



Pitt Collaborators:

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Pier Mastroberardino - Erasmus MC
Takao Yagi - Scripps

Current Funding: NINDS

NIEHS
Veterans Administration
American Parkinson Disease Association
Michael J. Fox Foundation

Prevalence: 1% of people over age 55

(I million in North America)

Inheritance: Sporadic and Familial

Etiology: Environmental toxins

Complex I defects?

Single gene mutations

 α -synuclein dupli- & triplications

Cardinal Signs: Tremor, rigidity, bradykinesia,

postural instability

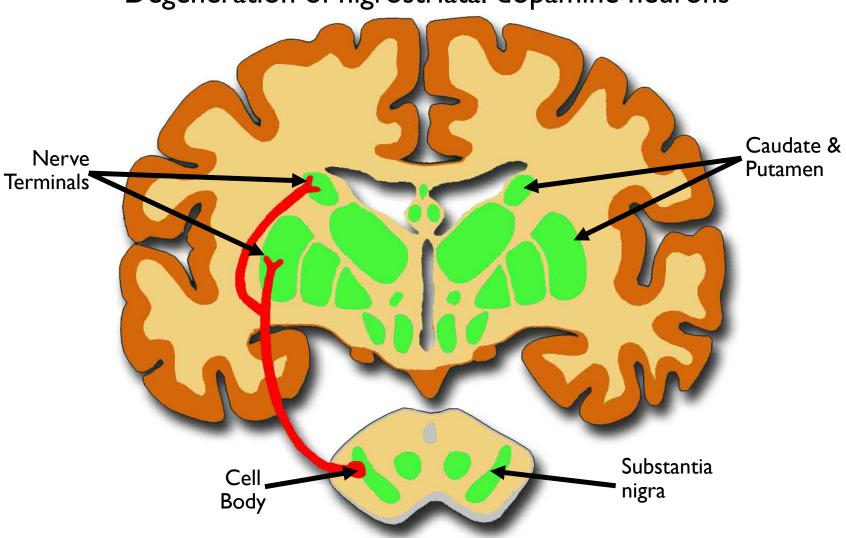
Other Signs: Shuffling gait, masked facies,

deceased blink rate

Classical Pathology:

- Loss of dopamine neurons in the substantia nigra pars compacta
- Lewy bodies/neurites
- Loss of neurons in locus ceruleus, dorsal vagal nucleus, dorsal raphe and nucleus basalis of Meynert
- Microglial activation

Degeneration of nigrostriatal dopamine neurons





Lewy Bodies

The pathological hallmark of Parkinson's disease. Among the proteins they contain:

- Phosphorylated neurofilament proteins
- Ubiquitin
- α -Synuclein
- Parkin
- Proteasome subunits

Biochemical Pathology in Substantia Nigra:

- Loss of reduced glutathione (GSH)
- Increased levels of malondialdehyde & lipid hydroperoxides
- Oxidative DNA & protein damage
- Oxidative (nitrative) modification of α -synuclein
- Iron accumulation

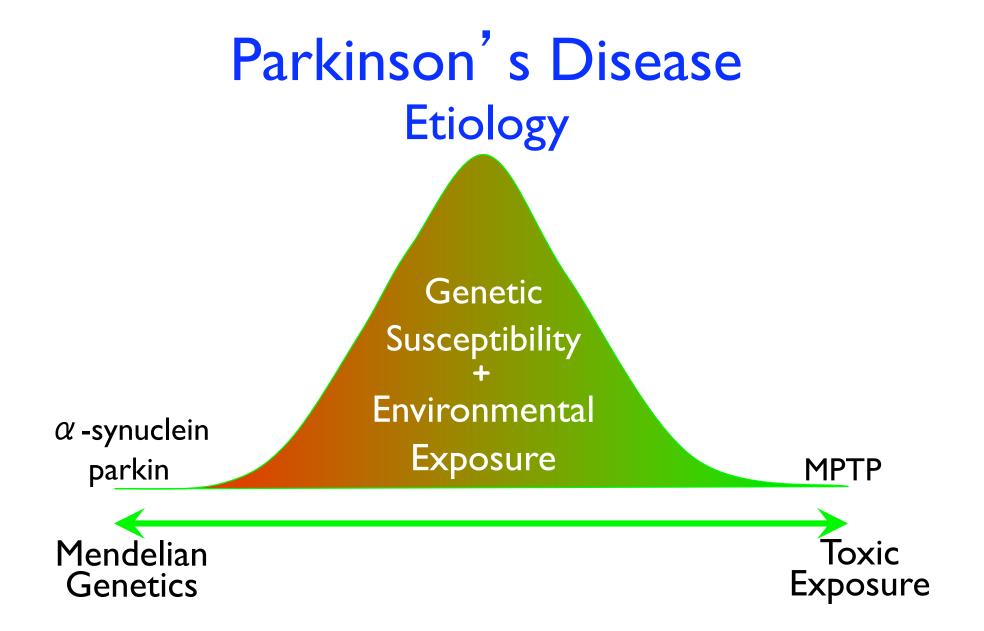


Table Different monogenic forms of parkinsonism						
Acronym	Mode of inheritance	Locus	Gene/protein	Mutations		
Monogenic	confirmed					
PARK1/PARK4	Autosomal dominant	4q21-q23	SNCA/lpha-synuclein	3 missense mutations, ^{9,135,136} whole gene duplications/ triplications in <10 families ^{10,12}		
PARK8	Autosomal dominant	12q12	LRRK2/dardarin	>50 variants, >16 of them pathogenic ^{3,24}		
PARK2	Autosomal recessive	6q25.2-q27	Parkin	>100 different mutations (gene dosage alterations and small sequence changes) ^{53,58}		
PARK6	Autosomal recessive	1p35-p36	PINK1	40 small sequence change, ⁷¹ rarely large deletions ¹³⁷		
PARK7	Autosomal recessive	1p36	DJ-1	10 mutations (point mutations and large deletions) ^{87,138}		
PARK9	Autosomal recessive	1p36	ATP13A2	3 different mutations that lead to premature protein truncation ⁹⁵		
Monogenic	single cases					
PARK5	Autosomal dominant	4p14	UCHL1 /ubiquitincarboxyterminalhydrolase I	1 mutation found in single family 139		
PARK13	Unknown	2p12	Omi/HtrA2	1 point mutation in four families; 1 disease-associated variant ¹⁴⁰		
Not assigned	Unknown	5q23.1-q23.3	Synphilin-1	1 missense mutation in two patients ¹⁴¹		
Not assigned	Unknown	2q22-q23	NR4A2/Nurr1	3 different mutations, 1 of them in coding region ^{142,143}		
Not assigned	Unknown	15q25	POLG/DNA polymerase γ	1 family with compound heterozygous mutations ¹⁴⁴		

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Mutations and Mitochondria

- PINKI a nuclear-encoded, mitochondrial protein kinase (Valente et al, 2004; Rohe et al, 2004)
- **Parkin** mitochondrial quality control; knock-out results in disruption of mitochondrial function (Greene et al, 2003; Palacino et al, 2004)
- **DJ-I** under conditions of oxidative stress, DJ-I translocates to mitochondria (Canet-Aviles et al, 2004)
- Omi a mitochondrial protease (Strauss et al, 2005)
- **POLG** mitochondrial DNA polymerase gamma

Parkinson's Disease Etiology

Genetic
Susceptibility
+
Environmental
Exposure

Mendelian Genetics Toxic Exposure

Associated with pesticide exposure:

Butterfield et al., (1993) Neurology, 43, 1150-8.

Fall et al., (1999) Mov Disord, 14, 28-37.

Flemin et al., (1994) Ann Neurol, 36, 100-3.

Hertzman et al., (1994) Mov Disord, 9, 69-75.

Hubble et al., (1993) Neurology, 43, 1693-7.

Liou et al., (1997) Neurology, 48, 1583-8.

Menegon et al., (1998) Lancet, 352, 1344-6.

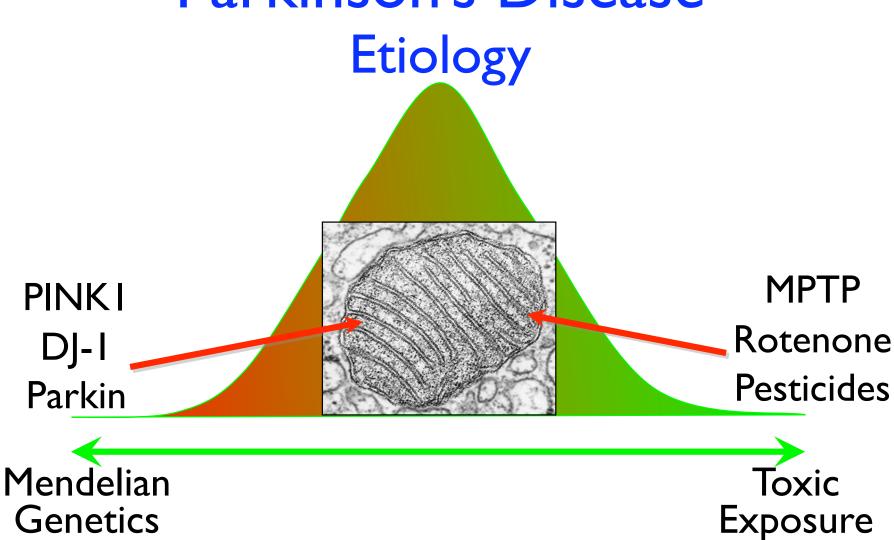
Seidler et al., (1996) Neurology, 46, 1275-84.

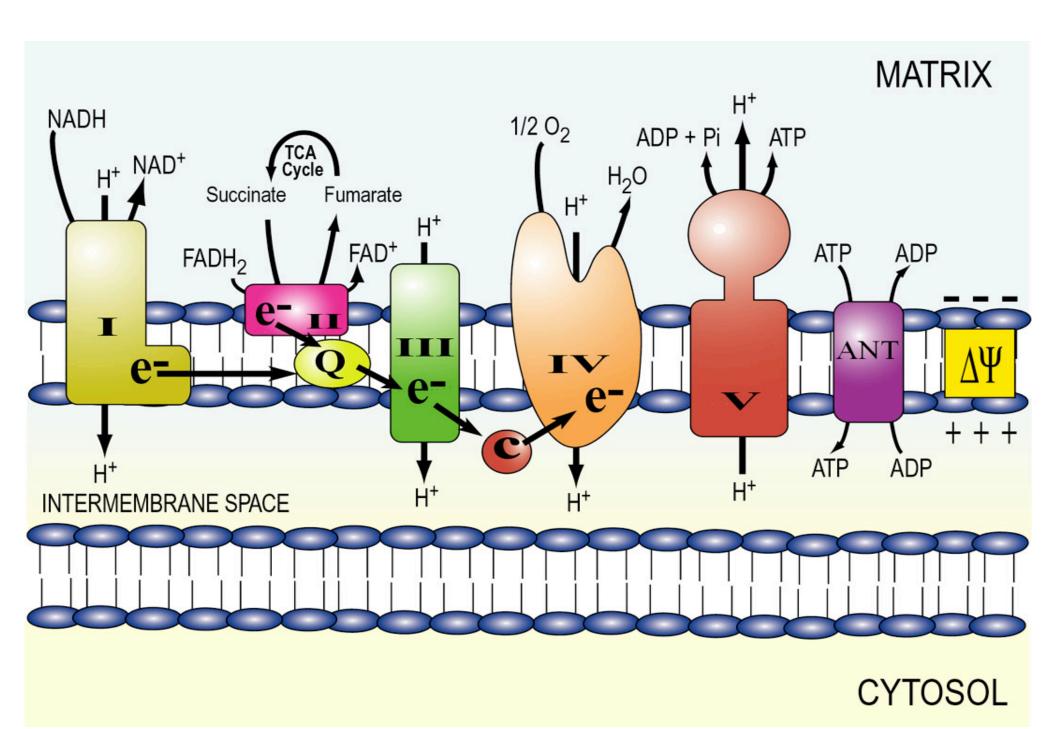
Fong et al., (2007) Clin Chim Acta 378, 136-41.

Ascherio et al., (2006) Ann Neurol, 60, 197-203.

Frigerio et al., (2006) Mov Disord, 21, 1688-1692.

Tanner et al., Envir. Health Perspect, 2011





MPTP

- In 1982, IV drug users present with an acute parkinsonian syndrome
- Astute medical detective work identifies the toxin as MPTP
- MPTP is metabolized to MPP⁺, a substrate for the dopamine uptake transporter (DAT)
- Mechanism of action is inhibition of mitochondrial respiration at complex I
- Mitochondrial dysfunction can cause a parkinsonian syndrome

A defect in mitochondrial complex I After the discovery of MPTP and its mechanism:

- 1989-92: A selective decrease in complex I activity in PD brains (Mizuno et al, Schapira et al)
- Complex I activity is reduced by 16 55% in platelets of PD patients (Yoshino et al, Parker et al, Mann et al, Haas & Shults et al)

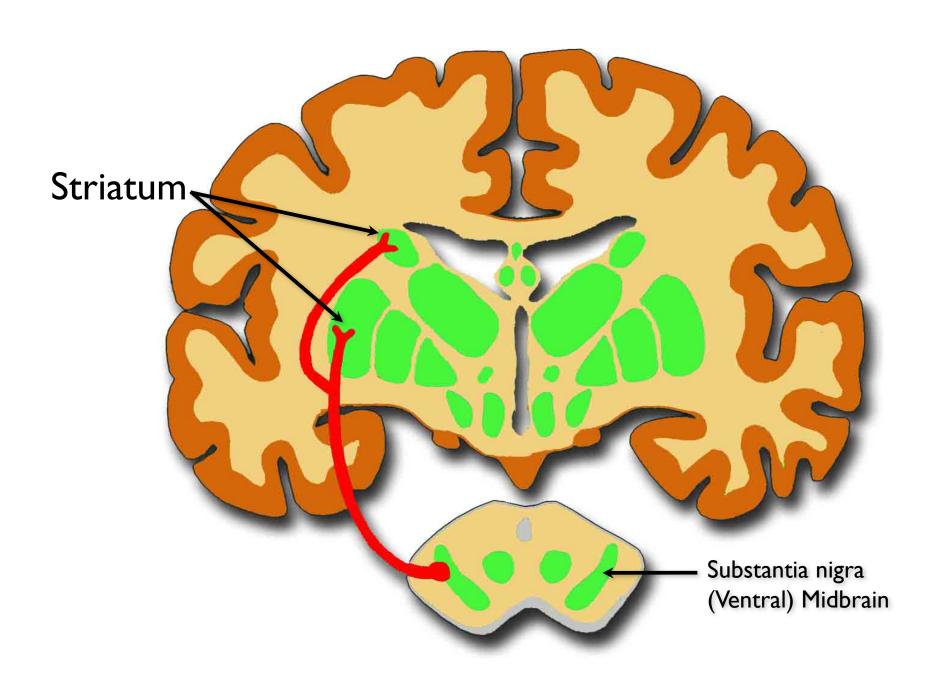
Parkinson's disease is associated with a systemic complex I defect, yet dopaminergic neurons of substantia nigra degenerate selectively.

Is the complex I defect relevant?

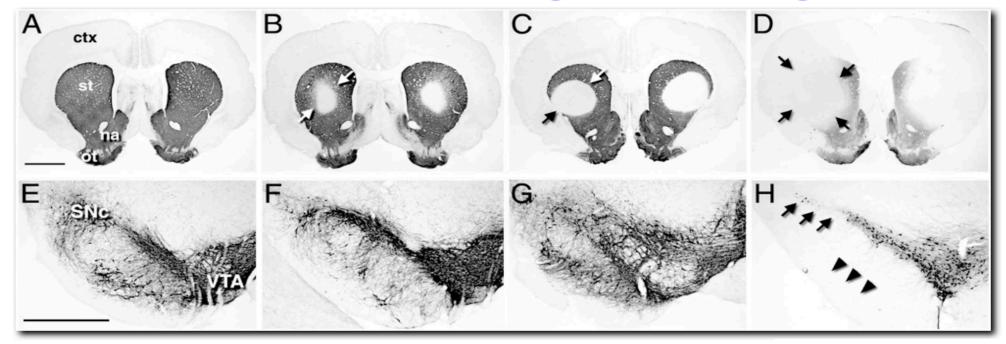
Hypothesis: An experimentally-induced, chronic, systemic inhibition of complex I can reproduce the behavioral, neurochemical and neuropathological features of PD in an animal model.

Rotenone

- Classical high-affinity inhibitor of complex I of the mitochondrial electron transport chain
- A natural product from several plant species
- Common <u>pesticide</u>; the "organic" (natural) alternative to synthetic pesticides
- Used to sample fish populations in reservoirs & kill nuisance fish in lakes
- Highly lipophilic; crosses biological membranes easily & independent of transporters



Rotenone causes selective nigrostriatal degeneration

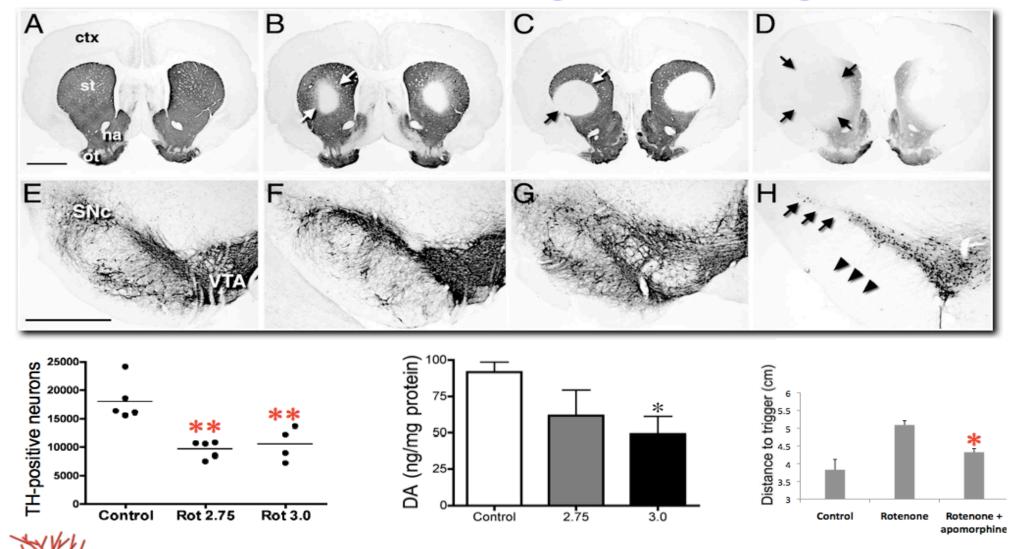




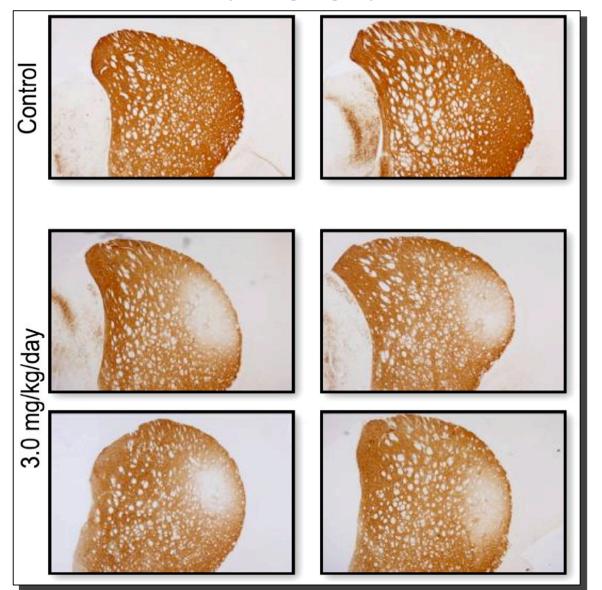


Betarbet, Sherer et al, <u>Nature Neuroscience</u>

Rotenone causes selective nigrostriatal degeneration

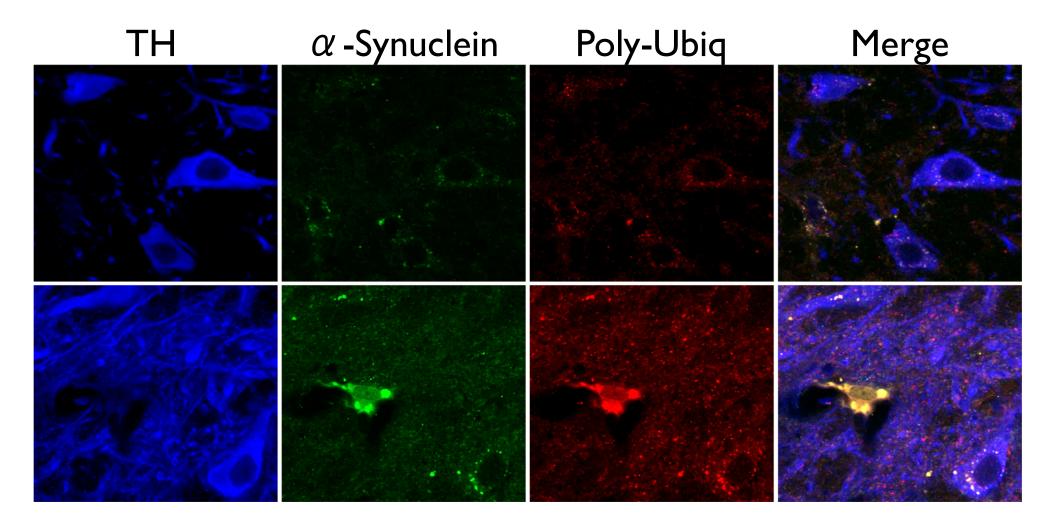


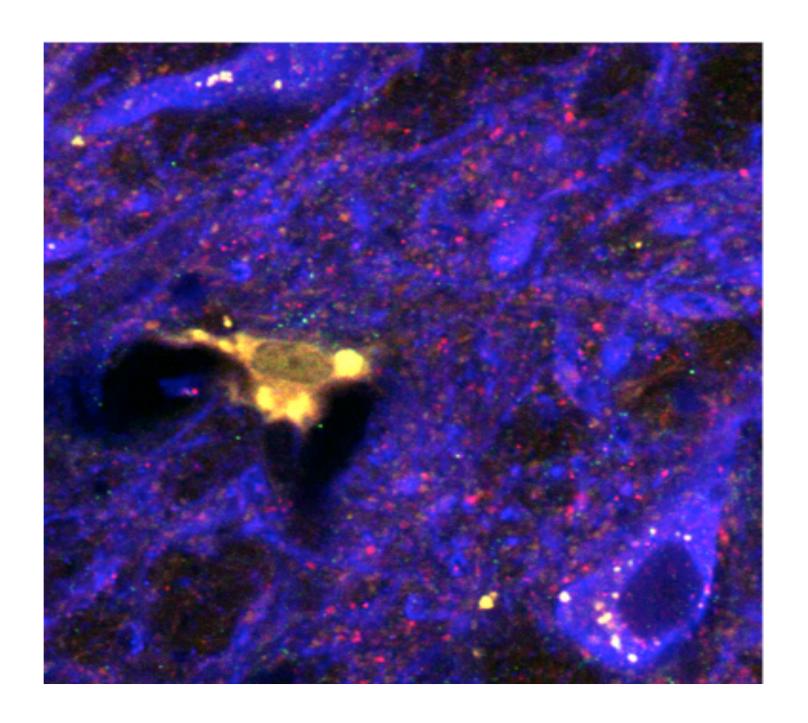
Refinement of the rotenone model (3 mg/kg/d)

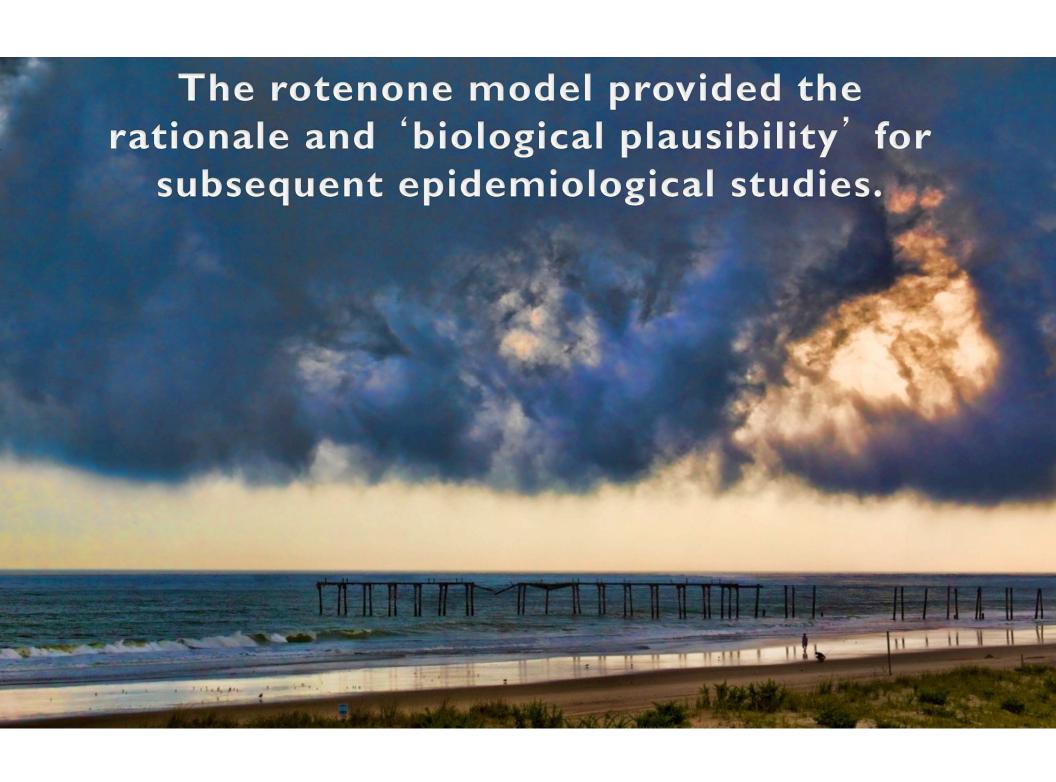














FAME Study: PD in Agricultural Health Study Tanner, Kamel et al

Nested, case-control study of 52,000 licensed pesticide applicators and 32,000 spouses

Rotenone ---- Increased Risk of PD:

All OR = 2.3 (95% C.I.: 1.2, 4.3)

Men OR = 2.8 (95% C.I.: 1.4, 5.8)

Model adjusted for age, gender, state, ever smoking, ever pesticide use



Plant derivative (worldwide)

Crop insecticide (mid-1800's - present)

Fish poison (ancient)



Home gardening

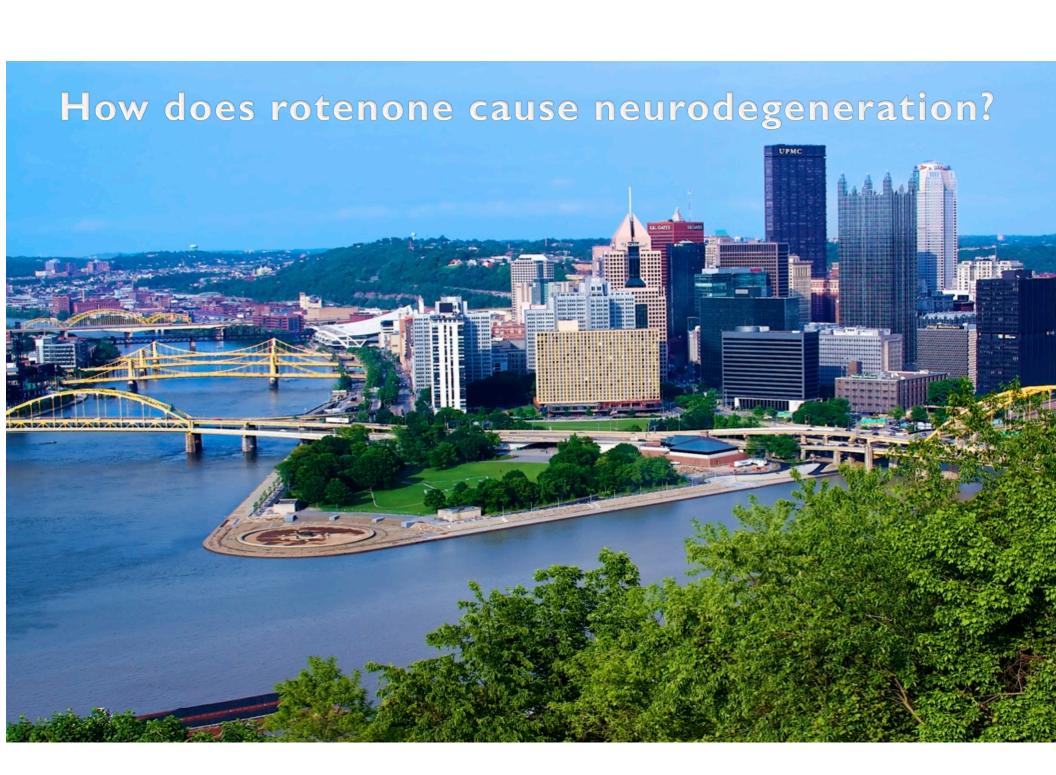


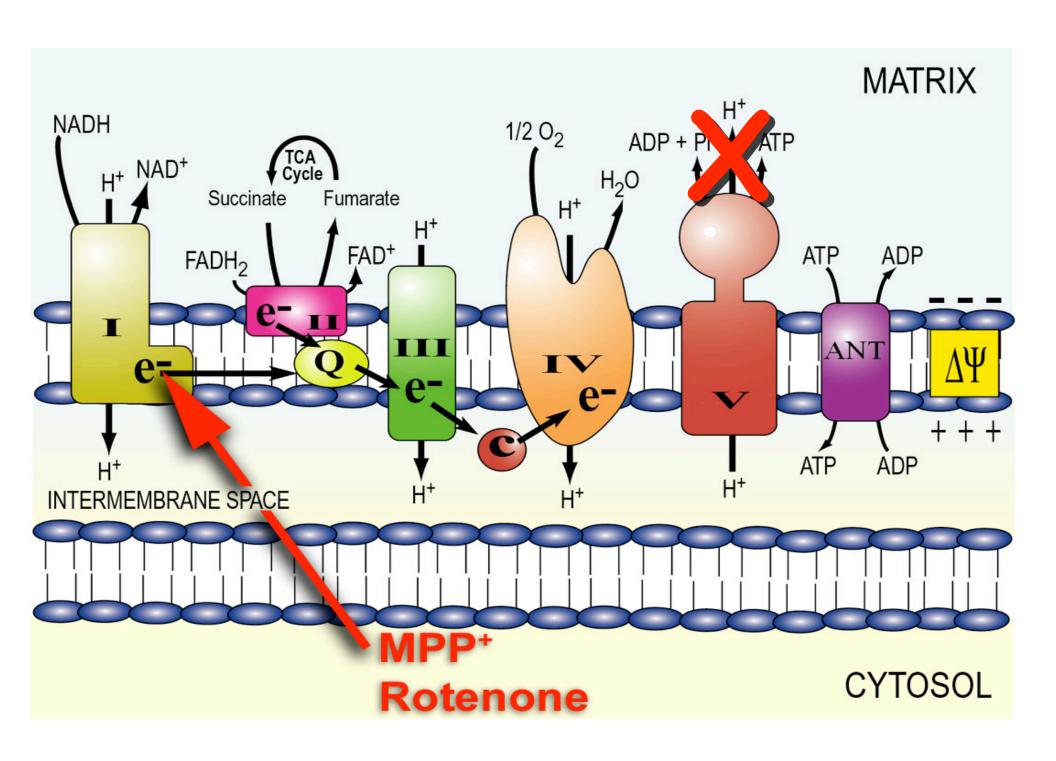


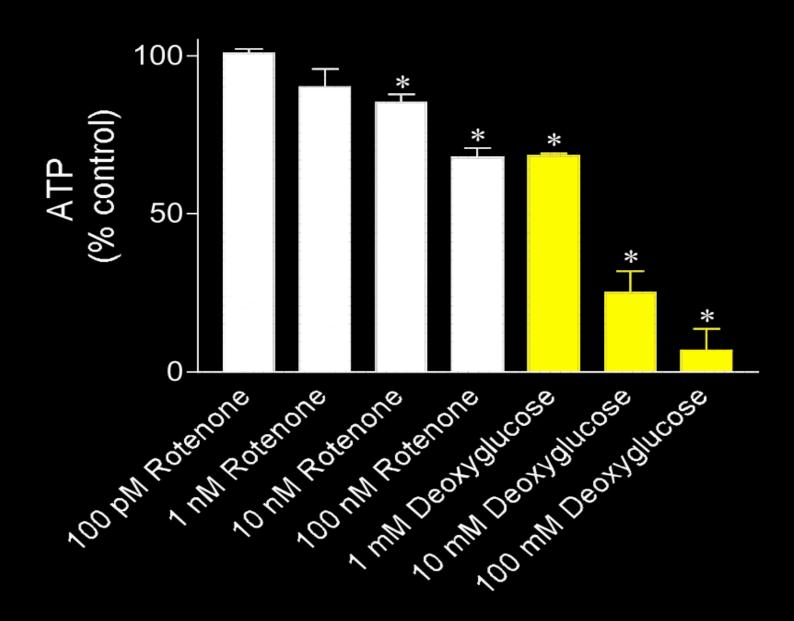
Pest Control

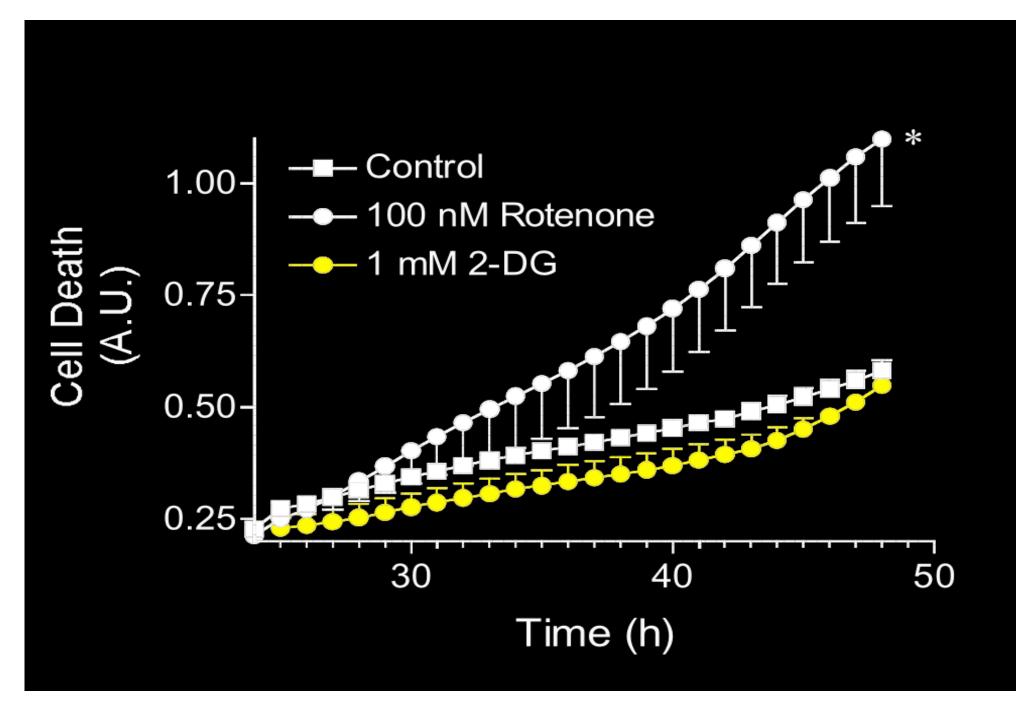


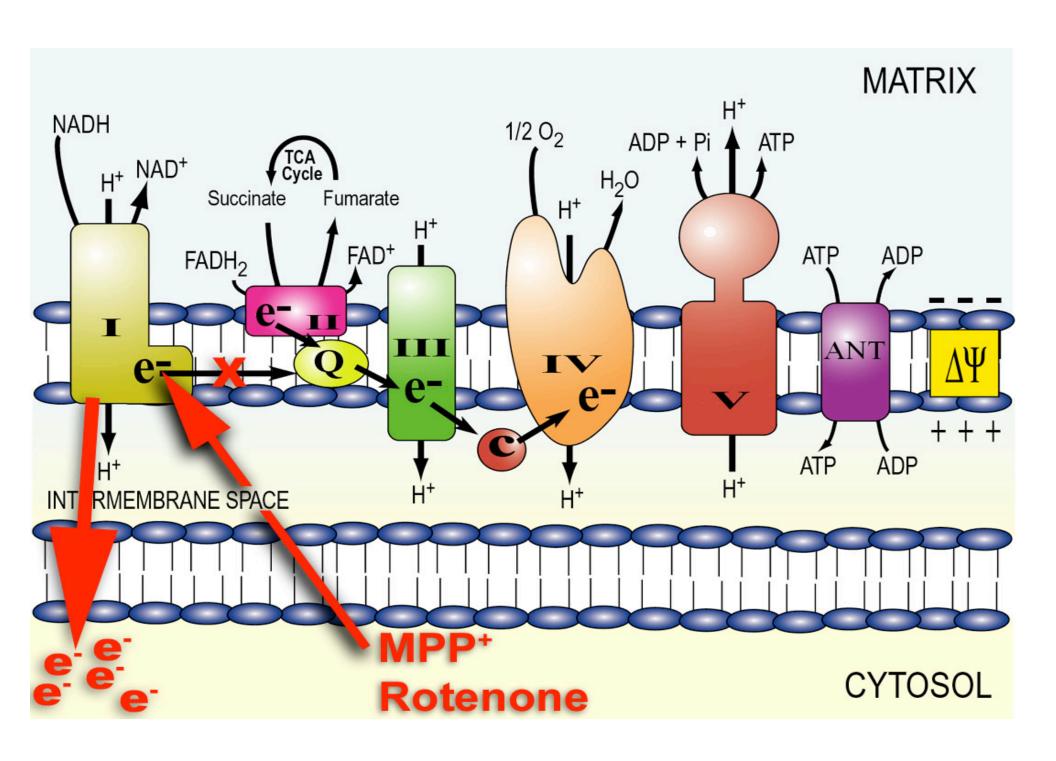
Tanner et al, Environ Health Perspect, 2011

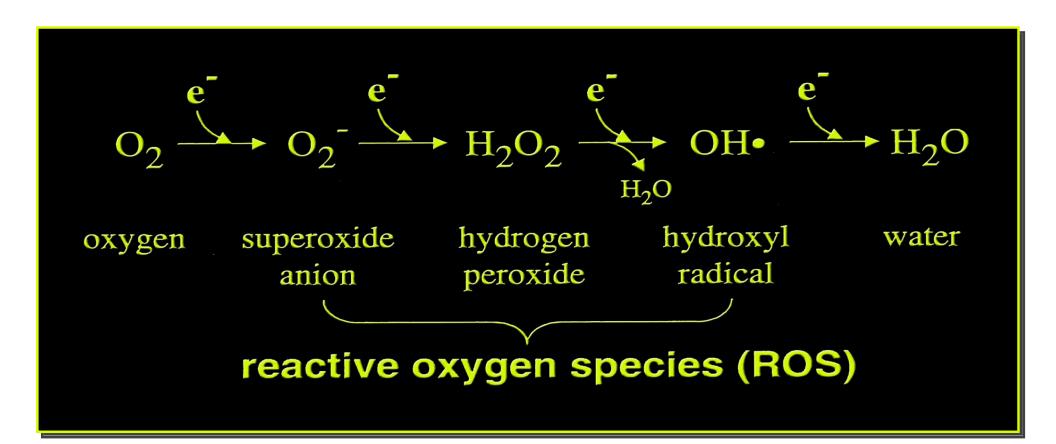




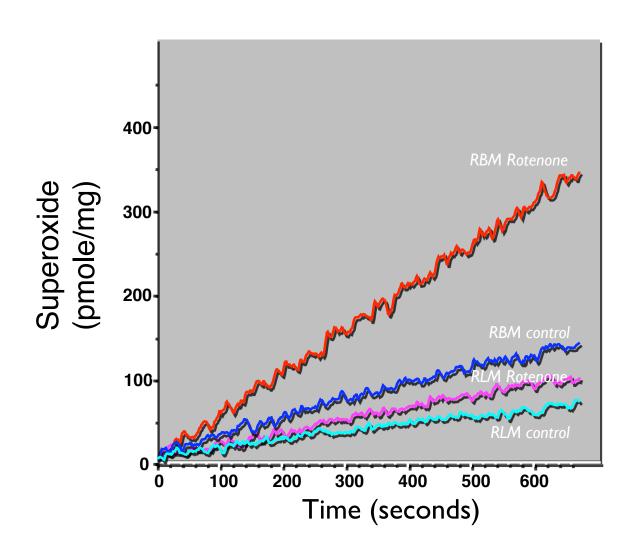


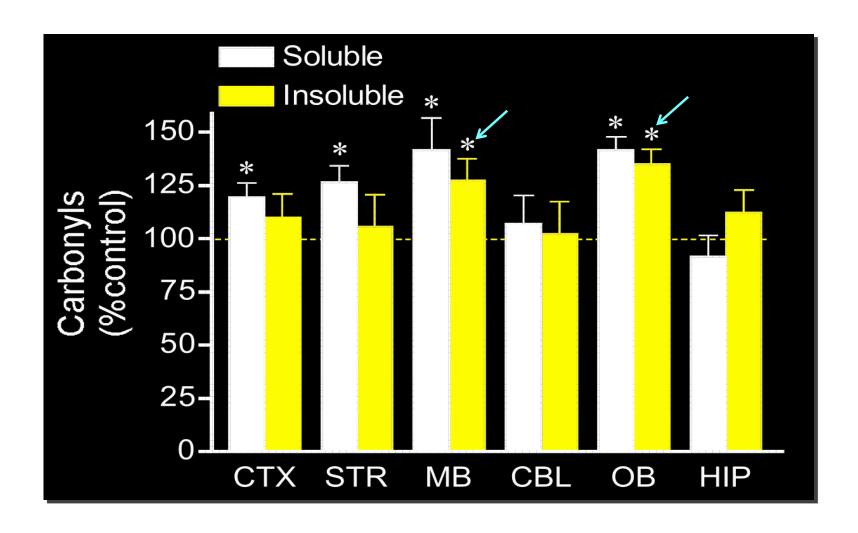


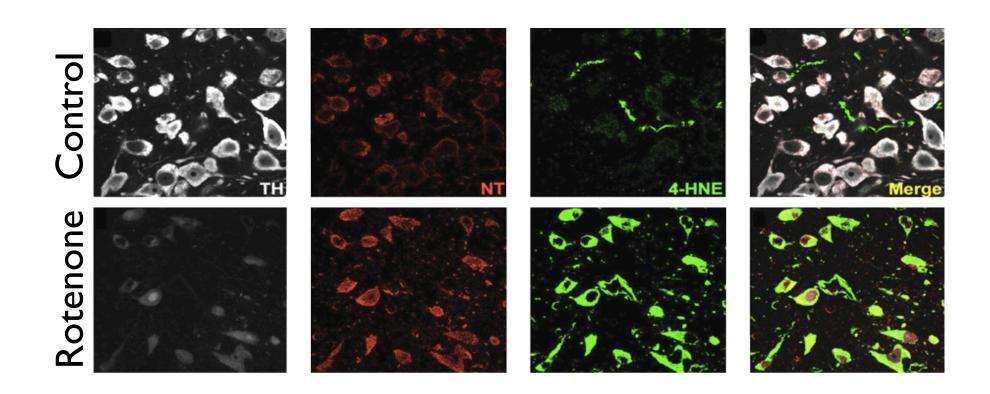




Superoxide production in the mitochondria of rotenonetreated rats measured with electron spin resonance





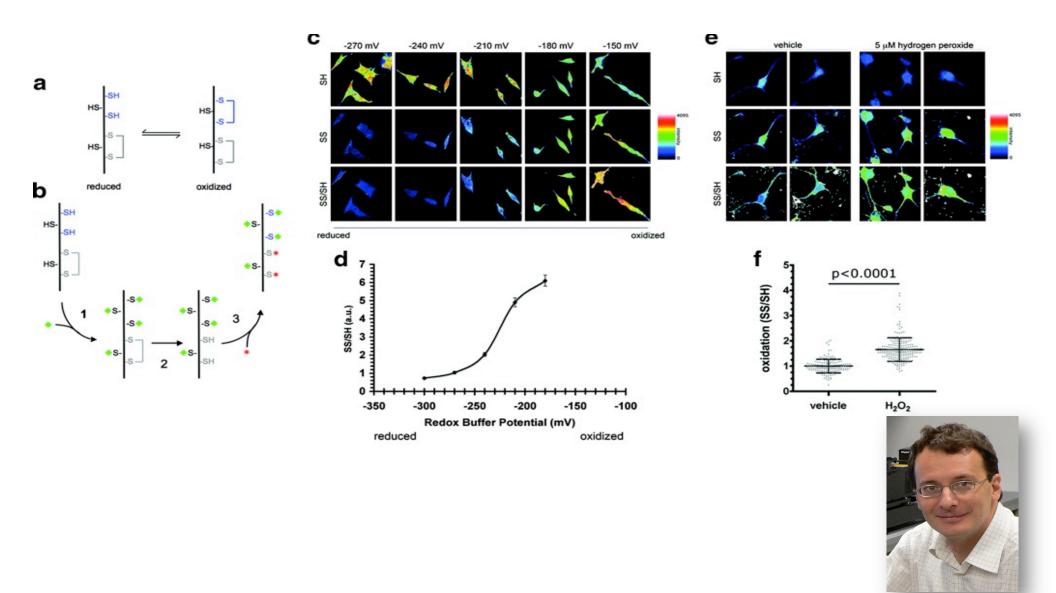


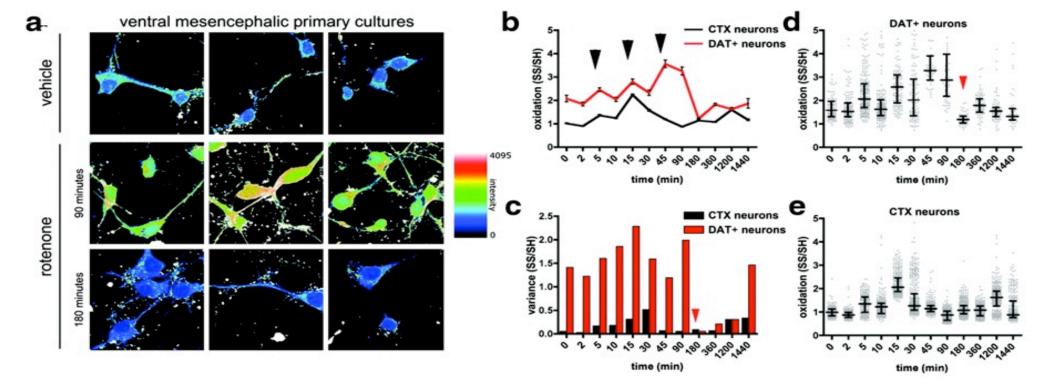
ORIGINAL RESEARCH COMMUNICATION

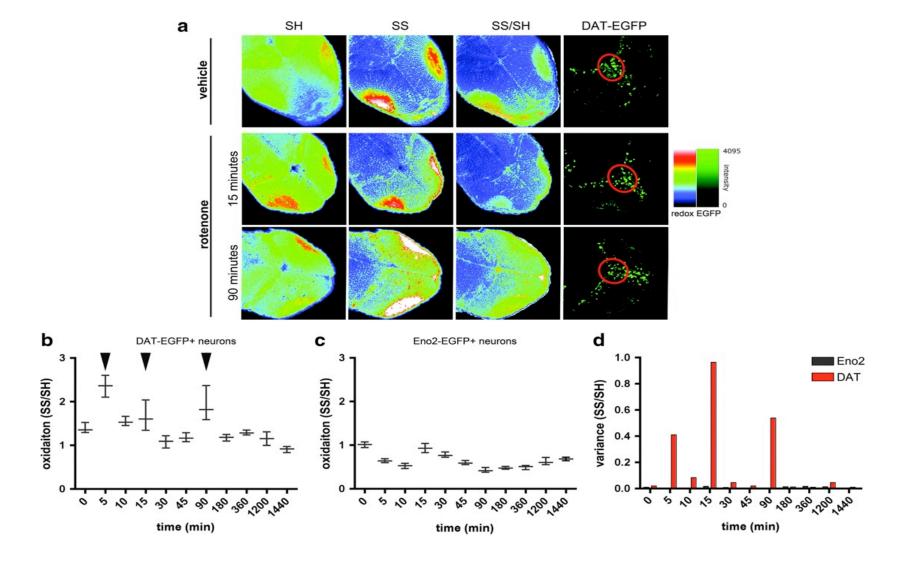
ANTIOXIDANTS & REDOX SIGNALING Volume 15, Number 4, 2011 © Mary Ann Liebert, Inc. DOI: 10.1089/ars.2010.3629

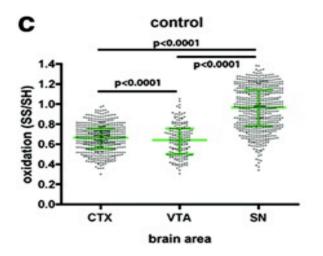
Single-Cell Redox Imaging Demonstrates a Distinctive Response of Dopaminergic Neurons to Oxidative Insults

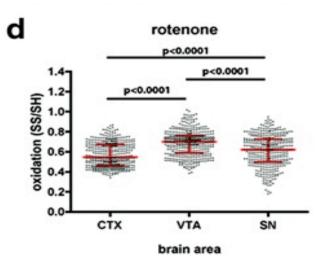
Maxx P. Horowitz, Chiara Milanese, Solvento Di Maio, Xiaoping Hu, Laura M. Montero, Laurie H. Sanders, Victor Tapias, Sara Sepe, Wiggert A. van Cappellen, Edward A. Burton, John Timothy Greenamyre, and Pier G. Mastroberardino

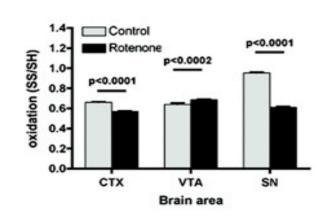




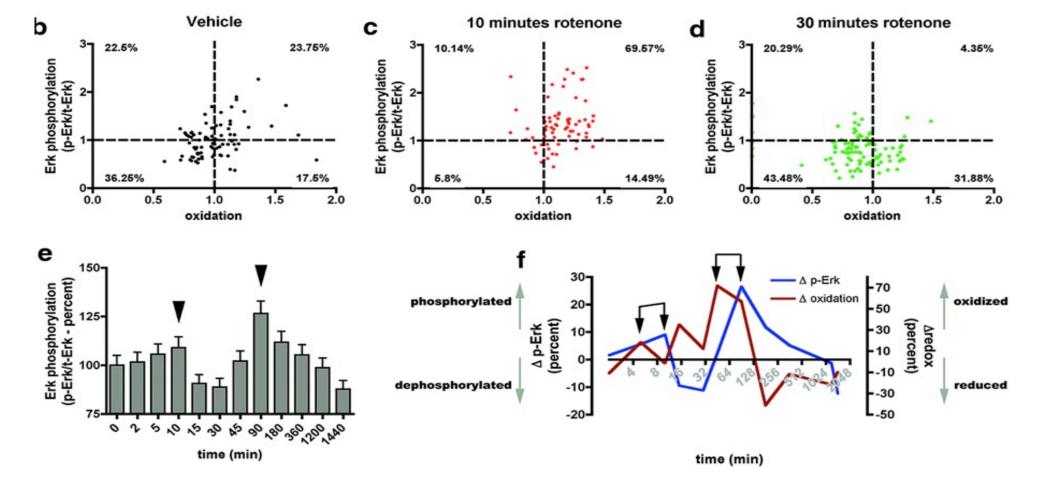


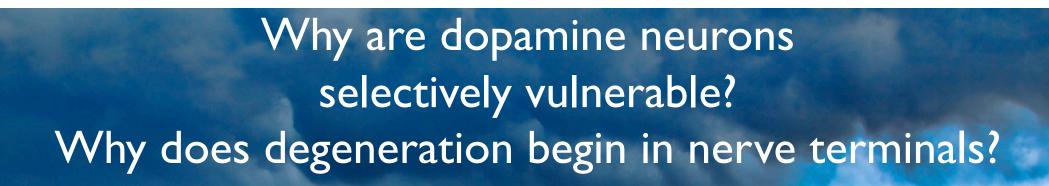


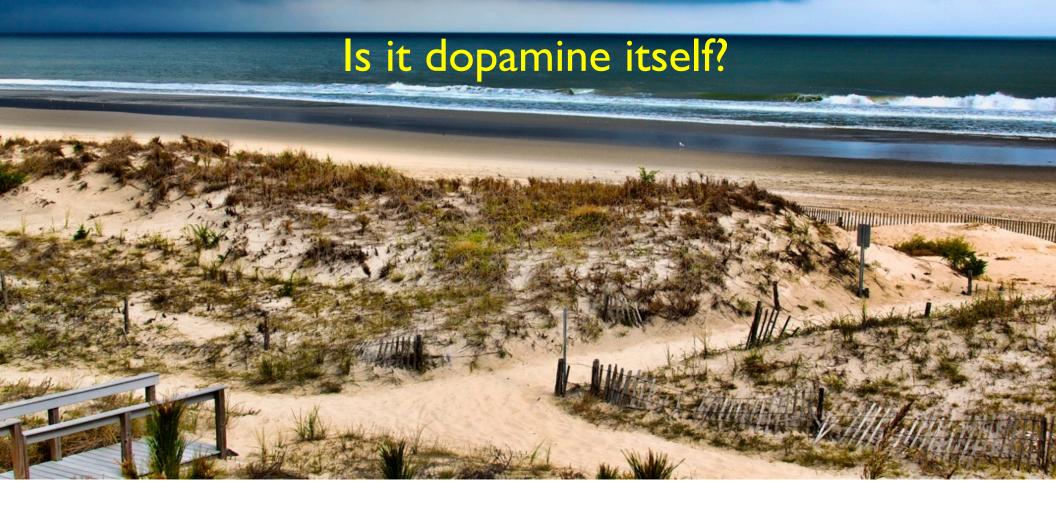




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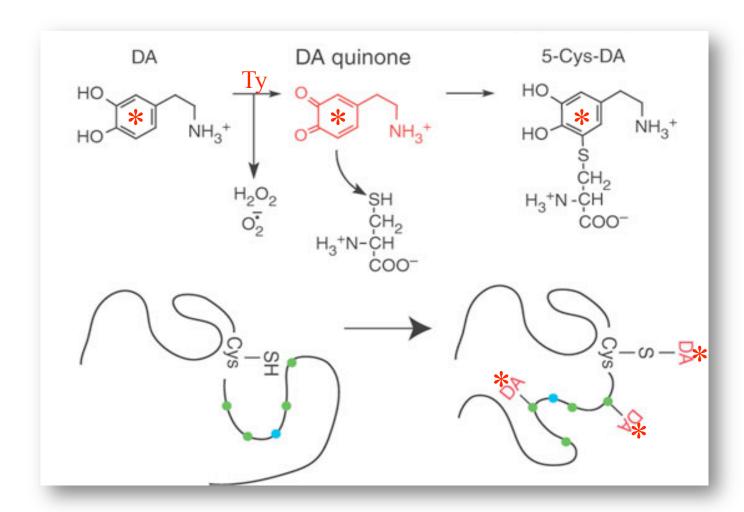


Terri Hastings

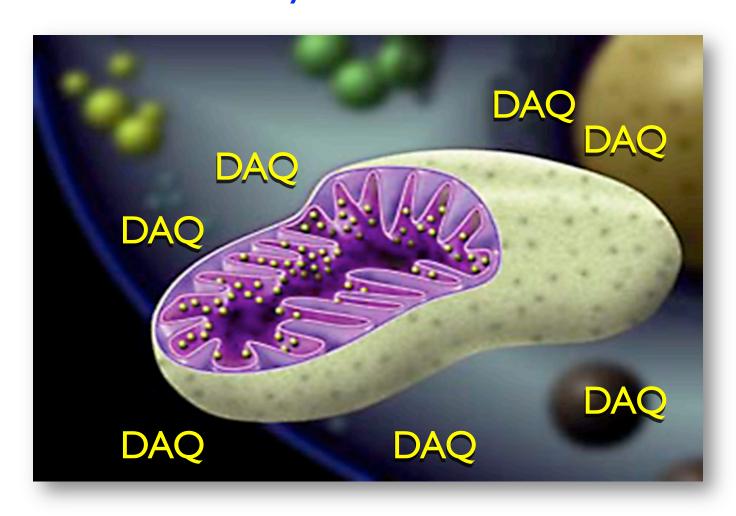
DA quinone 5-Cys-DA

HO

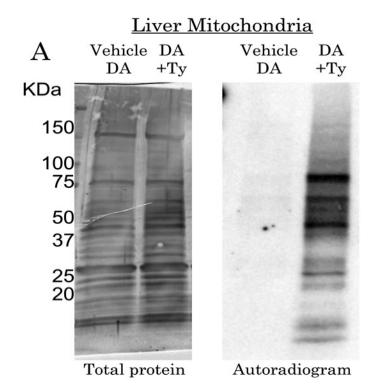
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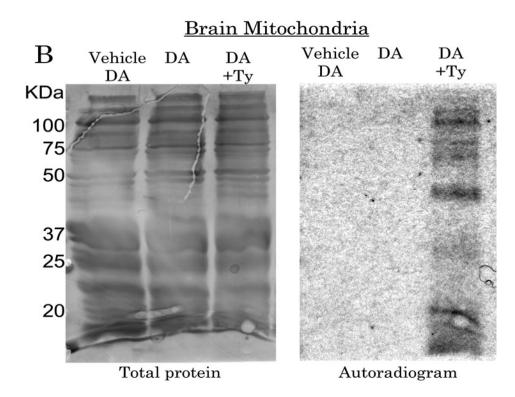


What is the effect of cytosolic DAQ on mitochondria?

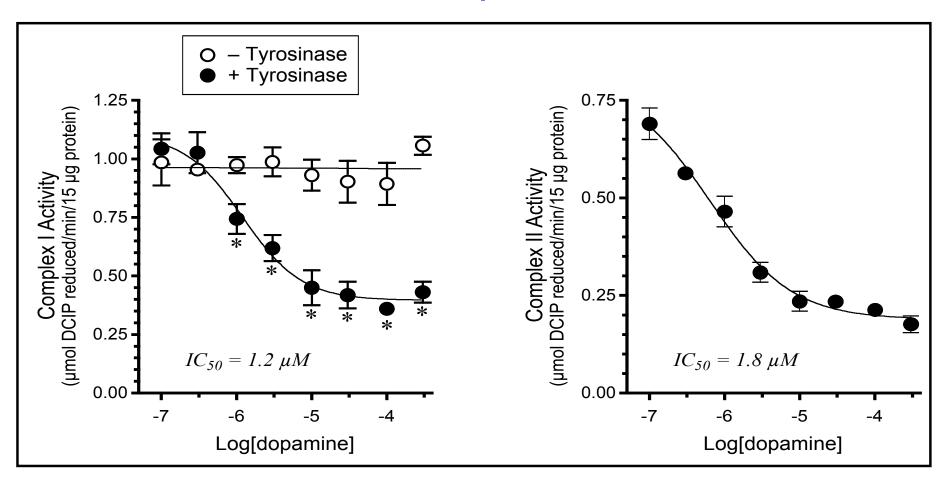


DAQ penetrates intact mitochondria and binds covalently to complex I subunits





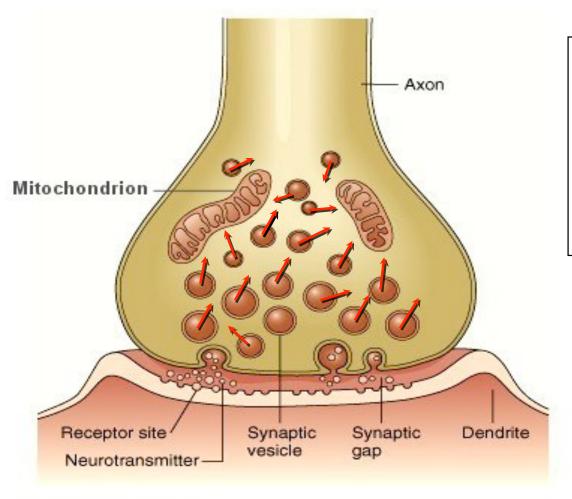
DAQ penetrates intact mitochondria and inhibits complexes I & II



The effect of DAQ is blocked by glutathione



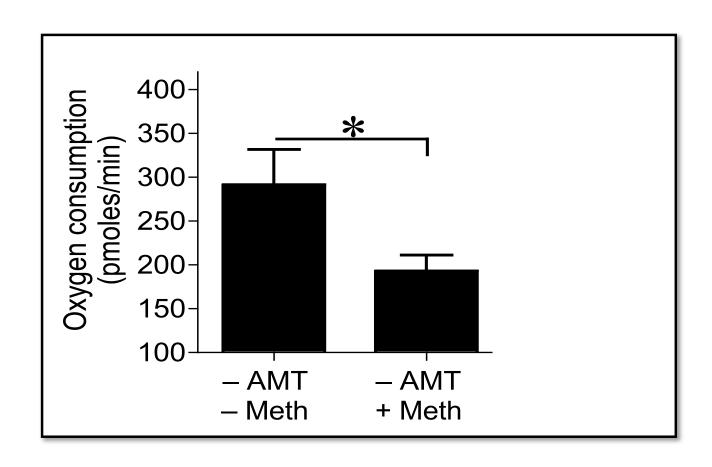
Methamphetamine releases DA from vesicles



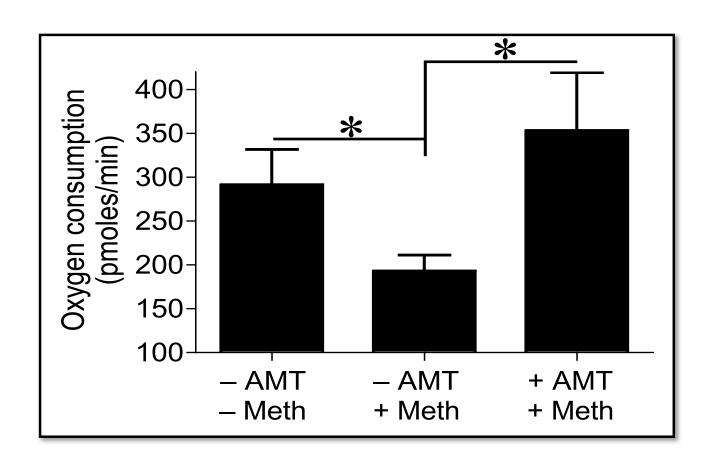
Relevance:

- alpha-synuclein increases cytosolic dopamine
- Complex I dysfunction increases cytosolic dopamine

Cytosolic DA inhibits mitochondrial respiration in intact cells

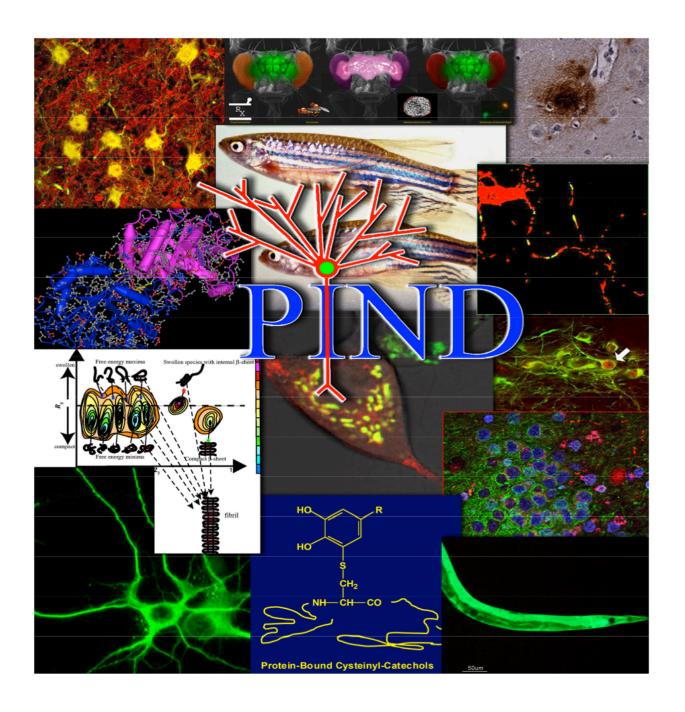


Cytosolic DA inhibits mitochondrial respiration in intact cells





- Systemic mitochondrial impairment
 - Pesticide exposure
- Selective nigrostriatal dopamine cell loss
- \checkmark Lewy body formation (α -synuclein accumulation)
 - Oxidative damage
 - Microglial activation (inflammation)
 - Proteasome dysfunction
 - Cardiac sympathetic denervation
 - Gl pathology/constipation
 - ✓ Iron accumulation



We'll do whatever it takes to cure Parkinson's.

(So, who had the decaf?)