Presentation 5 – Bellina Veronesi

Particulate matter and neurogenic inflammation … oxidative stress-mediated toxicity

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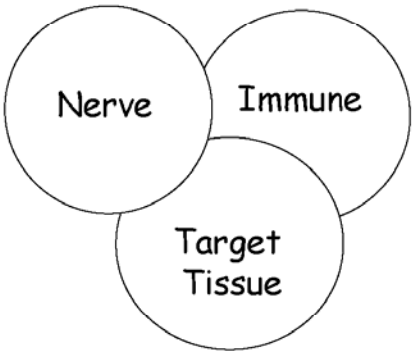
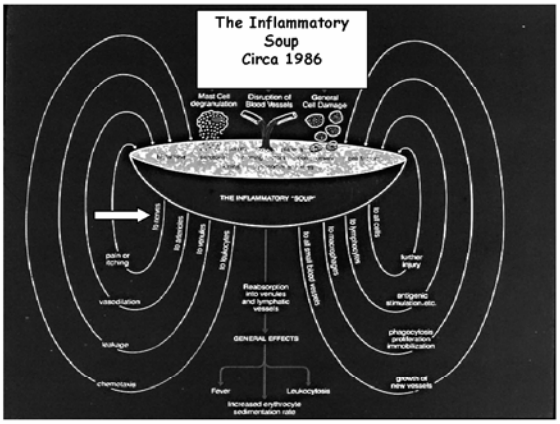
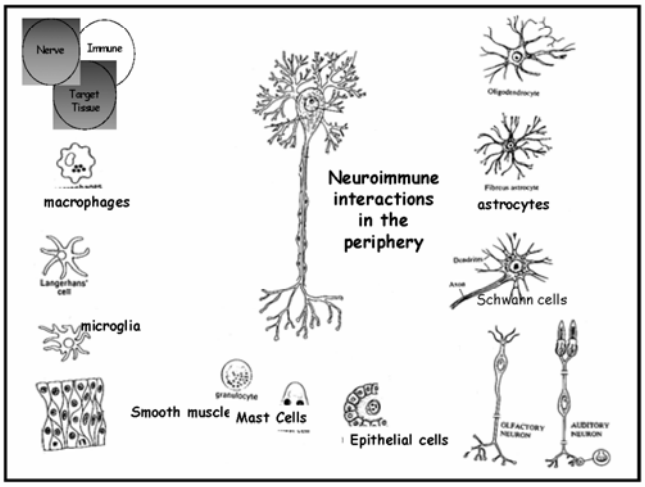
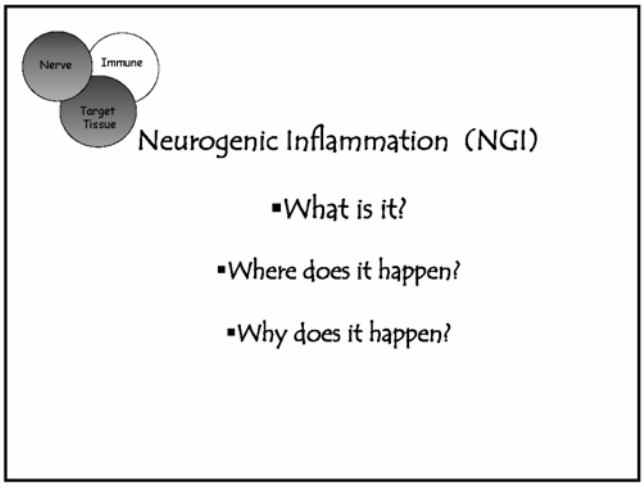


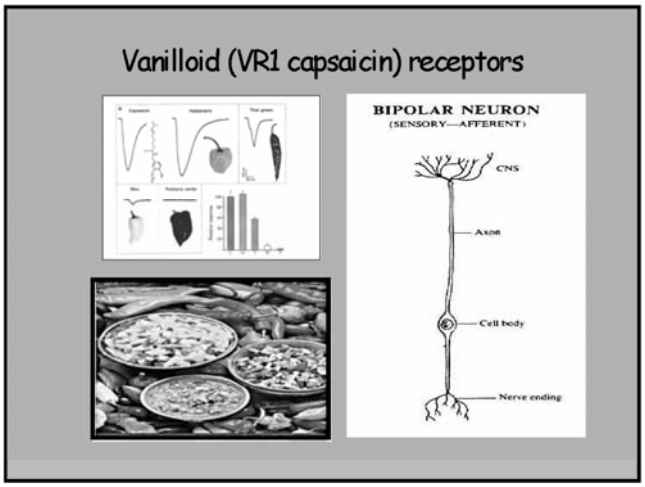
"Ruber et tumor cum colore et dolore"

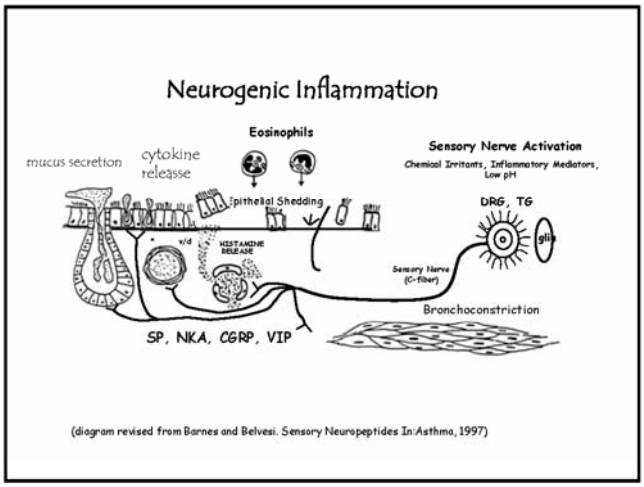
-Cornelius Cesus 35 B.C.




Nerve
Immune
Target Tissue
macrophages
Langerhans' cell
microglia
Smooth muscle
granulocyte
Mast Cells
Epithelial cells
Neuroimmune interactions in the periphery
Oligodendrocyte
Fibrous astrocyte
astrocytes
Dendrites
Axon
Schwann cells
OLFACTORY NEURON
AUDITORY NEURON

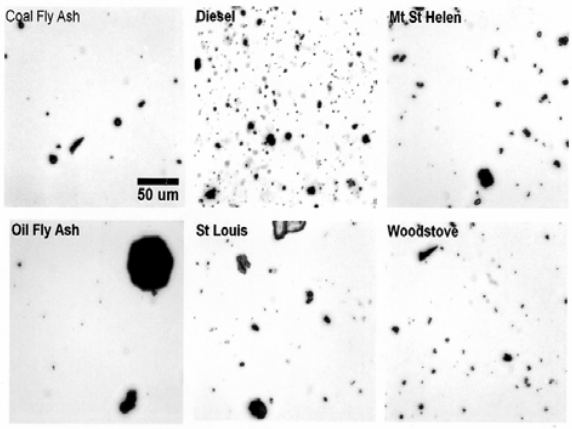

Nerve
Immune
Target Tissue
Neurogenic Inflammation (NGI)
▪What is it?
▪Where does it happen?
▪Why does it happen?


Vanilloid (VR1 capsaicin) receptors
BIPOLAR NEURON
(SENSORY—AFFERENT)
CNS
Axon
Cell body
Nerve ending


Neurogenic Inflammation
Eosinophils
mucus secretion
cytokine releasse
Sensory Nerve Activation
Chemical Irritants, Inflammatory Mediators, Low pH
Epithelial Shedding
DRG, TG
HISTAMINE RELEASE
Sensory Nerve (C-fiber)
Bronchoconstriction
SP, NKA, CGRP, VIP
(diagram revised from Barnes and Belvesi. Sensory Neuropeptides In:Asthma, 1997)

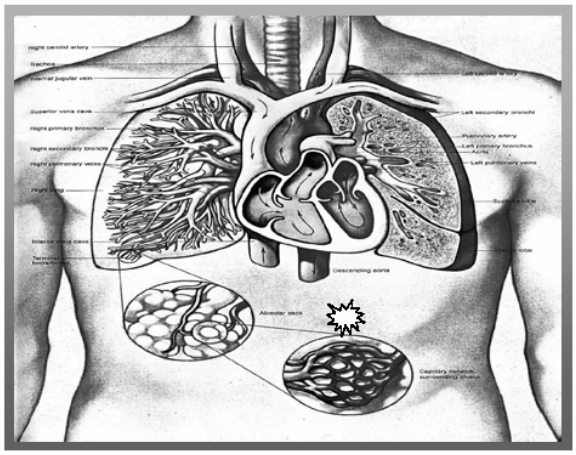
Particulate Matter (PM)

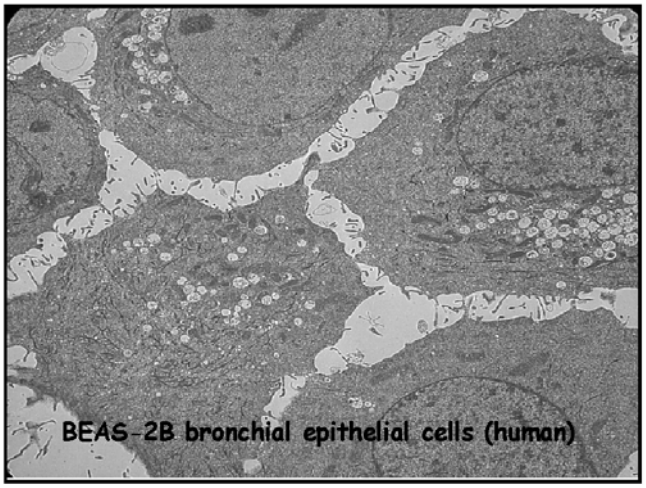
- A major concern of the US EPA
- Epidemiologically associated with increased respiratory symptoms and mortality, world-wide…cost burden
- Strong susceptible population (e.g. elderly, young, pre-existing conditions like asthmatics, cardiopulmonary, smokers)
- Multi-source PM (industrial emission, naturally occurring, botanical, ambient) with different pollutants
- Uniform degree of mortality and morbidity

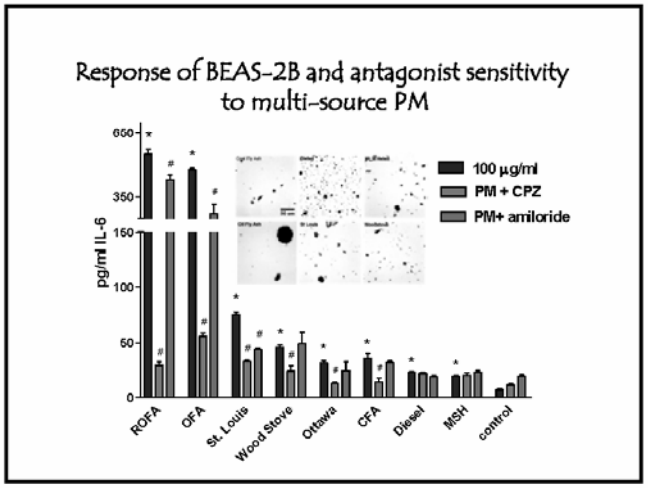


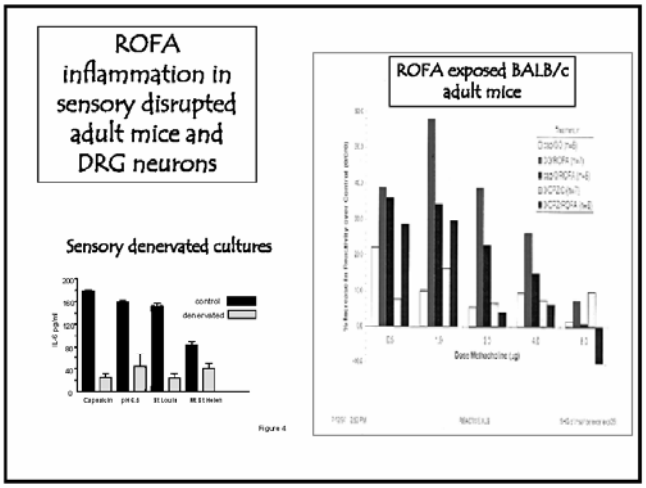
Particle size and number important

- Surface area
- Deposition
- Proximity
  - CO_2/O_2 interface
  - venous circulation

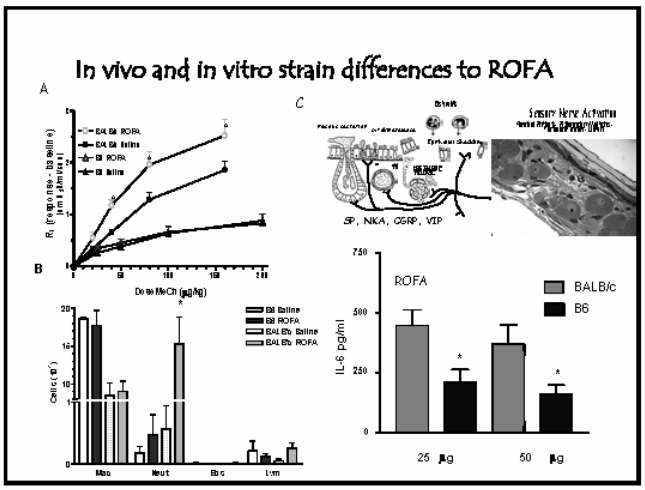


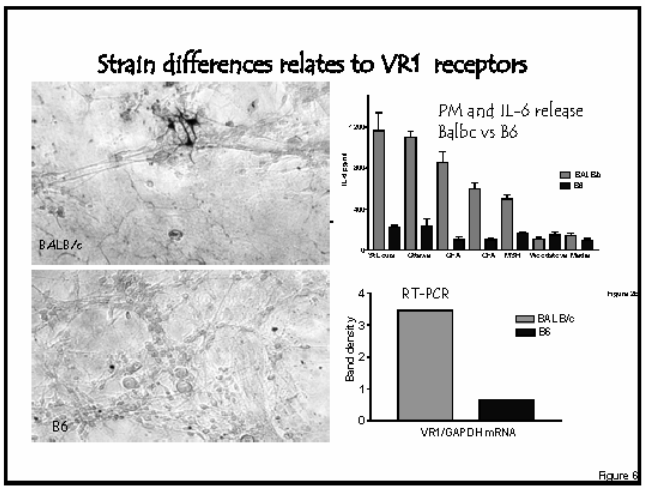

BEAS-2B bronchial epithelial cells (human)


Response of BEAS-2B and antagonist sensitivity to multi-source PM
pg/ml IL-6
650
350
150
100
50
0
100 µg/ml
PM + CPZ
PM+ amiloride
ROFA
OFA
St. Louis
Wood Stove
Ottawa
CFA
Diesel
MSH
control


ROFA inflammation in sensory disrupted adult mice and DRG neurons
Sensory denervated cultures
control
denervated
ROFA exposed BALB/c adult mice


Sensitive sub-populations





PM Research Summary 1999-2003

PM airway inflammation

- Non-neural cells have VR1 receptors
- PM toxicity is neurogenically mediated via VR1
- susceptible populations: genetic component, VR1 mediated

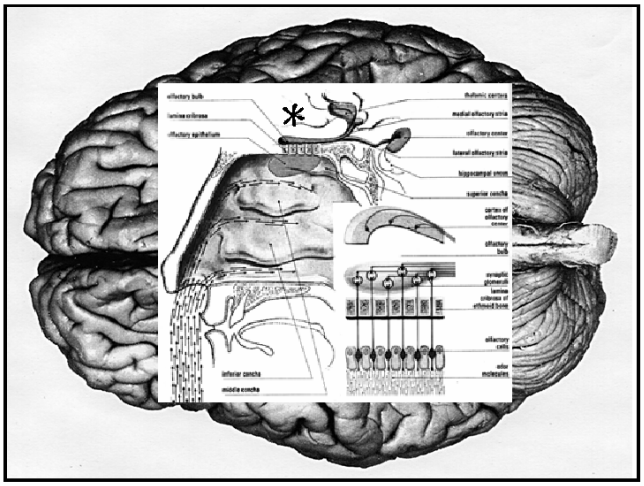
Particulate matter and CNS neurodegeneration

oxidative stress ..a culpable mechanism

Particulate matter and CNS entry

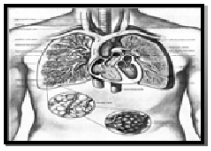
Oberdorster G. and Utell M. (2002)
"UF in the Urban Air: to the Respiratory Tract…and beyond"

- CNS another of PM's target
- TiO3, Ag UF found throughout extra-pulmonary organs
- Lilian Calderon-Mexican studies

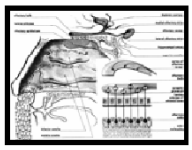


Particulate matter and CNS entry

- PM-UF can exit through CO2:O2 barriers
- PM-UF travels via olfactory route



Pulmonary vasculature



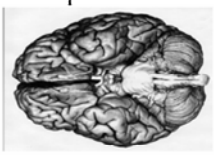
Olfactory bulb-translocation

Particulate matter and CNS entry

UF: SMALL-DENSE-CHARGED

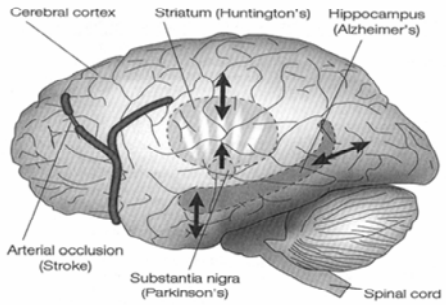
- Small enough (<100 nm) to pass through biological barriers
  - eg BBB and CO2:O2 interface using caveolae
- Dense – enter CNS in significant quantities
  - $10^6/cm^3$…..20^3 L/day
- Charged – carry free radical activity on their surface
- Above properties – central to PM toxicity
- *Could inhaled PM deliver (sustain) low levels of oxidative stress to the CNS?*
-

"The perfect storm"



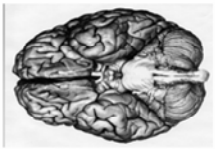
- High energy demands (transmission, conduction)
- Low levels endogenous scavengers
- High lipid content
- Non-replication of neurons
- Highly reactive cells (microglia)-differentially distributed

Oxidative stress and selective neurodegeneration



Also ALS, Picks, variants

Particulate matter and CNS neurodegeneration



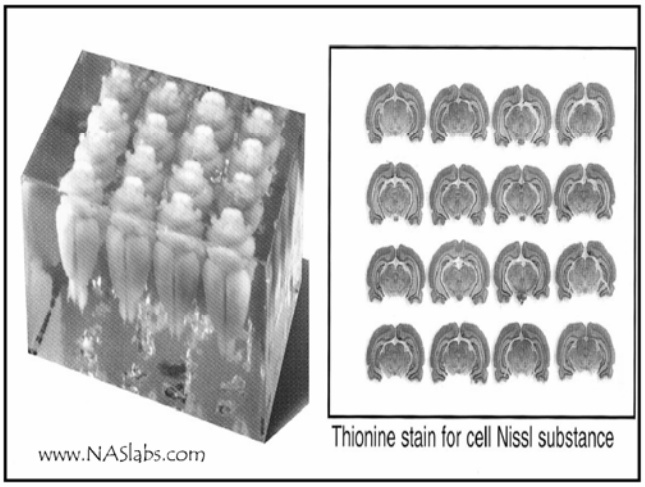
Oxidative stress
△
Culpable CNS cell (e.g., microglia)
△
Selective neuronal damage
△
Neuropathology
△
PM pollutant

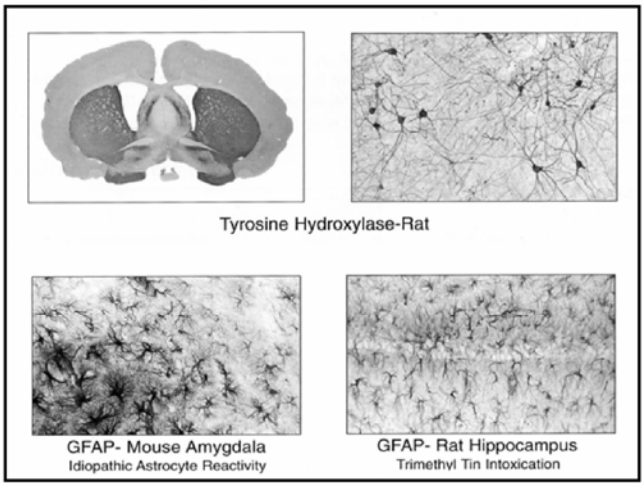
Prediction

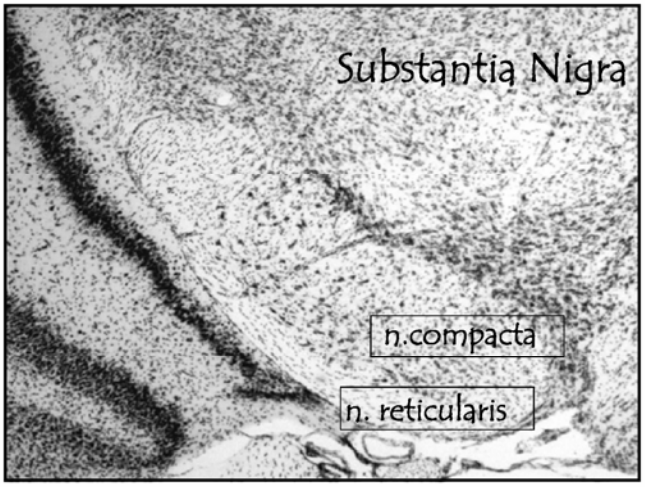
Normal individual + sub-chronic exposure=marginal

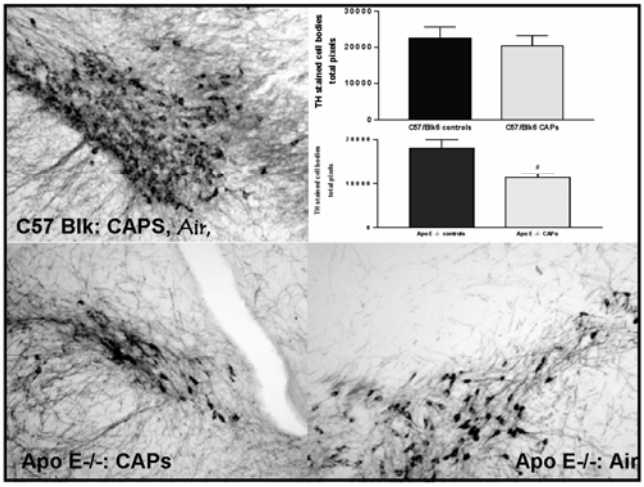
OS "compromised" + sub-chronic exposure=damage.

- ApoE-/- (KO) transgenic mouse predisposed to OS
- concentrated NYC ambient air
- 4-6 months exposure
- histopathology-special stains


www.NASlabs.com
Thionine stain for cell Nissl substance


Tyrosine Hydroxylase-Rat
GFAP- Mouse Amygdala
Idiopathic Astrocyte Reactivity
GFAP- Rat Hippocampus
Trimethyl Tin Intoxication

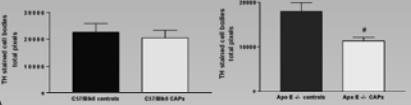

Substantia Nigra
n.compacta
n. reticularis


C57 Blk: CAPS, Air,
Apo E-/-: CAPs
Apo E-/-: Air

Prediction

Normal individual

+ sub-chronic exposure = marginal

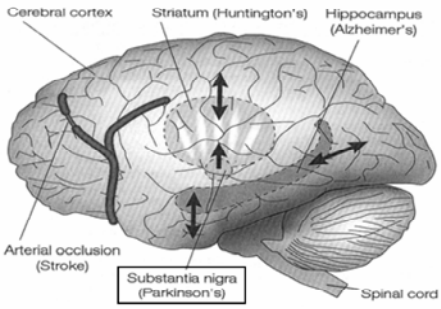


Compromised

+ sub-chronic exposure = damage.

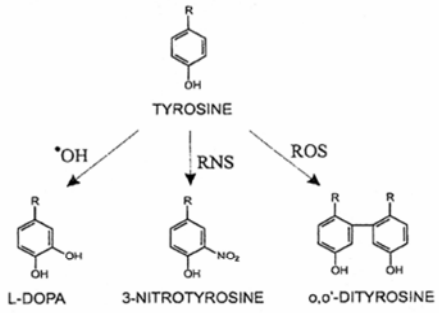
ApoE-/- transgenic mouse predisposed to OS

Oxidative stress and selective neurodegeneration



Also ALS, Picks, variants

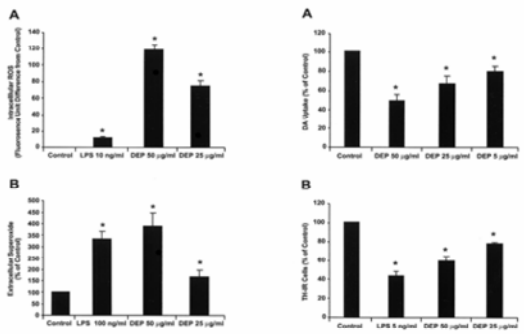
DA metabolism produces free radicals



Block et al., *The FASEB Journal* 18(13):1618-20 (2004).

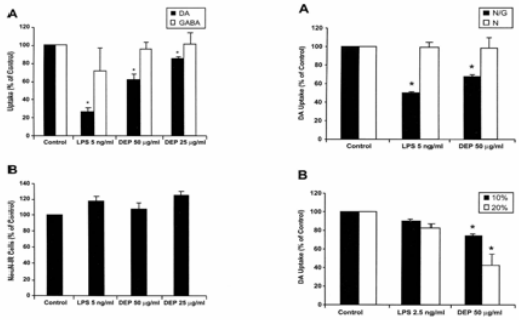
Diesel produces ROS

Diesel is neurotoxic

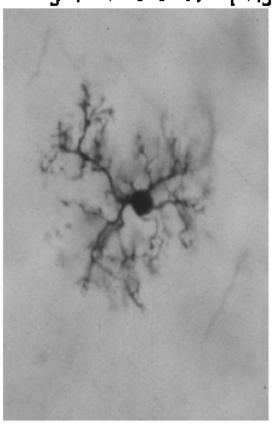


Block et al., *The FASEB Journal* 18(13):1618-20 (2004).

Diesel is selectively neurotoxic Microglia are critical



Microglia–The CNS macrophage

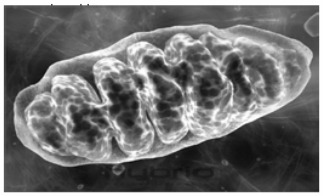


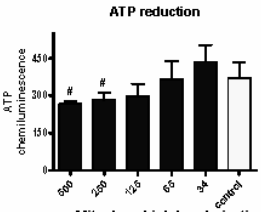
- Oxidative Burst
- *(NAPDHox mediated)*
- *Free radicals, super-oxides*
- *ROS, RNS, iNOS, NO*
- *over-expression of transcription factors e.g., AP-1, NFkB, Sp1, p-CREB*
- *Release of inflammatory cytokines,*
- *(innate immunity-neurotoxic)*
- *Glial proliferation-clusters, scarring*
- *ROS damage to energy-sensitive neurons*
- *(mesencephalic, SN, CA1)*

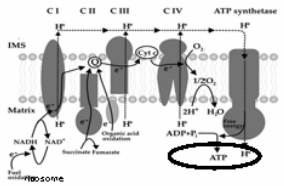
MATERIALS AND METHODS

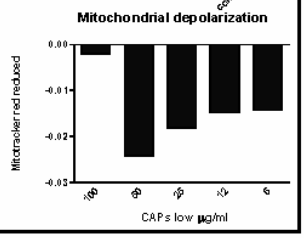
- CAPs collected on site and ranked (high, low) NFkB increases Immortalized mouse CNS microglia (BV2)
- Exposed and assayed for immediate, delayed OS changes cytokine release
- Exposed and examined with TEM
- Universal (affymetrix) microarray
- Bioinformatics

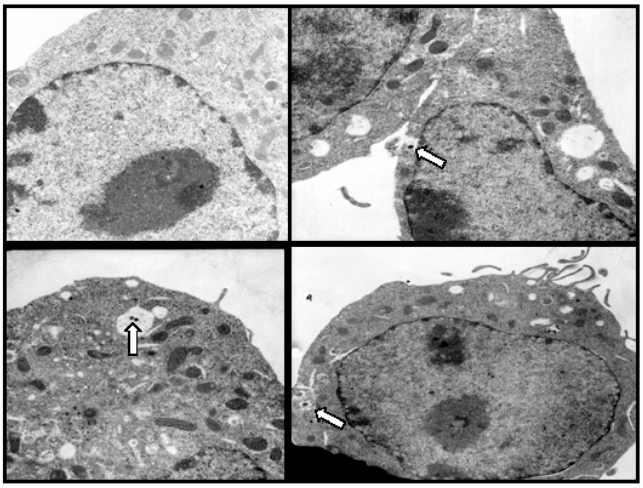
Mitochondrial Bioenergetics

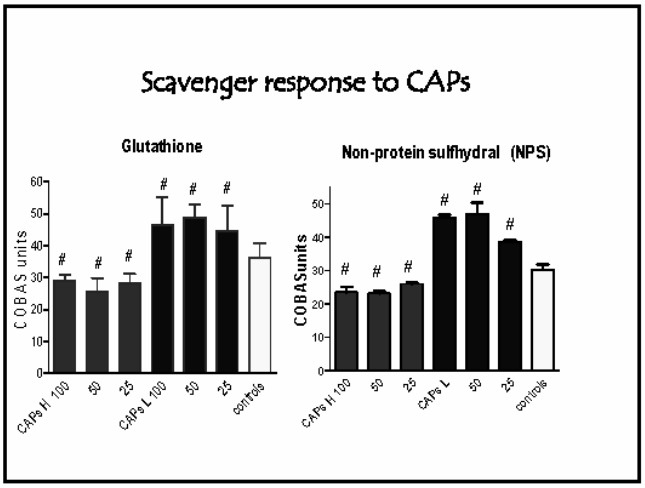


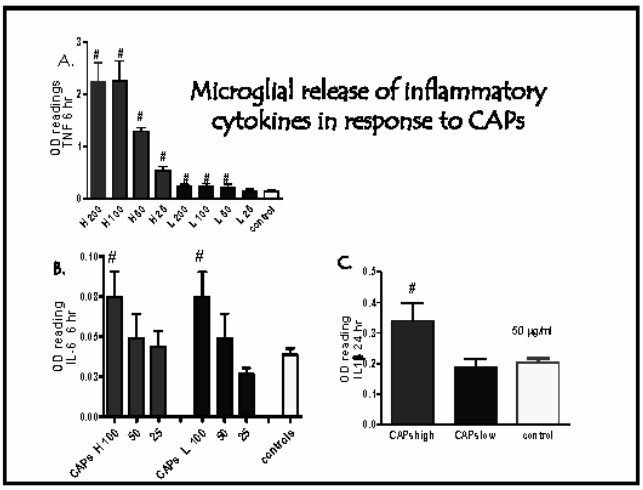


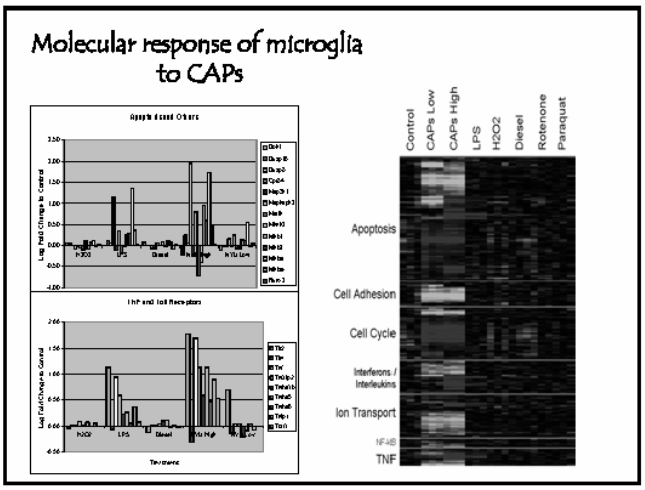


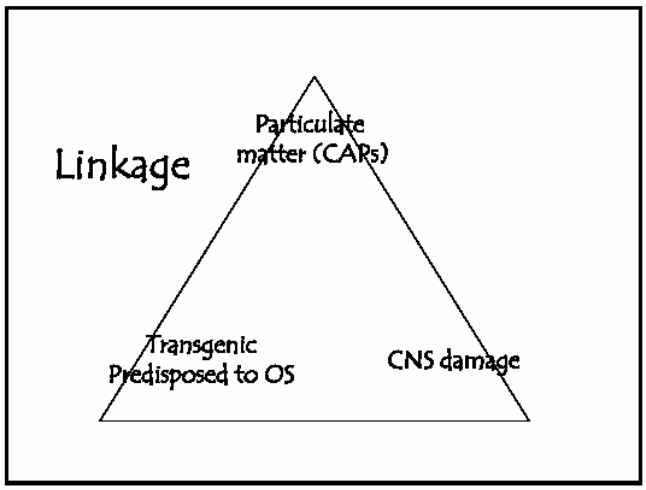


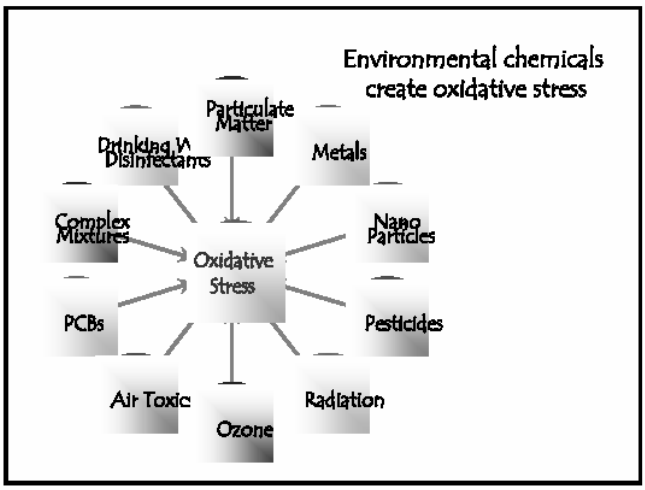


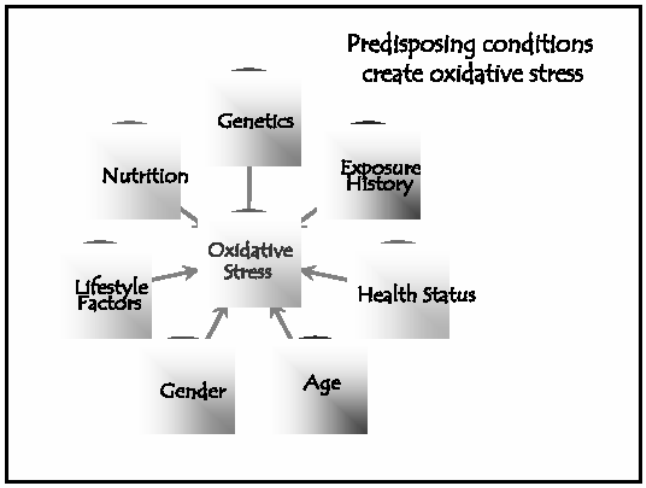

Scavenger response to CAPs
Glutathione
Non-protein sulfhydral (NPS)
COBAS units
COBASunits
CAPs H 100
50
25
CAPs L 100
50
25
controls
CAPs H 100
50
25
CAPs L
50
25
controls

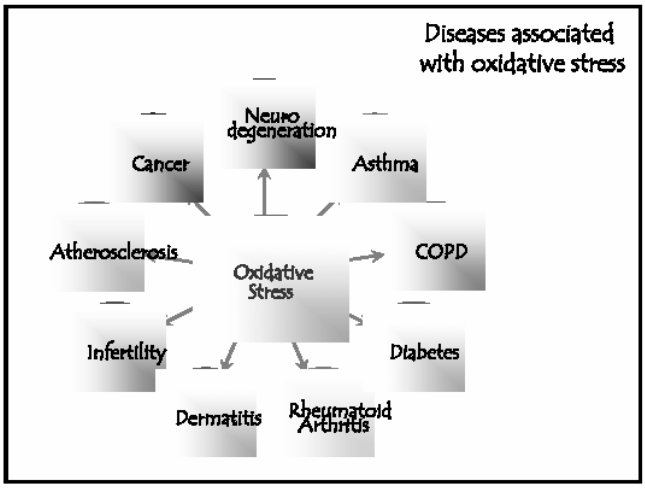

Microglial release of inflammatory cytokines in response to CAPs
A.
OD readings TNF 6 hr
H100
H50
H25
L100
L50
L25
control
B.
OD reading IL-6 6 hr
CAPs H 100
50
25
CAPs L 100
50
25
controls
C.
OD reading IL-6 24 hr
50 µg/ml
CAPs high
CAPs low
control

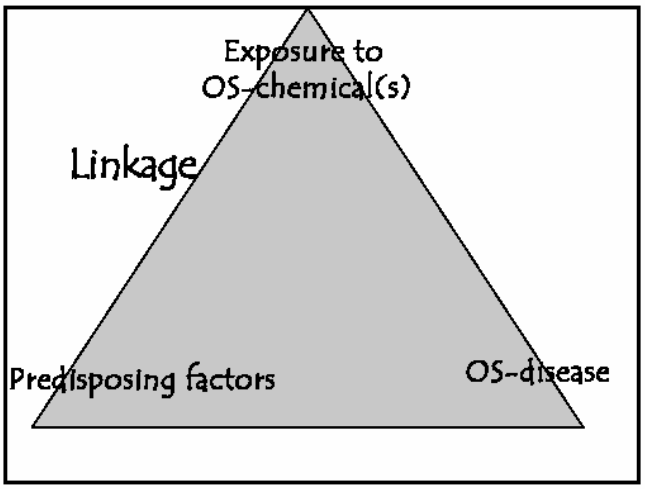

Molecular response of microglia to CAPs
Control
CAPs Low
CAPs High
LPS
H2O2
Diesel
Rotenone
Paraquat
Apoptosis
Cell Adhesion
Cell Cycle
Interferons / Interleukins
Ion Transport
NF-kB
TNF


Linkage
Particulate matter (CAPs)
Transgenic Predisposed to OS
CNS damage


Environmental chemicals create oxidative stress
Particulate Matter
Drinking W Disinfectants
Metals
Complex Mixtures
Nano Particles
Oxidative Stress
PCBs
Pesticides
Air Toxic
Radiation
Ozone


Predisposing conditions create oxidative stress
Genetics
Nutrition
Exposure History
Oxidative Stress
Lifestyle Factors
Health Status
Gender
Age


Diseases associated with oxidative stress
Neuro degeneration
Cancer
Asthma
Atherosclerosis
COPD
Oxidative Stress
Infertility
Diabetes
Dermatitis
Rheumatoid Arthritis



Toxicity Pathway of PM-CNS neurodegeneration

Gene, protein family

△

Immortalized single cell type

△

Target culture

△

Biochemical, morphological, functional changes

△

Normal vs "compromised" animal model

△

Epidemiology-Clinical outbreaks

Colleagues

NYU-Sterling Forest (LC Chen et al.,)

NIEHS-Pharmacology (J. Hong et al.,)

Duke University-Neurobiology. School of Medicine
(S. Simon, M. Oortgiesen et al.,)