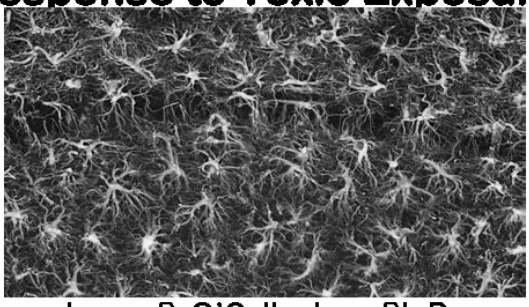
Presentation 5 – Jim O'Callaghan

Biological Mechanisms Potentially Associated with GWI: Neuroinflammation/Cytokine Activation in Response to Toxic Exposures



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Outline

- GWI & Neuroinflammation: links/definitions
- Neuroimmune vs. Immune-neuro
  - "Hostage Brain" (McEwen)
  - Autonomic nervous system (Tracey)
- Glia as targets/mediators/modulators
  - Role of TNF-α
- Modulation of "inflammatory" signaling as therapy

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Definitions/Links

- Neuroinflammation: hard to define
  - Synthesis/release of proinflammatory mediators (cytokines/chemokines)
  - Monocytes/Neurotrophils/Macrophages in the periphery/microglia in the CNS
- Links to GWI symptoms????
  - Chronic fatigue
  - Neurocognitive effects/depression
  - Chemical sensitivity
  - PTSD-like symptoms
  - Pain
  - Persistence after initiating event ("memory")
- Answer: yes…implicates cytokines and glia
  - altered cytokine profiles already reported in GW vets

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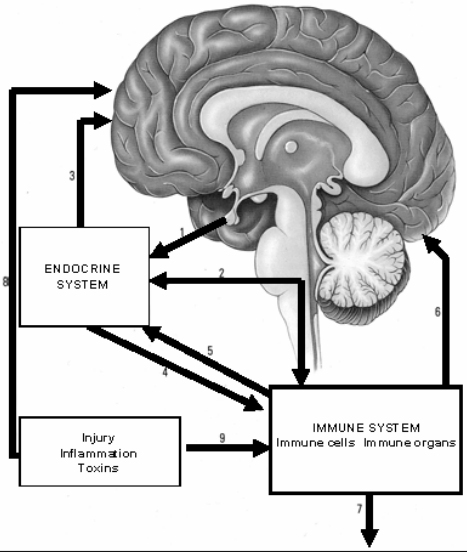
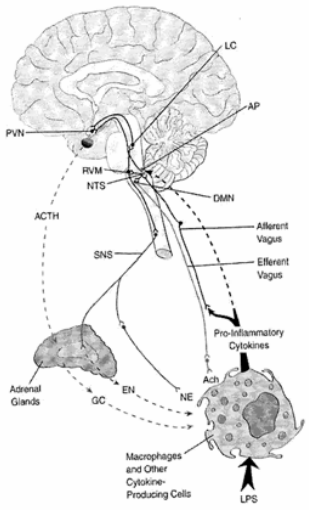
Neuro-Immune/Immune-Neuro Relationships



1. Pituitary-endocrine stimulation
2. Neuronal regulation of hormone secretion
3. Endocrine control of brain
4. Endocrine control of immune system
5. Immune system control of endocrine function
6. Immune system control of brain (Tracey's Immune-Neuro)
7. Immune surveillance
8. Stimulation of brain response by injury, inflammation and toxins
9. Stimulation of immunity and cytokine production by injury, inflammation and toxins

Adapted from McEwen BS and Schmeck HM "The hostage brain" Rockefeller University Press, NY (1994)

Autonomic Nervous System as Target and Regulator of Immune Function: Central Nervous System and HPA Remain Key Players



Adapted from Pavlov VA and Tracey KJ "Neural regulators of innate immune responses and inflammation", Cell Mol. Life Sci. 61, 2322-2331 (2004)

Pro-inflammatory cytokine cascade

Insult	Inflammatory response	Cellular response
Trauma, Stroke, Infection, Toxins	↑ TNF-α, IL-1β Other Cytokines/Chemokines	NF-κB → ↑ IL-6 Tissue damage

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Cytokine Theory of Disease

- **Immune and Nervous System Communicate Via Cytokine Signals**
- **Cytokines are "Proinflammatory" in nature (but some anti-inflammatory)**
- **Dysregulated Cytokine Signaling (usually viewed as an increase) leads to debilitating immune related disease**
  - Rheumatoid arthritis as extreme example
  - Depression as a potentially more subtle example (sickness behavior)
- **Regulation and termination of cytokine signaling is mediated via HPA**
  - Glucocorticioids suppress cytokines (clinically and experimentally)
  - Dexamethasone suppression test used to test HPA axis

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Cytokine Theory of Disease and GWI

- Is there a GWI cytokine "phenotype"?
- There Are Some Supporting Data:
  - **Elevated IL-2, IL-10, TNF-α and IFN-γ (Th1 phenotype)**
  - **Th2 phenotype not prevalent (glucocorticoid responsive)**
- Is it PTSD?
  - **No, not associated with elevated serum IL-6**

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Cytokine Theory of Disease and GWI

- Data Gaps:
  - Complete serum cytokine profiles
  - Glucocorticoid Responsiveness (Dexamethasone Suppression Test)

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Cytokine/Chemokine Blockers as Therapeutics

1. Original Hypothesis: One disease, one ("bad") cell, one cytokine
2. Reality: many cells, many cytokines, many therapeutic targets

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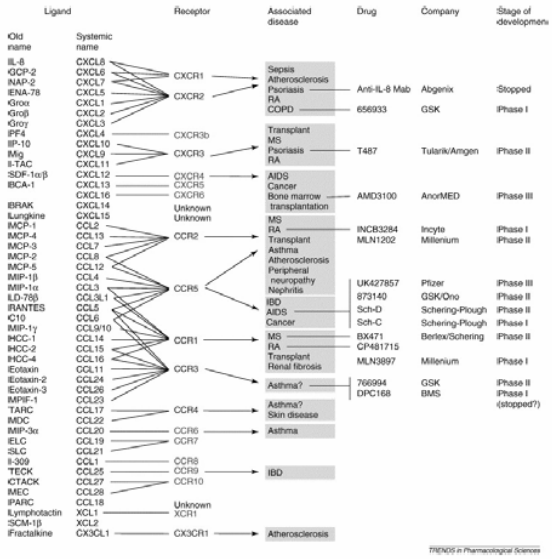
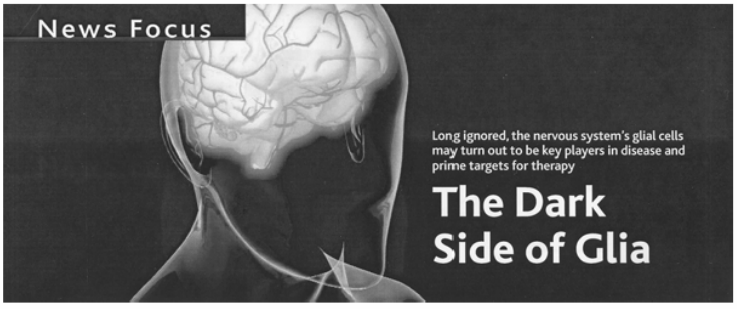
Chemokines as Examples of Immune System Complexity: Diverse Targets Dictate Diverse Therapies



Adapted from Wells et al, "Chemokine blockade – time to therapeutic trials in the making?" Trends Pharmacol Sci, 27, 41-47 (2006)

Cytokine/Chemokines, Glia and Neurotoxicity



Science 308: 778-781, 205

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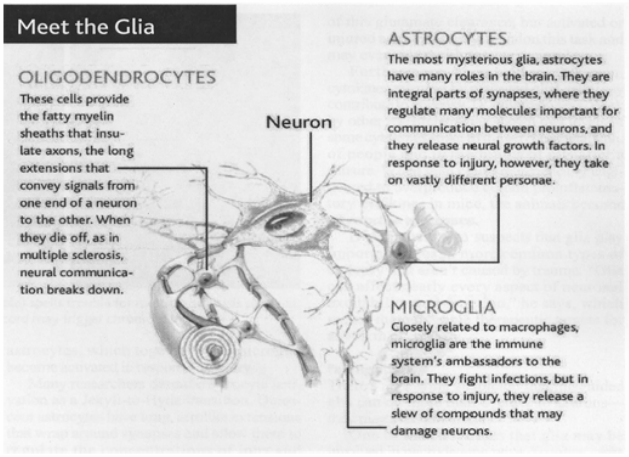
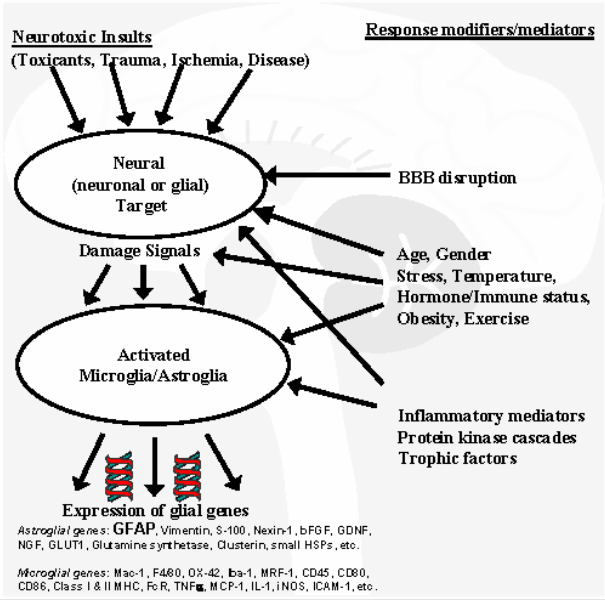
More Background on the "Dark Side"

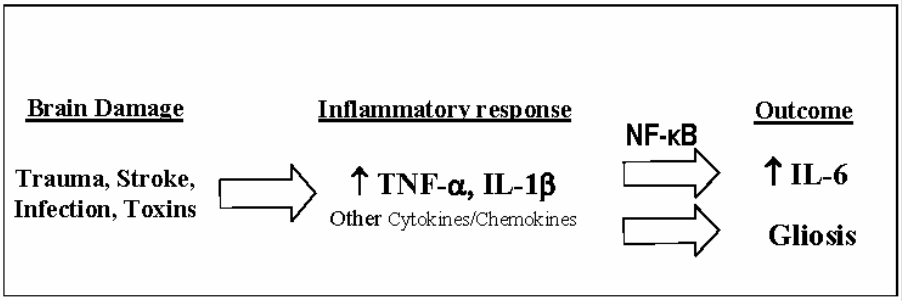


Science 308: 778, 2005

CELL-SIGNALING EVENTS ASSOCIATED WITH INJURY-INDUCED NEURAL DAMAGE & GLIAL ACTIVATION



Pro-inflammatory cytokine cascade and gliosis



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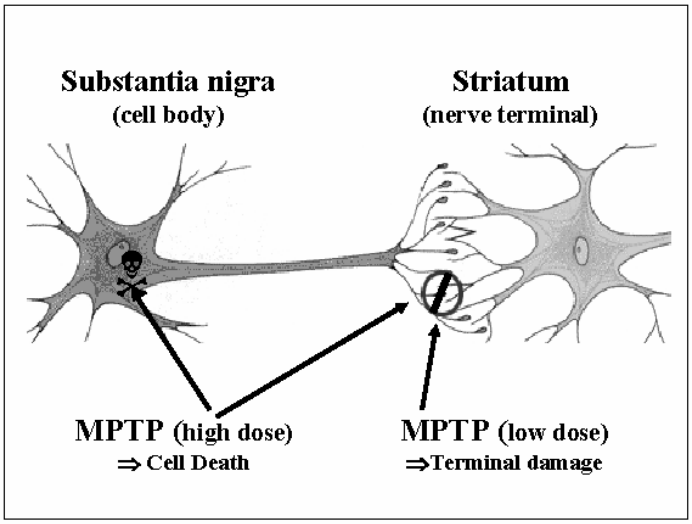
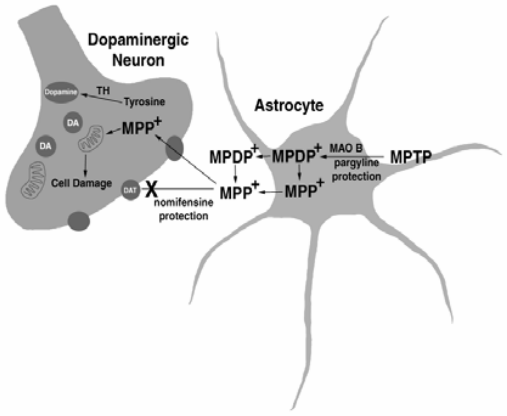
Dose-Related Targets for MPTP (The nigro-striatal dopaminergic pathway)



CARTOON REPRESENTATION OF THE MECHANISM OF MPTP-INDUCED NEUROTOXICITY

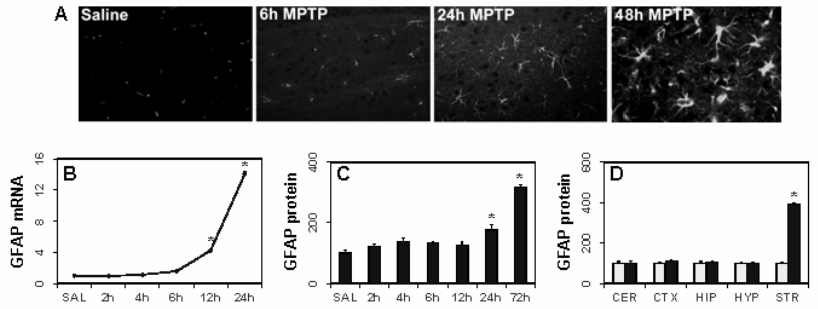


Adapted from R.E. Heikkila

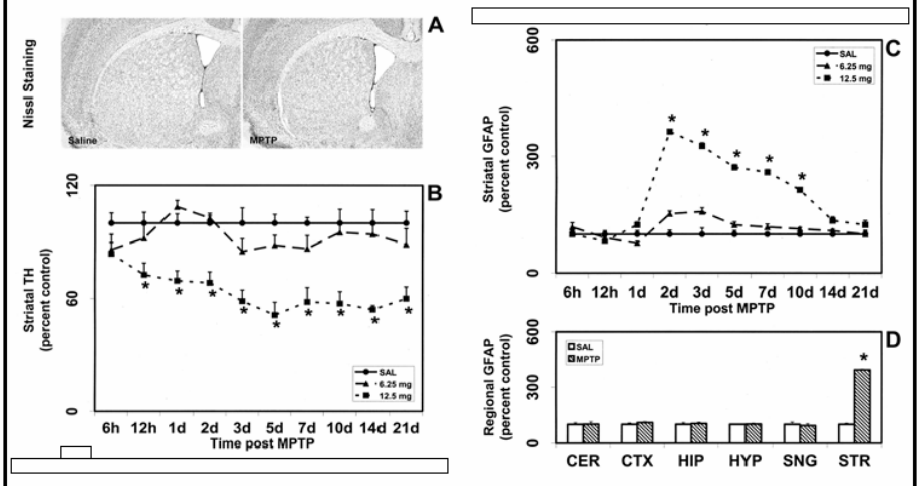
Indices of Neurotoxicity

- **Generic:**
  Cell loss-NOT seen in our dosing models
  Gliosis (GFAP assay/isolectin staining)
  Silver degeneration/Fluoro-Jade staining
- **Dopaminergic (When combined with above):**
  Dopamine
  Tyrosine hydroxylase (levels, immunohisto)

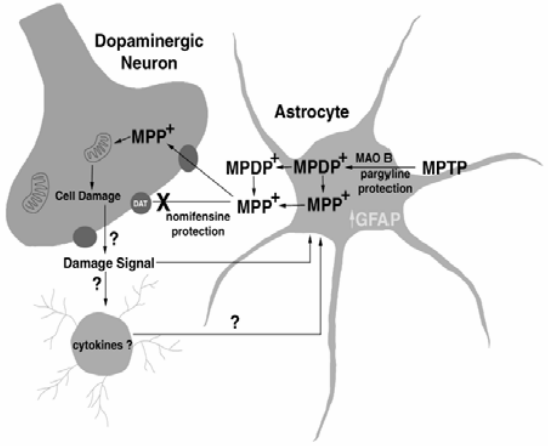
TIME COURSE AND LOCATION OF GFAP INDUCTION FOLLOWING TREATMENT WITH MPTP



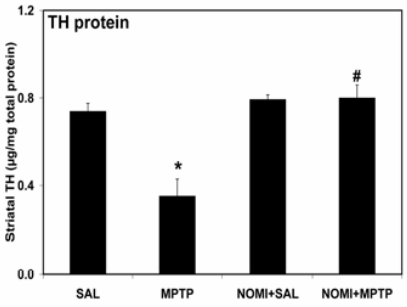
TIME COURSE AND TARGET SPECIFICITY OF MPTP NEUROTOXICITY

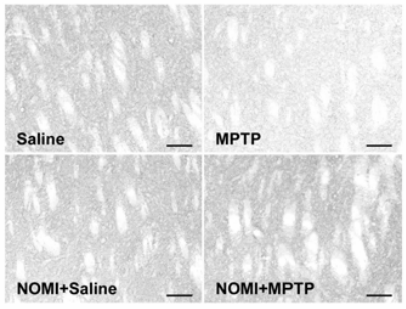


MPTP MODEL OF REACTIVE GLIOSIS

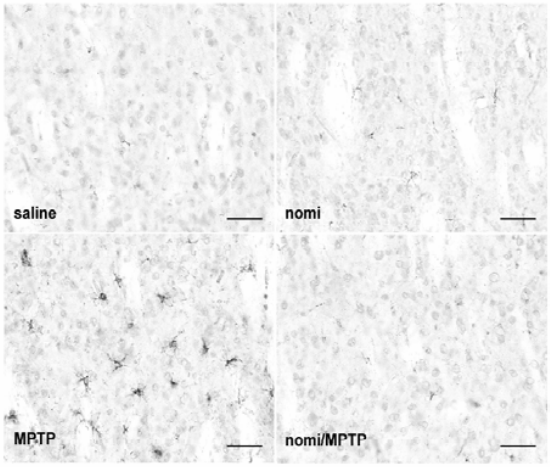


Inhibitors of Dopamine Uptake Protect Against MPTP-Induced Decreases in TH





Nomifensine Protects Against MPTP-Induced Activation of Microglia



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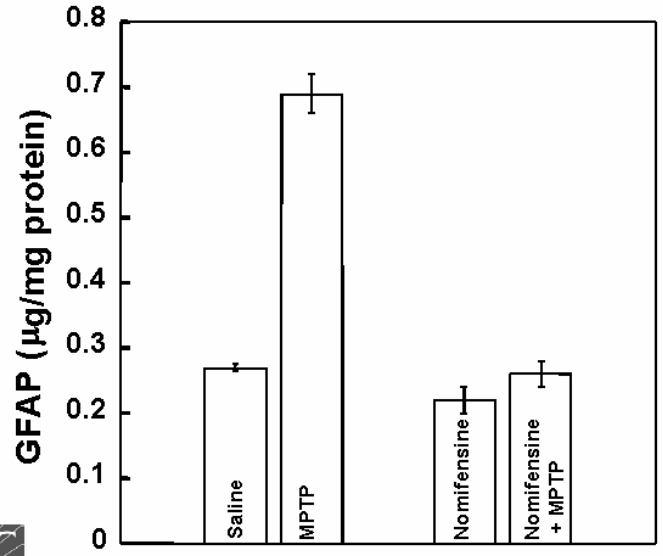
Inhibitors of Dopamine Uptake Protect Against MPTP-induced Increase in GFAP



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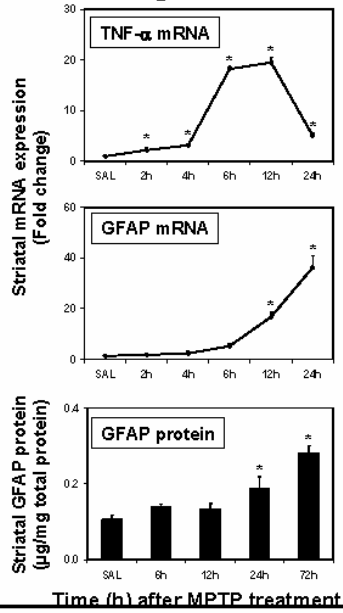
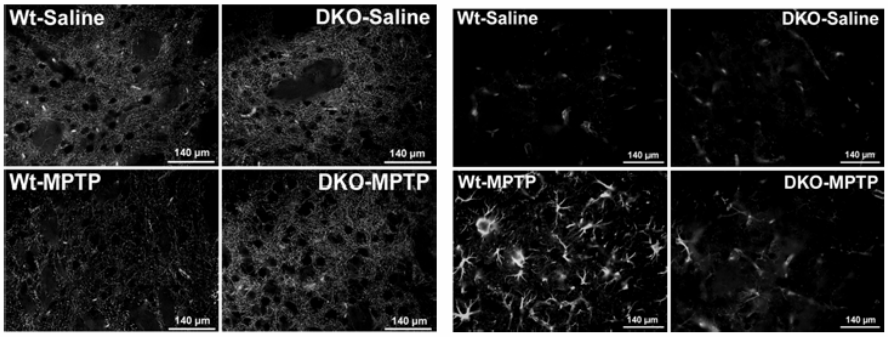
Tumor Necrosis Factor-α

- Proinflammatory Cytokine in the Periphery
- Effects Mediated through 2 receptors
- Role in CNS unknown
- Enhanced Expression in Brain Linked To:
  1. Parkinson's Disease
  2. HIV-dementia
  3. Activation of Microglia

MPTP-mediated expression of TNF-α precedes gliosis



Loss of striatal TH and GFAP immunoreactivity caused by MPTP is abolished in TNF receptor-deficient mice



Tyrosine Hydroxylase GFAP

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OK, TNF-α is "Bad" but can suppressed in the periphery by Glucocorticoids

Will Glucocorticoids suppress brain damage and TNF-α in the CNS?

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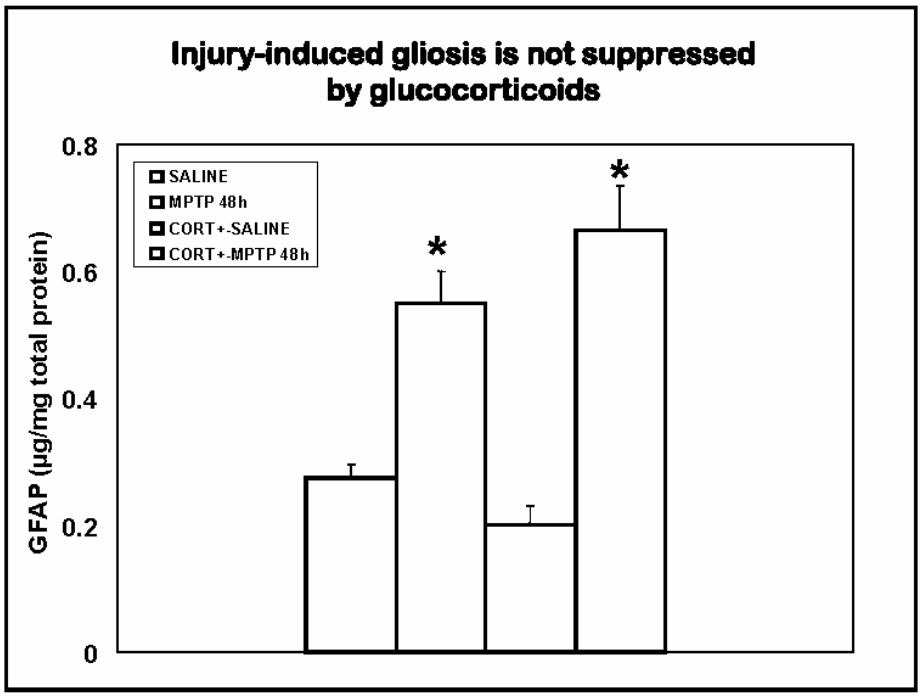
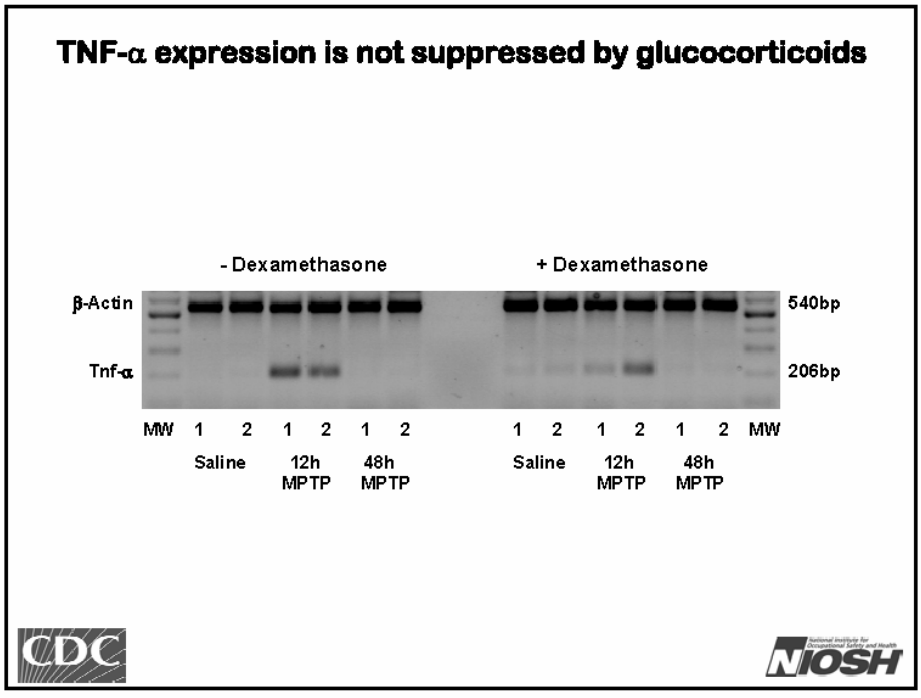

Injury-induced gliosis is not suppressed by glucocorticoids
GFAP (µg/mg total protein)
0.8
0.6
0.4
0.2
0
SALINE
MPTP 48h
CORT+-SALINE
CORT+-MPTP 48h
*
*


TNF-α expression is not suppressed by glucocorticoids
- Dexamethasone
+ Dexamethasone
β-Actin
Tnf-α
540bp
206bp
MW 1 2 1 2 1 2 1 2 1 2 1 2 MW
Saline
12h MPTP
48h MPTP
Saline
12h MPTP
48h MPTP
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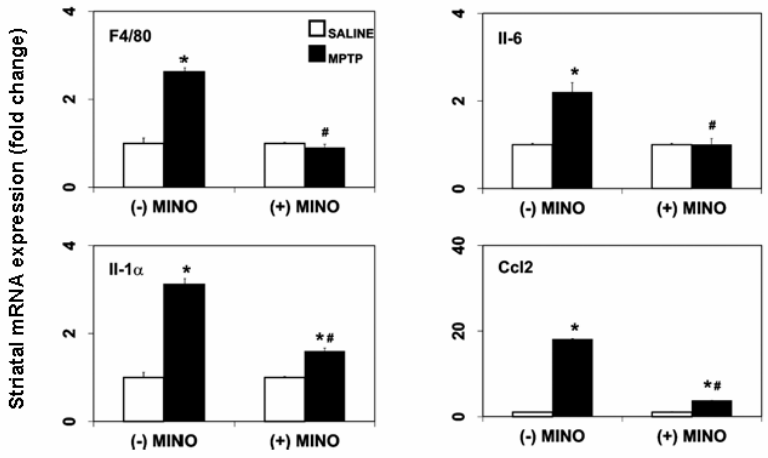
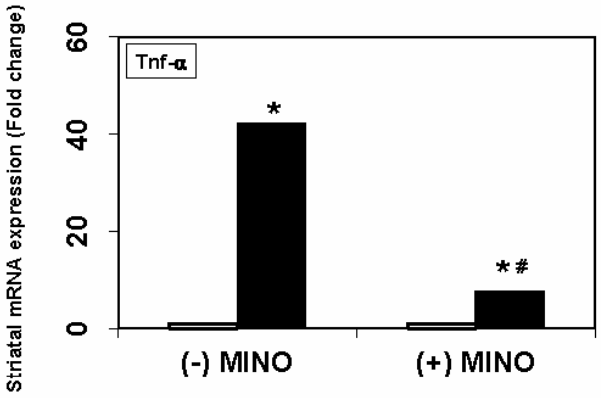
Use minocycline to block microglial activation?

- Broad spectrum tetracycline derivative antibiotic
- Anti-inflammatory properties
- Reported to block microglial activation
- Reported to block nigral cell loss after (high dose) MPTP

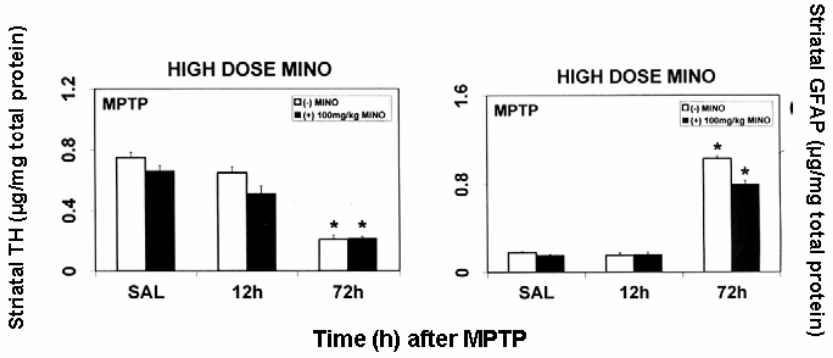
Minocycline suppresses the striatal expression of microglial factors following MPTP



Minocycline attenuates striatal TNF-α expression after MPTP



Minocycline Does Not Affect MPTP Neurotoxicity and Gliosis



OK…TNF is bad but…not so fast..

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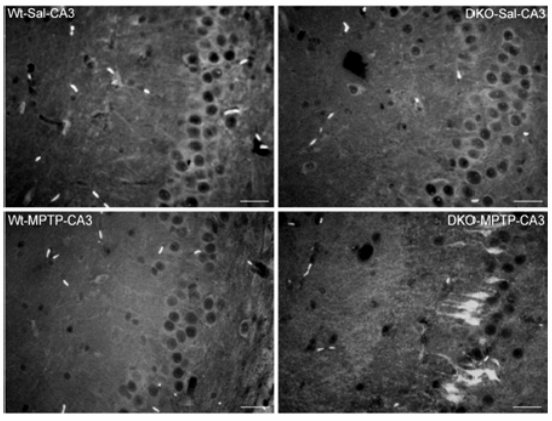
Role of TNF-α in Neurodegeneration is *NOT* Simple: F-J Staining is seen in hippocampus of TNF receptor-deficient mice treated with MPTP



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A few words from Richard Ransohoff on *in vivo* vs. *in vitro* cytokine data

"…..comparisons of *in vitro* studies of explanted CNS cells with *in vivo* date (e.g. in-situ hybridization) show that tissue disruption and cell culture dysregulates the chemokine system. Therefore, it is Perilous to extrapolate the situation *in vivo* from results *in vitro*."

Ubogu, et al., Trends in Pharmacol. Sci. 27: 49, 2006

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Some take home messages

1. Data exist suggestive of in involvement of dysregulated immune signaling in GWVI
2. Neuro-immune and immune-neuro interactions are complex and reciprocal; they pose multiple targets for diagnosis and therapy
3. Expanded serum cytokine profiling and immune function tests of GW veterans may aid in revealing the GWI phenotype
4. Therapeutics that affect immune signaling in the periphery may not modulate CNS immune signals or they may inappropriately disrupt normal beneficial effects of cytokine signals in the CNS

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