Presentation 7 – Jau-Shyong Hong

Role of Inflammation in the Pathogenesis of Neurodegenerative Diseases

Models, Mechanisms, and Therapeutic Interventions

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NIEHS / NIH

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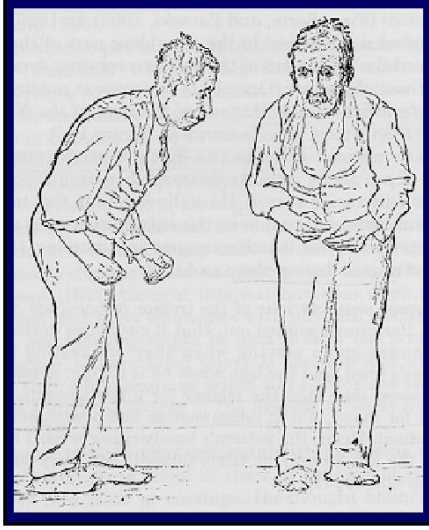
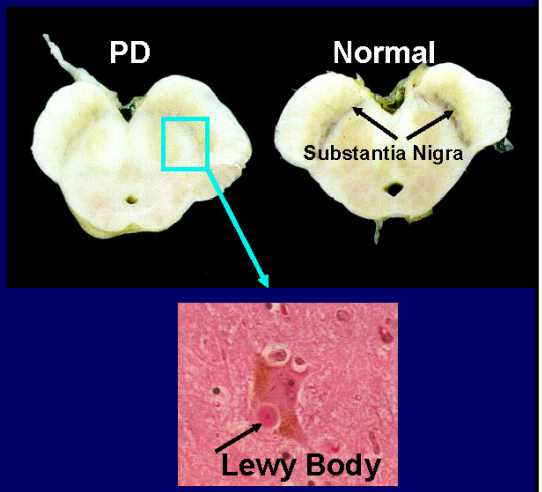
Parkinson's Disease





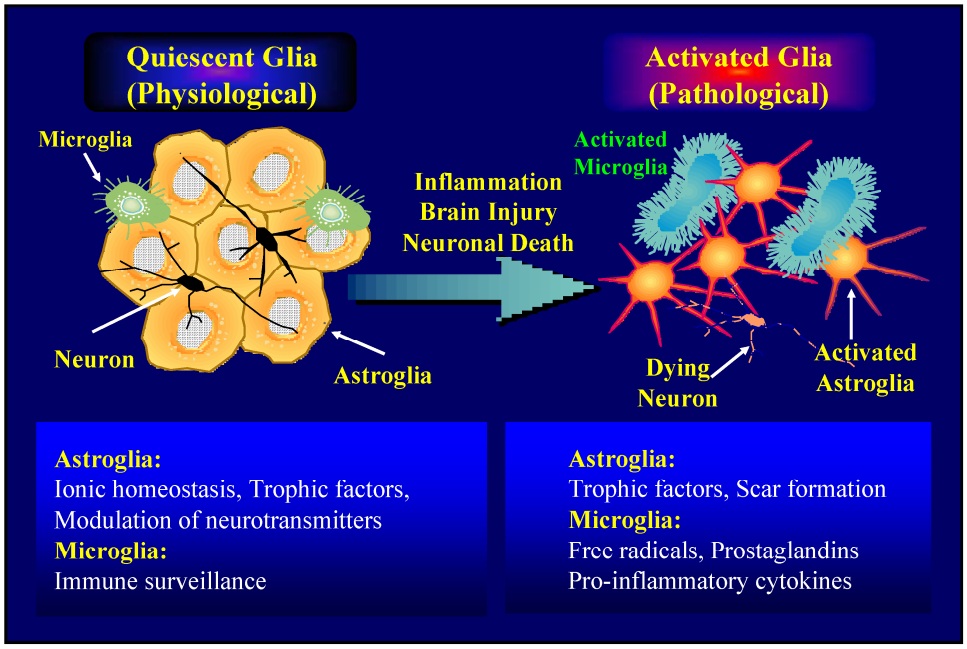
Parkinson's Disease (PD)

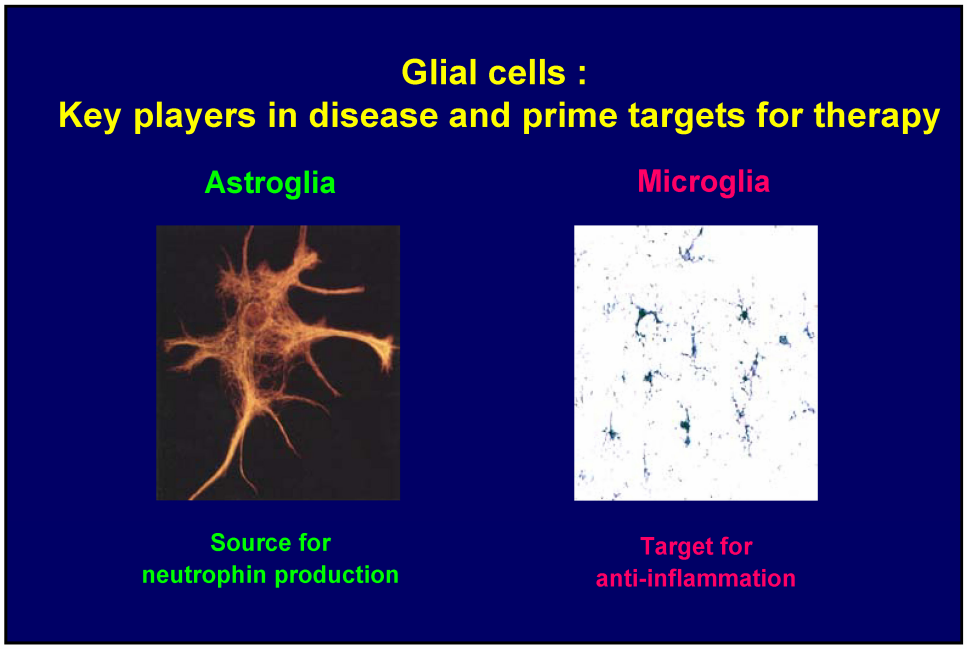
1. PD is an age-related, progressive (8-10 years), and self-propelling neurological disease with a selective loss of dopaminergic neurons in the substantia nigra.

2. Less than 5% of PD patients show genetic mutations (α-synuclein, Parkins). In contrast, 95% of PD cases are sporadic. Twin studies suggest that environmental factors are closely associated with PD.

3. Among the environmental toxins, the following have been considered important risk factors:

   (a) Infectious agents: Bacteria (LPS), Virus (HIV)
   (b) Pesticides & Herbicides: Rotenone, Paraquat


Quiescent Glia
(Physiological)
Activated Glia
(Pathological)
Microglia
Activated
Microglia
Inflammation
Brain Injury
Neuronal Death
Neuron
Astroglia
Dying
Neuron
Activated
Astroglia
Astroglia:
Ionic homeostasis, Trophic factors,
Modulation of neurotransmitters
Microglia:
Immune surveillance
Astroglia:
Trophic factors, Scar formation
Microglia:
Free radicals, Prostaglandins
Pro-inflammatory cytokines


Glial cells :
Key players in disease and prime targets for therapy
Astroglia
Microglia
Source for
neutrophin production
Target for
anti-inflammation

Research Aims

1. Creation of progressive and inflammation-mediated rodent Parkinson's disease models
2. Elucidation of mechanisms of inflammation-mediated degeneration: role of microglia
3. Development of novel anti-inflammatory therapy for Parkinson's disease

AIM 1

Creation of Progressive and Inflammation-mediated Rodent Parkinson's Disease Models

Rationale:

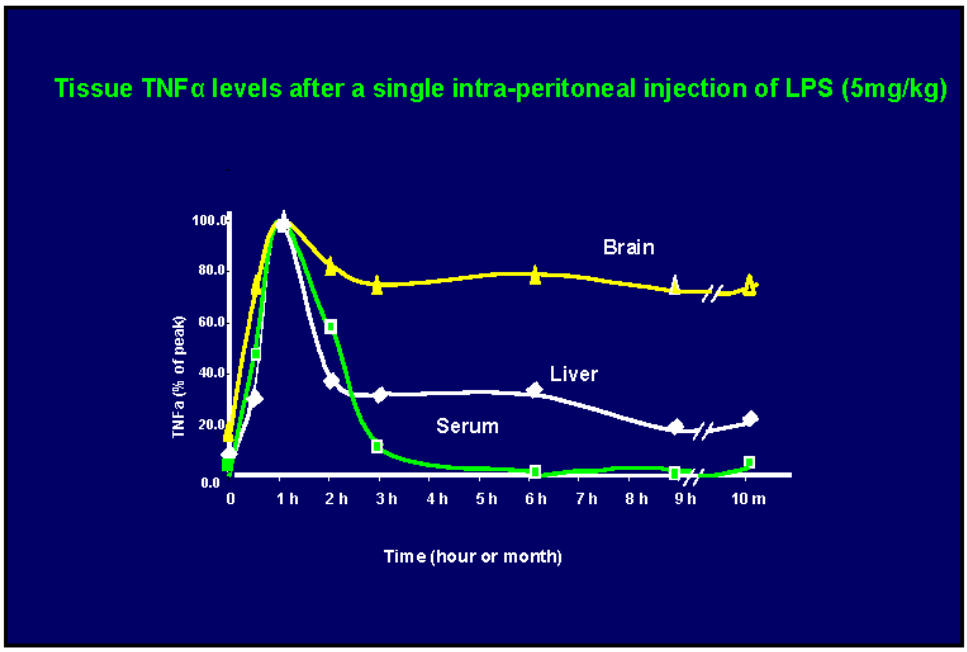
The existing models did not address the role of inflammation in the pathogenesis, nor reflect the delayed and progressive nature of PD.

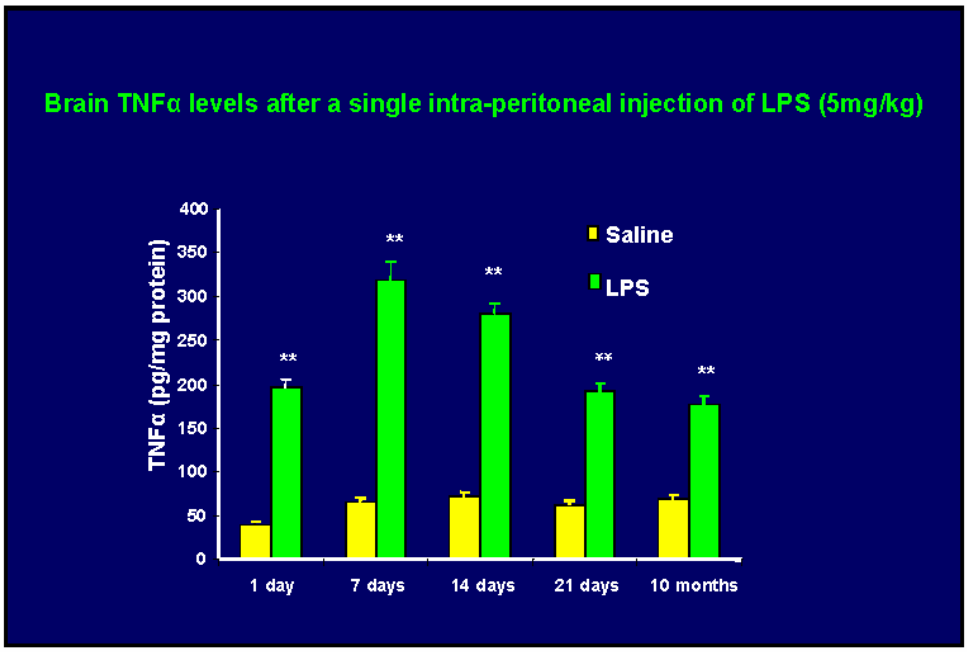
a) *In vivo* model:

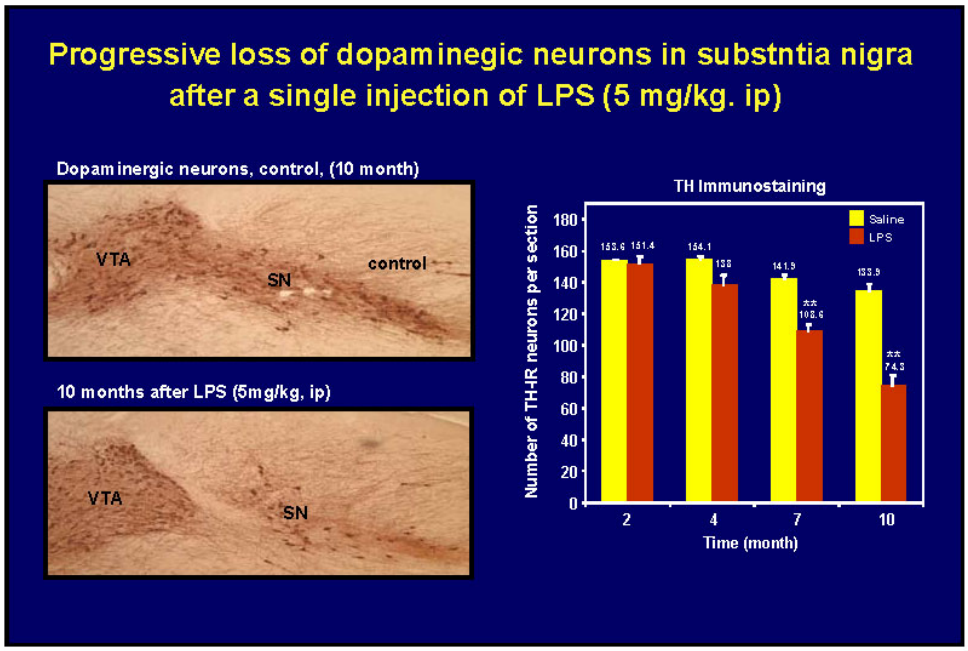
1. *Intra-nigral infusion of lipopolysaccharide (LPS, an inflammagen from bacteria)*
2. *Systemic injection of LPS*

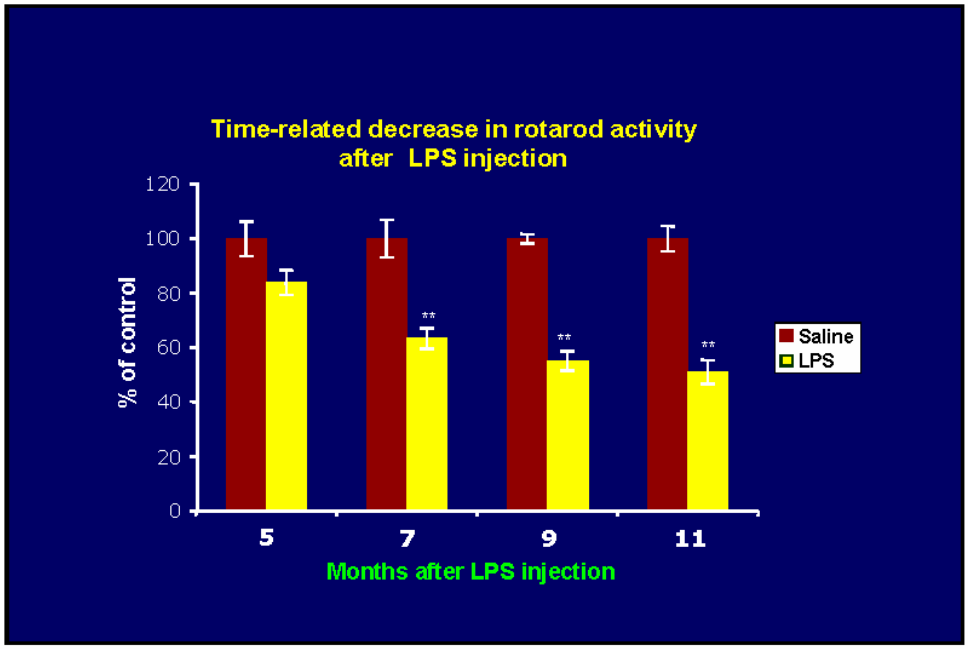
b) *In vitro* model: (Primary cell cultures)

Bin Liu, MD, Ph.D, Huiming Gao, MD, Ph.D, Liya Qin, Ph.D.


Tissue TNFα levels after a single intra-peritoneal injection of LPS (5mg/kg)
TNFa (% of peak)
100.0
80.0
60.0
40.0
20.0
0.0
Brain
Liver
Serum
0
1 h
2 h
3 h
4 h
5 h
6 h
7 h
8 h
9 h
10 m
Time (hour or month)


Brain TNFα levels after a single intra-peritoneal injection of LPS (5mg/kg)
TNFα (pg/mg protein)
400
350
300
250
200
150
100
50
0
Saline
LPS
**
**
**
**
**
1 day
7 days
14 days
21 days
10 months


Progressive loss of dopaminegic neurons in substntia nigra after a single injection of LPS (5 mg/kg. ip)
Dopaminergic neurons, control, (10 month)
VTA
SN
control
10 months after LPS (5mg/kg, ip)
VTA
SN
TH Immunostaining
Saline
LPS
Number of TH-IR neurons per section
180
160
140
120
100
80
60
40
20
0
153.6
151.4
154.1
138
141.9
108.6
133.9
74.3
2
4
7
10
Time (month)


Time-related decrease in rotarod activity after LPS injection
% of control
120
100
80
60
40
20
0
5
7
9
11
Months after LPS injection
Saline
LPS

AIM 2

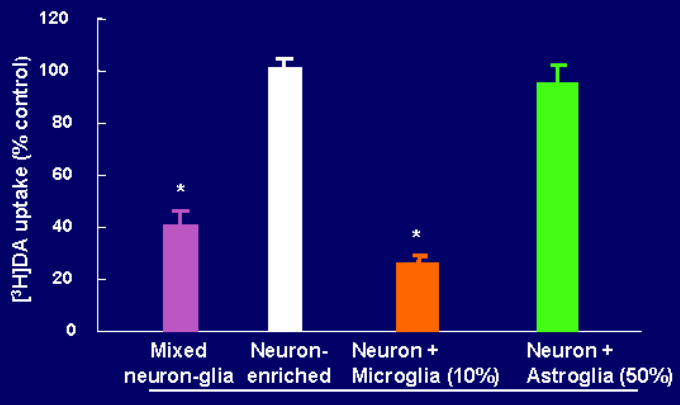
Elucidation of Mechanism of Inflammation-mediated Dopaminergic Neurotoxicity: Role of Microglia

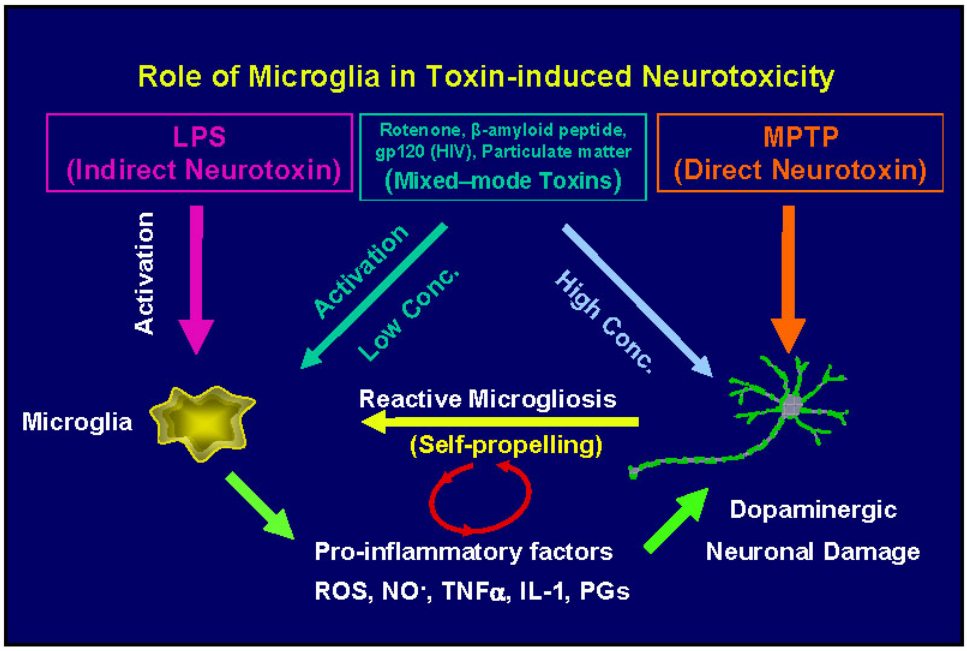
Rationale:

The role of inflammation and the cell type responsible in the pathogenesis of PD was not clearly defined .

Liya Qin, Ph.D. Wei Zhang, MD, Ph.D, Yuxin Liu, Ph.D.

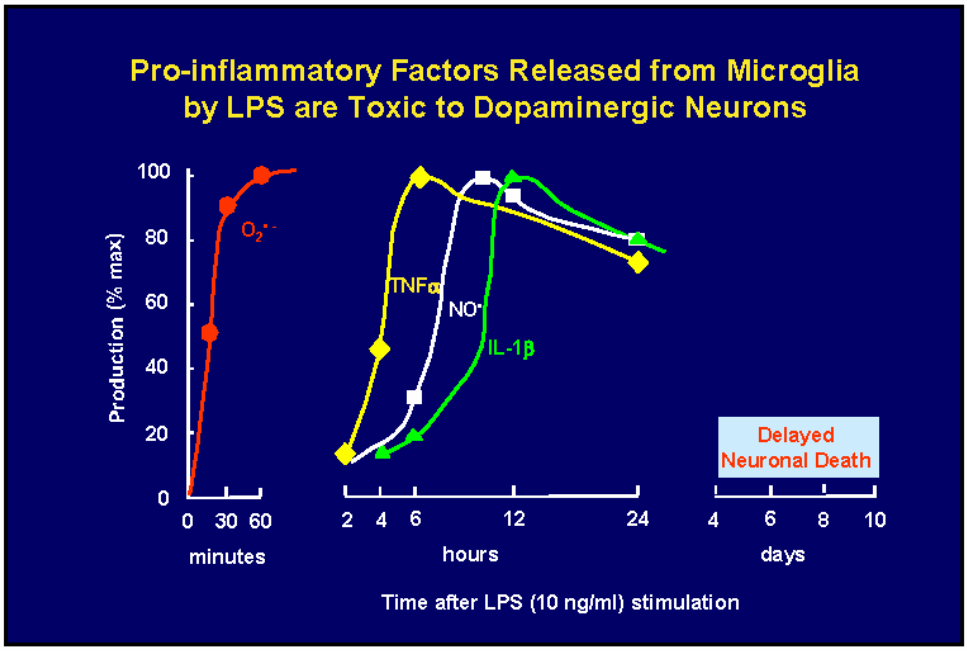
LPS-elicited Dopaminergic Neurotoxicity is Microglia Dependent

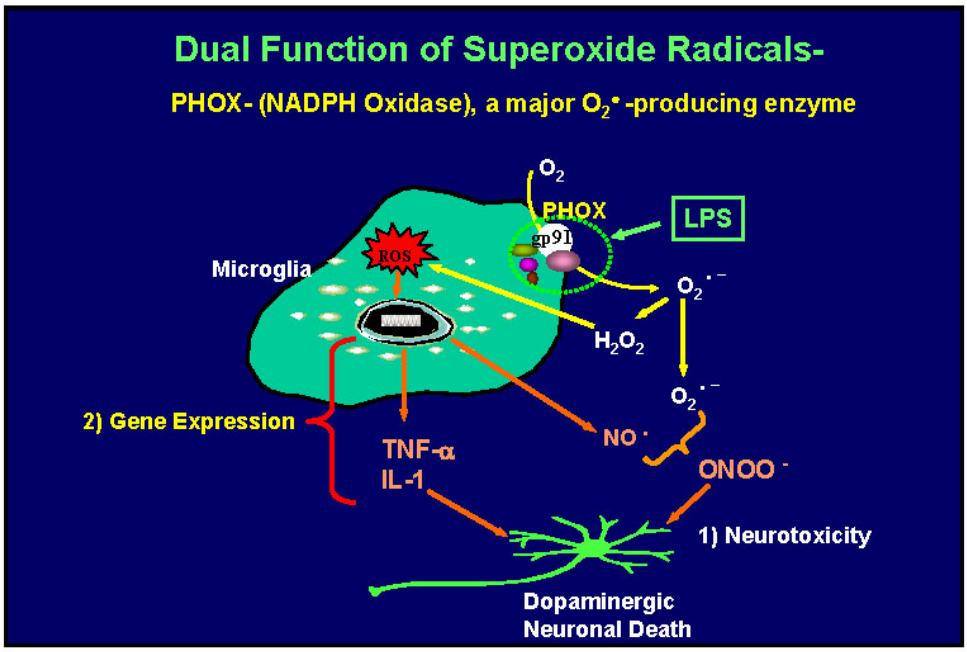


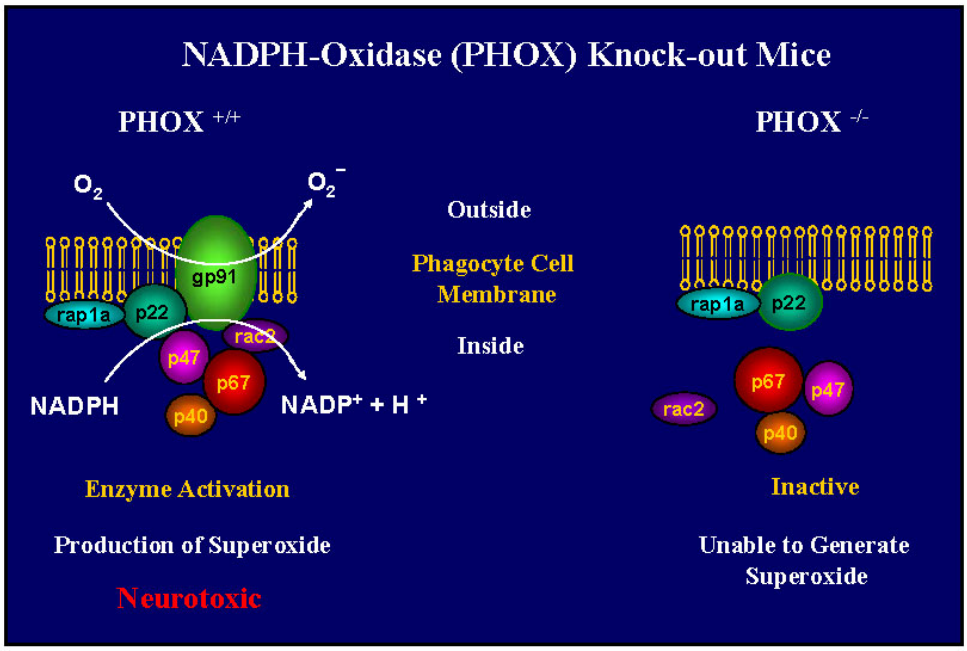


Mixed-mode Toxins

- Infectious agents:
  Bacteria, Fungus, Virus (gp-120, HIV coat protein)
- Pesticides:
  Rotenone, Paraquat
- Heavy metals:
  Manganese, Cadmium etc.
- Air pollutants:
  Particular matter, Diesel engine exhaust, Nano-particles


Pro-inflammatory Factors Released from Microglia by LPS are Toxic to Dopaminergic Neurons
Production (% max)
100
80
60
40
20
0
$O_2^{\cdot -}$
TNFα
NO·
IL-1β
0 30 60
minutes
2 4 6 12 24
hours
Delayed Neuronal Death
4 6 8 10
days
Time after LPS (10 ng/ml) stimulation


Dual Function of Superoxide Radicals-
PHOX- (NADPH Oxidase), a major $O_2^{\cdot -}$-producing enzyme
O_2
PHOX
gp91
LPS
ROS
Microglia
$O_2^{\cdot -}$
H_2O_2
$O_2^{\cdot -}$
2) Gene Expression
TNF-α
IL-1
NO·
ONOO⁻
1) Neurotoxicity
Dopaminergic Neuronal Death

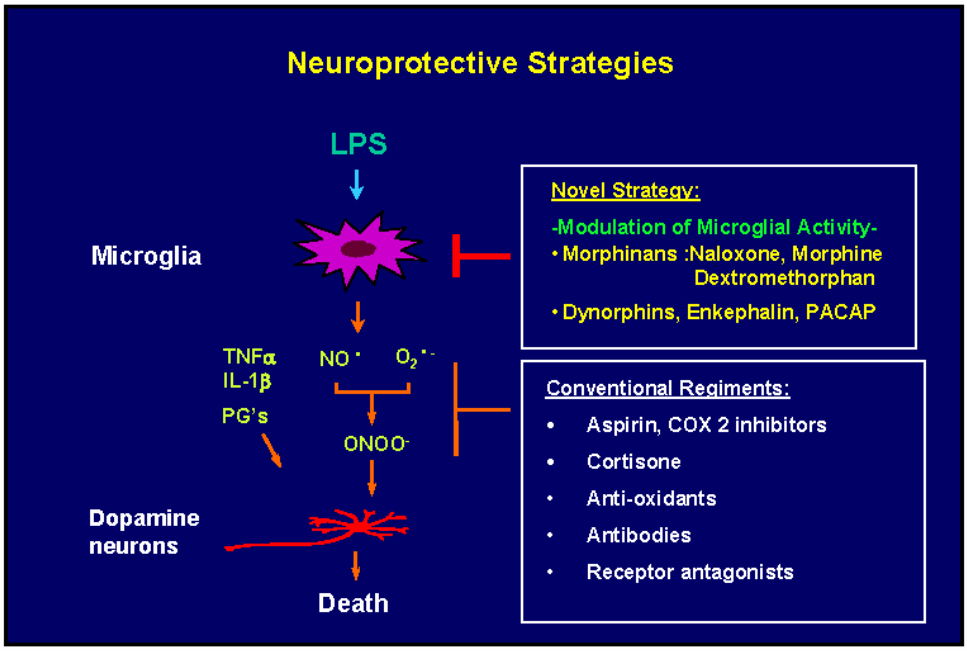


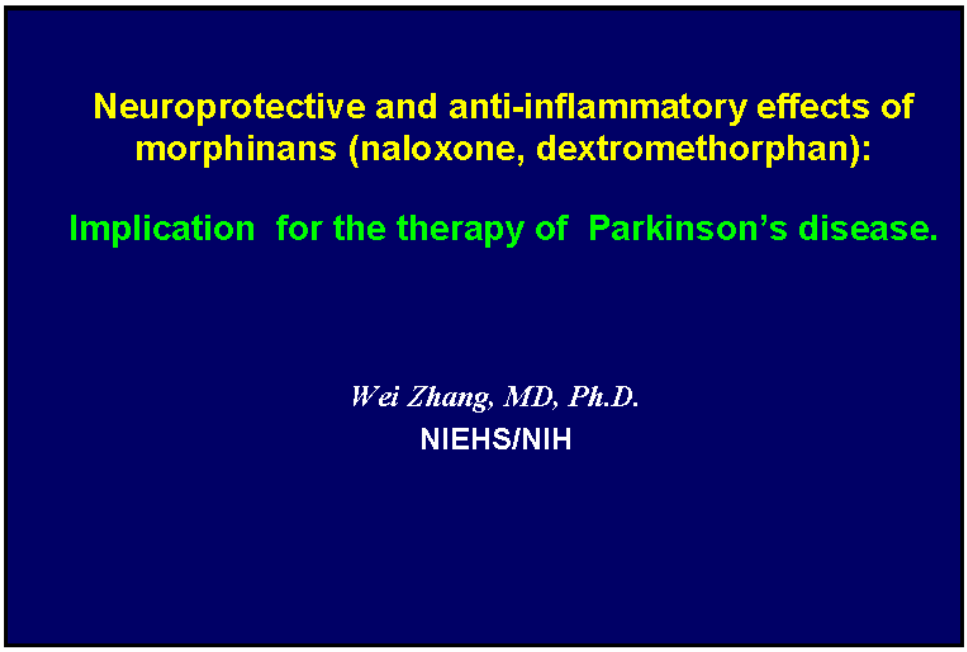
AIM 3

Development of Novel Anti-inflammatory Therapy for Parkinson's Disease

Rationale:

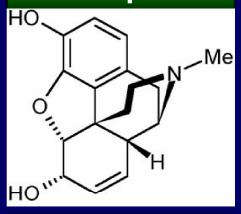
1. Current therapy (L-DOPA) does not slow the progression of PD.
2. Clinical trials show that anti-inflammatory therapy of PD is effective. However, due to their low potency and safety issues, current anti-inflammatory drugs are not suitable for long-term therapy.
3. More potent and safer anti-inflammatory drugs are urgently needed.


Neuroprotective Strategies
LPS
Microglia
TNFα
IL-1β
PG's
NO·
$O_2^{\cdot -}$
ONOO⁻
Dopamine neurons
Death
Novel Strategy:
-Modulation of Microglial Activity-
• Morphinans :Naloxone, Morphine Dextromethorphan
• Dynorphins, Enkephalin, PACAP
Conventional Regiments:
• Aspirin, COX 2 inhibitors
• Cortisone
• Anti-oxidants
• Antibodies
• Receptor antagonists

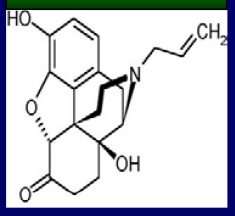

Neuroprotective and anti-inflammatory effects of morphinans (naloxone, dextromethorphan):
Implication for the therapy of Parkinson's disease.
Wei Zhang, MD, Ph.D.
NIEHS/NIH

Morphinans which are anti-inflammatory and neuroprotective

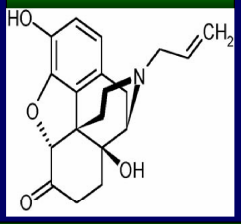
Morphine



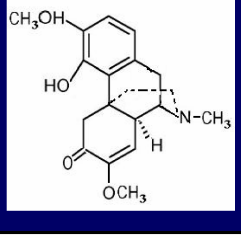
Naloxone



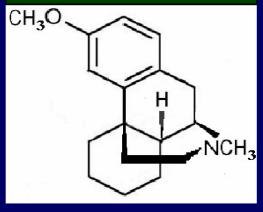
Naltrexone



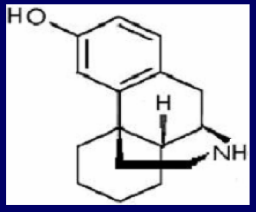
Sinomenine



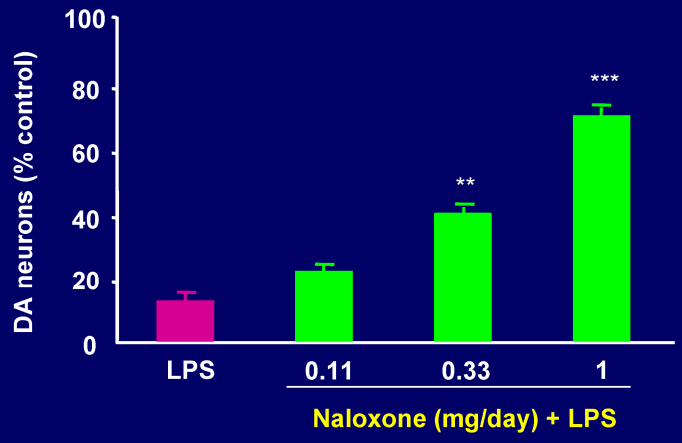
Dextromethorphan

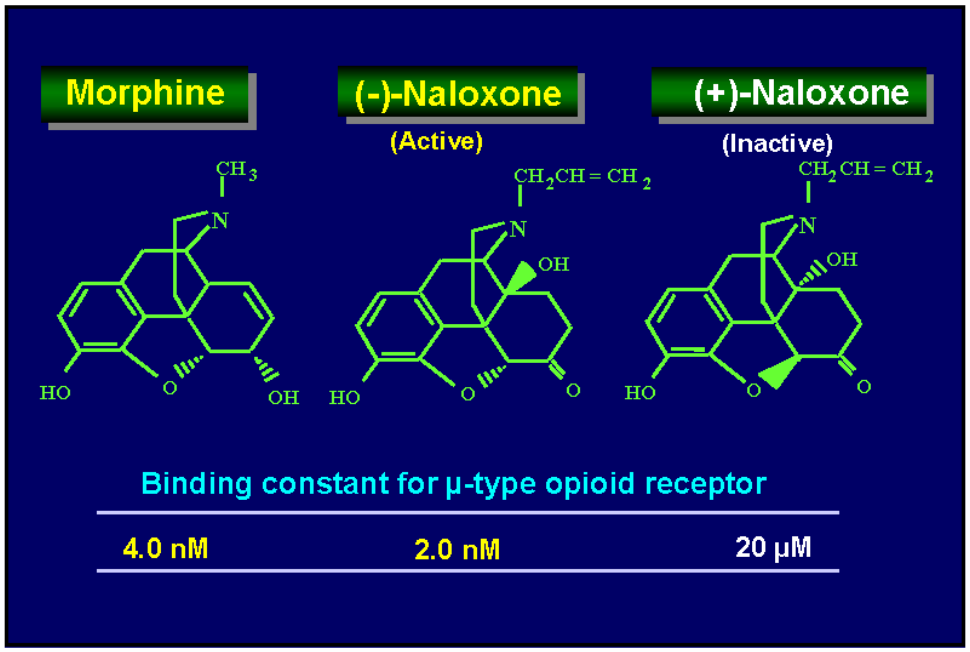


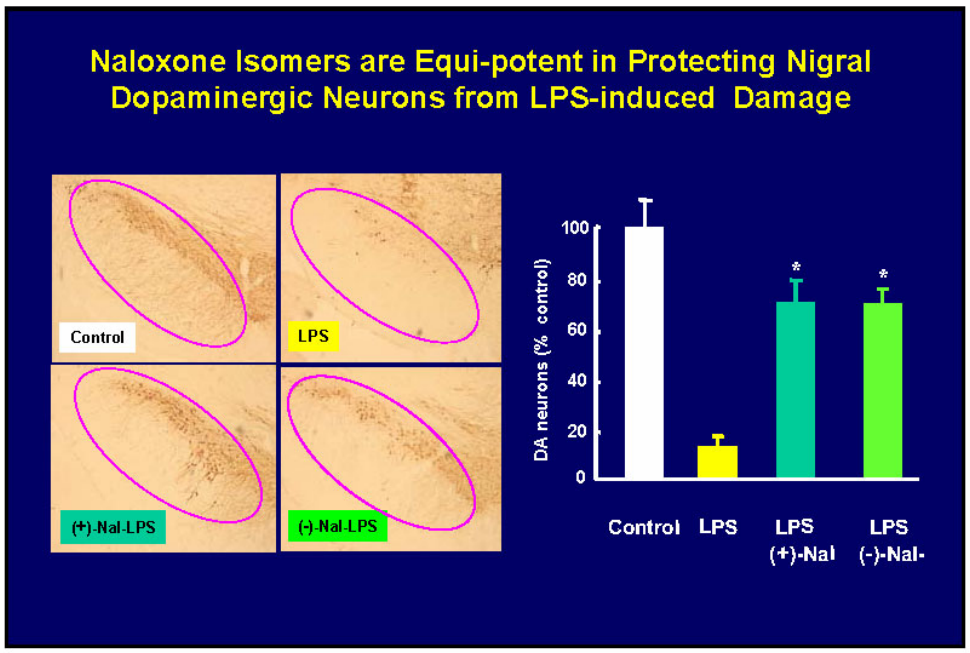
3-Hydroxymorphinan



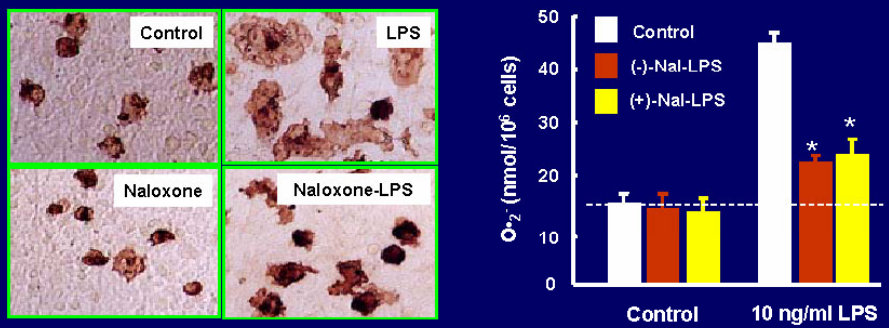
Naloxone Reduces LPS-induced Loss of Nigral Dopaminergic Neurons (*in vivo* study)



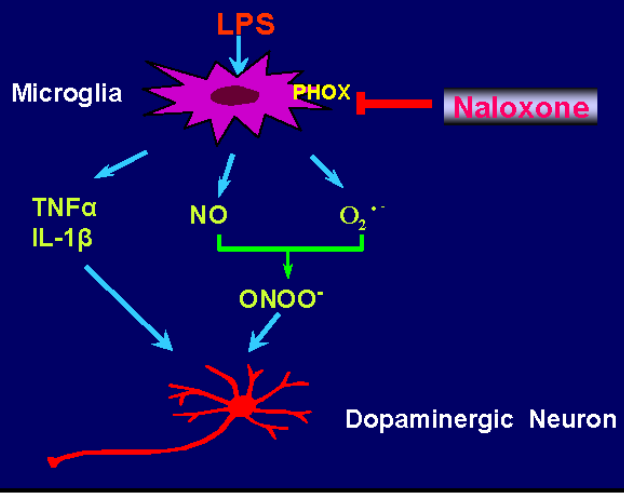

Morphine
(-)-Naloxone
(Active)
(+)-Naloxone
(Inactive)
Binding constant for μ-type opioid receptor
4.0 nM
2.0 nM
20 μM


Naloxone Isomers are Equi-potent in Protecting Nigral Dopaminergic Neurons from LPS-induced Damage
Control
LPS
(+)-Nal-LPS
(-)-Nal-LPS
DA neurons (% control)
100
80
60
40
20
0
Control
LPS
LPS (+)-Nal
LPS (-)-Nal-

Naloxone Inhibits LPS-induced Activation of Microglia and Production of Superoxide



Microglial PHOX is the Site of Action for Naloxone-elicited Neuroprotective Effect



What is LDN (Low Dose Naltrexone)

1. Low Dose Naltrexone (LDN) is a treatment for MS and other neurological diseases. This method was devised and developed by Dr. Bernard Bihari, a neuro-physician in New York.

2. It has been reported that naltrexone (3 mg per day, in contrast to 150 mg per day a day) is beneficial for patients who suffer from cancer, AIDS and neurological disorders, such as MS, PD.

3. How Naltrexone Works: The benefits are due to the temporary inhibition of brain opioid receptors and production of endorphins. This results in the reduction of painful symptoms and an increase sense of well-being.

Low-dose Naltrexone and Pain
(Pain Therapeutic Inc.)

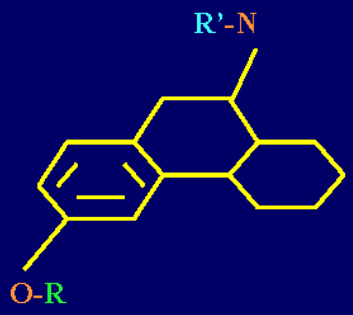
1. Inhibition of pain by opioid painkillers is achieved by inhibiting nerve cells that have opioid receptors

2. Opioid painkillers also activate an excitatory signaling pathway linked to opioid receptors, thereby stimulating the transmission of pain.

3. Tolerance and physical dependence can be prevented by co-administration of ultra-low-dose naltrexone , an opioid antagonist. We believe ultra-low-dose naltrexone blocks the excitatory pathway, but not the inhibitory pathway, on opioid receptors.

4. The inhibition of excitatory signals enhances analgesia and attenuates tolerance, physical dependence and addiction.

Structure of 3-hydroxy-morphinan (3-HM): a metabolite of DM



	R	R'
DM	CH_3	CH_3
3-HM	H	H

Potential Beneficial Effect of Morphinans

Opioid-related?

- *Alcohol abuse*
- *Compulsive eating disorder*
- *Opiate addiction*
- *Smoking*

Non-opioid and/or inflammation-related

CNS

- Alzheimer's dis.
- Brain Ischemia.
- Parkinson's dis.
- MS
- Spinal injury

Peripheral

- Asthma
- Arthritis
- Arteriosclerosis
- Cancer
- Diabetes
- Heart attack
- Hepatitis
- Inflammatory pain
- Irritable bowl dis.
- Lupus
- Sepsis


NIEHS


NIEHS