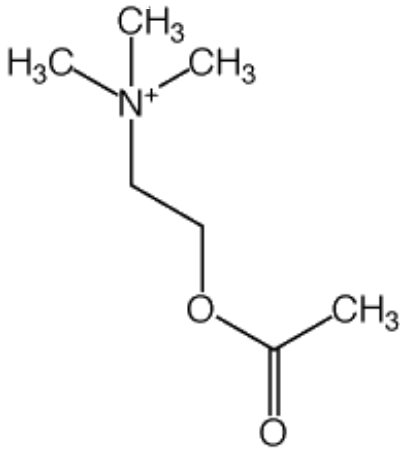


# **Axonal Transport in Living Rats Exposed to Gulf War Relevant Pesticides**

**Alvin V. Terry, Jr., Ph.D.**

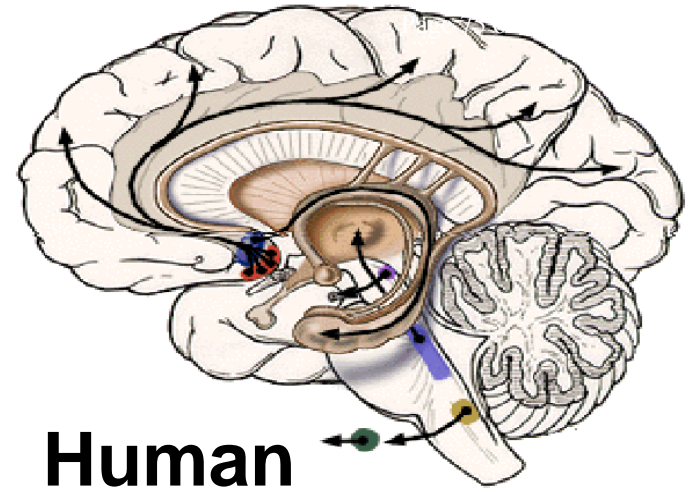
**Department of Pharmacology and Toxicology**



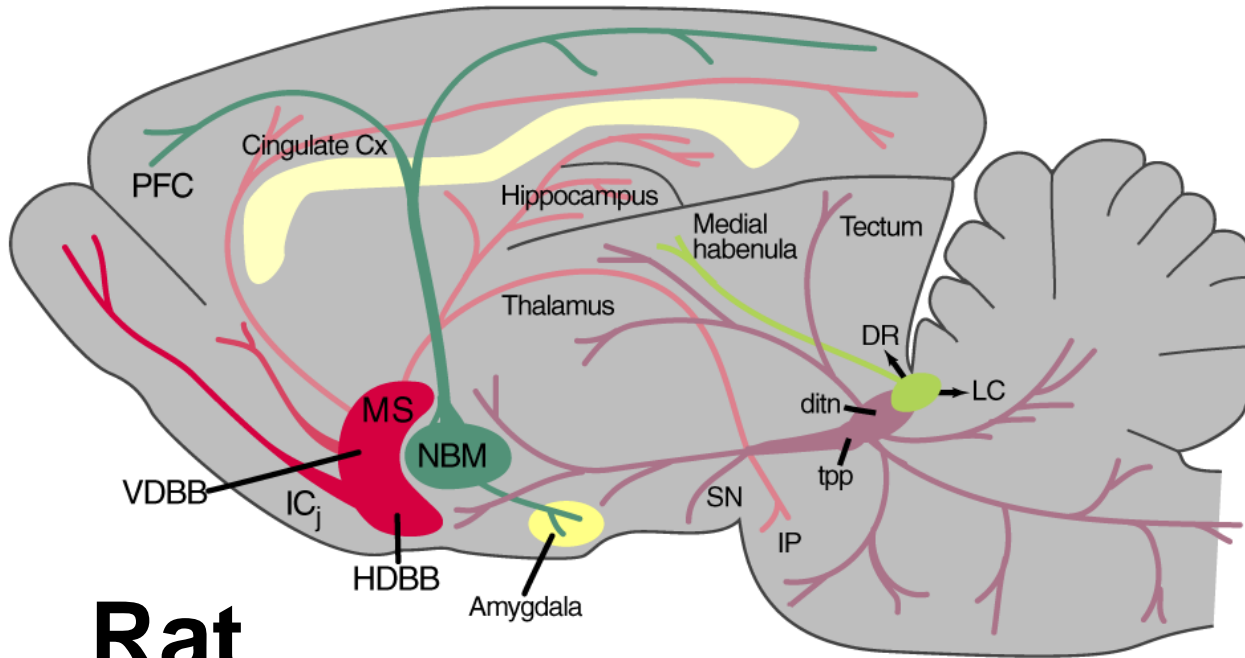


Acetylcholine

# Central Cholinergic Pathways

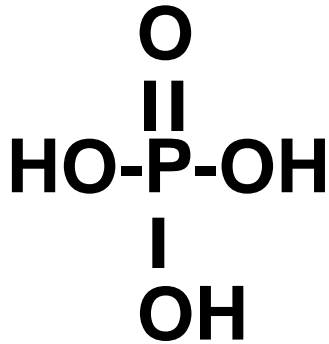


Human

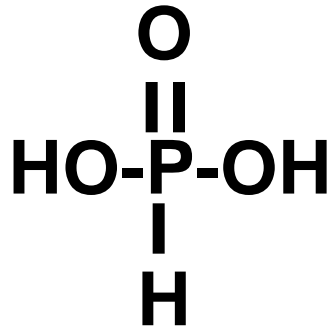


Rat

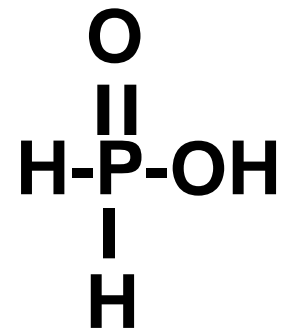
# Organophosphates



phosphoric acid



phosphonic acid



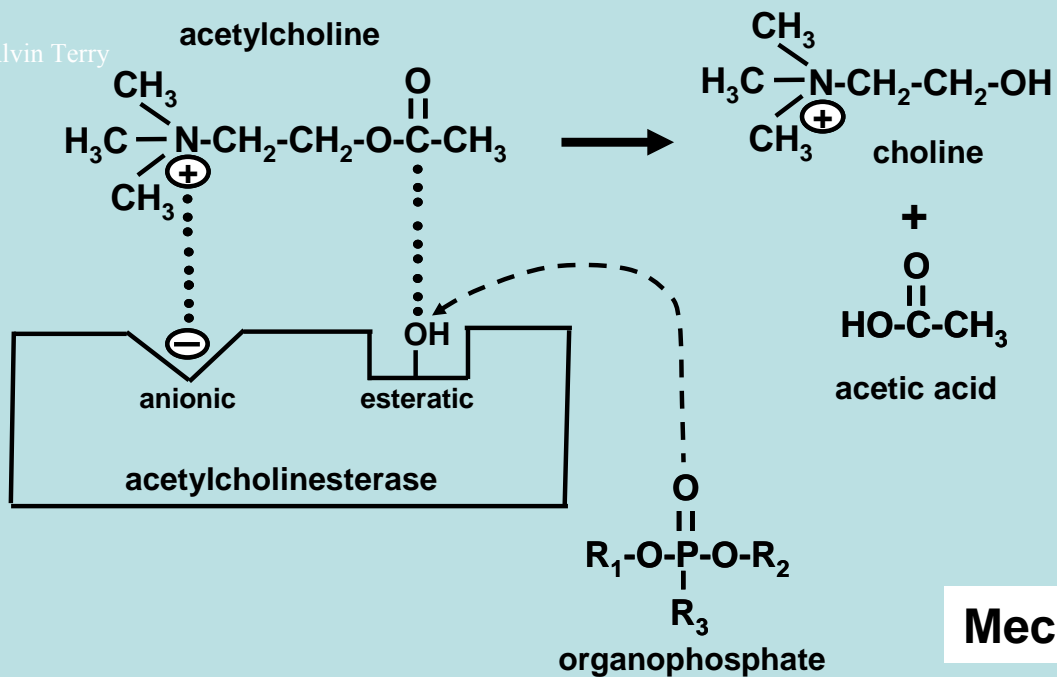
phosphinic Acid

- Esters of phosphoric, phosphonic & phosphinic acid
- First synthesized in the 1800s
- Insecticide Potential Recognized in the 1930s
- Further developed as “Nerve Agents” by the Germans and British during WWII

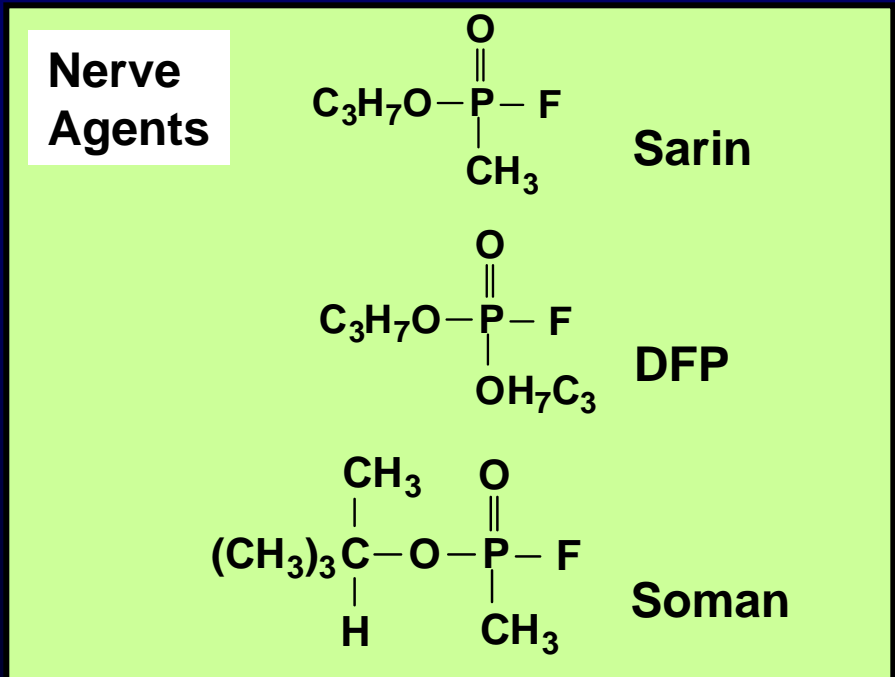
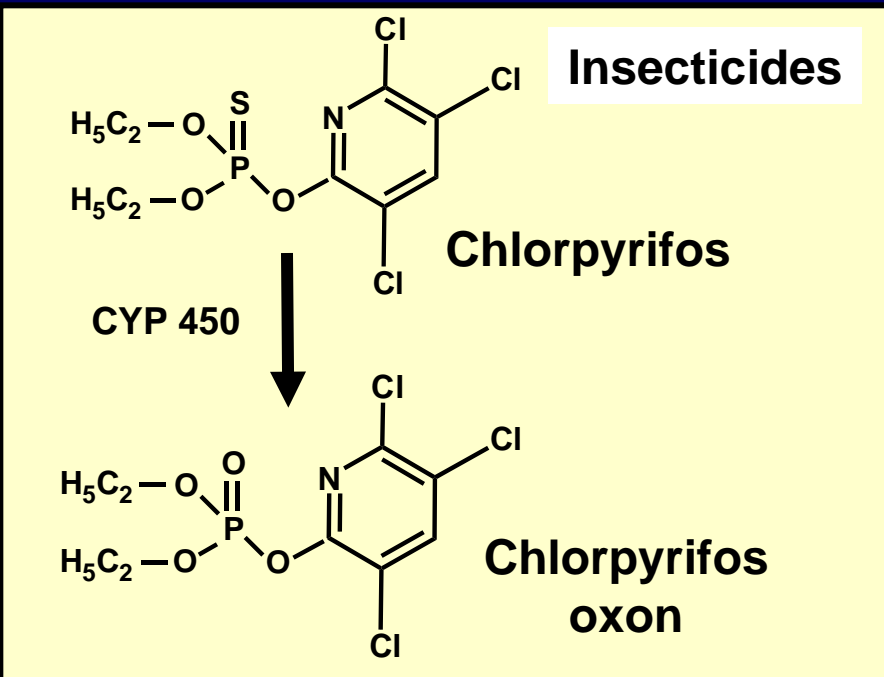
# Organophosphate-Based Chemicals

## Found in:

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



**Mechanism of Action**



# 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/Repeated Subthreshold 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.

# **AChE inhibition may not be the whole story....**

- **Different OPs can have markedly different toxicological profiles despite having similar effects on AChE activity.**
- **Both AChE knockout mice and their wild type controls exposed to OPs exhibited similar signs of neurotoxicity.**
- **Reports in both the human and animal literature indicate that OP toxicity (especially associated with chronic exposure) can occur in the absence of AChE inhibition.**
- **Human studies of occupational exposures to OPs often fail to find a significant association between blood AChE activity and neurobehavioral deficits.**

**Reviewed in Banks and Lein, *NeuroToxicology* 33 575–584, 2012**

# Overall Objectives

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



# 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 relatively high levels of 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**



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

---

### Continuous Performance Task (CPT) AX Type

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

Hit Lever



# 5C-SRTT

## Dependent Measures

### Task Measure

Accuracy (% correct)  
% Omissions  
Premature Responses  
Perseverative Responses  
Timeout Responses

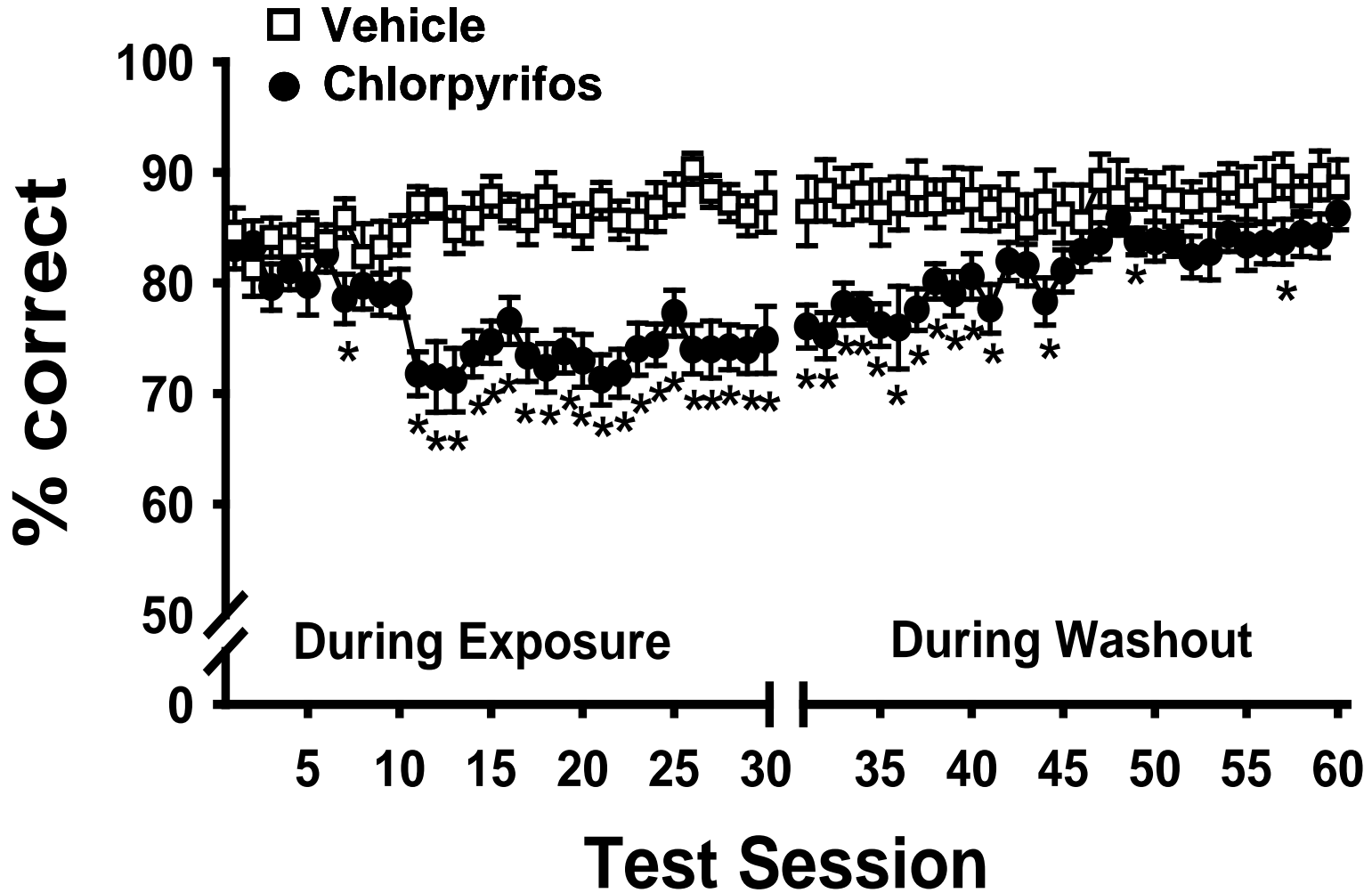
Correct Latency (mean) }  
Incorrect Latency (mean) }  
Reward Latency (mean) }

### Behavioral Domain

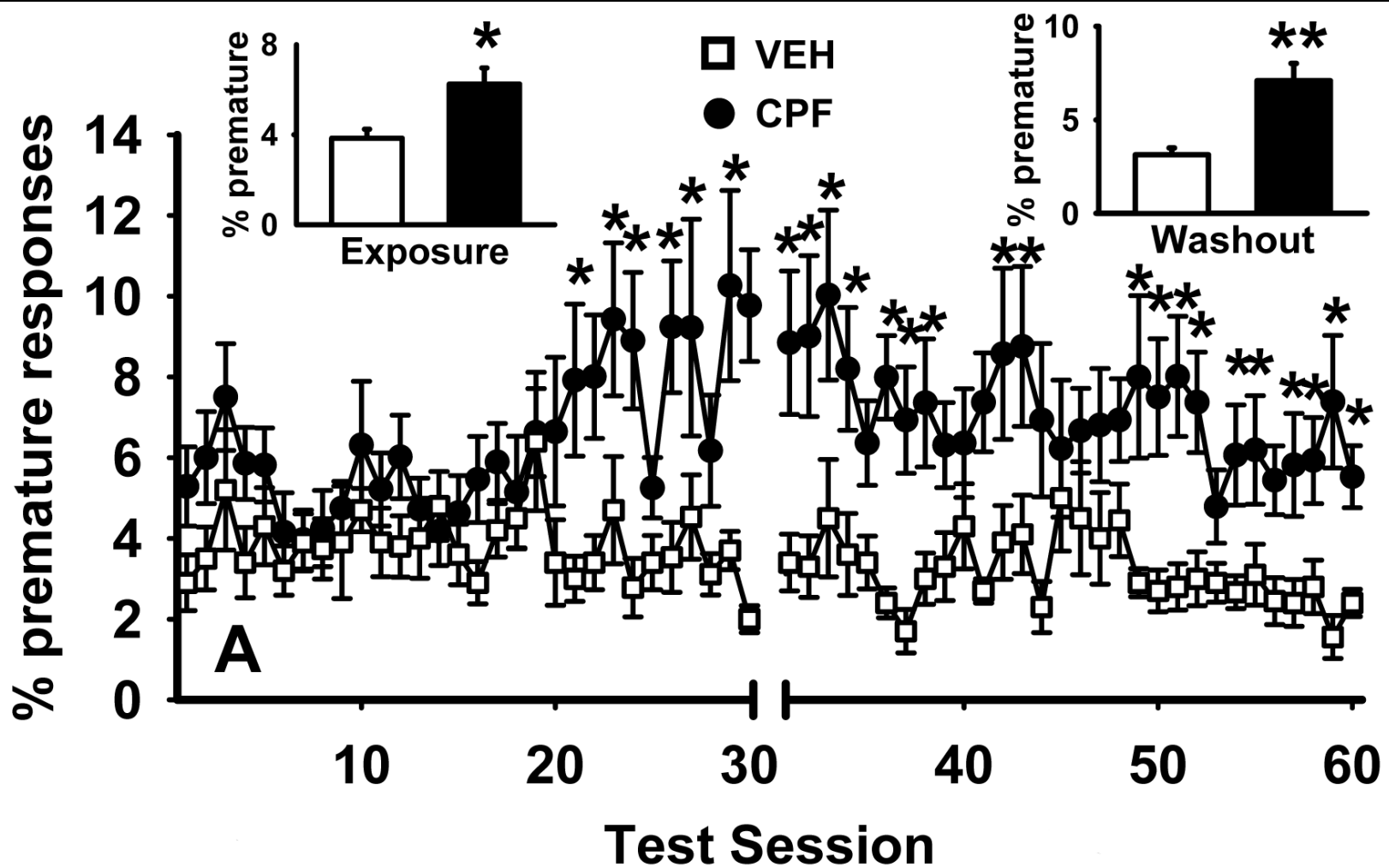
Sustained Attention  
Attention/Motivation  
Impulsivity  
Compulsivity  
Compulsivity/Cognitive  
Flexibility

Processing Speed  
and/or Motivation

# 5C-SRTT-Chlorpyrifos (Alternate Day Exposures)



# 5C-SRTT-Chlorpyrifos (Alternate Day Exposures)



# 5C-SRTT-Chlorpyrifos Experiments

## Conclusion

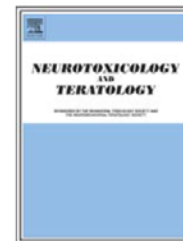
Neurotoxicology and Teratology 32 (2010) 415–424



Contents lists available at [ScienceDirect](#)

Neurotoxicology and Teratology

journal homepage: [www.elsevier.com/locate/neutera](http://www.elsevier.com/locate/neutera)



Repeated exposures to low-level chlorpyrifos results in impairments in sustained attention and increased impulsivity in rats

M.L. Middlemore-Risher<sup>a</sup>, J.J. Buccafusco<sup>b,c</sup>, A.V. Terry, Jr<sup>a,b,d,\*</sup>

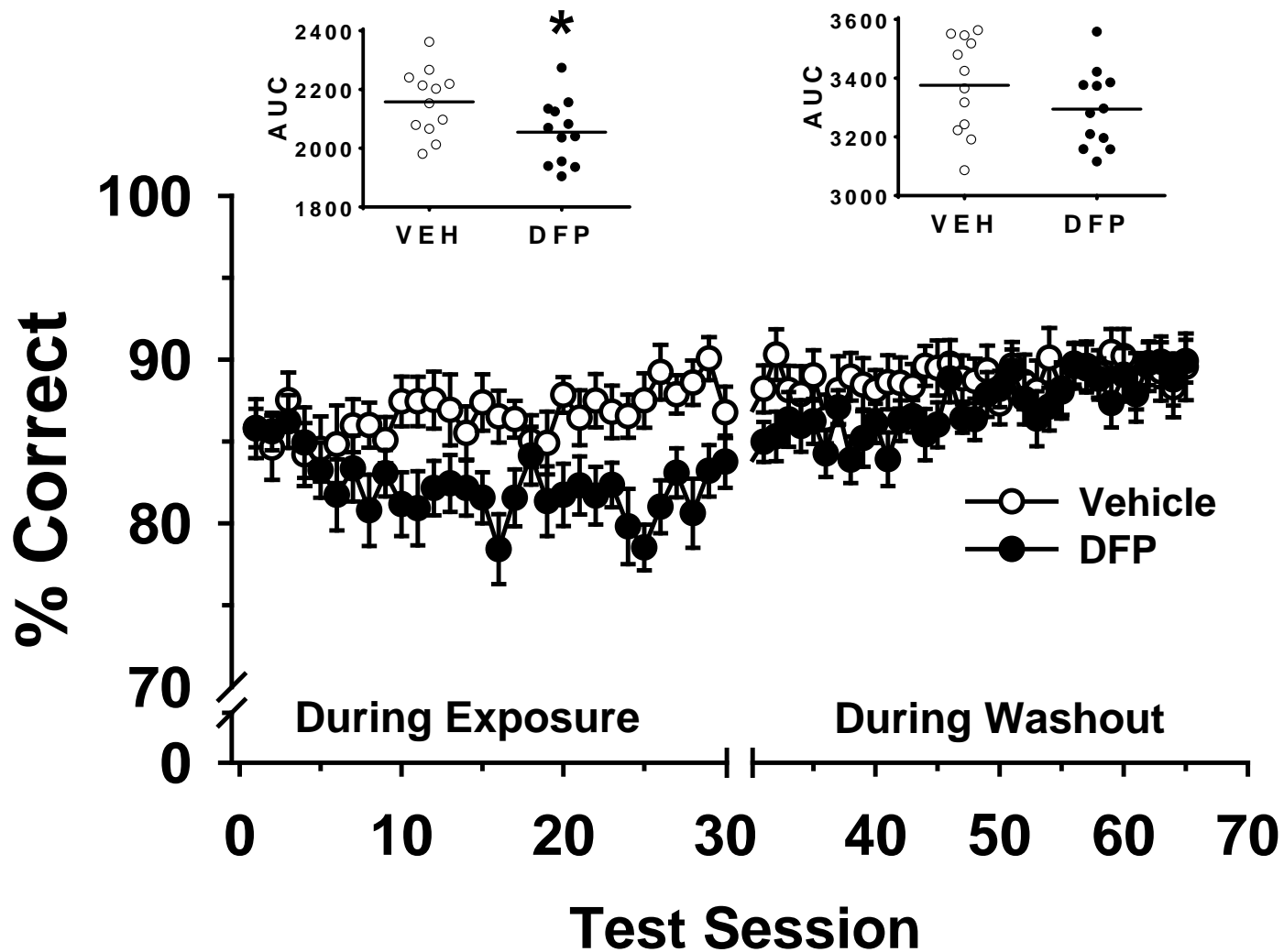
<sup>a</sup> Program of Clinical and Experimental Therapeutics, University of Georgia, College of Pharmacy, Augusta, GA, 30912, United States

<sup>b</sup> Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, Georgia, 30912

<sup>c</sup> Charlie Norwood Veterans Administration Medical Center, Augusta, Georgia, 30912, United States

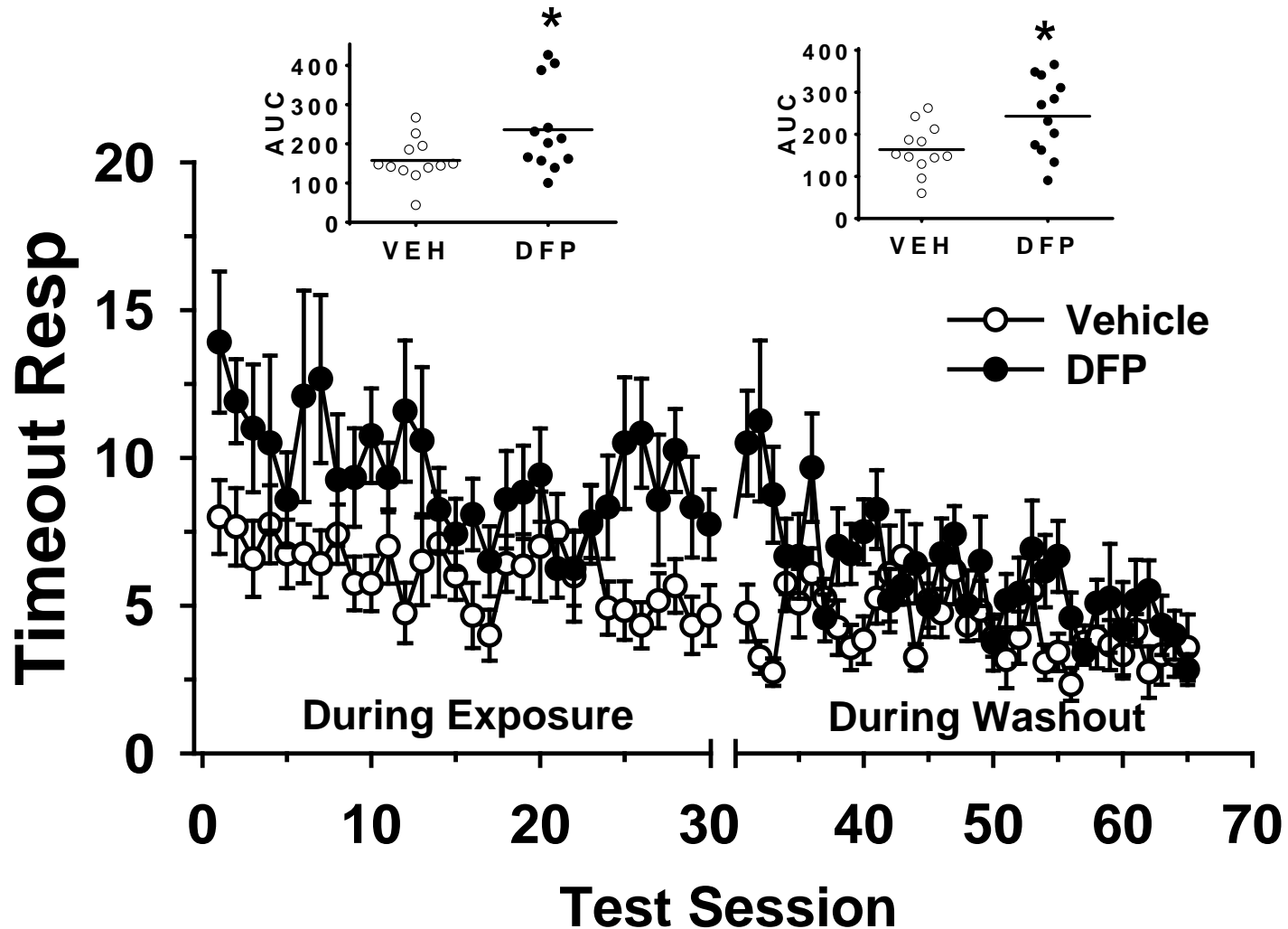
<sup>d</sup> Small Animal Behavior Core, Medical College of Georgia, Augusta, Georgia, 30912, United States

# 5C-SRTT-DFP 0.5 mg/kg (Alternate Day Exposures)





# 5C-SRTT-DFP 0.5 mg/kg (Alternate Day Exposures)



# 5C-SRTT-DFP Experiments

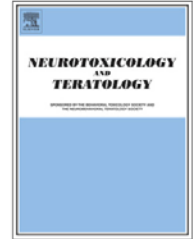
## Conclusion

Neurotoxicology and Teratology 44 (2014) 18–29

Contents lists available at [ScienceDirect](#)

Neurotoxicology and Teratology

journal homepage: [www.elsevier.com/locate/neutera](http://www.elsevier.com/locate/neutera)



Repeated exposures to diisopropylfluorophosphate result in impairments of sustained attention and persistent alterations of inhibitory response control in rats



Alvin V. Terry Jr. <sup>a,b,\*</sup>, Patrick M. Callahan <sup>a,b</sup>, Wayne D. Beck <sup>a</sup>, Leah Vandenhuerk <sup>b</sup>, Samantha Sinha <sup>b</sup>, Kristy Bouchard <sup>b</sup>, Rose Schade <sup>b</sup>, Jennifer L. Waller <sup>c</sup>

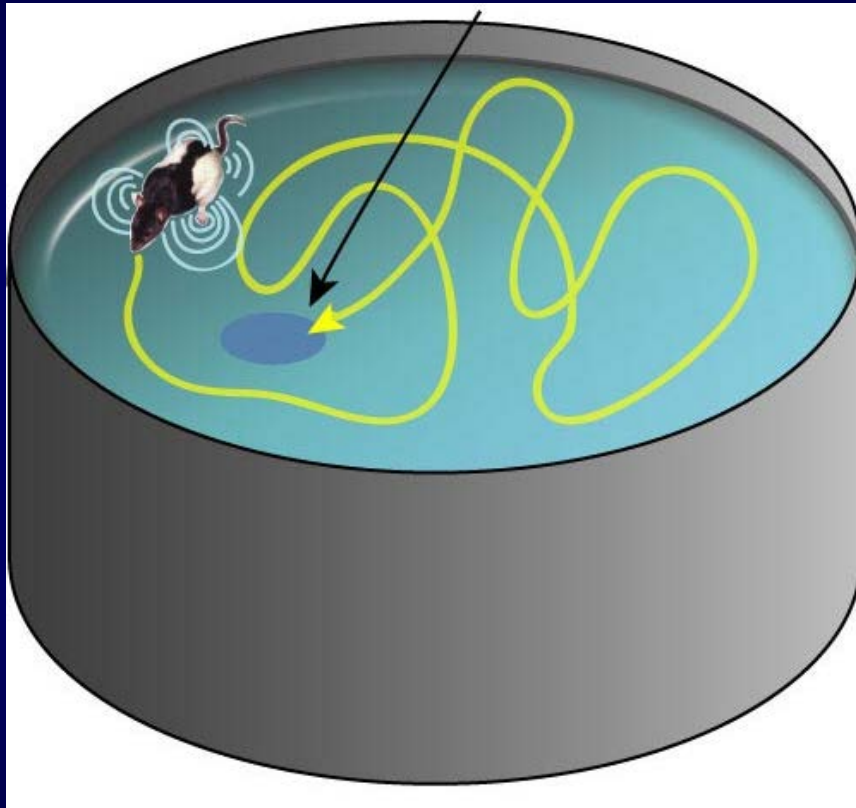
<sup>a</sup> Department of Pharmacology and Toxicology, Georgia Regents University, Augusta, GA 30912, United States

<sup>b</sup> Small Animal Behavior Core, Georgia Regents University, Augusta, GA 30912, United States

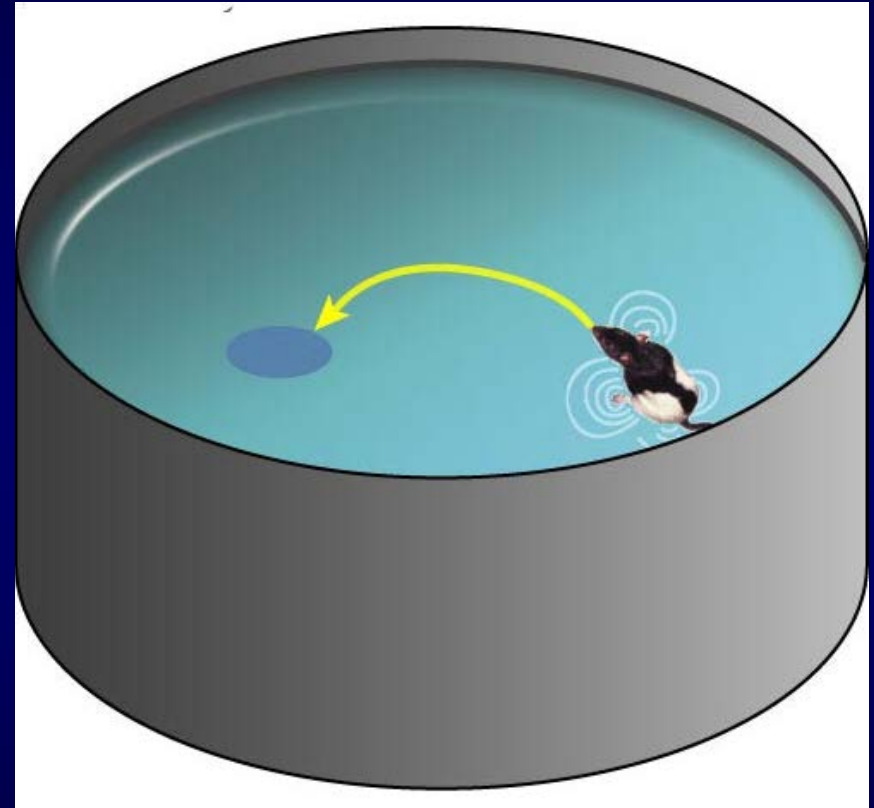
<sup>c</sup> Department of Biostatistics, Georgia Regents University, Augusta, GA 30912, United States

# Water Maze Hidden Platform Test

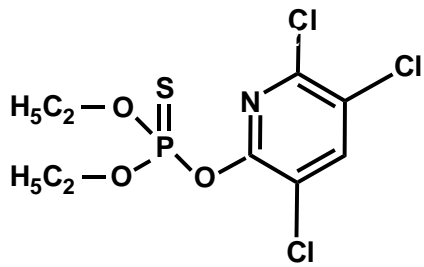
## Hidden Platform



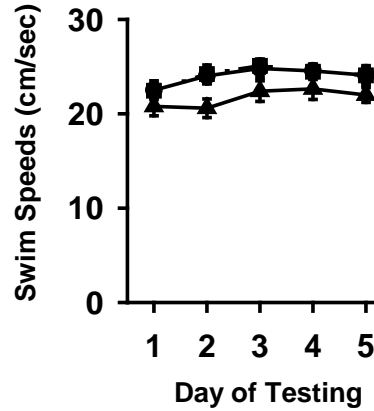
**During Learning**



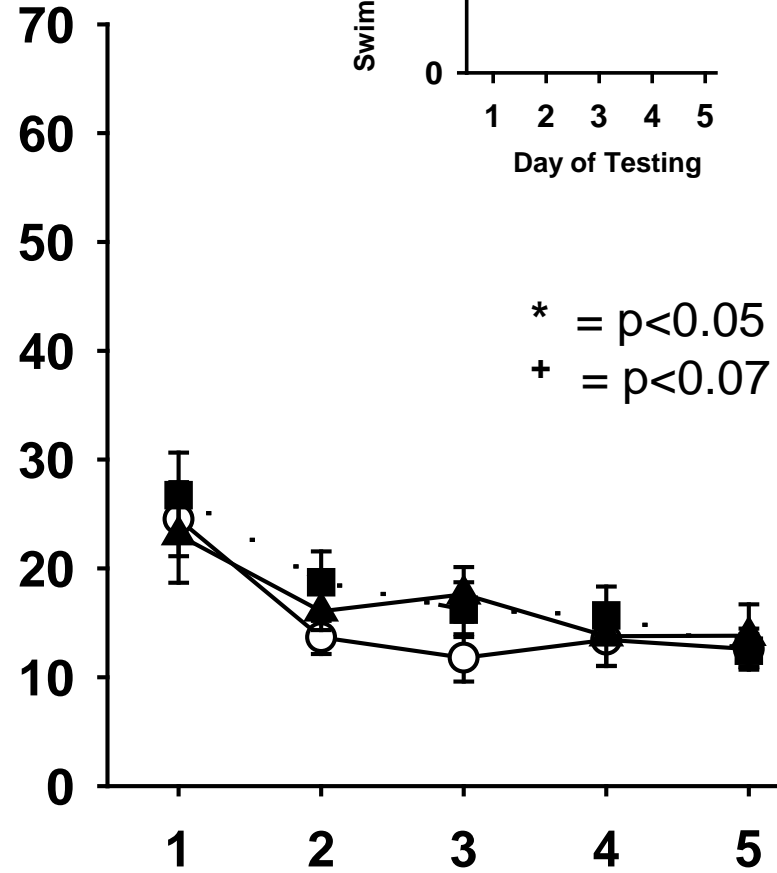
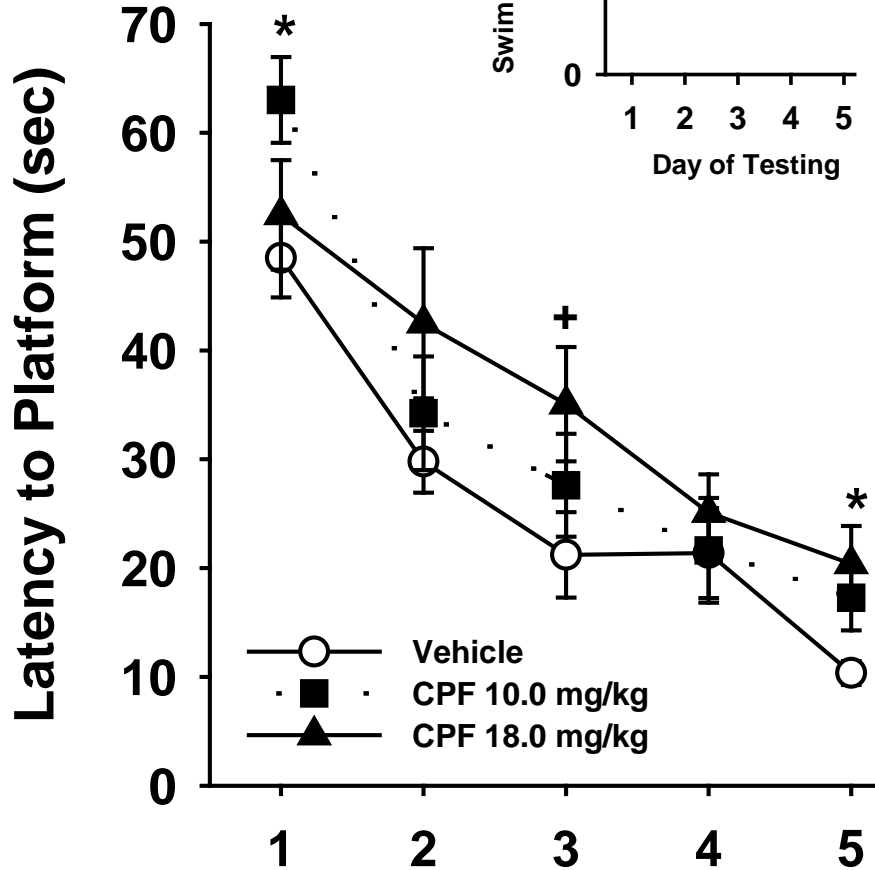
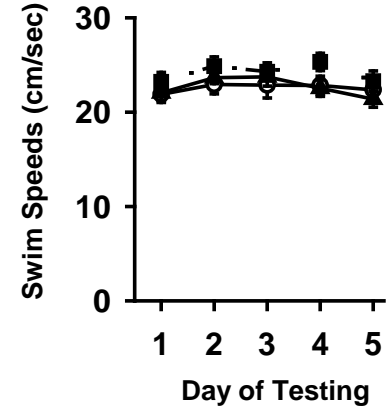
**After Learning**



### Phase 1



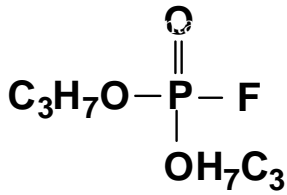
### Phase 2



\* = p<0.05  
+ = p<0.07

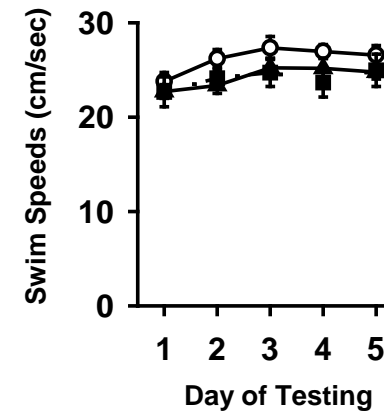
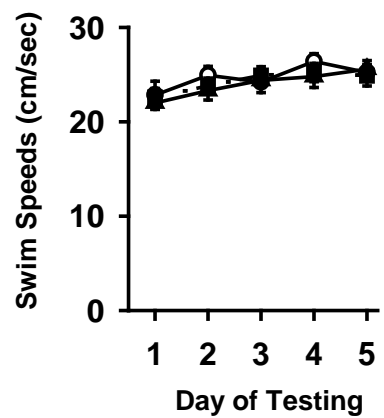
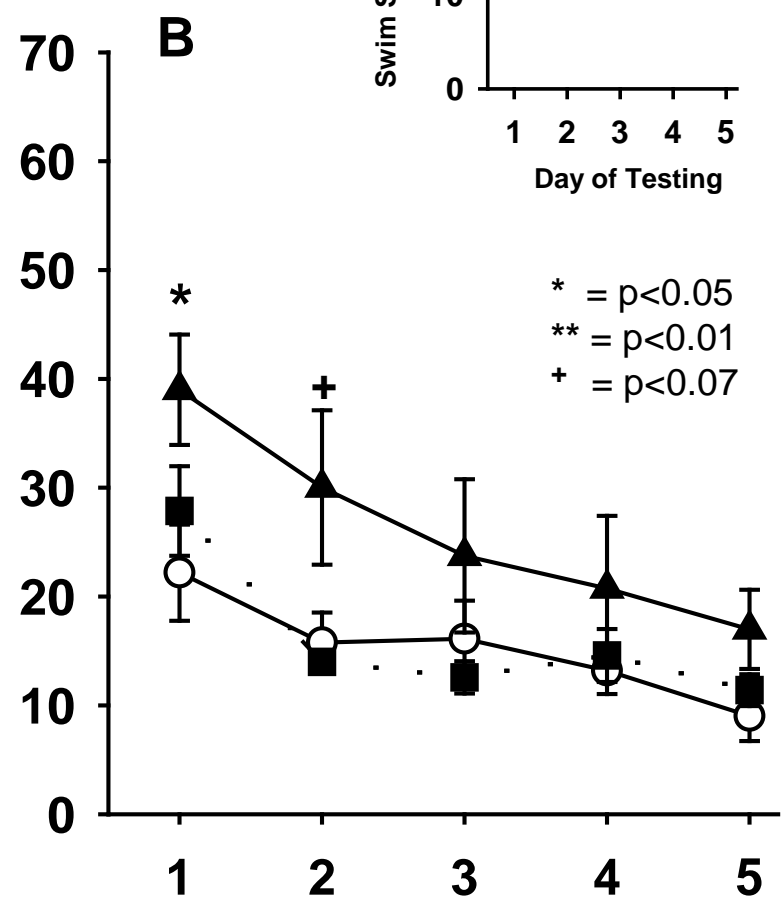
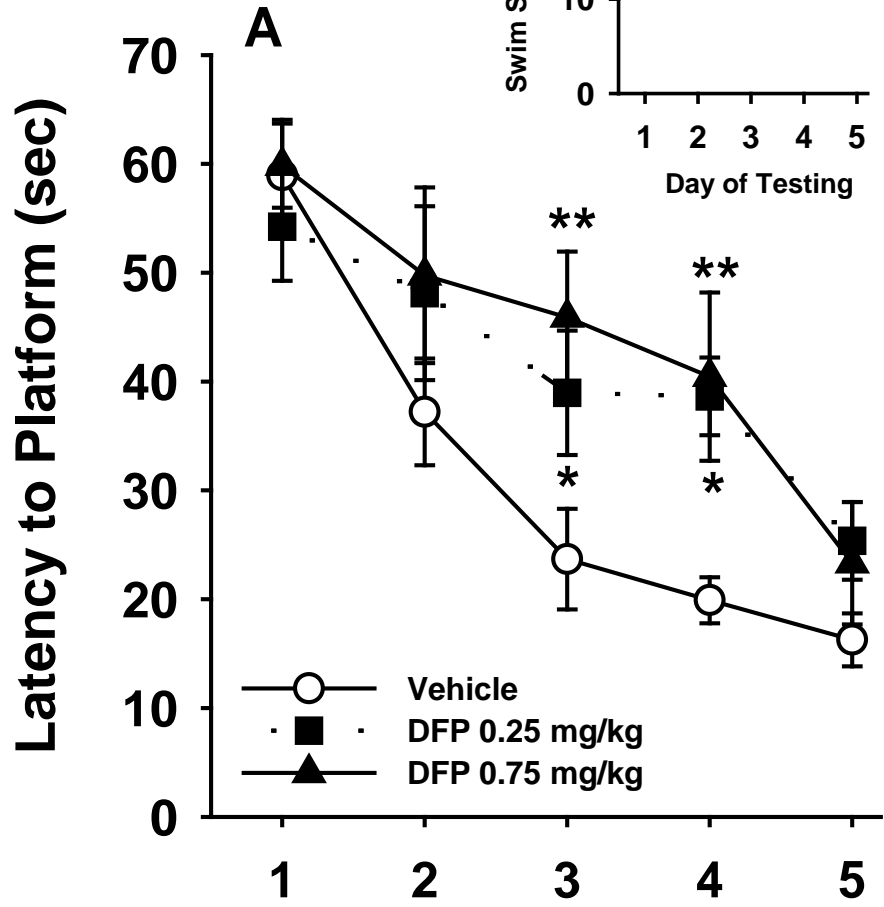
140 days post OP exposure

Day of Testing



Phase 1 

Phase 2 



\* = p<0.05  
 \*\* = p<0.01  
 + = p<0.07

**140 days post OP exposure**

**Day of Testing**

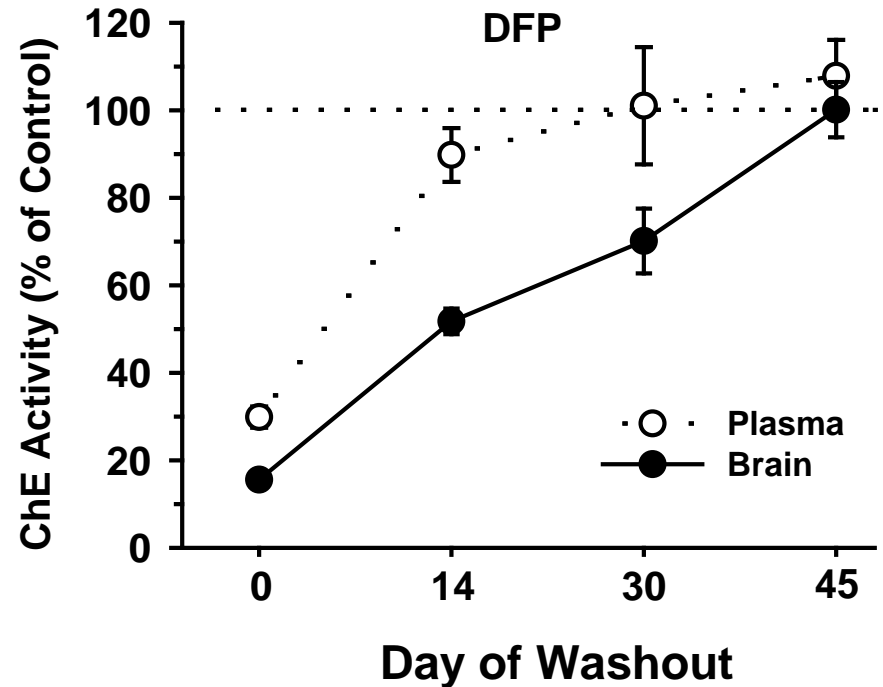
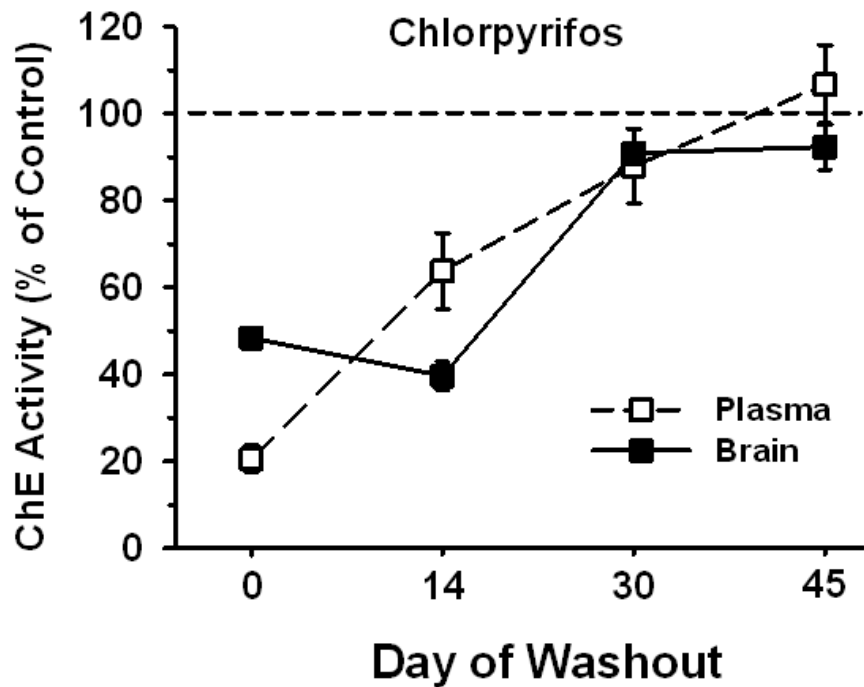


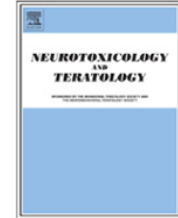
Fig 1. The effects of repeated exposures to CPF 18.0 mg/kg (Left) or DFP 0.75 mg/kg (Right) on cholinesterase activity in the plasma and brain at various time points during a 45 day OP-free washout period. Data (mean  $\pm$  SEM) are presented as % of vehicle-matched control levels. (N=3-6).



Contents lists available at SciVerse ScienceDirect

## Neurotoxicology and Teratology

journal homepage: [www.elsevier.com/locate/neutera](http://www.elsevier.com/locate/neutera)



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

A.V. Terry Jr. <sup>a,b,\*</sup>, W.D. Beck <sup>a</sup>, S. Warner <sup>b</sup>, L. Vandenhuerk <sup>b</sup>, P.M. Callahan <sup>a,b</sup>

<sup>a</sup> Department of Pharmacology and Toxicology, Georgia Health Sciences University, Augusta, Georgia, 30912, United States

<sup>b</sup> Small Animal Behavior Core, Georgia Health Sciences University, Augusta, Georgia, 30912, United States

# Conclusion

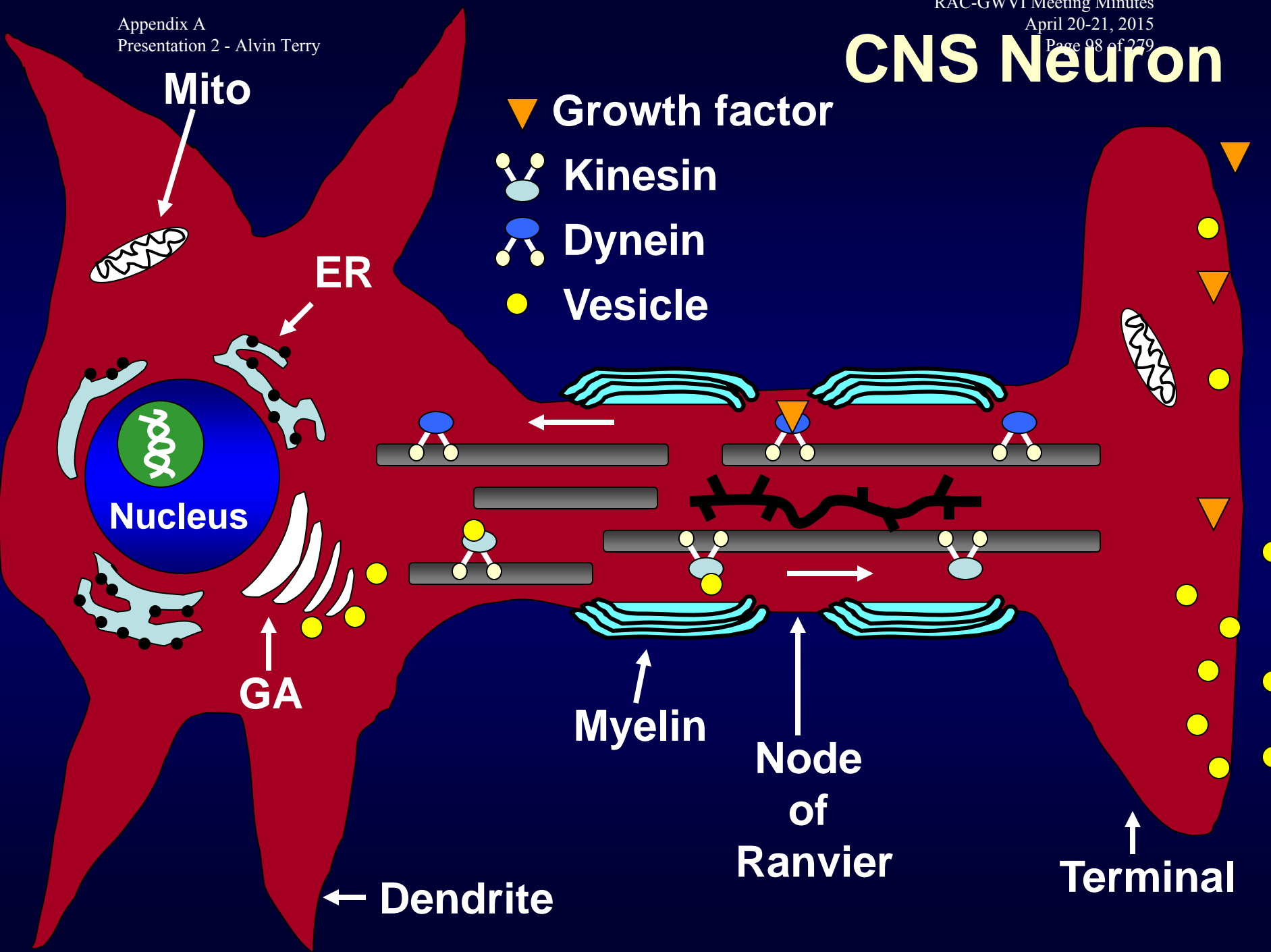
**“Repeated, subthreshold exposures to CPF and DFP may lead to chronic deficits in spatial learning and memory (i.e., long after cholinesterase inhibition has abated) and that insecticide and nerve agent OPs may have differential effects depending on the cognitive domain evaluated”.**

# OP Effects On Axonal Transport?

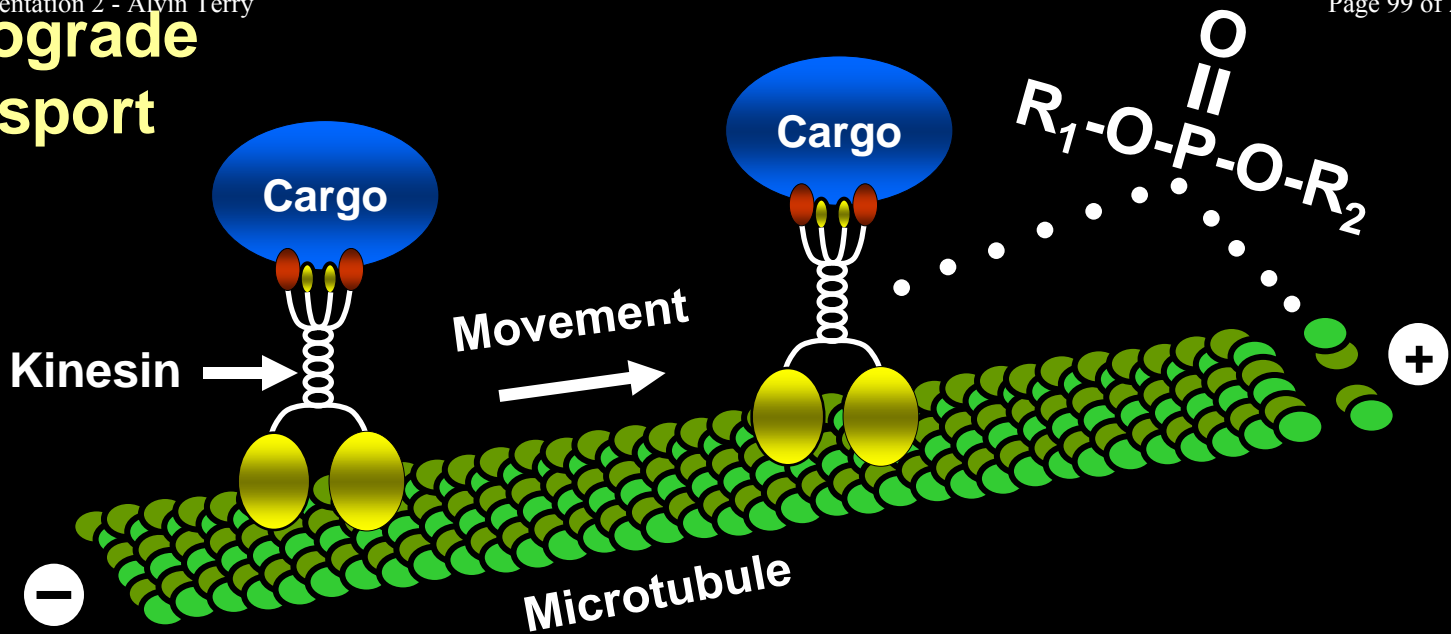
- **Decreases in presynaptic cholinergic receptors in the rat brain (Stone et al., 2000)**
- **Impairments in axonal transport of vesicles in sciatic nerves in rats (Terry et al., 2003; 2007)**
- **Disruption of tubulin polymerization & microtubule formation in vitro (Prendergast et al., 2007)**
- **Inhibited kinesin-dependent microtubule motility in vitro (Gearhart et al., 2007)**
- **OPs Covalently modify tyrosines on tubulin (Grigoryan et al., 2009; Bartlett et al, unpublished)**



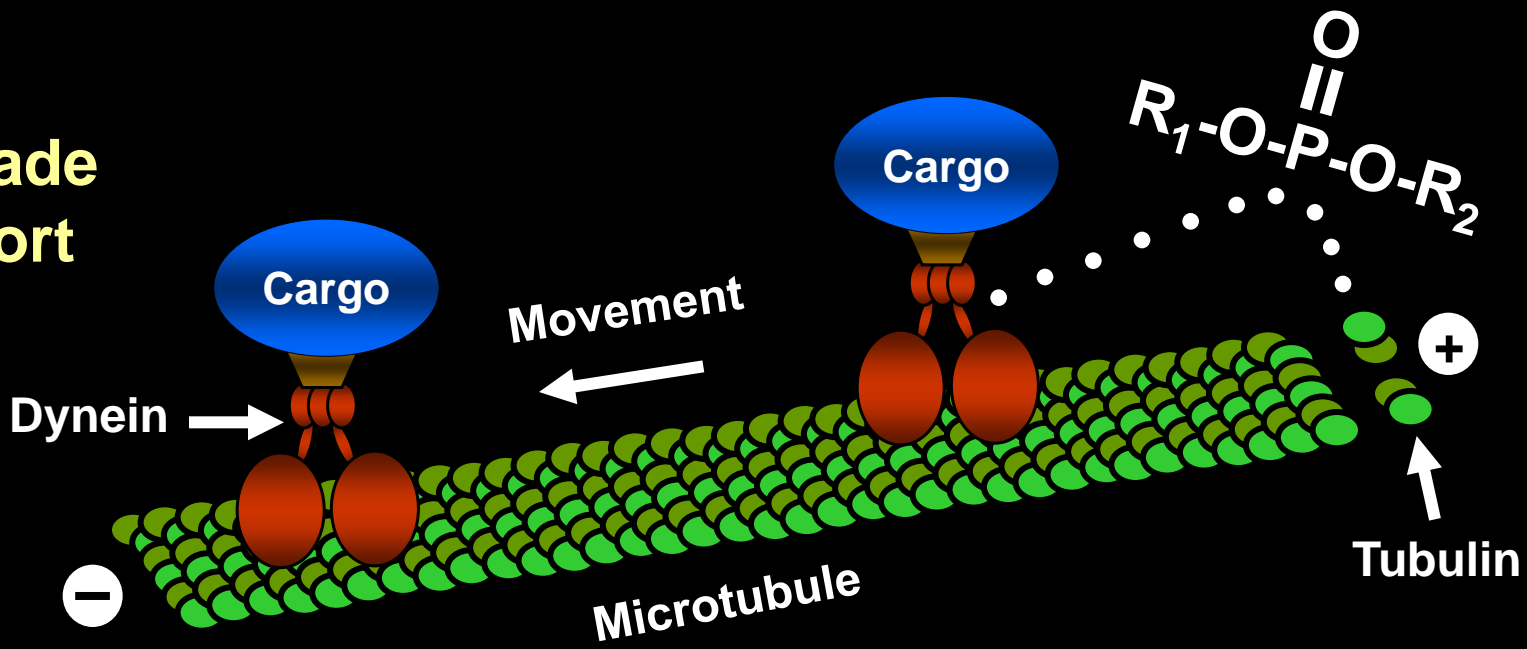
# CNS Neuron



# Anterograde Transport



# Retrograde Transport

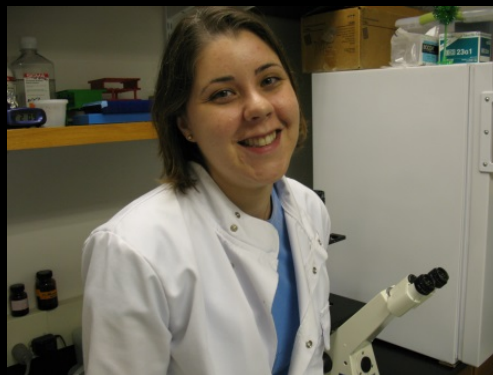


# Effects of Chlorpyrifos and Chlorpyrifos-Oxon on the Dynamics and Movement of Mitochondria in Rat Cortical Neurons<sup>S</sup>

Mary-Louise Middlemore-Risher, Bao-Ling Adam, Nevin A. Lambert, and Alvin V. Terry, Jr.

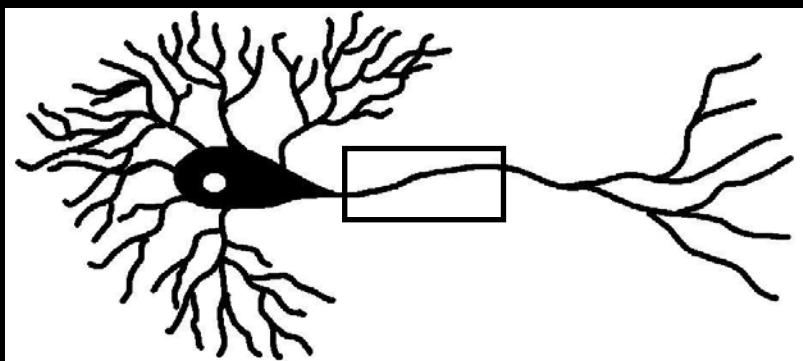
*Program in Clinical and Experimental Therapeutics, University of Georgia, College of Pharmacy, Augusta Georgia (M.-L.M.-R.); and Department of Pharmacology and Toxicology, Georgia Health Sciences University, Augusta, Georgia (B.-L.A., N.A.L., A.V.T.)*

Received June 3, 2011; accepted July 26, 2011



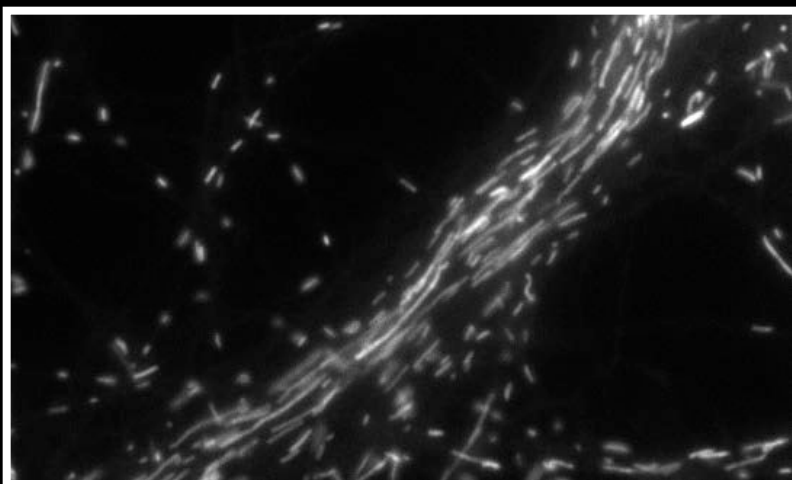
**Louise Middlemore-Risher**

# MitoTracker<sup>®</sup> Imaging Measurements

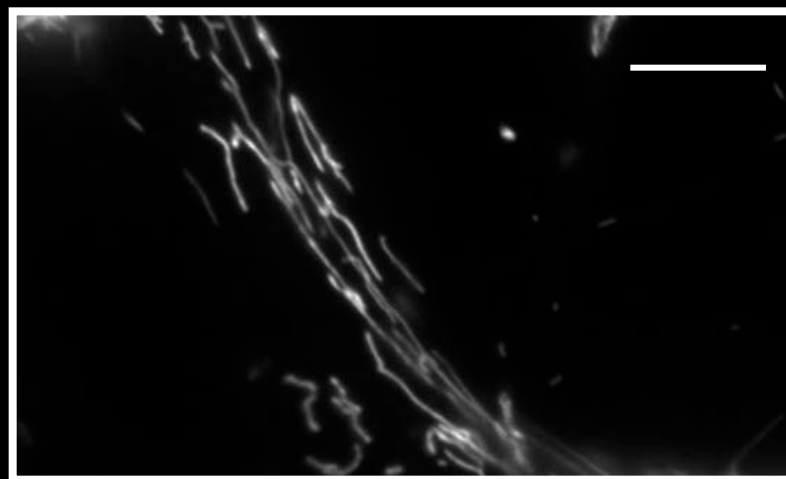


**Movement = mean # moving/ $\mu\text{m}$**   
**Length = average length in the ROI**  
**Number = # of mitochondria/ $\mu\text{m}$**

Scale bar = 100  $\mu\text{m}$



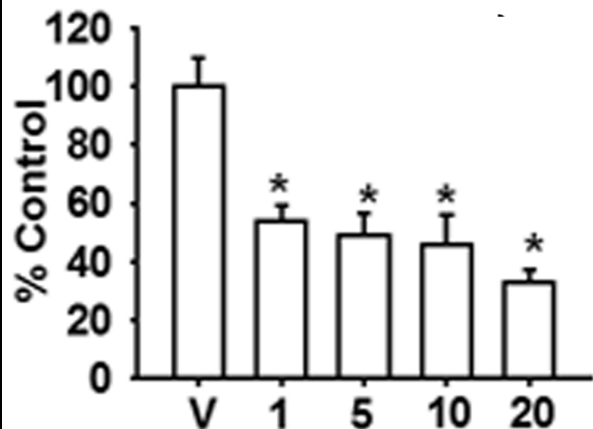
**Vehicle**



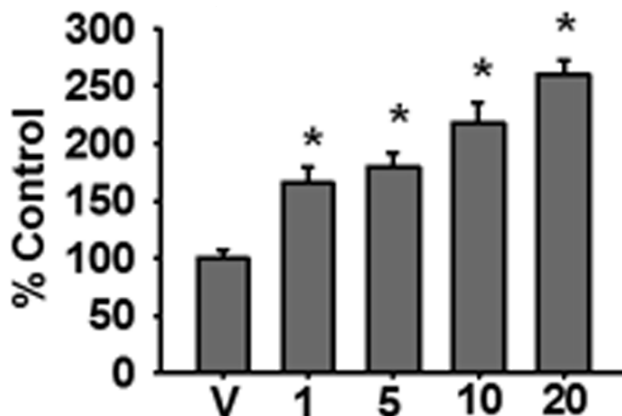
**CPF oxon (5.0 nM)**

# MitoTracker® Imaging Measurements

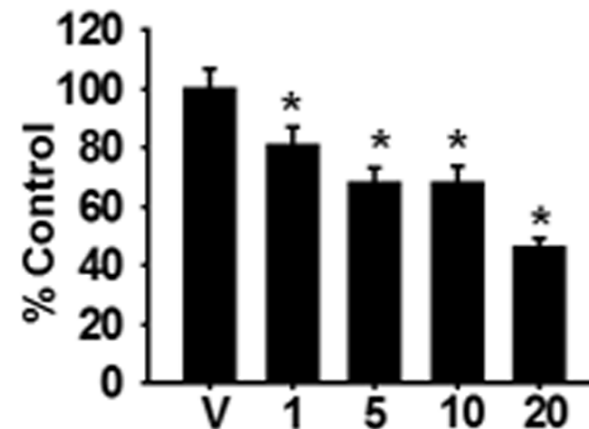
## Movement



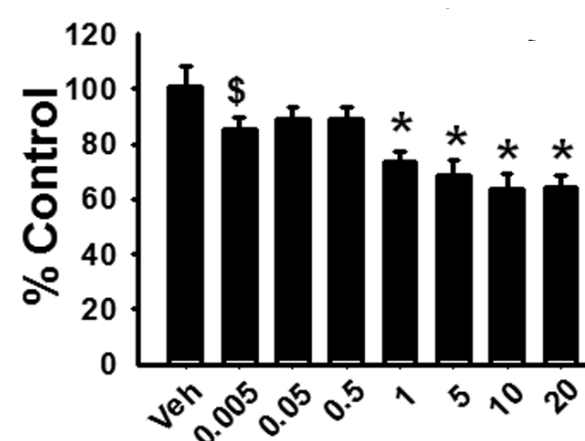
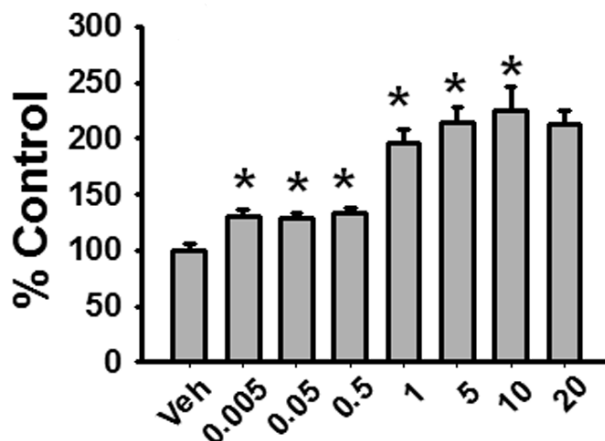
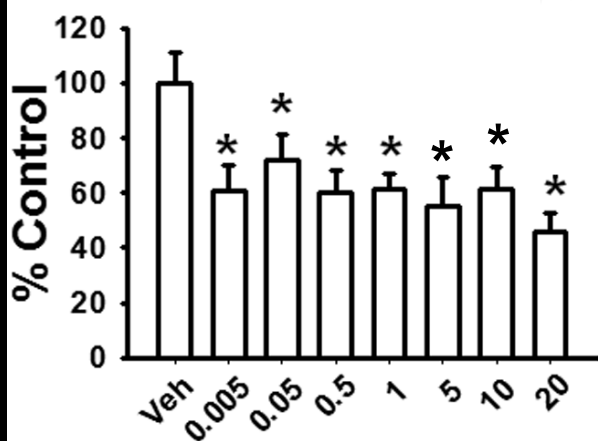
## Length



## Number

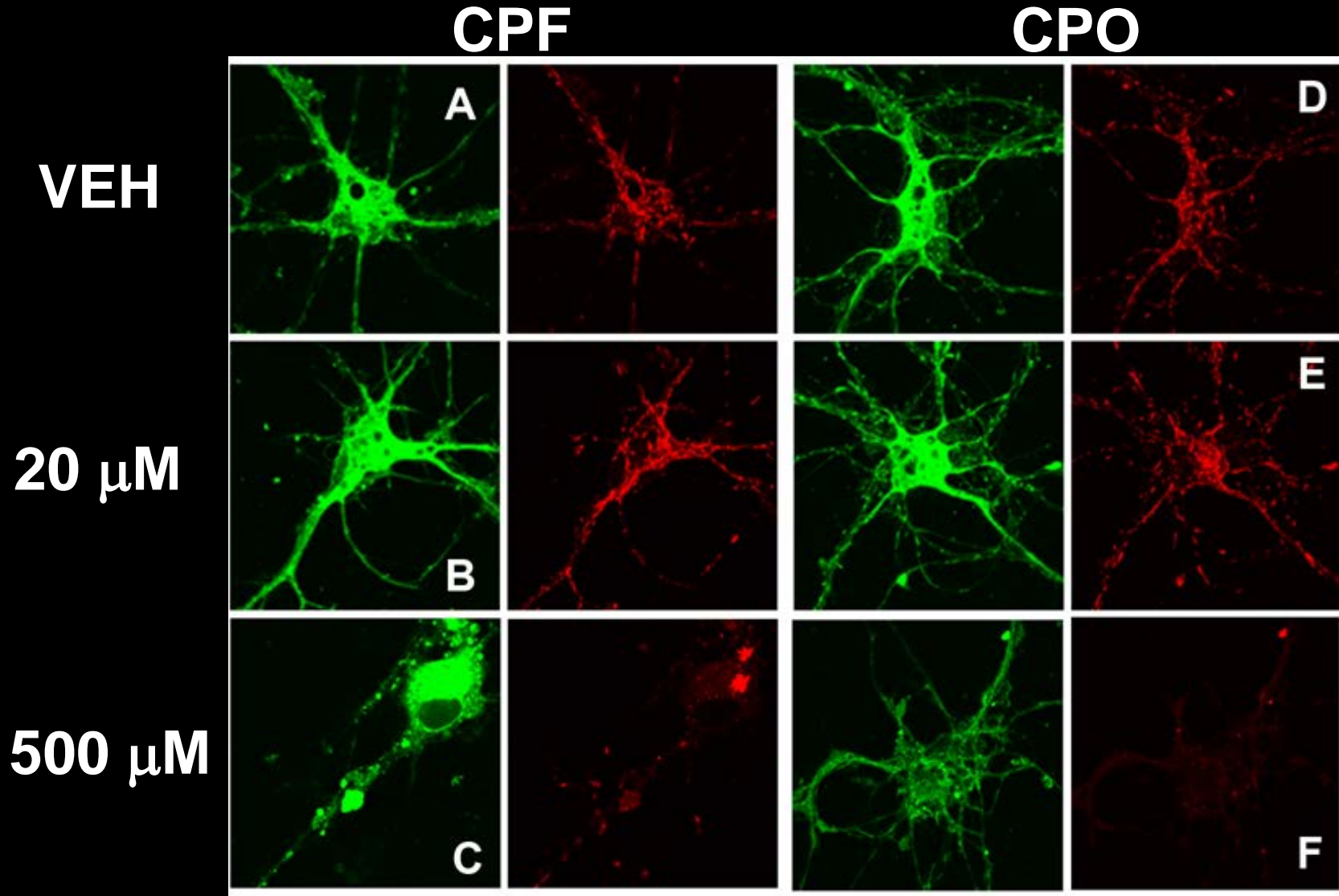


### Chlorpyrifos (μM)



### Chlorpyrifos oxon (μM)

# DePsipher™ Assay

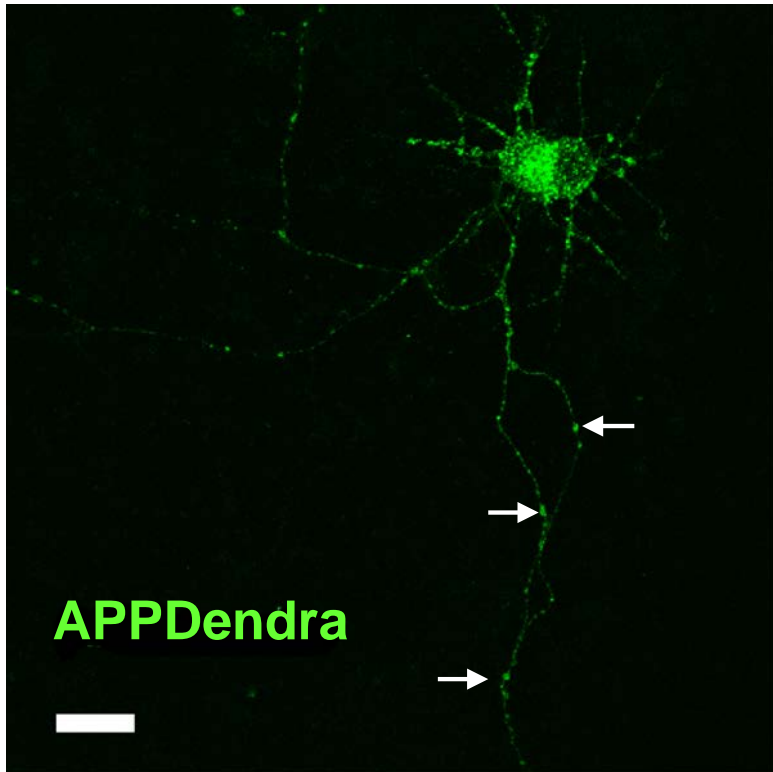
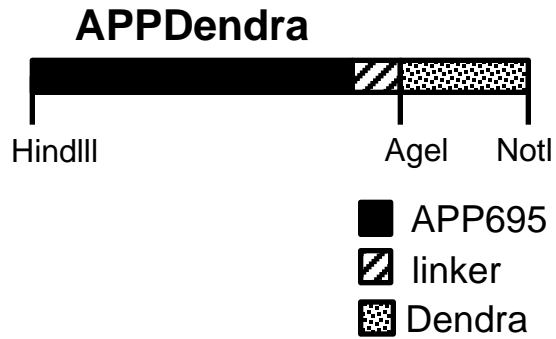


## **Summary (CPF & CPO in Neuronal Culture)**

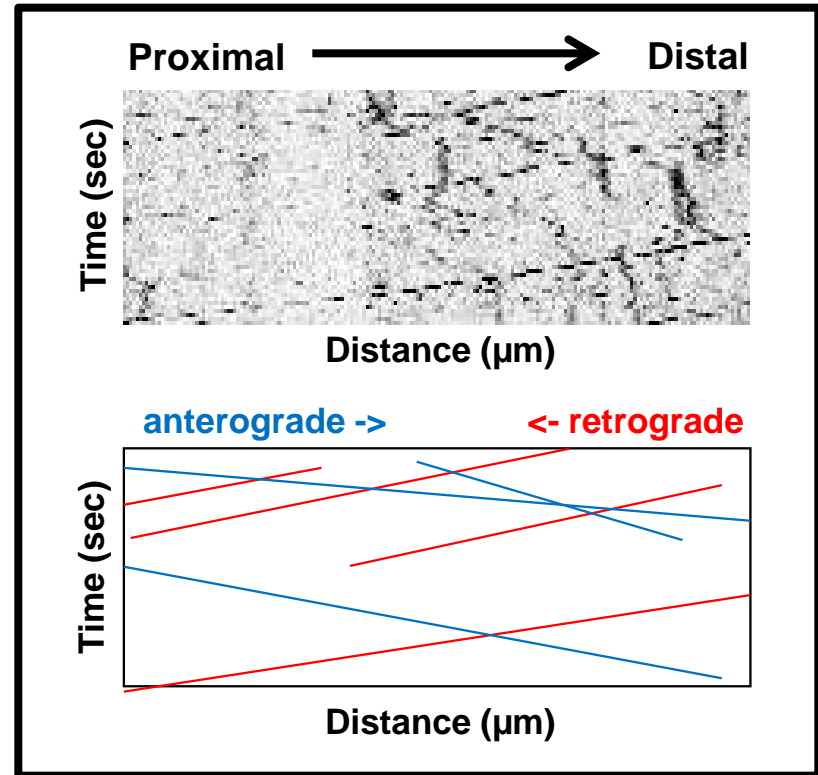
- **Concentration-dependent decrease in the transport of mitochondria in axons, an increase in mitochondrial length, and a decrease in mitochondrial number (indicative of increased fusion versus fission events)**
- **The neuronal changes occurred at OP concentrations that did not inhibit AChE, they were not blocked by cholinergic antagonists, and they did not appear to be associated with directly toxic effects on mitochondria (i.e., 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.**



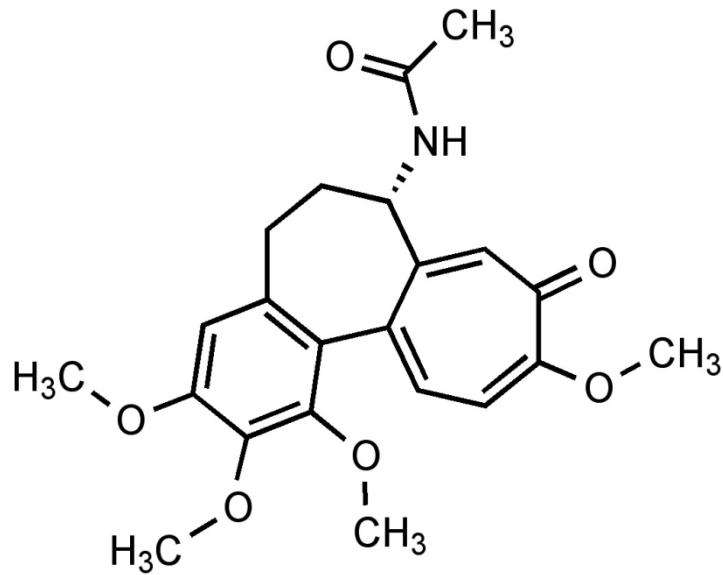
# APPDendra Tracking Studies (Primary Cortical Neurons)



## Kymograph







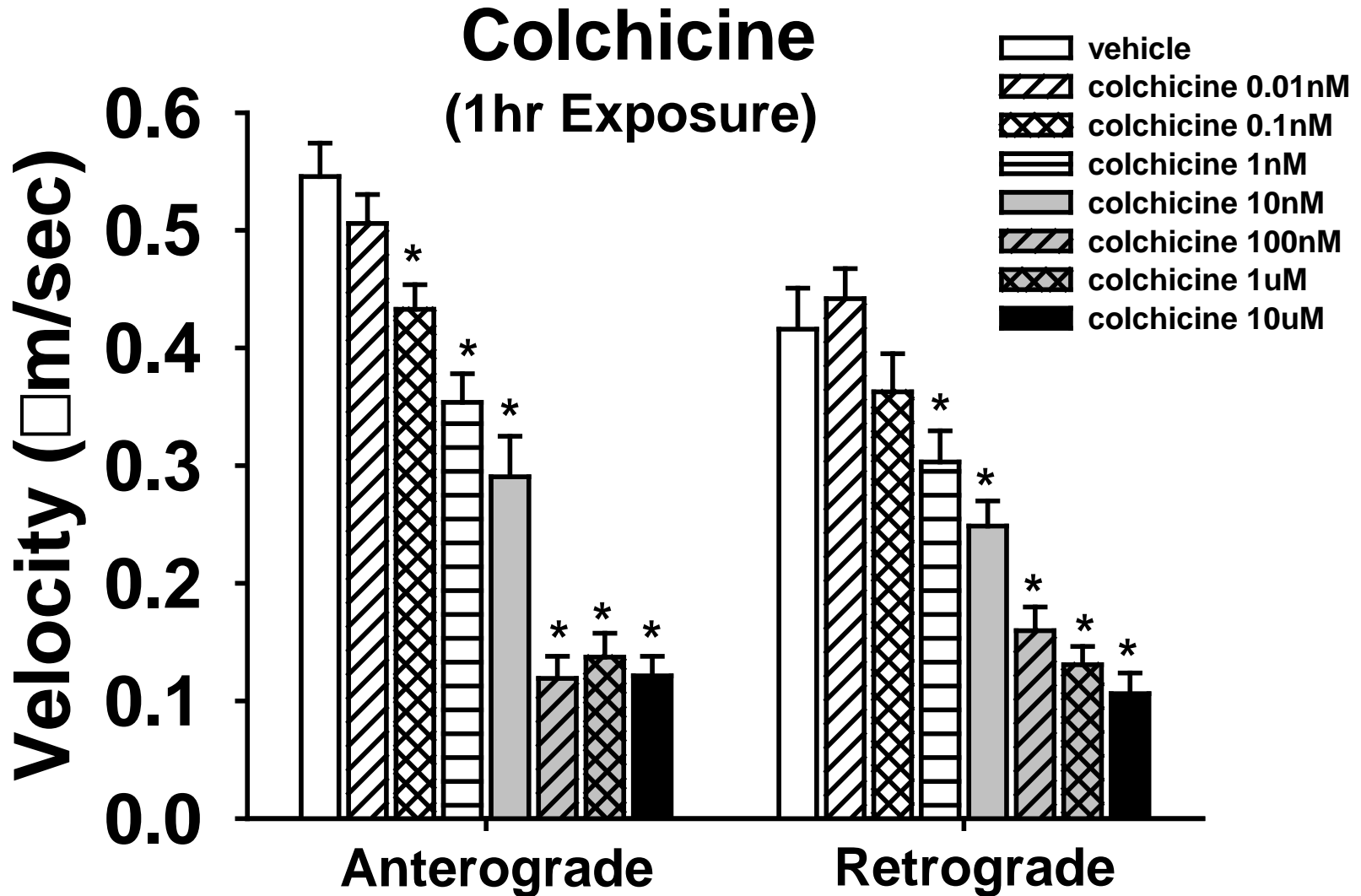
## Colchicine



*Colchicum autumnale*

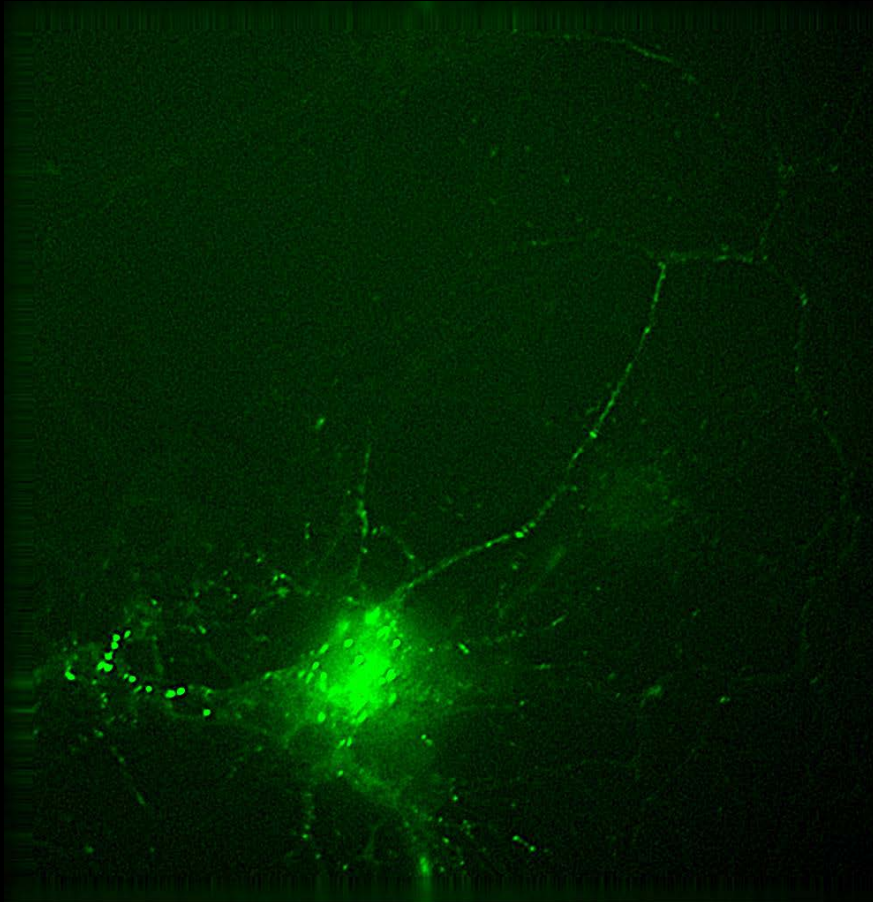
- Tropane alkaloid used since ancient times to treat rheumatism, swelling, and gout .
- Binds tightly to tubulin, impairs tubulin polymerization, disrupts microtubule assembly and consequently impairs the ability of motor proteins to transport cargo in axons (Hastie, 1991; Uppuluri et al., 1993, Han et al., 1998)

# APPDendra Tracking Studies

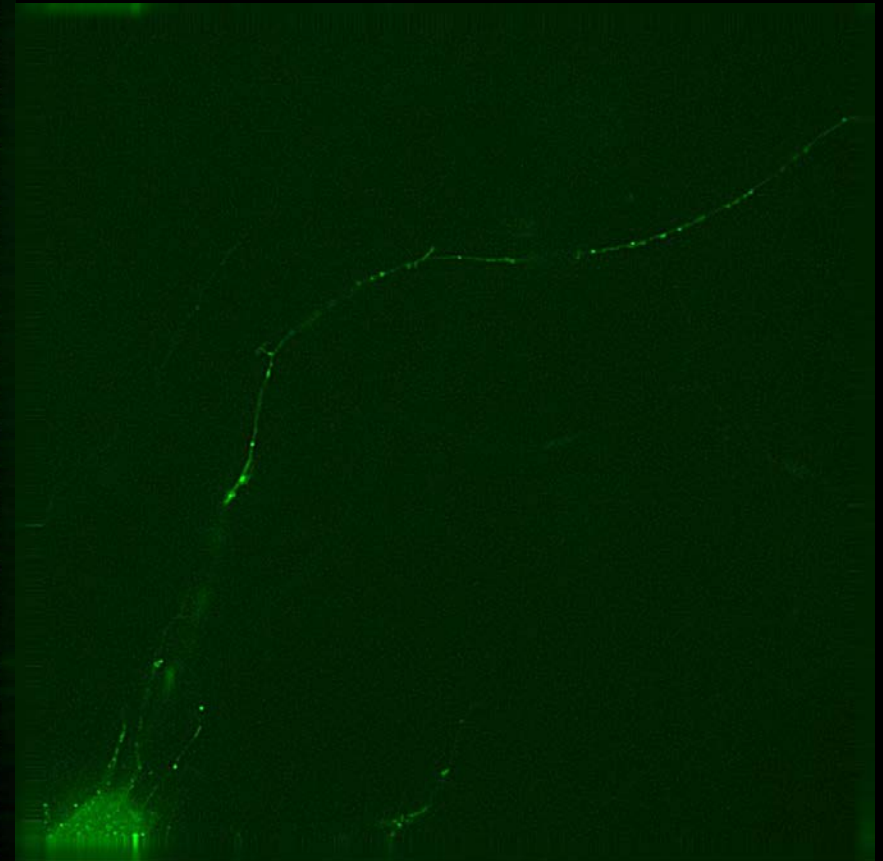


\*  $p < 0.05$  versus vehicle control velocity

# APP Dendra Tracking Studies (Primary Cortical Neurons)

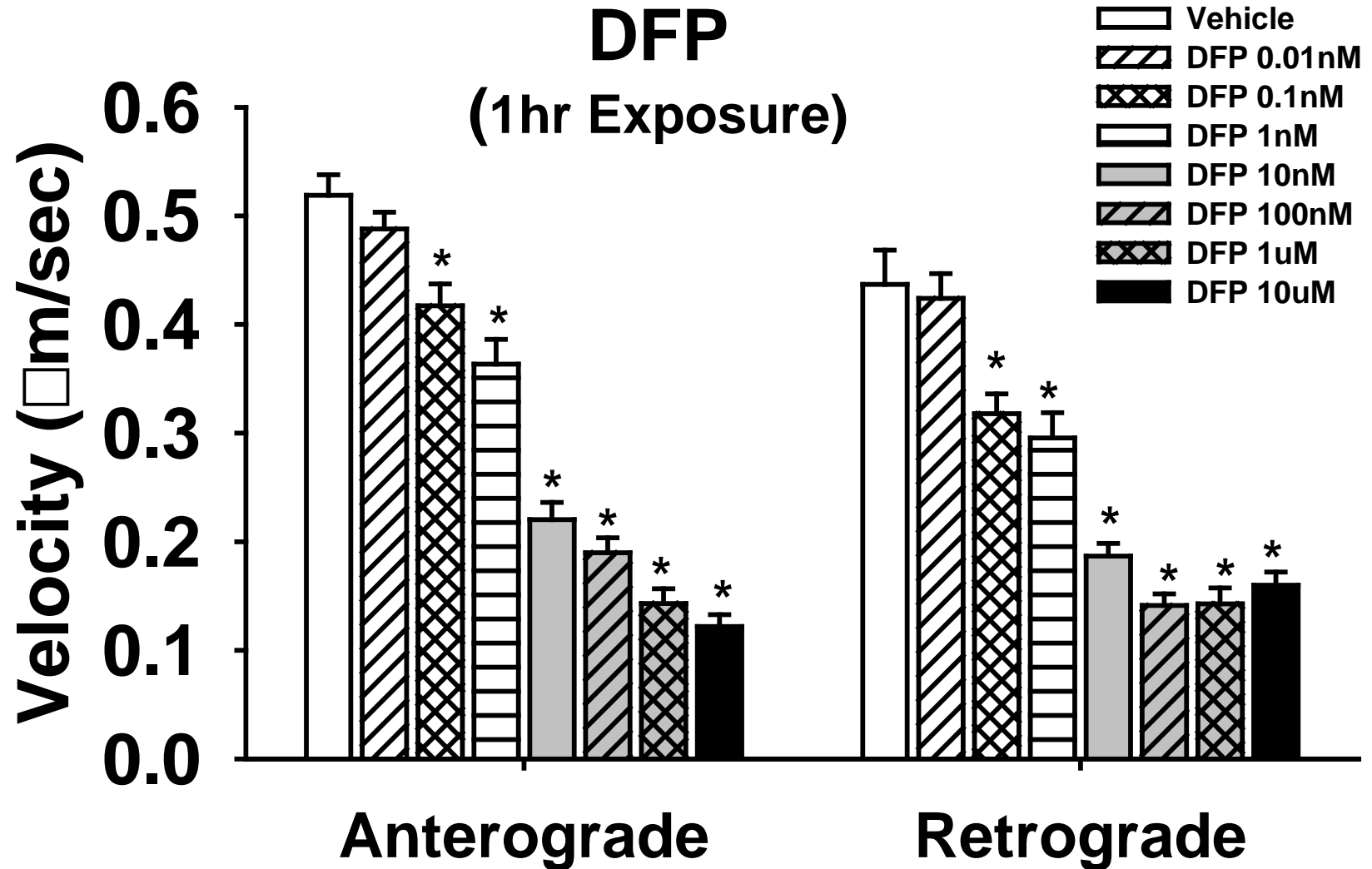


**Vehicle**



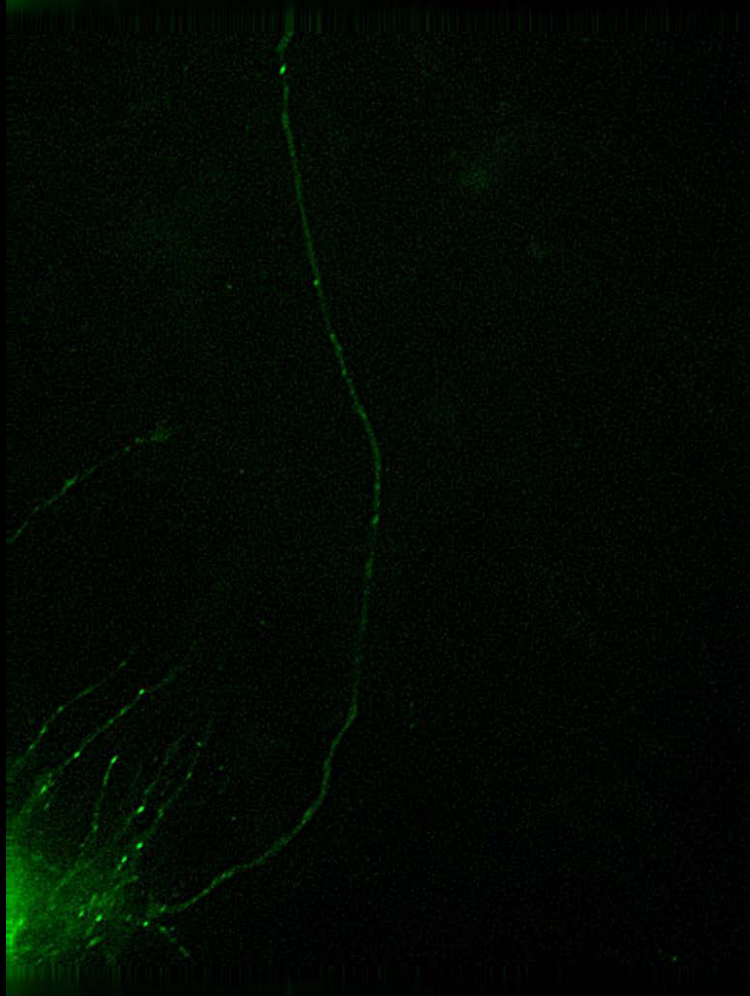
**Colchicine 10 nM**

# APPDendra Tracking Studies

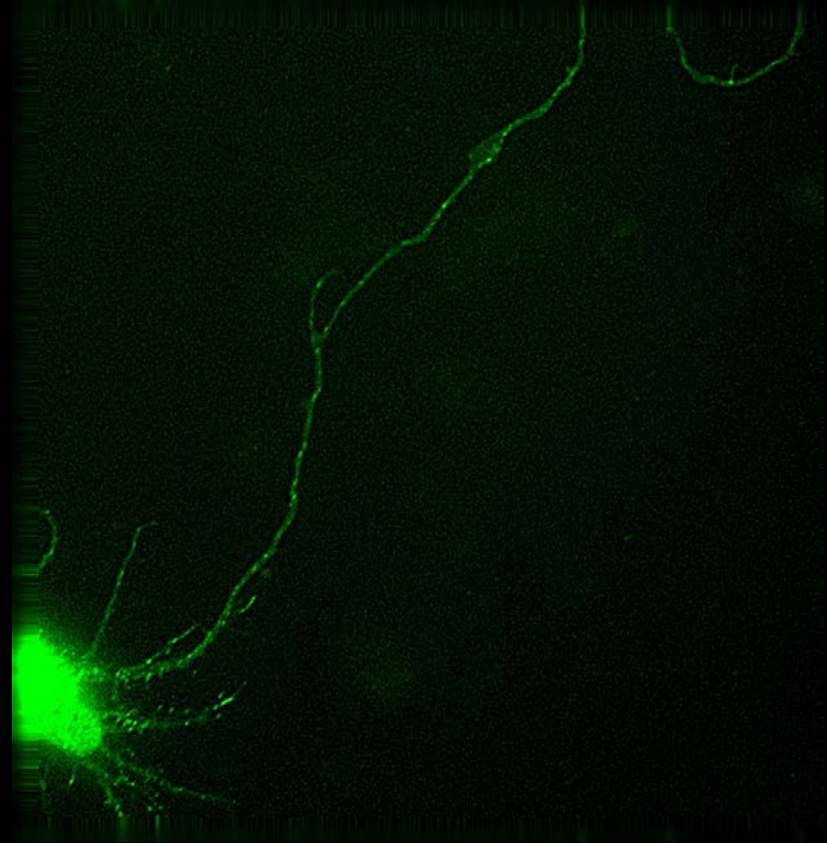


\*  $p < 0.05$  versus vehicle control velocity

# APPDendra Tracking Studies (Primary Cortical Neurons)



**Vehicle**

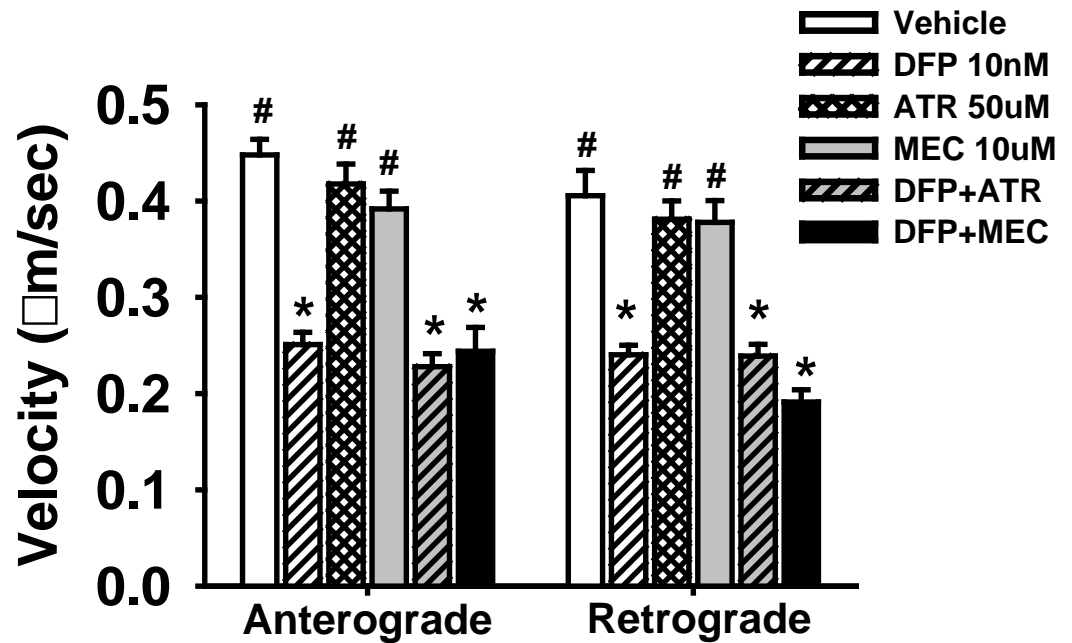
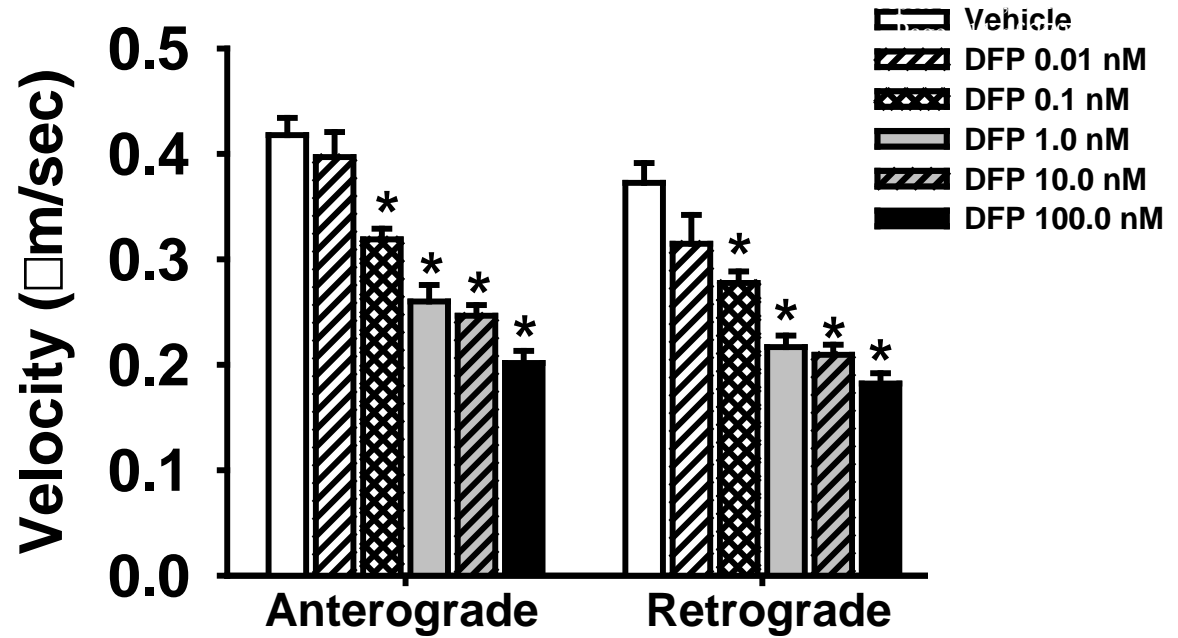


**DFP 10 nM**



# APPDendra Tracking Studies

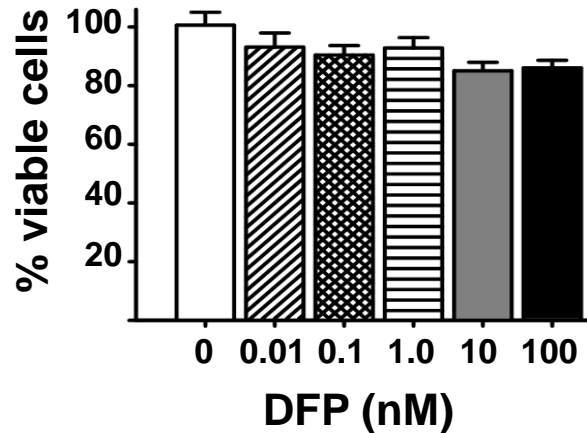
(24 hr Exposure)



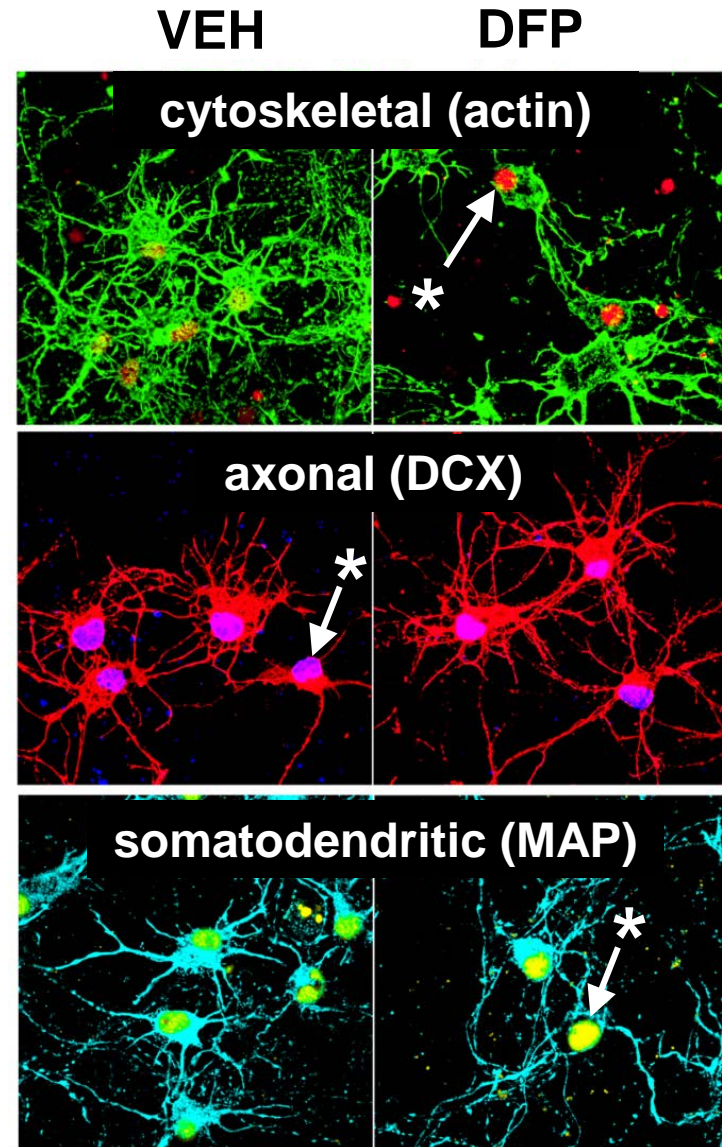
\*  $p < 0.05$  versus vehicle control velocity

#  $p < 0.05$  versus DFP related velocity

## A. Cell Viability Assay



## B. Cell Structure & Morphology



**Fig 3. Cell viability, structure and morphology are not compromised after 24 hr exposure to DFP.** **A.** MTT assay results indicate that 24 hr exposure to concentrations of DFP up to 100 nM does not compromise neuronal cell viability. **B.** Pseudo-colored confocal images of cultured neurons indicate that the integrity of cytoskeletal (Acti-stain, green), axonal (anti-doublecortin (DCX), red) and somatodendritic (anti-microtubule associated protein 2A/2B (MAP), blue) structure morphology were also not compromised [DFP=100nM]. Asterisks (\*) indicate nuclei, pseudo-colored for contrast/clarity. VEH = vehicle

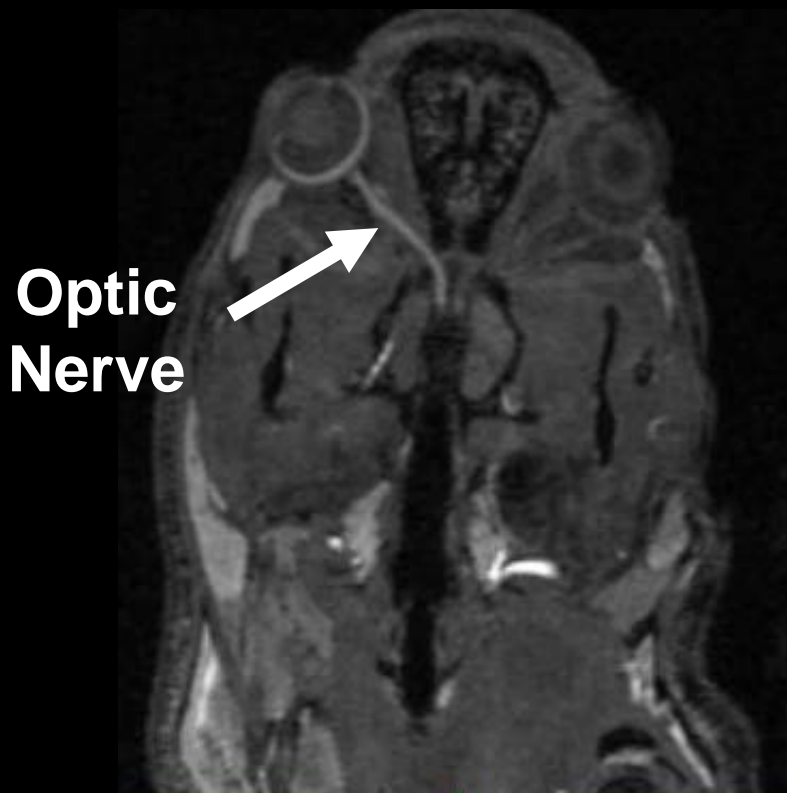
# Conclusions

## APPDendra Tracking Studies

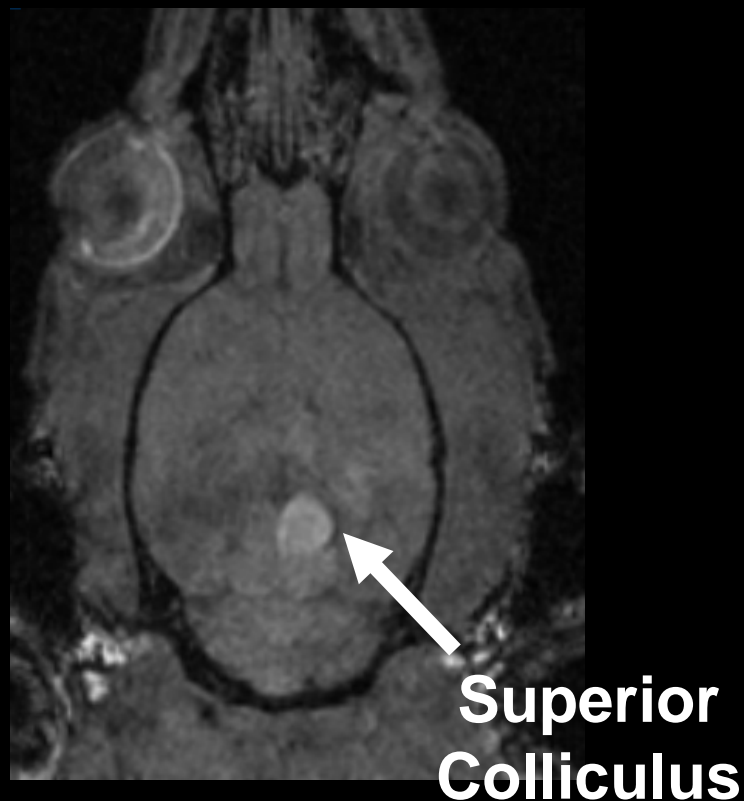
- **DFP-related impairments of axonal transport:**
  - ◆ **Were concentration dependent.**
  - ◆ **Occurred at concentrations that did not inhibit AChE activity.**
  - ◆ **Were not blocked by cholinergic receptor antagonists.**
  - ◆ **Were not associated with compromises in cell viability.**



# Manganese-Enhanced Magnetic Resonance Imaging (MEMRI) Studies

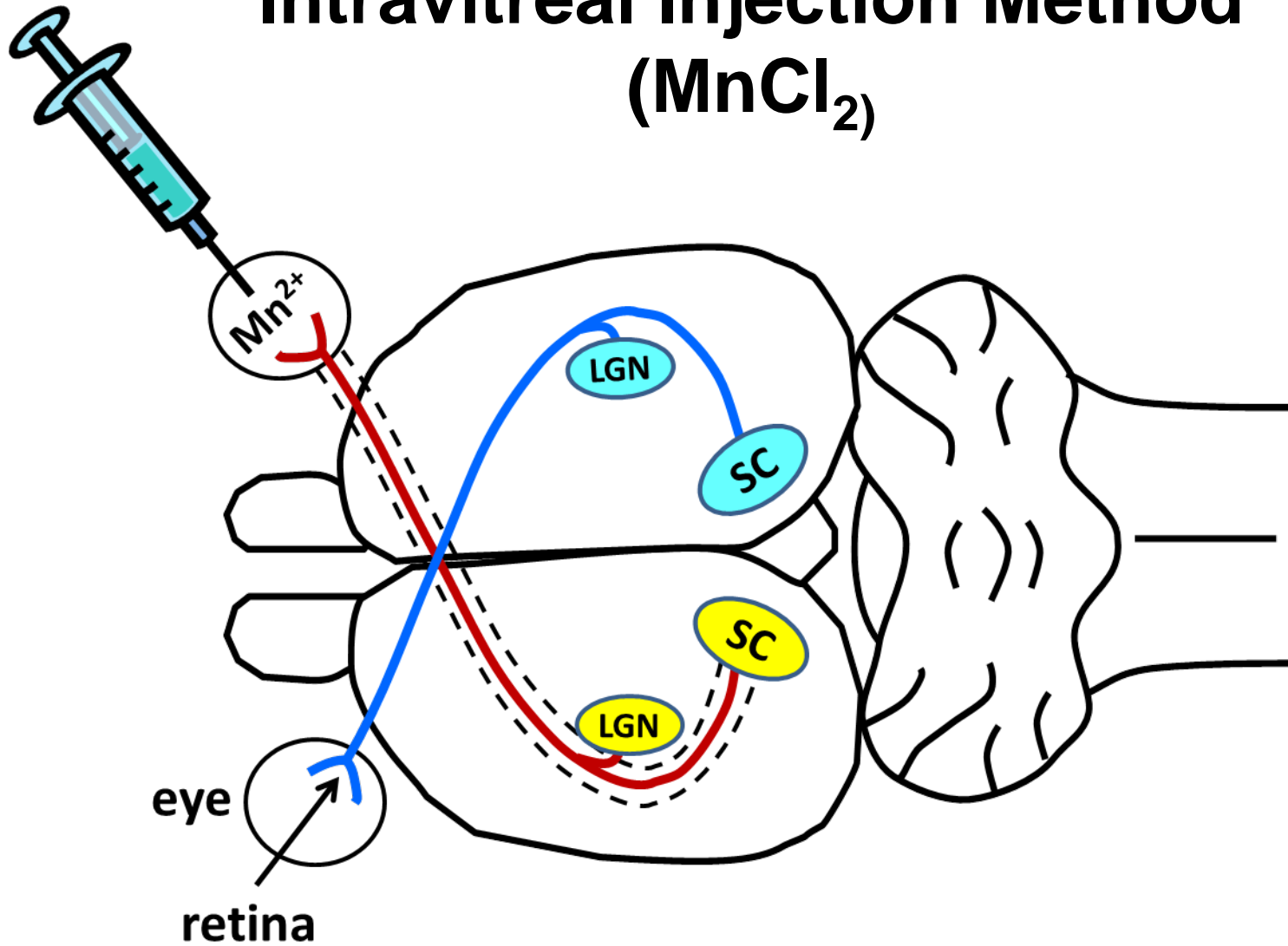


6 hrs



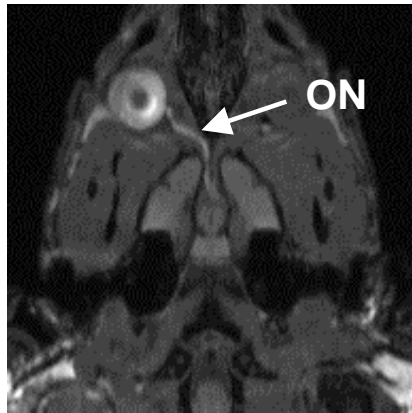
24 hrs

# Intravitreal Injection Method ( $MnCl_2$ )

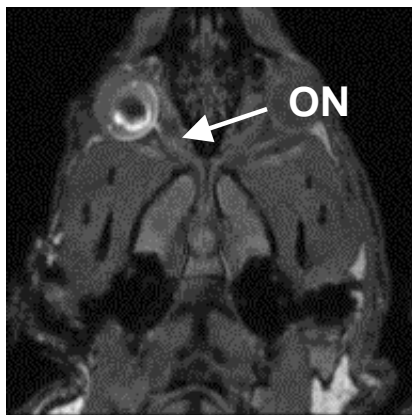


**Rat Brain (Top View)**

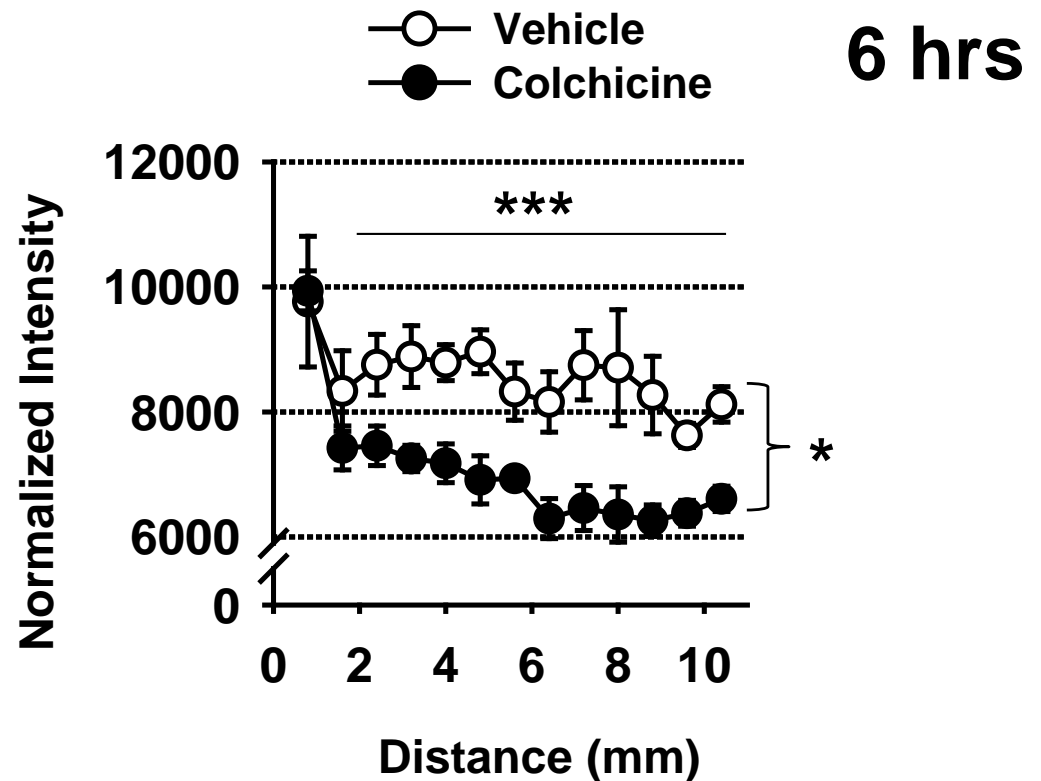
# MEMRI-Colchicine Validation Studies (N=3)



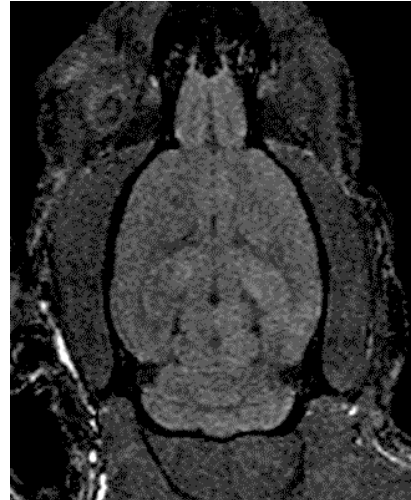
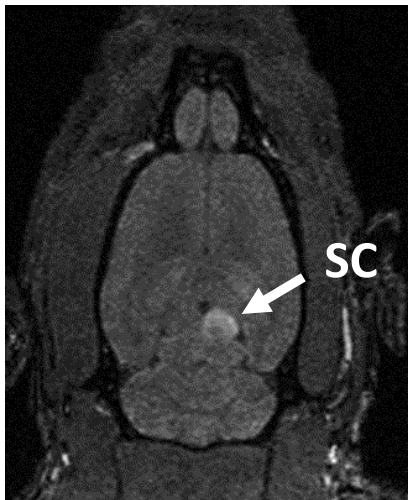
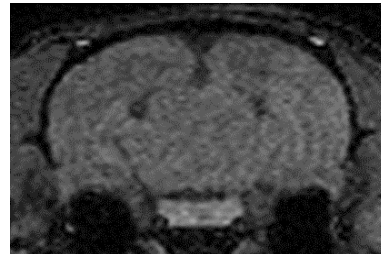
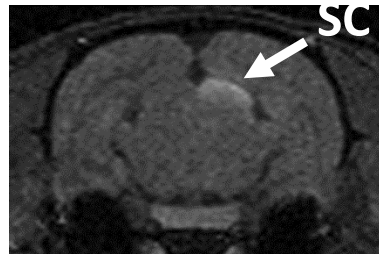
Vehicle



Colchicine

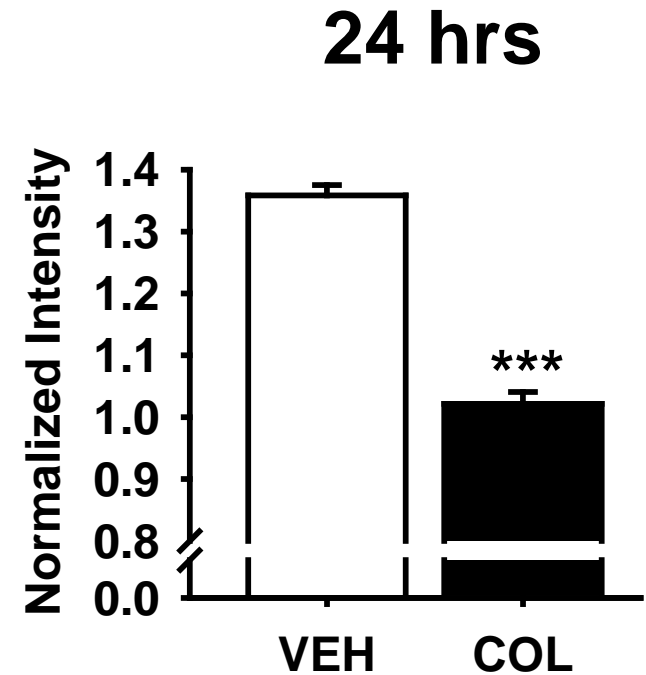


# MEMRI-Colchicine Validation Studies (N=3)

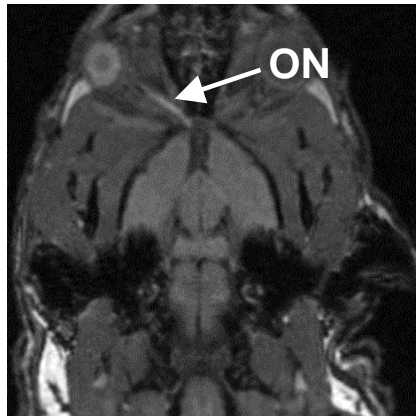


VEH

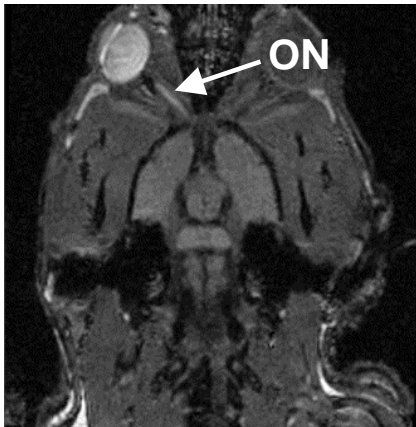
COL 2.5  $\mu$ g



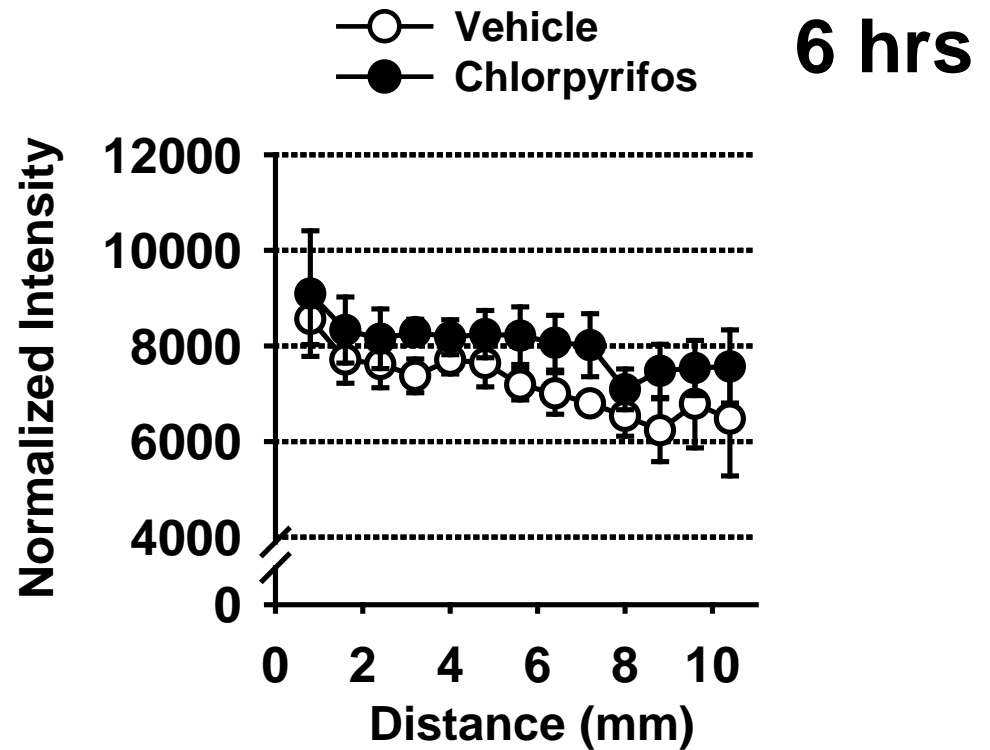
# MEMRI-Acute CPF Exposure (18.0 mg/kg Injection, N=6)



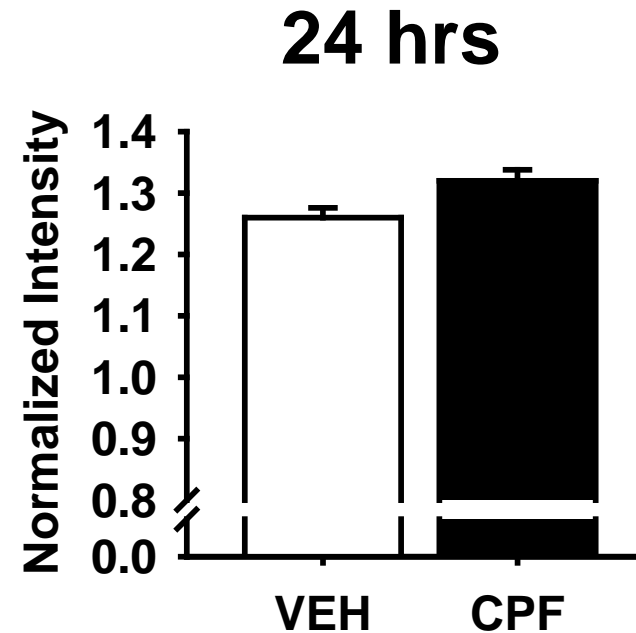
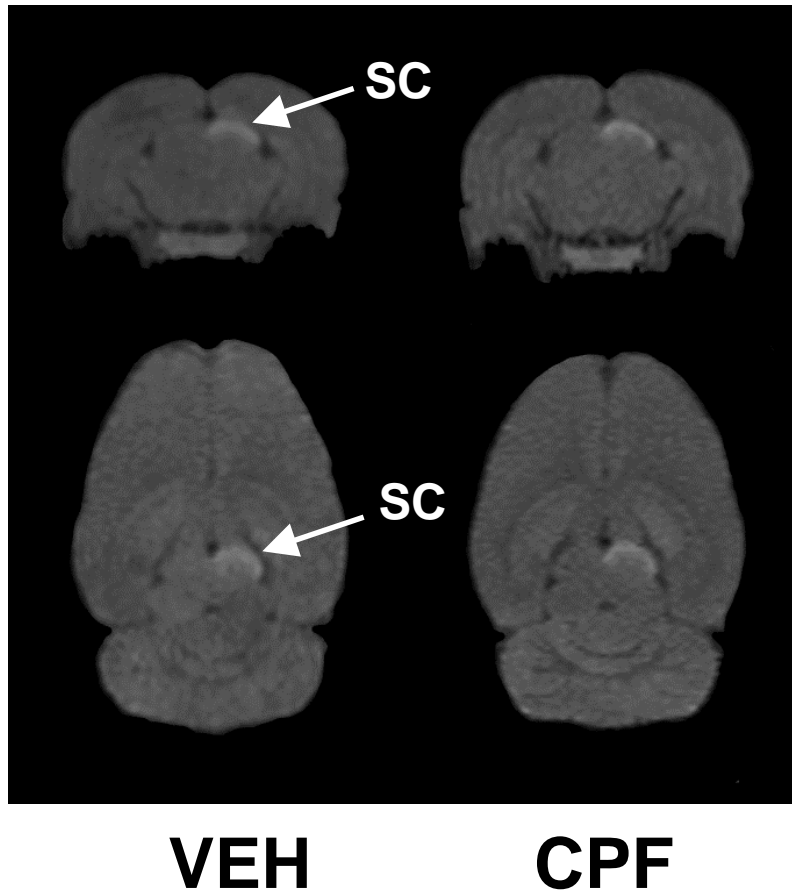
Vehicle



Chlorpyrifos

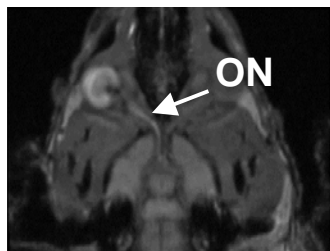


# MEMRI-Acute CPF Exposure (18.0 mg/kg Injection, N=6)

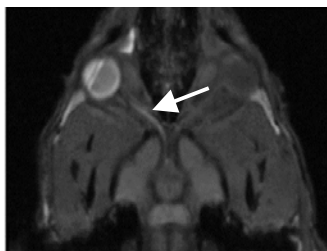


# CPF 18.0 mg/kg -14 Day Repeated Exposure Study (N=6)

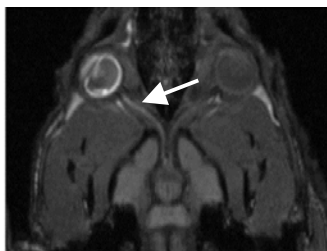
Baseline



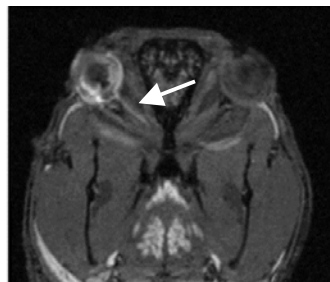
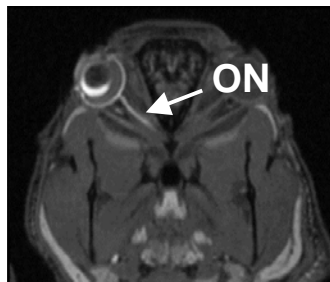
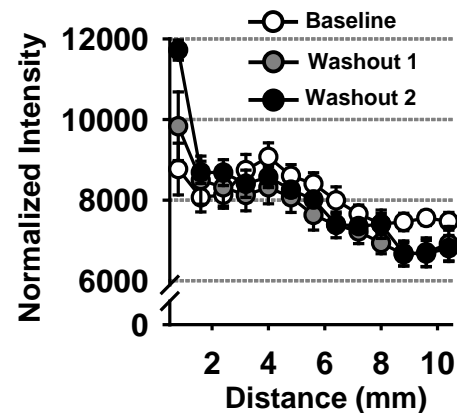
WO 1



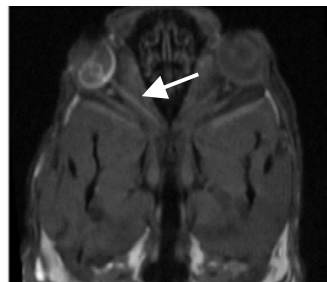
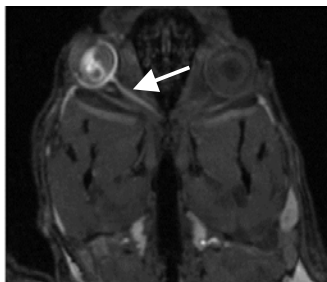
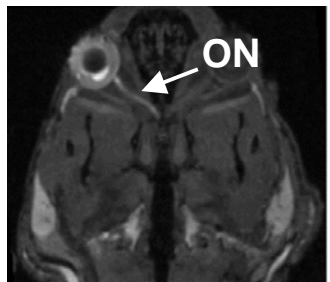
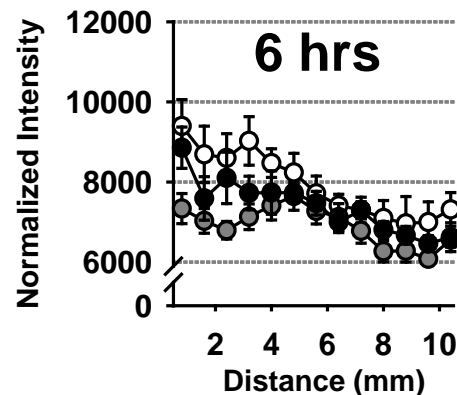
WO 2



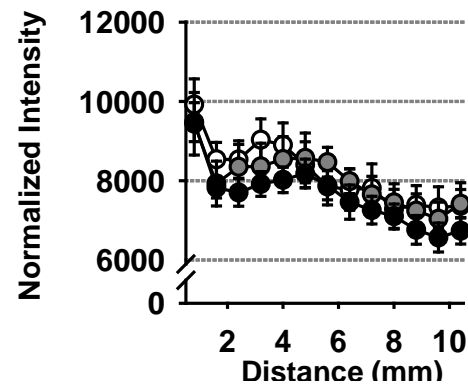
Vehicle



Chlorpyrifos 3.0 mg/kg



Chlorpyrifos 18.0 mg/kg



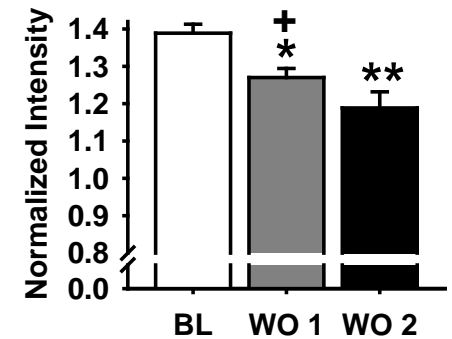
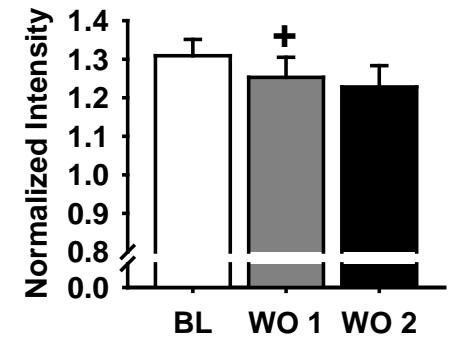
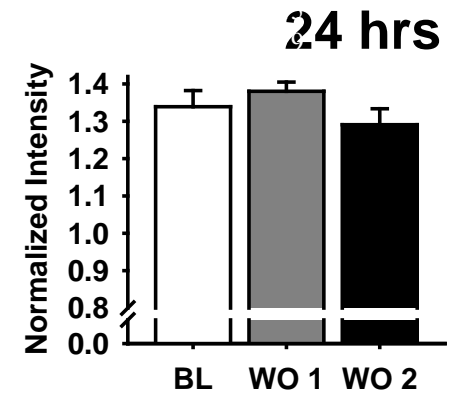
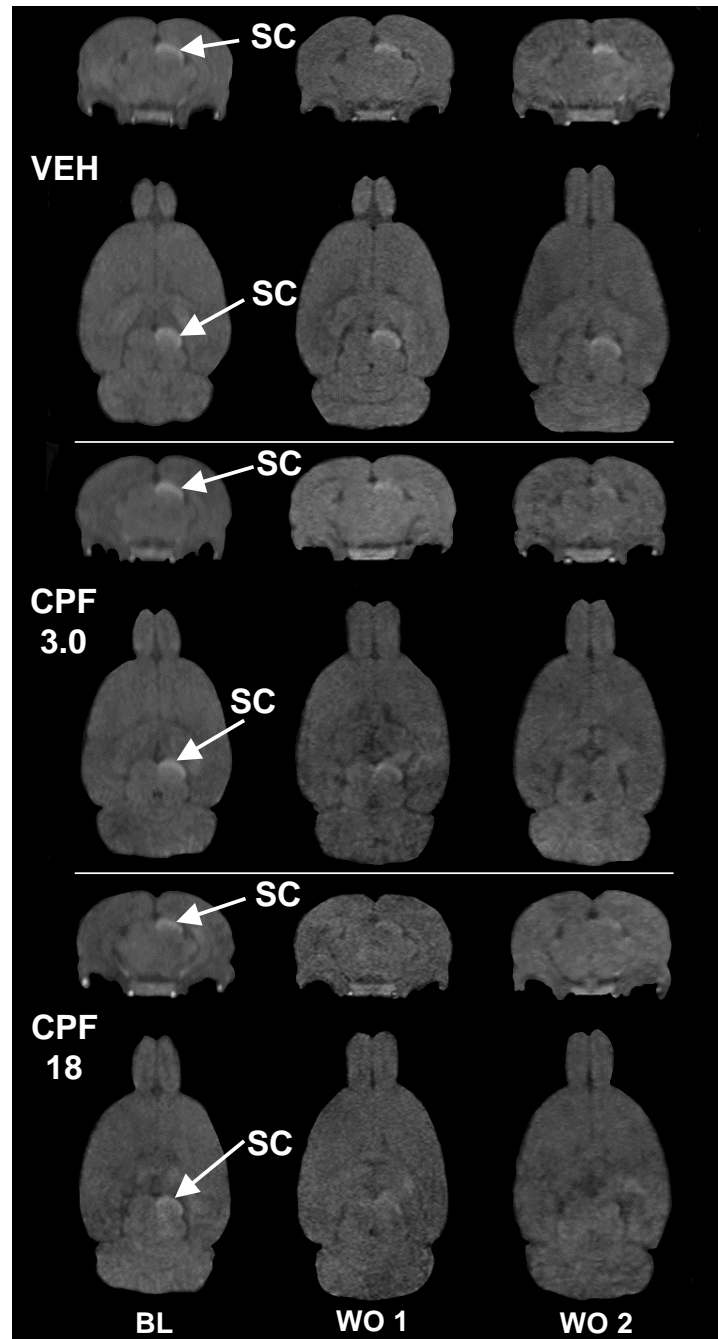
# Chlorpyrifos (CPF)

## 14 Day Exposure Study (MEMRI-Superior Colliculus)

BL= Baseline  
 WO1= 24 hr after last Injection  
 WO2 = 30 Days after last injection

\* $p < 0.05$ ; \*\*  $p < 0.01$  CPF- WO1 and  
 WO2 versus BL, respectively

+  $p < 0.05$  versus vehicle at the  
 same washout period. VEH =  
 vehicle; CPF = chlorpyrifos. N=6.





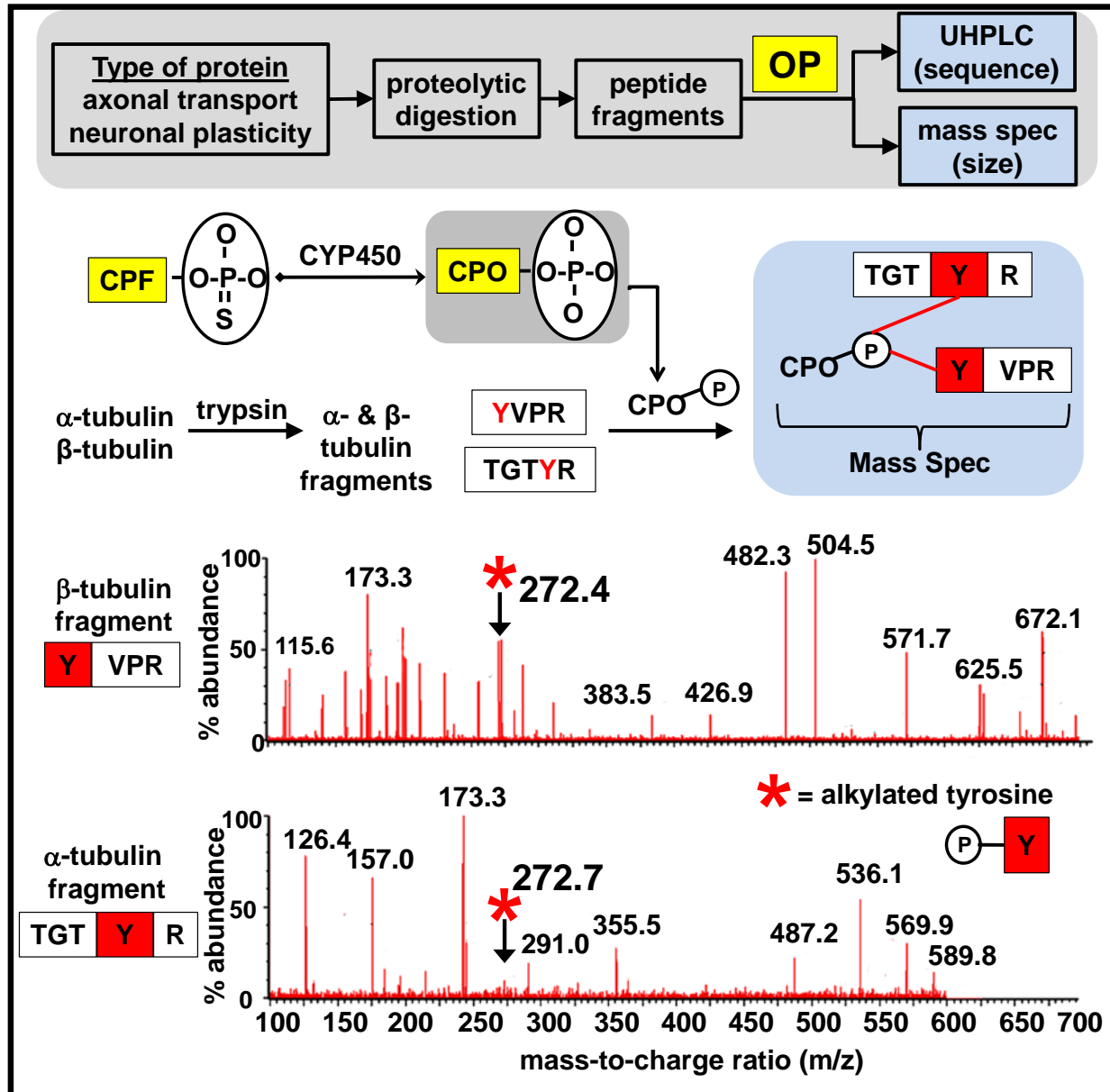
# Conclusions CPF-MEMRI Study

- **Intravitreal injection of the tropolone alkaloid colchicine led to marked impairment of axonal transport of  $Mn^{2+}$  in the brain of rats.**
- **A single subcutaneous injection of the commonly used insecticide, chlorpyrifos (i.e., at a subthreshold dose) did not appear to affect axonal transport in the brain of rats.**
- **Conversely, repeated, subthreshold exposures to the commonly used insecticide, chlorpyrifos led to prolonged impairments of axonal transport in the living rodent brain.**

# Overall Summary/Conclusions

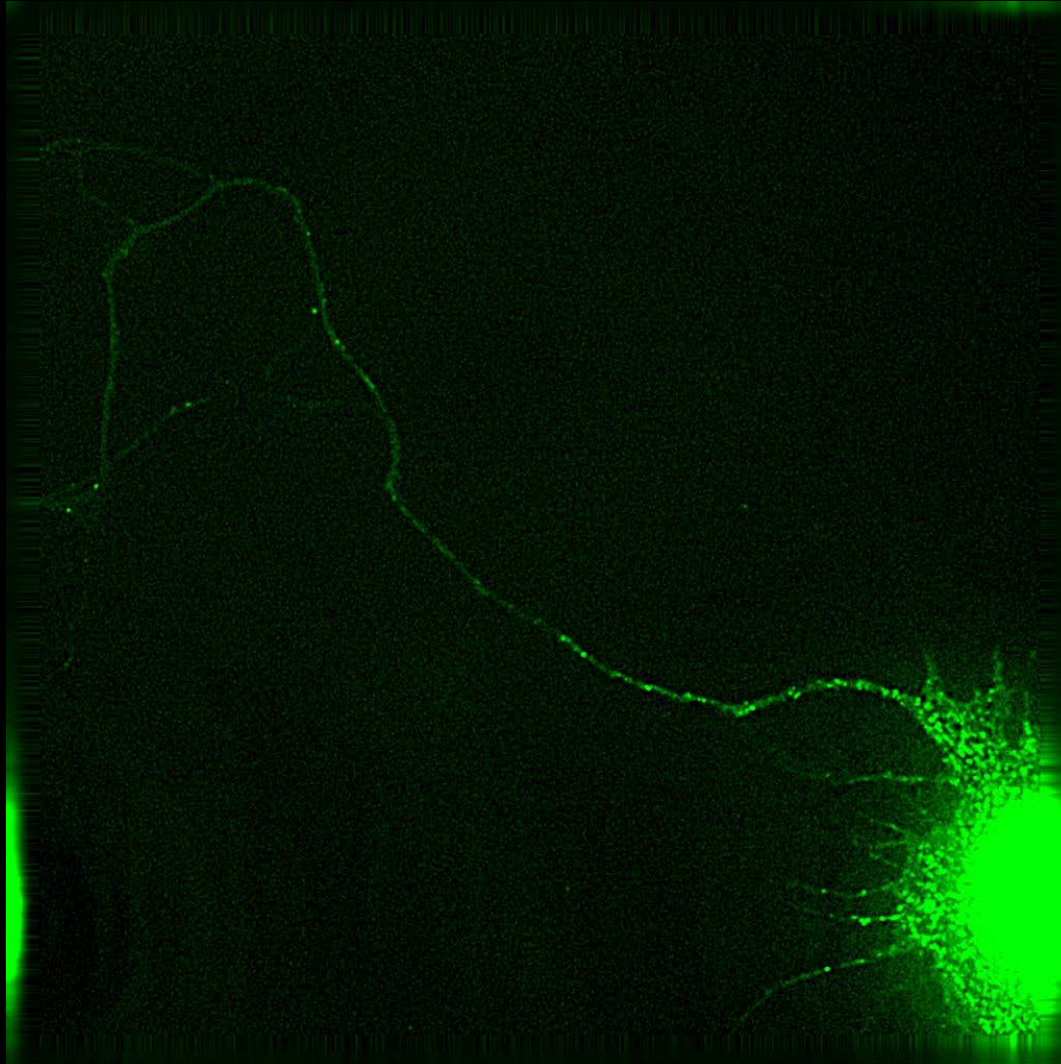
- **Repeated, subthreshold exposures to both insecticide and nerve agent OPs lead to protracted impairments of memory-related behavioral tasks in animals.**
- **Insecticide and nerve agent OPs may have differential effects on specific domains of cognition.**
- **One (non-cholinesterase) mechanism underlying long term (OP-related) impairments of cognition may involve deleterious effects on axonal transport.**

# Current/Future Studies: Proteomics



# Current/Future Studies

## Septal Neuron Imaging



# Current/Future Studies

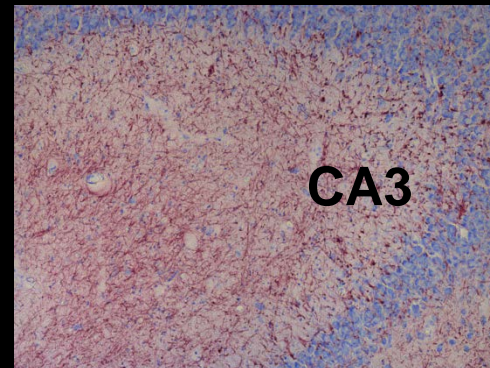
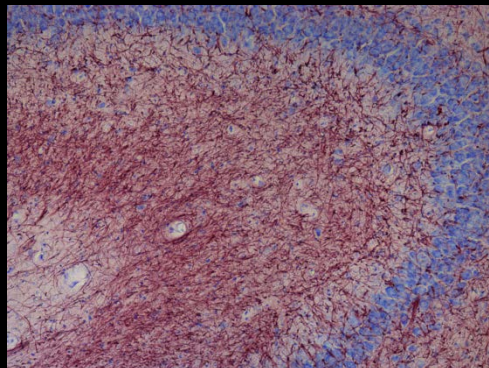
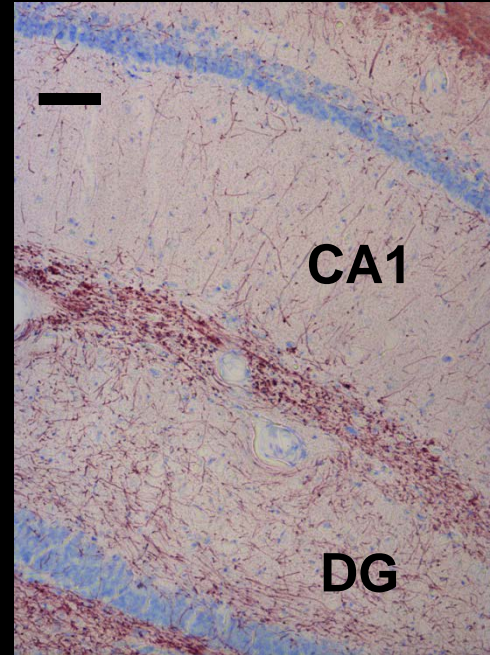
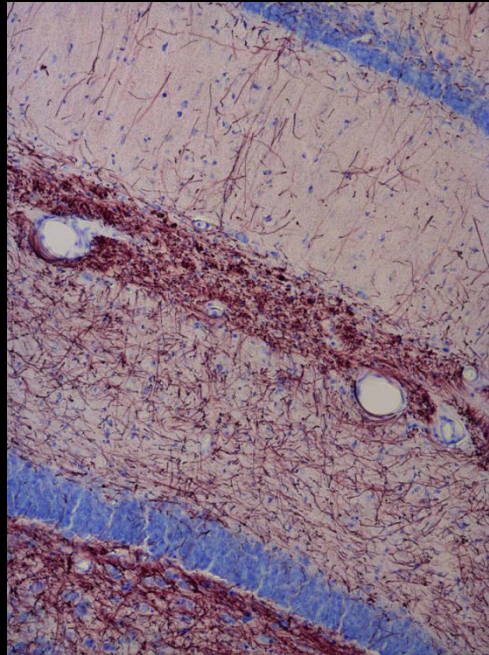
- ◆ **Specific Aim #1: Determine the consequences of repeated subthreshold exposures to representative OPs on axonal transport in the living rat brain.**
  - **Manganese-Enhanced Magnetic Resonance Imaging (MEMRI) Studies**
  
- ◆ **Specific Aim #2: Determine the consequences of repeated subthreshold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

**DOD-CDMRP Project “Organophosphate-Related Alterations in Myelin and Axonal Transport in the Living Mammalian Brain”**



# Black Gold II Histology

## Posterior Hippocampus



**Vehicle**

**DFP**

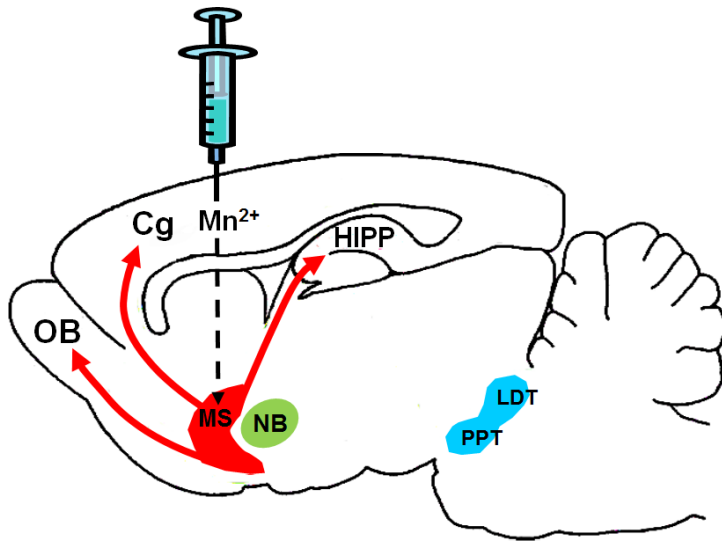
Scale Bar = 100  $\mu$ m

# Current/Future Studies

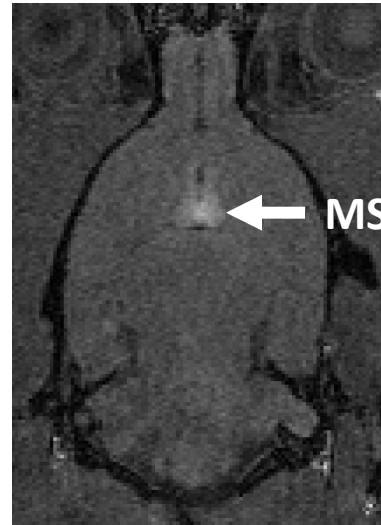
- **OP Effects on Cytoskeletal Proteins**
  - ◆ **Acetylated  $\alpha$ -Tubulin**
- **Therapeutic Interventions in vitro**
  - ◆ **Microtubule Stabilizers**
  - ◆ **Selective HDAC Inhibitors**
- **OP Effects on Signaling Proteins Involved in Neuronal Plasticity and Cognition**
  - ◆ **ERK**
  - ◆ **Akt**
  - ◆ **CREB**
- **Development of a Novel (Operant-Based) Test of Executive Function for Rats**
  - ◆ **Attentional Set Shifting**

# Future Studies

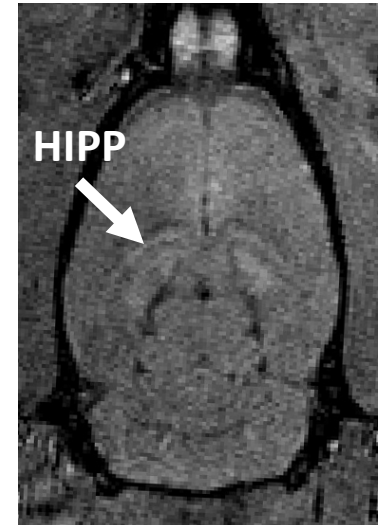
## Mn<sup>2+</sup> Enhanced MRI (MEMRI) Septohippocampal Pathway



Rat Brain (Sagittal View)



Mn<sup>2+</sup> Injection Site



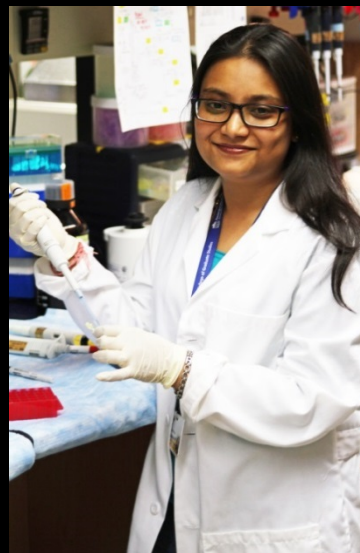
Target Region



# Pharmacology Lab



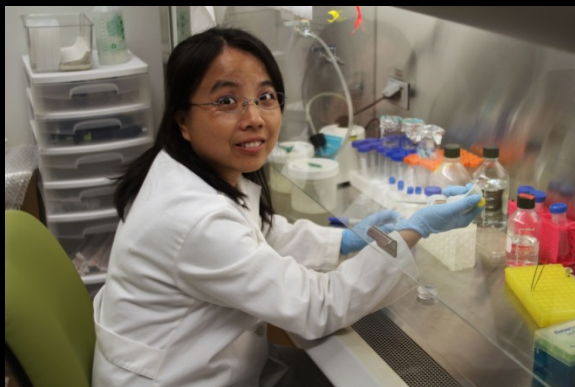
**Caterina Hernandez, Ph.D.**



**Indrani Poddar, MS**



**Sean Naughton**

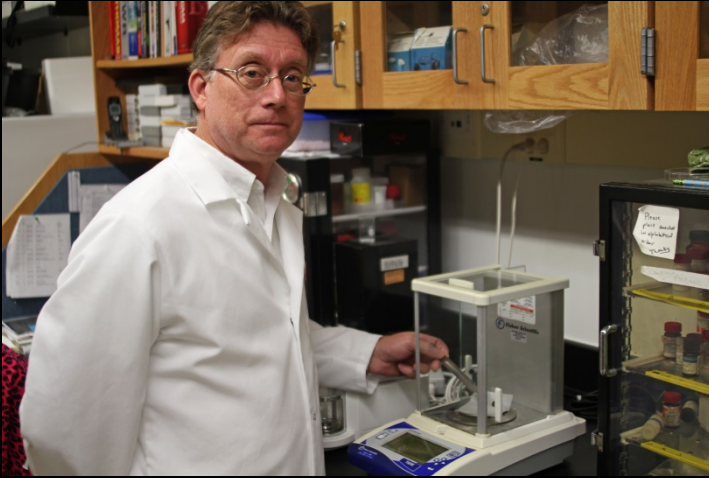


**Jie Gao, Ph.D.**



**Dan Beck**

# Small Animal Behavior Core



**Patrick Callahan, MS**



**Kristy Bouchard**



**Samantha Sinha**



**Leah Vandenhurk**



# Large Animal Behavior Lab



**Nancy Kille**



**Marc Plagenhoef**

# Office



**Ashley Davis**

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## ■ Collaborators

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- ◆ Jordi Magrane, Ph.D.

## ■ Departmental Colleagues

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- ◆ Bobby Thomas, Ph.D.
- ◆ Navneet Kaidery, Ph.D.

## ■ Previous Lab Members

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- ◆ Bao-Ling Adam, Ph.D.

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