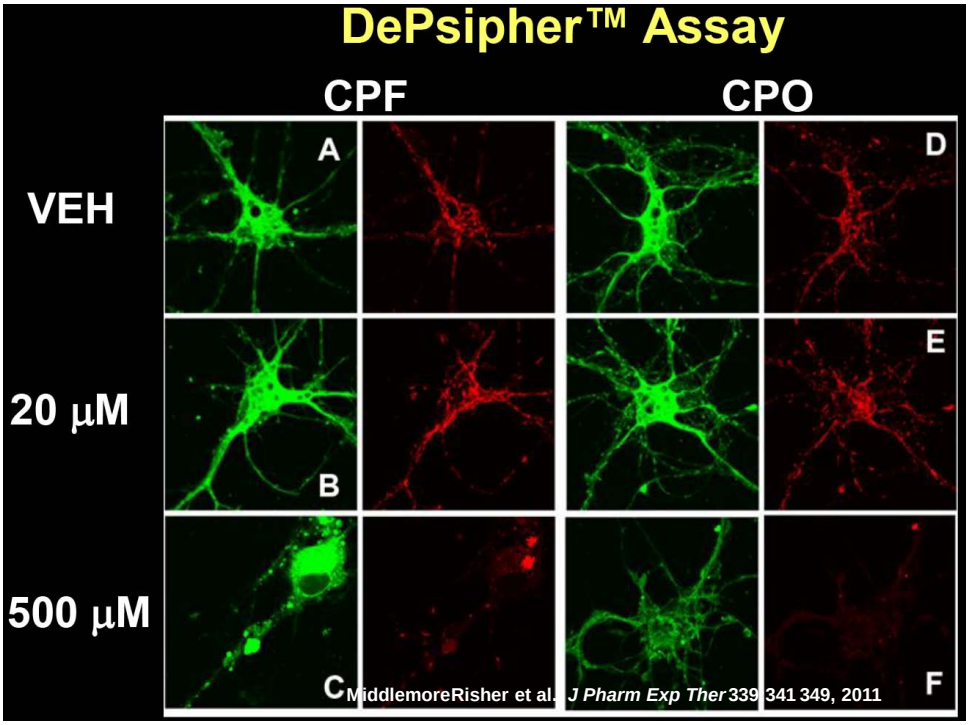


Chlorpyrifos (μM)



Chlorpyrifos oxon (μM)

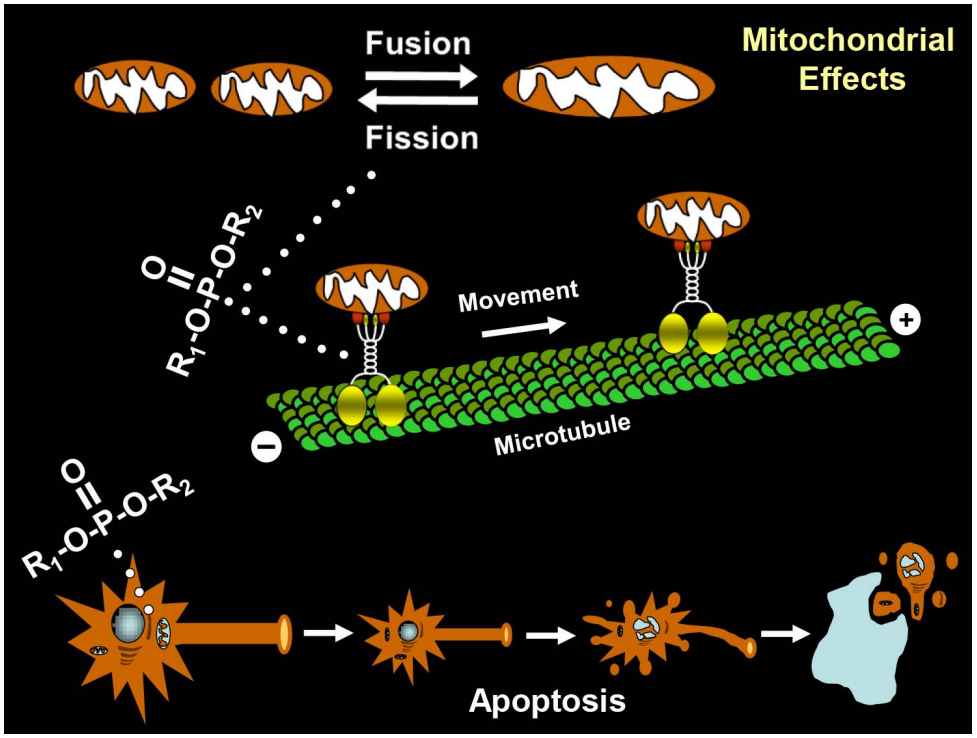
Middlemore-Risher et al., *J Pharm Exp Ther* 339:341-349, 2011

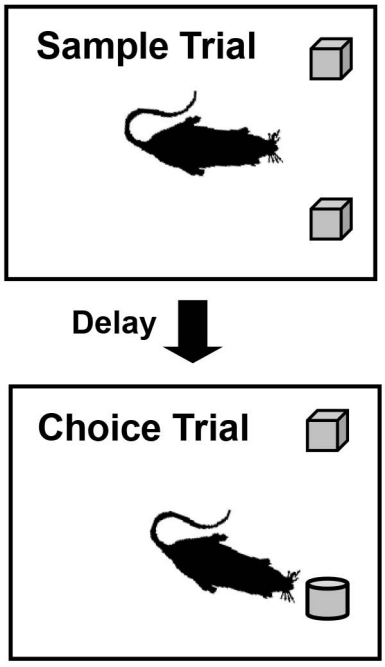


Summary (CPF & CPO in Neuronal Culture)

- Concentration-dependent decrease in the transport of mitochondria in axons, an increase in mitochondrial length, and a decrease in mitochondrial number (indicative of increased fusion versus fission events)
- The neuronal changes occurred at OP concentrations that did not inhibit AChE activity, they were not blocked by cholinergic antagonists, and they did not appear to be associated with directly toxic effects on mitochondria (i.e., alterations in ATP production, mitochondrial membrane potential, superoxide production).
- The results suggest that an underlying mechanism of OP-based alterations in neurological function might involve alterations in mitochondrial dynamics and/or their transport in axons.

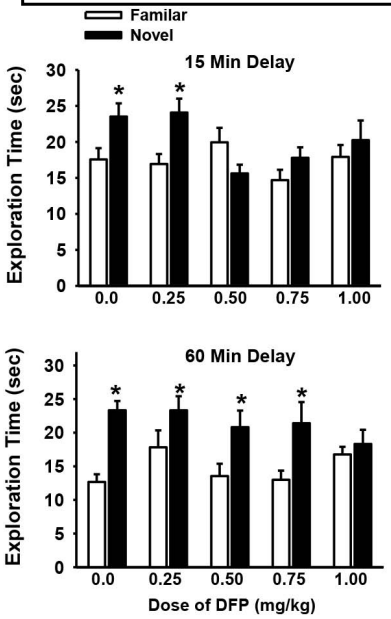
Middlemore-Risher et al., *J Pharm Exp Ther* 339:341-349, 2011

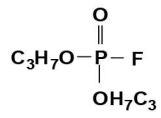




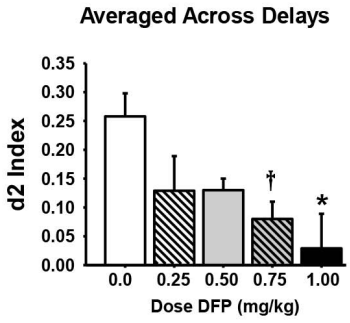
Spontaneous Novel Object Recognition

Spontaneous Novel Object Recognition



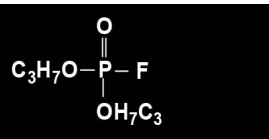


N=11-24

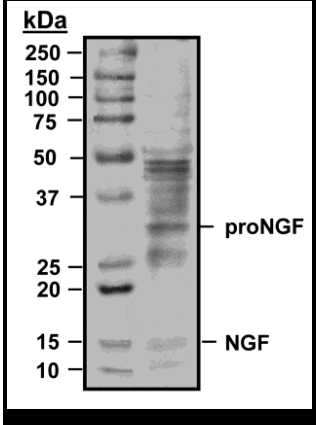


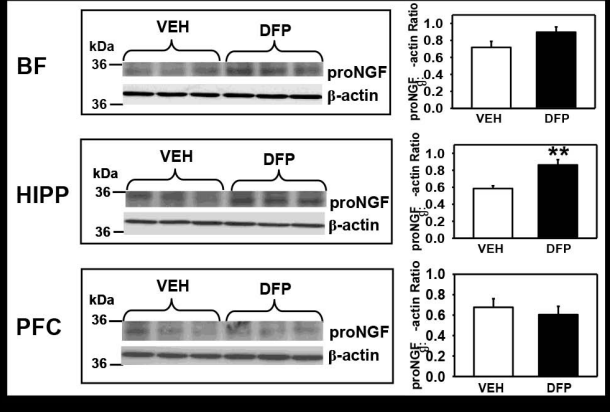
d2 index = (novel - familiar)/(novel + familiar)

Terry et al., *Neuroscience* 176: 237–253, 2011

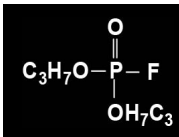


proNGF

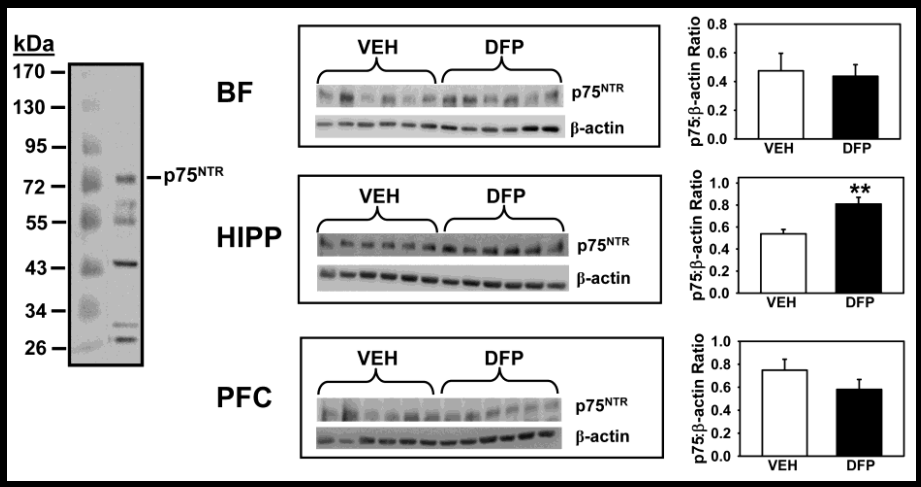




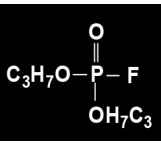
Terry et al., *Neuroscience* 176: 237–253, 2011



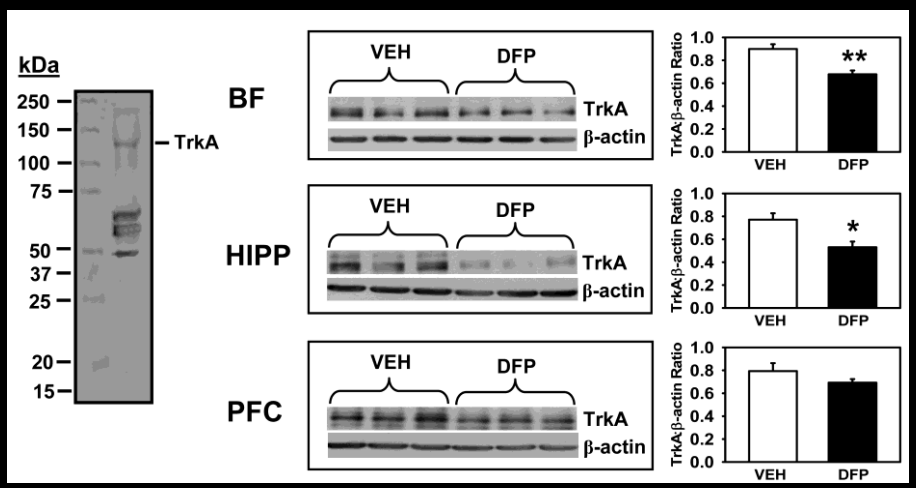
p75 neurotrophin receptor ($p75^{NTR}$)



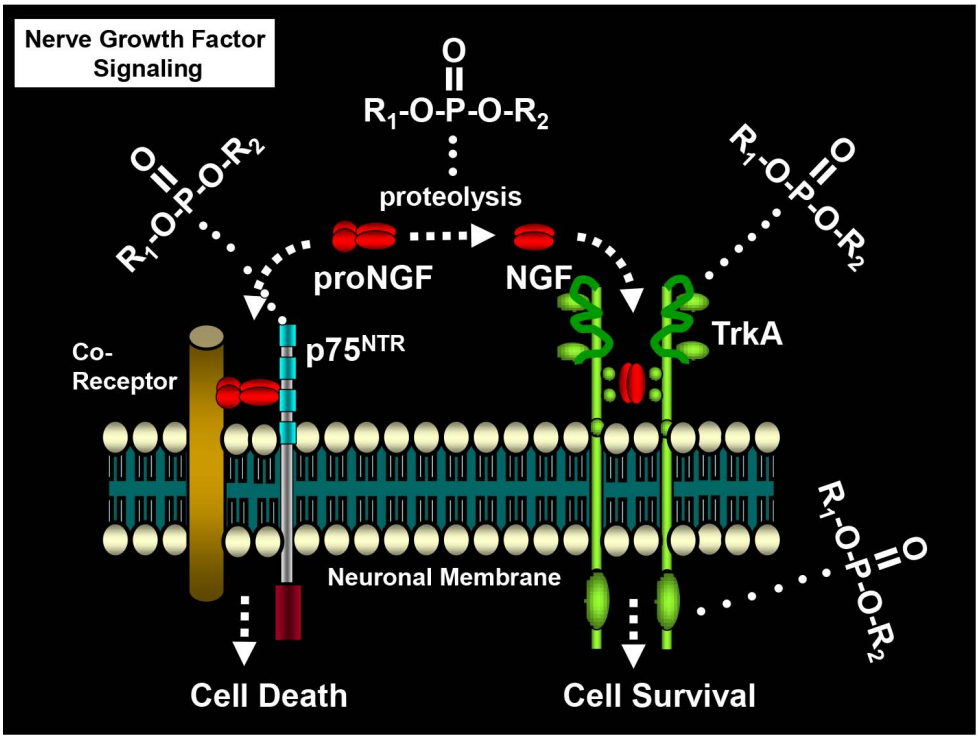
Terry et al., *Neuroscience* 176: 237–253, 2011



TrkA Receptor



Terry et al., *Neuroscience* 176: 237–253, 2011



DFP Experiments (Conclusion)

- Intermittent, subthreshold exposures nerve agent OPs can lead to protracted deficits in specific domains of cognition (i.e., spatial learning and recall, recognition memory)
- The cognitive deficits may be related to persistent functional changes in brain neurotrophin and cholinergic pathways.

Terry et al., *Neuroscience* 176: 237–253, 2011

Neurotoxicology and Teratology 34 (2012) 1–8

Contents lists available at SciVerse ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Chronic impairments in spatial learning and memory in rats previously exposed to chlorpyrfos or diisopropylfluorophosphate

A.V. Terry Jr. [a,b,*], W.D. Beck [a], S. Warner [b], L. Vandenhuerk [b], P.M. Callahan [a,b]

[a] Department of Pharmacology and Toxicology, Georgia Health Sciences University, Augusta, Georgia, 30912, United States
[b] Small Animal Behavior Core, Georgia Health Sciences University, Augusta, Georgia, 30912, United States

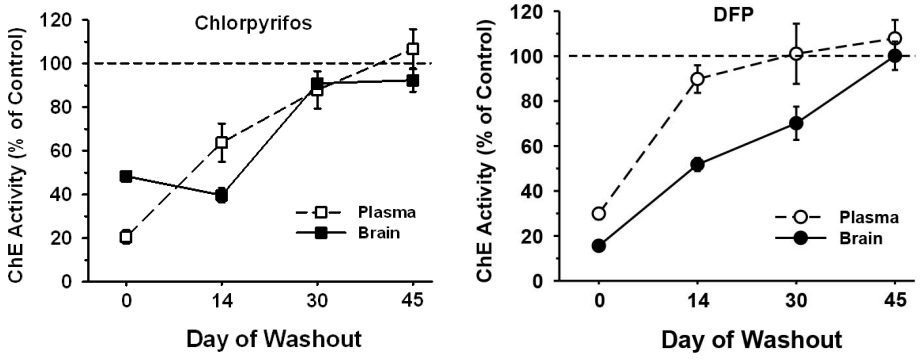
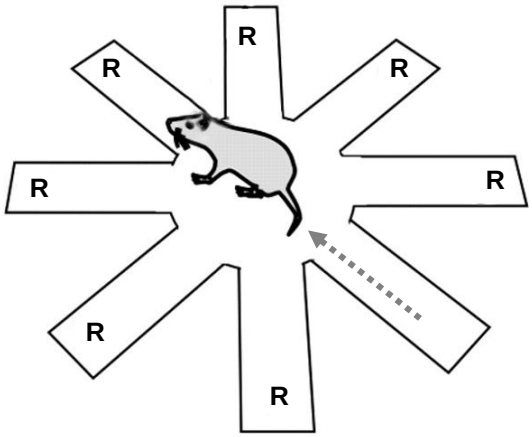


Fig 1. The effects of repeated exposures to CPF 18.0 mg/kg (Left) or DFP 0.75 mg/kg (Right) on cholinesterase activity in the plasma and brain at various time points during a 45 day OP-free washout period. Data (mean ± SEM) are presented as % of vehicle-matched control levels. (N=3-6).

Terry et al., *Neurotoxicology and Teratology* 34:1-8, 2012

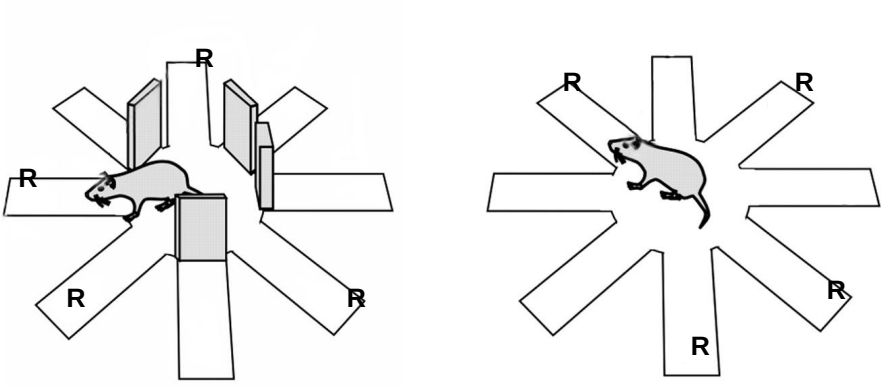
8-Arm Radial Maze

(Win-Shift Task)



8-Arm Radial Maze

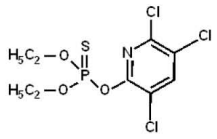
(Delayed Non-Match-to Sample)



Training Phase
(Forced 4)

Delay

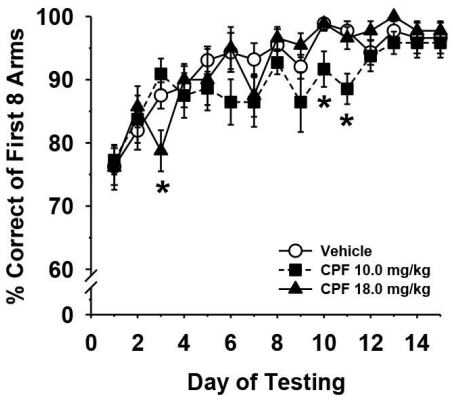
Test Phase
(Free 8)



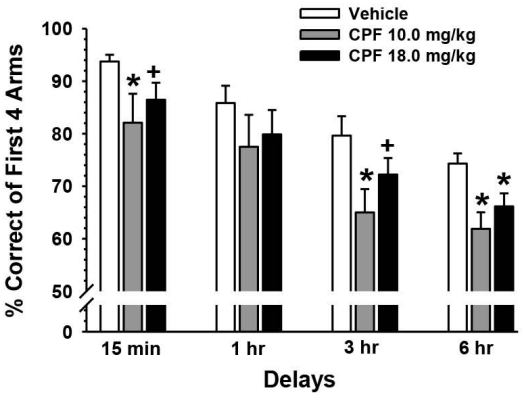
Radial Arm Maze

N=8-12

Win-Shift

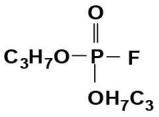


DNMTP



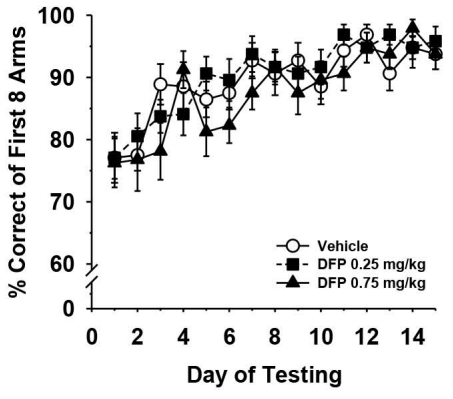
* = p<0.05
\+ = p<0.07

*** Behavioral Testing Began on day 50 of the OP-washout period**

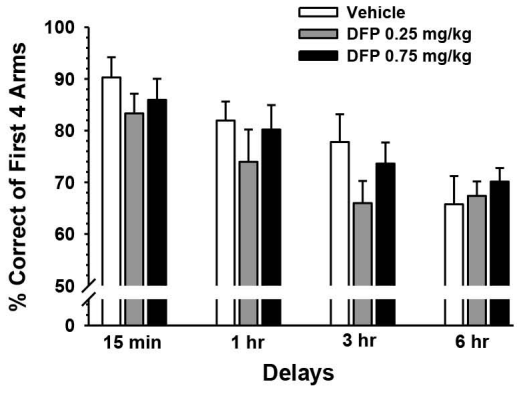


Radial Arm Maze

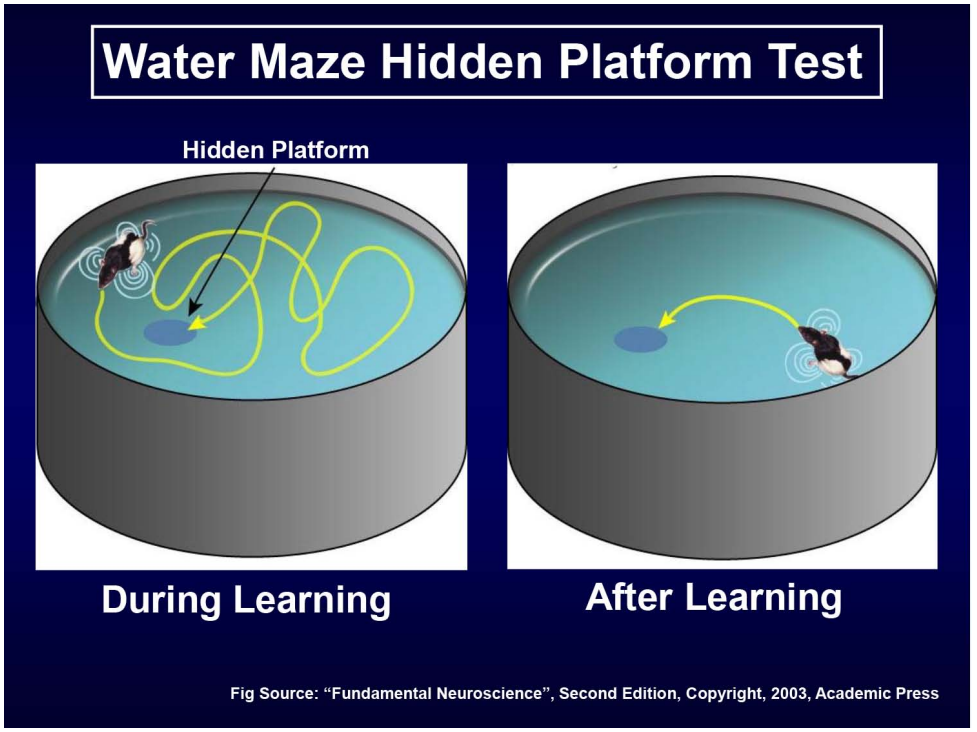
Win-Shift

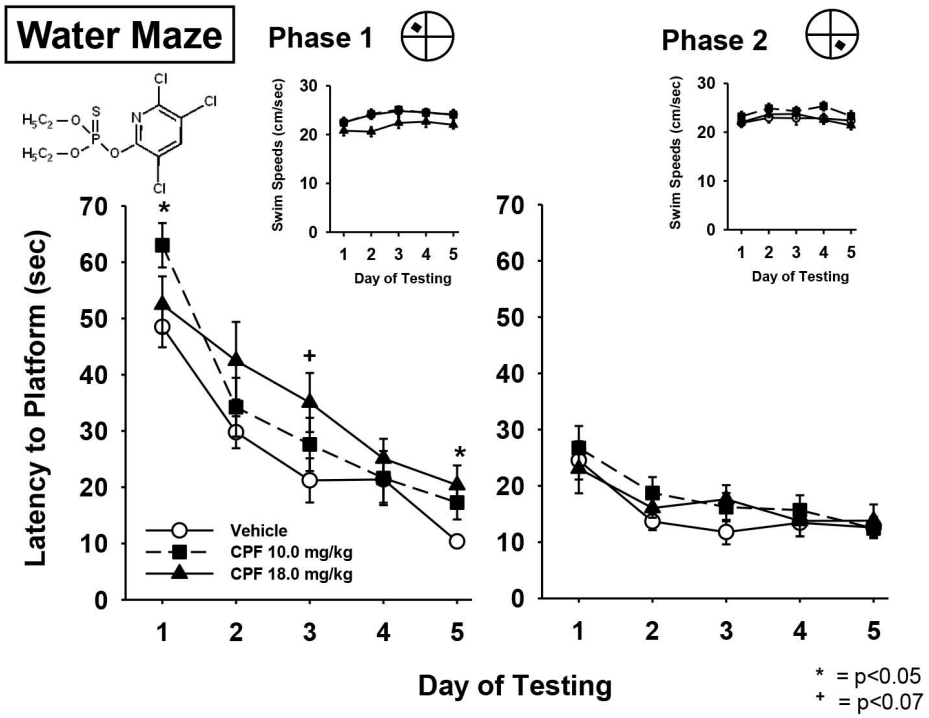


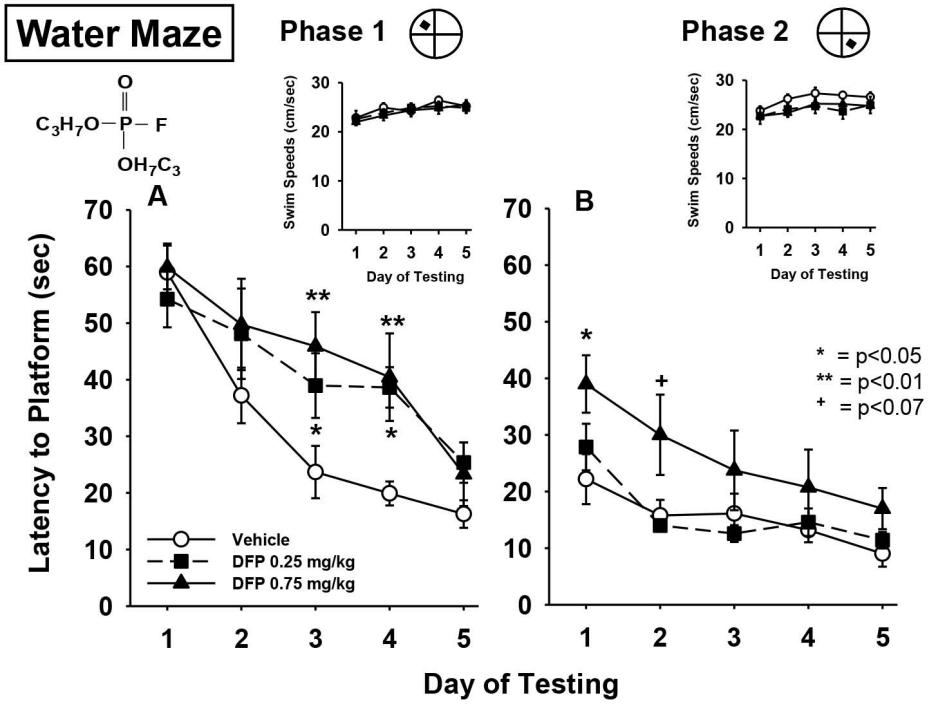
DNMTP



*** Behavioral Testing Began on day 50 of the OP-washout period**


Water Maze Hidden Platform Test
Hidden Platform
During Learning
After Learning
Fig Source: "Fundamental Neuroscience", Second Edition, Copyright, 2003, Academic Press


Water Maze
Phase 1
Phase 2
Swim Speeds (cm/sec)
Day of Testing
Latency to Platform (sec)
Vehicle
CPF 10.0 mg/kg
CPF 18.0 mg/kg
Day of Testing
* = p<0.05
+ = p<0.07



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Chronic impairments in spatial learning and memory in rats previously exposed to chlorpyrfos or diisopropylfluorophosphate

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Conclusion

"Repeated, subthreshold exposures to CPF and DFP may lead to chronic deficits in spatial learning and memory (i.e., long after cholinesterase inhibition has abated) and that insecticide and nerve agent OPs may have differential effects depending on the cognitive domain evaluated".

Current and Future Studies

- Specific Aim #1: Determine the consequences of repeated subthreshold exposures to representative OPs on axonal transport in the living rat brain.
  - Manganese-Enhanced Magnetic Resonance Imaging (MEMRI) Studies
- Specific Aim #2: Determine the consequences of repeated subthreshold exposures to representative OPs on myelin in the living rat brain.
  - Diffusion tensor imaging (DTI)
  - Black Gold II Histology

Approved DOD-CDMRP Proposal (GW110073) "Organophosphate-Related Alterations in Myelin and Axonal Transport in the Living Mammalian Brain"

Summary/Conclusions

- Repeated, subthreshold exposures to both insecticide and nerve agent OPs lead to protracted impairments of attention and memory-related behavioral tasks in animals.
- Insecticide and nerve agent OPs may have differential effects on specific domains of cognition.
- The mechanisms underlying OP-related impairments of cognition may involve deleterious effects on mitochondrial morphology and movement, axonal transport, and neurotrophin signaling.

Potential Therapeutic Strategies

- Cholinergic-Based Compounds
- Glutamate Receptor Antagonists
- Mitochondrial-Targeted Antioxidants
- Drugs that Increase Axonal Transport?
- Drugs that Improve Neurotrophin Function
- Cytokine-Based Treatments

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